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## Detection of approved and unapproved GMO by the "matrix approach"

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**Co-existence between GM  
and non-GM based agricultural supply chains**

14-15 November 2005, Montpellier (France)



**Edited by Antoine Messéan**

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PROCEEDINGS

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**Co-existence between GM and  
non-GM based agricultural supply chains**

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A. Messéan

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## Foreword

The PDF version of the proceedings of the "second international conference on co-existence between GM and non-GM based agricultural supply chains (GMCC-05)" includes some minor editing changes as well as two significant modifications :

- in the paper by T. Hirzinger *et al.* "Effects of the Regulation (EC) No 1829/2003 and 1830/2003 on the German Food Industry" pp. 125-129, the correct summary has been included;
- the paper by Matthews *et al.* "Predictive models for field separation, based on outcrossing data on oilseed rape and maize from the UK Farm Scale Evaluation programme" pp. 193-197, has been revised as the printed version does not clearly acknowledge CSL (York, U.K.) which generated the data used in the paper."

# Preface

The new European legal framework aims at reinforcing the assessment of GMOs, ensuring the traceability of products and clearly informing consumers through reliable labelling. In addition, there are EU guidelines designed to allow for the co-existence of various kinds of agriculture by ensuring that “farmers should be able to cultivate freely the agricultural crops they choose, be it GM, conventional or organic”. Such GMO dedicated regulations are part of a more general international trend to enable reliable co-existence and traceability for delivery of food and feedstuffs complying with the consumer requests.

Meeting this co-existence requirement is a real challenge for scientists, stakeholders and policy-makers. Indeed, on-farm gene flow through pollen or dispersal and commingling within supply chains highly depends on crop biology, farming systems and industrial processes. Ensuring co-existence requires changes in management practices from seeds to fork.

What are the implications for the on-farm management of agricultural landscapes? What kind of coordination between local actors and activities – agricultural and non-agricultural – within rural areas could be applied? What traceability, control and certification schemes could be implemented along the chains? How

to ensure supply chains co-existence not only in space but also over time? What are the legal and economic implications of co-existence in the framework of an open market facing globalization and international regulations? More generally, co-existence has various technical, economic, environmental, social and legal implications that should be addressed under a generic perspective as they would equally apply to a wide range of conventional agricultural systems. Multi-disciplinary research should highlight the issues at stake by a better understanding of biological, technical, legal, economic and social processes, designing biological systems and management scenarios for co-existence as well as by providing methods and tools for assessing their relevance and implications.

After GMCC-03 the first international conference on co-existence, held in Denmark in November 2003, GMCC-05 gives the opportunity to the scientific community, the regulators and the stakeholders to share experiences on co-existence and scientific findings from ongoing research projects as well as to discuss new scientific issues resulting from co-existence.

**Antoine Messéan**

*Chairman of the programme committee*



# **ORAL PRESENTATIONS**



# **INVITED PAPERS**

**Plenary session 1:  
GLOBAL OVERVIEW**





# International legal aspects of the co-existence between GM and non-GM products: approaches under international environment law and international trade law

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## INTRODUCTION

The development and commercialization of GMOs raises the broadest and most controversial array of issues concerning food and agriculture today. This takes place in a context of sharp contrast at present between the widespread international acceptance of biotechnology's benefits in pharmaceuticals and in industrial products, and the rapidly growing concerns about its possible dangers in agricultural and food production. Countries' positions on agro-biotechnology depend on many factors, such as their policy awareness, the level of risk they are willing to accept, their capacity to carry out risk assessments in this sector and to implement adequate legislation, their perception of the benefits they could gain from biotechnology, their dependence on agricultural exports, their reliance on food aid, or the investments they have already made in the sector<sup>1</sup>.

Assessments of the risks and benefits related to agro-biotechnology vary substantially between countries and regions, and so do the regulatory approaches (rules on GM approval, marketing, import regulations, labelling, documentation)<sup>2</sup>. When GM products are commercialized internationally, as has been the case since the second half of the 1990s, the diverging domestic requirements may hamper international trade in agro-biotechnology products and further complicate an already difficult regulatory trade system in the agricultural sector. While the estimated global area of transgenic or genetically modified crops continues to increase, the vast majority of acreage (around 99%) remains confined to just four countries, namely the US, Argentina, Canada and China.<sup>3</sup> In most developing countries it is still not legal to plant GM crops on a commercial basis, largely due to barriers in the approval process. Even countries that have in the past moved rapidly on the adoption of GMOs, including China and Argentina, are now

slowing down the approval processes. While the regulatory blockages are usually justified on biosafety grounds, trade concerns appear to play an increasing role with countries fearing export losses in markets such as the EU, Japan and Korea where the import regulations for GMOs continue to be tightened<sup>4</sup>.

These elements raise the issue of the “co-existence” between GM and non-GM products in legal terms. Co-existence raises some pragmatic questions at the domestic level: would it be viable for the commercial production of GM crops to coexist with existing conventional and organic systems of agricultural production in a way that assures continuing real choice to consumers? Could realistic measures be devised and implemented to ensure that these different sorts of farming can coexist, with domestic agriculture continuing to offer consumers the present choice of conventional and organic products side by side with GM products?<sup>5</sup> At the heart of the co-existence debate in industrialized countries with lower risks of cross-pollination, the key issue is more likely to be consumer choice, predominantly expressed as domestic consumers being able to continue to choose to eat non-GM or organic food products<sup>6</sup>.

Paradoxically, the concept of «co-existence» is not defined under national and international legal instruments. However, «co-existence» is a concept used more and more in the political arena<sup>7</sup> and through different bodies which are working on biotechnology issues<sup>8</sup>. “Interference” is sometimes used to express the opposite of co-existence<sup>9</sup>.

To our knowledge, the only legal trace of “co-existence” can be found, without a specific definition, in the European Community Law. A legal basis for EU Member States to take national measures to promote co-existence of organic and conventional crops with GM crops is introduced in Regulation 1829/2003 of the European Parliament and of the Council on genetically modified food and feed:

Member States may take appropriate measures to avoid the unintended presence of GMOs in other products. The Commission shall gather and coordinate information based on studies at Community and national level, observe the developments regarding co-existence in the Member States and, on the basis of the information and observations, develop guidelines on the co-existence of genetically modified, conventional and organic crops<sup>10</sup>.

In this perspective, the European Commission published guidelines in 2003 “for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming”<sup>11</sup>. Under the guidelines, it is said: “Co-existence 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”. The issue of co-existence addressed in the European Commission Recommendation concerns the potential economic loss and impact of the mixing of GM and non-GM crops, and the most appropriate management measures that can be taken to minimize mixing. Indeed, the Recommendation reads as follows:

The adventitious presence of GMOs above the tolerance threshold set out in Community legislation triggers the need for a crop that was intended to be a non-GMO crop, to be labelled as containing GMOs. This could cause a loss of income, due to a lower market price of the crop or difficulties in selling it. Moreover, additional costs might incur to farmers if they have to adopt monitoring systems and measures to minimize the admixture of GM and non-GM crops. Co-existence is, therefore, concerned with the potential *economic impact* of the admixture of GM and non-GM crops, the identification of workable management measures to minimize admixture and the cost of these measures.

Thus, under the European system, co-existence is seen as an «economical» issue and not as an «environmental» issue.

The European Commissioner for Agriculture has also expressed the principle that «farmers should be able to cultivate freely the agricultural crops they choose, be it genetically modified crops, conventional or organic crops». He recognized that, unless special measures were taken, the commercial cultivation of GM crops might result in the «adventitious presence of GM crops in non-GM crops and vice-versa»: in other words, GM plant material might turn up in a crop which was intended to be non-GM, or vice versa<sup>12</sup>. Co-existence gives rise to potential economic consequences for farmers, because as a result of adventitious presence a crop might fail to meet the relevant standards<sup>13</sup>, and therefore command a lower price on the market.

The Cartagena Protocol on Biosafety for its part, emphasizes the «environmental» and residually the «sanitary» aspects of co-existence between GM and non-GM products or crops<sup>14</sup>.

This difference of emphasis is only the tip of the iceberg. Indeed, the problem of co-existence raises broader issues which will be dealt with in the present contribution, *i.e.* co-existence between different regulatory strategies and co-existence between different technical and procedural frameworks.

### **Co-existence between different «regulatory» strategies**

*Co-existence between different national legal frameworks: The “Conventional risk” approach vs. The “No risk” approach*

GMO regulations are based on an assessment of the actual or potential risks that those products may bring about. Such assessment can be a “conventional” risk assessment or a risk assessment based on the precautionary approach. The former is about relevant scientific evidence, which means that there is sufficient scientific evidence for the perceived risks underlying the measure. Conversely, the “precautionary approach” to risk assessment is concerned with scientific uncertainty, where there is no “adequate

theoretical or empirical basis for assigning possibilities to a possible set of outcomes”<sup>15</sup>. Three basic conditions may thus trigger the application of protective measures: uncertainty, risk, and lack of proof of direct causal links. With respect to GMOs, the problem of defining the relationship between science and policy in risk regulation is by and large a matter of regulatory culture deeply embedded in underlying socio-economic settings<sup>16</sup>.

The United States, Canada and Argentina, major agricultural exporters, have substantially applied the conventional risk assessment approach, especially during the first years of the agro-biotechnology revolution, and have widely authorized most GM products for production and consumption.

Regulators in Europe and Japan, on the other hand, have taken up a more cautious approach based on guaranteeing a very low level of risk to human health and the environment. They have therefore imposed strict control measures on approval and marketing of GMOs and GM products. They have also imposed mandatory labelling schemes to allow consumers to make an informed choice in the market place. For instance, further to the ratification of the Cartagena Protocol on Biosafety, Japan promulgated in June 2003 ‘The Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms’ (LMOs)<sup>17</sup>. The law establishes an approval system for the use of LMOs and includes requirements for the exports of LMOs. Australia and New Zealand have processes for pre-market approval and implement mandatory labelling of GMOs.

Many developing and least developed countries, especially in Africa, still lack, or are in the process of developing, comprehensive regulatory systems to deal with the challenges of agricultural biotechnology. Developing a regulatory framework concerning GMOs may be a costly and lengthy process. Areas for regulation include: (a) research and development (R&D), for example conditions under which laboratory

experiments take place and conditions for testing in contained facilities or in the field; (b) approval processes for commercial release, including prior scientific assessment of risks to human and animal health and the environment, the minimum distance from organic agriculture or non-GM fields, labelling, post-commercialization monitoring, and liability; and (c) import regulations. In setting up domestic legislation, developing countries seem to be paying increasing attention to international trade concerns.

### **Co-existence between different multilateral legal frameworks: The “Cartagena Protocol” Approach vs. The “WTO law” Approach**

The Cartagena Protocol on Biosafety of the Convention on Biological Diversity is the only international regulatory instrument which deals specifically with the potential adverse effects of genetically modified organisms (known as living modified organisms (LMOs) under the Protocol) on the environment, taking also into account effects on human health. The Protocol covers transboundary movements of any genetically modified foods that meet the definition of an LMO. The Protocol distinguishes three categories of LMOs: LMOs for voluntary introduction into the environment – such as seeds for planting, live fish for release, micro-organisms for bioremediation; LMOs destined for contained use, contained use being defined in Article 3(b) of the Protocol to include activities in which LMOs are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment; and LMOs intended for direct use as food or feed, or for processing (LMO-FFPs). The latter represent the large majority of LMOs, *i.e.* genetically modified crops, such as soybean, maize, canola, cotton, etc. The Protocol does not cover consumer products derived from LMOs, such as corn flakes, flour, starch, seed-oil, tomato paste or ketchup, and pharmaceuticals.

Annex III of the Protocol specifies general principles and a methodology for risk assessment of LMOs. The Protocol establishes a harmonized set of international rules and procedures designed

to ensure that countries are provided with the relevant information, through an information exchange system called ‘Biosafety Clearing-House’. This Internet-based information system enables countries to make informed decisions before agreeing to the importation of LMOs. It also ensures that LMO shipments are accompanied by appropriate identifying documentation. While the Protocol is the key basis for international regulation of LMOs, it does not deal specifically with GM foods, and its scope does not consider GM foods that do not meet the definition of an LMO. Furthermore, the scope of its consideration of human health issues is limited, given that its primary focus is biodiversity, in line with the scope of the Convention itself. Consequently, the Protocol alone is not sufficient for the international regulation of GM foods.

The issue of co-existence is not dealt with directly and explicitly by the Protocol. It is particularly seen through the blueprint of «liability and redress». The issue of liability and redress was perhaps the most controversial issue discussed, with developing countries, especially from Africa, pressing for its first Meeting of the Parties (MOP-1) to adopt a strong international regime. They argued, in general, that in the event of accidents or incidents where LMOs cause damage to farmers’ crops, to human health or to the environment, there should be a legally binding regime to determine who is responsible and how redress or compensation can be made. MOP-1 eventually decided to set up a working group of experts on liability and redress. The group will analyze potential and actual damage scenarios of concern in order to identify situations for which international rules may be needed, and how international rules and procedures on liability and redress can be applied to the damage scenarios. It will also elaborate options for rules and procedures, including definition, nature and scope of damage, valuation of damage to biodiversity and human health, threshold of damage, causation, channeling of liability, roles of parties of import and export, standard of liability, mechanisms of financial security and standing or right to bring claims<sup>18</sup>.

An appropriate regime should theoretically be developed by 2008. Unfortunately, this year's MOP-2 has shown that progress on this issue is very difficult to achieve.<sup>19</sup>

The co-existence between GM and non-GM products may also be impeded by the difference between the "environmental approach" of the Cartagena Protocol and the "trade approach"

of the WTO legal system<sup>20</sup>. It seems there are four aspects of the Protocol that might give rise to overlaps with WTO law: (i) the scope for legitimate government action in the absence of conclusive scientific evidence; (ii) risk assessment and risk management; (iii) the socio-economic factors that may be taken into account in the decision-making process; and (iv) documentation obligations. It should also be noted, however, that member countries' obligations under the trade system should be read together and considered cumulative. Thus, WTO rules should be interpreted with a view to avoiding conflicts between them and those included in the Biosafety Protocol.

## **CO-EXISTENCE BETWEEN DIFFERENT "TECHNICAL" AND "PROCEDURAL" FRAMEWORKS**

### **Co-existence between different labelling systems: The "Mandatory" Approach vs. The "Voluntary" Approach**

The co-existence between GM and non-GM products raises the issue of the right to informed choice. This right can be applied, for example, in the debate on labelling food derived from GMOs to ensure that consumers know what they are consuming and are able to make informed decisions. Informed choice and resulting actions require access to information and resources. Consumers do not all have the same access to information and resources to make informed decisions about GMOs. Particularly in developing countries, the very poor (both women and men) may lack the most basic information to make decisions that may affect their health and capacity

to sustain themselves. Appropriate methods to reach the least educated, the poorest and the most disadvantaged groups should form part of any strategy to inform the public so that individuals are able to choose according to their needs<sup>21</sup>.

The differences between the United States' and the European Union's perspectives on the labelling of GMOs illustrate the difficulties of co-existence. In the United States, the law requires information on food products to be clear and unambiguous. Labels are intended to provide meaningful information and to warn and instruct the consumer. Further misleading or unnecessary information is believed to conflict with the right of consumers to be able to choose wisely, and to lessen the effectiveness of essential label information. If GMOs are not different from their traditional counterparts in terms of nutrition, composition or safety, labelling is considered to be unnecessary and perhaps misleading. In the European Union, labelling is viewed as a way to ensure the consumers' right to know any fact that they deem important; it is a way to give consumers a choice and to inform them about GMOs. The European Union's approach to labelling attempts to reach a compromise among the industrial, scientific and public sectors. In the European Union, the question is not *whether* to label products of biotechnology, but *how* to label them<sup>22</sup>.

EU legislation<sup>23</sup> establishes a threshold for the percentage content of GM material above which foods must be labelled as *containing* or being *produced from* a GMO. Food has to be labelled as containing GM material if it has a content of GM elements of 0.9% or more (previously the threshold was 1%). Below that level, it does not have to be labelled, provided that the GM content has been authorized for use in the EU and can be shown to be adventitious and technically unavoidable. Previously there was no tolerance threshold for the adventitious presence in food or feed of GM material that has not been authorized in the EU. The new food and feed regulation provides that there should be a threshold of 0.5% for the adventitious or technically unavoidable

presence of such “unauthorized” GM material, provided that the material has received a favorable EU scientific risk assessment and the operator can demonstrate that its presence was technically unavoidable. But this threshold can only be enforced where it is possible to test for the presence of the material in question; this may not be the case if the nature of the relevant GM material is not known. The new legislation extends the current labelling provisions to all food and feed produced from GMOs, even if they are analytically equivalent to those derived from non-GM sources (that is to say, even if DNA or protein of GM origin is not detectable anymore in the final product). It is argued that this responds to the need to enable consumers to exercise choice.

Some form of mandatory labelling standards for food products produced using gene technology have been adopted or planned by over 30 countries worldwide. These standards generally require a declaration of health and safety characteristics related to the GM commodity, and identification of the use of gene technology in the food production. The most frequently legislated requirement is for the words “genetically modified” to be used in association with the name of the food or the relevant ingredient<sup>24</sup>.

The range of actual (or proposed) GM food-labelling regulations includes<sup>25</sup>:

- voluntary labelling that indicates that a product may contain GMOs or products derived from GMOs (under development in Canada and South Africa);
- compulsory labelling of products that are derived from modern methods of biotechnology or contain products from GMOs (currently in the EU, Australia, Japan and New Zealand, Switzerland);
- regulations which enforce labelling when a product is likely to contain ingredients derived from genetic modification (EU); and

- labelling of products where consumers are informed that production methods are likely not to involve any steps which involve genetic modification (so called «negative claims»).

The discussions at the Codex Committee on Food Labelling (CCFL) demonstrate also the difference of visions with regard to labelling. At the last session of the CCFL, the Delegation of the European Community, supported by other delegations, proposed to restructure the guidelines on Draft Recommendations for the Labelling of Foods obtained through certain techniques of Genetic modification/Genetic engineering into two parts: one for mandatory labelling provisions relevant to changes in nutrient content, product composition, end use, and the other for optional labelling provisions linked to labelling of method of production, following the proposal by Canada. Some delegations pointed out that clear labelling on the method of production would facilitate consumer acceptance of biotechnology and would ensure fair practices in international trade. Several other delegations and some observers expressed their opposition to the inclusion of method of production labelling in the Proposed Draft Guidelines for the following reasons: such labelling did not address food safety issues and was not based on scientific evidence; it would not provide useful information to consumers but rather increase confusion; and it would create barriers to trade. These delegations proposed to focus on the provisions that reflected consensus on the need for mandatory labelling in cases where significant changes in the product composition, nutritional value or intended use existed. Several delegations and some observers stressed that the information on labelling should be accurate, verifiable and should not mislead consumers. In this respect, it was pointed out that labelling two identical products based only on method of production would convey misleading message that these products were different and many consumers would perceive this as a safety warning although safety evaluation had been conducted before these products were placed in the market<sup>26</sup>.

### **Co-existence between different «traceability» schemes: The «Traceability» approach vs. The «Product tracing» Approach**

The divisions regarding the need for traceability are reflected in discussions at the Codex Alimentarius Commission and were until recently a major stumbling block at the Codex Intergovernmental Task Force on Foods Derived From Biotechnology. In the end, countries agreed at the third meeting of the Task Force (4-8 March 2002) to include the “tracing of products” and food labelling as risk management tools in the *Principles For The Risk Analysis Of Foods Derived From Modern Biotechnology* of GM foods. The principles were subsequently adopted by the Codex Alimentarius Commission in July 2003. Some believe that this agreement might mark a major breakthrough in international negotiations on the use of traceability systems and at least partially vindicates the EU’s insistence on introducing such requirements for GM foods. The 14th edition(2004) of the Procedural

Manual of the Codex Alimentarius<sup>27</sup> contains a definition of traceability which reads as follows: «*Traceability/Product Tracing*: the ability to follow the movement of a food through specified stage(s) of production, processing and distribution».

This official definition of the Codex Alimentarius Commission presents ‘tracing of products’ and ‘traceability’ as synonymous terms. The US, however, continues to insist that the two terms are not equivalent, arguing that ‘product tracing’ is limited to ‘one step forward and one step back’<sup>28</sup> whereas ‘traceability’ of products refers to the whole production chain of a product. The US Food and Drug Administration (FDA) proposes the following definition of “product tracing” : “the ability to identify by means of paper or electronic records a food product and its producer, *from where and when it came, and to where and when it was sent*”. On the contrary, the European legislation, traceability<sup>29</sup> means “the ability to trace GMOs and products produced from GMOs at *all stages* of their placing on the market through the production and distribution

chains”. This definition goes even further than the one retained by the Codex Procedural Manual as the latter mentions only the “specified stage” of production , processing and distribution. Some countries even questioned the definition as it did not specify how the stages of production, processing and distribution would be specified and the current text might result in potential barriers to trade.

### **Co-existence between different approaches of «likeness»: The «Substantial Equivalence» Approach vs. The «Process and Production Method» Approach**

The concept of *substantial equivalent* has a particular meaning when it is used by the Codex Alimentarius. Of particular importance here is the *Guideline for the Conduct of Food Safety Assessment of Foods Derived From Recombinant DNA Plants* adopted at Codex Alimentarius Commission in July 2003, which presents the concept of ‘substantial equivalence’ as the «starting point» for safety assessment rather than a safety assessment in itself. The standard also includes a footnote stating that «in the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts».

It should be emphasized that the Codex has developed a very unique and specific definition of this concept which goes back to a wording arrived at in 2000 during joint FAO/WHO expert consultations. It should not be forgotten that this whole debate takes place in the context of fundamental differences of views primarily between the EU and the US, and of the need to arrive at a diplomatic consensus. The Codex therefore arrived at a definition of substantial equivalence not as it is usually understood as a statement of *sameness* or of *equality*, but as a *process* or a *method*:

The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its

conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart.<sup>30</sup> It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA plants. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart.<sup>31</sup>

Substantial equivalence acknowledges in this sense that the goal of the assessment is not to establish absolute safety but to consider whether the GM food is as safe as its traditional counterpart,

where such a counterpart exists<sup>32</sup>. It is generally agreed that such an assessment requires an integrated and stepwise, case-by-case approach. Factors taken into account when comparing a GM food with its conventional counterpart include:

- identity, source and composition;
- effects of processing and cooking;
- the transformation process, the DNA itself and protein expression products of the introduced DNA;
- effects on function;
- potential toxicity, potential allergenicity and possible secondary effects;
- potential intake and dietary impact of the introduction of the GM food.

If the GMO-derived food is judged to be substantially equivalent to its conventional counterpart, then it is considered to be as safe as the counterpart. If it is not, further tests are conducted. The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food

and its conventional counterpart. It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA plants. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart. In practice, very few foods consumed today have been subject to any toxicological studies, yet they are generally accepted as being safe to eat. In developing a methodology for the safety assessment of new foods, it was essential to establish a benchmark definition of safe food. This was taken up by OECD in 1991 who said that food is considered safe if there is reasonable certainty that no harm will result from its consumption under anticipated conditions of use. The difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, meant that an alternative approach was required for the safety assessment of genetically modified foods. This led to a preliminary development of the concept of *substantial equivalence*<sup>33</sup>.

The concept of substantial equivalence is close to the concept of likeness developed under the WTO Agreements<sup>34</sup>. The Technical Barriers to Trade (TBT) Agreement, for instance, stipulates that Members are not allowed to give less favorable treatment to any products «than that accorded to *like* products of national origin and to *like* products originating in any other country» (Article 2.1). Some argue that import regulations that impose special risk assessment, traceability and/or labelling requirements for 'substantially equivalent' GM products might contravene this provision as they discriminate against 'like' products. The new EU regulations, for example, require all foods and feeds to be subject to the full authorization procedure as well as traceability and labelling requirements, including those that are substantially equivalent. According to established practices under the GATT, likeness is determined on a case-by-case basis according to four criteria, *i.e.* the products'



physical properties, end-uses, tariff classification and consumers' tastes and habits. Given the strong physical similarity between traditional foods and substantially equivalent GM foods, the latter are likely to be viewed as 'like' under the first three criteria.

In this context, one can see that co-existence between GM and non-GM products depends mostly on the perception of countries on what can be considered as a «novel food» or a «novel food ingredient». In Australia and New Zealand, for instance, the standard for novel food is broadly-based – rather than characterizing the food in terms of physical or technological properties, novel food is defined in terms of the level of knowledge and understanding which exist in the community regarding the potential public health risk posed by these foods. Such an approach provides an opportunity for government to undertake a scientific risk assessment for those foods for which the health risk is unknown or uncertain. Novel food in Australia and New Zealand is defined as follows: 'novel food' means a non-traditional food for which there is insufficient knowledge in the broad community to enable safe use in the form or context in which it is presented, taking into account: (a) the composition or structure of the product; (b) levels of undesirable substances in the product; (c) known potential for adverse effects in humans; (d) traditional preparation and cooking methods; or (e) patterns and levels of consumption of the product. 'Non-traditional food' means a food which does not have a history of significant human consumption by the broad community in Australia or New Zealand.

Novel food in Australia and New Zealand does not include food *produced using gene technology* or food that has been irradiated, both of which also require a pre-market assessment, but under separate food standards.

By contrast, in Europe, the definition of novel food takes gives a stronger consideration to the «process and method of production» of a given food or food ingredient but does not give any weight to «substantial equivalence» derived

from the history of consumption of a given food or food ingredient. Indeed, novel foods or novel food ingredients are defined, *inter alia* as «foods and food ingredients containing or consisting of genetically modified organisms», «foods and food ingredients produced from, but not containing, genetically modified organisms», «foods and food ingredients with a new or intentionally modified primary molecular structure». Contrary to the Australian and New Zealand legislation which refers to the concept of «non-traditional food», the European legislation refers to the concept of «conventional counterpart» which is defined as a similar food or feed *produced* without the help of genetic modification and for which there is a well-established history of safe use».

This means that even if a food has already a «history of safe use» and/or will have been «used for human consumption to a significant degree» in other countries outside the European Community, it will still be considered as a novel food under European legislation if it contains or is produced

from GMOs<sup>35</sup>. Thus, co-existence between GM and non-GM products may vary according to the qualification that is given to a particular food under regulatory systems.

## NATIONAL AND INTERNATIONAL LEGAL TOOLS FOR CO-EXISTENCE

### Harmonization/Standardization process

At the international level, instruments have been agreed upon that implicitly promote the harmonization of regulatory systems<sup>36</sup>. The *Codex Principles for the risk analysis of foods derived from modern biotechnology* are available to guide the safety assessment of GM food, but they have no binding effect on national legislation. Their function is essentially to form the basis for harmonization under the SPS Agreement. Other important standards of the Codex which promote co-existence through harmonization of international standards are the *Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants*

and the *Guidelines for the Conduct of Food Safety Assessment of Foods Produced using Recombinant-DNA Microorganisms*.

The Codex Alimentarius Commission has worked since the mid-1990s to achieve consensus on international standards for safety assessment and labelling of foods produced through modern biotechnology. Codex standards, guidelines and recommendations are increasingly used as benchmarks under international trade agreements (e.g. the SPS Agreement). Thus strong incentives exist to establish and conform to such standards. Codex has initiated two streams of work with respect to food produced from GM commodities. The first, established in 1999, is the Ad Hoc Intergovernmental Task Force for Foods Derived from Biotechnology to develop standards, guidelines and recommendations regarding safety and nutritional evaluation for these foods. The Task Force completed the development of risk assessment principles in 2003, aided by a number of expert consultations run jointly by FAO and WHO. The work on food derived from biotechnology is being continued by a new Task Force. The second Codex initiative is being addressed by the Codex Committee on Food Labelling (CCFL) which since 1991 has intensively debated the nature and extent of labelling for foods produced through biotechnology, at meetings and through working groups. While there is general agreement on the need for food labelling standards addressing health and safety issues arising from the use of gene technology (such as altered allergenicity, composition, nutritional value or intended use), divergent views exist among Member States on appropriate guidelines for process-based labelling of such foods. As positions on process-based labelling are as divergent as national regulatory approaches, progress in achieving consensus is likely to be slow. In 2001, the Codex Alimentarius Commission agreed to a proposal by the CCFL to adopt mandatory labelling of allergens in foods derived from biotechnology in the general food labelling standard for prepackaged foods. However, the Commission could not reach agreement on a definition for biotechnology foods and on draft guidelines on labelling provisions.

The CCFL continues to address these and other labelling issues.

On the other hand, the Cartagena Protocol has established legally-binding rules for environmental risk assessments. In addition, OECD has experience in promoting international harmonization in the regulation of biotechnology by ensuring efficiency in the evaluation of environmental and human health safety, through its working group for harmonization in biotechnology and its task force for the safety of novel foods and feeds.

Harmonization can be achieved at several levels. The countries of the Association of South-East Asian Nations (ASEAN), for instance, have come together to cooperate on various levels including: (i) harmonization of legislation for products derived from modern biotechnology and intellectual property rights; (ii) research and development in biotechnology; and (iii) environmental protection. ASEAN is also looking at a regional approach to biosafety, although it is not clear what is intended, i.e. whether regional assessment and national decision-making would be considered. Those countries in the region that have made some progress have gone as far as developing labelling regulations.

After the 2002 humanitarian crisis in southern Africa, where a number of countries experiencing severe drought and food shortages questioned the use and safety of GM food aid, a Council of Ministers of the Southern African Development Community (SADC) established an Advisory Committee on Biosafety and Biotechnology to develop a common position on biotechnology and to harmonize biosafety legislations in the region. The objective is to facilitate the movement of food products that may contain GMO material across the region in future.

### **A Systemic Risk Analysis**

There is broad international agreement that food safety standards and related guidelines must have an objective basis in science<sup>37</sup>. Many risk assessors now agree that risk analysis,

and especially risk management, requires consideration of numerous more subjective and value-laden factors, to determine the appropriate level of protection and to choose the preferred risk-management option(s). The scientific community has developed ways to resolve disagreements over scientific facts, but disagreements over the value and ethical components of food safety decisions are often much harder to sort out. Internationally, food safety agencies also agree on the value of science as a significant tool in food safety policy-making and the development of food standards. The general policy guidelines of the Codex Alimentarius Commission contain statements of principle concerning the role of science in the Codex decision-making process and the extent to which other factors are to be taken into account. The first two of these statements are as follows:

«1. The food standards, guidelines and other recommendations of Codex Alimentarius shall be based on the principle of sound scientific analysis and evidence, involving a thorough review of all relevant information, in order that the standards assure the quality and safety of the food supply.

2. When elaborating and deciding upon food standards, Codex Alimentarius will have regard, where appropriate, to other legitimate factors relevant for the health protection of consumers and for the promotion of fair practices in food trade.»<sup>38</sup>

While risk assessment is based on science, scientific evidence and analysis cannot always provide immediate answers to questions posed. Much scientific evidence is tentative, as the established processes of science include checking and rechecking outcomes in order to obtain the required level of confidence. Decisions usually are defended as based on «science» and sometimes on economic costs and benefits as well, which offer seemingly objective, verifiable evidence that the policy choice is «correct.» Decisions explicitly based on ethical principles and value preferences can be just as defensible, if the society agrees broadly on the ethical assumptions used to make policy. The emphasis on science and the exclusion

of ethical argument as the basis for decisions may polarize the scientific debate. Stakeholders who find that risk managers will not entertain a serious discussion of, for example, their right to avoid consuming a food they believe is not safe enough, may argue instead that the food is not safe, exacerbating technical disagreements about inherently ambiguous evidence of risks.

That is why some regulations in view of promoting co-existence, insist on the necessity of integrating other factors in risk assessment and risk management of GMOs. This is the case of EC Regulation 1829/2003 which states:

it is recognized that, in some cases, scientific risk assessment alone cannot provide all the information on which a risk management decision should be based, and that other legitimate factors relevant to the matter under consideration may be taken into account.

This approach is also subscribed to by the Cartagena Protocol in its Article 26 which reads as follows:

The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities.

### **Transparency<sup>39</sup>**

Consumers have a legitimate interest in and right to information with regard to GMOs in agriculture<sup>40</sup>. This begins with rules for the transparent sharing of relevant information and the communication of associated risks. Science-based risk analysis seeks to enable experts to take decisions that minimize the probability of hazards in the food supply system and the environment. Consumers, however, may also

wish for more transparency to protect their right to exercise informed consent on their own. An often-discussed set of instruments intended to protect these rights is the labelling of products, whether or not they are derived from GMOs. An important aspect of science-based food safety assessments is that they involve a measure of uncertainty. These uncertainties should be presented by risk assessors and addressed in a transparent manner by risk managers if the assessments are intended to be a useful and responsible basis for societal decision-making. Currently, this need is not sufficiently realized within the scientific community.

## Accountability

On September 29, 2000, the United States District Court for the District of Columbia dismissed the challenge to the Food and Drug Administration's (FDA) regulatory policies concerning genetically engineered foods. The Alliance for Bio-Integrity and other public interest and religious groups had made allegations about the legality of FDA's 1992 Policy Statement, "Foods Derived from New Plant Varieties". The court ruled that the Agency was not required to prepare an environmental assessment or environmental impact statement because it was not a "major federal action" within the meaning of the National Environmental Policy Act. The court deferred to FDA's view that genetically engineered foods as a class do not require a pre-market review and approval of a food additive petition. The court also accepted the FDA's view that special labelling for genetically engineered foods as a class is not required solely because of consumer demand or because of the process used to develop these foods<sup>41</sup>.

Such a position does not promote co-existence between GM and non-GM products. On the contrary, consumers may wish to be more involved in local, national and international debates and in policy guidance. At present, there are very few fora available to the public to discuss the wide range of issues relating to GMOs<sup>42</sup>. It is the approach advocated by the Aarhus Convention on Access

to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (1998). Indeed, the First Meeting of the Parties (MOP-1) adopted in 2003, the *Guidelines on Access to Information, Public Participation, and Access to Justice with respect to Genetically Modified Organisms*<sup>43</sup>. One of the objective of the Guidelines is to "stimulate open, transparent, efficient and accountable decision-making on activities with GMOs". Measures relating to co-existence are foreseen in the Guidelines. It is recommended that public participation should be provided for as appropriate in the following GMO-related decision-making procedures:

- «(a) First-time deliberate release into the environment of GMOs in any new location;
- (b) First-time placing on the market of GMOs not exclusively intended for research or for culture collections;
- (c) Procedures for determining whether sufficient experience has been obtained with respect to deliberate releases of certain GMOs in certain ecosystems and simplified procedures could therefore be followed».

<sup>1</sup> These developments are quoted from S. Zarrilli, *International Trade in GMOs and GM Products: National and Multilateral Legal Frameworks*, Policy Issues in International Trade and Commodities Study Series, N. 29, UNCTAD, 2005, p. 2.

<sup>2</sup> *Ibid.*, p. 3.

<sup>3</sup> In 2004, the global area of GM crops amounted to 200 million acres, up 20% from 2003, according to industry source International Service for the Acquisition of Agri-Biotech Applications, available at [http://www.isaaa.org/kc/CBTNews/press\\_release/briefs32/figures/Biotech\\_map\\_acreage.jpg](http://www.isaaa.org/kc/CBTNews/press_release/briefs32/figures/Biotech_map_acreage.jpg)

<sup>4</sup> Regarding these developments, see H. Baumuller, *Domestic Import Regulations for Genetically Modified Organisms and their Compatibility with WTO Rules. Some Key Issues*, ICTSD, August 2003, p. 3, available at

[http://www.ictsd.org/ministerial/cancun/docs/TKN\\_Baumuller.pdf](http://www.ictsd.org/ministerial/cancun/docs/TKN_Baumuller.pdf)

<sup>5</sup> The notion that co-existence is possible is contested by some researchers, e.g. Prof. Miguel Altieri, professor of agroecology at the University of California, Berkeley, considers that the large-scale use of GM crops will exacerbate the ecological problems already associated with Green Revolution monocultures, especially in developing countries because of their high levels of agricultural biodiversity where he considers hybridization with weedy relatives and contamination of non-GM crops is a virtual certainty. In view of the fact that removing or recalling genes once they have escaped into natural gene pools is impossible, he

concludes that non-GM seed lineages may be threatened with serious consequences, especially where extensive monocultures may result in serious social and environmental problems. See Miguel A. Altieri, University of California, Berkeley: *The Myth of Co-existence: Why Transgenic Crops Are Not Compatible With Agroecologically Based Systems of Production*. *Bulletin of Science, Technology & Society*, Vol. 25, No. 4, August 2005, 361-371. From: Third World Network [twnet@po.jaring.my](mailto:twnet@po.jaring.my), 4 Oct. 2005.

<sup>6</sup> See, e.g., *GM Crops? Co-existence and Liability: A Report by the Agriculture and Environment Biotechnology Commission*, November 2003, United Kingdom, p. 25, available at: [http://www.aebc.gov.uk/aebc/co-existence\\_and\\_liability\\_aebc\\_1.pdf](http://www.aebc.gov.uk/aebc/co-existence_and_liability_aebc_1.pdf).

<sup>7</sup> See Communication from Commissioner Fischler to the European Commission, Brussels, SEC(2003) 258. 25/02 2003. Co-existence of Genetically Modified, Conventional and Organic Crops.

<sup>8</sup> See, e.g., *GM Crops? Co-existence and Liability: A Report by the Agriculture and Environment Biotechnology Commission*, November 2003, United Kingdom, available at: [http://www.aebc.gov.uk/aebc/co-existence\\_and\\_liability\\_aebc\\_1.pdf](http://www.aebc.gov.uk/aebc/co-existence_and_liability_aebc_1.pdf). See also, Food Safety Department, World Health Organization, *Modern food biotechnology, human health and development: an evidence-based study*, 23 June 2005, p. 52, available at: <http://www.who.int/foodsafety>: "The potential risk of outcrossing and contamination by dispersed material from GM plants can pose problems for organic farming, as defined in Codex Guidelines. Dispersal of materials from GM crops (e.g. seeds) can occur over wide distances depending on the plant characteristics and climatic conditions. Outcrossing and dispersal are natural phenomena that can affect the production of conventional seeds. The future prospects of providing GM-free seeds and crops have been debated as a solution for addressing consumer choice. Co-existence of agricultural practices must respect the threshold limits set for contamination of organic products and realize the difficulty of adhering to this goal for certain plants (257-259). Contamination of honey with GM constructs as a result of insect vectors has also been identified. Agricultural practices that include GMOs may need to develop improved agricultural or molecular systems which enable a *benign* co-existence of GM and GM-free agriculture, in which a limited level of outcrossing is accepted. Otherwise, separation of GM plants with a significant potential for outcrossing from conventional or organic farming may be necessary».

<sup>9</sup> See FIBL Dossier, *Agriculture biologique et génie génétique*, n°3, février 2003, p. 13.

<sup>10</sup> Article 43 of Regulation (EC) No 1829/2003 (Food and Feed Regulation).

<sup>11</sup> Commission Recommendation of 23 July 2003 notified under document number C(2003) 2624 available at: [http://www.fsai.ie/legislation/legislation\\_update/July%2003/Rec%202003.556.EC.pdf](http://www.fsai.ie/legislation/legislation_update/July%2003/Rec%202003.556.EC.pdf)

<sup>12</sup> Communication from Commissioner Fischler to the European Commission, Brussels, SEC(2003) 258. 25/02 2003. Co-existence of Genetically Modified, Conventional and Organic Crops.

<sup>13</sup> These standards could include both thresholds of permissible GM presence in conventional or organic crops, and standards of purity in high-value specialist GM crops.

<sup>14</sup> One should take into account that according to the European Commission Recommendation, environmental and

health aspects of co-existence are already dealt with under Directive 2001/18/EC on the deliberate release of GMOs into the environment.

<sup>15</sup> See, Th. Christoforou, "The Precautionary Principle, Risk Assessment and the Comparative Role of Science in the European Community and the US Legal Systems, in *Green Giants? : Environmental Policies of the United States and the European Union*, ed. by Norman J. Vig and Michael G. Faure, Cambridge, MIT Press, 2004, pp. 17-51. By the same author, "The Regulation of Genetically modified Organisms in the European Union: the Interplay of Science, Law and Politics", *Common Market Law Review*, vol. 41, n°3, 2004, pp. 637-709.

<sup>16</sup> Regarding the following developments, see S. Zarelli, *International Trade in GMOs and GM Products: National and Multilateral Legal Frameworks*, Policy Issues in International Trade and Commodities Study Series, N. 29, UNCTAD, 2005, pp. 4-9.

<sup>17</sup> See WTO Document *G/SPS/N/JPN/107*, Committee on sanitary and Phytosanitary Measures, *Notification from Japan*, 25 September 2003.

<sup>18</sup> See on this point, S. Zarelli, *International Trade in GMOs and GM Products: National and Multilateral Legal Frameworks*, Policy Issues in International Trade and Commodities Study Series, N. 29, UNCTAD, 2005, p. 26.

<sup>19</sup> Earth Negotiation Bulletin (ENB/IISD), Vol. 9 No. 320 COP/MOP-2 (Biological Diversity) - Summary and analysis, Available at <http://www.iisd.ca/>

<sup>20</sup> See L. Boisson de Chazournes, M. M. Mbengue, "GMOs and Trade: Issues at Stake in the EC Biotech Dispute", *Review of European Community and International Environmental Law*, vol. 13, n°3, 2004, pp. 289-305.

<sup>21</sup> Regarding these developments, See *Genetically modified organisms, consumers, food safety and the environment*, FAO Ethics Series 2, 2001, p. 6.

Available at: <ftp://ftp.fao.org/docrep/fao/003/x9602e/x9602e00.pdf>

<sup>22</sup> See, H. Baumuller, *Domestic Import Regulations for Genetically Modified Organisms and their Compatibility with WTO Rules. Some Key Issues*, ICTSD, August 2003, p. 27, available at

[http://www.ictsd.org/ministerial/cancun/docs/TKN\\_Baumuller.pdf](http://www.ictsd.org/ministerial/cancun/docs/TKN_Baumuller.pdf)

<sup>23</sup> Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed; and Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning traceability and labelling of genetically modified organisms and traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. The food and feed regulation entered into force on 7 November 2003 and applies since 18 April 2004. The labelling and traceability regulation also entered into force on 7 November 2003, applying 90 days from publication of a system for development and assignment of unique identifiers for GMOs.

<sup>24</sup> See Food Safety Department, World Health Organization, *Modern food biotechnology, human health and development: an evidence-based study*, 23 June 2005, available at: <http://www.who.int/foodsafety>

<sup>25</sup> *Ibid.*, p. 50.

<sup>26</sup> See, Codex Alimentarius Commission, Twenty-eighth Session, Rome, Italy, 4 – 9 July 2005, Report of the Thirty-Third Session of the Codex Committee on Food Labelling, Kota Kinabalu, Malaysia, 9 – 13 May 2005, doc. ALINORM 05/28/22, available at : <http://www.codexalimentarius.net/web/reports.jsp?lang=en>

<sup>27</sup> Available at [http://www.codexalimentarius.net/web/procedural\\_manual.jsp](http://www.codexalimentarius.net/web/procedural_manual.jsp), p. 45.

<sup>28</sup> This principle of 'one step forward and one step back' is contained for example in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (Bioterrorism Act) and pursuant to its title III, subtitle A sec.301, steps were taken to protect the public from a threatened or actual terrorist attack on the US food supply. In the event of a potential or actual bioterrorism incident or an outbreak of food-borne illness, the aim is to help the FDA to determine the location and source of the incident and to enable the agency to quickly notify facilities that may be affected. Under the proposed rule related to record keeping (which follows the "one step back, one step forward" principle), manufacturers, processors, packers, distributors, receivers, holders and importers of food will be required to keep (paper or electronic) records: *identifying the immediate source from which they have received the food; identifying the immediate subsequent recipient of the food.* See, FAO, *Traceability implementation in developing countries, its possibilities and its constraints. A few case studies*, 2003, available at : <ftp://ftp.fao.org/es/esn/food/traceability.pdf>

<sup>29</sup> See, Article 3 of Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.

<sup>30</sup> The concept of *substantial equivalence* as described in the report of the 2000 joint FAO /WHO expert consultations (Document WHO/SDE/PHE/FOS/00.6, WHO, Geneva, 2000).

<sup>31</sup> Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, *CAC/GL 45-2003*, para. 13.

<sup>32</sup> Regarding the following developments, see Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety, Rome, Italy, 30 September to 4 October 1996, pp. 4-10. Available at : <http://www.fao.org/es/ESN/food/pdf/biotechnology.pdf>. This report is particularly interesting as it distinguishes between "Products that are shown to be substantially equivalent to existing foods or food components", "Products that are substantially equivalent to existing foods or food components except for defined differences" and "Products that are not substantially equivalent to existing foods or food components".

<sup>33</sup> For an evaluation of the concept of substantial equivalence, see *Safety aspects of genetically modified foods of plant origin*, Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, 29 May – 2 June 2000, pp. 7-8. Available at: [http://www.who.int/foodsafety/publications/biotech/en/ec\\_june2000\\_en.pdf](http://www.who.int/foodsafety/publications/biotech/en/ec_june2000_en.pdf)

<sup>34</sup> Regarding the following developments, see H. Baumüller, *Domestic Import Regulations for Genetically Modified Organisms and their Compatibility with WTO Rules. Some Key Issues*, ICTSD, August 2003, pp. 28-29, available at: [http://www.ictsd.org/ministerial/cancun/docs/TKN\\_Baumuller.pdf](http://www.ictsd.org/ministerial/cancun/docs/TKN_Baumuller.pdf)

<sup>35</sup> See, e.g., Commission Decision of 19 May 2004 authorizing the placing on the market of sweet corn from genetically modified maize line Bt11 as a novel food or novel food ingredient under Regulation (EC) N. 258/97 of the European Parliament and of the Council (doc. C(2004) 1865); See also, Commission Decision of 3 March 2005 authorizing the placing on the market of foods and food ingredients derived from genetically modified maize line NK 603 as a novel foods or novel food ingredients under Regulation (EC) N. 258/97 of the European Parliament and of the Council (doc. C(2005) 580).

<sup>36</sup> Regarding the following developments, see *Modern food biotechnology, human health and development: an evidence-based study*, Provisional edition, 23 June 2005, pp. 30-31.

Available at: [http://www.who.int/foodsafety/publications/biotech/biotech\\_en.pdf](http://www.who.int/foodsafety/publications/biotech/biotech_en.pdf)

<sup>37</sup> The following ideas are quoted from Food Safety Department, World Health Organization, *Modern food biotechnology, human health and development: an evidence-based study*, 23 June 2005, p. 56, available at: <http://www.who.int/foodsafety>

<sup>38</sup> Codex Alimentarius Commission Procedural Manual Fourteenth Edition *op. cit.*, 188.

<sup>39</sup> Regarding the following developments, see *Genetically modified organisms, consumers, food safety and the environment*, FAO Ethics Series 2, p. 7. Available at: <ftp://ftp.fao.org/docrep/fao/003/x9602e/x9602e00.pdf>

<sup>40</sup> See also *FAO Expert Consultation on Food Safety: Science and Ethics*. FAO Readings in Ethics 1, Food and Agriculture Organization of the United Nations (FAO), Rome, 2002, pp. 33-34. Available at : <ftp://ftp.fao.org/docrep/fao/006/J0776E/J0776E00.pdf>

<sup>41</sup> Decision available at <http://www.dcd.uscourts.gov/district-court-2000.html>

<sup>42</sup> See *Genetically modified organisms, consumers, food safety and the environment*, FAO Ethics Series 2, 2001, p. 8. Available at: <ftp://ftp.fao.org/docrep/fao/003/x9602e/x9602e00.pdf>

<sup>43</sup> The Guidelines are available at: <http://www.unece.org/env/pp/documents/gmguidelinesenglish.pdf>

# Technical and economic issues related to co-existence supply chains

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It is usually said that under coexistence farmers should be able to choose the agricultural production system they prefer –be it conventional, organic, or one that includes genetically modified (GM) crops. Yet, under co-existence unintended or adventitious presence (AP) of GM material in conventional and organic (non-GM) systems can not be excluded during cultivation, harvest, transport, storage, and processing. In this context, persistent questions have been raised about whether it is technically and economically feasible for the various production systems to co-exist.

There is, however, extensive experience of co-existence in the US and in a few other countries with substantial GM crop production. Over the past ten years, the US has seen rapid expansion in the cultivation of GM crops (e.g. maize, soybeans, canola, and cotton) with multiple traits and events. Amid increasing GM production and in close proximity, various identity-preserved (IP) non-GM production systems have also continued

to grow and prosper. These include IP non-GM corn and soybean systems for domestic processing (e.g. in the production of starches and oils); IP non-GM corn and soybean systems for export markets (e.g. Japan); certified organic production systems; conventional seed production systems; and others. Valuable experience on co-existence has also been accumulated from supply chains designed to channel GM crops with unapproved events away from certain export markets and to keep regulated crops with food, feed, industrial, and pharmaceutical traits in thousands of field trials away from the agrifood supply chain.

Using its PRESIP modeling platform, the Economics and Management of Agrobiotechnology Center at the University of Missouri –Columbia has analyzed the technical challenges and the economics of parts or whole IP supply chains under conditions of co-existence. Some general conclusions can be readily gleaned from such analyses.

There is significant variety among IP non-GM supply chains in the US. Key differences can be found in their degree of vertical control, the ways they employ land and capital assets, as well as in their operating standards and compliance systems. The choice of structure and standards is typically market-driven, determined by end user needs and willingness to pay.

A key consideration for all IP non-GM supply chains is AP of GM material. AP can occur throughout the supply chain. However, the level of AP and its probability of occurrence are not evenly distributed across the chain and neither are the associated costs for controlling it. Generally, the need to meet lower AP thresholds implies stricter supply chain practices and higher costs. Incremental costs are typically non-linear, rising sharply at very low AP thresholds. Some parts of the supply chain (e.g. seed production) experience fast-rising incremental costs at low AP levels (e.g. below 0.5%). Other parts of the supply chain (e.g. commercial storage and processing) experience more tempered cost increases at similar AP levels.

The additive nature of AP implies interdependencies in IP non-GM supply chains. Such interdependencies are well recognized and are managed through market transactions or coordination. End users demanding low AP levels must typically reach all the way to the beginning of the supply chain and secure high seed purity and strict agronomic practices through contractual arrangements with seed firms and agricultural producers. Strict IP programs regularly meet AP thresholds between 0.1 and 0.9% under conditions of co-existence. Less strict programs are less coordinated and depend mostly on market transactions and testing practices to ensure that minimum standards are met.

IP premiums paid by the end users closely match the IP costs incurred in the supply chain suggesting that local non-GM markets are efficient in pricing effort and assets required to manage co-existence. IP costs and premiums are not static however. Trends in the US indicate that

they tend to increase beyond a certain level of GM adoption, due to increased competition for suitable land and other capital assets, increased testing, larger discards and other factors.

In all, empirical evidence from the US implies that co-existence of GM and non-GM production can be achieved, securing even low AP levels in the presence of high levels of GM crop adoption. And for the most part, co-existence has been achieved at practical cost increases. This success can, in part, be attributed to the process-focus, flexible structure, and market orientation of the IP non-GM supply chains as well as to the limited size of the IP non-GM markets in the US. Whether such experience can be replicated in markets where production and supply chain standards are broadly imposed through regulation is unclear.



# An overview of past and on-going co-existence studies

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The new European legal framework aims at reinforcing the assessment of GMOs, ensuring the traceability of products and clearly informing consumers through reliable labelling (Regulations (EC) No. 1829/2003 and No. 1830/2003). In addition, there are EU guidelines designed to allow for the co-existence of various kinds of agriculture by ensuring that “farmers should be able to cultivate freely the agricultural crops they choose, be it GM, conventional or organic” (Recommendation 2003/556/EC). Such GMO dedicated regulations are part of a more general international trend to enable reliable co-existence and traceability for delivery of food and feedstuffs complying with consumer requests.

On-farm co-existence is usually defined as the ability to keep adventitious presence of GM material in non-GM production below a certain level. Adventitious presence at the farm level could have several causes:

- Crop-to-crop pollination between neighboured fields;
- Presence of volunteers in conventional fields resulting from former GM crop cultivation in the field;
- Pollination from feral GM plants occurring in field borders and resulting from seed dispersal during transportation;
- GM impurities in seed lots (cross-pollination during seed production or admixture in the post-harvest process);
- Adventitious admixture due to machinery during sowing and harvesting operations.

On-farm co-existence is thus highly related to gene flow. Although gene flow is a common phenomenon for crop species, its implications for Genetically Modified Plants have raised new concerns. Undesirable effects related to gene flow may result in ecological or agronomic considerations (persistence of resistant volunteers, creation of new weeds, multiple resistance) as well as commercial considerations (unintended presence of GMOs in conventional crop production affecting its competitiveness in the marketplace). Consequently, the co-existence between different types of crops has become a major issue and has to be addressed *per se* whatever the actual ecological, agronomic and safety impacts may be.

## Several co-existence studies have been performed over the last years

In recent years, several studies have been carried out to address the scientific issues related to co-existence within the EU in order to help both public and private decision-making.

- A research group coordinated by INRA has carried out a study of the economical relevance and technical feasibility of a non-GM supply chain involving the input of various stakeholders. Four topics were addressed:

willingness of consumers to buy non-GM products (Ruffieux & Robin, 2001), management of on-farm co-existence (Le Bail & Meynard, 2001), detection methods of GMOs (Bertheau, 2001), and economic assessment of non-GM supply chains (Valceschini & Avelange, 2001). The study clearly stated that the feasibility and the costs of co-existence depend on the threshold for GM presence and pointed out the great variability of situations among crops (given maize and soybean), cropping systems, and supply chain organisations.

- A JRC-IPTS and ESTO network consortium conducted a study approaching the estimation of adventitious presence by using computer modelling and expert opinion as well as selecting typical EU "type farms". The main conclusion of the study (Bock *et al.*, 2002) was that co-existence measures and their costs depend on the given crops, agricultural landscape, farm typologies, and crop rotations.
- The Danish report on co-existence (Tolstrup *et al.*, 2003), aimed at undertaking a scientific evaluation of the possible sources of adventitious presence of GM material in conventional and organic productions, evaluating the extent of adventitious presence and the need for control measures as well as identifying and evaluating the measures deemed necessary to ensure co-existence of GM crop production with conventional and organic productions. The study concluded that co-existence of many crops (e. g., maize, beet, potatoes, etc.) is achievable at the existing threshold level for food and feed with some control measures when the adoption of GM varieties by crop growers is moderate. Nevertheless, for cross pollinated crops and/or species with long seed survival in the soil, (such as rape, clover and grasses) more rigorous measures will be required.
- Two Swiss studies have been recently published (Schlatter & Oehen, 2004; Sanvido *et al.* 2005). Based on recent scientific results on

gene flow, and taking into consideration the specificity of Swiss agricultural systems, they differed in their conclusions as they considered different thresholds for assessing co-existence feasibility. While Sanvido *et al.* state that co-existence would be possible for maize, wheat, and rapeseed, taking into account the official threshold of 0.9% for labelling, Schlatter & Oehen focused on organic farming and lower levels of GM material.

Several other reports have been issued, collating available information on gene flow and its implications for co-existence (Eastham & Sweet, 2002; AEBC, 2003, Brookes *et al.*, 2004). Outside the EU, various reports have also been published, though their focus was slightly different (Christey & Woodfield, 2001; Glover, 2002).

Even if the conclusions drawn are quite different, common features can be highlighted:

- The major focus has been on technical aspects of on-farm co-existence and the implications of gene flow while organisational, economic, and legal aspects have been less at issue and require further investigation;
- Adventitious presence of GM in non-GM production strongly depends on crop biology, farming systems, agricultural practices, and regional adoption of GM crops; therefore, no general co-existence recommendation can be made at the EU level;
- For crops such as maize or beets, co-existence at the 0.9% threshold value for labelling is technically possible, as long as co-existence measures are adopted while long-term co-existence for crops like oilseed rape remains difficult to predict, far less implement;
- The technical and economic feasibility of co-existence at thresholds much lower than 0.9 % (for organic or conventional crops) is much more speculative and often thought to be not possible without a strong spatial organisation;

- A large amount of data has been made available on gene flow. The FP6 research project SIGMEA<sup>1</sup> aims at collating and structuring existing datasets and will provide added value by carrying a meta-analysis at the European level;
- Due to the high variability of agricultural conditions and long-term effects of gene flow for some crops, co-existence cannot be assessed only on a case-by-case basis through field experiments, therefore decision-support systems should be designed.
- re-assessing the overall balance of the impacts of GM crops when new results are available (from trials as well as from monitoring).

Modelling for forecasting the behaviour of transgenes has been in development for some years. It has mainly focused on crop-to-crop gene flow, and 22 models have been published so far for different types of crops. However, only a few of them, such as GENE<sup>®</sup> for rapeseed (Colbach *et al*, 2001a & b) and MAPOD<sup>®</sup> for maize (Angevin *et al*, 2001), actually take into account the spatial patterns of landscapes and agricultural practices and are thus able to forecast the behaviour of transgenes within the landscape. GENE<sup>®</sup> takes into account crop rotations as well as seed persistence over time. An adaptation of GENE<sup>®</sup> for sugar beet is under progress and validation using a wide range of available data is being carrying out (Sester *et al*, 2003; Sester, 2004).

### **Models are necessary for helping decision-makers to set up co-existence measures**

Forecasting the fate of GM crops at the landscape level by taking into account the various cropping systems and agricultural practices that may occur across Europe is necessary for helping in the elaboration of co-existence rules as well as for assessing their feasibility and their consequences as well as for setting up monitoring and control schemes. Indeed, for such a perspective, specific field experiments, even if necessary, are not sufficient as their predictive value remains restricted to general rules. For forecasting the spread and behaviour of GM plants and seeds as well as their impacts in a wide range of agro-ecosystems, modelling is a key element. Models reproduce the functioning of agro-systems and take into account the relevant factors and processes as well as their interactions. They thus allow simulating the behaviour of agro-systems in non-observed situations and on a long term basis.

Models help in:

- structuring knowledge, identifying gaps and reducing research fragmentation;
- ranking farming systems according to adventitious presence in non-GM production;
- forecasting the behaviour of transgenes in cultivated and non-cultivated lands;
- testing *a priori* the efficiency of co-existence measures or regulation schemes;
- implementing monitoring schemes by identifying high risk situations;

Models have been used to underpin the co-existence studies carried out by INRA in France (Le Bail & Meynard, 2001) and by JRC/IPTS (Bock *et al*, 2002). They also have been used in a second JRC/IPTS study, launched in 2004, aiming at updating and expanding the findings of the first report, namely, taking advantage of Geographical Information Systems (GIS) describing actual agricultural landscapes. Combining spatially-explicit models with such GIS makes it possible to assess the feasibility of co-existence at the individual farm or small region levels. Results will be made available by the end of 2005.

### **CONCLUSIONS**

Results from co-existence studies have raised several issues that research should further address:

- There exists a wide range of farming systems within Europe that could not be fully addressed through specific studies;
- The landscape fragmentation has a great influence on gene flow and ecological impacts and its effect should be taken into account in modelling;

- Induced costs due to indirect effects of co-existence rules are difficult to estimate and are highly dependent on the local regional variability of landscapes and on agricultural farming systems;
- Available models for gene flow mainly focus on the field level or on a small region (group of fields). However, co-existence measures and monitoring schemes should not only involve the field level with crop management practices and the “cropping system” within the farming systems strategy but also the regional level. Thus, up-scaling of models at different biogeographical levels should be made possible, keeping user friendliness in mind.

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<sup>1</sup> “Sustainable introduction of GMOs into European Agriculture”



# **INVITED PAPERS**

**Plenary session 2:**  
INTERNATIONAL EXPERIENCE  
ON CO-EXISTENCE





# Co-existence of GM and non-GM crops in Canada: current status and future direction

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**Abstract:** Transgene escape has been happening in Canada and it can cause problems. A recent Canadian Supreme Court ruling on the case of a Canadian farmer sued by Monsanto for possession of their patented GM canola has made the liability situation related to transgene escape less clear for non-GM farmers and the grain and food industry in Canada. The Canadian government has initiated mechanisms for consultations on the issue of transgene confinement and its implications but it has not created new regulation or new laws to address either the recent Supreme Court Ruling or the Royal Society of Canada precautionary action recommendations (released in 2001). The position of the Canadian government as a promoter of biotechnology and the lack of mandated thresholds in Canada for the adventitious presence of transgenes may be affecting governmental will to produce concrete action on transgene confinement. In this environment, research in Canada into the means and mechanisms of transgene escape will likely remain ad hoc. In the longer term, as pharmaceutical and industrial traits are introduced into crop plants more Canadians will be directly affected by transgene escape. At that time the political will may be created to formally address the issue of transgene confinement in Canada.

## INTRODUCTION

Genetic engineering (GE) is truly novel technology which allows for the inclusion of almost any trait imaginable into crop plants to serve all manner of desired functions and end-uses (Tolstrup *et al.*, 2003). In Canada, farmer adoption levels of GE or genetically modified (GM) crops have been high with more than 75% of the canola grown in 2004 being GM while GM soybean and corn crop acreages represent over 60% of total acreage (ISAAA briefs, 2004). Although GM crops are registered for unconfined release in Canada they continue to be a concern

in countries where GM crops are not yet registered for unconfined release. In addition, because GE allows for the realization of truly extraordinary traits in crop plants, it can also produce novel and unexpected risks. Most risks related to the release of GM crops are related to transgene movement, which remains relatively poorly understood and has been studied to only a very limited extent (Marvier & Van Acker, 2005). The exploitation of GM crops will require responsible introduction which, in turn, requires the creation of effective and acceptable transgene confinement protocols. These protocols must be based on knowledge of the nature and interaction of those factors which

contribute to transgene movement and a realistic consideration of the cooperation required to make confinement effective (Tolstrup *et al.*, 2003). In order that they are effectively administered the protocols must include the assignment of responsibilities for transgene confinement which are enforced through law.

## **GM CROP CONFINEMENT FAILURES IN CANADA**

In North America where there have been a high number of documented cases of transgene escape (Marvier & Van Acker, 2005). Among all documented cases, intraspecific transgene movement in canola (*Brassica napus* L.) has been the most common. In western Canada, there has been so much intraspecific transgene escape in canola that farmers in this region have come to expect the unintended appearance of transgenes in their canola (Van Acker *et al.*, 2004). Even after only 3 years of commercial production of GM canola, Hall *et al.* (2000) found that the specific transgenes encoding for different herbicide tolerance traits were stacking within individual volunteer canola plants, giving rise to multiple herbicide resistant volunteer canola plants. By the year 2000, only 5 years after the initiation of commercial production of GM canola in western Canada, farmers began to complain about the appearance of volunteer glyphosate herbicide tolerant canola in their fields, even when they had not intentionally sown glyphosate tolerant canola in these fields the previous year. Independent testing of certified canola seed lots from western Canada revealed that the majority of tested seed lots contained at least trace amounts of genetically engineered herbicide tolerance traits Friesen *et al.* (2003). The source of the adventitious presence for these seed lots was never determined. The high level of adventitious presence of unintended transgenes in pedigreed certified seed lots was disturbing because it showed that stringent seed production segregation systems were not sufficient to prevent significant transgene movement (Friesen *et al.*, 2003).

The most troubling examples of transgene escape are those that involve human error because they are so unpredictable. In the US the most famous of these was the 'Starlink' case where corn, engineered to express an insecticidal protein, was approved for animal feed but not human consumption. There was insufficient segregation oversight between food and feed streams in the US bulk commodity handling systems and the insecticidal protein was found in a number of processed foods (Marvier & Van Acker, 2005). Three years after this discovery and after the execution of a massive recall effort, traces of the Starlink protein could still be commonly found with both food and feed handling streams in the US (USDA, 2003). The Starlink case showed not only that human error can result in problematic transgene escape, but full retraction of transgenes (and their products) from complex and massive commercial food and feed systems is difficult, and perhaps impossible.

## **CURRENT LEGAL SITUATION IN CANADA**

The case in Canada of Monsanto versus Percy Schmeiser, tried under patent law, was settled at the Canadian Supreme court level in May 2004. This case has many interesting legal and liability implications for co-existence management. Mr. Schmeiser lost his case in final appeal to the Supreme Court of Canada, which ruled that Monsanto could retain the full rights and privileges of patent ownership, as well as the right to sue farmers for the possession of this transgene, regardless of how it came to be in their possession and regardless of whether or not they profited from possessing it (Supreme Court of Canada, 2004). The ruling is problematic because it does not explicitly consider the case of innocent infringement and because the Roundup Ready (GM) transgene is now present in the majority of certified non-Roundup Ready canola seed sold in western Canada. Any farmer in this 40 million ha region who grows canola has a better than 50% chance that Roundup Ready canola will be on their land even if they purposely choose not to

grow it. Monsanto can choose to sue anyone of these farmers yet Monsanto is not bound to any responsibility for the uncontrolled movement of their Roundup Ready transgene. In an academic legal assessment of this case, Cullet (2005) makes special note that the outcome of the case points to a real need to assign liability and responsibility in regard to transgene ownership and the effects of transgene escape, and that in the current context all burdens resulting from transgene escape are shifted to the users of GM-crops and those potentially affected by their unconfined cultivation. In the absence of formal co-existence legislation which clearly assigns liability and responsibility, recourse for damage suffered by transgene escape will be difficult to achieve. For example, recently in Canada, the Saskatchewan Organic Directorate (SOD - an association of organic farmers in the province of Saskatchewan) attempted to set precedence in civil law in Canada by suing in class action, Monsanto Canada Ltd and Bayer CropScience for the ubiquity of GM transgenes in canola in western Canada and the resultant inability of organic farmers in this region to produce GM-free organic canola. The case was denied class action status by a Canadian federal court judge because the plaintiffs had failed to adequately prove that the entire class (all farmers in SOD) was suffering damage (Smith, 2005). The lack of clear assignment of liability related to problems arising from transgene escape has created risk for the agri-food industry in Canada. The recent voluntary withdrawal of glyphosate-tolerant spring wheat from the regulatory process in Canada was likely related to political pressure from the grain and food industry whose members were fearful of the liability they would hold in the event of intraspecific transgene escape in wheat after the commercial release of glyphosate tolerant wheat.

There is one effort in Canada to create a GM crop exclusion zone. In Canada's smallest province, the Prince Edward Island Certified Organic Producers Co-op is assessing a market for agriculture products produced in an Island GMO free grow zone (PEI COPC, 2005). There

are political and legal efforts challenging the validity of the arguments being used to establish this zone. In Canada, GM crops are not regulated *per se* because Canada subscribes to the notion of substantial equivalence between GM and non-GM crops. In addition, where there are concerns about transgene (trait) movement the regulatory body in Canada is only allowed to regulate on the basis of human health or environmental risk and not economic risk. In addition, it is not yet certain whether such GM free regions can prevent the adventitious presence of transgenes. However, such regions are changing the concept of co-existence from spatial differentiation at the farm level to the county level and to larger more isolated regions including islands.

Thresholds for the presence of transgenes are only useful to business entities within the agri-food industry (including farms and farm organizations) if the thresholds are set within law. Thresholds for transgene presence may be set by organizations, such as organic certification agencies, but they must be recognized in law within the political jurisdiction within which that agency is functioning if there is to be any enforcement of the threshold or recourse in the event that the threshold has been exceeded. A good example of this is the fact that the EU has established a transgene thresholds in law while in Canada, the right of organizations such as the Saskatchewan Organic Directorate's to no threshold ('zero threshold') for transgene contamination of organic crops has not yet been recognized in Canadian law (Cullet, 2005). In the context of this issue it is worth noting that the International Federation of Organic Agriculture Movements (IFOAM) adopted the position in 2002 that organic certification is a certification of a process of production and as such does not imply an end product guarantee (ISF, 2004). In this sense, IFOAM does not necessarily support *de minimis* threshold levels ('zero thresholds' or minimal testing level thresholds). This creates a challenge for organic farmers who are trying to keep their products 'GM-free' because "GM-free" has not be defined.

## GOVERNMENTAL APPROACH IN CANADA

The government of Canada promotes biotechnology as an opportunity for Canadian industry and Canadians. It has positioned itself as a “catalyst, reasoned advocate, interlocutor and facilitator in advancing Canada’s plant and animal molecular farming sector” (Industry Canada, 2004). This sentiment affects how the Canadian government approaches the regulation of GM crops. The Canadian government does not have regulation specific to GM crops because it regulates on the basis of the product not the process. This decision is based on a belief in substantial equivalence where GM crops are deemed to be substantially equivalent to non-GM crops with respect to regulatory requirements. The government of Canada uses the term “adventitious presence” in the context of GM crops and defines it as “the unintended, technically unavoidable presence of genetically engineered material in an agri-food commodity” (CFIA, 2005). Without a formal recognition that transgene escape can lead to contamination situations there will be little or no progress towards Canadian regulation for transgene confinement. The Royal Society of Canada released a formal report on the future of food biotechnology in Canada (Royal Society of Canada, 2001) which called for more direct regulatory oversight for biotechnology in agriculture and food products as well as a significant intensification of research into the potential effects of consumption of GM crops as well as the potential effects (primarily environmental) of transgene escape. The government of Canada has developed some initiatives related to the introduction of GM crops. Environment Canada has created an interdepartmental committee which has been charged with developing a research strategy, the purpose of which is to generate knowledge on long term ecosystem effects of novel living organisms (NLOs) (including GM crops), in order to strengthen the sound scientific basis for policies, decisions and management of NLOs (Environment Canada, 2005). The government has also created an industry consultation

initiative called “responsible introduction of novel agricultural products” (RIONAP) (Industry Canada, 2004), but no formal commitment has been made to acting on recommendations that may come from the RIONAP activities. As of the summer of 2005, the government of Canada is still submitting progress reports on their actions related to the Royal Society report (Health Canada, 2005). The most common governmental action has been consultation but no new regulations or laws have been created yet and there has been no dedicated allocation of governmental funding to achieve the research recommendations. There has also been no governmental response to the Supreme Court ruling on Percy Schmeiser nor has there been any response to the seed contamination issues. The Canadian Seed Growers Association (CSGA), along with the Canadian Seed Trade Association (CSTA), has initiated a review of seed production regulations, with specific consideration of genetic purity issues (CSGA, 2005). The CSGA along with international seed industry argues in support of an update of seed and varietal purity notions within the context of GM crops but they also express strong concern over the ability of the seed industry to meet absolute genetic purity standards and the tremendous cost of meeting such standards (ISF, 2004).

## CONCLUSION AND FUTURE DIRECTIONS

Transgene escape has been happening in Canada and it has caused some problems. The position of Canada as a promoter of biotechnology means that there is little governmental will to produce concrete action and there are no signs that specific regulation or law related to transgene escape and its potential effects will be created any time soon in Canada. Within this political environment, research into the means and mechanisms of transgene escape will remain an *ad hoc* activity in Canada, performed primarily by academics using funding acquired through existing programs. The information this research provides will be useful and welcomed by those who may be directly affected by transgene escape. In the immediate term this would include non-GM

farmers. In the longer term, as pharmaceutical and industrial traits are introduced into crop plants, more Canadians will be affected by transgene escape. At that time the political will may be created to formally address the issue of transgene confinement in Canada.

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# Co-existence: an Australian perspective

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**Abstract:** Despite approval from the Australian Commonwealth Office of the Gene Technology Regulator (OGTR) for open release of Roundup Ready®, InVigor® and Liberty Link® canola varieties, the introduction of the technology into canola production in Australia has been indefinitely delayed by 'moratoriums' set in place by State Governments on the grounds of potential adverse market impact on non-GM production systems and markets.

The moratoriums span all food crops, but the immediate industry focus is on canola. The canola industry has sought to work with Government and the technology providers to establish the principles and practices of co-existence between GM and non-GM supply chains to address these concerns. The broad principles and detailed protocols for a co-existence strategy for canola have been developed but the State Governments are concerned about the market impact of proposed purity standards which allow low levels of adventitious presence (AP) in non-GM canola, rendering the concept of 'GM-free' redundant. The debate on AP is currently highlighted by the discovery of trace levels of a GM event (~0.01%) in the Australian canola crop.

The industry is targeting standards that meet market expectations and the ability of the market to offer premiums in line with the increased cost of quality assured segregation. The standards proposed are in line with those under discussion in the EU, 0.9% AP in non-GM produce and 0.5% in planting seed. If the industry's proposed standards and co-existence protocols are accepted as addressing the State Government concerns on market impact, then the industry will seek to scale-up evaluation of the coexistence protocols, still under contained conditions. In a stepwise process, it is hoped that further evaluation and refinement of co-existence principles will lead eventually to the removal of the current moratoriums on GM canola production.

## INTRODUCTION

Through GM cotton, Australian agriculture has been a major beneficiary of GM technologies for nearly a decade. The first insect-resistant Bt cotton was introduced into commercial production in 1996, to be followed by GM herbicide-tolerant cotton varieties (glyphosate-tolerant) in the 2000/01 season and, in turn, the twin Bt 'Bollgard II®' constructs commenced production in 2003/04 (Higgins & Constable, 2004). It is anticipated that in the current (2005/06) season, 80% of the Australian cotton crop will be sown to Bollgard II® varieties and 60% to 'Roundup Ready®', cottons for a total of over 85% GM (G. Strickland *pers. com*).

The cotton industry has elected not to segregate the crop on GM and non-GM lines. A low level of segregation has been practiced with cottonseed oil (widely used domestically as a commercial frying oil) and cottonseed meal (in livestock rations). In consultation with the livestock industry users of cottonseed meal, the cotton industry examined the costs of strict segregation and agreed that there were no premium prices that would balance the costs. A low cost system based on grower declarations and quality assurance principles has been in place, but as the percentage of GM in the crop has risen, the cottonseed oil and meal has largely been marketed as undifferentiated products in line with the fibre.

While GM research proceeds at an increasing pace within Australian scientific institutions, the only other open release of GM plant technologies in Australia has been a blue carnation with extended vase life, introduced by Florigene back in 1996. Debate on the merits of GM technologies ranges over a wide area in the media in Australia. From a practical farming community point of view, the predominant focus of attention over the last decade has been on GM canola. In 2004, the Federal Government regulator, the Office of the Gene Technology Regulator (OGTR), granted open release approval of 'Roundup-Ready®',

'Liberty Link®' and 'In-Vigor®' GM canola varieties on the basis of science-based criteria for human health and safety and environmental impact. However, commercial production was subsequently prevented by 'moratoriums' established by State Governments in all canola growing States on the basis of uncertainties over potential adverse economic impact of GM canola introduction on production costs and market access/prices for non-GM production.

In the medium term, further progress in the adoption of GM technologies in Australia hinges on the case of GM canola. In the short term, the solution demanded is to develop an effective framework for GM and non-GM co-existence that enables all sectors of the supply chain to exercise choice without occasioning excessive costs of segregation.

## PROGRESS TOWARDS A CO-EXISTENCE FRAMEWORK FOR AUSTRALIAN CANOLA

The Australian grain supply chain has long recognised the need for a coordinated approach to meeting the management challenges of GM crops. In early 2000, the Gene Technology Grains Committee (GTGC) was formed, initially in Western Australia, and extended to the eastern states in 2001. The GTGC brought together the grower representative bodies, grain handlers, processors and marketers, the biotechnology industry and government.

The GTGC initially identified an information gathering role to identify issues of concern held by farmers and the public and to provide factual information to government, the industry and the public. Focussing on canola, the Committee recognised at an early stage that in the short to medium term, the integrity of both GM and non-GM supply chains would need to be maintained. The key issues were identified as agronomic risk management and market impact, which were addressed as below.



- Risk management in farming systems

In examining farming systems issues, the Working Group identified 'gene flow' as requiring further clarification. Two areas emerged:

- *The weediness potential of volunteer herbicide-tolerant canola and the potential for outcrossing into weedy relatives (such as wild radish, wild turnip).* The consensus established in this area is that the risks in Australian farming systems are acceptably low and can be managed without adverse environmental impact. (Salisbury & Downey, 2002).
- *The potential for cross-pollination between canola fields.* Under Australian broadacre production systems, with large fields and hot dry conditions at flowering, cross-pollination occurs at low incidence (Rieger et al. 2002).

- Market access issues

As with most broadacre agricultural production in Australia, canola production is strongly export oriented. Approximately 80% of the crop is exported (Foster et al. 2003), the majority (88%) to Japan, China, Pakistan and Bangladesh, markets in which Australia competes predominantly with Canada (Apted et al. 2005). The most recent study by the Department of Agriculture Western Australia (Morcom & Fernandez, 2002) concluded that the potential loss of export sales in 2002/03 from the introduction of GM canola was 0.4%.

Domestic consumption in Australia presents a contrast to the export scenario. Australian canola crushers have demanded non-GM product, based on current consumer preferences. This is also the case for livestock producers accessing canola meal.

Consultations between the GTGC and State and Commonwealth Governments identified the need to develop formal protocols for the GM and non-GM supply chains to practice co-existence. After a series of workshops the GTGC released for public comment the 'Guidelines for Industry

Stewardship Programs and Crop Management Plans' (Anon., 2002). After further process, the 'Canola Industry Stewardship Protocols for Co-existence of Production Systems and Supply Chains' was released in July 2003 (Anon., 2003).

The 'Stewardship Protocols' provided hazard analysis of the canola supply chain from crop breeding and seed production and supply (pre-farm) through to crop production, storage and management on-farm and concluding with post-farm grain receipt, marketing and processing. Throughout the document, references were made to pre-existing standards, protocols and responsible agencies and organisations. Key components in the three operational stages included:

- *Pre-farm*

The Seed Industry Association of Australia (SIAA) holds responsibility for compliance with internationally recognised standards for seed quality and purity and for testing protocols. With GM crops, the SIAA published a Code of Practice in plant breeding, seed production and marketing and has set purity standards at 0.5% adventitious GM presence in non-GM seed.

- *On-farm*

'Crop Management Plans' prepared in consultation with industry by the biotechnology company were identified as providing the basis for on-farm management and containment of GM crops. Components included separation distances from non-GM crops and rotational requirements to avoid problems with volunteers in following crops. The 'Technology Use Agreements' signed by growers with the biotechnology companies formed the basis of quality assurance documentation, audit and sanction.

Both GM and non-GM growers additionally have access to quality assurance programs and supporting documentation that underpins their variety declarations at grain receipt points.

- *Post-farm*

The post-farm protocols provide the industry standard for the management of traceability and identity preservation through receipt, handling and storage to the point of dispatch from the handling facility.

The legal implications of the Stewardship Protocols are variations on current fair trading laws, subject to the standards, contracts and dispute resolution processes of the Australian Oilseeds Federation (AOF) and the National Agricultural Commodity Marketing Association (NACMA). When drafting the Gene Technology Act 2000, the legislature chose not to implement a specific liability regime for damage caused by GMOs. Ultimately, where activities of one farmer affect a neighbour, recourse is to existing statutes and common law (Dalton *et al.* 2003).

## **THE TRANSITION FROM CO-EXISTENCE PRINCIPLES TO CO-EXISTENCE PRACTICE**

The GTGC Stewardship Protocols were passed on to the three responsible industry sectors for further development and implementation – pre-farm to the SIAA, the on-farm to the biotechnology companies (through their Crop Management Plans) and the post-farm to the AOF. In particular, the post-farm bulk handling and marketing groups needed to work up further in-house systems development (enhanced data management, quality assurance, testing protocols) and to implement a series of ‘beta testing’ using model systems.

The post-farm systems development by the grain handling sector was enhanced by a program of project investments from the Commonwealth Department of Agriculture, Fisheries and Forestry Australia (AFFA). A series of reports emerged, including a gene flow study by the Bureau of Resource Services (Glover *et al.* 2002), a review of testing technologies from the Australian Government Analytical Laboratories (Griffiths *et*

*al.* 2003), a ‘Good Agricultural Practice’ analysis of existing quality assurance systems for handling GM and non-GM products (Lovell *et al.* 2003) and a simulation study on segregating GM and non-GM grain in the Australian grain storage system (Viljoen *et al.* 2004) and the ‘Eyre Peninsula Study’ on the development of a protocol for accreditation of non-GM grain produced in a designated non-GM region (Boyce, 2005).

With the regulatory approval for open release of Roundup Ready®, InVigor® and Liberty Link® canola varieties in 2003, the industry hoped to further test the expanded Stewardship Protocols in commercial scale trials in which the produce would be tracked through to market.

These plans were overtaken by State Government decisions in the canola growing states exercising their rights under State-Commonwealth agreements to legislate ‘moratoriums’ on GM technologies on the grounds that there might be adverse economic impact on the State’s economy. These decisions have been taken despite an unbroken series of reports that there would be no significant adverse impact on markets. Apter *et al.* also calculate that the current moratoriums and extension to other transgenic broadacre crops are expected to result in a loss of GNP in NPV terms of A\$3 billion. Norton (2003) estimated that the loss of access to the current GM canola varieties alone represents a cost to the grains industry of the order of A\$135 million annually.

## **CURRENT AND FUTURE CO-EXISTENCE ISSUES FOR THE AUSTRALIAN CANOLA INDUSTRY**

While there are further research challenges ahead to refine co-existence principles, two issues require resolution before any steps can be made to satisfy State Governments that the canola industry can manage the market challenges of co-existence of GM and non-GM supply chains – the primary condition for lifting the moratoriums currently in place. These are:

- *Acceptance of realistic thresholds for adventitious presence*

Uncertainty remains over purity standards to apply to planting seed and production. The broad approach by industry is to manage AP in non-GM canola to standards defined by the market and at a cost that the market will bear. Opponents of GM production quote the 'GM-free' terminology as the justification for demanding zero GM adventitious presence. The industry regards the GM-free concept and zero AP as unmanageable. The Co-existence Protocols propose definitions for three grades of canola receiptal:

- *'Canola'*: Meets all commodity trading standards and importing country requirements. May or may not contain GM events (approved under a science-based process – ie, may not be approved in Australia). No differentiation for production systems.
- *'Non-GM Canola'*: Meets commodity trading standards. Within market specifications for adventitious presence (AP). Implicitly excludes canola produced under a GM production system
- *'GM-free Canola'*: Meets all commodity trading standards. Market specification for 'nil' AP (based on a testing protocol that would provide an agreed level, eg 95% confidence, that it does not exceed 0.1% AP). Must be produced under a GM-free production system that meets customer specification or export standard requirement.

Further work will be necessary to establish a base for non-approved events, which are banned under OGTR regulations.

- *Establishing industry-based dispute resolution processes*

Proposals are being considered by some State Governments to introduce strict liability legislation on GM risks, as are in place in Germany and Austria. The industry believes that the current industry processes for managing standards,

contracts and dispute resolution are adequate and that any outstanding legal matters can be managed by common law and existing statutes.

Since mid-year 2005, the industry has been dealing with the discovery of AP at or near the limit of detection (0.01%) in the Australian crop. It is currently believed that the AP has arrived from Canada in material used in a non-GM breeding program. The details and eventual industry and Government response is still under development, but the incident has highlighted the fact that zero thresholds present problems. Co-existence will require that the market accepts the nexus between high standards and high price and the appropriate balance is struck.

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# Co-existence of GM and conventional soybean productive chain: experiences of cooperatives and processing firms

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**Abstract:** This article aims to demonstrate seven experiences of Identity Preservation in processed soybeans by the Brazilian processing industry. The firms and cooperatives reported here have their soybean meal certified by international auditing firms like SGS-International Certification Services, Genetic ID and ECOCERT. The analyzed experiences reveal that Brazilian crushing industry is investing heavily in order to guarantee that soybean meal exports are free from GM organisms. This initiative has been a result of consumer requirements in EU and Japan. However, Brazil has only a small portion of its soybean production certified as to comply with that market condition. Currently, the co-existence of two soybean productive chains has been determined by the fact that the product is sold to two different markets, either domestic or international. The only institutional project for segregation of soybean in Brazil has been carried out by the government of the state of Paraná.

## INTRODUCTION

The first attempt to introduce GM for commercial purpose in Brazilian agriculture occurred in 1998, when Monsanto requested Biosecurity National Technical Commission, CTNBio, for liberalisation of production of soybean GM/RR, which was approved of in September the same year. This attempt was, however, frustrated by the actions of consumer movements and environmentalist organisations. Moreover, Brazilian justice refused the authorisation for cultivation until constitutional clauses protecting consumer rights were fully attended. Nevertheless, Ministry of Agriculture has always positioned favourably to such an approval. This ambiguous situation led to the smuggling<sup>1</sup> of

soybean seed from neighbouring, with neither certification nor plan for co-existence. National production of soybean might increase to 58.2 million tons in 2005/2006<sup>2</sup>, from which about 25% will be genetically modified.

Brazilian farmers produced 4.1 million tons of genetically modified soybeans in 2003/2004<sup>3</sup>, which was 8.2% of the whole production of soybean in the country. The GM cultivated area was 2.78 million hectares, corresponding to 13.2% of the total soybean area. The main states producing GM soybeans are Rio Grande do Sul (88.1%), Paraná (1.8%), Minas Gerais (1.7%), Goiás (1.4%), Piauí (1.4%) e Santa Catarina (1.4%).

## APPROACH

In this paper we present some experiences of co-existence of productive chain within the same company or cooperative, highlighting the

processes used in order to preserve the identity<sup>4</sup> of non-GM grains. In other words, attention is given to the process through which non-GM soybean is isolated. Experiences of co-existence within cultivation are not discussed, once field research agriculture on farming activity has not yet been carried out.

## RESULTS AND DISCUSSION

### **COTRIMAIO – Cooperativa Agro-Pecuário Alto Uruguai Ltda.**

COTRIMAIO is an agro-industrial cooperative located in Rio Grande do Sul, having nearly 6.500 members, the majority of them small and medium sized. Besides soybean, COTRIMAIO has also been involved with producing and selling other products, both vegetal and animal.

The first impulse to produce non-GM soybean meal was given by negotiations aiming at exporting it to French cooperatives, whose demands that certification procedures should be adopted, triggered off changes in the management of the productive chain and most of all in the relationship with the farmers.

The adoption of an identity preserving system was a precondition for certification. As COTRIMAIO deals with GM, non-GM and organic farming, traceability and certification of its products are conducted by ECOCERT. Traceability is performed from seed selection through sowing, harvest and final delivery of crops into the cooperative facilities. Farms are visited by professionals who give orientation about harvest techniques, cleaning of equipment and transportation.

That company operates according to an exclusive non-GM soybean regime in which its

own processing plant as well as their suppliers follows with a contract followed by all plants involved. When grain crushing is transferred to other firms, contracts set down the terms to preserve the grains' identity, like those related to the procedures of cleaning the operating equipment and silos. In such circumstances certification is given to each produced batch, though the critical points on which control is made and genetic identity exam is undertaken, are stipulated and inspected by ECOCERT, the certifying agent, which gathers samples in the properties and analyse them in laboratories. COTRIMAIO stores its exporting product in appropriate silos of CESA (State Company of Silos and Storehouse) in Rio Grande Port, where conditions for preserving identity are better, given that it is hardly used for exportation of soybean grains.

### **IMCOPA – (Imports, Exports e Oil Industry Ltd).**

IMCOPA was installed in Ponta Grossa (State of Paraná) in 1967, with a daily production capacity of 20 tons. Ten years later another plant was built whose daily capacity is as follow: 2.000 tons of processed soybean; 1.500 tons of soybean meal; 370 tons crude oil; 400 tons of refined oil; 200.000 cans/hour of refined oil; 20 tons of lecithin; 500 kg of greasy acid. The company also has a specific plant used for crushing non-GM grains. IMCOPA has two silos with a total capacity of 120.000 tons. The company uses also two rented silos in Paranaguá harbour to store its exported products transported by railroads. Those silos belong to COTRIGUAÇÚ and are rented by IMCOPA according to a contract establishing procedures of manipulation and transportation of grains.

The present soybean storage capacity of IMCOPA is of 240.000 tons daily. Also, the company will have processed 850.000 tons of and marketed more than one million tons of products<sup>5</sup>.

As IMCOPA processes non-GM products only, traceability is more easily carried out by giving

priority to soybean produced in Paraná where production of GM products is smaller. Control over products is achieved through inspection and tests by a well trained group of experts that travel all over the year, visiting crops, cooperatives and ports.

Soybean processed by the company is bought, from five cooperatives, following contracts which require that the product is non-GM. Beside an inspection by IMCOPA, production is controlled by the cooperatives themselves. On the other hand, during the sowing period, technical staff of IMCOPA visits the cooperatives and their suppliers in order to test the seeds. During the harvest new tests are made, on both plants and product.

In order to control the unloading process of a product a test is carried out through the SDI method before it starts. Part of the sample is then taken to the Genetic Laboratory ID in the US where a quality is tested through the PCR method. Additionally, grains are tested by IMCOPA's laboratory to identify the levels of humidity, impurity, quality and acidity.

Control of storage is made possible by segregating grain into portions and storing them in separate silos of up to 15,000 tons. Before starting the crushing process the results of the PCR are assessed in order to guarantee that GM products are not present.

IMCOPA still controls its final products by adopting the following procedures:

- Meal: quality test 3 times a day of samples collected every hour.
- Oil: sample to be tested collected every two hours.
- Lecithin: samples collected from each produced lot.

All samples collected by the company are stored for 180 days, so that if a client wishes to review data of a material kept up to five months, the original sample with all the previous analysis will be provided.

IMCOPA has sheer control over the identity of its products, which are traced down to the raw material supplier.

### **Caramuru Alimentos Ltd.**

Caramuru set up its activities in March 1964, in Maringá, north of Paraná. Nowadays, its headquarters are located in the state of São Paulo with branches in the states of Paraná, São Paulo, Goiás, Mato Grosso, Bahia, Pernambuco and Ceará. The company is involved with activities from processing grains, production of seed, storage, de-germination, corn pre-cooking, extraction and refinement of special oil from soybean, corn, sunflower and oilseed, and meal production. Its products are exported to the EU, Asia and Africa.

### **Hard IP System (Hard Identity Preserved System):**

The plants of São Simão and Itumbiara (State of Goiás) are involved with non-GM grain processing and the production of Hi-Pro soybean meal, pellets of soybean meal, hi-fiber meal, coarse and refined oil, and soybean lecithin.

All items produced by Caramuru follow a negative standard of non-GM at a limit of 0.1% (PCR - Polymerase Chain Reaction with a detection limit of 0,01%), including raw material. In order to so, the company's non- GMO identity preserved system with traceability is independently certified, involving inspection, tests, control and analysis at different stages of grain production, processing, logistics, storage and loading.

There is thus a programme, which initially consists of checking the seeds' source, the sowing stage and the subsequent development of soybean plants. Harvest, storage, industrial processing, port haulage and international logistics are also taken into account.

All the processes are separate, as analysis of seeds, leaves in the field, soybean before loaded

into the silos and before being taken in the processing plants, is carried out through fast tests of enzymes. It is important to stress that 100% of soybean is analysed before being loaded into the silos and the crushing industry, accepting only those lots of soybean whose test result is negative.

PCR analyses are carried out during the industrial process as well as in port silos and shipment of Hi-Pro soybean meal and pellets.

The team from the company is fully committed to the programme, adopting operational procedures according to what is determined by the terms of approval and the corresponding certificates. Presently, two separate companies control the programme of non-GMO: Cert ID and SGS of Brazil. The former also provides a demonstration of traceability by issuing Certificates of Transaction (TCC) for each delivery to its clients.

Caramuru produces the following: soybean lecithin, Hi-Pro soybean meal, pellets of soybean meal and Hi-Fibber meal.

### **OLVEBRA Industrial S.A.**

This company started its activities in 1957 in the State of Rio Grande do Sul, pioneering soybean industrialization in Brazil. Nowadays it only operates with non- GM soybean.

Besides the functional quality of its raw material, that company provides a guarantee that its products are non-GM, thus suitable for the production of other different products such as milky specialties, fruit juice, proteins, canned meat, bread, biscuits, pasta and chocolate.

Ovelbra pays a bonus for conventional soybean. However, in 2005, due to a drought, the company had to bring soybean from outside Rio Grande do Sul, raising costs – freight and higher price – by around 12%. Costs of certification have also increased, since, instead of the previous suppliers already known and inspected, this year had to test

and analyse all acquired lots of soybean. About 80% of that company's production – a total of 600 to 700 tons – comes from the states of Paraná and Mato Grosso.

### **Cooperativa Agropecuária CASTROLANDA**

This cooperative was founded by Dutch immigrants settled in the Centre-Southern region of Paraná just after World War II. Nowadays its storage capacity is 285,000 tons, its total turnover US\$ 200 million, and the total number of associates is 634 farmers. Nearly 50% of the cooperative's turnover comes from the sale and industrialisation of dairy products, since its foundation in 1954. The other 50% derives from different activities and products, mainly soybean. Castrolanda supply its members with animal feedstuff, selling the surplus in the domestic and foreign markets.

Since 2001, the cooperative has followed international rules to give support to production and distribution of non-GM soybean certification. According to a cooperative representative, Mr.

Sinohe Guerreiro de Oliveira<sup>6</sup>, the first stage towards complying with those rules is the control of seeds through the PCR test – which identify GM soybean – in order to make sure that there is no contamination before sowing. As plants start to grow, samples are collected and the farmers have the visit of technicians who test the plants to detect traces of genetic modification on the leaves. In the processing units, the vehicles used to deliver soybean are inspected, and after the transportation of every 5 thousand tons a new PCR test is applied. The whole process is registered, and based on the reports of inspection a certificate of traceability is issued.

### **Selecta**

SELECTA is a family company placed in the state of Goiás. Production of high quality seed is its main activity. Since 2001, the company has also produced grains according to systems of identity preservation, traceability and certification.



As the company already had a large control over seed production and groundwork for crop fields, running the grain business with preserved identity became highly advantageous. These activities were then adapted for the production of non-GM grains with preserved identity.

Among those companies buying soybean from SELECTA are suppliers to large companies, like Nestlé and Carrefour, which use non-GM soybean as raw material.

Soybean grain is thoroughly controlled, from the seed produced by the company and delivered to farmers to its final shipment. Besides, the company runs a database of registered farmers, in which information about crop production are stored and constantly monitored. Therefore, the seed's genetic origin is guaranteed and the development of crop fields is strictly controlled. In the end, farmers rewarded with a price higher than that prevailing in the market.

In order to guarantee quality and to preserve soybean identity, the company has its own logistics. All soybean grain from SELECTA is stored in separate and private silos. Owing to an agreement with Vale do Rio Doce Company, after being stored in 13 silos located in the state of Goiás, soybean is carried on trucks, provided by the latter, to a 12 tons terminal in Uberlândia (State of Minas Gerais) on trucks belonging to the latter. From there the product is transported, on separate wagons properly identified, by Ferrovias Centro Atlântica (FCA) and Estrada de Ferro Vitória-Minas, down to Tubarão harbour, where Vale do Rio Doce owns a 50 thousand tons silo for soybean.

### **Brejeiro**

This group, created in 1944, originally processing and trading rice, started the business of trading and crushing soybean in 1983. Its headquarters are located in Orlandia (State of São Paulo), 400 km from the capital, with branches scattered over the main soybean producing states: São Paulo, Mato Grosso, Goiás and Rio Grande do

Sul. In 2005 the company had its HACCP System of Food Safety Management of soybean meal certified according to the guidelines of Codex Alimentarius.

That system was certified by SGS, a European company, after a process of assessment in which Brejeiro had all the stages of its soybean meal industrial processing approved of. The certificate lasts for three years, being revalidated every six months.

This certification is thus a guarantee that Brejeiro soybean and soybean meal are free from physical, chemical or biological risks, and also that the meal raw material is not genetically modified.

Having the endorsement of Genescan, a German inspection and auditing company, Brejeiro joined the IP (Preserved Identity) programme, which can prove that only non-GM soybean grains have been used, after which contracts were made to sell lecithin to Japan and Europe.

## **PERSPECTIVES**

The predicted expansion of genetically soybean area after the bio-safety law was approved of by the congress authorising its cultivation and trade, led the cooperatives to negotiate a bonus for the non GM soybean. Directors of three major cooperatives in Paraná, Cocamar Cooperative from Maringá, Coamo Cooperative from Campo Mourão, and Agrária Cooperative from Guarapuava, believe there will be favourable conditions for an increase in value of non-GM soybean. For them, market will regulate production. If the commodities system prevails, there is no room for differentiated products, obtained by certification processes, identity preservation and traceability.

In Rio Grande do Sul, third major producer, with an area of 4.1 million hectares, 90% of total production is of GM crops, according to the president of Cotrimaio cooperative. As the

cooperative buys its product from small farmers, there is a great difficulty for them to separate crops. Furthermore, not having their own tractors and harvest machines, they need to borrow them from their neighbours. Therefore, it becomes very difficult carry out segregation, once the machines are used on GM and non-GM crops. In Rio Grande do Sul, it becomes also increasingly hard to plant non-GM soybeans because seeds are very scarce.

The unique institutional initiative on non-GM certified soybeans is the State of Paraná. Since 1998, the local regulations discipline the GMO production, transport and commercialization. Is the State controller, CLASPAR (Classification Paraná), charged to inspect inbound freight for quality and presence of biotech grain prior to arrival at the terminal in Paranaguá harbour. However, this police are countercurrent to the central government's position about GMO crops that approved a complaisant Biosecurity Act to biotech interests on March 2005.

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<sup>1</sup>In 1999 Brazilian Association of Seed Producers denounced the sale of smuggled seeds from Argentina into Rio Grande do Sul. In that year, the cultivated area of GM soybean in that State should reach 1 million hectares, one third of the total area cultivated with soybean.

<sup>2</sup> According to the first estimate for the season made by Brazilian Association of Vegetable Oil Industry (ABIOVE), released by the end of September.

<sup>3</sup> According to Farming Defense Secretary – Agriculture Ministry

<sup>4</sup> Identity Preservation Systems aim to ensure that product integrity and/or purity is maintained (within given standards) along the supply chain. Tests to verify product identity can be conducted at any stage of the supply chain from seed selection, sowing, grain production and delivery. Associated documentation is critical to demonstrate that Identity Preservation has occurred.

<sup>5</sup> For more details see <http://www.imcopa.com.br>, where data and information on the company's history, its activities and the way its products are traced are available. Information can be assessed in various languages.

<sup>6</sup> Agrolink, 2005-08-19

# **INVITED PAPERS**

**Plenary session 3:**  
IMPLICATIONS OF CO-EXISTENCE  
ON RESEARCH ACTIVITIES



# Plurality of agricultures in Europe: from research on co-existence to plurality in research policy?

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**Abstract:** European agriculture is experiencing a major shift with a departure from a mode of subsidising based on volumes of productions and from the mode of standardization emblematic of agrofood post-World War 2 modernization. Product differentiation has emerged as a central dynamic in contemporary agrofood systems (economists talk about a new “economy of quality”, see Allaire 1996). Rural spaces are now viewed and ruled as more than spaces of agricultural production. The biological and socio-cultural diversity of farming systems is seen as a public good and policy goal that contributes to the sustainability of agriculture (Fischer Boel 2005). Promoting diversity instead of standardisation plays therefore at least three majors functions within the new agricultural policy: it has an environmental value, it allows the exploration of various possible agricultural and rural futures, and it helps the successful integration of European agricultures in the localized/globalized “economy of quality.”

What are the challenges for science policy to support the diversity of agricultures in Europe? Are co-existence measures enough? Are thresholds and identity preservation (with all the research and modelling necessary to provide rational basis for such policies), be they technically achievable and seriously implemented (liability regime, coordination at local and regional scale, etc.), enough to ensure co-existence between GM, non GM and organic crops? Certainly not. This communication will attempt to shift upstream the reflection on co-existence, from the issue of “adventitious presence” to issues of science policy. Two starting points will provide basis for our reflexion.

First, recent history provides numerous examples of failures in maintaining diversity in agriculture and socio-technical lock-ins:

- the wheat/corn breeding contrasted history and progresses in USA and Europe (contrasted balance in research investment);
- the decrease of systemic and integrative approaches in favour of molecular approaches in agricultural research (Joly & Hervieu 2003), especially in the 1980's;
- the reduction of the number of crops whose biology, genetics and plant breeding are investigated by private and public research organisations, and the reduction of intra-varietal genetic diversity cultivated on farm in developed countries;
- the weakness of organic agriculture and organic food and agriculture research in some European countries, as compared to steady development in other countries and at the EU level (see Figures 1 and 2).

*N.B. The specialisation index is the ratio of the world share of a country in a sub-domain (here, publications on organic agriculture and food) per its world share in a wider domain (here «applied biology and ecology» which is an ISI statistical category). Specialisation indexes far above 1 indicate a high degree of prioritization of research on/for organic agriculture and food in science*

*policy, whereas indexes below 1 indicate that the domain is a neglected one.*

Analysing these cases will illustrate that the plurality of farming systems goes well beyond issues of identity preservation, and is strongly linked to strategic choices in science policy. For instance, the existence of organic agriculture beyond niches certainly implies, among other policies, a stronger prioritization of this domain by science policy-makers in some European countries like France, that may have to follow the successful Danish model (DARCOS).

The second «food for thought» on agriculture plurality's implications for research, is provided by sociology and anthropology of science. Michel Callon (1994) has argued that science is not intrinsically a public good in itself, since it is neither intrinsically nonexclusive (it can be appropriated in many forms) nor intrinsically nonrival (nonrivalry in science is limited to small specialists communities and is the result of a series of strategic and costly investment in capacities that make knowledge mobilizable). It is not the intrinsic public-good-nature of science that justifies a strong implication of the governments in research, argues Callon, but rather the fact that science is a source of diversity of socio-technical dynamics that can be harnessed by policy to avoid lock-in and maintain a plurality

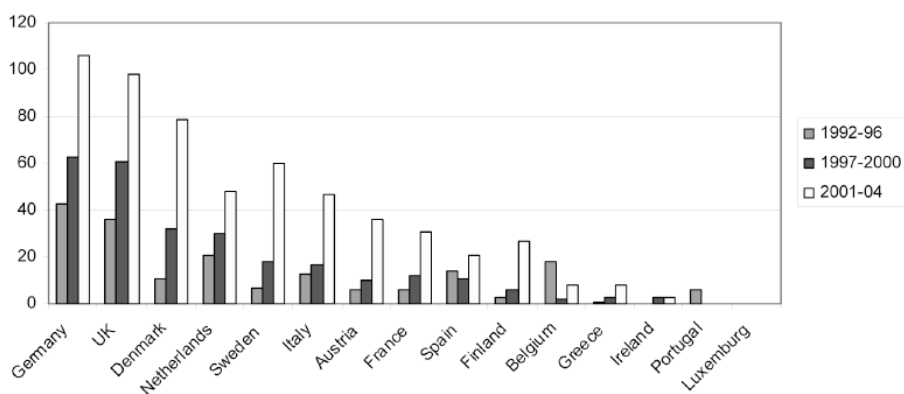


Figure 1. Organic food and agric. research in Europe: publication output in ISI.

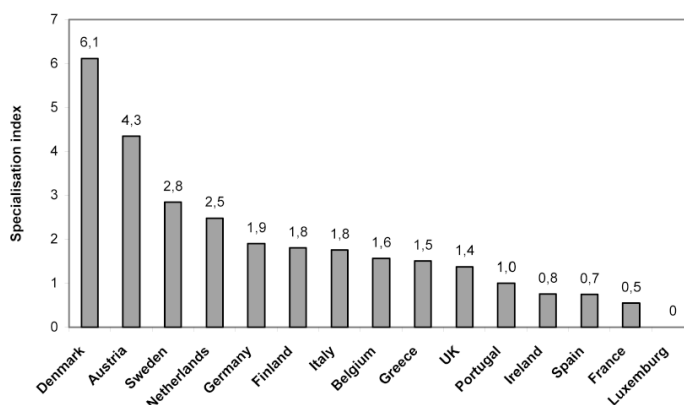


Figure 2. Organic food and agric. Research in Europe: prioritisation within applied biology and ecology.

of possible futures in democratic societies. From this perspective, science and science policies hold great responsibilities for constantly «lending support to emergent collectives [i.e. heterogeneous networks of scientific and «lay» actors, objects and statements] and encouraging their proliferation» so as to explore a plurality of socio-technical futures (Callon 1994, p. 417).

From a one-dimensional «plants for the future» motto to «various plants in complex agroecosystems for richer futures and better quality of life in a variety of rural areas» what would it mean for European agricultural research to follow Callon's proposal, both in terms of research priorities and in terms of modes of partnerships?

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# Research activities on co-existence between GM and non-GM crops at the European Commission's Joint Research Centre

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**Abstract:** The European Commission's Directorate-General known as Joint Research Centre (JRC) is involved in research relevant for coexistence mainly through the work of its Institute for Prospective Technological Studies (IPTS). IPTS provides customer-driven support to the European Union policy-making process, by researching science-based responses to policy challenges that have both a socio-economic as well as a scientific/technological dimension. Coexistence between Genetically Modified (GM) crops and non-GM crops is a clear example of a policy dossier with substantial techno-economic implications. IPTS has been active in providing scientific support to other European Commission services at all stages of the development of the coexistence policy, since the conception phase (2000-2003) that ended with the publication of a Commission Recommendation in 2003, to the current stage of implementation of the European Commission's role of supporting Member States in designing science-based strategies for coexistence. During this period several scientific questions with relevance for policy have been addressed by research and new questions in the fields of agronomy and economics of coexistence have arisen due to policy and scientific developments.

## INTRODUCTION: THE EUROPEAN COMMISSION'S JRC AND IPTS

The mission of the Joint Research Centre (JRC) is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

The Institute for Prospective Technological Studies (IPTS) is one of the seven Research Institutes of the JRC. The mission of IPTS is to provide customer-driven support to the EU policy-making process, by researching science-based responses to policy challenges that have both a socio-economic as well as a scientific/technological dimension. The IPTS main activities relate to provide strategic support for the conception and development of EU policies. Its core competence is the ability to work at the intersection between the socio-economics of an issue and the science and technology involved.

In addition to its staff, IPTS makes extensive use of its research networks to reinforce its core competences. Through its networks IPTS can provide high-quality advice at the European level over a whole range of policy fields. IPTS has established a number of networks, most notably the ESTO network (the European Science and Technology Observatory).

The ESTO network, now in its final year, has been instrumental in the research on coexistence undertaken by IPTS. ESTO has a core membership of around 20 institutions and is a mechanism to complement and expand IPTS' internal capabilities. Two research projects on coexistence have been developed by IPTS and ESTO consortia during 2000-2002 and 2003-2005, and will be described below.

In 2005, the IPTS created a new network known as the European Techno-economic Policy Support Network (ETEPS). The new ETEPS Network brings together the leading national counterparts of the IPTS throughout Europe and will place more emphasis on policy support services to the Commission.

## SCIENTIFIC SUPPORT FROM JRC-IPTS IN THE CONCEPTION OF COEXISTENCE POLICY (2000-2003)

The need for a coexistence "policy" in agriculture arose after the decision by the European Union of introducing a 0.9 % labelling threshold for the adventitious presence of GM crops in non-GM crops. Since agriculture is not done in a closed environment, suitable technical and organisational measures during cultivation, harvest, transport, storage may be necessary to ensure co-existence. These coexistence measures should make it possible for farmers growing non-GM crops to keep the adventitious presence of GM material in their harvest below the labelling thresholds established in Community law. Two techno/economic questions were relevant for the initial stages of policy action in coexistence:

(1) What is the estimation for adventitious presence of GM crops in non GM crops in Europe if GM crops are introduced and current farming practices continue without significant changes? In other words, are coexistence measures necessary for EU agriculture to comply with the 0.9% threshold?

(2) If there are cases where estimations for adventitious presence are above the 0.9 % threshold, what are the agronomical measures needed to meet the threshold and at what cost?

A JRC-IPTS and ESTO network consortium<sup>1</sup> was formed in 2000 to address these questions. Since there was almost no experience of commercial GM crop growing in the EU, estimations of adventitious presence could not be addressed by direct measurement. The consortium approached

the estimation of adventitious presence by using computer modelling and expert opinion. The models used integrate both gene flow pollen dispersion curves and agronomical practices, including crop rotations. This allowed comparing the effects of changing farming practices. Several model "type farms" were defined trying to represent the variability found in the EU regions selected for the study.

A final report was published in 2002<sup>2</sup>. The main conclusion was that the need for coexistence measures in the EU was not general and depended on the agricultural landscape, farm typologies and crops considered (maize, oilseed and potato were studied). Model estimations showed how factors such as the size and form of the plot, the meteorological conditions, and the farming practices, all influence the final adventitious presence level. For maize, the only major GM crop authorized in the EU for cultivation, the sensitivity analysis showed that a major factor contributing to adventitious presence was the relative positions of GM and non-GM plots with respect to dominant winds, and their size and shape. In other words, the landscape pattern was a determinant factor for the feasibility of coexistence. Other factors showed less than expected influence, for example the absolute share of GM crops in the landscape (scenarios of 10% and 50% studied).

For those farm types/crops combinations identified in the report as needing coexistence measures, a number of possible measures (isolation distances, cleaning of machinery, using GM and non-GM varieties with different flowering dates) were model-simulated for their technical efficiency in achieving desired thresholds. Limited cost analysis of some measures and impact on farm revenues was included.

The working hypothesis in the 2002 report was that coexistence measures were to be taken (and therefore the costs borne) by non-GM crop farmers, if they wished to avoid the economic consequences of labelling their crops as GM. In 2002 there were still no policy guidelines on co-existence, therefore GM crop farmers

had no obligation to take any measure to avoid adventitious presence, making the approach of the report realistic.

## **POLICY DEVELOPMENTS AND THE NEED FOR NEW RESEARCH (2003-2005)**

The 2002 JRC report showed that many factors that determine efficient and cost-effective measures for co-existence are specific to national and regional characteristics and farming practices. On 23 July 2003, the Commission adopted Recommendation 2003/556/EC on guidelines for the development of national strategies and best practices to ensure the coexistence of GM crops with conventional and organic farming, reaffirming that measures for coexistence should be developed by the Member States. In the Recommendation, the Commission committed itself to support and advise the Member States in the process of developing and implementing measures for coexistence.

Also, the European Commission started discussions on setting specific thresholds for the adventitious presence of GM seeds in conventional seeds, stricter than those allowed in the final crops (0.9%). Several threshold figures were proposed in a species-specific way. Therefore seed production might operate under different coexistence requirements than crop production. These discussions are still on-going.

Taking into account these policy developments and the experience gained with the 2002 report, the need for new research was considered by the JRC and other relevant European Commission services. A new consortium IPTS-ESTO was

formed in 2003<sup>3</sup> to undertake this research and deliver a final report by end 2005. The main objectives of the research are

- ***Identify and allocate technical measures for coexistence that could be implemented by GM crop farmers.*** The 2002 JRC report assumed

that all measures and costs were borne by non-GM crop farmers. The guidelines on co-existence published by the Commission in July 2003 specify that the *farmers who introduce the new production type in a region* should bear responsibility for implementing the farm management measures necessary to limit gene flow. Following these guidelines, coexistence measures currently being designed by Member States are targeted for GM crop farmers. Therefore, in the new study, the working hypothesis is that any additional technical measures needed is taken by (and costs allocated to) GM crop farmers.

- **Evaluate specific co-existence measures (and costs) needed to meet the new thresholds being discussed for seed production.** Also, the study should look at how different levels of initial seed purity effect on the final level of adventitious presence in the crops produced. In other words, to produce evidence on whether strict seed purity would be a cost-efficient measure to achieve coexistence.

- **Introduce the landscape-scale when estimating levels of adventitious presence of GM crops in non-GM crops.** The 2002 JRC report and other research suggest that *adventitious GM presence at a large scale cannot necessarily be predicated from small scale, experimental plot studies*. For example, research was performed at landscape level in Australia on the adventitious presence of Herbicide Tolerant (HT) canola on harvests of non-HT canola<sup>4</sup>. Direct measurements of GM adventitious presence, once averaged by field, were much lower than those suggested by previous studies based on experimental fields, and well below the 0.9 % level set by the EU.

This objective is achievable since detailed, digitalized versions of many EU agricultural zones are available. Here, another JRC Institute, the Institute for Protection and Security of the Citizen (IPSC) has provided the IPTS-ESTO consortium with expertise and digital data on a maize landscape from France as case study, to feed the computer models estimating adventitious presence of GM crops.

- **Review existing models of gene flow and provide information on the level of validation of these models,** in particular for the two models used in the 2002 JRC report, namely MAPOD® and GeneSys®.

- **Study the effects of time on the levels of adventitious presence of GM crops.** This is relevant for crops producing seeds with long life and dormancy period, which can build banks of GM seeds in the soil. Computer models will be used to understand the evolution of adventitious presence in long temporal series, and subsequently to identify coexistence measures that have a sustained effect over time.

The study is focused on seed and crop production of maize, sugar beet and cotton (plus oilseed rape for analysis of coexistence over time). Maize is the only major GM crop authorised for cultivation in the EU and thus a priority for policy-oriented research. The other crops are among the list of GM varieties in the development/authorisation pipeline<sup>5</sup>.

Most of the results of this research will be presented in this conference GMCC-05 in the relevant sessions. The final publication of a report is expected before the end of 2005.

## **OUTLOOK: NEW OBJECTIVES FOR RESEARCH ON COEXISTENCE AT THE JRC**

The scientific programme of GMCC-5 shows the wealth of information on the agronomical aspects of coexistence generated since the first conference GMCC-03 took place only two years ago. Research with different degrees of policy relevance has been started by Member States agricultural services, university departments and research centres including the JRC.

Also, a relevant policy development is that some Member States have already decided (or are about to decide) on the coexistence measures targeted for GM crop farmers (some general measures and others specific for GM maize as the

priority crop). In practical terms, these measures will first be in place for the 2006 maize harvest.

In this situation, and given its techno-economic expertise, IPTS-JRC research is moving into two new areas

- Develop decision tools, science-based, to help decision makers when establishing coexistence measures

As stated there has been a lot of novel research on adventitious presence of GM maize in non-GM maize submitted to this conference. Some are based on field trials, others on models. Many EU regions have been covered. It is time to gather this information and set a multidisciplinary research team that develops it into a tool for decision makers. Such exercise should start with GM maize as a priority and be followed by oilseed rape.

- Understand the socio-economic consequences of coexistence measures

Compared with the wealth of information on the technical aspects of coexistence, our understanding of the economic effects is still very limited and restricted to few examples at the farm level. Now that coexistence measures are no longer hypothetical and have been spelled out by some Member States, the economic impact of such measures at the farm and aggregated level must be properly studied. Coexistence measures are specific of EU agriculture and do not operate currently (except for speciality crops) elsewhere. The benefits provided by coexistence measures (freedom of choice) may have a cost. In particular, coexistence measures may reduce the gross margin of GM crops, which in turn may affect the rates of adoption of the technology by EU farmers. The aggregated effects on the EU economy and competitiveness of different rates of adoption of these technologies have not been studied.

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<sup>1</sup> Members of this Consortium were IPTS, INRA (France); NIAB (UK); CEST (UK); Fraunhofer ISI (Germany); ADAS Consulting Ltd (UK)

<sup>2</sup> Scenarios for co-existence of genetically modified, conventional and organic crops in European Agriculture. (2002) DG JRC-IPTS-ESTO Technical Report. European Commission (EUR 20394 EN)

<sup>3</sup> Members of this consortium were JRC-IPTS, Empresa Publica Desarrollo Agrario y Pesquero-DAP (Spain), University of Applied Sciences of Weihenstephan and Fraunhofer-ISI (Germany) and INRA (France).

<sup>4</sup> Rieger M. A., Lamond M., Preston C., Powles S. B., and Roush R. T. (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296: 2386-2388.

<sup>5</sup> "Review of GMOs under research and development and in the pipeline in Europe" (2003) DG JRC-IPTS-ESTO Technical Report. European Commission (EUR 20680 EN)



# Community research programmes to support co-existence in Europe

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The European Commission under the sixth framework programme has recently funded three important GMO co-existence research projects. These projects will provide guidance, and develop and validate methodologies, for tracing GM materials along the food chain and for facilitating the co-existence of genetically modified, conventional and organic crops. They will demonstrate and validate practical systems of sampling, tracing, labelling and documenting GMO content of foods and feeds, and will be suitable for use by all stakeholders in the food chain. This work will require the integration of new or existing systems for detecting and quantifying GM content of foods and feeds with other component elements of the traceability process. The projects also develop an EU-wide networking structure and information system on co-existence which will be used for consultation, sharing, assessing and disseminating existing data, experiences, and codes of best practice on facilitating the co-existence of genetically modified, conventional and organic crops. The acronyms of the three projects are SIGMEA, COEXTRA, and TRANSCONTAINER respectively. Together they involve almost 100 scientific partners, across Member States and INCO countries such as Russia, Argentina or Brazil with a total budget of 35 M€ of which the EU contributes 20 M€. All three projects build

upon the substantial GMO safety research effort which has been funded across the five previous framework programmes.

The expected deliverables include: a landscape generator simulating agricultural landscapes; dynamic and generic gene flow modelling platforms at the landscape level; an integrated and dynamic decision-support system for assessing the sustainability of regional farming systems; practical recommendations for the decision-making processes relating to the market release of GM crops; a long-term monitoring strategy for the EU through on-site GMO detection identification and quantification, as well as routine sampling procedures for maize, oilseed rape and sugar beet; scenarios ensuring co-existence in six regional case studies; concise information for decision-makers about gene flow and its implications in terms of co-existence. Results from these actions will be disseminated online on the respective project websites. This represents a very significant initiative on behalf of EU research into finding ways for different agricultural systems to coexist here in Europe. Important policy developments surrounding the issue of co-existence and traceability for food and non-food uses will be further supported through technological developments exploiting existing genomic knowledge under theme 2 "food agriculture and biotechnology" of the seven framework programme (2007-2013).





# **PARALLEL SESSION**

GENE FLOW



# Contribution to gene flow by seed and pollen

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**Abstract:** Gene flow frequencies are compared for seed and pollen in maize and oilseed rape. Where volunteer plants germinate and survive, gene flow beyond a few metres from the plant is typically 10 to 100 times greater by seed than by pollen. Seed-mediated gene flow will make current GM thresholds difficult to achieve on a farm that wishes to grow GM and non-GM crops, either in the same year, or in the same field later in the rotation. However, if seeds of GM origin can be excluded from a farm, pollen-mediated gene flow into the farm from a low density of GM fields (as may occur in the early stages of commercialisation) is unlikely to breach current thresholds. There is little data to show whether or not thresholds would be breached if the combined area of GM fields in the landscape was similar to or higher than that of non-GM fields. Research collated within the EU SIGMEA project will answer some remaining major questions of seed persistence and landscape-scale gene flow in maize and oilseed rape, but further studies are needed to assess whether a long-term rise in the background GM frequency would occur following commercialisation of GM crops.

## INTRODUCTION

An international symposium held over six years ago (BCPC, 1999) identified many possible routes through which genes might move between crops in pollen or seed. The probability that gene flow between fields would occur was amply demonstrated, but the magnitude of gene flow in realistic agricultural landscapes could not be predicted from knowledge at that time. Gene flow has since been studied in depth in several countries, mostly over short ranges of up to a few hundred metres, but in some instances between fields in the landscape and over cropping sequences. By 2004, the available information was extensive but uncoordinated. Accordingly, the EU project, SIGMEA, was funded to assemble

and validate the known information on spatial and temporal gene flow, concentrating on maize, oilseed rape and beet. The aim of the project was to collate information in greater detail than usually available in refereed publications. The data would be used to populate and validate models of regional gene movement, then the models themselves used to explore the possibilities and methods of coexistence between GM and other crop varieties in various European agricultural landscapes (SIGMEA, 2004).

The results being brought together by SIGMEA are likely to elucidate many important physical, biological and human factors in crop-to-crop gene flow. More generally, SIGMEA will lead to better methods for integrating effort and

funding on questions that cross wide spatial and temporal scales. The results from some of the largest studies are not yet complete, but the main factors in pollen- and seed-mediated gene flow are now known and there is enough data from both SIGMEA and other work (e.g. Damgaard & Kjellsson, 2005; Eastham & Sweet, 2002; Ramsay *et al.*, 2003) to allow some interim quantification.

## GENEFLOW BY SEED AND POLLEN AMONG CROP PLANTS

The generalised differences between gene flow by seed and by pollen are summarised in Figure 1. Gene flow is expressed as a fraction (e.g. 1/1000, or 0.1%) of the plants being considered within an area, such as a patch or a field, that contains genes through ingress of pollen or seed. Gene flow is also sometimes expressed as the number of gene copies in a batch of seeds or plants, but while the two measures (gene copies, individual plants) may differ, individual plants are the accounting unit for agronomy and are used here. Genes can move in through impurities in sown seed, as volunteers from a previous crop and as pollen from another crop simultaneously in flower. To give the following arguments context, the horizontal line in both parts of Figure 1 indicates a frequency of 3/1000, which is the highest gene flow that can occur by each of these mechanisms if the overall commercial target of 0.9% (i.e. 9/1000) is to be achieved.

### By pollen

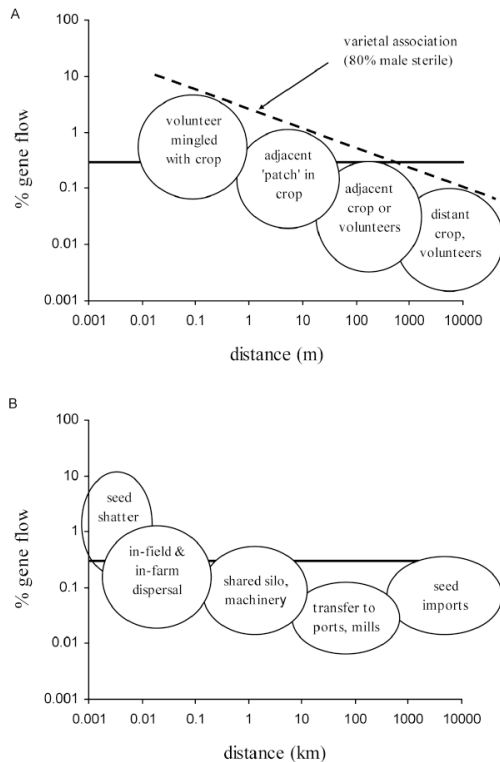
Gene flow by pollen in oilseed rape and maize occurs over a smaller total range than gene flow by seed. The range is limited by how far wind or pollinating insects can transport the pollen, mostly 1 to 10 km, and by how long the pollen remains alive (several hours, a day). At very close range, 0.1 to 1 m, pollen is exchanged at high frequency (1/10, 1/100) between adjacent sexually compatible plants. These include crop plants, and if they occur, volunteers of the same species. A main feature of gene flow by pollen is that it declines very steeply with distance, simply a result of the

incoming pollen being at a much lower density than local pollen. By 100 m, whether within the same crop or between adjacent crops, gene flow by pollen will have decreased to around 1/1000. Predictions by extrapolation, backed by emerging findings, indicate the frequency averaged over whole fields 1 km apart will be around 1/10,000, decreasing to 1/100,000 or less when fields are 1 to 5 km apart. Higher values may occur throughout the range if a large proportion of the recipient plants are male-sterile (do not produce their own pollen) as in the 'varietal associations' of oilseed rape, in which only 20% of plants produce pollen. For these, a tentative upper maximum of crop-to-crop gene flow is indicated by the diagonal line sloping down to the right in Figure 1A. Otherwise the measured frequencies beyond 100 m are mostly well below the line for 3/1000. Caution is advised because nearly all published data giving rise to Fig. 1A are for gene flow from single donor fields or blocks (e.g. GM) to recipient fields (e.g. non-GM) in landscapes containing other non-GM fields from which they can receive pollen.

### By seed

Seed, being persistent, can travel farther than pollen and over a much longer period of time. Seeds are also more effective in moving genes around since they can contain twice the number of copies of the genes in pollen. (But note there are exceptions, as for example after segregation of some GM herbicide-tolerant oilseed rape.) If seed is dropped or shattered at harvest, it can join volunteer in-field, or feral wayside, populations. Seed can, in principle, be contained within a few metres of the mother plant, or at least its movement can be restricted within or between farm holdings. However, where the same machinery for harvesting, soil cultivation and spraying is used in several fields, the shed seed will be readily moved around. Seed-mediated gene flow therefore does not have such a steep, short-range, distance-dependence as pollen. Over intermediate distances (e.g. 100 km) at which harvested produce is transported to ports and processing plant, gene flow between crop fields is likely to dip (Fig. 2A) since, while such transport

might give rise to wayside ferals, it will largely bypass crop fields along the way. At a larger scale still, inter-continental transport of genes will occur through imported seed containing impurities, which if sown at 1/100 to 1/1000 can be as great as gene flow by seed movement between fields on a farm.



**Figure 1.** Representation of ranges of values for gene flow frequency by (A) pollen, distance axis in m, and (B) seed, distance axis in km. The horizontal line = 0.3% (see text) and the dashed line in A indicates the likely maximum when the recipient crop is a varietal association containing 80% male sterile plants.

## GENEFLOW AND SEPARATION OF CROPS

If GM and non-GM crops are to be grown together in an agricultural system, then the degree of seed movement that presently occurs will be a problem. The general features of seed movement apply to both oilseed rape and maize; but maize being tropical in origin does not persist

during the cold winters of central and northern Europe, where it rarely contributes volunteers. If it contributed volunteers, for example after climatic warming, then it would pose problems similar to those of oilseed rape (Gressel, 2005). Volunteer oilseed rape is now one of the most frequent arable weeds in Europe, and its seedbank population in most arable fields is typically of a similar order (e.g. 100 m<sup>2</sup>) to the sown population of the crop (Squire *et al.*, 2005). The decay rates of seed in soil are highly uncertain, since they are affected by interactions between the weather, soil cultivation following seed drop and secondary dormancy of the seed. Growers will find it very difficult, though not impossible, to achieve frequencies lower than 3/1000 after about 4 years, and much longer intervals may be required between a GM and non-GM crop on the same field (Sweet *et al.*, 2004).

The more contentious issue is whether non-GM farms can expect to achieve an agreed GM threshold if the neighbouring farms grow GM crops of the same species. There is little evidence from realistic landscapes to inform this question, since where GM is already widely grown the question has rarely been asked, and in Europe GM crops have not been so widely grown as to offer natural experiments for study. The evidence from past records of varietal impurity in crops is less relevant than might be expected, since the acceptable thresholds are much higher than those for the presence of GM. If we can surmise nevertheless that gene flow by seed into a farm can be prevented, by sowing seed from certified non-GM sources and using separate machinery and buildings, the main source of gene flow into such a holding will be by pollen. As described earlier, pollen movement alone will not generally challenge a marketing threshold of 0.9% while the donor type (e.g. GM) occupies a relatively small area in the landscape. This condition should hold during the early stages of commercialisation of a GM crop, but might not if GM varieties became widespread. It is not yet clear whether, and under which circumstances, gene flow to a field might increase above 1/1000 if several donor fields were grown in its vicinity.

**Table 1.** Examples from the SIGMEA database of studies in maize and oilseed rape, unpublished or in progress, that will provide information or context for medium- to long-range pollen-mediated gene flow in landscapes.

Donor / recipient	Features / contribution of experiment	Organisation
Maize: Bt and non-Bt	Multiple donor and recipient commercial fields in an agricultural landscape	IRTA, Spain
Oilseed rape: high erucic (HEAR) donor, low erucic recipient	Multiple commercial donor and recipients fields in four experimental domains in UK.	SCRI, CEH, Rothamsted Research, NIAB, ADAS & CSL, UK.
Maize: various non-GM cultivars	From several single-field sources over a few km in realistic agricultural landscapes in UK	IGER & University of Wales, UK.
Oilseed rape: glufosinate ammonium tolerant GM donor, commercial non-GM recipients	Up to 5 km from single-field sources to surrounding fields, 7 locations in arable landscapes in UK as part of the Farm Scale Evaluations.	SCRI, CEH, Rothamsted Research, NIAB, ADAS, CSL.
Oilseed rape: glufosinate and glyphosate resistant donors, non-GM recipients	Donor plots in centre of, and recipient samples taken over, about 8 ha of experimental land near Braunschweig, Germany	BBA, Germany.
Maize: landraces and non-GM varieties	Estimate of transfer rates of genes from varieties to landraces by comparing samples from <1950 and 2000.	Universita Politecnica delle Marche, Italy.
Oilseed rape: demographic studies	Distribution of crops, volunteers, ferals and wild relatives in landscapes of Germany, France and UK: data for modelling and scenario-testing.	University of Bremen, Germany; University Paris-Sud, Orsay, France; SCRI, UK.

## FILLING THE GAPS IN KNOWLEDGE

The following are therefore the main factors that need to be resolved for a thorough assessment of gene flow issues in coexistence.

1) The decay rate and persistence times of volunteer seed over 2 to 10 years as affected by field management, soil and stochastic weather variables.

2) The frequency of gene flow at distances over

100 m when donor and recipient types are in comparable proportions in the landscape.

3) The possibility of a long-term rise in impurities in a region as a result of pollen- and seed-mediated gene flow interacting at the scale of the landscape.

Substantial information, probably sufficient to answer question 1 will be provided during the next few years from a large body of research collated in SIGMEA, primarily in Germany, France, Denmark and the UK. For question 2, only two primary

studies, one in each of maize and oilseed rape, will examine gene flow in landscapes having similar areas of donor and recipient crops, but these findings will be augmented by other work on landscape-scale gene flow from single donor fields and by demographic studies (Table 1). Some contextual information will be forthcoming on question 3, at least for non-GM plants, but the factors and mechanisms that determine the long-term interactions between crops, volunteers and feral plants in the landscape need further integrative study, beyond SIGMEA.

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# Origin of oilseed rape feral populations in a farmland area

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**Abstract:** A four-year large-scale survey of the main processes involved in the presence of oilseed rape feral populations at a landscape level in a farmland area was conducted. The results were subjected to statistical methods suitable for analysing large data sets and showed that the feral populations do not rely only on seed immigration from neighbouring crops and seed transport, but also result from self recruitment and from seed banks. The local production of seeds combined with seed survival in the soil indicates a potential for the persistence of transgenes and the possible emergence of new combinations of genes.

## INTRODUCTION

Feral populations of oilseed rape are widespread in field margins, roadside verges or waste ground (Charters *et al.*, 1999; Pessel *et al.*, 2001; Crawley & Brown, 1995, 2004). If GM oilseed rape crops were introduced, feral populations would behave as agents for gene flow *via* seeds and pollen, thus increasing the frequency of the unintentional spread of transgenes from crop to crop and making the coexistence management of GM and non-GM crops more difficult (Colbach *et al.*, 2001). If feral populations show ability to persist many years, they could furthermore behave as a reservoir of transgenes.

Feral populations are often considered as resulting mainly from spillage from farm or transport machinery or from cultivation in the

current or previous year in neighbouring fields (Lutman, 2003). However there are also indications that feral populations could persist many years in self-sustaining populations *via* local recruitment (local production of new individuals) or *via* seed banks (Charters *et al.*, 1999; Pessel *et al.*, 2001).

A survey was conducted in the centre of France at a landscape level in a farmland area from 2000 to 2003 in order to study the likely origin of feral oilseed rape populations. We present here the methods used to deal with the extensive data set obtained. For each road segment, we model the relationship between the probability of feral population presence in 2003 (the last year of the survey) and a set of selected explanatory variables, based on observations of the study area in the previous years. Finally we address the relative importance of the main processes involved in the presence and persistence of feral population.

## METHODS

The study area was a 41 km<sup>2</sup> production area of winter oilseed rape centred on the village of Selommes and a seed collection site (Loir et Cher, France). Twice a year we monitored GPS coordinates of all cultivated oilseed rape fields and feral plants on a road network of about 110 km.

To create the data set, we used the roads as the reference system. We divided them in three-meter long oriented segments, distinguishing the two roadsides. It thus defined a one-dimensional spatial system composed of 74002 segments. The GPS coordinates of fields and feral populations collected between 2000 and 2003 were projected onto these segments, together with their attributes. We consider 18 explanatory variables, including different types of oilseed rape presence in the past (cultivated fields, feral populations from 2000 to 2002) and permanent features characterising the segment

(road type, road number, vicinity to a junction, to a village). Each road segment was thus characterised by the presence or not of a feral population in 2003 and by the value taken by each of the 18 explanatory variables at that segment. We first selected the most relevant explanatory variables with a bagging algorithm (Breiman, 2001) and then introduced them in a mixed-effect logistic model to predict the probability of feral population presence in 2003 in various combinations of explanatory variables of biological interest. We tested the significance of the effect of each explanatory variable, whether alone or accounting for interactions.

The original data were collected in a database created with PostgreSQL DBMS, coupled with ArcView GIS software, used for data and prediction visualisation (figure 1). The preliminary data treatments, the projections as well as the statistical analyses, were handled using R-software interfaced with PostgreSQL via RPostgreSQL library.



**Figure 1.** GIS map of feral populations monitored in 2003 (black points) and rape fields monitored in 2002 (light-grey polygons). The black thin lines represent the road network.

## RESULTS AND DISCUSSION

The bagging algorithm showed that rape fields cultivated in 2002 and 2003 were relevant causes of feral populations present in 2003. Many feral populations would thus have resulted from direct sowing, harvesting, and natural losses around these fields. However feral populations and rape fields present before 2002 were also important explanatory variables, indicating that feral populations can persist, either through local recruitment or seed banks.

The mixed-effect logistic model allowed us to deal with additional sources of variability such as variation in seed transport intensity and weed management among roads. The random road effect is significant and the remaining overdispersion shows that the model does not take into account all the unknown sources of variability. Using GIS maps, we noticed that roads with high positive effects were mainly directed towards the silo. On such roads, the frequency of feral populations is higher than explained by past land use (presence of fields and/or feral populations), possibly indicating seed shedding during transport towards or from the silo. The residuals do not show any particular spatial pattern.

When compared to segments where no oilseed rape had been observed before 2003, road segments which had harboured oilseed rape only once in the past (specifically: a field or a feral population in 2000) had a significantly higher probability of harbouring a feral population in 2003. This result is most likely explained by the presence of a seed bank from these past fields or populations.

In general, our results thus show that feral populations present in 2003 result both from seed immigration (mainly from field losses but also during seed transport) and processes of persistence. In particular, they indicate that the seed bank plays a major role in the dynamics of oilseed rape feral populations and could be responsible for many feral populations observed in this study site. This major role of seed bank in explaining the persistence of feral populations was unexpected,

because, although frequent in fields, seed banks have not been documented yet on road verges where the soil is more compacted and seed banks are thus less likely (Gruber *et al.*, 2004). However, it tends to confirm that feral populations should not be considered absent if plants fail to appear in any one year (Charters *et al.*, 1999).

## CONCLUSION AND PERSPECTIVE

The dynamics of oilseed rape feral populations is an important factor in the distribution of gene flow over an agro-ecosystem (Claessen *et al.*, 2005). While many feral populations result from annual seed dispersal, a significant number also result from seeds that have remained in the soil for more than one year. This contribution of seed banks to the dynamics of feral populations needs to be taken into account in models of gene flow at the landscape scale. The establishment of persistent populations of plants of cultivars that had been grown in different years will allow the production of hybrids between these cultivars and could result in transgene-stacking when different GM varieties are grown.

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# Pollen mediated gene flow in maize in real situations of co-existence

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**Abstract:** The rate of cross-fertilization was evaluated in several non-GM maize fields in two different regions of Spain where *Bt* and conventional maize are commonly cultivated. A map was designed using land registry and aerial photographs from the Spanish GIS and the different crops identified, as well as sowing and flowering dates from *Bt* and conventional maize fields. These data was used to choose the non-transgenic fields for sampling and analysis by the RT-PCR technique. In 11 of the 12 analysed fields the rate of cross-fertilization was higher at the borders and decreased towards the centre of the field. The results obtained are dependent on the specific situation of the field, the presence of natural or physical barriers to prevent pollen movement, the prevalent wind direction and the coincidence of flowering with the surrounding *Bt* fields. In the particular situations studied, where the size of cultivated fields were small and without any containment strategy, we found some with an adventitious content higher than the 0.9% threshold due to pollen gene flow. However, the results obtained in the other fields in this study suggest that co-existence between GM and conventional fields can be achieved by establishing simple rules that take into account the flowering coincidence or buffer zones, rather than establishing security distances, because the efficiency of these varies dramatically, depending on what is being cultivated in between the fields.

## INTRODUCTION

Co-existence between conventional and GM maize can be affected by the coincidental presence of one crop with another, which can be due to a variety of reasons. In most field trials especially conducted for quantifying the adventitious presence of GMOs (Bénétrix, 2004; Bénétrix & Bloc 2003; Fabié 2004; Foueillassar & Fabié 2004; Henry *et al.*, 2003; Ma *et al.*, 2004; Messeguer *et al.*, 2003; Melé *et al.*, 2004; Ortega

Molina 2004; Weber *et al.*, 2005) a nucleus of pollen donor maize has been planted and then the rate of cross-fertilization in the surrounding or adjacent field determined. However, more data is needed to determine to what extent results encountered in these field trials can be applied in real situations, where GM and non-GM maize fields are sown at different sowing dates, mixed with other crops, and with different barriers that could influence pollen dissemination.

## MATERIAL AND METHODS

Two crop regions had been chosen during the growing season of 2004, where transgenic *Bt* and conventional maize fields already coexisted with other crops. The first one is located in T ermens (Lleida) and the second in Pla de Foix a (Girona), both in Catalonia, Spain. Both of these regions are characterized by the small size of the fields. Wind speed and wind orientation data during the flowering period were taken from a meteorological station in the crop areas. In both regions, two areas were defined: the central area where conventional fields were selected for sampling, and the surrounding area that may influence the rate of pollen gene flow. The total surface studied in T ermens was of 300 Ha with the central area of 43 Ha, whereas in Pla de Foix a the area was of 400 Ha with the central area of 100 Ha. A map was designed using the land registry and aerial photographs from the Spanish GIS. Then the different crops (cereals, fruit trees, maize, etc.) were identified as well as the maize cultivar (either *Bt* maize or a conventional cultivar), and the sowing

and flowering dates. All these data were used to choose the non-transgenic fields for sampling.

Taking into account that the risk of cross pollination is higher in the borders than in the centre of the field (due to the buffer effect of maize plants), we applied a stratified sampling system, dividing the fields into different zones according to the distance to the borders, in such a way that it would be possible to estimate the GMO presence in each zone (in the borders, from 0 to 3 m, from 3 to 10 m and in the inner part of the field). The number of samples analyzed by RT-PCR differed from one field to another (from 18 to 37) depending on the size and the particular shape of the field. Each sample (three cobs) was threshed by hand and the kernels were ground to a fine powder for analysis by RT-PCR to quantify the relative content of GM-DNA with respect to the total DNA.

By using the results of RT-PCR analyses, the total content of GM-DNA/total DNA in yield production was estimated, taking into account the different rates and areas corresponding to the borders.



Figure 1. Sampled non-transgenic Fields in the Foix a Zone.



Figure 2. Sampled non-transgenic Fields in the Tércens Zone.

## RESULTS AND DISCUSSION

Both areas studied had *Bt* and conventional maize fields distributed at random and co-existing with cereals, fruit trees, or non-cultivated fields. During the 2004 growing season, there were a wide range of conventional maize varieties and 16 *Bt* varieties on the market. In the Tércens area, the most cultivated *Bt* maize was Compa CB (*Bt*176 event) whereas in the Foixà area, *Bt* varieties with the Mon810 event were the most used. The geographical position of the *Bt* transgenic maize fields, conventional maize fields, fruit trees, cereals and non-cultivated fields, as well as geographical and physical barriers that could disturb pollen flow, are shown in Figures 1 and 2.

The sowing period in the Tércens region was quite similar for *Bt* and conventional maize, and nearly all fields were sown early, between March 18<sup>th</sup> and April 28<sup>th</sup>. In the Foixà region, there were two different sowing periods (from March 15<sup>th</sup> to April 20<sup>th</sup>, and from May 10<sup>th</sup> to May 30<sup>th</sup>) due to the fact that heavy rainfall flooded the fields in

the middle of April and growers had to wait at least three weeks until the fields dried. Bearing in mind that the attack of corn borers is more severe as the temperature rises, most of the growers that had to sow their crops later decided to use *Bt* maize, whereas those that sowed earlier, used conventional maize.

According to its position with respect to the surrounding *Bt* fields, and taking into account the flowering dates of all of them, five conventional fields in Tércens and seven fields in Foixà were chosen, in order to be able to study a wide range of different real situations of co-existence, depending on the area and the shape of the field. All samples from the 4 fields in Tércens were analyzed for both the MON810 and *Bt* 176 event, whereas in Foixà, the analysis was performed only for the MON810 event, because neither the fields of the selected zone nor the fields of the surrounding one had been sown with a *Bt* 176 maize. A total of 488 DNA quantifications were done.

In all the analysed fields, with the exception of field 7 from Foixà, the rate of cross-fertilization

was higher in the borders and decreased towards the centre of the field. An estimation of the percentage of GM-DNA/total DNA of each field is shown in Table 1. Nine of the 12 analysed fields gave values much lower than 0.9%, whereas in the other three the values were higher than 0.9%. For 11 of the 12 fields, the results were dependent on the specific situation of the field in the landscape, its relative size, the presence of natural or physical barriers to prevent pollen movement, the prevalent wind directions and the coincidence of flowering with surrounding *Bt* fields. The coincidence of flowering was the factor that most clearly determined the content of *Bt* maize in the conventional fields studied (fields 2, 4 and 6 from Foixà). This is demonstrated by the fact that the field 4 from Tèrmens and field 5 from Foixà were surrounded by *Bt* fields, without any physical barrier or distance separating them, but with high coincidence of flowering, which explains the high content of *Bt* maize. However, in the case of field 7 from the Foixà area, these factors do not fully explain the results obtained because the percentage of GM-DNA/total DNA was high in the borders (due to the coincidence of flowering with surrounding fields) but did not decrease towards the centre of the field. This

suggests that the high adventitious GM content in this field may not be only due to pollen gene flow, but also to factors such as crop management. This field was not irrigated, had lower plant density and the flowering was not uniform.

In the particular situation studied, where the size of cultivated fields is small and without any containment strategy, we found some with an adventitious content higher than the 0.9% threshold due to pollen gene flow. However, the results obtained with the other fields in this study suggest that co-existence between GM and conventional fields can be achieved by establishing simple rules that take into account the coincidence of flowering or buffer zones, rather than establishing security distances, because the efficiency of these varies dramatically, depending on what is being cultivated in between the fields.

Results obtained in this study will be very useful for validating mathematical simulations at the landscape level, such as the MAPOD<sup>®</sup> model (Angevin et al., 2001), in the framework of the SIGMEA project.

CONVENTIONAL FIELD			% GM-DNA/total DNA		
Zone	Nº	Area (Ha)	Mon 810	Bt 176	Total
Tèrmens	1	0,5	0,03	0,01	0,04
Tèrmens	2	3,08	0,02	0,51	0,53
Tèrmens	3	0,97	0,04	0,03	0,07
Tèrmens	4	1,89	0,01	2,28	2,29
Tèrmens	5	2,55	0,01	-	0,01
Foixà	1	1,89	0,05	-	0,05
Foixà	2	4,63	0,00	-	0,00
Foixà	3	3,56	0,11	-	0,11
Foixà	4	0,58	0,00	-	0,00
Foixà	5	1,10	1,22	-	1,22
Foixà	6	1,07	0,00	-	0,00
Foixà	7	1,5	1,89	-	1,89

**Table 1.** Estimated % of GM-DNA/total DNA for *Bt176* and *Mon810* events in each analysed field.



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# Long-term monitoring of GM winter oilseed rape volunteers and the implications for co-existence

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**Abstract:** This paper summarizes current results of an ongoing long-term monitoring study of the incidence of genetically modified winter oilseed rape (GM OSR) volunteers and their possible impact on eight selected field areas in the Czech Republic (CZ). The survey covers mainly experiment and surrounding areas where herbicide-tolerant genetically modified (HT-GM) OSR was grown during 2000/01. The study aims are to analyze the influence of various environmental conditions and agricultural practices on the dynamics of GM OSR incidence and survival, its possible spread within and beyond experiment sites, and where appropriate the degree of transfer of the bar transgene coding for herbicide tolerance to OSR wild relatives.

The experimental fields were monitored at regular intervals for the presence of OSR survivors and volunteers. Data are presented from eight different locations for four seasons after the GM-HT OSR was grown (2002-2005). Results obtained to-date indicate that GM-OSR plants originating from the soil seed bank could be still present in experimental fields four years after GM rape cultivation. Their frequency can be suppressed efficiently using proper agronomy measures to minimize possible risks of contamination of non-GM rape or to reduce to an acceptable level the likelihood of gene transfer to related species. The study has helped to improve sampling methodology and HT transgene detection in OSR as well as to refine the new oversight systems (and policies) in preparation for the introduction of co-existence rules by the national GMO authorities in the Czech Republic.

## INTRODUCTION

Winter oilseed rape (*Brassica napus* L. var. *napus*) is one of the main crops for which genetically modified cultivars have been developed and released for commercial use in some countries. As an invasive species, grown usually on broad acreages and producing huge amounts of pollen and seeds, OSR can persist in fields for years and slowly spread to surrounding ecosystems (Pessel *et al.*, 2001; Richter *et al.*, 2004; Legere, 2005). Therefore OSR is the subject of intensive risk assessment research with the aim of identifying possible risks and consequently define basic rules for transgene confinement (e.g. Morris *et al.*, 1995; Timmons *et al.*, 1995; Fargue *et al.*, 2004; Poppy, 2004). Nevertheless, the interpretation of outcomes of such studies should reflect the requirements of local authorities, specific geographical and biological conditions and local agricultural practices. Such studies can serve as the basis for local oversight and monitoring systems and the preparation of Co-existence rules.

OSR represents one of the most important agricultural crops in the Czech Republic (CZ). Its cultivation area ranges annually between 250 to 350 thousands hectares and a great deal of the harvested crop is exported to other EU countries for processing to oil. As a result, the national authorities within the Czech Republic are very interested in creating relevant rules for co-existence in various agricultural systems. These rules would be based also on the results of studies performed under the specific geographic conditions and within the agricultural systems typical of the Czech Republic.

## APPROACH AND METHODS

This study was conducted on eight OSR experimental fields in CZ, ranging from ca 2500 m<sup>2</sup> to 72250 m<sup>2</sup>. Each field was treated in a different way following GM OSR cultivation to determine optimal combinations of agronomy measures for safe GM crop cultivation. Fields were regularly monitored from 2001 to present for the presence of OSR volunteers and the possible transfer of the

HT transgene to wild relatives of OSR. Polymerase-chain reaction (PCR) sampling was performed annually on samples from three to five selected locations with the aim of gradually gaining insights into the dynamics of OSR volunteer persistence over time in areas next to sites where GM OSR had been grown. To distinguish among GM and non-GM plants, a molecular method based on PCR was used to confirm/ exclude the HT transgene presence. Whole seedlings and/or young leaves of OSR and related wild plant species were collected from fields and used for DNA isolation. Selected primers specific to the *bar* gene sequence, together with the primers for an internal control (gene for UDP-glucose:sinapate glucosyltransferase), were used in the PCR.

## RESULTS AND DISCUSSION

In the first year after GM OSR cultivation there was a substantially decline in the frequency of GM OSR volunteers if proper agronomy measures were taken. This result confirms the findings of other authors (e.g. Gruber *et al.*, 2005). One of the most effective means for OSR volunteer control is to allow seed arising from the harvest to germinate directly in a field immediately after the harvest, and to follow this with skimming(s) and/or herbicide treatment. Use of high-density- or competitive crops (e.g. cereals, alfalfa) in the following years helped to further suppress volunteers. In this way, a zero or low-frequency of OSR volunteers appearance could be achieved within two to three years following a GM OSR harvest.

DNA analyses showed the presence of both GM and non-GM genotypes, which was expected given the original design of the field experiments. However, the GM/non-GM ratios usually did not correspond to those expected, indicating the possibility of either GM - non-GM winter rape hybridisation and/or seed movement caused by agriculture machinery. So far there have been no indications of transgene movement to both cultivated (turnip rape and black mustard volunteers put in scientific names?) and wild

(yellow charlock, wild radish and yellow field cress put in scientific names?) OSR-related species. However, the numbers of wild-related plants useful for transgene detections were still insufficient to reveal low transfer frequencies due to measures taken to make the original GM experiments as safe as possible.

## PERSPECTIVES

Based on our studies, suggestions have been submitted to the Czech national GMO regulatory authorities (Ministry of Agriculture, Ministry of Environment) to broaden the science-based platform for the preparation of co-existence rules aimed at possible future winter GM HT-oilseed rape cultivation and handling. The recommendations are based on the specific geographical and biological conditions and agricultural practices used in the Czech Republic and do not necessarily relate to the geographic and climatic conditions and/ or different agricultural production systems used in other countries. Based on data given by, e.g. the Central Institute for Supervising and Testing in Agriculture (CISTA), the State Phytosanitary Administration of CZ, and particular national acts and EU legislation, both Ministries will specify cultivation conditions including the following recommended agricultural steps: A period of at least 6 years for conventional OSR repeated cultivation on the same field should be kept in the case of GM OSR. Because one of the most effective steps in controlling OSR volunteers is to allow seed (arising from the harvest) germination directly on a field immediately after the harvest, skimming(s) and/or herbicide treatment (if used) should be postponed to the treatment-sensitive OSR plant developmental stage. This is imperative for farmers in seasons when there are weather or other circumstances which do not allow for a complete GM-OSR harvest in a given field. The cultivation of high-density- or competitive crops (e.g. cereals, alfalfa) in years following GM OSR crops can also help to further suppress volunteers. Various crop rotation schemes should be recommended by the agriculture specialists (CISTA) for years following GM OSR cultivation

(it seems that at least two years of suppressive crops are necessary following a GM OSR crop) to satisfy both the needs of biosafety and farmers. Any change in agricultural practices should be carefully assessed in advance and modifications made (if necessary) to co-existence rules.

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# An update on the persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*)

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**Abstract:** Field experiments started in 1998 and 1999 have studied the persistence of seeds of conventional and herbicide tolerant oilseed rape at five sites. Initial seed losses were assessed at harvest and subsequent seed survival in the soil has been measured from soil cores taken approximately yearly, thereafter. Mean seed losses at harvest were 3575 seeds/m<sup>2</sup>. Seed losses were monitored on seven experiments at the five sites and regressions were fitted to six of them. Seed declines in the first few months were rapid but were subsequently much slower. Estimates based on samples collected up to 2003 predicted 95% seed loss in 3-20 years (mean 9 years). Addition of data from 2005 to three of the data sets indicated 7.6 years for 95% seed loss. Seed persistence poses a clear problem for the temporal co-existence of conventional and GM rape, assuming the continuation of the EU 0.9% threshold of GM presence in non-GM seeds. The results suggest that a farmer who had sown GM rape and created a GM seedbank would have to wait perhaps 8 years before planting a non GM crop.

## INTRODUCTION

One of the key issues associated with the coexistence of conventional and GM crops relates to the temporal persistence of seeds, which provides a mechanism for gene escape in time. This is particularly an issue with oilseed rape (*Brassica napus* L.) which can form persistent seedbanks (Schlink, 1998; Lutman *et al.*, 2003). Although, there are published data on the short-term persistence of rape seeds, there is only limited information on the long-term survival of seeds, particularly seeds of GM rape cultivars (Gruber *et al.*, 2004). As part of a detailed investigation of the agronomic and environmental impacts of herbicide tolerant (HT) crops in the UK, a series of four-year rotation experiments with conventional and HT oilseed rape and sugar beet (*Beta vulgaris* L.) was started in the season 1998/99 (Sweet *et al.*, 2004). Conventional cultivars of both crops were compared to genetically modified varieties tolerant to glyphosate or glufosinate. A fourth cultivar of rape, developed by mutation breeding to be resistant to the imidazolinone herbicides, was also included. The experiments were carried out at five sites, but each site included more than one rotation. This paper reports on the persistence of seeds of oilseed rape shed at harvest in 1999 and 2000 at 7 site/rotations.

## MATERIALS AND METHODS

This paper reports the results of seven experiments; Rothamsted (RES99, RES00),

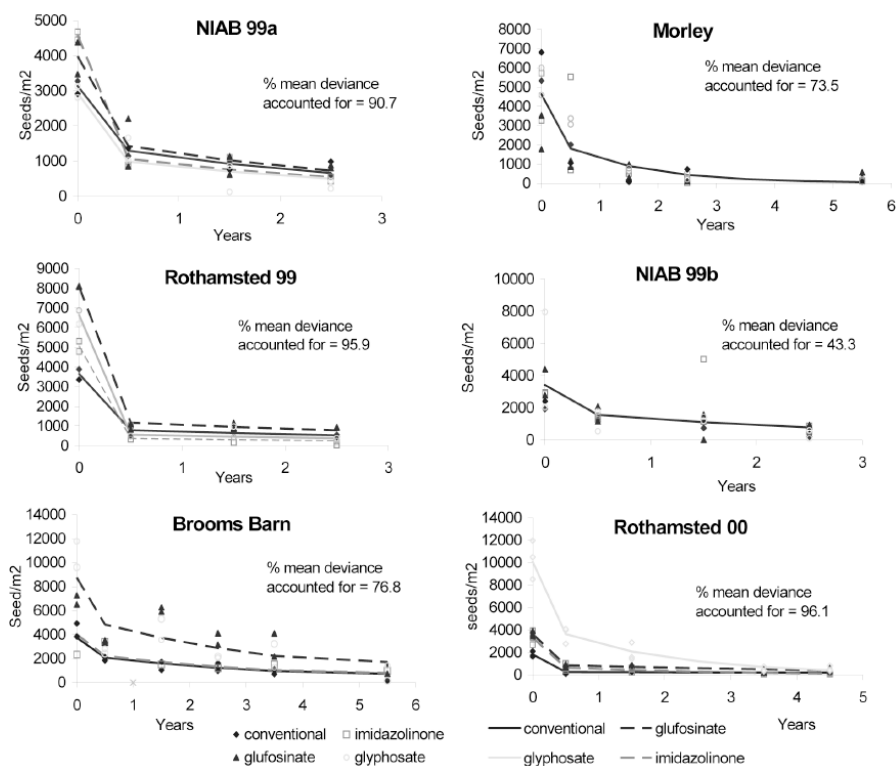
Broom's Barn (BB99), NIAB (NIAB99a, NIAB99b), The Arable Group (MOR99) and Scottish Agricultural College (SAC99) (the numbers refer to the harvest years; two experiments were included from NIAB and RES). Sizes of plots varied between experiments from 12x12m to 72x92m, depending on the site and the rotation. The rape was grown as a commercial crop, apart from the herbicide treatments, and was harvested with standard commercial, or small plot, combine harvesters. A range of crops have been grown since the harvesting of the oilseed rape (Sweet *et al.*, 2004) but no further seeding has occurred, except at SAC99 where poor weed control in 2001 resulted in some seed return.

Seed losses were assessed at harvest either by counting small quadrats placed on the stubble immediately after harvest, or by putting plastic gutters in the crop, just prior to harvest. Soil cores, 2.5 cm diameter and c. 25 cm deep (24-80/plot), were taken approximately 6 months after harvest from each plot, then annually for the next 2-3 years, and then less frequently thereafter (Table 1). Cores/plot varied between sites/rotations and was increased in later samples (Sweet *et al.*, 2004; Lutman *et al.*, 2005). Seeds were extracted from the soil by wet sieving, using a 4mm and 1mm sieve. The seeds were collected from the 1mm sieve and viability was assessed by squeezing. All data were analysed by GenStat, using generalised regression techniques, assuming a Poisson distribution for the observed numbers of seeds. The fitted model was as follows:  $Y = N \times P1 \times P2^{(T-0.5)}$  where N is the number

Table 1. Details of the sites and sampling dates

Site	Harvest year	Number of replicates	Soil type	Sampling dates (months after harvest)
RES99	1999	2	Silty clay loam	0, 6, 18, 30
RES00	2000	3	Silty clay loam	0, 7, 19, 43, 55
BB99	1999	2	Sandy loam	0, 4, 17, 30, 43, 66
NIAB99a	1999	2	Stoney clay	0, 4, 16, 28
NIAB99b	1999	2	Stoney clay loam	0, 6, 18, 29
MOR99	1999	2	Sandy clay loam	0, 8, 19, 31, 67
SAC99	1999	2	Sandy clay loam	0, 7, 20, 30





**Figure 1.** Modelled decline curves for four types of oilseed rape seeds (conventional (Apex) (◆), and tolerant to imidazolinone herbicides (□), glufosinate (▲) & glyphosate (○)) from harvest for up to 66 months, at six sites.

of seeds shed at harvest, P1 is proportion of the seeds remaining after the first 6 months, and P2 is the proportion remaining after each subsequent year. T is time in years. The optimum model was selected for each data set, and used to determine years to 95% and 99% predicted seed loss.

## RESULTS

There was site to site and treatment to treatment variation in seed losses at harvest. Mean losses were 3575 seeds/m<sup>2</sup> (range 1219-10893 seeds/m<sup>2</sup>). Regressions of seed declines were created for 6 of the seven data sets; the SAC99 data would not fit the regression. All six sites showed a steep decline in the first year and a long 'tail' with a slower

decline rate, thereafter (Fig. 1). Although numbers of seeds lost at harvest varied between the four cultivars, subsequent decline rates did not differ greatly at most of the sites. Results from the first 3 years suggested a fast decline rate at Morley and a slower decline at Broom's Barn. Data collected in 2005 at three of the sites (BB99, MOR99 and RES00) indicated that the decline rates were beginning to become more consistent. Overall estimates of numbers of years to lose 90 and 95% of the seeds are shown in Table 2. Loss of 95% of seeds took between 3 and 19 years (mean 7.6 yr), whilst loss of 99% of seeds was predicted to take up to 49 years (mean 14.1 yr). The most persistent seeds, where there were detectable differences between cultivars, were from the conventional cultivar (Apex), especially at Rothamsted.

## DISCUSSION

Population dynamics models have highlighted the importance of factors determining the longevity of the seedbank (Claessen, *et al.*, 2005), and the significance of post-harvest cultivations to minimise seed numbers in the seedbank (Pekrun *et al.*, 2005). The data presented in this paper clearly show that low levels of seeds will persist for at least 10 years. This may not be a major concern in standard cropping systems, but if GM/non GM crops are involved and the grower has to meet the EU limit of 0.9% GM seeds in conventional seeds, the presence of even low numbers of volunteers is important. Even a 99% loss of seeds would leave perhaps 40 seeds/m<sup>2</sup> following average seed losses at harvest of the previous rape crop and 'normal' agricultural practice in subsequent years. This could result in more than 1 GM volunteer plant/m<sup>2</sup>, which could cause the harvested rape seed to exceed the EU labelling threshold of 0.9%. It seems that a farmer who wished to switch back to non GM rape would have to wait at least 8 years before he/she could consider growing a non GM crop. This assumes no subsequent seed return from the volunteer rape, no crops where the control of oilseed rape can fail, such as field beans (*Vicia faba*) and no poorly managed uncropped areas. A further critical management issue is ensuring that cultivation practices, especially after the harvest of the rape crop, minimise incorporation of rape seeds into the soil. Seeds should be allowed to germinate, cultivation delayed and ploughing avoided.

There is no evidence in the data that the herbicide tolerant cultivars were more persistent than the conventional one. This concurs with the conclusion of previously published work by Gruber *et al.*, (2004). However, the genetic background of a cultivar can affect its persistence (e.g. Gruber *et al.*, 2004) and so it would seem prudent for plant breeders to use less dormant cultivars when creating HT types.

**Table 2.** Calculated number of years to lose 95% or 99% of rape seeds shed at harvest.

Site	Number of years to achieve seed losses of	
	95%	99%
RES99 (2.5 years' data)	3-9	11-17
RES00 (3.5 years' data)	4-13	7-31
RES00 (4.5 years' data)	5-19	8-49
BB99 (3.5 years' data)	20	34
BB99 (5.5 years' data)	11	17
NIAB99a (2.5 years' data)	6-8	11-12
NIAB99b (2.5 years' data)	8	12
MOR99 (2.5 years' data)	3	5
MOR99 (5.5 years' data)	4	7
Mean (longest data set/site)	7.6	14.1

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# Reproduction capacity of oilseed rape volunteers and potential gene flow

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**Abstract:** The capacity for actual gene dispersal of oilseed rape (OSR) volunteers, via pollen and seed production, was evaluated in this study. Surveys on two farmers' fields and on two experimental fields in winter cereals resulted in data about flowering periods, mortality and seed set by OSR volunteers. Oilseed rape volunteers flowered in a density of 0.004–0.02 plants m<sup>-2</sup> on farmers' fields when herbicides were used, and in a density of 0.03–1.01m<sup>-2</sup> on experimental fields where herbicides were not used. The flowering period of volunteers and sown oilseed rape overlapped on three of the four fields. Nearly 50% of all flowering volunteers on farmers' fields are lost between onset of flowering and harvesting of cereals. Seed set of OSR was observed on all fields with a maximum of 320 germinable seeds per plant on farmers' fields. Because of the high volunteer density, the number of seeds m<sup>-2</sup> was highest on experimental fields with a maximum of 13 germinable seeds m<sup>-2</sup>. Main mortality factors were damage by flea beetles in juvenile growth stages and by hares in later stages. A self-reproducing population of OSR volunteers on arable land is unlikely since seed production of volunteers is low compared to seed losses of sown OSR crop during harvesting. However, transgenes can be preserved on an area by volunteers, which act as link to other OSR crops. The results are a first step towards the quantification of the actual reproduction capacity of volunteer OSR, and can be used for modelling gene dispersal by genetically modified oilseed rape.

## INTRODUCTION

Oilseed rape volunteers emerging from a soil seed bank are a potential source for gene flow in the context of risk assessment of genetically modified (GM) plants. Several important aspects of seed persistence have been studied, i.e. duration of seed persistence (Schlink, 1998; Lutman *et al.*, 2003), effects of tillage on the size of the soil seed

bank (Pekrun, 2004; Gruber *et al.*, 2004a; 2005) and genotypic variation of dormancy and seed persistence (Gulden *et al.*, 2004; Pekrun *et al.*, 1997; Momoh *et al.*, 2002; Gruber *et al.*, 2004b). However, there is little data on volunteer flowering patterns and seed set. Data about flowering periods and number of seeds from sown oilseed rape cannot simply be transferred to volunteers since growth conditions and competition are

different for both plant groups. On the other hand, these data are essential for a reliable prediction of gene dispersal in decision support systems or for risk assessment of GM oilseed rape. To close this gap of information in a first approach, data about volunteers reproduction were collected on two farmers' fields and on two experimental fields between 2002 and 2004.

## MATERIALS AND METHODS

Winter oilseed rape was grown on two farmers' fields (Hohenheim 1 and Hohenheim 2) and harvested in 2001 and 2002. The soil was tilled using a rototiller, a rigid tine cultivator and a harrow for seedbed preparation. No deep soil inversion by ploughing was performed at anytime within the crop rotation except on field Hohenheim 2 for primary tillage prior to field bean seeding in December 2002. Crops in rotation included winter wheat and field beans, and winter barley on one field (Table 1).

These fields were managed according the best management practice including the use of herbicides. In the years when volunteers flowered in winter wheat or winter barley, the herbicides Pendimethalin, Flufenacet and Isoproturon have

been applied previously. The experimental fields 3 and 4 were located on the experimental station Ihinger Hof of the University of Hohenheim, in the south west of Germany. Rape seed was broadcast on a cereal stubble in summer 2001 and 2002 to simulate harvesting seed losses of oilseed rape. After immediate stubble tillage in summer and primary tillage using a cultivator in the autumn, winter wheat was sown as the following crop without herbicidal weed control. Flowering volunteers were tagged on farmers' fields and experimental fields as well, and counted once (Hohenheim) or several times until July (Ihinger Hof). Shortly before harvesting the cereals the oilseed rape volunteers were cut and the number of seeds determined. A germination test determined the viability of seeds.

## RESULTS AND DISCUSSION

A number of volunteers emerged from the soil seed bank 1–3 years after harvest losses or artificial seed input. Volunteers flowered synchronically with sown oilseed rape in 2002 (Ihinger Hof 3) and 2004 (Hohenheim 1 and 2; Table 2). Consequently, intraspecific outcrossing was possible only in these years, whereas interspecific outcrossing with wild relatives was possible in all 4 years.

**Table 1.** Management history of fields with volunteer oilseed rape in winter wheat and winter barley. WOSR: winter oilseed rape; WW: winter wheat; WB: winter barley; FB: field beans.

Location	Hohenheim (farmers' fields)		Ihinger Hof (experimental fields)	
	1	2	3	4
<b>Crop rotation</b>				
2001	WOSR		WOSR <sup>2)</sup>	
2002	WW	WOSR	WW <sup>1)</sup>	WOSR <sup>2)</sup>
2003	FB	WW/FB		WW <sup>1)</sup>
2004	WW <sup>1)</sup>	WB <sup>1)</sup>		
Herbicides <sup>3)</sup>	Pendimethalin + Isoproturon	Pendimethalin + Flufenacet	None	None

1) crop with OSR volunteers; 2) oilseed rape seed artificially broadcast; 3) in years with OSR volunteers

**Table 2.** Flowering periods of oilseed rape volunteers in winter wheat and winter barley on four locations and three years.

Year	Location	Sown crop	Flowering period of oilseed rape
2002	3 (Ihinger Hof, experiment)	Winter wheat	8 May–2 August
2003	4 (Ihinger Hof, experiment)	Winter wheat	10 June–30 July
2004	1 (Hohenheim, farmers' field)	Winter wheat	End of April–28 July
2004	2 (Hohenheim, farmers' field)	Winter barley	End of April–7 July

As shown by the appearance, OSR volunteers on farmers' fields (Hohenheim) had obviously germinated before winter and survived the herbicide application in autumn. Although volunteers on location Ihinger Hof 4 did not emerge until spring 2003, these plants set flowers and seeds. More volunteers emerged over the whole cropping period on all locations, particularly in wheel tracks, but did not reach the generative stage. Volunteer plant density was highest on location Ihinger Hof with a maximum of 1 flowering plant m<sup>-2</sup> (Table 3).

A high seed input (10,000 seeds m<sup>-2</sup>) and the short period for seeds to persist in the soil (only about 6 months) may have been the reason for the high volunteer density at Ihinger Hof. In contrast only 0.02 to 0.004 plants m<sup>-2</sup> flowered on farmers' fields in Hohenheim. This may be an indication of the seed bank decline over 2 and 3 years (Lutman *et al.*, 2003), and of the lower level of seed return. Harvest losses are usually between 1,000 to 8,000 seeds m<sup>-2</sup> (Pekrun, 2004; Gruber & Claupein, 2005). This level of harvest loss could be assumed for the Hohenheim site since no extraordinary weather conditions occurred before and during harvest. Compared with this generally high seed input, seed production of volunteers was very low and reached a maximum of only 320 seeds per plant or 13 seeds

m<sup>-2</sup>. Thus, a long lasting, self-reproducing volunteer population cannot be expected. Seed losses of volunteer × sown oilseed rape hybrids would nevertheless replenish the soil seed bank with partly transgenic seeds and maintain transgenes in the environment.

Only up to the half of flowering and tagged plants set mature seeds, either due to the time-limited growth period or due to plant losses by damage. The limiting conditions for volunteers are also evidenced by the small number of seeds per plant, and the reduced viability of seeds at Ihinger Hof.

During the entire volunteer life cycle the first filter the volunteers had to pass was the herbicide application. Another filter was damage by flea beetles (*Phyllotreta* spp.) in the juvenile plant stage. Later on, flower beetles (*Meligethes aeneus*) and cabbage aphids (*Brevicoryne brassicae*) affected buds and pods, particularly at Ihinger Hof, where most of the volunteer plants were large and attractive to insects. The loss of whole, adult OSR volunteer plants, at least at Hohenheim, was due mainly to feeding by hares (*Lepus europaeus*) which had selectively fed on OSR volunteers within the cereal crop.

## CONCLUSIONS

The mortality of oilseed rape (OSR) volunteers is relatively high and the potential for reproduction and gene dispersal may be low, as a result. If herbicide application in winter cereals is not effective, OSR volunteers can use nearly the whole cropping period to grow. Outcrossing of OSR volunteers in winter cereals is generally possible with a sown rape crop due to an overlap of flowering periods. Nevertheless, pollen flow from a few volunteers to adjacent oilseed rape fields may be small. Outcrossing with wild relatives is possible until the sown crop is harvested. Volunteers still flowering up until approximately one month before the sown crop is harvested are still able to set seed, but seed production is limited compared to sown rape plants. A self-reproducing population of OSR volunteers on arable land is unlikely since the potential for

**Table 3.** Number and seed production of flowering oilseed rape (OSR) volunteers in winter cereals; tillage after OSR harvest: stubble tillage 1-7 days after harvest, primary tillage by rigid tine cultivator.

Location	Hohenheim (farmers' fields)		Ihinger Hof (experimental fields)	
	1	2	3	4
Field				
Seed input of OSR (year)	2001	2002	2001	2002
OSR volunteers (year)	2004	2004	2002	2003
Sown crop	Winter wheat	Winter barley	Winter wheat	Winter wheat
Flowering volunteers m <sup>-2</sup>	0.004	0.015	0.03 – 0.09	0.32 – 1.01
Mature volunteers m <sup>-2</sup>	0.002	0.010	n.d. <sup>1)</sup>	0.11 – 0.47
Plant loss %	52	49	n.d. <sup>1)</sup>	34–47
Germinability %	92	91	52	87
Germinable seeds/volunteer	320	265	n.d. <sup>1)</sup>	15-21
Germinable seeds m <sup>-2</sup>	0.6	2.7	6.5–12.6 <sup>2)</sup>	1.6 – 7.5 <sup>2)</sup>

1) not determined; 2) depending on variety

replenishing the soil seed bank is low compared to seed input via harvest losses. The reproduction potential of OSR volunteers is limited by several mortality factors and these have to be considered in future modelling efforts if the aim is to obtain sound and reliable predictions of gene dispersal. More data are still required. For example, the effectiveness of the OSR volunteer mortality factors considered in this paper have not been quantified and the reproductive capacity of OSR volunteers in other crops has also yet to be determined.

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# Using the GeneSys–beet model to evaluate and manage populations of Herbicide-Tolerant weed beet, and implications for coexistence of Herbicide Tolerant and conventional sugar beets

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*The views expressed in this paper are those of the authors and do not necessarily correspond to those of their institutions.*

**Abstract:** Simulations were carried out with the GeneSys sugar beet model to assess the impact of introduction of GM herbicide-tolerant beet varieties into actual European cropping systems. Problems related to the appearance of HT weed beet and related practices were identified and further evaluated. As final step, the efficiency of different mitigation measures applied by the GM crop farmer to reduce the content of GM seeds with tolerance to herbicide in the seed bank of the neighbouring non-GM fields was compared.

## INTRODUCTION

Sugar beet gives rise to a very specific case of coexistence because the roots, not the seeds, are harvested. Accidental bolting due to vernalisation or cross pollination during seed production could result in the presence of GM weed beets in non-GM fields but not in co-mingling of GM and non-GM sugar beet roots beyond the field. Indeed, annual weed beets are unlikely to be harvested with sugar beet roots in the subsequent crops because:

- most bolters are located outside of the sowing row and are therefore not harvested;

- if some bolters were to be caught up by the root harvester, they would probably be eliminated due to their small root size;
- bolters are often pulled up before harvesting in order to avoid seed production and because their long stems could cause problems with the machinery.

Consequently, the only significant source of adventitious presence of GM sugar beet in non-GM sugar-beet crop harvest would be the GM traces found in the seeds sown (assuming that no commingling occurs during sowing and harvesting nor admixture between piles). As long as the adventitious GM presence in non-GM seed

used remains below the legal threshold, there would be no co-existence issue in sugar beet crop production.

Nevertheless, agronomic issues arising from the development of weed beets in the case of GM herbicide-tolerant (HT) varieties should be considered from a long-term perspective. The presence of weed beet in sugar beet crops results in decreases in sugar yield (approximately 10% sugar yield loss per weed beet plant per m<sup>2</sup>) and difficulties with harvest and sugar extraction. These problems are due to differences in the reproductive cycle, as sugar beet is biennial and weed beets are annuals. Therefore, appearance of a HT weed beet population in a non-GM field and the subsequent weed control problems could create coexistence conflicts between non-GM crop and GM crop farmers different from those related in the absence of adventitious presence of GM in the final crop.

## MATERIAL AND METHODS

GeneSys®-beet (Sester, 2004, Sester et al., 2003) is an adaptation of GeneSys®-rapeseed (Colbach et al, 2001). The aim is to compare cropping systems according to their effect on gene flow from sugar beet to weed beet. The input variables are the regional field pattern, the crop rotations, the cultivation techniques of each crop and certain meteorological data. Output variables are, for each field and each year, adult plants, newly produced seeds as well as density and genotype proportions in the seed bank.

GENESYS Sugar Beet is derived from the well known GENESYS Oilseed Rape (Colbach et al., 2001) which has been quite thoroughly tested and found to give globally reasonable results (Colbach et al., 2005). The quantitative validation of the sugar beet version is on-going but preliminary analysis demonstrated a general agreement of the model outputs with expected behaviour.

In a study dealing with co-existence in sugar beet crop and seed productions we determined

typical model farms (conventional and organic) for two representative sugar beet crop production regions (Picardy in France and Lower Bavaria in Germany).

As a working hypothesis, the reason for adoption of GM varieties was a need to simplify weed control (including ending of hand pulling). In a first set of simulations, best practices for weed beet management were applied in non GM fields surrounding the GM beet fields. Other situations were tested in a second set of simulations representing the various potentially problematic situations in the non-GM neighbouring fields (e.g. poor quality of bolter management). In another step of the project, the impact of different measures applied by the GM farmer on the content of GM seeds in the seed bank of the GM field as well as neighbouring non-GM fields were simulated.

## RESULTS

The impact of various levels of adventitious GM presence in seed lots (0.1%, 0.3%, and 0.5%) on seed bank composition was simulated. No significant differences were observed, regardless of the situation tested. Consequently, agricultural practices seem to be the main driving force behind the development of HT weed beet populations, rather than initial seed purity

When best practices for weed beet management are applied (baseline situation), simulations show that the problem of HT weeds appeared to be well controlled in the fields of conventional sugar beet. Nevertheless, in other simulations representative of less strict weed management regimes, HT weed beet infestation was a real problem.

Several adjustments of GM crop farmer practices were tested to decrease HT weed beet population in non GM fields.

Ploughing has a marked effect on the development of the weed beet population: indeed, fields with simplified cropping practices had smaller seed banks than fields with the



conventional cropping system. Furthermore, yearly ploughing of the fields with GM sugar beet in the rotation generally resulted in higher levels of infestation than the basic cropping system (baseline), certainly because of the long seed survival in the soil.

Plots with organic cropping systems had the least infested seed banks in each simulation. This could be due to the presence of alfalfa for three years in the rotation. Weed beets cannot survive this long and therefore cannot produce seeds.

Having the transgene present in the pollinator during seed production appears to be an effective way to manage bolters generated by accidental pollination by annual beets; indeed the resulting bolters are susceptible to the non-selective herbicide. This method was generally the best way to limit infestation. However, this means of introducing the transgene creates other problems with the management of seed production (results not shown).

In the final analysis, hand pulling of bolters appeared to be one of the best solutions for farmers with GM sugar beet, to prevent gene dispersal from their field. This practice is highly feasible as it is already used in current cropping systems but could be in conflict with the reduction of labour expected when using HT GM varieties.

## CONCLUSION AND PROSPECTS

Modelling gene flow provides a means of testing wider range of variations in cropping systems than in field experiments. Individual quantification of the effects of each component is essential in order to synthesise and combine these effects, and the model approach is well suited to this purpose. Moreover, simulations carried out with a model allow a priori evaluation of the advantages and disadvantages of innovating cropping systems, of strict practices for managing weeds and volunteers as well as of their long-term consequences. Last, but not least, comparable long-term field experiments

would require much more time and would be expensive.

The GeneSys Sugar Beet model is a tool proposed specifically for decision support for questions concerning the agronomic consequences of the use of transgenic sugar beet. The model is destined for various users such as technical institutes, scientists, agronomists, and seed producers. It allows separating, at the scale of a small farming region, the problems due simply to weed beet invasion from those particularly due to the use of transgenic varieties.

In the future, such models will make it possible to establish a system of cost prediction for each cropping system and thus to evaluate the real, long-term economic balance before deciding on the use of varietal innovations.

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# Biological containment systems for genetically modified plants

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## INTRODUCTION

Plant biotechnology has the potential to produce a wide range of products and foods in more sustainable ways for the benefit of both mankind and the environment. New crops will undoubtedly develop in the coming decades that will achieve improved pest and disease resistance, replace non-renewable resources and will provide novel products such as pharmaceuticals and industrial raw materials. Many of the developments planned will make significant contributions to environmental sustainability.

However, development of the technology is being delayed by both real and perceived threats to human and animal health, and to the environment. Development of techniques for significantly restricting or preventing gene flow between plants would significantly improve the

food and environmental safety of GM plants and their products, and remove many of the human health and environmental concerns. In addition European society is concerned that the introduction of GM foods and products will remove their ability to make informed choices about the food that they purchase and eat. Development of techniques for significantly restricting or inhibiting gene flow between plants would go a long way towards removing societal concerns and allow choices to be made. In addition, the coexistence of different forms of agriculture in the EU should be developed. It is important for the advancement of agricultural biotechnology and for Europe's international competitiveness in this rapidly evolving area. Development and application of gene flow restriction and crop containment measures will be of importance to facilitate co-existence of farming systems.

## CONTAINMENT METHODS

Special attention has to be paid to the environmental assessment questions posed by the EU 2001/18 Directive and the EFSA guidance on the Risk Assessment of GMOs. The assessments must be properly conducted for each individual transgenic event. Should risks associated with transgene flow into non-target recipients be identified, or estimated to be probable, adequate containment measures must be taken. The risks associated with GM crops are considered to be the product of the potential hazard, and the exposure to humans, animals and environment. The research on Biological Containment Systems seeks to reduce risks by reducing exposure (through restricting/inhibiting gene flow) while maintaining, or even increasing, the potential hazard through the development of novel GM crops. Thus, in order to reduce overall risk, the methods developed must significantly reduce or prevent gene flow into foods and feeds, and reduce impacts on the agriculture and ecosystems of countries in which the GM crops will be grown.

Practicability and economic impacts are key concerns for the further development of these containment methods. Technical and economic evaluations should be made at all stages of the production chain to insure that containment systems remain appropriate for the breeding methodologies, the seed multiplication and the agricultural production of the respective crops. Different requirements must be met at different phases of crop propagation and utilization, and these must be practicable.

Exposure of transgenes to the environment can be reduced by removing superfluous transgenes and associated sequences, e.g., antibiotic selection markers, or by expressing a transgene conditionally, or by creating biological and physical barriers to gene-flow. The probability of transgene escape is dependent on the crop, its location, the presence of out-crossing wild relatives, or sexually compatible crops, and

the competitive nature of the introduced trait. However, in all cases, pollen dispersal and seed dissemination remain the major routes of transgene escape.

### Cytoplasmic male sterility

Biological containment strategies, which attack the problem of gene flow at source, have been developed. Currently existing methods of blocking viable pollen production take advantage of naturally occurring cytoplasmic male sterility (cms), a maternally inherited inability to produce functional pollen. Natural sources of cms are available for many crops (40 sources are available in maize). Breeding companies first made use of cms as a dominant tool for hybrid seed production in maize, sunflower and rape seed, introducing fertility restorer genes (which also occur naturally) to provide farmers with male fertile commercial seed. To exploit naturally occurring cms as a tool for transgene containment, the GM hybrid version must not contain the fertility restorer genes. The pollen necessary to fertilise the GM stand would be supplied by non-transgenic male-fertile hybrids. The success of such a strategy depends entirely on the reliable detection of fertility restorer genes (i.e. their absence in GM hybrids and their presence in non-GM hybrids). Therefore, further investment must be made to identify tightly linked molecular markers to the fertility restorer genes to ensure that individuals containing these genes can be excluded from the breeding process, thus maintaining male sterility.

The introduction of genetically engineered male sterility was an early strategy for gene containment that has some advantages for species where no natural cms systems have been found. Due to a considerable extra effort for the seed production it has found some use for high seed prize species, for example chicory. These genetically engineered male-sterile crops frequently rely on non-specific nucleases, driven by cell-specific promoters that destroy the tapetum, thus preventing pollen development. Viable seed production from male-sterile flowers can be achieved by pollination with exogenous pollen. The recent development of inducible promoters could provide a means

of controlling fertility by conditionally expressing 'restorer genes', when required, in a male-sterile background.

### **Cleistogamy**

Another potential means of gene containment is offered by the phenomenon of cleistogamy, a process whereby self-pollination and fertilisation occurs within an unopened flower. Cleistogamy occurs to different degrees in many species of flowering plants, including agricultural crops such as wheat and soybean. Cleistogamous traits have been induced by mutation in oil seed rape. The trait is controlled, in the main, by one gene, CLG1, which is currently the subject of a positional cloning project. In field experiments studying the impact of cleistogamy on pollination in oil seed rape, some results showed a lack of stability of the trait. Since then, new, more stable cleistogamous lines have been generated.

## **NEW TECHNOLOGIES**

With our increasing understanding of gene regulation in plant development and physiology come new opportunities for the containment of transgenes. A number of new technologies are currently under investigation. These include the exploitation of apoptosis, split gene technology, plastid transformation, apomixis, and transgene mitigation.

### **Split gene technology**

Over recent years trans-splicing inteins, which allow the reconstitution of functional proteins from non-functional protein fragments, have been developed for use in plants. This technology offers a means by which to prevent the production of functional proteins in non-target plant varieties. Future generations of transgenic crops could be developed to contain split genes, linked in repulsion, so that the desired trait will be present only in the target crop plants. Incomplete, and therefore non-functional, protein fragments would be generated in hybrids resulting from out-crossing, with reduced environmental impact.

### **Plastid transformation**

Recent technological advances have made it possible to integrate and express foreign genes in the plastome. Chloroplasts are usually inherited maternally, providing a vehicle in which to express foreign genes, whilst minimising the risk of gene flow through pollen. Integration of foreign genes can be achieved in chloroplasts through homologous recombination, so that superfluous vector DNA sequences can be excluded, and undesirable alterations in the expression of native genes can be avoided (sometimes caused by the random integration of transgenes in the plant nuclear genome). Using this technique it is possible to express several linked genes in a single operon (transgene stacking), and avoid the possibility of epigenetic effects that can cause silencing of transgenes expressed in the nuclear genome. Although this technology may not be universally applicable, since chloroplasts can be inherited through the pollen of some plant species, its potentially broad application has been limited by the lack of universal plastid transformation methods. While reliable methods are available for tobacco and tomato, and some progress has been made in rice and potato, more methods are needed.

### **Apomixis**

The development of apomixis technology (asexual seed production) is an attractive approach to produce seed in the absence of pollen, thereby reducing gene flow from genetically modified crops. Application of this technology has broad implications for agriculture. Its potential benefits for hybrid seed production and maintenance of heterosis have attracted the interest of laboratories worldwide. Apomixis occurs naturally in many plant species, but in very few of agricultural importance. Recent technological advances make it feasible to identify components of the process through genetic modification of sexually reproducing plants. It is widely accepted that apomixis results from the deregulation of gene expression both temporally and spatially in reproductive tissue. The identification of genes

that enable asexual seed production is essential to the development of apomixis technology as a superior method of transgene containment.

### Transgene mitigation

An alternative approach to transgene containment is transgene mitigation (TM). TM prevents the spread of a transgene by linking it to a mitigator gene (segregation of tightly linked genes is very rare), thus lowering the fitness of the recipient to below that of the wild-type population. The TM traits chosen are neutral or favourable to crops, but deleterious to non-crop progeny due to a negative selection pressure. Such traits include dwarfism, uniform seed ripening, non-shattering seed pods, anti-secondary dormancy, and non-bolting genes. The TM concept was initially demonstrated in tobacco, and has now been transferred successfully to oilseed rape. The TM plants show reduced fitness when competing with wild-type weedy relatives in simulated greenhouse containment trials.

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# **PARALLEL SESSION**

DETECTION OF GMO  
AND CONTROL





# Key issues and open questions in GMO controls

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**Abstract:** The introduction of genetically modified organisms (GMO) induced the implementation of a set of regulations in Europe as well as in other countries. These regulations were meant to provide a freedom for the consumers to choose the products they want. Analytical methods and traceability shall ensure the reliability of the labelling, a requirement that is not always easy to meet. Technical, financial and other challenges to labelling reliability are among the key issues in the GMO detection area and several of these are discussed here.

## INTRODUCTION

Developments in biotechnology over the last decades have resulted in the release of a range of GMOs, particularly of GM plants (GMP) into the environment. Many European citizens are expressing a strong reluctance to the release of GMPs as well as to the commercialization of their derived products, although a discrepancy between expressed opinions and attitudes of consumption can be observed. Whatever their opinions and attitudes are, European consumers, like consumers in other countries, have a desire for and a right of freedom of choice. This right can

only be realized by clear and reliable labelling of products.

The European Union (EU) legal framework has imposed mandatory labelling of food- and feedstuffs above certain thresholds of fortuitous presence. Enforcement laboratories have been appointed and are responsible for control and implementation of the EC legislation, including controlling that feed and food producers comply with the legislation. Recent amendments of the EU legislation include (i) the establishment of a Community Reference Laboratory (CRL) that is officially supported by the European Network

of European Laboratories (ENGL), (ii) mandatory traceability of food- and feedstuffs, and (iii) GMO notifiers now have to provide the CRL with specific and quantitative detection methods that need to meet specific quality criteria, as well as appropriate reference materials (control samples). These amendments have taken into account several of the major problems that enforcement and private laboratories were facing before.

However, still a number of serious challenges are not covered by the current European legislative framework, e.g. (i) lack of methods for non-EU approved GMOs, (ii) methods to detect unknown GMOs, (iii) cost-effective screening methods (targeting P35S, Tnos, etc.) and appropriate controls (CaMV, *Agrobacterium* spp., etc.), and (iv) more rapid and cost-effective, such as multiplex, detection/ identification methods.

One of the most important consequences of the recent regulatory amendments is that method development work can focus on fit for purposes strategies and concepts rather than on development of single GMO detection methods for EU authorized GMOs.

This lecture will try to highlight key issues and address some of the still open questions in the GMO detection area. We also take as a postulate that the modular approach is used (at least partially) in the routine laboratories, as indicated by the wide acceptance of this approach in Europe, Japan, China and the USA, including method validation studies organized by e.g. the AACC.

## KEY ISSUES

The impact of the cost of controls and traceability is the first easily discernable issue for the consumers. Consumers are generally looking for the lowest prices and the highest quality. The cost of analyses is also a concern for enforcement laboratories whose budgets are generally not extensible while the number of new detection areas is still growing (allergens, mycotoxins, etc.).

Two of the regulatory steps taken by the EC may contribute to reduce these costs. Detection methods and reference materials, as well as a large part of the expenses for method validation, shall be provided by the GMO notifiers. Mandatory traceability may also lead to a reduced need for analytical controls. Further cost reducing measures are constantly being sought by method developers, analysts and others dealing with labelling and traceability. As the number of GMOs on the global market increases, so does the need for high-throughput, low-cost methods and efficient traceability tools.

GMO detection can be carried out for several purposes. The aims and habits of detection differ between e.g. seeds producers, competent authorities in charge of environment monitoring or food and feed controls, and other stakeholders (e.g. food manufacturers). These differences may strongly influence the sampling plan and the chosen detection methodology, e.g. a low-cost protein based method vs. a more expensive quantitative event-specific real-time PCR method.

Given the different contexts, the analytical target generally also differs between these different actors. As no internationally agreed unit is defined to express the GMO content, some actors may prefer to refer to kernels (also called "contamination unit") while others may prefer to refer to haploid genomes (DNA, also called "traceability unit"). The latter approach has recently been recommended by the EC. Unfortunately, this lack of international consensus could induce costly technical changes and duplicated analyses to comply with different aims and expressions of GMO content. Thereof, the result may be disputes and costly lawsuits.

Costly disputes may also result from uncertainties in analytical measurements, stemming e.g. from inherent differences or weaknesses of individual methods, analysts or laboratories. Reliable and traceable analytical results, including their known measurement uncertainty, are reliant on method comparison, evaluation, validation and harmonization. Due to the specific nature of

a GMO and the typical character of their bio-analytical detection techniques, the application of internal and external quality control measures and compliance to the ISO 17025 principles are not straightforward. Indeed, GMO detection was the first application of PCR based methods to the whole supply chains, without any solid feedback from other detection areas.

Method developments are also costly. Generally, specific and quantitative GMO detection methods are now provided and validated through a regulatory process. However, this process does not ensure availability of methods to detect the GMOs authorized only outside the EU. As already mentioned, globally the number of commercialized GMOs is constantly increasing, and the analytical costs from testing for single GMOs might becoming prohibitive. Methods that allow for multi-GMO screening and identification at a reasonably low cost must be developed.

Availability of appropriate Decision Support Systems (DSS) that would assist the stakeholders/users in selecting the most appropriate analytical strategy in a certain environment and at a certain moment could also be a major step forward. The need for DSS may also increase in relation to interpretation of data and response to particular results in unstable situation, e.g. when suspecting the presence of unknown GMO.

## OPEN QUESTIONS

The lecture will address the following items in relation to the key issues listed above.

- Sampling issues: costs vs. lots representativeness, alternative sampling plans.
- Unit of measurement or expression of GMO quantity: the pros and cons of proteins and DNA, and bioassays; genome-based vs. seed-based quantification and the consequences for decision making.
- Modular approach: commutability of modules, combined measurement uncertainty, consensus modules e.g. for matrices and taxa.

- Reference materials: continuity, availability and stability, commutability and alternative types of reference materials.
- Measurement uncertainty: how to assess and how to minimize?
- Reducing analytical costs: threshold based determination, screening approaches, qualitative vs. quantitative approaches, possibilities for multiplexing, choice of chemistry and apparatus, etc.
- Unauthorized and unknown GMOs: how to detect and characterize, considerations in relation to costs.
- Alternative technologies for GMO detection: physico-chemical methods.
- Decision support systems (DSS): what is needed, how should it work, for whom?

## CONCLUSION

Current GMO regulations and the development and implementation of coherent GMO control systems leave a number of problems open for discussion.

With a few exceptions, most of the issues and open questions discussed here are common to several areas of analytical detection. Our aim is to provide a basis for fruitful discussions between method developers, analysts and stakeholders.



# Decision Support Systems (DSS) and GMO detection

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**Abstract:** Genetically modified organisms (GMO) and plants (GMP) became a part of our environment and the food chain. GMO handling is strictly regulated in EU and up to now only a few GMOs have been approved for marketing and even less for culture. Products below a 0.9% level of fortuitous presence of approved GMO are exempted of labelling. Enforcement laboratories have been therefore established in EC to control the implementation of these European regulations. GMO analysis includes several steps ranging from sampling, sample preparation, choice of fit for purpose analytical method(s), analytical procedure itself (DNA isolation, screening and/or GMO identification and quantification) and results interpretation. Uncertainty of the measurement is relatively high and several factors have been identified that increase the uncertainty degree. Appropriate decision support systems are thus, more or less implicitly, used by the laboratories and the Competent Authorities to ensure the quality of the analyses and appropriate interpretation in the most cost-effective ways.

## INTRODUCTION

The rapid development of biotechnology in the last decades has launched genetically modified plants (GMP) into the environment and on the market. Only a few of GMPs were approved according EU rules. The GMPs handling is driven by EC regulations, directives and harmonized national acts. In particular, labelling and traceability of GMPs and GM based food and feed is required by EU regulations and directives (e.g. directive 18/2001/EC, regulations 1829/2003/EC, 1830/2003/EC) beside more general regulation on traceability (e.g. 178/02/EC).

Moreover, the EC released recommendations (03/556/EC) on crops co-existence while some European countries established laws for co-existence of different types of agriculture (GM versus conventional and organic).

National control laboratories established to control the implementation of European laws in food and feed supply chains belong to the European network of GMO laboratories (ENGL) which supports the Community Reference Laboratory (CRL) in its validation tasks. Stakeholders also operate laboratories for GMO identification to check the quality of their own production.

Beside requirements on quality management (e.g. EN ISO 17025) GMO laboratories are asked to provide precise, reproducible results with adequate interpretation at the lowest costs. If several factors, such as ways to master uncertainty measurements, most of the laboratories are trying to master the analyses costs by balanced applications of fit for purpose procedures.

They are thus using, in a more or less formalized way, decision support systems (DSS) to (i) determine the appropriate analyses methods to be applied to known or unknown simple or complex sample to be analyzed, and to (ii) analyze results of e.g. screening methods and to (iii) decide next steps of analyses, for instance in front of unexpected results or when unapproved GMO are suspected to be present which then induce data analyses.

There is thus a clear need of standardized and cost-effective procedures, not only of standardized and validated analytical methods such as DNA extraction or PCR, but also to determine the fit for purpose detection methods and adapt analyses to (i) decrease price and duration of analyses which can impact the ability of the States to make controls and of the consumers to get access to products they wish, (ii) decrease the possibility of litigation, between both stakeholders and States.

## DECISION SUPPORT SYSTEMS

A decision support system (DSS) can range from a simple decision tree with branching steps to a sophisticated computer software that analyzes data and presents it so that users can make corresponding decisions more easily. In some cases, such a software can go up to proposing solutions and decisions. Generally speaking, it is an "informational application".

Typical information that a decision support application might gather and present would be:

- a deeper insight into the quantified impact of drivers;

- statistically validated, or alternatively probabilities based, hypothesis for accurate decision making
- a 'test-lab' for evaluating alternative strategies before one makes a final decision.

A decision support system may present information graphically and may include an expert system or artificial intelligence. It may be aimed to executives, some other groups of knowledge workers or policy makers such as Competent Authorities.

Several decision support tools can be identified such as expert systems and decision trees (Coleman & Khanna, 1996; Dhar & Stein, 1997; Marakash, 1999). All of them are able to more or less user-friendly support decision in complex situations.

## Theoretical considerations

In many issues chance (or probability) plays an important role. Decision analysis is the general name that is given to techniques for analyzing problems containing risk/uncertainty/probabilities.

A decision tree takes as input an object or situation described by a set of properties and outputs a yes/no decision. Decision trees therefore represent Boolean functions. Functions with a larger range of outputs can also be represented.

DSS help to choose between several courses of action. They provide a highly effective structure within which one can lay out options and investigate the possible outcomes of choosing those options.

## Decision theory

Decision theory is normative or prescriptive, i.e. it is concerned with identifying the best decision to take, assuming an ideal decision taker who is fully informed, able to compute with perfect accuracy, and fully rational what people

will actually do. Decision theory is only relevant in decisions that are difficult for some reasons. The choice under uncertainty of GMO content is a typical example in the case of GMO testing.

### Choice under uncertainty

This area represents the heartland of decision stated that, when faced with a number of actions each of which could give rise to more than one possible outcome with different probabilities, the rational procedure is to identify all possible outcomes, determine their values (positive or negative) and the probabilities that they will result from each course of action, and multiply the two to give an *expected value*. The action to be chosen should be the one that gives rise to the highest total expected value.

Decision trees are often used by decision makers and possess many advantages over competing procedures:

- Decision trees are easy to build.
- Decision trees are easy to understand.
- Decision trees handle both continuous and categorical variables.
- Decision trees can perform classification as well as regression.
- Decision trees automatically handle interactions between variables.
- Decision trees identify important variables.

Several other specific tools are available, for information one consult for e.g.:

- Precision Tree  
<http://www.palisade-europe.com/html/ptree.html>
- Decision Pro <http://www.vanguardsw.com/decisionpro/jdtree.htm>
- Trial Pro <http://www.treeage.com/products/download.html>
- Tree Boost and Decision Tree Forest  
<http://www.dtree.com/sstreeboost.htm>

### STEPS IN GMO DETECTION

GMO identification and quantification require several steps.

First appropriate sampling plans are essential for representative results. Different matrices and lots have to be analyzed according to numerous possibilities taking into consideration reliability, cost-effectiveness and practicability, thus “fitness for purpose”. As it was shown by KeSTA and KeLDA projects of the JRC, the distribution of unintended GMO contamination in soybeans shipments is random. Available normalized sampling procedures are not fully applicable especially in case of huge lots or highly processed food and feeding stuff. A DSS is thus needed since this first step, since analytical methods (protein or DNA based) or sample preparation (simple or multiple control plans by attributes) have to be considered very early.

The second use of DSS can be observed when facing the laboratory samples and the detection strategy and methods to be applied. The analytical procedure to be applied will depend for instance on the matrix (pure, such as seeds, or mixtures of ingredients, raw material or matrix without presumed detectable presence of analytes, etc.), the purpose (purity of plants for seeds production, separation of crops in silos before exports or analyses of imported shipments in Europe, etc.), the available methods (quantification of approved GMO or detection of unknown GMO thus without specific detection methods, etc.) and economic considerations (budgets of enforcement laboratories, impact for consumers of analyses costs on end-products).

Inadequate analytical sample preparation, and analytical procedure itself, can then lead to false results. Advantages and disadvantages of analytical procedure based either on protein identification or gene amplification (PCR) as well as, for instance, properties of *Taq* polymerases used in PCR have been discussed elsewhere. Due to different nature of matrices entering food and feed chains the use of the “modular approach” is

an adequate theoretical reply to large costs and duration of methods validation. It however has as yet not been accompanied with guidelines on measurement uncertainties and assessment of the entire commutability between sets of theoretically similar modules. Real-time PCR used for GMO quantification can be affected by many factors/ inhibitors resulting from un-appropriate DNA extraction. Moreover, new technologies such as DNA chips, MALDI-TOF, SNPlex become available owing their advantages and disadvantages.

The detection of unapproved GMO is a quite new analytical aspect of GMO detection which will probably greatly reinforce the use of DSS since legal actions have to be taken on solid and consensus bases, at least in EU. Matrix approach, Quantitative differential PCR and polymorphism studies, such as anchored PCR, all the techniques would need to be standardized at least for a robust interpretation. Could these aspects of disputes be

taken into consideration by the Codex documents on the ways to solve disputes based on analytical results?

Finally, based on analytical procedure and data, decisions are issued by e.g. Competent Authorities. The responsible person must thus be highly trained to achieve correct interpretation and decision. Again, as new GMOs – either EU approved and unapproved - are expected to appear on EC market suitable, consensus DSS must be made available to decision makers (Fig 1).

**CONCLUSION**

Numerous DSS are routinely used, under a more or less formalized state, at each step of an analytical process such as GMO detection.

As in other fields needing a choice between sampling and detection methods, not leading

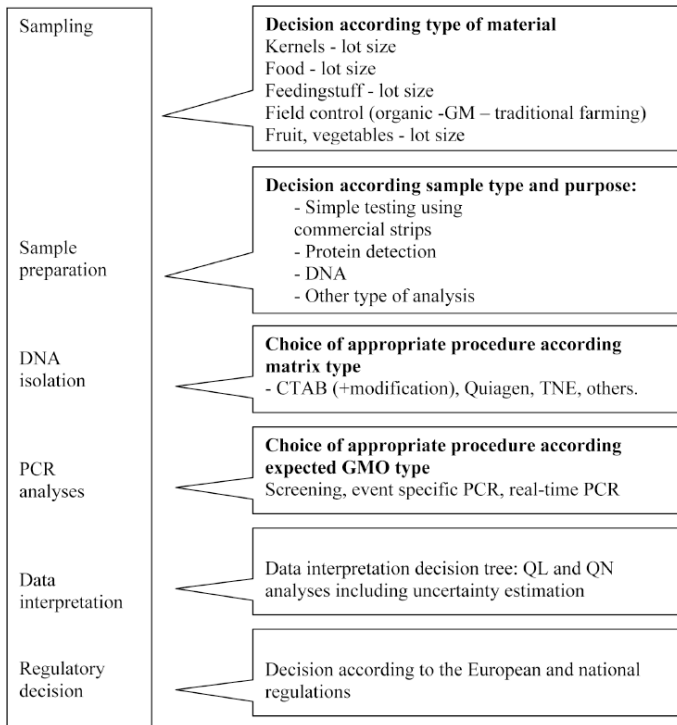


Figure 1. General procedure on which decision tree should apply.



sometimes to consensual analyses of results and decisions, DSS would greatly benefit from international "standardization" for improving the efficiency, cost-effectiveness and rationality of decisions on a subject still sensitive for consumers.

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# Heteroduplex mobility analysis – an approach to monitor transgenic crop sequences

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**Abstract:** Stability of transgene sequences is a crucial prerequisite for application of the DNA based methods that are used for detection, identification and quantification of GM crops.

A new technique for mutation screening based on the heteroduplex formation is tested and optimized for the model species *Arabidopsis thaliana*. The optimized procedure was then used to screen target sequences in some commercial transgenic events. The suitability of this new approach for high throughput screening for single nucleotide polymorphisms (SNPs) and its applicability for molecular monitoring of transgene events are discussed. Besides labelling and traceability of GMOs, post-market monitoring is a very important issue in the field of GMO analysis and monitoring.

## INTRODUCTION

It is generally assumed that transgenic sequences are “hot spots” for mutations in GMOs. Some studies show that transgenes have stable inheritance over the generations (Armstrong *et al.* 1995, Padgete *et al.* 1995). Point mutations are also an important source of genetic variability. Some recent data display that in the transgenic loci they seem to occur with a rate comparable

with this of the endogenes of the same species (Ogasawara *et al.* 2005). This question is becoming very important nowadays, as there is a high demand for monitoring the stability of the transgenic sequences of the commercial crops. In the context of the EU requirements for labelling of GM derived foods the stability of the transgenic insert and the junction regions appears to be an important issue. This is a crucial factor regarding the application of event specific assays.

The development of high throughput and sensitive approaches for mutation scanning has been accelerated during the last years and improved systems are becoming available. Recently Applied Biosystems developed a new polymer that is able to distinguish the wild type and mutation molecules based on their heteroduplex mobility differences ([www.appliedbiosystems.com](http://www.appliedbiosystems.com)).

Heteroduplex Mobility Analysis (HMA) is a new technique that has not been tested yet in the field of plant genetics. We tested the sensitivity and the throughput of the technique on the model plant *Arabidopsis thaliana*. Our research program is aimed at application of HMA in the field of GMO research to monitor the stability of the sequences in transgenic crops.

## RESULTS AND DISCUSSION

HMA is based on formation of homo- and heteroduplexes between a 'reference' (wild type) DNA molecule and a corresponding molecule containing one or few mutations. The homo- and the heteroduplexes display different mobilities when they are loaded on a capillary array. As a consequence homo- and heteroduplexes appear as different fluorescent peaks. This is observed in the pattern of the pooled leaf samples from *Arabidopsis*. This pattern is reproducible in all the pooled samples, in which different proportions of the two plant DNAs were mixed. The results obtained in our lab indicate that one homozygous mutation can be detected in a pool of 10 plants. Other heteroduplex based techniques can detect in *Arabidopsis thaliana* 1 mutation in 8 (McCallum *et al.* 2000).

Additionally, the method is suitable only for low size fragments up to 500 bp. These characteristics make the method suitable to examine the stability of sequences in the transgenic plants that are used for event-specific detection and quantification. We initiated a study on the stability of the sequences in commercial transgenic crops like maize and soybean, in order to establish a method that can be used for post-release monitoring of GMOs.

## PROSPECTIVE

HMA gives the opportunity to detect mutations in known sequences in a sensitive and high throughput manner. A simultaneous analysis of a significant number of samples and targets is possible. An advantage of the method is the possibility to make a database of reference wild type and mutation patterns. In this way, on one hand new mutations can be detected in comparison with the reference wild type pattern and on the other hand already existing mutations in the analyzed sequence can be found. Based on the database of the validated methods for GMO detection and quantification the patterns can be generated for every event specific assay. A similar database can be created for endogene sequences as well.

Transgenic plants have to be monitored after the deliberate release. A specific aspect of this post-release monitoring is the stability of the DNA sequences that are used for event-specific identification and quantification. Therefore, methods for mutation detection, such as the proposed HMA based approach have the potential to be used in the field of GMO research for monitoring purposes. Applying this methodology in the frame of suitable monitoring schemes can provide extensive information about *on field* behaviour of transgenic crops.

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**PARALLEL SESSION**  
SOCIO ECONOMICS OF  
CO-EXISTENCE



# Effects of the Regulation (EC) No 1829/2003 and 1830/2003 on the German Food Industry

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**Abstract:** This survey analyses the effects of Regulation (EC) No 1829/2003 and 1830/2003 on the German food industry. Genetically modified organisms (GMOs) are already traded worldwide and the food industry in Germany has to undertake organisational efforts and is facing costs to fulfil this legal requirements.

Until now there are no studies available analysing the effects of Regulations (EC) No 1829/2003 and 1830/2003 and how they can be implemented efficiently in food industry. According to this survey 89% of the food producers in Germany are affected by those two Regulations, although no food producer in Germany processes GMOs which would require labelling. Furthermore the German food industry developed quality management systems to exclude GMOs of production. In this context 82% of the food producers mentioned to demand a written affirmation from suppliers on the GMO-free status of deliveries and 77% mentioned to make enquiries back to suppliers on the GMO-status of raw materials, aside other measures. Increasing costs of implementing Regulations (EC) No 1829/2003 and 1830/2003 mainly result from higher costs of GMO free raw materials, costs for analytical testing of GMO contents in raw materials and additional personnel costs. Measures to avoid GMOs in the German food production seem to be successfully.

Labelling and traceability requirements of Regulation (EC) No 1829/2003 and 1830/2003 have been fulfilled According to first results of control agencies in Bavaria and Baden Württemberg in the year 2004. There was nearly no GMO admixture detected which would require labelling. In cases were GMO admixture was detected, this was under compliance of legal thresholds.

## APPROACH

In contrast to the growing use of genetic modified plants in agriculture, the acceptance of genetically modified food in the European Union (EU) and in Germany is still low (Frank, 2004). Due to this low acceptance the EU passed regulations to ensure the safe use of GMOs in the EU market. The food industry is mainly affected from Regulations (EC) No 1829/2003 and 1830/2003. These regulations provide a framework for processing and trading of genetically modified food in the European food industry. However, labelling of GMOs in food is required since passing the „Novel Food Directive“ in the year 1997: any food product contained more than 1% of genetically modified ingredients was obliged to label, a label which food producer and retailers have strived to avoid. This policy gave a free ride to highly processed food products where the presence of GMOs is not any more detectable by analytical testing (Transgen, 2005b). Therefore Regulation (EC) No 1829/2003 and 1830/2003, which entered into force in April 2004, obtain exceeded labelling and traceability requirements for GMOs with following key components:

- **Traceability:** Mandates product traceability through documentation and implementation for the entire supply chain.
- **Labelling:** Products containing GMOs must be labelled as such, even when undetectable by

tests. Products containing traces of GMOs below the appropriate regulatory tolerances thresholds are exempt from labelling, provided that compliant traceability systems are in place and traces of GMOs are adventitious and technically unavoidable.

- **Thresholds:** 0.9% tolerance for EU authorized GMOs and 0.5% for unauthorized GMOs if they have already received a favourable EU risk assessment. Compliant traceability systems must be in place and must demonstrate that any traces of GMO are adventitious and are technically unavoidable (Fagan, 2004).

Until now there are no studies available analysing the effects of Regulations (EC) No 1829/2003 and 1830/2003 and how they can be implemented efficiently in the food industry. To investigate this issue a questionnaire was developed, based on previewing existing literature as well as interviewing experts and representatives of the food industry. The questionnaire was sent to 1,714 factories of the German food industry in May 2005. Emphasis laid on efforts to reach all branches of the food industry by considering different sizes (depending on staff and total revenue) and brand strategies. The number of returns was about 20% what can be interpreted as a „good result“. Before starting this activity there was a lot of scepticism from members of the food industry about getting any feedback due to the fear in the German food industry that, who is talking

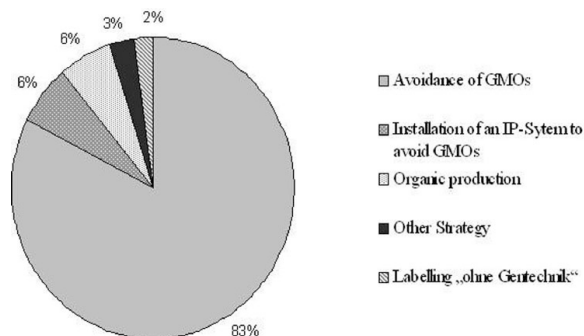


Figure 1. Strategies in the German Food Industry towards GMOs.  
Source: Own investigations.



**Table 1.** “Appropriate steps” to comply labelling and traceability requirements for GMOs.

Source: Own investigations.

<b>Measures, “appropriate steps” of the German food producers</b>	<b>Percentage</b>
Written affirmation on GMO-free status from suppliers of certain products	82%
Enquiry to supplier on general GMO-status of raw materials	77%
Analytical GM-testing of raw materials and end products	28%
Checking if GM-plants are already existing for raw materials	19%
Checking if raw materials derive from countries with GM-plants	16%
Demand of IP-certificates on GM-free-status of suppliers	2%
No additional measures caused by GMO-legislation	11%

publicly about GMOs will lose consumers trust and sales. The data gathered in this inquiry have been analysed with SPSS.

## **STRATEGY OF THE GERMAN FOOD INDUSTRY TOWARDS GMOS**

The results of this survey show that the food industry in Germany avoids GMOs in production processes and no producer uses GMOs which would require labelling. There are different strategies towards GMOs in the food industry as it is shown in (Figure 1). 83% of the answering food producers mentioned to take all appropriate steps to avoid GMOs which would require labelling. 6% of the food producers installed an Identity Preservation System (IP-System) to avoid GMOs. Another 6% mentioned to produce under organic rules and regulations which forbid the use of GMOs. Since October 1998 the German legislation offers the label „ohne Gentechnik“ (without genetic modification) under compliance stricter labelling regulations and 2% of the food producers mentioned to use this label.

## **THRESHOLDS OF GMOS IN THE CONTEXT OF PRODUCT LIABILITY AND WARRANTY**

If GMO contents exceed the legal thresholds of GMO adventitious presence, Regulation (EC) No 1829/2003 and 1830/2003 require an active

duty of forwarding information on GMO contents by suppliers (EC 2003a; EC 2003b). Besides this legal duty of forwarding information article 12 and article 47 of Regulation (EC) No 1829/2003 require that „operators must be in a position to supply evidence to satisfy the competent authorities that they have taken appropriate steps to avoid GMOs“. This means that in case of detecting GMOs exceeding legal thresholds the burden of proof is shifted. Thus food producers are obliged to submit evidence that they have undertaken appropriate steps to avoid the presence of GMOs in production processes (BLL, 2004). The wording of this Regulation (EC) No 1829/2003 is very general and food producers enquire definitions of appropriate steps to comply those requirements. Due to this lack of information several institutions of the food industry developed guidelines for their members, according to product liability and warranty. The results of this survey show that the food industry in Germany is considering different „appropriate steps“ to comply article 12 and article 47 of Regulations (EC) No 1829/2003 (Table 1).

Around 11% of producers in the German food industry mentioned that no additional „appropriate steps“ are necessary in context of GMO-legislation. This mainly refers to breweries and non-alcoholic beverages producing industry which seem to be less stressed by potential GMO admixture.

**Table 2.** Selected Costs related to GMO legislation in the German food industry.

Source: Own investigations

	<b>Costs for analytical GMO testing</b>	<b>Higher costs of raw materials</b>	<b>Additional personnel costs</b>
Milling industry	57%	29%	29%
Confectionary industry	44%	26%	30%
Other food industry	38%	22%	40%
Bakery industry	36%	23%	29%
Dairy industry	28%	17%	38%
Fruit/Vegetable processing	42%	8%	54%
Meat industry	23%	8%	40%
Non alcoholic beverages	5%	-	10%
Brewery	-	-	8%
Fruit juice industry	-	-	35%
Food industry in total	28%	14%	33%

## **ECONOMIC IMPACTS OF LABELLING AND TRACEABILITY REQUIREMENTS**

Additional personnel costs throughout Regulation (EC) No 1829/2003 and 1830/2003 mentioned 33% of all food producers, these costs range from 1,000 € up to 50,000 € per year. Additional costs for GMO free raw materials mentioned 14% of all food producers and they range from 16,000 € up to 28,000 € per year. Analytical testing of GMO contents in raw materials or end products is feasible using a quantitative or qualitative testing regime. Some producers do just quantitative GMO testing, some do qualitative GMO testing and some apply both. In average companies test about 39 times per year and one test is about 174 € what leads to average costs for analytical GMO testing of 6,786 € per year. In Table 2 additional efforts and costs of the different branches in the food industry are illustrated.

## **CONCLUSION**

The food industry in Germany is affected of the worldwide increasing use of GMOs and of requirements of Regulations (EC) No 1829/

2003 and 1830/2003, although German food companies avoid GMOs which would require labelling. Measures and costs for avoiding GMOs in German food production depend strongly on branches and raw materials. But it seems that the applied measures to avoid GMOs in food production fulfil their purpose. Labelling and traceability requirements of Regulation (EC) No 1829/2003 and 1830/2003 have been fulfilled, according to first results of governmental control agencies in Bavaria and Baden Württemberg in the year 2004. Just in some cases there was GMO admixture in food products, but this was mostly under compliance of legal thresholds without labelling obligations (Transgen, 2005a).

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# A multidisciplinary discussion on the co-existence draft regulations in Spain: case study in Lleida (Catalonia region)

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**Abstract:** The Spanish and the regional Catalonia's Governments are currently developing legislation in order to regulate the co-existence between organic and conventional agriculture and genetically modified (GM) varieties. Essentially, the issue is still one of controversy and far from being closed yet.

In this study, the issue of co-existence is analysed in the following ways: first, the current state of GM and organic agriculture in Spain is reviewed, with special emphasis to the challenges of co-existence and GM contamination of organic crops. Second, a comprehensive review of the co-existence debate as developed in Spain and Catalonia over the last years is provided. On the one hand, legal, economic and technical aspects which have emerged are analysed. On the other, the major stakeholders and participants are described, as well as their main discourses and the issues related to the public debate on co-existence.

Finally, as genetically modified organisms (GMOs) have been growing at a commercial scale in Spain since 1998, this experience can be used in order to assess strengths and pitfalls of the proposed legislations on coexistence in Spain, particularly in the Catalan province of Lleida.

## INTRODUCTION

In July 2003, the European Commission issued the "Guidelines for the Development of National Strategies and Best Practices to Ensure the Co-existence of Genetically Modified Crops with Conventional and Organic Farming" (Commission of the European Communities, 2003). Following the principle of subsidiarity established in these recommendations, Spain is now developing

national legislation on the issue. As Catalonia has competences over agricultural issues, is now readying its own legislation simultaneously with the Spanish one.

Spain has been until now the solely country within the European Union with GMOs grown at a commercial scale since 1998. This unique characteristic allows analysing the legal, social and economic processes emerging during the

introduction of genetically modified crops. The most relevant aspects of the draft legislations can be assessed on the basis of on the ground experience.

Until the present season (2005), five varieties with the modification Bt-176 and eleven of Mon 810 have been approved. In July 2005<sup>1</sup>, 14 varieties with the modification Mon810 were registered in the Register for Commercial Varieties, but those with the modification Bt-176 were excluded as they carry antibiotic-resistant marker genes (European Food Safety Authority, 2004).

Farmer's acceptance of GM maize has been substantially heterogeneous in the Spanish State but globally represented about 10% of the total surface planted with maize in 2004 (Ministry of Agriculture, 2004a). The percentage increased up to 40% in Catalonia, mainly in the province of Lleida (Northwest of Catalonia), with more than 16.000 ha cultivated with GM varieties (Serra & Salvia, 2004). Moreover, Spain became the European country with the largest number of field trials (SNIF<sup>2</sup>).

As a result of WTO tariff quotas, Spain imports 2 million tonnes of maize from the two largest GM maize producers, Argentina and USA. Although Spain produces maize seeds, it also imports a substantial quantity (Ministry of Agriculture, 2004b).

Simultaneously, organic agriculture is in expansion, with a rapid increase both in the number of producers, manufacturers and hectares. Despite of this, the actual market share of the organic farmers is still reduced, without big farmer corporations influencing the market. It should also be noticed that traditional products –organic although sometimes not certified- are frequently sold through short distribution chains, such as direct selling in local markets or in the field. Although distribution infrastructures are weak in a formal sense, it is estimated that about 80% of the organic food produced in Spain is exported to other European countries, mainly Germany and United Kingdom (Junta de Andalucía, 2004).

With the increase of land with GMOs and organic agriculture, controversy has also grown. However, public participation in Spain during the introduction of GM crops has been inexistent. Just recently, discussion has been timidly opened to relevant stakeholders such as environmentalist or farmer associations, especially within the framework of negotiations on the co-existence legislations.

## APPROACH AND METHODS

The methodological approach can be divided in two sections. The first one consisted in a review of the comprehensive official information available on this issue (European Commission's press releases and communications, legislative documents, technical reports) and also documents produced by other stakeholders. Other secondary sources that have contributed to shape the question of co-existence are the results of different research projects, scientific meetings and round tables conducted at the European, Spanish and Catalan level. This section also included an extensive literature review on key issues such as: co-existence technical measures and ethical aspects, liability, testing or complexity.

The second part of the study is based on an on-going field research in the province of Lleida (Catalonia), started in year 2002. The aim is to incorporate stakeholders' viewpoints and to systematically assess the main aspects in relation to the feasibility of implementing the proposed co-existence measures. To collect this information, qualitative techniques including workshops, group and individual in-depth interviews are being administered, in addition to participant observation. To date 21 farmers, 6 managers of agricultural cooperatives and 3 technicians were interviewed in the field; also, 5 discussion groups with farmers and 3 with regional politicians were performed. Moreover, participant observation was used in several workshops and meetings as a means of collecting first hand information from stakeholders.

## RESULTS (WORK IN PROGRESS)

A first assessment of the practical implications of the draft legislation to regulate co-existence in Spain is being conducted. It includes stakeholders' contributions and perceptions on the issue. The following paper will look at:

a) Spanish and the regional Catalan governance related to the introduction of GMOs was lacking on previous public debate as evidenced by the deficiency of studies monitoring the agronomic, environmental and socio-economic situation after six years of GMO plantation (Brookes & Barfoot, 2003). The publication of the draft decrees on the coexistence measures of the Catalan and Spanish governments in 2004 has initiated some debate on the GMO issue. This discussion is focused "officially" on which technical measures are needed to achieving the co-existence between transgenic, conventional and organic agriculture below an agreed threshold (once a vast extension of GM maize is already planted). However, it seems difficult to concur on these measures without a previous discussion on the underlying practical problem(s) (Ravetz, 1971; Strand, 2002; Cañellas *et al.*, 2004). Furthermore, without a transparent discussion on the purpose to be achieved (e.g. which agricultural model), the legitimization of policy frameworks through scientific and technological models that promote a determinate socio-politic and economic agenda can drive to a legitimacy crisis if their prescriptive agendas are in confrontation with other social interests (Levidow & Marris, 2001; Wynne, 2001; Clark & Lehman, 2001). Such as in the case of the so-called "science based regulation", of which a paradigmatic example could be, precisely, the basis of "*strictly scientific criteria*" measures on co-existence as stated in the Catalan proposal of co-existence normative.

b) There is also a lack of common understanding on the definition of the co-existence concept (see, i.e. European Parliament, 2003; European Economic and Social Committee, 2005). A formulation closer to the definition of coexistence used by the European Commission has been

adopted by the institutional stakeholders<sup>3</sup> and the main agrarian union. However, other stakeholders shift the focus from the farmer to consumer rights. This difference in the understanding of the concept makes difficult reaching an agreement on the measures to be applied (e.g., the focus will be put at different stages of the supply chain or different thresholds would be used).

c) The analysis of the technical measures within the coexistence legislation reveals that a local approach should be promoted, based in ground experience:

- Interviews with local stakeholders reveal the existence of large social pressure against farmers growing organic. This, altogether with the fact that there is a need for social cohesion (especially in small villages) obstacles the ability to reach balanced agreements between farmers, as stated in the legislation. Some of the organic farmers growing maize have already shifted the crop as they wish to avoid direct confrontations with their neighbours. This also applies for liability issues, which are addressed by the Spanish Civil Code and the Catalan civil legislation on neighbourhood.

- The proposed legislation establishes isolation distances of 50 m for maize. In despite the fact that most stakeholders declare that they believe isolation distance are small, all interviewed farmers declare that they are very difficult to accomplish due to the agrarian structure, which is very fragmented.

- According to the EC recommendations, the management measures and instruments adopted should be subjected to ongoing monitoring and evaluation to verify their effectiveness and to obtain the information necessary for improving the measures over time. Member States should establish adequate control and inspection systems. However, for conventional crops there are no systematic monitoring programmes while for organic crops monitoring programmes have been subjected to protocol changes, leading to inconsistent and incomparable results. Moreover,

GM contamination events have not been profoundly analysed.

## PERSPECTIVES

The aim of the present study is to reach a better understanding of the relationships between biological, technical, legal, economic and socio-cultural processes in order to assess the limitations and strengths of the proposed coexistence legislation in Spain, with special emphasis in Catalonia. It becomes evident the need for more specific studies, i.e. analysing the economic feasibility to fulfil, on the one hand, the traceability and labelling normative and, on the other, the proposed coexistence measures. An extended assessment of the GM material (seeds, grain, fodder) flows is also be required for these purposes.

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<sup>1</sup> Order APA/2628/2005, 28<sup>th</sup> July, BOE num. 191, pp. 28268-28269.

<sup>2</sup> The SNIF (Summary Notification Information Format) database, managed by the Joint Research Centre can be consulted at: <http://gmoinfo.jrc.it/>

<sup>3</sup> E.g. the Catalan draft of the Decree on Coexistence defines coexistence as "the ability of the farmers for freely chose the use of organism genetically modified, organic agriculture or conventional systems, in compliance of the rights and obligations for each type of exploitation, making possible the coexistence among different exploitations".



# Danish farmer's perception of GM-crops

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**Abstract:** This paper presents a study of 185 farmer's perception of GM-crops in Denmark. The respondents' attitude to GM-crops mainly reflects a conservative view of the adoption of GM-crops. Among farmers only the exciting crops in rotation is seen as their future potential GM-crops. Findings from this study show that more the 60% do not expect any or less than 13 EUR increase in gross margins on their farms from adopting GM-crops. This assessment illustrates that the farmers regard the GM-crops more as a way of "staying in business" than a technology to increase their profits.

## BACKGROUND

In 2002 the Danish Minister for Food, Agriculture and Fisheries initiated a strategy work on co-existence with participation from scientific, legal and administrative experts and a broad group of stakeholders. Based on the recommendation from the strategy work a co-existence act was passed through the Danish Parliament in June 2002 (act. No. 436 of June 9. 2004). The act has been well received by both consumers and the agricultural sector and is seen as a transparent regulatory framework. On the one hand it gives the opportunity to grow GM-crops and on the other hand it fulfils the demands for a continued non GM-production.

However, no commercial scale GM-crop production has been initiated for the growing season 2005.

This paper is the first Danish investigation of farmer's perception of genetically modified (GM) crops. So far the debate and studies of perception of GM-crops and GM-food has mainly been among consumers and public stakeholders. A significant number of these studies have been made to determine the consumers' attitude towards GM-crops.

In 2001, Kronberger et al. published "The Train Departed Without Us" which presented a study of public perceptions of biotechnology in 10 European countries. The study was based on a number of focus groups in each of 10 European countries. A number of similar studies concerning the European public perception of GM has been made on biotechnology by the European Commission in Eurobarometer. Moreover, a significant number of studies dealing with these issues are represented in: Bredahl, 1999; Spetsidis, 2001; Jonas et al., 1998.

In Denmark the latest publication about public perception of-GM crops was based on a citizens' jury consisting of 16 laymen. This study discusses advantages and disadvantages of the adoption of GM-crops in relation to health and environmental issues and economic prospects and consequences of growing new GM-crops. Moreover, issues about Danish citizens' assessment of these crops were also addressed in this study (The Danish Board of Technology, 2005). This publication underlines the fact that Denmark in some areas is pro-GM oriented and do see GM crops as a potential benefit for Denmark. The relatively low focus on divers side effect on the present Danish non-GM production do not seem to be an issue. This can in some point be due to the fact that consumers and stakeholders in general trust the further practice with co-existence. A successfully introduction of GM-crops is therefore, if the co-existence systems works, seen as a potential benefit for Denmark. The form of production a the GM attributes is however another issue.

## METHODS

In this study, a questionnaire has been forwarded to 400 farmers (respondents) by mail. The anonymous respondents were asked to answer a number of questions concerning environmental considerations, expected cost and benefits of GM-crops and there own expectations in relation to the growing of GM-crops in their rotation. Furthermore the farmers' economical expectations about the introduction and use of GM-crops were addressed.

The selection of respondents was taken from an existing database administrated by the Danish Institute of Agricultural Sciences (DIAS) containing farm statistics in Denmark. The starting point for the selection was that all farm types and farm sizes, in all regions was relevant for the investigation. We knew that a large group of the respondents had grown rape seed within the last five years or at least had the potential to grow rape seed. This criterion was made in order to broaden the number of crops in the survey to other crops than cereals.

The other criterion was that there should be an equal distribution of farms with more then 100 ha and less than 100 ha in rotation to include both part time and full time farmers.

The selected farms had no special background in relation to GM. Their knowledge in the area of GM can be based on many sources. In this respect, this study may show a snapshot of the present perception of GM-crops among Danish farmers.

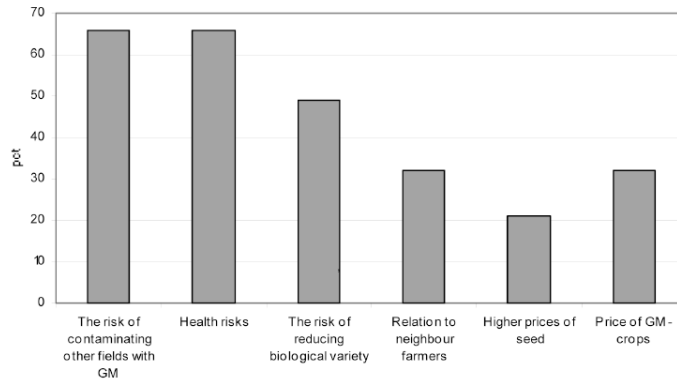
Mainly closed questions were used in the questionnaire in order to make the respondents answers as clear as possible and to ease the statistical handling of the data. In total 185 respondents returned a completed questionnaire (46%) which is regarded acceptable for this type of survey. Among these respondents 50% were above 50 years. Farming practices also differs among the respondents. 60% had either pigs or milk production along with crop production and only 35% didn't grow fodder crops on their farms. The division of labour also differs among the respondents. 35% did all the work in the field by them selves and 35% had help to some activities. The most frequent crops in rotation were wheat, rape seed and barley.

Multisided pivot tables have been made in Microsoft Excel to handle and analyse the open survey questions. However, the main results presented in this paper are based on results from the closed questions part of the survey. In order to validate the composition of the respondents a cross checks with Danish farm statistics have been made. The presentation form in this paper is based on the use of tables and diagrams, in order to visualise and give the best virtual understanding of the collected data.

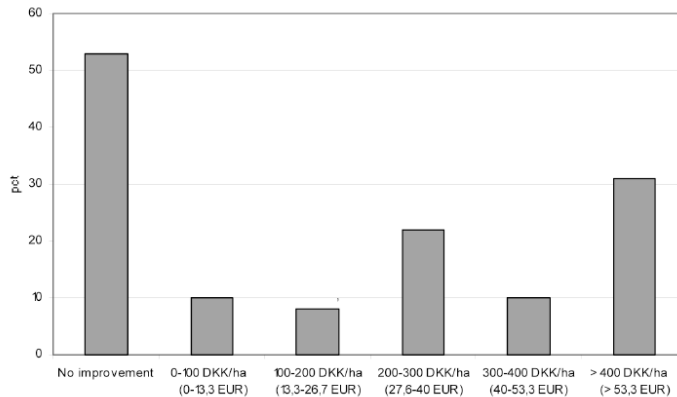
## RESULTS

Findings from this survey show that GM-crops seem to divide farmers in two groups. A significant share (46%) of the farmers who responded in this survey felt that they would like to grow GM-crops while the rest were more reluctant.

**Figure 1.** Issues that will prevent farmers from adopting GM-crops.  
 Note: The respondents had the opportunity to answer in several categorie



**Figure 2.** Expected potential impact on gross margins per hectare, N=134  
 Note: 1 EUR = 7,5 DKK



Most farmers find it important that GM-crops are safe with little health risk and minimal environmental risk involved with adopting GM-crops. 66% of the respondents were not interested in adopting GM-crops if there are either health risks or risk of contaminating other fields with GM material. Moreover 49% of the respondents are reluctant to percept GM-crops if there are risks of reducing the biological variety. (see figure 1)

There seems to be a link between the incentive to adopt GM-crops and expected economic returns.

Farmers are generally quite sceptical about the potential profitability of GM-crops and its impact on gross margins. For the majority of farmers the willingness to adopt GM-crops seems to relate to this perception. 53 farmers among 134 respondents expect no improvement of gross margins while 31 respondents believe that gross margins will be improved with more than 400 DKK (53,3 EUR) per hectare with GM-crops (see figure 2). The economic benefits are according to most farmers related to input savings and only a few farmers believe that the price of GM-crops will increase compared to conventional crops.

**Table 1.** GM crops that farmers believe to have the highest economic potential

	1. priority	2. priority	3. priority	Total
Beets	27	13	11	51
Wheat	20	19	12	51
Rape seed	21	12	18	51
Maize	19	16	11	46
Barley	1	12	10	23
Potatoes	0	0	5	5
Grass seed	0	3	1	4
Plants for medicin	1	0	0	1
Fruit/vegetables	0	1	0	1
Other	1	2	12	15

Note The respondents were asked to mention 3 crops that they believed would be most beneficial as GM-crops on their farms

When asking about the economic potential for specific GM-crops, many farmers tend to focus on crops that they already use in conventional varieties in their crop rotation. One exemption is GM sugar and fodder beets, which are believed to have a significant economic potential among farmers – despite the fact that only a minority of the respondents have beets in their crop rotation.

As shown in table 1, farmers tend to believe that sugar beets and cereals like winter wheat, barley and maize for silage are GM-crops that may improve the economic profitability. Moreover GM rape seed are also expected to improve the economic yield. The apparent lack of economic yield from GM rape seed as outlined in several economic studies (see Hasler et al. 2005) seems not to have any influence on the farmers' perception of this crop.

Finally, most farmers believe that economic yields will be gained from input savings rather than increased outputs and prices. The majority of farmers expect that GM-crops will enable farmers to reduce the application of pesticides and growth regulators whereas only few farmers believe that the application of fertilisers will be reduced with GM-crops. In general 45% of the respondents felt that GM-crops will improve the environment.

The high ranking of sugar beets supports this assumption, sugar beets have rather high costs for

herbicides and the right timing of applications is of vital importance for the weed control.

## CONCLUSION

Danish farmers have mainly a positive pragmatic attitude to the adoption of GM-crops.

The issue of co-existence has a very high priority and the main concern among farmers is whether they are being able to grow GM-crops without affecting other productions of non GM-crops, conventional as well as organic. The farmers seem to be fully in line with the consumers' demand of "free choice" between GM and non GM production.

About 30% of the farmers from the survey expect an improvement in gross margins while more than 50% do not expect any improvement in overall profitability. Most farmers seem to consider GM-crops as a mean to stay competitive more than a possibility to improve profits in the long run. It seems obvious from the survey that the farmers tend to assess GM-crops based on the crops they currently grow. The farmers assessments are based on GM agronomic traits in the crops while the second generation GM-crops with improved product quality traits doesn't seem to be seen as a new production possibility in the near to medium range future.

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# **PARALLEL SESSION**

ORGANISATION AND COSTS  
OF CO-EXISTENCE





# Co-existence of GM and non-GM winter oilseed rape: estimating costs for regulatory impact assessment in the UK

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**Abstract:** This study estimates the costs that farmers in the UK might incur by applying measures to facilitate the co-existence of GM, conventional and organic winter oilseed rape (WOSR). This is one of the GM crops that are in line for possible commercial cultivation. Data on price, yield, field size and additional costs associated with co-existence were collected in 2003 for organic and conventional WOSR in the UK. Scenario analysis was used to evaluate options of co-existence. A “geometrical approach” was applied to estimate the total costs of co-existence for each of the scenarios. It has been presumed that any farm-to-farm co-existence arrangements are likely to involve farmer-to-farmer crop notification of the intention to sow GM seed; control of ‘volunteer’ crop weeds to limit these acting as a source of GM presence; cleaning of farm machinery to prevent seed transfer and separation distances to limit crop-to-crop cross-pollination (other measures are possible to minimise cross-pollination, but separation distances are the most obvious and well understood mechanism for this). Opportunity costs derived from contamination are presented as well as the additional costs associated with avoiding seed transfer and GM presence (i.e. cleaning machinery and volunteers control). Results show that the cost of implementing preventive measures for contamination/cross pollination (separation distances) for oilseed rape farmers varies depending upon the field size, field shape and the farm practice (GM, conventional or organic).

## INTRODUCTION

Co-existence 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.

Research on the costs of co-existence has been conducted in the last few years. Bock *et al.* (2002)

prepared a report based on studies conducted in different European countries. This report shows the costs of changes in farming practices, costs of monitoring systems and costs of potential insurance systems using different scenarios covering three arable crops (winter oilseed rape, grain maize and potato). For oilseed rape, Tolstrup *et al.* (2003) the extra costs were estimated to be 3-9% of the total costs of growing in conventional production, whereas they are at 8-21% of the

total costs of growing in organic production. Brookes and Barfoot (2004) concluded that GM, conventional and organic maize crops in Spain have co-existed without economic and commercial problems. Finally, an economic cost-benefit analysis for sugar beet, wheat, barley and potato in Ireland conducted by Flannery *et al.* (2004) has shown that GM crops can provide savings for the producer.

This study identifies and estimates the costs (including opportunity costs) associated with implementing co-existence measures for conventional, organic and GM farmers; identifies which production system (i.e. conventional, organic or GM) is most likely to incur the lowest costs of co-existence measures; and investigates the efficacy of the methodology based on the design of different scenarios (i.e. field shape, forms and production system).

## METHODS

Data on price, yield, field size, production costs for organic and conventional WOSR in 2003 were collated from the UK Department for Environment, Food and Rural Affairs (Defra), the UK Soil Association and Nix (2003). Defra also provided information about the likely separation distances to be implemented. Costs associated with cleaning machinery and volunteer control were obtained by contacting agronomists and farming service companies in the UK between August and September 2003.

A “geometrical approach” was used to calculate the separation areas affected. This accounted for the location of the GM and non-GM WOSR fields under different field shapes (i.e. square and rectangular field). Different scenarios covered the various permutations for WOSR; the separation distances to avoid contamination of organic and conventional WOSR fields, and whether it is the GM, conventional or organic grower who has to observe the specified distance (Figure 2). Options available to farmers were identified and assessed for each scenario.

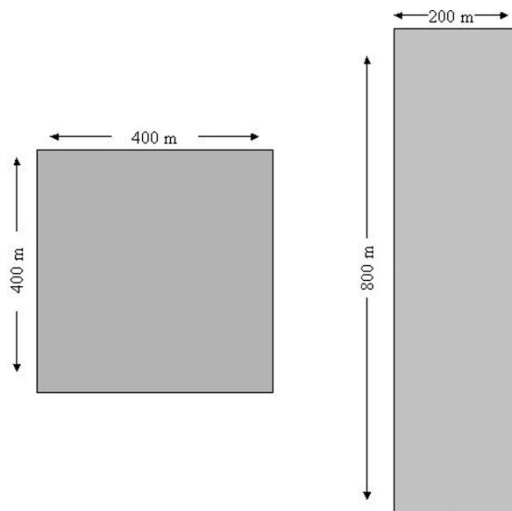


Figure 1. Field shapes for a 16 ha oilseed rape field.

Six general points need to be emphasised:

(i) The scenarios assume in every case that there are neighbouring farmers who want to grow a GM and non-GM crop respectively of the same species, in close proximity. In practice, if GM crops are grown, it is possible that in many instances there will be no direct co-existence ‘conflict’ between neighbouring farms.

(ii) The scenario analysis assumes average values for parameters such as field size and crop price. Clearly, the impact on individual farms would depend upon the specific circumstances arising in each case.

(iii) Two field size (16-hectare and 57-hectare) and a 3.5% premium for conventional WOSR are assumed. These assumptions are based on the average UK organic and conventional WOSR field size and on surveys in the US which identified premia for segregated non-GM maize of around 3.5% (Foster *et al.*, 2003).

(iv) Two different field shapes were assumed (squared and rectangular) (Figure 1). This in combination with the separation distances assumed, allowed the estimation of conflicting areas.

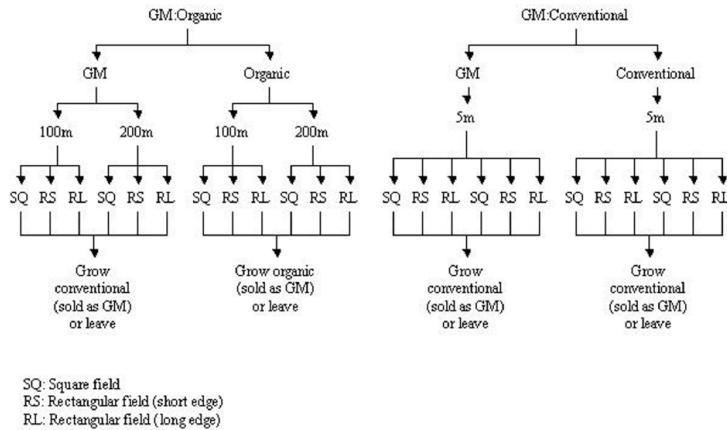


Figure 2. Flow diagram of scenarios and options

(v) This study does not include costs of monitoring systems to certify crops or insurance costs.

(vi) A parametric model was not used in this study due to time restrictions.

Total co-existence costs comprise of opportunity costs and additional costs. Opportunity costs for each scenario and option were estimated using the equation below:

$$\text{Opportunity Cost} = (\text{Organic/conv.Price.Average Organic/conv. Yield} - \text{GM price. Average Organic/con. Yield})$$

## RESULTS AND DISCUSSION

The opportunity costs are extremely sensitive to separation distances (Table 1), average field size, field shape, yields and prices. Since these variables may differ between regions, costs may also vary, which makes it impossible to obtain a precise general conclusion.

Organic farmers have incentives to grow WOSR in the entire field and collect the potential contaminated crop and the organic crop separately. Conversely, for the conventional farmer to meet a 10m separation distance (assuming a 5m cropped area within the effected field and 5m between

fields) it is not realistic to grow a crop in such an area (unless the costs associated to cleaning machinery are very low). Table 1 shows that the costs of co-existence are high for organic farmers. However, there is currently only one organic WOSR grower in the UK, which means there is a low risk of contamination of organic WOSR.

In the UK there are approximately 2.5 OSR fields per farm (varying between 1.7 and 3.2) on an average farm size of 57 ha. If the conventional farmer intends to grow WOSR on two (or more fields), then this could mean that he does not have to apply any additional separation distance. A 0.9% threshold could be achieved through mixing the harvested WOSR seed from additional fields.

Table 1 shows that it is advantageous for the GM WOSR grower to keep the separation distance by growing conventional WOSR. To produce conventional WOSR the GM farmer will incur additional chemical costs (i.e. conventional spray regime -£41/ha-).

This study estimates the additional costs for a conventional WOSR grower are between 1-4% of the total costs. The additional costs for an organic WOSR grower are between 8-63% of the total income. Bock *et al.* (2003) estimated additional costs associated with co-existence to be €126/

ha (£88/ha) for conventional oilseed rape and €345/ha (£241/ha) for organic oilseed rape. Although these estimates are different to those shown in this study, they are highly dependent on the assumptions used.

Further investigation should focus on the distribution of WOSR farm shapes, yields, farm size and farming practices by region. This will identify the type of distribution for each of these characteristics by region, and therefore allows the use of probabilities into the calculation of costs of co-existence measures. Farmers' attitudes to GM crops, and WOSR in particular, must be studied in order to identify where GM WOSR is most likely to be adopted.

A modelling approach accounting for field characteristics (size, yield, shape), prices and costs of production as well as gene flow may be a better approach to estimate the costs of co-existence for different farm types.

To conclude, further research is needed based on geographical crop data to identify areas that are likely to grow GM crops and assess local effects.

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**Table 1.** Co-existence cost comparison between options for WOSR growers

	Opportunity cost (£/ha)	Cleaning machinery		Volunteers control*	Total (£) -16 ha-	Total (£) -57 ha-
		Farmer (£/time/yr)	Contract (£/time/yr)			
Org. grow 100-200m	403	72-126	256-448	30-36	938-7,472	2,237-10,545
Org. leave 100-200m	707	-	-	30-36	1,474-11,888	3,685-17,089
Conv. grow 5m	16	72-126	256-448	21-23	78-467	81-469
Conv. leave 5m	458	-	-	21-23	48-192	120
GM grow 100-200m	41	-	-	21-23	155-1,275	338-1,883
GM leave 100-200m	442	-	-	21-23	926-7,691	2,393-11,010
GM grow 5m	41	-	-	21-23	8-32	19-43
GM leave 5m	442	-	-	21-23	48-191	120-263

\* Assuming following crop is wheat (£/ha)

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# Supply of non-GM feed in consumer-driven animal production chains

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**Abstract:** We studied the economic effects, the risks and the practical bottlenecks of the supply of non-GM feed for animal production in 4 different scenarios. To this purpose, we formed a project team with experts on plant sciences, genetic modification, analytical techniques, animal nutrition, chain management, and agro-economics. Design and progress of the project was evaluated with stakeholders in policy making, consumer organisations and feed and food producers. The scenarios were production of organic feed, and of feed with threshold levels of unintended GMO content at < 0.9%, <0.5% and 0.0%, respectively. Results were compared to those of conventional feed manufacture. The estimated extra costs per metric ton of raw material ranged from €36 (<0.9% GM) to €82.50 (almost 0.0% GM and organic), adding 41-92% to the market value of maize (€ 95.40/metric ton). The global increase of cultivation of GM crops that are not yet approved in the EU was considered to be a major risk in all scenarios, including that of conventional feed manufacture. In all non-GMO scenarios, unintentional mixing with GM materials during cultivation, transport and processing is an important risk. For crop production aiming at 0% GMO (including organic farming), insufficient protection of the crop against dispersion of seeds and pollen may add to these risks. In all scenarios, practical bottlenecks included: 1) the availability of appropriate sampling strategies and adequate techniques for detection and analysis of GMOs; 2) uncertainties with regard to liability in case of contamination of non-GM feed; and 3) implementation of standards. These issues have to be solved in national implementation programmes, but also require international consensus and further interactive and interdisciplinary research.

## INTRODUCTION

Food products derived from animals kept on rations containing genetically modified (GM) crops are not considered GM under the European Regulation for GM Foods and Feed (EC 1829/2003) and, therefore, do not require labelling. However, consumers may demand products from

animals raised and maintained on non-GM feed. Examples are found in organic production chains (EEC 2092/1991), and in some conventional production chains for consumers in NW-Europe, especially the UK, Norway and Germany (van Vliet, 2004). This trend may be expected to grow in Europe (Halman *et al.*, 2005).

The area of GM-based agriculture is increasing world-wide (James, 2003), based on a growing number of different GM crops and varieties, many of which are not yet approved, or known, in the EU. Moreover, the cultivation of GM-crops in the EU is increasing, in line with the implementation of the co-existence regulation. Due to these developments, the production of non-GM based animal feeds will be facing extra costs due to growing scarcity of raw materials, to necessary adaptations in chain management, guarantees, and testing protocols for the maintenance of the non-GM status. The increase in GM varieties also poses new risks to non-GM feed and food production chains. Further the practical implementation of co-existence imposes specific bottlenecks with regard to non-GM animal feed production. The aim of this study was to assess the risks and bottlenecks associated with the production of non-GM animal feeds under current EU legislation and to estimate the extra costs thereof.

## APPROACH

### Project design

A project group was formed consisting of experts on plant sciences, genetic modification, analytical techniques, animal nutrition, chain management, and agro-economics. The project design included a steering group with policy makers from the Dutch Ministry of Agriculture, Nature and Food Quality, business and R&D managers from the feed and food industry, and spokesmen from non-governmental organisations. The latter group consisted of, amongst others: Platform Biologica, the Dutch Organisation for the Certification of Organic Agricultural products (SKAL), the Commodity Board of the Feed Industry (PDV), and the Dutch Association of Feed Producers (NEVEDI). The steering group was consulted in the definition phase of the project and for advice and suggestions during the finalisation of the report. The results of the study were reported to the principal, the Dutch Ministry of Agriculture, Nature and Food Quality (Kok *et al.*, 2004) and to all stakeholders in a workshop.

### Scenarios

Four scenarios were chosen, to cover the full range of current legal and potential consumer demands. Organic farming was included because it represents current consumer demands for a minor but growing part of the market and because it has its own requirements for quality assurance and the maintenance of the organic status. Three (non-organic) non-GM feed scenarios were added, one at the level of <0.9% unintended inclusion, in line with current legislation, one at 0.0% GM inclusion as a parallel to organic farming, and one intermediate scenario (<0.5%). The latter was considered a possible scenario for the future, where producers, in a competitive market, would accept new criteria for non-GM feed production, providing sufficient consumer demand. Conventional feed production was added to these non-GM scenario's, for comparison.

### Economic effects

The economic effects were related to the costs of the required quality assurance systems, costs of associated analysis protocols, and necessary management adjustments to guarantee the non-GMO status. Quality assurance systems included were:

- nonrecurrent declaration of non-GM: Per metric ton of maize or soya these costs were considered to be negligible;
- declaration of origin: As in latter case, the costs were also considered negligible;
- non-GM declaration with associated analysis certificate: Costs for analysis (PCR) were based on € 320, for each GM 'event' (Bock *et al.*, 2002), conducted every year for each lot, and estimated € 3 per metric ton (including sampling costs of € 0.50);
- supply chain certificate and identity preservation: The costs for monitoring systems in arable crop farms were based on a study of Bock *et al.* (2002). The monitoring costs in other parts of the production chain were considered to be much lower.



Necessary adaptations in chain management, in order to achieve the level of unintended inclusion of GM material, were estimated for the subsequent production phases of seed cultivation (Colon & Dolstra, 2003), arable farming (Bock *et al.*, 2002), trade, storage and processing (Coppola, 2002), and transport (Wolf *et al.*, 2003).

Changes in market prices for raw materials, due to the separation of the total market in a GMO and a non-GMO market were not taken into account, nor were substitution of specific raw materials in feeds by other non-GM crops.

### **Risks and practical bottlenecks**

The results with regard to the risks and practical bottlenecks were obtained by a desk study, complemented by interviewing feed producers and in an interactive workshop with stakeholders. The desk study summarized developments in EU regulations up to 1830/2003, and the implementation thereof in the Netherlands. Further, production of GMO-products, supply and processing of feedstuffs in the Netherlands was summarized, specifically for maize and soy beans. Potential risks of co-existence were analyzed and precautionary measures were presented where possible. Also, developments of different quality systems in the feed industry were described, including the control and management of non-GM feedstuffs. The results were partially, obtained by interviewing 12 feed manufacturers, and evaluated in a workshop with feed manufacturers, policy makers and ngo's.

### **RESULTS**

The estimated extra costs per metric ton of raw material ranged from €36 (<0.9% GM) to €82.50 (0.0% GM and organic), adding 41-92% to the market value of maize (€ 95.40/metric ton). These extra costs were mainly associated with necessary management changes (31-77%), further with quality guarantees (3.8-9.4%) and testing (6.3%). The global increase of cultivation

of GM crops that are not yet approved in the EU was considered to be a major risk in all scenarios, including that of conventional feed manufacture. In all non-GMO scenarios, unintentional mixing with GM materials during cultivation, transport and processing is an important risk. For crop production aiming at 0% GMO (including organic farming), insufficient protection in the case of co-existence of the crop against dispersion of seeds and pollen may add to these risks. In all scenarios, practical bottlenecks included: 1) the availability of appropriate sampling strategies and adequate techniques for detection and analysis of GMOs; 2) uncertainties with regard to liability in case of contamination of non-GM feed; and 3) implementation of standards.

### **PERSPECTIVES**

The results of our study show that the production of non-GM feed for animal production may have a large impact on the price. Together with the increase of GM-based arable farming in the world this may hamper the co-existence of conventional feed and non-GM feed. Under the current EU legislation a market for non-GM feed can only develop from a sound and substantial demand from consumers. Better information for consumers may stimulate this. We used an estimate of €320, for the cost of PCR analysis per event. Although this price may decrease in the future, the number of events to be tested will increase.

Furthermore, this study revealed risks and practical bottlenecks, associated to non-GM feed production, which can not be tackled by the free market or feed industry alone. Amongst these are the risks associated to the rise of GM crops in the world and to unintended contamination of crops by dispersion of seeds and pollen. The practical bottlenecks mentioned in above paragraph have to be solved in national implementation programmes, but, also, require international consensus and further interactive and interdisciplinary research.

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# Using Identity Preservation to meet market demands: the case of Canadian non-GM IP soybeans

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**Abstract:** Traditionally, Canadian grains and oilseeds have been commodity products, shipped on a large scale, high throughput basis. Bulk shipment shipments of Canadian grains meet the quality needs of many international buyers, but an increasing number of processors are seeking crops with specific quality traits essential to the quality of their end product. The private sector has responded to the marketplace requirement for the delivery of specific traits through tightly controlled supply chains known as identity preserved (IP) programs. This paper reviews the history, development and success of the IP programs developed by Canadian soybean exporters to meet market demands for non-GM (genetically modified) soybeans. The on-farm and post-farm procedures adopted within these programs are described and the role of government in providing third party audits, verification and certification of IP processes is also examined.

## INTRODUCTION

Traditionally, Canadian grains and oilseeds have been commodity products, shipped on a large scale, high throughput basis. The quality of these commodity crops has been based on grade standards set by the Canadian Grain Commission (CGC). The CGC is an agency of the Canadian federal government which derives its authority from the *Canada Grain Act* (1912). Its mandate as set out in the Act is to “establish and maintain standards of quality for Canadian grain and regulate grain handling in Canada, to ensure a dependable commodity for domestic and export markets.”

Bulk shipment shipments of Canadian grains meet the quality needs of many buyers, but an increasing number of processors are seeking crops with specific quality traits. One important example is the demand for non-GM soybeans which results from consumer demands for choice regarding the consumption of products derived from biotechnology.

This paper describes the history of how the Canadian soybean industry responded to the demand for non-GM soybeans by developing identity preserved (IP) programs and the role that the CGC, an agency of the government of Canada, has played in providing assurances of the integrity of these programs.

## **DEVELOPMENT OF IDENTITY PRESERVATION TO DELIVER NON-GM SOYBEANS**

IP programs can be defined as process based systems developed by industry as a method to capture the value associated with a specialized commodity. These systems involve three key steps: (1) identifying the particular quality requirements of a customer, (2) contracting with farmers to produce a crop with that particular quality, often with particular production and management practices, and (3) establishing and implementing processes to segregate and preserve the identity of that crop through the grain handling and transportation system. The goal is to deliver a product that meets the specific quality requirements of the customer, thereby providing added value and an ability to extract a price premium.

The Canadian soybean industry has a long history of using IP processes to segregate varieties. Markets for IP varieties began to develop in the 1980's as processors found that specific varieties performed better than others. By the late 1990's the non-GM market developed in Europe and Japan because of consumer perceptions and concerns about the safety of GMs. It was at that point that the industry realized that there was a need to introduce more rigor to the IP processes that they had been using in order to supply these emerging non-GM markets. Under the auspices of the Canadian Soybean Export Association (CSEA), industry cooperated to develop a set of IP procedures in order to achieve three goals:

1. to provide guidance on best IP practices that could be used by their members in developing their IP programs,

2. to serve as a basis for certification of private sector IP programs in order to provide assurances to buyers that their Canadian suppliers have verified processes in place that can deliver the products with their specific quality requirements, and

3. to help protect the reputation of Canadian IP soybeans by encouraging exporters to adopt the best IP practices laid out in the standard.

The IP procedures developed by CSEA are designed to minimize the risk of GM contamination to the lowest feasible level. Soybeans present no risk of contamination through pollen transfer, but isolation distances from other soybean fields of at least three meters and field history records are required to minimize the risk of volunteers. Only certified seed can be used by farmers, and all planting, harvesting and transporting equipment as well as storage bins must be thoroughly cleaned and inspected prior to use. Farmers are also required to keep records to ensure traceability from the field to the storage bin and then to the delivery load to the elevator.

Post farm, elevators are required to have documented IP procedures for receiving, storage, processing and loading. Incoming loads must be identified and are not eligible for IP certification unless its identity is verified. Although there are over 150 GM varieties of soybeans registered for commercial production in Canada, they all contain the same GM event for which an inexpensive and quick strip test is available. Therefore, elevators can verify the non-GM status of each incoming and outgoing load as part of their IP processes.

The CSEA procedures require that all elevator legs, conveyors, augers and storage bins must be either dedicated to non-GM IP or be thoroughly cleaned before used for non-GM loads. Records must be kept to provide evidence that all procedures have been followed and to track the flow of IP shipments through the facility. This minimizes any risk of adventitious presence of GM soybeans and provides traceability to identify the source and correct any problems that may arise prior to shipment.

In 2000, CSEA recognized that credible third party audits and certification against their standard was essential to achieving their goals. They approached the CGC as the preferred audit and certification body because of its mandate for

grain quality assurance and its credibility and reputation in the international marketplace. In response to this and other market requirements for IP products, the CGC developed the Canadian IP Recognition System (CIPRS) which requires that IP procedures be developed and implemented with the context of a quality management system.

To participate in CIPRS, companies must write a quality manual compliant with the CGC Quality Management System Standard for IP Programs. The manual specifies the IP production and management program and processes they have implemented within their own facilities. Also under the requirements of the CIPRS standard, companies must develop methods of evaluating and approving their suppliers. These suppliers include the farmers and other suppliers (grain handling facilities and transportation providers, etc.) they contract with in order to coordinate the supply chain. This ensures that the company has adequate controls over the IP processes used through out the supply chain.

CGC accredited auditors conduct quality system assessments against the CIPRS standard and verify that the requirements of the CSEA IP procedures have been consistently followed. Auditors prepare an audit report that includes a recommendation on whether or not the IP program should be certified and submit it to the CGC. The CGC conducts a technical review of the audit report and makes the certification decision. If the decision is positive, CIPRS certification is issued indicating that the requirements of the CGC IP standard and the CSEA procedures have been met.

## RESULTS

The first CIPRS certification was granted in February 2004. Sixteen additional companies received CIPRS certification during the spring of 2004 when the program was first launched. There are now 20 companies with CIPRS certification and four more currently in the certification process. The 20 CIPRS certified companies operate a total of 57 grain handling and processing facilities in

Quebec, Ontario, Manitoba and Saskatchewan and are estimated to contract with over 3,500 farmers. Most of these companies are exporting non-GM soybeans.

Canada produces an average of about 2.5 million metric tons of soybeans annually, of which an estimated 60 to 65% are GM varieties. Canadian soybean exports over the past 3 years have been in the range of 725,000 to 975,000 tons. Of this total, approximately 35%, or an average of about 300,000 tons are non-GM soybeans destined for food grade markets. An estimated 30% of Canadian non-GM IP soybeans are exported under a CIPRS certified IP program. The rest of Canada's food grade non-GM soybeans are produced, processed, and exported under less rigorous IP systems that do not include third party audits and certification.

The main markets for Canadian IP non-GM soybeans are Southeast Asia and Western Europe. Buyers in these markets demand non-GM purity levels of 0.5% to 2.0%, with an average of about 1.0%. Premiums are paid for non-GM food grade soybeans, but it is not always possible to extract additional premiums for the extra measures taken in order to achieve CIPRS certification. One exception is Japan because it is the most quality conscious market in S.E. Asia. Other markets in this area, such as Hong Kong, Singapore and Malaysia, tend to be more price sensitive making premiums more difficult to obtain. However, some Canadian exporters believe that these markets are also becoming more quality conscious and therefore more interested in the higher levels of assurances. In Europe, the United Kingdom, Belgium, Netherlands, France, Germany and other countries are all important markets for Canadian IP non-GM soybeans. Canadian exporters believe that CIPRS certification is an important marketing tool, but price premiums are only available under certain market conditions.

The added value generated from IP programs for non-GM soybeans allows premiums of about C\$0.60 to C\$4.00 (about 15% to 60% over the prices available for crush soybeans) to be paid

to farmers. These premiums compensate the producer for the additional work and record keeping as well as the added costs of producing non-GM soybeans, such as the reduced yield of non-GM varieties.

## **CONCLUSIONS**

The Canadian soybean industry has been proactive in meeting the market demands for non-GM soybeans by developing IP programs that consistently meet market tolerances for non-GM purity. This history of success is augmented by CIPRS, a program that helps to ensure that risks of GM contamination are mitigated through documented processes, records to provide traceability, and third party verification. The slow but steady growth of the number of companies adopting CIPRS is an indication that industry expects that credible third party audits and certification will become increasingly important to maintain market access and price premiums.

Buyers familiar with CIPRS are pleased with the assurances it provides and appreciate the credibility that CGC, as a government agency, brings to the certification process. However, because it is a new program, CIPRS is not yet widely recognized in the non-GM soybean markets. As knowledge of CIPRS expands and the success of the Canadian industry in meeting market requirements continues, it is hoped that the premiums that offset the added costs of IP will also continue. Premiums for non-GM soybeans are critical to the continued co-existence of GM and non-GM production of soybeans in Canada as they provide the economic incentive to take the measures needed to maintain the segregation of non-GM products.

# Seed certification as a model for managing co-existence: results of an OECD Workshop on Seed Certification and Modern Biotechnology, Sept. 2005

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**Abstract:** Seed certification is a model of co-existence in which the identity and varietal purity of seed is maintained through cycles of reproduction by strict adherence to segregation procedures in production and handling. The Organization for Economic Cooperation and Development (OECD) Seed Schemes offer a system of harmonized seed certification procedures to facilitate international trade of seed with assurances of identity and varietal purity. The recent introduction into the international marketplace of varieties derived from modern biotechnology, coupled with some buyer demands for GM-free products, raises issues about the characterization of varietal identity and purity, assurances of purity and adventitious presence, as well as the role of government in seed certification. The OECD hosted a “Workshop on Seed Certification and Modern Biotechnology” in September 2005 to address these issues. The outcomes of this workshop are discussed here.

## INTRODUCTION

The processes of seed production and certification have long incorporated the principles of co-existence in that they maintain the segregation of one variety from another. Maintaining the identity and varietal purity of seed are essential components of a modern, efficient and effective agricultural production system. Seed producers observe rigorous, crop-specific land-use restrictions, isolation distances and other co-existence measures to mitigate against physical and biological mixtures of varieties to meet varietal purity and other certification requirements. Field inspections and post-control plots, supplemented

in some cases with laboratory tests, act as checks to the system.

A proliferation of varieties in recent years, resulting from greater investment in plant breeding by the private sector and opportunities presented by techniques of modern biotechnology, means that varieties are no longer as easily distinguished from one another as had been the case previously. This calls for a review of procedures that have been used to develop and maintain varieties and to certify seed of some varieties in order to ensure that the goals of seed certification are still being met. Furthermore, the introduction of genetically engineered or genetically modified (GM) varieties

and their lack of acceptance in certain markets, means that the identity and varietal purity of seed are more important than ever before.

Internationally agreed-upon definitions, standards and procedures are required to realize the benefits of variety development and to facilitate national and international trade in seed. The Organization for Economic Cooperation and Development (OECD) Seed Schemes have provided such a framework for over 40 years. It is appropriate, in light of recent seed technology developments, that our common understanding of the principles of seed quality assurance be reviewed to ensure that they are still valid. New challenges for seed certification systems have arisen related to: 1) changes in the understanding of varietal identity and varietal purity; 2) new emerging expectations for monitoring and measuring purity and adventitious presence; and 3) questions about the respective roles of government and the private sector in the seed certification process.

On September 27-28, 2005, the OECD Seed Schemes held a Workshop on Seed Certification and Modern Biotechnology to address these issues. The Workshop was attended by approximately 100 representatives of about 50 countries. Speakers from international organizations, academia and OECD Seed Scheme participating countries expressed a wide range of viewpoints. Participants broke into smaller discussion groups following the presentations in order to delve further into the issues.

## VARIETAL PURITY

Standards for varietal purity are required to facilitate trade in seed of modern agricultural varieties. Varietal purity standards for seed vary depending on the biology of the species in question, and the methods and procedures for production, sampling, testing, reporting results, interpreting results and application of associated tolerances need to be transparent to ensure confidence in the seed certification system.

Control of varietal purity in seed certification is, for the most part, based on phenotypic, usually visually distinguishable, characteristics. In recent years, varieties that are visually indistinguishable but that have significant phenotypic trait differences have been developed, recognized and certified. Examples include varieties of herbicide tolerant maize, soybean and canola and insect resistant varieties of potatoes, maize and cotton.

The terms “varietal purity” and “genetic purity” were once synonyms and are sometimes still used as such, but there are reasons to clearly differentiate between the two. Varietal purity is perhaps best described as relative phenotypic uniformity whereas genetic purity relates specifically to the DNA of plants. Again, while plant breeding, seed certification and definitions of varietal purity have, in the past, relied heavily upon visually distinguishable traits, genotype by environment interactions give rise to a wide range of attributes that may or may not be visual. Thus, primary proteins (being the product of genes) and secondary proteins and other metabolites are valid distinguishing phenotypic characteristics of varieties. In the absence of visually distinguishable morphological characteristics, biochemical tests to ascertain varietal traits have, therefore, been integrated components of seed certification for some crop kinds and/or varieties.

It is increasingly important that there is common understanding of the standards and procedures for measurement and the application of varietal purity standards to Certified seed. Under the OECD Seed Schemes “varietal purity standards apply to all seed producing fields and shall be checked at field inspection”. Questions arise, however, as to whether these same standards should apply in post-control or whether a more generous standard should be allowed, and whether biochemical test results should be interpreted in the same manner as field results. Some countries are on the verge of developing true genetic purity standards and some customers require that specific genes be absent or present. This is new territory for variety verification systems.



## VARIETAL IDENTITY

A basic requirement for certification of a seed lot is that the seed be of a variety that has been determined to be eligible for certification. The OECD maintains a list of varieties eligible for seed certification, derived from the lists of eligible or registered varieties in each of the Seed Schemes participating countries. Traditionally, the eligibility of a variety was based on agronomic characteristics, disease reactions and end-use quality, as well as visually-distinguishing botanical traits. These characteristics are included in official descriptions of variety. The GM status of a variety may or may not be indicated in the official description of the variety. To facilitate the co-existence of GM varieties with conventional varieties, the OECD Seed Schemes are exploring questions such as the information about method of development or particular traits on the OECD list of eligible varieties or on descriptions of variety, and the availability of methods and procedures for determining specific traits that are not visual during field inspections.

## MEASURING PURITY AND ADVENTITIOUS PRESENCE FOR INTERNATIONAL SEED TRADE

The emergence of GM varieties, and the establishment of regulatory frameworks based solely on the method of development of these varieties, has affected agriculture profoundly. The rejection of GM varieties and their products by certain international markets, and the consequent development of markets differentiated between GM and non-GM varieties, has had dramatic effects on the international trade in seed and challenged seed certification systems. More sophisticated seed and crop production and handling systems have, therefore, been developing to ensure that customer requirements are being met, as freedom from specific genetic modifications or from GM varieties or off-types in general has emerged as a factor that determines the value of the end product.

Increasing commercialization of GM varieties means that seed of non-GM varieties may have

small quantities of adventitious (unintended) GM material. GM varieties may also test positive for other GM material not intended to be present. Adventitious presence at low levels may not be an issue provided varietal purity standards are achieved and the marketplace accepts unintended low levels of approved GM seed in seed of non-GM varieties. Problems arise, however, when there are expectations of, or requirements for, complete freedom from the adventitious presence of GM seed either in the domestic seed, feed and food markets or in export markets. In these cases, buyers require assurances not only that seed meets varietal purity standards but also meets buyer-specified levels of genetic purity. It may be a particular challenge to provide these assurances when the exporting country has no regulatory requirement to segregate non-GM and approved GM varieties.

The OECD Seed Schemes are exploring ways to manage, monitor, measure and report adventitious presence in seed for international trade to meet the needs of countries with different regulatory systems and international obligations.

## THE ROLE OF GOVERNMENT IN SEED CERTIFICATION

Over the past 20 years many national seed certification systems, from field inspections to sampling and testing to post-control monitoring, have evolved into public/private partnerships. Some countries maintain official control over most of the system, others allow nearly exclusive private involvement, while some have established independent, accredited third party agencies for some roles. Countries also differ in their approaches to co-existence of GM and non-GM crops and to regulation of GM or plants with novel traits. Official government involvement is required where co-existence measures are imposed or legislated; in other cases, private or third party mechanisms may be more efficient or cost-effective. The OECD Seed Schemes are addressing questions about the role of governments in certification of seed for international trade relative to its GM status.



# Managing the co-existence of conventional and genetically modified maize from field to silo - a French initiative

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**Abstract:** A three-year study into the co-existence of transgenic Bt maize and non Bt maize was conducted in France between 2002 and 2004, under controlled field conditions from seed to storage. The Operational Program for GM Crops Evaluation (POECB) studied the traceability of crops and analysed the different conditions governing co-existence, contributing to the development of co-existence guidelines. The programme was led by a scientific committee working in partnership with maize growers. The committee comprised project managers and scientific experts from various fields. The programme involved two other committees: a management committee responsible for financial and political decisions, and a communication working group. The research took place in seven locations, with 80 ha dedicated to the programme and a total of 10 ha of Bt maize (each plot measuring between 0.5 and 2 ha). The same plot configuration was used each time, with conventional maize surrounding the GM crop. All the research conditions were designed to assess the worst-case scenario: hybrids all flowering at the same time and non-GM crops planted downwind of the GM plot, in order to identify effective co-existence measures.

## INTRODUCTION

At each stage of the maize supply chain, procedures have to be implemented to ensure the traceability of maize production, with all operations and key points listed. Traceability was formalised using a suitable documentation system, as well as suitable controls. The aim of the programme was to identify parameters essential for successful segregation, and for the control of factors critical in minimising GM adventitious presence. Specific procedures for harvest, transport, drying and storage have been developed to make co-existence possible and manageable.

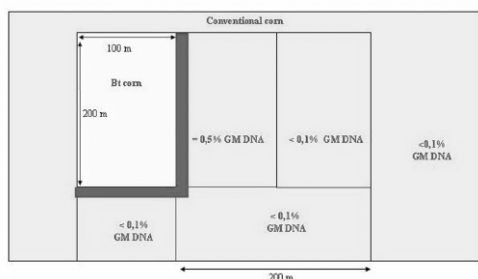
## APPROACH

The survey on cross-pollination was a major part of the programme (Bénétrix *et al.*, submitted). Samples from the non-GM maize fields were taken at different distances from each side of the GM plot, and a mosaic sampling was also applied where samples were taken in squares. Samples were then dried and analysed using PCR techniques to establish the level of GM contamination (measured as the ratio of transgenic DNA against total DNA).

## RESULTS AND DISCUSSION

Analysis of the results showed how much GM transfer had resulted from pollen flow from one crop to the other. GM contamination due to gene flow found in the non-GM maize crop decreased rapidly as the distance from the GM emitter crop increased. Significant amounts were only detected in a 10 metre strip immediately bordering the other crop. Variation in cross-pollination levels in the first few rows was mainly due to different wind intensities. Where the prevailing wind at flowering time blew from the GM crop towards a non-GM crop, beyond 20 – 25m the level of GM contamination found in the non-GM crop was less than 1%.

At field level, GM DNA content in excess of the 0.9% EU threshold was only found in the outer rows of the non-GM maize closest to the GM emitter crop. The level of GM contamination likely to be found in non-GM maize crops planted adjacent to a GM plot is on average 0.5%, measured over the whole crop (Figure 1).



**Figure 1.** Square sampling cross pollination means observed in 2003 location (% modified DNA).

This falls to 0.3% when a 10m gap is maintained between the GM and non-GM crops.

The results demonstrated that field size is not the only criterion that has to be taken into account. The width or length of the field must also be taken into consideration. The average level of cross pollination from GM DNA in conventional maize fields at a distance of 50m is very close to the 0.9% threshold. When eliminating the first rows bordering the GM maize plot, the level

of GM DNA in the whole field of conventional maize is far below this threshold (Table 1).

**Table 1.** Cross-pollination levels (% modified DNA) at different distance from the Bt corn plot with and without a 10m strip.

Field depth	GM content (% GM DNA)	
	Total field area	After eliminating 10 m border strip
40 m	1,00 %	0,75 %
50 m	0,81 %	0,50 %
75 m	0,59 %	0,39 %
100 m	0,52 %	0,29 %
150 m	0,25 %	0,14 %
200 m	0,25 %	0,17 %
300 m	0,19 %	0,13 %

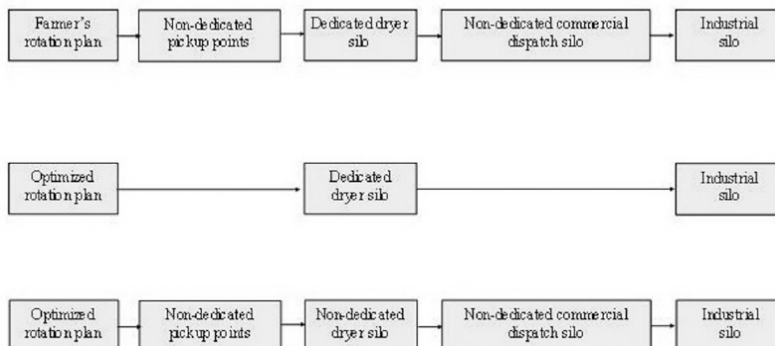
In this programme, we also worked with storage operators to set up several possible scenarios for organising production processes, and to better identify critical control factors. The scenarios included dedicated and non-dedicated dryer silos and compared farmers' rotation plans with rotation plans optimised to minimise AP (Figure 2).

The cost of successful segregation varied considerably depending on whether or not dedicated structures and equipment were used. The trial programme indicates that precise measures can therefore be adapted to suit local circumstances applying to different sectors of the industry, regions and harvest parameters (geographical location, number of dryers, etc.).

## OUTCOME

The implementation of good farming practices was usually shown to be sufficient to ensure that GM adventitious presence in non-GM maize is below the 0.9% EU labelling threshold. Based on these scientific results, a methodology guide has been produced to specify the commitments that

### Examples of process organization scenarios



**Figure 2.** Hypothesis for process organization based on (1) realistic agricultural plan, (2) risk minimization, (3) cost minimization.

GM producers should make in order to ensure crop segregation.

In this guide, the GM producer must inform the French government, stakeholders and neighbouring farmers of his wish to grow a GM crop. A buffer zone of 12 non-GM maize rows in the GM field has to be implemented when the distance between GM and non-GM crops is less than 25m. This guide also contains procedures for cleaning equipment (drill, harvester) and a quality management system for implementation throughout the maize production chain.

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# Co-existence of GM and non-GM Cotton crops in Andalusia (Southern Spain)

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*The views expressed in this paper are those of the authors and do not necessarily correspond to those of their respective institutions.*

**Abstract:** Following the recent reform of the Community aid scheme, improving competitiveness by reducing production costs is crucial to guaranteeing the sustainability of the cotton sector (including farms, upstream and downstream industries). Some authors have suggested that the introduction of genetically modified (GM) varieties of cotton with tolerance to insect pests would be a key element of a cost reduction strategy. The potential introduction of GM cotton in the European Union (EU) makes it necessary to study the possible situations that might arise due to the co-existence of GM and conventional crops. This paper studies how GM and conventional cotton (for both seed and fibre production) can coexist in the same region/landscape while minimising the possible levels of adventitious GM presence in non-GM cotton by adapting current agricultural practices. The Andalusia region (Southern Spain) is used as a case study. About 92 475 hectares of cotton were grown during the 2003/2004 seasons. Using information from expert panels, a literature review, a dedicated farmers' survey and a probabilistic model, the possible sources of adventitious GM cotton presence in non-GM cotton are identified. The probabilistic model is used to estimate the maximum levels of adventitious admixture and the probability for this admixture to occur. Agricultural practices adapted to different co-existence scenarios are proposed and an economic assessment is carried out to estimate the impact on the GM grower's gross margin.

## INTRODUCTION

Cotton is a very important industrial crop for many countries in the world. It is the world's leading non-food crop in terms of both the amount of land cultivated and the economic turnover it generates. The fruit of the cotton plant consists of a seedpod (boll) containing oil-bearing seeds whose epidermal cells produce cellulose fibres. Cotton is mainly grown for fibre production. In addition, cotton seed is a by-product used to produce edible oils or cosmetics; it is also processed into meal cakes for animal feed.

The importance of cotton in large areas of the world has made it one of the main objectives of traditional genetic improvement, and, in recent years, of biotechnology.

Cotton production in the European Union (EU) is hardly relevant on a world scale. The crop is grown in Greece and Spain and amounts to 460,000 hectares, representing only 1.3% of the world total. Nevertheless, the crop is economically and socially significant in those European regions where it is produced, such as Andalusia (Southern Spain), where it covers around 92,475 hectares according to 2003/04 data. In this region, cotton cultivation involves 1.5 million man/days per year, of which 85% is family labour (Bilbao *et al.*, 2004).

The EU has not authorised the cultivation of any of the different types of GM cotton, but five marketing applications have been received by Community authorities. Of these, two refer to all uses, including cultivation.

The introduction of GM varieties in the EU could be of interest for the Community's cotton-producing sector, since the sector is keen to improve its productive competitiveness by reducing production costs, especially after the recent reform

of the Community aid scheme<sup>1</sup>. This fact makes it necessary to study the possible situations that might arise as a result of the co-existence of GM and conventional crops under local conditions.

The general objective of this study is to analyse how GM and conventional cotton (for both seed and fibre production) can coexist in the same region/landscape while minimising the possible levels of adventitious GM presence in non-GM cotton by adapting current agricultural practices, when needed. The economic feasibility of co-existence is also considered, with a calculation of its impact on the GM farmer's gross margin.

## METHODOLOGY

This paper is an *ex ante* analysis since GM cotton has not yet been grown commercially in the EU. Data is based on existing knowledge of the performance of cotton and the GM varieties cultivated in other countries and on the cultivation practices and the structure of farms in Andalusia, one of Europe's leading cotton-producing regions. To gather all this information, a panel of experts was formed, a review of the existing literature was carried out, a survey on cotton producers was conducted (sample size 8.81% of the target population) and a model was built to characterise Andalusian cotton farms (Gómez-Barbero & Rodríguez-Cerezo, 2005).

The technical and economic feasibility of co-existence of GM and non-GM cotton in Andalusia was studied as follows: (1) definition of cotton cultivation practices, (2) definition of representative farm types based on agronomic variables which affect co-existence, (3) identification of possible sources of adventitious GM presence in non-GM cotton, (4) estimation of the maximum level of adventitious admixture according to the defined farm types and current agricultural practices (worst case scenario), (5) estimation of levels of adventitious admixture for each farm type and scenario, (6) proposal to adapt practices to ensure co-existence, and (7) estimation of the economic impact of ensuring co-existence for the GM farmer.

The scope of this research includes:

- The whole production cycle from farm to the ginning factory.
- Seed production farms and fibre production farms.



Table 1. Cotton farm types studied.

Production	Farm types		Characteristics			
	10% scenario farm types	50% scenario farm types	Production system	Average farm size	Average cotton crop area on farm	Average size of cotton plot
Seed	1	1'	Conventional (100% non-GM)			2.77 ha
	2	2'	Co-existence (10%-50% GM)			2.77 ha
Fibre	3	3'	Small conventional (100% non-GM and shared machinery)	16 ha	5 ha	2.77 ha
	4	4'	Large conventional (100% non-GM and own machinery)	160 ha	30 ha	9.70 ha
	-	5'	Small co-existence (50% GM and shared machinery)	16 ha	5 ha	2.77 ha
	6	6'	Large co-existence (10%-50% GM and own machinery)	160 ha	30 ha	9.70 ha

- Presence of GM cotton in the region (in all cases) and on the farms (in some cases): 10% and 50%.

- Tolerance thresholds of adventitious GM cotton presence in non-GM cotton production: 0.1% and 0.5% for seed production; 0.1% and 0.9% for fibre production<sup>2</sup>.

Currently, no organic cotton is grown in Andalusia. Council Regulation (EEC) No 2029/91 only contemplates seed cotton and pressed seed cakes, as raw material of vegetable origin for animal feed, but not as fibre, which is the main destination of Andalusian-produced cotton, with seed as a by-product<sup>3</sup>.

## PRELIMINARY RESULTS AND DISCUSSION

Table 1 describes the cotton farm types studied. The first column classifies farms by output produced, seeds or fibre. The second column lists the types of farm studied, separated according to the adoption rate of GM cotton in the total GM cotton area and in some cases within the

farm (10% and 50%). The last column shows the characteristics of the farms (percentage of conventional cotton on the farm, cotton plot size and use of shared or own machinery). For example, farm 1 produces conventional cotton seeds in a landscape where 10% of the total cotton area is cultivated with a GM variety. The average size of the cotton plot is 2.77 ha. Farm 6' is cultivated 50% with GM cotton and 50% with conventional cotton. The adoption rate in the landscape is also 50%. This farm uses its own machinery.

Possible sources of admixture presence between GM and non-GM-cotton were identified, namely:

- (1) Seeds for sowing, which may contain GM cotton seeds as an impurity.

- (2) Sowing, where admixture could take place between the seeds remaining in the drill after sowing of GM cotton on another plot.

- (3) Harvesting. Cotton residue (both seeds and fibre) from the previous plot remains in the harvester.

- (4) Transport. Cotton is transported from the plot where it has been harvested to the intermediate

warehouse or directly to the ginner, with cotton residue (both fibre and seeds) remaining in the back of the truck or in the trailer after unloading. If the trailer is cleaned, which is already done in seed-producing fields, the amount of cotton remaining is negligible, and is considered here to be zero.

Other sources of admixture are considered to be negligible by the experts consulted, namely: seeds from the previous year's harvest, seed storage, cross-pollination, and non-harvested. Intermediate storage is not considered in this research.

A probabilistic method was used to estimate the levels and probabilities of adventitious admixture for each farm type and each scenario. The model determines the maximum level of adventitious presence of GM cotton in non-GM cotton caused by each of the possible sources mentioned above. The expert panel selected those agricultural practices that are easy to implement and ensure adventitious presence below the targeted thresholds.

Finally, using the farmer's gross margin as an indicator, the economic impact of introducing or changing current agricultural practices is calculated.

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<sup>1</sup> Council Regulation (EC) No 864/2004 of 29 April 2004 amending Regulation (EC) No 1782/2003 establishing common rules for direct support schemes under the common agricultural policy and establishing certain support schemes for farmers, and adapting it by reason of the accession of the Czech Republic, Estonia, Cyprus, Latvia, Lithuania, Hungary, Malta, Poland, Slovenia and Slovakia to the European Union.

<sup>2</sup> On 2 July 2003, the European Parliament and the Council adopted two European Commission proposals establishing an EU system to trace and label GMOs and the labelling of food-feed products derived from GMOs. The regulations set a threshold no higher than 0.9% for adventitious presence of GM material.

<sup>3</sup> Council Regulation (EEC) No 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs. Official Journal L 198 , 22/07/1991 P. 0001 - 0015

# Segregation of GM and non-GM production at the primary production level

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**Abstract:** The objective of this study is to propose management scenarios for segregating GM and non-GM crops for country elevators for high production volume. To do so we made a case study segregating two types of corn (starch and meal) in country elevators in the Alsace region. This revealed the difficulties in segregating these two forms of production. We then turned to GM and non-GM crop segregation and propose 4 segregation scenarios. One is based on chronological segregation of collection; two are based on geographical segregation decided either before or after sowing, taking into account farmers' preferences. The last one proposes collaboration between country elevators in a given region in order to share collection silos and to allocate one silo to each type of crop.

## INTRODUCTION

The problems of coexistence between GM and non-GM production give rise to questions about agricultural production, crop transformation and transport. The first type of question is concerned with agronomic work which proposes biotechnical models of gene dissemination which allow cropping plan modifications to be tested and contamination risks between GM and non-GM crops in various regional configurations of production systems to be evaluated (Angevin *et al.*, 2003). For crop transformation and storage, Le Bail (2003) proposed different forms of segregation management strategy. These propose to manage the coexistence between GM and non-

GM crops by dedicating infrastructure or space to a specific product: at the farm level using the storage and drying capacity of the farmer; at the supply zone level using a contractual strategy between farmers and country elevators to define islands of production, or at a regional level defining GM-free regions by agreement of all those involved in the region (country elevators and farmers). However, these three scenarios are limited for significant volume or quality production, at least for corn. Storage and drying on the farm do not present the quality guarantees needed for profitable markets. In addition, the management of small islands of production does not allow large volume demands to be catered for.

Thus, management of GM and non-GM segregation for significant volumes requires either two independent industrial infrastructures (Valceschini, 2003) or a collection procedure that allows this segregation. The first would need significant investment which is not compatible with production which has already peaked. We then propose to explore organisational solutions which would allow country elevators to ensure separation between GM and non-GM crops for significant-sized batches and a fixed industrial infrastructure for GM or non-GM dispersed production. We present here the first results of a research program based on a case study of separation for a supply chain with significant volume in a French agricultural cooperative and the extrapolation we made for GM and non-GM segregation. We first present our working method and then the studied case and the various possible management scenarios for the separation we deduce.

## MATERIALS AND METHOD

This work is based on a case study (Dubois & Gadde, 2002) made during the year 2005 on a cooperative in Alsace. We studied the collection management from sowing to marketing and specifically the mechanisms that ensure segregation between two products: corn starch and corn meal. Corn meal is made from specific varieties while corn starch may be made from any type of variety bought by the cooperative. Corn meal purity rate is 85% and is achieved with difficulty. We interviewed several managers in charge of different levels of crop collection: crop planning management and relationships with farmers, collection and transport scheduling, marketing and managers of collection silos, storage silos and dryers. From these discussions we worked out a representation of the collection of corn. This representation is then used to extrapolate scenarios for the management of GM and non-GM segregation.

## RESULTS

### The case studied

250,000 tons of corn is collected and mainly sold for human consumption. Collection is distributed between starch corn (190,000 t) and meal corn (20,000 t). The remainder is sold for animal feed.

The cooperative has various collection silos distributed over its supply zone. These silos have a low storage capacity (about 400 t divided into 4 cells). After temporary storage in these small silos, crops are transported to drying sites according to product type. After drying, the products are stored in storage silos, in different cells for each product.

### Segregation between meal corn and starch

It is possible to discern two stages in the collection management. A scheduling stage which goes from sowing to harvest and a monitoring stage which begins at harvest time and lasts for 4 to 6 weeks. The scheduling stage's objective, involving segregation, is to determine the volume to collect for each production type and to allocate the infrastructures (collection silos, storage silos, dryers) to the different products according to their geographical origin. During the monitoring stage the problem is to manage the flows in order to ensure segregation between corn types whilst accepting the deliveries of farmers when they arrive. Mixing of the two products could occur at each of the three collection stages:

- At the collection silo level: if the rate of arrival of meal corn does not fill a cell fast enough, the silo manager has to mix the two products.
- At the drying level: if the corn meal volume is less than the drying capacity, the drying manager has to mix the two products to use the dryer to full capacity.
- At the storage silo level, if flows of the two products are not sufficiently separated in time the continuous filling of the cells does not permit segregation.

Thus segregation of the two products depends on a prior identification of the production zones according to the areas sown by farmers and an estimated flow distribution of the two types of corn. It is still possible that the cooperative cannot ensure segregation between products, as was the case in 2004. From these experiments, we propose 4 scenarios of segregation management of GM/non-GM crops.

### **Scenarios for segregating GM and non-GM crops**

The scenarios we present are based on grouping product type deliveries on a geographical or chronological basis, leaving farmers free to choose their type of corn production. These scenarios may or may not use horizontal coordination between country elevators.

- Product discrimination over time: delivery dates are imposed on farmers according to their product (certain weeks reserved for GM or non-GM crops for example). Some collection silos are dedicated, for a given period, to a single product. In this case there is no need for segregation at collection silos. In addition, by concentrating the collection of one product over a short period of time it is possible to have a sufficient flow to dedicate a dryer to this product and to fill one or more storage cells. However farmers may choose to deliver to another country elevator that has fewer constraints on delivery. This then causes a loss of collected volume and so a loss of market shares.

- A collection silo allocation, made by taking into account the area sown. The collection silos are allocated to GM or non-GM crops, taking into account the proportion of GM and non-GM crops in their supply zone. It is then possible to concentrate the deliveries of one product to specific collection silos and thus to separate the two products. If the supply zone produces a sufficient volume it is possible to dedicate a dryer to each product over the collection season and so ensure separation during drying and final storage. But a farmer whose product is not collected by his usual silo will have additional transport costs or will

deliver to a competitor.

- A collection silo allocation made before sowing. The collection silos are allocated to a given product. The farmers are informed of this allocation before sowing so they can choose their production taking into account the one accepted by the nearest collection silo and the transport costs to deliver to other silos. Farmers are thus indirectly encouraged to choose a particular kind of production. The country elevator can change its allocation after sowing if the amount of the selected product is likely to be less than expected. However, it does not prevent farmers from choosing to deliver to a competitor closer to their farm which accepts both products.

- A geographical or chronological allocation, with compensation between operators. This scenario is based on a sharing of the collection silos between the various country elevators in a given zone. If the collection silo of a country elevator accepts only GM products, the competitor will accept non-GM production. The farmer will deliver his product to the appropriate silo, indicating the country elevator for which his load is intended. The two country elevators then have to arrange the concentration of the two products before drying or at final storage, compensating each other as necessary. This scenario limits the problems of competition between operators, but it involves significant transaction costs related to the compensation management.

### **CONCLUSION**

The scenarios we present here are a first step in on-going work. They should be discussed with cooperative managers in order to evaluate their feasibility. They can however be used as a basis for management scenarios for coexistence on a territory scale, combining them with a model of gene flow within a territory (Angevin, *et al.*, 2003) and with models of farmers' choices according to distances to collection silos, their loyalty to their country elevator, and the profit expected from GM or non-GM production.

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# Grain trader implements advanced “grain-matching” system

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**Abstract:** In 2005, the German seed and grain trading company Maerkische Krafftutter GmbH (Maerka) took an initiative around the issue of coexistence of genetically modified (GM) and conventional products. Together with Monsanto and Pioneer, Maerka started a pilot project in the region of Golzow (Brandenburg) which allows GM maize grain to be commercialized while, at the same time, meeting different labelling standards (GM or conventional) for domestic grain. TUEV NORD EnSys Hannover was assigned the task to set up a comprehensive documentation for the quality assurance in the project, including a quality manual and a description of operational procedures.

The Maerka initiative was based on the premise that “Good Agricultural Practices” (GAP) for growing GM crops are not currently regulated by German law. However, to ensure an outlet for their production, farmers growing either GM maize or conventional maize in the vicinity of GM maize may rely on contractual or voluntary measures agreed with their seed/grain trading companies. The project was carried out using Bt maize line MON 810, an insect resistant GM maize approved for commercialisation in the European Union since 1998, and took into account recent scientific data on coexistence especially from Spain, France and Germany. The Maerka system guarantees farmers that conventional grain maize grown adjacent to fields with Bt-maize is accepted, regardless of possible traces of MON 810 and without any price reduction. Maerka is responsible for ensuring the proper labelling of the goods. Bt maize is also accepted, separately stored and brought to market after being labelled accordingly.

## INTRODUCTION

The overall objective of the Maerka “grain-matching” system was to create a scheme for selling and buying GM maize satisfactory for all its customers, e.g. Bt maize growers as well as farmers growing conventional maize adjacent to the Bt maize fields.

GM farmers implement voluntarily GAP to reduce to a minimum the presence of GM material in adjacent fields of non GM maize. Maerka in turn agrees buy both the GM maize and the maize from adjacent fields, providing an outlet for the production of all types of farms. The trader is responsible for separation and labelling of the different types of grain, according to current regulations.

In Germany, Bt maize is grown on an area of some hundred hectares since 1998 (Degenhardt *et al.*, 2003) with an increase in the last years. Farmers interested in using biotechnology are faced with problems due to special liability requirements specific to the cultivation of GM crops. The liability rules in Germany are set by the amended “German

Law on Genetic Engineering” whereby growers of GM-crops are liable for any economic loss encountered by their neighbours and resulting from the movement of pollen from their Bt-maize fields. Even if farmers respect GAP, they are not exempt from third party liability claims. This problem calls for a solution that provides legal certainty for each stakeholder.

To overcome farmers’ worries about the assumed unpredictable financial risks connected with the cultivation of GM maize, Maerka created its concept for the uptake of conventional grain maize grown next to GM maize. This guarantees farmers, worried about potential losses from being located next to fields where GM maize is cultivated, a fair sales potential for their products without any price reduction. This offer to purchase conventional maize on customary market terms (for maize not subject to mandatory labelling) does not abolish the statutory liability claim against the grower of GM-crops opens a practicable way which could prevent a damage event in the first place.

**Table 1.** Maize pollen dispersal, viability and cross-pollination in conventional maize crops: summary of research findings (from Brookes *et al.* 2004)

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



## APPROACH AND MEASURES

The basis for the required GAP measures in the Maerka grain matching project is the scientific outcome of the 2004 trials in Germany (Weber *et al.*, 2005a, b), as well as other publications (Brookes *et al.*, 2004; Melé *et al.*, 2004) on pollen movement and cross fertilization in neighbouring maize fields (Table 1). According to these studies, applying a separation distance of 20 m between GM and adjacent conventional maize should ensure levels of GM material in conventional maize lower than 0.9%. Maerka additionally asks farmers to:

- inform them about neighbouring maize fields in less than 100m distance to the Bt plot,
- adhere strictly to the following GAP measures:
  - . separately store and transport GM and non-GM maize,
  - . separate at harvest,
  - . clean machinery adequately.

Both the flow of information and necessary procedures will be regulated in line with the previously mentioned agreements between maize growing farmers and the receiving trader, which also apply to the receiver's internal standard operational procedures (SOPs).

### Quality assurance and management measures

The SOPs are embedded in the quality assurance system specially implemented at Maerka to ensure separate commodity flow and guarantee adequate labelling. The quality assurance system sets strict rules for:

- Control of documents and records
- Appropriate personnel training
- Separation of goods
- Sampling
- Nonconforming products and corrective action
- Auditing

The whole path of Bt maize, from seed supply to the farmers to uptake of the harvest and transport of the maize away from the receiving

location, is covered by SOPs. They are focused especially on handling of the different maize batches as well as sampling procedure with the corresponding control of records. The sampling procedures are based on DIN EN ISO 21568 (see also Lischer, 2001) and the LMBG L 15.01/02-1 (Sampling procedure according to German Food Law). The sampling takes place from trucks while delivering incoming and outgoing goods.

### Assessment of the system

The end of this scheme will see an evaluation about the practicability of GAP measures by farmers growing GM maize in terms of a general no-labelling-policy for neighbouring conventional maize fields. It is expected that the GM Bt maize content in the conventional maize batches from adjacent fields do not exceed 0.9%. This could make an obligatory inspection of the GMO content by the receiver unnecessary in the future. At the same time the project should identify which measures the elevators need to adapt in order to allow for separate commodity flows (GM and non-GM) within their facilities.

A second party audit will be conducted after implementation of the quality assurance system during the maize harvest period 2005 at the grain traders receiving location.

The last point to assess in the system is whether or not the maize batches from adjacent fields are to be labelled as GM. When grain maize is delivered from areas where GM maize is grown the receiving company needs reliable information about the GMO content of the batches. Therefore different laboratories accredited for GMO analytics are chosen to conduct the investigation of the maize batches from adjacent fields. If one of the delivered maize batches proved to contain more than 0.9% GM Bt-maize, the complete storage silo will be labelled according to the EU Regulations No. 1829/2003 and 1830/2003. Samples of the outgoing maize will provide results about the successful separation of the GM-maize and the conventional maize during storage and transportation at the receiving facility.

## RESULTS AND PERSPECTIVES

In 2005, the seed and grain trading company Maerka implemented a pilot project as a further development of a "grain matching" concept described in Tencalla *et al.* (2005). The quality assurance system was implemented at the grain trader facility in Golzow, Germany. First preliminary results of the project have shown that management measures and customer agreements are suitable to identify the neighbours of the Bt maize growers and to assure a comprehensive information supply. GM Bt maize and maize from adjacent fields can be accepted and handled by one elevator. The system has to prove itself in 2005 under difficult legal conditions in Germany. Given that we are dealing with commercially managed grain maize farms that are located in the neighbourhood of GM farms for the first time, scientific guidance and monitoring are essential. This scientific accompanying is conducted in the project by the University Halle, Germany, as in the test trials 2004 (Weber *et al.*, 2005b) and will provide further information about practicability, separation success and potential improvement of the system.

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**PARALLEL SESSION**  
NATIONAL IMPLEMENTATION



# An evaluation of measures to ensure agricultural coexistence of GM and non-GM crops in Switzerland

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**Abstract:** The aim of the present study was to analyze if coexistence of both genetically modified (GM) and non-GM crops was technically feasible in Swiss agricultural production. Using maize, oilseed rape and wheat as examples, technical and organizational measures were identified, which will ensure that adventitious GM-contents in food and feed do not exceed the threshold of 0.9% specified by EU and Swiss legislation. In order to determine the required isolation distances between fields of GM and non-GM maize and oilseed rape, an analysis of available out-crossing data was performed. Two different approaches were used to assess the feasibility of spatial co-existence of GM and non-GM based farming in Switzerland. 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 measures as well as the exchange of information and agreements among farmers are necessary. The approach developed in this study may be of assistance for evaluations in other countries or for evaluations based on lower threshold definitions.

## INTRODUCTION

The European Union (EU) recently adapted the current legislation regulating the commercial cultivation of GM crops (European Union, 2003a, 2003b). In September 2004 the EU approved the first GM Bt-maize varieties and entered them into the Common EU Catalogue of Varieties (European Commission, 2004). It is generally expected that Bt-maize will be cultivated commercially in EU countries other than Spain where Bt-maize is grown since 1998. As a consequence, the coexistence of GM and non-GM crops is highly debated, both in politics and in public. There is an

urgent need to discuss and define the conditions and measures required to ensure coexistence on a scientific, legal, and public basis.

Although commercial cultivation of GM crops in Switzerland seems to be rather unlikely in the near future, Swiss farmers and consumers might one day become interested in GM crops with specific properties. Similar to the EU, Swiss legislation stipulates that protection of GM-free production and consumers' freedom of choice must be guaranteed if GM crops were commercially cultivated. Agroscope FAL Reckenholz – the Swiss Federal Research Station for Agroecology and

Agriculture – was commissioned in December 2003 by the Swiss Federal Office for Agriculture to evaluate possible measures that would allow coexistence of GM and non-GM agriculture in Switzerland. The study (Sanvido *et al.*, 2005) was confined to agricultural 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. Based on the results of the study, the Swiss Federal Council's subsequently formulated an official statement to the initiative 'For GM-free Food', an initiative which has been put forward by environmental, consumers and farmer associations.

### APPROACH CONSIDERED FOR THE EVALUATION

The evaluation was based on the principles and methods 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 study that evaluated coexistence in Denmark (Tolstrup *et al.*, 2003). Isolation distances between fields of GM and non-GM maize and oilseed rape were determined based on an analysis of the recent scientific literature. The feasibility of spatial co-existence of GM and non-GM based farming in Switzerland was assessed using statistical data of an agricultural farming data survey as well as Geographic Information Systems (GIS).

### RESULTS

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:

- Introduction via seed impurities
- Volunteers from GM pre-cultures
- Out-crossing (fertilisation by pollen from GM plants)
- Mixing in machinery during sowing and harvesting

- Dispersal of GM seeds via straw, manure, etc.
- Mixing during post-harvest handling (transport to the farmyard, storage)

Several technical and organizational measures can help to minimize mixing during each of these six steps. Dispersal from seed impurities can be minimized by using certified seeds. Volunteers can be controlled by ensuring optimal soil preparation techniques after harvest and before sowing (Gruber *et al.*, 2004; Pekrun *et al.*, 1998), as well as by using cropping intervals. The extent of out-crossing between fields of GM and non-GM crops can effectively be reduced by respecting isolation distances (Eastham & Sweet, 2002; Ingram, 2000). The risk of mixing in machinery can be reduced by standardized cleaning practices 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 delivery of harvest can also minimize the risk of mixing.

The analysis of available out-crossing data was performed using twelve recent studies for maize and eleven studies for oilseed rape. Evaluation of data indicated that 25 meters for silage maize and 50 meters for grain maize would be sufficient to keep adventitious GM-contaminations below 0.5% at the border of non-GM crop fields. Since out-crossing is highest at the field borders close to GM crop fields, harvest of the entire field leads to a further reduction of adventitious GM-contents. This will ensure that GM-inputs through out-crossing will be below 0.2% (Sanvido *et al.*, 2005). For fertile oilseed rape varieties (conventional varieties and hybrids with restored fertility), the respective isolation distance was determined to be 50 meters. For oilseed rape with male sterile components (varietal associations), sufficient experimental data was lacking and therefore an isolation distance of 400 meters was recommended, as used for basic seed production 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 agricultural farming data survey carried out by the Swiss Federal Statistical Office in 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 pictures covering a 164-square-kilometre area in eastern canton Zurich (Schüpbach *et al.*, 2003). Geographic information systems (GIS) were used to calculate the shortest distance between two maize fields at a resolution of 50 meters. 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 and depended on the landscape structure. In the area investigated, half of the fields were more than 90 meters apart. The analysis suggested that establishment of isolated GM crop fields with the proposed distance of 50 meters should be possible for the majority of maize fields in this area, however, communication among farmers would certainly be a prerequisite for the implementation of such distances.

## CONCLUSIONS

The here presented evaluations of scientific information and legal frameworks indicate that from an agronomic point of view, the coexistence of GM and non-GM based agriculture is possible in Switzerland based on the current legal threshold of 0.9%. However, various technical and organizational measures are necessary to prevent or minimize the different GM-dispersal routes. These measures depend on the biological properties of the individual crop plants and the need for co-existence measures must therefore be assessed for each crop separately. Dialogue and a comprehensive information exchange between neighbouring farmers is also essential

for planning crop rotation, and especially for ensuring the necessary isolation distances between adjacent fields. A successful co-existence of various cultivation systems therefore calls for mutual respect of producers of all farming systems. Although, the responsibility for ensuring separation of both GM and non-GM crops lies with the farmers cultivating GM crops, ideally, non-GM farmers should also be disposed to assist co-existence measures.

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# Co-existence of conventional and organic farming with GMO-based agriculture in Bulgaria

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**Abstract:** The national policy of Bulgaria is strongly oriented through integration with the EU structures in line with the recently expected Accession to EU in 2007. In February, 2004, by request of the Bulgarian Parliament, a working group under the auspices of the Ministry of Agriculture and Forests has been convened to elaborate a national policy for the co-existence of conventional, organic and GM crops. It consists of recommendations given in accordance with several groups of economically important crops. Bulgaria was one of the first of the Central and East European (CEE) Countries to release genetically modified organisms (GMOs) in open field trials and a regional leader in plant biotech research. As a country with economy in transition however, Bulgarian agriculture still faces certain economical problems, which reflected also in a dramatic decrease of the pesticide use that has been thought favourable to future organic farming developments. Since July 2005, a GMO law, being considered as very restrictive to the potential of using new technologies, is in force. It contains certain provisions for liability with respect to co-existence, which will be overviewed and discussed in this paper.

## INTRODUCTION

Transgenic crops appeared for the first time on the market in USA in 1994. According to the latest statistics, the global area with commercially grown transgenic plants is 81.0 million ha by 8.25 millions of farmers from 17 countries on 6 continents. The global market value of GM crops is estimated to 4.70 billion USD that represents 16% of the global seed market. The global value of the biotech market is projected at more than 5 billion USD for 2005 (James, 2005), which ambiguously shows the considerable economical benefit of the modern biotechnologies.

In Europe, Romania grows transgenic soy on a commercial scale on area of about 100,000 ha and Spain grows Bt maize (resistant to European corn borer) on the same hectares. Germany, France and other countries from EU continue their field trials with biotech crops. In the period of 1999-2004, more than 17,000 field trials with transgenic plants have been performed in EU. Over the same period the organic area, as well as the market of organic products, has also increased. For example, in USA, the country which grows 42.8 million hectares (63% of global total) of transgenic crops, the organic area of maize and soybeans has increased from about 33,000 hectares in 1995 to

about 109,000 hectares in 2001. The value of the organic food market rose from US\$3.3 million in 1996 to US\$11 million in 2002 (Brookes & Barfoot, 2004).

Co-existence refers to the economic consequences resulting from adventitious presence of material from one crop in another and is related to the principle that farmers should be able to cultivate freely the crops of their choice using the production system they prefer (GM, conventional or organic) (Brookes, 2004). It arises logically after the decision-making process has taken place, when a given GM crop has obtained the permit for release into the environment and its safety does not pose concerns anymore. Co-existence of the three types of agriculture is not, consequently, a biosafety issue. It rather relates to the management measures that have to be taken in order to prevent the adventitious presence over a certain threshold of GMOs into other production systems such as organic farming and conventional agriculture. The EC legislation however is still unclear in terms of setting precise thresholds. Regulation 2092/91 on organic production states that organic products must not have been made using GMOs leaving open the question whether they can contain adventitious traces of GMO. Organic producers, driven perhaps by the consumers' interest, attempt to apply 0% threshold, notwithstanding with the legal basis, which does not specify a threshold for adventitious presence, different from the one for conventional products (0.9%). The last threshold should not be applied to seed production: a specific EU legislation on seeds is under elaboration.

One year after the first commercialization of a transgenic plant in 1994 (Flavr Savr tomato), Bulgaria signed an agreement with EU, which paved the way of the negotiations for EU accession. In this line, the country was strongly encouraged to harmonize its legislation with that in place in EU. On March, 15<sup>th</sup> 2005, the Bulgarian Parliament adopted a GMO law, enacted since July, 2005, that implements EC Directives 2001/18 on deliberate release

and placing on the market of GMOs, 90/219/EC, amended by 98/81 on contained use of GMOs and Regulation 1946/2003/EC on the transboundary movement of GMOs. Some of the provisions of the GMO Act encompass the issue of liability with regard to co-existence.

### **LEGAL BASIS OF CO-EXISTENCE OF CONVENTIONAL AND ORGANIC FARMING WITH GMO CROPPING IN BULGARIA**

Bulgaria was one of the first of the Central and East European (CEE) Countries to release genetically modified organisms (GMOs) in open field trials that regulated the releases under an interim regulation "Guideline for the dispersal of genetically modified higher plants, developed through DNA recombinant technology" has been adopted (1996) in accordance with the EU Directive 90/220 in place of that time. Notwithstanding with the fact that approvals for commercial planting of GMO have not been granted so far, these experiments allowed for a significant accumulation of scientific data with respect to biosafety and co-existence.

The Bulgarian GMO Act, in force since 1 July 2005 and discussed for more than two years by the Parliament, has been strongly influenced by non-governmental ecological organizations and organic farmers' associations (although the rate of organic farming in Bulgarian agriculture is less than 1% and the market of organic products is extremely limited). With relevance to co-existence, it goes much beyond the EU biosafety framework and particularly the EC recommendation on guidelines for co-existence as of July 2003 that states "No form of agriculture, be it conventional, organic or agriculture using genetically modified organisms (GMOs), should be excluded in the European Union", by putting a ban on:

- carrying on research that involves genetic modification with specific plant species: tobacco, vine, and oil rose;

- deliberate release into environment and placing on the market of tobacco, vine, cotton, damask rose, wheat, and all vegetable and orchard crops
- applying GMO-based farming “if organic farming is practiced on an adjoining field”
- the deliberate release of any GMOs into the areas included in the National Ecological Network, as well as into the adjoining areas within a zone of 30 kilometres around any such areas.

The GMO Law provides for public registers of permits for deliberate release of GMOs into environment and register of the areas wherein the deliberate release of GMOs is authorized. The GMO farmer is to inform the Ministry of Agriculture and Forestry of the location and size of the areas planted. The obligation to respect respective isolation distances (provided by the Law) is onus to the GMO grower. As an example, the distances found to assure sufficient level of protection to the conventional and organic fields planted with non-modified crops of the same species are for maize, soybean and rape 800, 20, and 400m, respectively. As a comparison, field trials with GM maize have been performed in the period of 1998-2004 on overall 428,005 ha in different landscape regions in Bulgaria. The distance between the GMO and the conventional fields was 50 m when the conventional fields were not used for seed production. The adventitious presence of GMOs in the non-transgenic fields was found to be under 0.5% (Atanassov *et al.*, 2003). The isolation distance for maize adopted by the GMO Act however, exceeds the scientifically approved one (50m) 40 times and thus, practically makes co-existence impossible.

One of the core questions with direct relevance to co-existence is how to comply with the threshold of 0.9% of adventitious presence of GMOs in conventional production systems. The economic studies performed so far have shown as well, that co-existence at the 0.9% level for certain

crops is possible but with different costs and needs for changing farming practices. Interestingly it has been found out that organics farms, as they already operate rules of segregation and traceability, will support less additional costs than conventional non-GM farms to achieve the 0.9% threshold (Bock *et al.* 2002; Rieger *et al.* 2002; Ramsley *et al.* 2003; Brookes & Barfoot, 2003a,b). According to Bulgarian GMO Act, the threshold of adventitious or technically unavoidable admixture is 0.5%. Bulgarian legislation, including the Ordinance on organic farming N.22/2001 does however not provide any definition of the notion of “GMO free” organic production. The above mentioned studies demonstrated also that apart from the inherent biological inability to obtain 100% pure crops, zero tolerance thresholds, when technically achievable, would be very expensive. It is therefore recommendable that a common threshold of above 0.5% for liability of GMO farmers independently on the production system they might affect, to be adopted in Bulgaria. In addition, amendments in the GMO Act have to be envisaged in order to ensure co-existence and thus to comply with the EC policy in this line. Such amendments should include provisions for science-based case-by-case risk assessment; adoption of realistic isolation distances and realistic liability and redress regime (according to the present GMO Act the GMO farmer may be finable with up to 500 000 Euro in case of causing adventitious GMO presence in organic production) among others. Possibility of establishment of fund with the participation of all farmers’ groups, which would serve the issue of liability and redress should also be considered.

## **CONCLUSIONS FROM THE REPORT OF THE WORKING GROUP ON CO-EXISTENCE, FEBRUARY 2004**

By request of the Bulgarian Parliament, a working group under the auspices of the Ministry of Agriculture and Forests has been convened to elaborate a national policy for the co-existence of conventional, organic and GM crops. The paper, revealing the strategic view for the next 5-10 years

of scientists and ministry officers actively working in the field of conventional agriculture, organic farming or biotechnology, is based on economical, political, geographic, biological, social & ethical factors and was prepared in February 2004. It consists of recommendations given in accordance with several groups of economically important crops. The basic concept in this document is that the conventional agriculture shall keep being the main production system for the staple crops like wheat, rye, barley, rice and almost all vegetables. Together with the principal role of the conventional methods for the crops maize and potato, some steps towards commercialization of legal GM varieties can be envisaged. At the same time, the local conventional breeding in maize and potato should receive stronger governmental/public support. For all fruits, with particular emphasis on small fruits as well as for the emblematic Bulgarian crops of oil rose and grapes a priority should be given to the organic farming with view of further consumer niche on the European market.

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# Agreement coexistence in the Netherlands 2004, where are we now?

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**Abstract:** On the 2<sup>nd</sup> of November 2004, a committee of stakeholders in the Dutch agriculture presented a compromise proposal on coexistence of GMO, conventional and organic agriculture to the Dutch Minister of Agriculture. The agreement proposes practical measures to ensure coexistence, to limit damage from admixture, ensuring liability and proposes a fund to be filled by all stakeholders.

The Minister sent the agreement of the participating stakeholders to the Dutch Parliament, where it was discussed intensively. In consequence it was decided to start implementation. The agreement will not lead to new legislation but will be enforced by the Productschap for Agriculture through an arbitration system that is currently set up. The EU Commission should approve the system.

## BACKGROUND

The Minister of Agriculture in the Netherlands supported by other Ministries does not intend to create special legislation for coexistence of GMO and non-GMO agriculture in the Netherlands; Parliament supports this approach.

In order to apply the guidelines (2003/556/EG) of the EU Commission, the Minister therefore initiated a small committee of stakeholders to work out an agreement for coexistence rules on a voluntary basis. In the committee conventional farmers, organic farmers and seed industry are

represented. Plantum NL represented the seed and biotechnology industry.

The committee was asked to create an approach to coexistence that all could agree on; which was practical, and balanced resulting in "freedom to operate" for all farmers with a minimum chance of economic damage by GMO-admixture, and legal action for claims of liability.

The scope of the agreement was to be limited to primary agricultural production only, hence not on seed production and food processing.

## KEY COMPONENTS OF THE AGREEMENT

- All stakeholders have agreed that interaction cannot be totally avoided, the measures must ensure that the damage is minimised (100% GMO-free cannot be guaranteed)

- On the other hand: damage is not directly linked to the 0.9% labelling threshold. Any additional costs or commercial loss of value to the production as a consequence of admixture is considered (based on the liability system in the Netherlands).

- Enclosed to the report is an analysis of the legal situation for liability for this kind of damage in the Netherlands. It was concluded that direct economic damage of direct loss of value (food value reduced to feed etc.) together with additional (testing) costs could be subject to a claim for liability. If the court will award such a claim very much depends on the situation and "behaviour" of the involved parties. Damage to the image of a product usually cannot be claimed.

- The agreement states that a GMO-farmer that follows the proposed measures correctly cannot be made liable for any economic damage of GMO admixture that occurs any way.

- In those cases that all necessary measures are taken and "residual" damages occur, they can be claimed for from funds that are set up on a per-crop basis. These funds are filled by conventional, organic and GM-farmers as well as other relevant biotechnology stakeholders in the chain. The government is asked to give support as well.

- A package of practical measures that the GMO and/or conventional/biological farmer will have to take has been agreed on. Farmers will have to register their actions in the general farm certification scheme that is enforced by the *Productschap*, a semi-official body between government and farmers.

- A key factor in these measures is the isolation

distance for admixture/cross pollination:

- The GMO-farmer must keep a distance to conventional farmers of 3 meters for potato fields, 1,5 meters for sugar beets and 25 meters for maize.

- For those farmers that can demonstrate that they deliver to a "GMO-free" market any GMO-grower must keep a distance of 10 meters for potatoes, 3 meters for sugar beet and 250 meters for maize.

The "GMO-free" farmer is mainly the certified organic farmer but can also apply to other farmers that produce for specific "GMO-free" markets and consistently take management measures in their enterprise based on a "zero tolerance" for AP (e.g. they cannot import feed at 0,9% levels.) In the Netherlands for these crops, biological farmers represent less than 1% of the average.

- Clustering of GMO and non-GMO production will be stimulated.

- GMO-farmers must inform all neighbouring farmers that have fields within a distance of minimal the isolation distance on the GMO-crops they plan to grow in the next season.

- GMO-farmers must register their plans to grow a GMO-crop and location in a register at the Ministry before the first of February; non-GMO farmers must react if necessary before 1<sup>st</sup> of February as well (This register is already functional).

- Farmers, including hobby farmers/gardeners will be subject to an education program and/or made aware of these measures.

- The effectiveness of the measures will be monitored; after three years the measures will be reassessed and the agreement can be adapted.

- It is proposed that GMO experimental field trials keep at minimum the same isolation distances.

- The agreement is a coherent package, all aspects must be taken care of.

## **IMPLEMENTATION PROCESS**

- A draft arbitration system has been set up, including all the measurements farmers should take. This will be put in the approval system of the Product Board starting in November. After approval it will be sent to the European Commission for final approval.

- The possibilities for setting up a fund are currently studied. On basis of a risk analysis the size of the fund will be determined. Afterwards it will be decided how the costs will be divided over the private partners of the agreement and the government.

- A research proposal is being developed on isolation distance in maize in the Netherlands.

- A start is made on developing a monitoring system of the effect of the measures that are agreed on.





# **PARALLEL SESSION**

MODELLING FOR CO-EXISTENCE  
DECISION-MAKING



# Predictive models for field separation, based on outcrossing data on oilseed rape and maize from the UK Farm Scale Evaluation programme

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**Abstract:** Data on crop to crop pollination of oilseed rape and forage maize collected during the Farm Scale Evaluation programme was statistically analysed. Four empirical models, one for each of winter and spring oilseed rape and grain and fodder maize, were derived. These models input orthogonal length of the field containing the conventional crop and the target level of admixture to be obtained and output a figure for separation distance that will allow this level of admixture to be achieved with 98% confidence based on mixing of the harvested crop within that field.

Data from FSE was considered suitable as the sole source of data for this study as it was derived from a range of trial sites in 3 seasons with different orientations, and spatial features of a size and shape that could collectively represent an average of realistic situations.

These UK models compare favourably with data and models from other countries and the results of this study will be used to derive recommendations for separation distances for coexistence of GM and conventional oilseed rape and maize crops in the UK.

## INTRODUCTION

In 2004 NIAB was commissioned by Defra to review the study conducted by J. Ingram for Defra (Ingram, 2000), examine new data available since the publication of this report and make recommendations for appropriate separation distances between GM and non-GM maize and oilseed rape in order to achieve given thresholds of admixture. Since the publication of Ingram's report a number of further large-scale studies have been published and/or conducted which add substantially to the body of data. The most important of those from a UK perspective, are the Farm Scale Evaluations (FSE) and specifically the

data generated on GM cross pollination in maize and oilseed rape. This study examines the FSE data in order to develop models that can be used to extend recommendations for separation distances to cover additional levels of permitted admixture.

### Oilseed Rape Sampling in the FSE

Gene flow was monitored at the FSE sites of winter and spring oilseed rape (OSR) and forage maize, genetically modified to be herbicide tolerant (HT) and released under the authority of the Genetically Modified Organisms (Deliberate Release) Regulations (Henry *et al.*, 2004 and Weekes *et al.*, 2005).

Sites comprised a split field design, half planted with Seedlink® OSR line RF3/MS8 and the other half with an equivalent conventional OSR variety.

Over 100 OSR FSE sites including both types of OSR were sampled in this study, from which seeds at over 2000 sample points within the conventional crop were collected and tested over three years. Each sample comprised seeds collected from plants within a 1m quadrat. Samples were collected from points along transects in the conventional crop.

For each sample DNA was extracted from 5g seeds using a standard CTAB (hexadecyltrimethylammonium)-based method as described by Weekes *et al.* (2005).

### Fodder Maize Sampling in the FSE

A total of 55 maize FSE sites were used in this study, from which cobs at 1152 sample points within the conventional crop were collected and tested during the course of three years. Each sample consisted of 3-5 cobs (>1000 seeds), each from a separate plant in the sampling location. Samples were collected from three transects in the conventional crop at distances of approximately a quarter, half and three-quarters (Figure 1) of the way across the field (6 transects were sampled in year 2000; see Figure 1). Along each transect, cob samples were collected at the following distances: 2m, 5, 10, 20 or 25m, 50 and 150m away from the junction with the GM crop.

Treatment of maize samples was similar to that of OSR except that the maize grains were removed from the cobs, ground up and DNA extracted using the Promega Wizard® Magnetic DNA purification system and the LabSystems KingFisher ml Magnetic Particle Processor.

In the case of maize TaqMan assays, Two sets of primers and probe were used. One set was specific for the pat gene (target gene; to detect the T25 transformation event) and the other set was specific to the *Zea mays cdc2* gene (the endogenous control). The calculation of target

concentrations was carried out as described for OSR.

### Calculation of Separation distances

The FSE studies, as with other similar studies in France and UK, of adjacent blocks of GM and non-GM crops, show the levels of pollen penetration and subsequent outcrossing from one block to another. There is little or no separation between the plots, so that these studies give an indication of the levels to be anticipated when there is no separation distance between fields.

The 98th percentiles of the full dataset from the FSE were used to derive the levels of cross pollination; from this it was possible to derive the expected levels of cross pollination for different GM events in terms of the zygosity of the transgenes introduced (GM index); one hemizygous GM locus would, for example, represent an Index of 1; a GM that was homozygous at a given locus would represent an Index of 2. Once these figures for cross-pollination had been derived they were converted back to %GM as expressed in the FSE data.

In order to calculate the average %GM (DNA/Genome) in seed to be expected from a field of a given size, for a crop of a given GM Index, a mathematical function of distance ( $f_0(x)$ ) that described a curve that followed a best fit line through the actual data was first plotted. This function was integrated between two distances  $x_0$  (the separation distance) and  $x_1$  (the orthogonal depth of the field) to arrive at the sum of GM (DNA/Genome) over that distance. By also integrating between  $x_0$  and  $x_1$  for a mathematical function of distance that describes a straight line at the 100% GM level ( $f_1(x)$ ) it was possible to find the mean level of GM DNA averaged over the whole of a given field from the following equation. (Equation 1)

**Table 1.** Summary statistics for the goodness of fit of the models

	R	R <sup>2</sup>	Adjusted R <sup>2</sup>	SE of the Estimate	Durbin-Watson
spring oilseed rape	0.993	0.985	0.983	1.84803	1.635
winter oilseed rape	0.982	0.965	0.960	3.23067	1.619
grain maize	0.946	0.895	0.887	1.91584	1.864
fodder maize	0.962	0.925	0.915	1.01961	2.338

$$\left[ \frac{\int_{x_0}^{x_1} f_0(x) dx}{\int_{x_0}^{x_1} f_1(x) - f_0(x) dx} \right] \cdot 100$$

**Equation 1.** This equation shows the general form of the models used in this study where  $x$  is distance;  $x_0$  is the separation distance;  $x_1$  the field depth;  $f_0(x)$  is the curve through the data and  $f_1(x)$  is the straight line function at 100%.

For each of the graphs of GM DNA/Genome against distance for crops of various GM index, it was found to be possible to obtain curves of the general form  $y = y_0 e^{(-ax)+b}$  that fitted the data well (Table 1). This form of exponential decline model, whilst simple, was felt to be appropriate as the data had been pooled and was not linked to any additional factors such as topology or prevailing wind direction. Table 1 shows the R<sup>2</sup> values and Durbin-Watson statistics for each of the models. The R<sup>2</sup> values are very high indicating a good fit between the fitted curves and the observed data. The Durbin-Watson statistics all lie between 1.6 and 2.6 indicating little autocorrelation among the residuals and little to be gained from further tuning of the models.

## DISCUSSION

For comparison, the recommendations of Ingram (2000) in terms of %GM are given in Table 2 together with figures calculated from the models derived in this study.

The models presented here constitute an evidence-based decision support tool derived from a large body of data relevant to the UK farming environment. This is in contrast to recommendations for coexistence that have been arrived at previously (e.g. Ingram, 2000; Tolstrup *et al.*, 2003) which tend to propose single highly conservative figures for each crop regardless of its genetics or the sizes of the fields involved. While a number of mechanistic models of gene dispersal are under development (Damgaard & Kjellsson, 2003; Walklate *et al.*, 2004), they are at present either incomplete or not tested under UK conditions.

The present models are empirical rather than mechanistic and are based on data from a large number of locations with differing geometries, topologies and prevailing climate. This allows separation distances to be arrived at with a high degree of confidence but with a minimum number of parameters being input by the user. This modelling approach and mechanistic computer models such as GENESYS-rape and MAPOD-maize (Colbach *et al.*, 2001 a & b; Angevin *et al.*, 2003) can complement one another. These models are based on numerous field experiments results.

**Table 2.** Comparison of separation distances recommended by Ingram with those derived from the models present here (in square brackets).

Crop	Threshold Levels Of %GM		
	0.51%	0.25%	0.05%
Oilseed Rape	0.51%	0.25%	0.05%
Conventional varieties and restored hybrids	1.5 [33]	10 [38]	100* [55]
Maize	0.40%	0.20%	0.04%
For grain	200 [64]	300 [84]	n/a [130]
For silage	130 [35]	200 [43]	420 [100]

In the framework of SIGMEA research program, they are currently refined and improved by the insertion of new data and by being tested against various data sets so that they are applicable to a wide range of scenarios. They take into account any actual landscape patterns, crop rotations, agricultural practices, as well as non-cultivated area management (for OSR at least), and precise climatic data (case of maize). Thus, they allow calculating gene flow for every individual situation. Therefore, they are necessary when the aim is to predict gene flow for an individual farm, a group of fields or a collection area while empirical models such as the one presented in this article, are useful to set up global recommendations for regional or national measures such as isolations distances.

## ACKNOWLEDGEMENTS

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# Validation of a model for pollen dispersal over heterogeneous landscapes

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**Abstract:** A numerical model has been developed within an Eulerian framework to allow a better understanding of the interactions between landscape heterogeneities and pollen dispersal. This model is validated against two field experiments where airborne concentration and deposition rate of pollen were measured downwind of maize plots. The model simulates correctly concentration profiles but underestimates the maximum of the deposition rate just behind the source plot. This model discrepancy seems to be due to an underestimation of the average maize pollen settling velocity which may be increased by the turbulence in the plot wake, as compared to its value in an undisturbed flow.

## INTRODUCTION

Since the introduction of genetically modified (GM) crops, predictive tools modelling dynamics, turbulence and pollen concentrations over heterogeneous canopies have been required to assess the cross-pollination rates between GM and conventional crops. Pollen dispersal is closely linked to flow dynamics and turbulence within and above crops, themselves depending on the landscape heterogeneities such as roughness changes, field discontinuities, gaps, roads, tree lines, forest plots, etc. It is therefore of primary importance to assess the influence of these heterogeneities on pollen dispersal. Lagrangian models are often used for modelling particle dispersal because they tend to mimic particle motion and are relatively easy to use. However they are unable by essence to calculate the

flow characteristics and require velocity and turbulence fields to be prescribed a priori, which is not possible in most heterogeneous, real-world situations.

To address this problem we have adapted a CFD type model, Aquilon, to canopy flow (Foudhil et al., 2005) and added an advection-diffusion conservation equation for pollen particles. Turbulence is modelled statistically with a  $k-\epsilon$  closure scheme. The flow equations in the canopy are modified to account for the drag forces and the production of turbulent kinetic energy by the vegetation. The relative velocity between air parcels and particles is represented through the addition of a particle settling velocity, and deposition is represented by a sink term accounting for impaction and sedimentation.

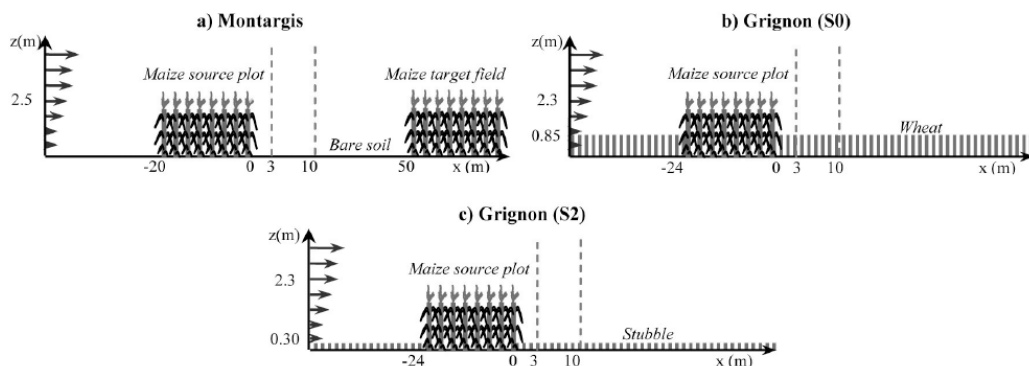


Figure 1. Scheme of the three numerical configurations.

The dynamic part of the model has been previously validated in 2D cases (continuous and discontinuous vegetation canopies), against wind-tunnel and *in-situ* measurements (Foudhil *et al.*, 2005), and in a 3D heterogeneous urban park (Dupont & Brunet, 2005). A first validation of the dispersive part of the model is presented here.

## VALIDATION

The model is validated against two field experiments, Montargis and Grignon, where airborne concentration at  $x = 3$  m and 10 m, and deposition rate of maize pollen (*Zea mays*) were measured downwind of maize plots (Jarosz *et al.*, 2005). Daily trials of 1 to 2 hours were recorded on 12 occasions during flowering in Montargis and 32 occasions in Grignon.

Two-dimensional simulations were performed under neutral atmospheric stratification in three different computational domain configurations as represented in Figure 1. The maize foliar density was deduced from the digitalization of maize plants; drag coefficients were assumed constant vertically and equal to 0.2; and an average settling velocity of  $0.31 \text{ m s}^{-1}$  was used for pollen grains as measured by Di-Giovanni *et al.* (1995).

Simulations were carried out for each trial by assuming that the incoming flow at the inlet boundary was in equilibrium with the ground. The mean velocity profile therefore followed a

logarithmic law, and the mean turbulent kinetic energy and its dissipation rate were deduced from the equilibrium relationships. The lower boundary conditions were given by wall laws at the ground. The friction velocity  $u_*$  and the flux of pollen released from the maize tassel source plot were estimated by “inversion” of the model, *i.e.* modelled wind velocity and pollen concentration profiles at 3 m were fitted against observations through linear regressions.

## RESULTS

The average non-dimensional vertical profiles of simulated wind velocity and airborne pollen concentration at  $x = 3$  m and 10 m, as well as the average simulated deposition rates downwind of the source plot, were compared with observations in Figure 2 for the three configurations. The wind velocity was made non-dimensional by the upwind source plot wind velocity  $u_{\text{ref}}$  ( $x = -40$  m) located at the maize plot height, and the pollen concentration by the concentration  $C_{\text{ref}}$  located at the top middle of the source plot, *i.e.* at the tassel level.

The wind velocity below 2 m height is smaller close to the source plot, due to the sheltering of the downwind area. The average wind velocity profiles are accurately simulated by the model with relative root mean square errors (RRmse) less than 0.12. From the maize tassel level a plume of high pollen concentration is transported and

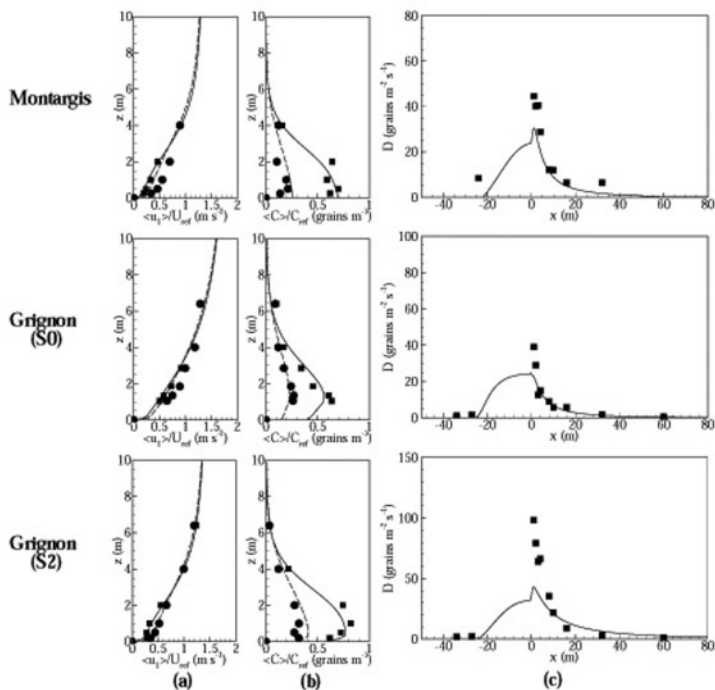


Figure 2. Comparison between model and measurements for the three configurations.

(a) Measured non-dimensional wind velocity profiles at  $x = 3$  m (black square) and  $x = 10$  m (black circle), and simulated profiles at  $x = 3$  m (solid line) and  $x = 10$  m (dashed line). (b) Same as (a) but for non-dimensional concentration profiles. (c) Measured (black square) and simulated (solid line) ground deposition downwind from the source.

diffused by the turbulent flow downwind of the source plot, with an average downward motion due to the large settling velocity of maize pollen. The airborne pollen concentration is larger at  $x = 3$  m than at  $x = 10$  m. The general pattern of concentration profiles is relatively well simulated by the model. Within the wheat and stubble canopies, the concentration decreases due to pollen deposition on plants as observed at  $x = 3$  m for S2 trials. At  $x = 10$  m the concentration is often overestimated by the model, except for S0, but the concentration RRmse remains reasonable, usually less than 0.20. Pollen deposition at the ground is maximum at  $x = 2$  m where the pollen plume interacts with the ground. The model nicely simulates the ground deposition rate pattern downwind of the source plot but the maximum is always underestimated by about 20-25% for Montargis and S2, and 30-35% for S0. Further downwind of the source plot, the deposition rate magnitude is relatively well simulated.

## DISCUSSION

A Lagrangian model, SMOP-2D, had been previously validated against the same dataset (Jarosz *et al.*, 2004). This Lagrangian model obtained similar performances as our Eulerian model when adjusted empirically for wind velocity and turbulence fields. Both models underestimate the maximum ground deposition rate behind the source plot. A sensitivity study made with Aquilon (results not presented here) showed that the model discrepancy on the deposition rate may be explained by an underestimation of the pollen settling velocity in the wake area behind the maize source plot. In this region, the maize pollen response time is in phase with the time characteristic of the turbulent structures since the Stoke number of pollen particles is around one. By extrapolating the results obtained in homogeneous isotropic turbulence at low Reynolds number on the dispersal of heavy particles by Wang and

Maxey (1993) to the turbulence in the wake region, we may expect to have a heterogeneous pollen distribution with accumulation areas in regions of low vorticity due to the inertial bias mechanism, and consequently a higher pollen settling velocity than that in an undisturbed flow. Wang and Maxey (1993) showed that the particle settling velocity could be increased in a turbulent fluid up to 40% to 50%, as compared with that in an undisturbed flow when the Stoke number is near unity.

Aquilon simulations with higher settling velocity ( $\sim 0.50 \text{ m s}^{-1}$ ) improve significantly the maximum pollen deposition rate simulation as well as the simulated airborne pollen concentration profile at  $x = 10 \text{ m}$  (results not shown). This increase of the maize pollen settling velocity due to turbulence in the wake area behind the maize plot is specific to the size and density of the maize pollen, and may not occur for smaller pollen grains.

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# Regionality as a key parameter for co-existence of genetically modified maize with conventional and organic maize

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**Abstract:** According to the recommendation on co-existence of the European Commission, workable co-existence measures should take into consideration the specificity of production systems, agricultural structures and topographic and climatic conditions of a member state. For genetically modified (GM) maize the use of isolation distances as a co-existence measure has been suggested. Although the extent of these distances is still controversial, their spatial impacts within a certain agricultural context are unknown. We evaluated these spatial effects of isolation distances between GM maize and non-GM maize on the loss of cultivation area in three different agricultural regions in Austria by the use of a GIS-based simulation model. In conclusion the simulations show that the structure of the landscape, the extent of the isolation distance and the proportions of GM maize and of organically grown maize are the most important factors for co-existence on a regional scale.

## INTRODUCTION

The recommendation of the European Commission 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” (EC 2003) is intended to help Member States to develop workable measures for co-existence in conformity with EU legislation. Within the framework of national catalogues of co-existence measures which have to be defined by the individual member states, the specificity of farm structures, production systems, agricultural structures and topographic and climatic conditions of a member state should be particularly considered.

Separation or isolation distances between GM and non-GM crops of the same species are used to create buffer zones thus reducing the chance of cross-pollination below a certain threshold. Although the extent of these isolation distances is still controversial and may depend on local agricultural and agronomic structures, their spatial impacts in a certain agricultural context may be substantial and have so far not been taken into consideration. The aim of this study was to evaluate the spatial effects of different isolation distances between GM maize and non-GM maize fields on the loss of maize cultivation area in three different agricultural regions in Austria by the use of a GIS-based simulation model assuming 10% and 50% GM maize cultivation.

## MATERIALS AND METHODS

Aerial views (2 x 2 km<sup>2</sup> or 4 x 2 km<sup>2</sup>) of three representative maize cultivation regions (test regions) in Austria were used as the basis for the mapping of maize fields and for the simulations. The test regions were located in the federal states of Lower Austria (Nitzing), Styria (Mehlteuer) and Burgenland (Halbturn). In a field survey every maize field was mapped by hand on the aerial view. These spatial data were stored and analysed by the use of a geographical information system application (ESRI®ArcMap8.2 and ArcView GIS 3.2). Each test area was characterised by calculating the proportion of maize area in the region, the average field size and width, orientation and aspect ratio of the maize fields. Then 10% or 50% of the mapped maize area was defined to be GM maize. These GM maize fields were chosen by random procedure until a proportion of 10% or 50% of the maize growing area was obtained. This procedure was repeated 3 times. Then buffer zones of 50 m, 100 m, 150 m and 200 m were generated around the virtual GM maize fields in order to obtain the isolation distances. Finally, the fraction of the non-GM maize area which was covered by a certain buffer zone of any GM maize field was calculated. This area is assumed to be lost for non-GM maize cultivation if a certain isolation distance between GM and non-GM maize has to be maintained. The proportion of this "area loss" of the total remaining non-GM maize cultivation area (total maize area minus GM maize area) was then calculated (loss of non-GM maize area). Additionally, for each test region the proportion of maize cultivation area used for organically grown maize or seed maize production was evaluated, representing the "GM contamination-sensitive" maize area. These fields are assumed to achieve very low GM contamination levels and will therefore have to maintain higher isolation distances than conventional non-GM maize fields. The data for the organically grown maize area and the maize seed production area was provided by the Austrian INVEKOS system (integrated administration and control system for certain Community aid schemes according to Regulation (EC) No 2419/2001). Protection areas

corresponding to buffer zones of 300 m and 800 m around these "contamination sensitive" maize fields were generated and the extent of maize area which has to remain free from GM maize cultivation was calculated. Area loss is calculated as the percentage of the remaining maize area (total maize area minus area of organically grown maize and maize seed production).

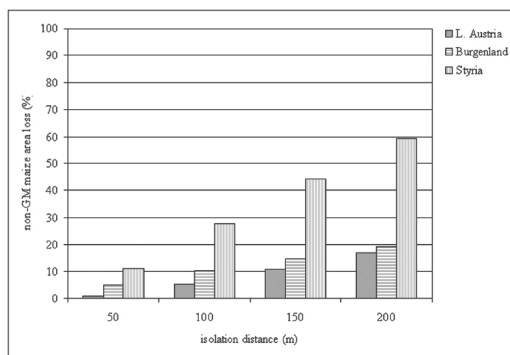
## RESULTS

### General description of the test regions

The comparison of the agricultural structures of the three agricultural regions shows that the test region in Styria has the highest percentage of agricultural area cultivated with maize fields (62%), followed by Burgenland (27%) and Lower Austria (24%). Although the test region in Styria has approximately the same area for maize cultivation as the other two regions (Styria: 157.5 ha, Burgenland: 171 ha and Lower Austria: 144 ha) this region has more than twice as many maize fields (Styria: 142, Burgenland: 57, Lower Austria: 65), indicating a rather small-scaled landscape structure. This small-scaled agricultural structure is reflected in the smaller field sizes (average 1.1 ha) and field widths (average 67 m). In contrast, in the test regions of Lower Austria and Burgenland only approximately 25% of the agricultural area is seeded to maize. Maize fields are larger and more rectangular in Burgenland (mean size 3 ha) while fields in Lower Austria are intermediate in size (mean size 2.2 ha).

### Loss of agricultural area for non-GM maize cultivation

Figure 1 shows the loss of agricultural area for non-GM maize cultivation due to the use of isolation distances when 10% GM maize cultivation is simulated. Loss of non-GM maize area increases with increasing isolation distance, with the least area lost with 50 m distances (1-11%) and the most area lost with 200 m distances (17-59%). Loss of non-GM maize area



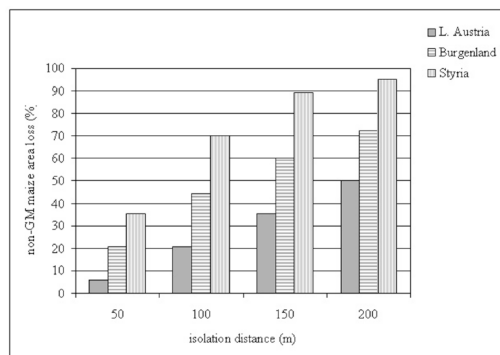
**Figure 1.** Loss of non-GM maize area in 3 different agricultural regions in Austria based on 10% GM-maize cultivation and different isolation distances. Values shown are means of 3 simulations. Mean area of non-GM maize was 141.7 ha (standard deviation = 12.1 ha).

is highest in Styria and lowest in Lower Austria for all isolation distances. Loss of non-GM maize area in Styria (11%) with isolation distances of 50 m is similar to maize area loss in Burgenland with 100 m isolation distance (10%) and Lower Austria with 150 m isolation distances (11%).

With the simulation of 50% GM maize cultivation the loss of non-GM maize area ranges from 6 to 35% when using 50 m isolation distance and reaches a maximum of 50-95% with 200 m isolation distance (Figure 2). As for the 10% simulation, area loss is highest in the test region of Styria where already 35% of non GM-maize cultivation area is lost with 50 m of isolation distance. When 200 m of isolation distance are chosen this area loss increases to almost 100%, corresponding to a complete GM maize cultivation area thus eliminating the possibility for conventional or organic maize production in this test region.

### Protection zone around “contamination sensitive” maize fields

For “contamination sensitive” maize fields such as organically grown maize or seed maize production the creation of “protection zones” is suggested. These maize fields could be protected by a buffer zone to other maize fields within



**Figure 2.** Loss of non-GM maize area in 3 different agricultural regions in Austria based on 50% GM-maize cultivation and different isolation distances. Values shown are means of 3 simulations. Mean area of non-GM maize was 78.5 ha (standard deviation = 6.7 ha).

which no GM or GM contaminated maize will be cultivated. In two of the three agricultural regions (Styria and Lower Austria) only a low percentage of maize (0.2% in Styria and 1.8% in Lower Austria) is grown organically which is represented by one single “sensitive” maize field in these two test areas. In these areas 10-17% and 27-37%, respectively, of the maize area would have to be defined as “protection area” when using buffer zones of 300 m or 800 m, respectively (see Table 1). In contrast, the third test area in Burgenland has the highest percentage of organically grown maize or maize for seed production (30.6%) which results in a high proportion of contamination sensitive maize fields (13 fields). In this region the loss of agricultural area for maize cultivation rises to almost two third of the remaining maize area when using a 300 m protection area. The use of larger buffer zones (800 m) will lead to the complete prohibition of maize cultivation and consequently to a complete GMO-free area.

**Table 1.** Loss of maize cultivation area due to protection zones (buffer zones) around “contamination sensitive” maize fields in three different agricultural areas in Austria.

Protection zone	Area loss (%)		
	Styria	Burgenland	Lower Austria
300 m	10,3	64,6	17,3
800 m	26,6	100,0	37,4

## DISCUSSION

If 10% of the area within the test areas considered in this project were seeded to GM maize the loss of the non-GM maize cultivation area due to isolation distances will be higher in agricultural regions with small field sizes, a high proportion of maize cultivation and small-scale structured landscapes than in agricultural regions with fewer maize fields, monotonous landscapes and larger field sizes. When considering 50% GM maize co-existence will be impossible in these small-scale structured regions. Already isolation distances of 50-100 m will block a considerable extent of agricultural area which cannot be used for the cultivation of non-GM maize. In contrast, in other regions non-GM maize production and thus co-existence of GM and non-GM maize will still be feasible, even at isolation distances of 200 m. If protection zones around organic maize fields are used (300 m), the majority of the remaining maize area will have to remain free from GM or GM contaminated maize cultivation in regions where the proportion of "contamination sensitive" maize is high which consequently can lead to a GMO-free area when the extent of the protection zones is increased to 800 m. In conclusion the simulations show that the structure of the landscape, the extent of the isolation distance and the proportion of maize grown organically or grown for seed production are the most important determinants for co-existence of GM and non-GM maize in an agricultural region.



# Analysing the effect of field characteristics on gene flow between oilseed rape varieties and volunteers with regression trees

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**Abstract:** The aim of the study was to analyse the effects of field characteristics (distance, areas, shapes, orientation) on gene flow between oilseed rape varieties and volunteers, in interaction with regional cropping systems. A large number of very contrasted field couples were created and used to calculate pollen and seed dispersal rates with GENESYS which quantifies the effects of cropping systems on gene flow between oilseed rape varieties and volunteers, in time and in space. In addition, contrasted cropping systems were simulated on these fields and the resulting adventitious presence of GM seeds in non-GM oilseed rape harvests calculated. This harvest contamination as well as the pollen and dispersal rates were linked to field characteristics, using regression trees. Cropping system was identified as the overall major factor. Field characteristics were only important if both GM and non-GM oilseed rape varieties were frequent in time and in space and if the non-GM varieties presented low self-pollination rates.

## INTRODUCTION

The model GENE<sub>SYS</sub> quantifies the effects of cropping systems (crop distribution, crop succession, cultivation techniques, oilseed rape varieties) on gene flow between oilseed rape varieties and volunteers, in time and in space (Colbach *et al.*, 2001a, 2001b). The model is used to study gene spread in time in a region, and, most importantly, how the characteristics of the farming region and the regional cropping system influence this gene spread. Among these, the characteristics of the regional field plan play a

major role in pollen and seed dispersal. However, their effects, in interaction with cropping systems, have not yet been precisely analysed. In addition, the calculations of pollen and seed dispersal as a function of the regional field plan characteristics are based on step-by-step algorithms estimating quadruple integrals which are highly time-consuming and need powerful computers.

The aim of the present study was to quantify the effects of the characteristics of the individual fields on pollen and seed dispersal, as well as the resulting adventitious presence of GM seeds

in non-GM oilseed rape harvests, in interaction with cropping systems. GENE<sub>SYS</sub> was used to run simulations where the characteristics of individual fields were made to vary in a large range of values while the simulated cropping systems were those identified by Colbach *et al.* (2004) for leading to contrasted situations of gene flow, ranging from maximum-risk to nearly zero-risk. The resulting pollen and seed dispersal proportions as well as the rates of harvest contamination were linked to these input variables, using regression trees. In addition to explaining the effects of the input variables, these trees could directly relate field plan characteristics to dispersal values and thus shorten calculation times in future GENE<sub>SYS</sub> simulations.

## MATERIAL AND METHODS

### Model presentation

The detailed structure of the GENE<sub>SYS</sub> model is presented by Colbach *et al.* (2001a, b). Only the main aspects will be described here. The input variables of the model are the regional field pattern comprising uncultivated road and field margins, the succession of crops of each field, the management of each crop defined by a series of cultivation techniques and rapeseed variety characteristics. The main output variables are, for each year and spatial unit, the density and genotype proportions of adult rapeseed plants, newly produced seeds and seed bank. The model comprises an annual life-cycle for the rapeseed plants, whether volunteer or cropped plants, that is simulated for each plot and year. The relationships between life-stages depend on crop type and management. Pollen and seed exchanges between plots depend on plot areas, shapes and distances as well as on rape flowering dates and harvest seed loss.

GENE<sub>SYS</sub> was evaluated by comparing its simulations to independent field data, which showed that the model correctly predicts volunteer densities, but that it underestimates harvest pollution by approximately half an order of magnitude (Colbach *et al.*, 2005a).

### Simulations

Three different output variables were analysed: the proportion of pollen dispersed from a donating to a receiving field, the same for seeds, and the proportion of GM seeds in non-GM oilseed rape harvests (hence harvest contamination).

In each simulation, the field plan was limited to two individual fields, which were obtained by combining (1) the distance between the plots (0, 10, 50, 100, 500, 1000, 1500, 2000 or 3000 m); (2) their areas (9, 100, 961 or 10000 m<sup>2</sup>); (3) their shapes (square, linear with 1-m-width or intermediate with length equating three times the width); and (4) the orientation of the two plots (parallel or perpendicular). In total, there were  $9 \cdot 4^2 \cdot 3^2 \cdot 2 = 2592$  couples of plots tested. For each of these couples, the proportions of pollen and seeds from the first plot to itself, from the first plot to the second plot, from the second plot to itself and from the second plot to the first plot were simulated and analysed, resulting into  $4 \cdot 2592 = 10\,368$  situations.

The same 2592 plot couples were used to simulate harvest contamination, but additional input variables were necessary, comprising cropping systems, initial seed bank and the characteristics of the oilseed rape varieties. In all cases, the initial seed bank of all plots was empty. In order to initialise a realistic seed bank for the simulation, the duration of the simulation was 25 years, but only the last simulated year was analysed. This pre-analysis simulation duration considerably exceeded the time period during which the initial seed bank influences harvest contamination (Colbach *et al.*, 2004). The simulated allele was a dominant *A*; transgenic varieties were *AA* and conventional ones *aa*. The other varietal characteristics were chosen according to the six contrasted cropping systems identified by Colbach *et al.* (2004), ranging from high-risk genotypes (low self-pollination of non-GM plants, large pollen emission of GM plants etc) to low-risk genotypes (high non-GM self-pollination, low GM pollen emission etc.).

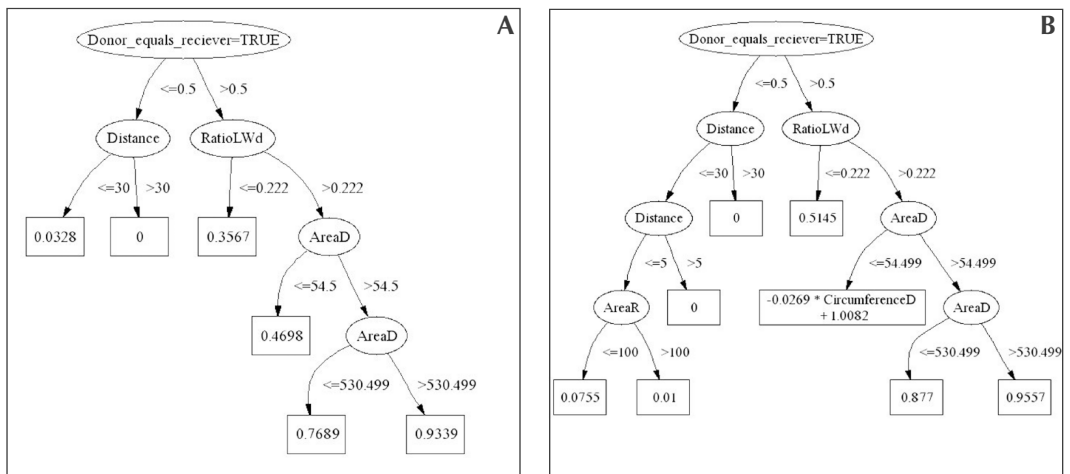
The remaining cultivation techniques were also chosen according to these contrasted cropping systems. They comprised two high-risk systems (with frequent GM rape), two intermediate systems and two low-risk systems (with GM rape only every 10 or 25 years).

For each of the 6 cropping systems × 2592 plot couples, 7 repetitions were simulated, resulting into 108 864 simulations. The 7 repetitions resulted from starting each time with a different crop from the 7-year rotation (e.g. rape/winter winter/spring barley/set-aside/rape/winter wheat/spring barley) simulated in the plot couples.

**Regression trees**

For each of the three output variables, a regression tree was fitted. Regression trees are a representation for piece-wise constant or piece-wise linear functions. Like classical regression equations, they predict the value of a dependent variable (called class) from the values of a set of independent variables (called attributes). Unlike classical regression approaches, which find one single equation for a given set of data, regression trees partition the space of examples into axis-

parallel rectangles and fit a model to each of these partitions. A regression tree has a test in each inner node that tests the value of a certain attribute, and in each leaf a model for predicting the class: the model can be a linear equation or just a constant. Given a new example for which the value of the class should be predicted, the tree is interpreted from the root. In each inner node, the prescribed test is performed and according to the result of the test the corresponding left or right sub-tree is selected. When the selected node is a leaf then the value of the class for the new example is predicted according to the model in the leaf. Tree construction proceeds recursively starting with the entire set of training examples. At each step, the most discriminating attribute is selected as the root of the (sub)tree and the current training set is split into subsets according to the values of the selected attribute. For discrete attributes, a branch of the tree is typically created for each possible value of the attribute. For continuous attributes, a threshold is selected and two branches are created based on that threshold. Technically speaking, the most discriminating discrete attribute or continuous attribute test is the one that most reduces the variance of the values of the class variable. For the subsets of training examples in each branch, the tree construction algorithm is



**Figure 1.** Regression trees for predicting pollen (A) and seed (B) dispersal (proportions dispersed from a donating field to a mean m<sup>2</sup> of a receiving field). Explanatory variables are distance between fields (in m), length/width ratio (RatioLWd) and circumference (CircumferenceD, in m) of donating field, areas of donating (AreaD, in m<sup>2</sup>) and receiving fields (AreaR, in m<sup>2</sup>), and dispersal type (identical vs. distinct donating and receiving fields).

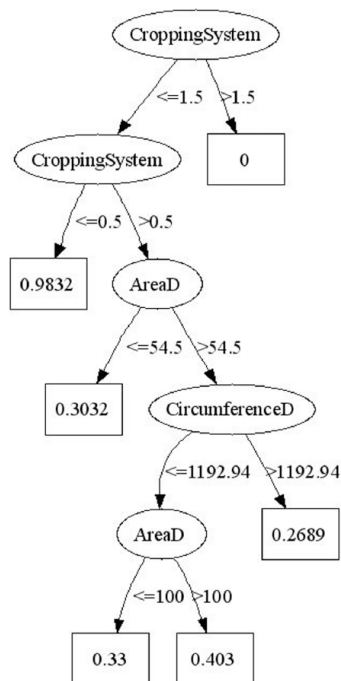
called recursively. Tree construction stops when the variance of the class values of all examples in a node is small enough (or if some other stopping criterion is satisfied). Such nodes are called leaves and are labelled with a model (constant or linear equation) for predicting the class value.

An important mechanism used to prevent trees from over-fitting data is tree pruning. Pruning can be employed during tree construction (pre-pruning) or after the tree has been constructed (post-pruning). Typically, a minimum number of examples in branches can be prescribed for pre-pruning and confidence level in error estimates in leaves for post-pruning. A number of systems exist for inducing regression trees from examples, such as CART (Breiman *et al.*, 1984) and M5 (Quinlan, 1993). M5 is one of the most well-known programs for regression tree induction. We used the system M5 (Wang & Witten, 1997), a re-implementation of M5 within the software package WEKA (Witten & Frank, 1999). The parameters of M5 were set to their default values.

## RESULTS

In all cases, the correlation coefficient  $r^2$  of the regression trees was extremely large (0.99). The structures of the pollen and seed dispersal trees were similar (Fig. 1). The main factor explaining the proportion of immigrating pollen was the "type" of dispersal, *i.e.* dispersal was larger for pollen movement from a plot to itself than from a plot to a distinct neighbour plot (Fig. 1.A). In the case of distinct plots, the only other factor was the distance between fields: below 30 m, mean pollen dispersal to a mean  $m^2$  of the receiving field was 0.03 of the production of the donating field; above 30 m, it was nil. In case of seed dispersal to neighbour fields, the area of the receiving plot also had an effect; the larger this area, the smaller the dispersal because the incoming pollen was distributed over a larger reception area.

In case of self-dispersal, *i.e.* dispersal from a plot to itself, the shape of the field was most important: self-dispersal was lower for rectangular



**Figure 2.** A regression tree for predicting harvest contamination (rate of adventitious presence of GM seeds in non-GM oilseed rape harvests). Explicative variables are area of donating field (AreaD, in  $m^2$ ), cropping system (numbered from 0=most favourable to 5=least favourable for gene flow), and circumference of donating field (CircumferenceD, in m).

(low width/length ratio) vs. square plots. This effect was amplified for seed dispersal onto small plots (area  $\leq 54.5 m^2$ ) where dispersal decreased with circumference. In the case of "squarer" plots, both pollen and seed dispersal increased with field area.

The structure of the regression tree for harvest contamination was very different (Fig. 2). The main factor was the effect of cropping system. In case of the maximum-risk systems ("0") as well as intermediate and low-risk systems ("2", "3", "4", "5"), field characteristics had no influence at all. Only in the case of the high-risk systems ("1") was there any effect of field characteristics, which were similar to those observed for pollen and seed dispersal: harvest contamination increased with the area of the gene-donating field and was more important in case of rectangular vs. square donating fields.

## DISCUSSION

The identified effects of field characteristics were consistent with previous sensitivity analyses (Colbach *et al.*, 2005b) and the knowledge on dispersal mechanisms: dispersal decreases with distance from the pollen or seed source, large areas emit more pollen or seeds and “dilute” the incoming material, rectangular plots emit more material because most of their surface is close to a neighbour field etc. In contrast to the previous study, the present work improves the knowledge on interactions, e.g. that the shape of fields is most important for small fields. The most interesting result was the interaction between cropping systems and field characteristics, showing that the latter were only important in certain situations such as the high-risk system in the present study. This system comprised frequent GM and non-GM oilseed rape crops both in space and in time and, most importantly, non-GM varieties with low self-pollination rates (50%) (Colbach *et al.*, 2004).

The present study again confirmed the overall importance of the cropping systems, overriding most of the field plan effects. To obtain satisfactory simulations with GENESYS, it is thus most important to concentrate on gathering input data on cropping system while errors on field coordinates should have less impact.

## PERSPECTIVES

Before using the present regression trees to directly predict pollen and seed dispersal rates from field characteristics, the sensitivity of the harvest contamination to these rates must be analysed more in detail. Furthermore, the present study showed the extreme importance of cropping system effects and oilseed rape varieties for harvest contamination and these factors should be studied in priority.

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## **PARALLEL SESSION**

SPATIAL ORGANISATION,  
GMO-FREE REGIONS AND  
TRANSBOUNDARY ASPECTS





# Spatial organisation, GMO-free regions and transboundary aspects

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**Abstract:** In the field of anti-GM protests, one observes that they have followed a traditional path, via parliamentary reports or NGO lobbying. However, such protests have also come to exist through deliberate illegal actions, which have, up to now, taken two paths: the destruction of GM trial crops and the creation of GM-free zones, at a municipal level, as well as on a greater scale. This movement is more important in certain countries, but not restricted to Europe. We shall first establish that these actions do clearly infringe the law, but we shall also show how, in this field, certain illegal actions have led to statutory changes. Within this framework, we shall ask ourselves what is the political and economic validity of this phenomena.



# Report on the workshop on transboundary pollen movement, June 13<sup>th</sup>-14<sup>th</sup> 2005, Neuchâtel, Switzerland: administrative and technical issues

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**Abstract:** On the request of the Swiss Minister, the four German speaking Environment ministers of Austria, Deutschland, Liechtenstein and Switzerland decided at the Potsdam meeting (16 September 04), to collaborate regarding the issue of unintentional transboundary pollen movement. The Swiss Agency for the Environment, forests and Landscapes undertook the organization of an experts meeting, in order to approach the problem regarding liability and GMOs release into the environment. Trying to find a common solution for transboundary cross pollination with all the neighboring countries, Italy and France were invited to join the meeting.

## HISTORY

Since 2004 a new law relating to non-human Gene technology (Federal law relating to non-human gene technology, GTL) came into force in Switzerland. The aim of the law is to guarantee protection and to serve the welfare of humans, animals and the environment from the abuses of gene technology. It stipulates that diversity, soil fertility and sustainability has to be conserved and consumers' freedom of choice should be enabled.

Practically, it means that GMOs shall be handled and marketed in such a way that dispersal of new traits in the environment could be excluded and that their metabolites and wastes do not impair non-GMOs production (conventional

and organic), material balance and function of the environment. Furthermore anyone handling genetically modified organisms (for the use in the environment: experimental release, cropping, marketing) should respect flow segregation, to avoid undesired mixing with non-GMOs. Concerning liability, the authorization owner is solely liable for damages that occur to agricultural or forestry enterprises, to consumers and to the environment for an extended period of 30 years at the latest.

At national level, the GTL gives sufficient protection to ensure coexistence of GMOs with conventional and organic farming as well as with the environment. The coexistence principles and measures which are currently being debated, will soon take shape.

The presence of seeds production and organic farming close to the Swiss border (ex: Rheinau ZH) raised the issue of the cross pollination of GMOs and spread of their genetic content, which is at the moment not a relevant issue within EU countries. Following European Recommendation 3003/556/CE, member states are currently establishing national strategies for coexistence and cropping management. Taking this opportunity the Swiss government would like to encourage discussion, communication strategies and developing instruments for guaranteeing GMOs and non-GMOs coexistence with its neighboring countries.

## RESULTS

Four areas of importance with respect to transboundary pollen movement were identified and need further development.

1) National registers: the participants agreed that the establishment and maintenance of national registers, containing information on the organisms, the precise sites and the time of their use, are essential tools to manage coexistence and monitoring. In principle, it is considered preferable to keep information about deliberate releases publicly accessible. Considering reasons such as risk of vandalisation or data protection, interests might be balanced by keeping part of the information confidential. It is essential that at least national competent authorities of the neighboring countries would get access to all informative and relevant data. National registers are currently under development in several countries where such a tool is not yet available.

2) Information exchange: high priority was given to the improvement of direct contacts between competent authorities of neighboring countries. It means that communication lines between authorities should be functional in order to ensure immediate and full information in case of experimental releases or cultivation in direct vicinity of a borderline. These communication lines should be developed and updated at

national and regional levels. For this purpose any national contact person should be identified and his coordinates should be available for other competent authorities. Websites links of neighboring competent authorities should be provided in any involved websites of the other competent authorities.

3) Notion of damage: It was recognized that, based on the Lugano convention, the legal framework for civil liability issues is set. Procedures for claims and redress in case of damage due to transboundary movement of transgenic pollen are clear in most countries. Particularly, there could be damage to persons (e.g. by increasing allergenicity), to property (e.g. by loss of bio label for crops) or to the environment (introgression of transgenes into genomes of wild flora). There is, however, a need for clarification with respect to the notion of what a damage is. For instance, concerning contamination of neighboring fields the notion of damage is not yet clear.

Taking into account that GMO contamination can occur after harvest during the processing of food and feed it is widely accepted not to tolerate a contamination of 0.9 %. It is currently debated what level of contamination in neighboring fields should be tolerated beneath this level.

4) Monitoring: Monitoring the effects of transgenic organisms in the environment is an important issue in all countries. With respect to transboundary pollen movement, it was recommended to continue and even to strengthen the research efforts. The participants pointed out mutual information on research projects dealing with monitoring issues, projects and methods for GMO detection and dispersal should be exchanged between involved interested parties. This would include e.g. the development of methods for the detection of GMO in the environment, as well as the identification of useful indicators for certain effects. In the context of monitoring, it is important to note that any other spread of transgene material such as seeds has to be taken into consideration, as it should be provided with every notification according to EU regulation.

## CONCLUSION

The transboundary pollen movement issue was put onto the agenda of the Environment ministers meeting (Austria, Deutschland, Liechtenstein and Switzerland) which took place in Liechtenstein, on the 5<sup>th</sup> October 2005. The final report of the workshop and the following proposals were submitted to them:

- the Ministers shall take note of the workshop report
- they would support the conclusions of the workshop
- they should stress existing information systems and count on further improvement in communication and transparency between neighboring countries, concerning transboundary pollen movement (and spread of transgenes) issues
- they should specifically encourage the development and maintenance of national registers for GMO experimental releases, cultivation and marketing
- they should encourage access to all relevant data to competent authorities of neighboring countries
- they should promote exchange on various issues such as monitoring programs for transgenes detection, transgenes dispersal as well as for environmental damage
- they support further discussions between neighboring countries on the notion of damage

The plenary recognized the need to keep authorities and all concerned parties informed of uses (cropping practices, experimental releases, cultivation) along the borderline and the potential of unintended transboundary outcrossing. Participants considered the meeting to be a first step to address this issue.



# Report on the workshop on transboundary pollen movement, June 13<sup>th</sup>-14<sup>th</sup> 2005, Neuchâtel, Switzerland: legal issues

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The risk of transboundary pollen movement (tpm) and the possibility of damages which can occur as a consequence of tpm raise various legal questions:

- Which national or international regime on liability and redress would apply if damages occur?
- Which types of damages would be covered under the relevant regulations?
- What is the relevance of different national regulations for farmers located near the borderline?
- What measures have to be taken to prevent tpm?

It is recognized that based on the Lugano Convention (Convention on jurisdiction and the enforcement of judgements in civil and commercial matters) the legal framework for civil liability issues is set. Procedures for complaints and redress due to tpm are in most countries clear.

Principally there could be damage to persons (e.g.

by increasing allergenicity), to property (e.g. by loss of bio label for crops) or to the environment (introgression of transgenes into genomes of wild flora).

Measures to prevent tpm were given high priority:

- The general obligation of states to prevent major damages in neighbouring countries was highlighted.
- It was stipulated that the environmental risk assessment procedures for the release of GMOs have to be carried out carefully.
- Problems around coexistence should be addressed in order to guarantee the freedom of choice for consumers.
- Prevention could also address handling with GMOs and should be observed and regulated at any early stage before using GMOs in the environment.
- Direct contacts between competent authorities of neighbouring countries with respect to handling with GMOs should be improved.





# **POSTERS SESSION**



# Use of coupled atmospheric and dispersion models to evaluate strategies for pollen confinement

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**Abstract:** A methodology for evaluating strategies to reduce fugitive pollen from genetically modified (GM) crops has been developed by coupling a hydrodynamic model of wind and turbulence around porous obstacles with a Lagrangian-stochastic model for particle motion and deposition. The coupled models are used as a preliminary screening tool to assess strategies that merit further testing in field studies. The conceptual framework and example results are shown, and a comparison with field observations is discussed.

## INTRODUCTION

Adoption of genetically-modified (GM) crops has motivated research on methods to reduce outcrossing with non-GM crops. Protocols for minimization of adventitious outcrossing due to windborne pollen transport often have been based on rules of thumb or results obtained from limited observations, and it is reasonable to ask whether improvements can be made to these approaches. Proposals for refined methods of pollen confinement require verification by field studies. Because field studies are expensive and time-consuming to perform, the number of approaches that can be tested is severely limited.

We have developed a framework for objective assessment of methods to reduce long-range transport of pollen by combining a numerical

hydrodynamic model of fluid (atmospheric) flow around obstacles and porous media with a Lagrangian model for transport and diffusion of particles. The modeling approach is used as a screening tool to evaluate hypothetical field designs, so that the most promising designs can then be evaluated in field studies. The model results also are used to guide sampling strategies by indicating the locations where pollen samples or meteorological measurements should be made.

## METHODS

Strategies for pollen confinement are assessed by coupling two mathematical models. The first is a hydrodynamic model that predicts airflow and turbulence expected for a hypothetical field design. This model is described in detail by

Wang and Takle (1995) and is based on the non-hydrostatic, anelastic equations of atmospheric motion. The second model is a Lagrangian-stochastic model that predicts the motions of tracer particles in a turbulent flow (Arritt et al. 2004). In the present application, these tracer particles are interpreted as a sample of the pollen grains that are shed from maize plants in a source plot. The Lagrangian-stochastic model includes both the mean and turbulent parts of the flow. For a sample of  $N$  particles identified as  $i = 1, 2, \dots, N$ , the turbulent component  $u'$  for the  $i$ th particle evolves from time  $t$  to time  $t+\Delta t$  as a first-order Markov process given by:

$$u'_{ik}(t+\Delta t) = u'_{ik}(t)R_{ik}(\Delta t) + u''_{ik}(t), \quad k = 1, 2, 3$$

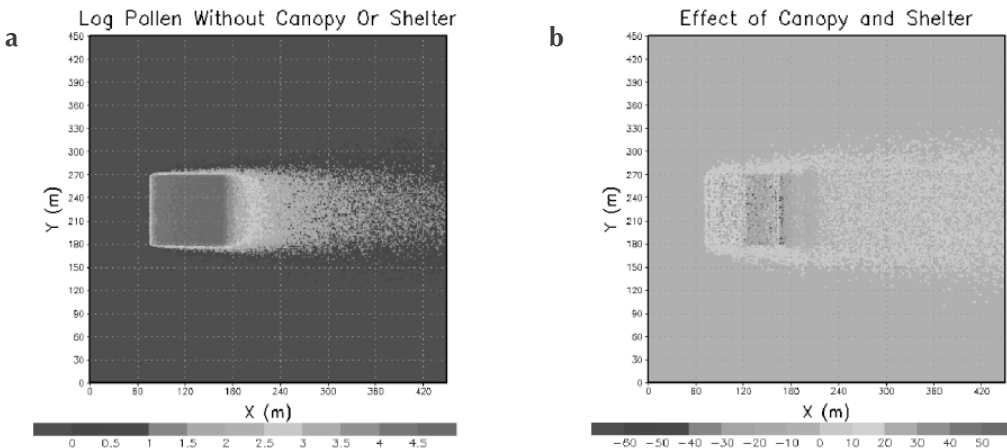
where  $k = 1, 2, 3$  represents the three dimensions of space and  $R_{ik}$  is the autocorrelation function for lag time  $\Delta t$ . The random component  $u''_{ik}(t)$  is

$$u''_k(t) = \gamma \sigma_k (1 - R_k^2(\Delta t))^{1/2}, \quad k = 1, 2, 3$$

where the standard deviation  $\sigma_k$  is obtained

from the turbulent kinetic energy predicted by the hydrodynamic model and  $\gamma$  is a unit random deviate with zero mean. We adjust the vertical (i.e.,  $k=3$ ) velocity for the well-mixed condition described by Thomson (1987), by including a drift correction term following Legg and Raupach (1982). The terminal fall speed of pollen grains also is superimposed on the vertical velocity.

We have used the combined models to study the effect of porous barriers (windbreaks) on the upwind and downwind sides of an isolated GM maize plot. First, predicted winds and turbulence are obtained from the hydrodynamic model. These wind and turbulence fields are then used in the Lagrangian-stochastic model in order to track the movement of a sample of pollen grains shed from maize canopy height (assumed to be 2 m) in a source plot. The Lagrangian-stochastic model usually employs about 500,000 test particles but we have recently implemented an increase in computing capability that will enable tracking of about 10,000,000 particles. Pollen deposition is computed as the particles are transported away from their source and fall to a predefined receptor height.



**Figure 1:** (a) Base-10 logarithm of pollen deposition (grains per square meter) from a 1 ha test plot without considering effect of canopy height or surrounding by a shelter; (b) Change in base-10 logarithm of pollen deposition when including the effect of canopy height and a surrounding shelter of 3 m height.

## RESULTS

We have used the combined hydrodynamic and Lagrangian-stochastic models to test the possible reduction of fugitive pollen by surrounding a small test plot with barriers of various heights. By comparing simulations with and without the barriers we can assess the effectiveness of this design for restricting transport of pollen. An unexpected result of the model assessment is that the barrier on the downwind side of the plot has more benefit than the barrier on the upwind side. That is, sedimentation of pollen in the calm zone to the lee of the downwind barrier is more beneficial in terms of restricting fugitive pollen than is the windbreak effect of the upwind barrier. It should be noted that the windbreak effect interacts with the turbulence and surface friction generated by the canopy itself; that is, the difference in roughness between the maize test plot and its surroundings contributes to alteration of the pollen deposition pattern.

## DISCUSSION

We recently completed a field experiment during the 2005 season that was designed to test the screening methodology. We planted barrier strips (4 m wide) of sudangrass on all sides of a 1 ha test field of maize, surrounded by approximately 260 ha of soybeans. A second field was located approximately 2 km away, of identical configuration except that it lacked the barrier strips. The effect of the barrier strips on pollen confinement is being evaluated by comparing deposition of pollen traps onto sticky traps at various distances around each field. Lofting of pollen by turbulence and organized vertical motions also is being evaluated from pollen traps that were hung on 15 m tall towers. If the model results are confirmed by the field results, the tested configuration could be recommended a simple and economical method for decreasing flow of pollen from GM crops to their surroundings. Such confirmation would also give confidence in the utility of the coupled numerical model as a screening tool for hypothetical confinement strategies.

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# Cross fertilization in maize under the Swiss agricultural conditions

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**Abstract:** In 2003 and 2004, we performed a study on cross pollination of maize in different regions representative for Swiss agriculture. We applied a straight forward method to study cross pollination, based on the use of yellow-kernelled maize (simulating transgenic maize) close to white-kernelled maize fields (simulating conventional maize). Due to this simple but meaningful experimental setup, a large number of samples could be collected across different climatic and topographic scenarios. From our first results, we can tentatively say that distances of 50 m between conventional and GMO fields should lead to outcross rates below the threshold of 0.9%.

## INTRODUCTION

The extensive adoption of commercial transgenic crops in many European countries is foreseeable. In Spain and Rumania, the growth of transgenic maize has already become a reality. In Switzerland, there are at the moment no special needs to grow transgenic crops. This situation, nevertheless, could change with changes in consumers and environmental demands.

In order to confront this eventual adoption of GM crops in Switzerland with defined rules of coexistence, different studies need to be done.

During the last two years, we focused our studies on the transgenic pollen flow and cross fertilization in maize under different environmental and topographic scenarios, in order to set secure isolation distances between a GM and a conventional maize field.

## MATERIAL AND METHODS

The cross pollination study was done without using transgenic plants, but using maize varieties with different kernel colors. A white-kernelled hybrid maize of the company DSP Delley seeds and plants AG was used to check the cross pollination of yellow-kernelled maize fields, because yellow kernel color is dominant to white kernel color. If pollen of yellow-kernelled maize fertilizes on white-kernelled maize, there will be a yellow kernel on the white-kernelled ear for each successful pollination. By just counting the yellow kernels on white-kernelled 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.

Long distance cross pollination was investigated in the Urner Reusstal (Switzerland, Canton Uri), a region with a low density of maize fields but high

**Table 1.** Rate of out-crossing, in dependence of the distance between the yellow- and the white-kernelled maize field.

Field No.	Distance [m]	Size of the field [ha]	Rate of out-crossing [% of yellow on total no. of kernels]
1	52	0.70	0.009
2	85	0.46	0.015
3	105	0.45	0.006
4	125	0.50	0.020
5	149	1.00	0.016
6	150	0.45	0.014
7	200	0.80	0.017
8	287	0.50	0.005
9	371	0.25	0.008
10	402	1.00	0.005
11	458	1.44	0.000
12	4125	0.50	0.011
13	4440	0.50	0.001

density of grassland. This region, surrounded by up to 3000 m high mountains and the Vierwaldstatter Lake, is representative for agricultural areas close to mountains with prevailing grassland use and a few fodder maize fields. Here, 13 field experiments were conducted. The distances between yellow and white pollen sources (with field sizes of 0.5 to 3 ha) were of 52 m to 4440 m.

## RESULTS AND DISCUSSION

In the case of the long distance cross pollination experiments, the cross pollination rate was always below 0.017%. The minimum distance between a yellow and a white maize field of 52 m was more than enough to ensure an extremely low rate of out-crossing (Table 1).

## PERSPECTIVES

In the frame of the EU project "SIGMEA" (Sustainable Introduction of GMOs into European Agriculture), further studies on short distance

cross pollination will be performed, and the dynamics of pollen flow, as affected by thermal up winds and further climatic phenomena, will be investigated. The flow of the existing and the new data into computer based models should contribute to define case by case scenarios of coexistence of GM and conventional crops.

## ACKNOWLEDGEMENTS

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# Co-existence of GM, conventional and organic crops: commercial experience

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**Abstract:** This paper provides an overview of co-existence experience of GM, conventional and organic crops in commercially grown crops. It presents the crop stewardship guidelines required of farmers in countries (notably North America) where GM crops are widely grown and summarises the experiences. It shows that co-existence is not only possible but is allowing farmers to grow crops using different production systems without causing economic problems.

## CO-EXISTENCE REQUIREMENTS IN NORTH AMERICA

All suppliers of GM seed to farmers in North America provide farmers with 'Technology Use Guides' or 'Crop Stewardship Guides'. These provide recommendations for use of the GM products (eg, herbicide use for weed control recommendations) and some advice on 'co-existence issues' that target maintaining the purity of non GM crops growing on GM crop planting farms, on nearby farms, in storage or when supplied to buyers. For example, in relation to GM corn, farmers are provided with information and advice to help them meet the requirements of different corn markets, including speciality markets (eg, seed, waxy, high oil), non GM and organic markets covering:

- Pollen movement: ways of minimising the chances of cross pollination through the siting of crops in relation to prevailing wind directions, use of buffer crops and barriers, timing of plantings, varieties planted (with different flowering times), separation distances and removal (ie, separate harvesting and segregation) of outer strips of crop in a field (eg, some speciality corn crops require the removal of the outer 9 metres (30 feet) of a crop to ensure the removal of impurities from adjacent (non speciality) corn crops);
- Holding discussions with neighbours about planting intentions;
- Holding discussions with grain buyers to ensure that contractual requirements are identified (eg, whether GM traits not yet approved for importation into the EU are accepted).

All farmers of herbicide tolerant crops are also provided with advice on managing volunteers in crops<sup>1</sup>. This advice covers aspects of an integrated weed management system, the majority of which is equally applicable to non GM varieties of these crops, and includes crop rotation, rotation of herbicides, rotation of herbicide tolerant traits, rotation of timing of herbicide applications, rotation of timing of tillage and use of certified seed.

## CO-EXISTENCE EXPERIENCE IN NORTH AMERICA

The evidence to date shows that GM crops, which now account for the majority (65%) of total soybean, corn and canola grown in North America, have also successfully co-existed with conventional and organic crops without significant economic problems.

Survey evidence amongst US organic farmers shows that the vast majority (92%) have not incurred any direct, additional costs or incurred losses due to GM crops having been grown near their crops. Only 4% had any experience of lost organic sales or downgrading of produce as a result of GM adventitious presence having been found in their crops (the balance of 4% had incurred small additional costs for testing only).

The US organic areas of soybeans and corn have increased by 270% and 187% respectively between 1995 and 2001<sup>2</sup>, a period in which GM crops were introduced and reached 68% and 26% shares of total plantings of soybeans and corn. The states with the greatest concentration of organic soybean and corn crops are also often states with above average penetration of GM crops (eg, the leading organic corn growing states are Iowa, Minnesota and Wisconsin, of which Iowa and Minnesota have above average penetration of GM crop plantings - 32% and 36% respectively of total corn plantings relative to the US average of 26% in 2001).

A small number of instances of adventitious presence of GM events have been found in non

GM and organic crops (and resulted in possible rejection of deliveries by buyers or imposition of contractual price penalties). Often this has been due to deficiencies in segregating/channelling crops once harvested, in storage or transport. Some instances may also have arisen from the use of conventional (non organic) seed with low levels of GM adventitious presence.

The only crop/sector where there appear to be disputes about the feasibility of co-existence between GM and non GM/organic crops<sup>3</sup> is canola, in Canada. Some analysts have suggested that the planting of GM canola has resulted in problems for both GM and non GM canola farmers mostly related to the control of volunteers, resistant to one or more of the herbicides used with GM (herbicide tolerant) crops<sup>4</sup> in subsequent crops. Two key points are important to recognise in respect of this issue:

- It relates to herbicide use and weed resistance (to specific herbicides). As such, it applies to all forms of canola on which herbicides are used. This includes conventional (non herbicide tolerant) canola, canola with non GM herbicide tolerance, and GM herbicide tolerant canola. In other words this is not a GM-specific issue, as illustrated by the provision of volunteer management advice to farmers;
- A number of bodies and published data suggest that this is not a major issue or problem. For example, research by the Canola Council (2001 and 2005) amongst canola growers found that farmers considered using strategies to minimise the development of weed/pest resistance as 'normal husbandry practice' and about 60% of adopters of GM canola perceived that herbicide management to avoid weed resistance had been made easier as a result of using GM canola. Only 7% perceived it had been made more difficult (the balance perceived no change). In terms of volunteer canola management in subsequent crops 60% perceived that management was about the same as before, 16% indicated it was easier and 23% thought it more difficult. The more

recent work from 2005 found that volunteer control measures taken by farmers are not materially different for GM or non GM canola crops. This suggests that volunteers do not appear to be a problem for most of the farmers. It is also interesting to note that one of the GM technology providers, Monsanto offers a free GM canola volunteer removal service but reports few calls and requests for this service.

It should also be noted that given the historically low area planted to organic canola<sup>5</sup> and the current existence of some organic plantings (about 2,000 hectares in Canada), GM and organic canola is co-existing. These organic growers may have made some changes to farming practices in order to successfully co-exist (eg, ensuring reasonable separation distances, testing seed prior to use, operating rigorous control of volunteers and sowing *brassica rapa* varieties).

## CONCLUSION

Overall, co-existence of GM and non GM, including organic, crops has been successfully occurring in countries where GM crops are commercially grown, without causing any economic problems.

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<sup>1</sup> See for example CropLife Canada, Controlling herbicide tolerant volunteers in a succeeding crop: a best practice guide. [www.croplife.ca](http://www.croplife.ca)

<sup>2</sup> Latest available data from the USDA

<sup>3</sup> This refers to presence of GM material being found that may impact economically on the grower. In other words, GM material may be found in non GM crops grown on adjacent land to a GM crop, but is not of relevance to the non GM farmer if the market the crop is sold into (or its use) is indifferent to whether it is GM derived or not, or the level of GM presence is below a contractual or labelling threshold (eg, 0.9% in the EU)

<sup>4</sup> This also applies to volunteers of canola crops resistant to herbicides in which the herbicide resistance has been incorporated into canola through non GM methods

<sup>5</sup> This essentially reflects difficulties in growing organic canola and the limited nature of the market



# Coexistence and landscape gene flow with threshold effect: the case of genetically modified herbicide tolerant oilseed rape (*B. napus*)

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**Abstract:** Contamination of conventional crops represents a “negative externality”. By taking genetically modified herbicide tolerant oilseed rape (GM HT OSR) as a model crop and starting from an individual pollen dispersal function, we develop a computer simulation to assess the effect of the size of the source/sink populations and the degree of spatial aggregation on the extent of crop contamination.

## INTRODUCTION

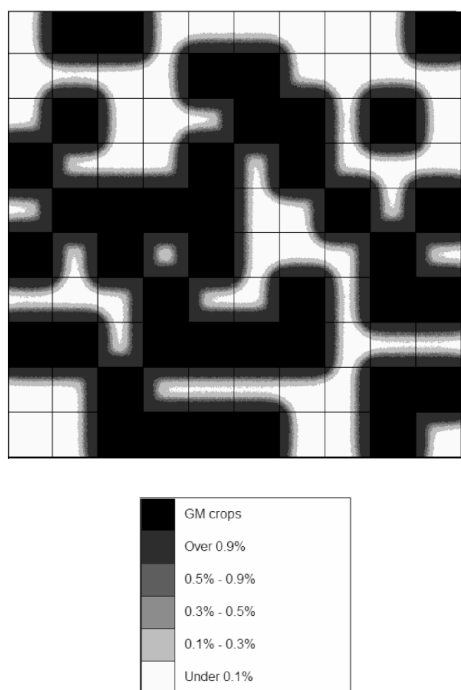
In Europe the main concerns associated with the introduction of GM crops are reflected in the coexistence debate (e.g. Bock *et al.*, 2002; Boelt, 2003). The ability of transgenic crops to produce pollen and to “contaminate” conventional (and organic) produces has led the European Council to adopt two important regulations on GM food and feed and to establish the maximum level of tolerance for adventitious presence of GM material in conventional product at 0.9%. Beyond this threshold, products have to be labelled as containing or originating from GM material. Therefore, should a premium for non-GM products appear on the market, contamination with GM will generate a negative externality on conventional growers. The external cost will exhibit a *threshold effect*: it will be zero for levels of contamination

below 0.9% and it will be positive for levels of contamination above 0.9%. In this paper we focus on oilseed rape, which is known to exhibit a variable level of outcrossing. gene flow through pollen is controlled by a number of factors (e.g. Eastham & Sweet, 2002) including level of outcrossing, mode of pollen dispersal, etc. A large body of research has established that pollen concentration decreases rapidly within a few metres from the source. This can be represented graphically by a leptokurtic curve (e.g. Lavigne *et al.*, 1998). The main objective of this work is to assess the level of contamination in the conventional fields as a function of the size of the source and sink areas (i.e. GM and conventional crop acreage respectively) and the level of aggregation. The hypothesis to be tested refers to the relevance of the above mentioned factors in explaining transgene presence in conventional crops.

## RESULTS

Starting from an individual pollen dispersal function, as obtained by Lavigne *et al.* (1998), we develop a computer simulation (Figure 1) to test our hypothesis.

The level of aggregation is computed using the aggregation index (AI) developed by He *et al.* (2000). The aggregation index (AI) is a number between 0 and 1. AI is equal to 0 (will be equal to 1) when the spatial configuration is completely disaggregated (aggregated)



**Figure 1.** Simulation results showing contamination of non GM fields with GM pollen

For each run of the simulation we retain data on the percentage of the total landscape planted with GM crops (PGM), the aggregation index (AI), the percentage of the total conventional crop contaminated above 0.9% (PC) and the average level of contamination for the overall conventional crop (AC). In order to test our hypothesis we use the dataset generated by our simulations to estimate the following relationships:

$$PC = \beta_0 + \beta_1(PGM) \quad (1.a)$$

$$PC = \alpha_0 + \alpha_1(PGM) + \alpha_2(AI) \quad (1.b)$$

$$AC = \lambda_1(PGM) \quad (2.a)$$

$$AC = \gamma_0 + \gamma_1(PGM) + \gamma_2(AI) \quad (2.b)$$

where from the theory of pollination ecology (e.g. Handel, 1986) we expect  $\alpha_1 > 0$ ,  $\alpha_2 < 0$ ,  $\beta_1 > 0$ ,  $\gamma_1 > 0$ ,  $\gamma_2 < 0$  and  $\lambda_1 > 0$ . The results are summarised in tables 1 and 2 respectively.

**Table 1.** Regressions (1.A) and (1.B)

Variable	Model 1.A	Model 1.B
	Coefficient	Coefficient
Constant	19.86***	80.91***
PGM	1.05***	1.04***
AI		-91.4***
Adj. R <sup>2</sup>	83.303	99.993

**Table 2.** Regressions (2.A) and (2.B)

Variable	Model 2.A	Model 2.B
	Coefficient	Coefficient
Constant		0.167***
PGM	0.033***	0.032***
AI		-0.199***
Adj. R <sup>2</sup>	98.930	98.797

\*\*\* Significant at 0.1% level

The results expressed in Tables 1 and 2 illustrate that the proportion of the conventional crop contaminated above 0.9% (PC) increases with the increase in the GM area and decreases when the spatial configuration becomes more aggregated. On the other hand, when looking at the average level of contamination among the whole conventional produce in the landscape (AC) (e.g. because of grain pooling) the level of spatial aggregation is not significant. This probably occurs because when looking at AC the effects of AI get diluted across all non-GM fields. In interpreting the results it is important to bear in mind that in our simulations, no specific practices to limit transgene contamination (e.g. adoption of buffer zones) are taken into account.

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# Case study of co-existence and Bt maize growing in the Czech Republic

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**Abstract:** Legislation including measures for genetically modified maize grown in the Czech Republic entered into force in April 2005. These measures result from Commission recommendation from 2003 dealing with the issue of co-existence. Isolation distances (different in relation to organic farming) and record keeping of GM maize were laid down as obligatory measures. In 2005, 52 farmers elected to grow Bt maize MON 810 that was sown for the first time on 270 ha. On more than half of the whole area, Bt maize was grown on parcels under 1 ha.

## INTRODUCTION

In the EU, a strong debate is currently being held on the use of GM crops and their co-existence with conventional and organic crops. With regards to the consumers' demand for products not containing GM crops or products not derived from them, a legal requirement on labelling of products derived from or containing more than 0.9% of GMO has taken effect. In addition to that, there is zero tolerance of GMO in organic products, in the Czech Republic. About 300,000 ha of maize is grown in the Czech Republic (75% of the area harvested as silage/green maize). A small acreage, about 400 ha, is grown as organic production.

## LEGISLATION FOR GM CROPS GROWING IN THE CZECH REPUBLIC

In 2003, the European Commission issued recommendation on co-existence issue. In order to ensure the co-existence in the Czech Republic, fundamental measures were implemented in the framework of a government decree No 145/2005 Coll. laying down conditions for complementary national direct payments in respect of the year 2005. Such conditions, which took effect in April 2005, are valid for GM maize in 2005 only. GM maize MON 810 is the only commercially grown GM crop in the country. Among the measures controlling GM crops are isolation distances

between different types of fields with the same crop and record keeping of the fields with GM crops. Isolation distances are set as follows: 100m between GM maize and conventional maize and 50m between GM maize and conventional maize with buffer zone of at least six rows of conventional maize around the GM maize. Similar to this, isolation distances towards the fields with organic maize are set as 600 and 300m with respect to the above mentioned zero tolerance in organic farming.

No payment is granted for the area with GM crops in case of not complying with the rules. General measures controlling the growing and handling of all the approved GM crops should be subject to amendment of general Act No. 252/97 Coll. "on agriculture" and should be followed by crop specific public notices. The above-mentioned general Act on agriculture should set more obligatory record keeping of and reporting on the fields with GM crops. Next year, we can expect shortening of isolation distances of maize based on new research results on co-existence within the EU.

## THE GROWING OF GM MAIZE IN THE CZECH REPUBLIC IN 2005

Bt maize MON 810 was sown by Czech farmers for the first time in 2005, except for small field trials in the past several years. Two seed companies offered varieties of Bt maize MON 810 to the farmers and Bt maize was sown on total of 270 hectares. Most of the fields sown with Bt maize were under 1ha (59%). More than one third of the total Bt maize was grown on three fields (see Table 1). A total of 52 farmers decided to grow Bt maize in 2005. More than half of the subjects growing Bt maize were corporate; almost 90% of the subjects growing Bt maize were legal persons (corporate + cooperative) with total acreage of 93%. Almost half of the Bt maize acreage was run by cooperatives (see Table 2).

**Table 1.** Bt maize growing in the Czech Republic depending on the field size.

field size	Nr. of fields	% of fields	ha	% ha
less than 1ha	37	59	14,32	5
1,1-10 ha	16	25	40,06	15
10,1-20 ha	7	11	113,01	42
20,1-30ha	0	0	0	0
more than 30 ha	3	5	102,59	38
<b>Total</b>	<b>63</b>	<b>100</b>	<b>269,98</b>	<b>100</b>

**Table 2.** Bt maize growing in the Czech Republic with respect to the business type.

business type	Nr.	% of fields	ha	% ha
corporate	27	52	122,17	45
cooperative	19	37	128,70	48
natural person	4	8	14,71	5
other	2	4	4,40	2
<b>Total</b>	<b>52</b>	<b>100</b>	<b>269,98</b>	<b>100</b>

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Commission Recommendation Nr. 2003/556/EC on guidelines for development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming.

# Detection of approved and unapproved GMO by the “matrix approach”

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The European directive and regulations 2001/18/EEC, 1829/03/EC and 1830/03/EC make mandatory the traceability and labeling of food products above a threshold of fortuitous presence. Two thresholds have been established: a threshold of (i) 0.9% for approved GMO and (ii) a threshold of 0.5% for GMO still unapproved GMO but with a positive safety's evaluation of EFSA. According to 1829/03/EC regulation, specific and quantitative detection methods have to be provided by GMO notifiers. However, cost-effective and reliable routine detection methods, such as screening methods or methods to detect unapproved GMO, are not covered by European regulation.

The development and application of such reliable, qualitative or quantitative analytical detection methods are thus essential in order to allow free choice for consumers by completing in a cost-effective way the regulatory methodological arsenal.

We describe in this poster a PCR based detection strategy called the “Matrix Approach” we previously described in the GMOChips research program. The matrix approach is based on the combined, and preferably simultaneous, detection of several short DNA sequences, some

of them already used for GMO screening, thus applicable to processed material. The balanced choice of such sequences lies on their occurrence frequency into worldwide growing GMO as well as the nature of the sample to be analyzed and can thus be readjusted according to the approval process.

This detection methodology, and its different ways of application, should be able to distinguish both approved GMOs, using previously established patterns of amplification, and unapproved GMO, by discrepancies of patterns between those of approved GMO and samples.

The use of DSS (decision support system) such as decision trees is highly desirable for complex situation of data analyses as it is in general for deciding which detection methods have to be applied to unknown complex samples.

This matrix approach can be used with both qualitative and quantitative PCR, into simple or multiple sampling plans by attributes, combined or not to micro-array hybridizations. For quantitative tests, the matrix approach can also be used for the “Quantitative Differential Analysis”, a detection strategy to detect unknown GMO also studied in our laboratory.



# Using microsatellite markers to estimate pollen dispersal patterns of oilseed rape at the landscape level

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**Abstract:** We analysed the progeny of male-sterile plants scattered over a French production area using a spatially explicit pollination model that accounts for pollen dispersal from (i) characterised fields grown in the study area through a dispersal kernel (i.e. a function that describes the probability that an efficient pollen grain falls at a given distance from its source) and (ii) uncontrolled pollen sources through a background pollen cloud. The dispersal kernel that we estimated predicts more long-distance pollination than all functions estimated at a smaller scale. We also found that the composition of the sampled pollen clouds was only partly explained by the position and composition of fields. About 50% of the pollen sampled by the male-sterile plants was common to all sites and attributed to uncontrolled sources meaning that information on the distribution and composition of fields grown in the study area is insufficient to accurately predict the composition of the pollen cloud at any position in the landscape. Nevertheless, we showed that the immigrant pollen cloud that would contribute to the progenies of feral populations would be composed of pollen from several distant fields.

## INTRODUCTION

Estimating the frequency of long-distance pollination, i.e. the shape of the tail of pollen dispersal kernel, is important to assess the risk of transgene transfer following the release of genetically modified crops. This kernel is a function that describes the probability that an efficient pollen grain falls at a given distance from its source. Its shape determines the composition of the fertilizing pollen clouds over a landscape and thus the pattern of pollution of conventional crops. Previous studies have mainly

focused on markers exhibiting low variability (such as herbicide resistance) and only few studies attempted to estimate pollen dispersal kernels. The challenge now stands in (i) checking if these small-scale estimates remain valid when extrapolated to larger distances and/or (ii) estimating pollination patterns directly from landscape-scale data. We answer here these two questions by estimating pollination patterns within a spatially explicit pollination model (Burczyk *et al.* 2002), that includes genotypic data previously collected in a French oilseed rape production area.

## MATERIAL AND METHODS

### Collecting experimental genotypic data

The study area was a 10 x 10 km square within a bigger production area of winter oilseed rape (Loir-et-Cher, France). In 2002, 82% of the fields were characterised (from both surveys and genetic analyses). We sampled 1960 seeds on 49 male-sterile oilseed rape plants (Yudal spring cultivar) positioned at 12 different sites during two successive replicates of 7 and 10 days, respectively. These sites were either 50 m or 300 m away from the closest oilseed rape field (details in Devaux *et al.* 2005).

We genotyped seeds of the male-sterile cultivar, seeds collected from the male-sterile plants and seeds of 20 cultivars at nine microsatellite loci specifically selected for discriminating the 17 cultivars grown in the 2002 study area. The three cultivars that were not grown in 2002 as fields within the study area were also considered as potential sires because they might contribute to pollination within the study area.

### Modelling the total fertilizing pollen clouds above male-sterile plants

We modelled the fertilizing pollen cloud above each site of male-sterile plants as composed of an expected pollen cloud (EPC), i.e. pollen coming from characterised fields, and a background pollen cloud (BPC), i.e. pollen coming from uncontrolled pollen sources (volunteers, feral plants and uncharacterised fields, within and outside the study area). The contribution of a field to the EPC above each site depended on (1) the distance between that field and the site through the dispersal kernel, (2) the surface area of the field and (3) the contributions of all other characterised fields. Three families of dispersal kernels were used: the exponential, the exponential power and the geometric kernels. These functions can predict different proportions of long-distance pollination according to their parameters. The BPC was common to all sites and composed of the 20 genotyped cultivars at estimated frequencies.

### Estimating the parameters of the pollen dispersal model

We assumed that each seed had been sired by a pollen grain randomly drawn from the fertilizing pollen cloud above its site. We performed a maximum likelihood estimation of the proportion and composition of the BPC and the parameters of the dispersal kernels.

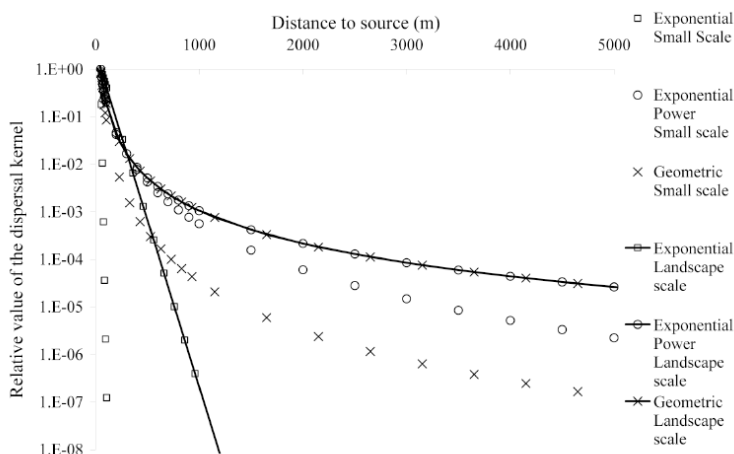
Using likelihood ratio tests, we compared the performance of four biological hypotheses by investigating the following cases. Case 1: there was no effect of distance to fields on the composition of the pollen clouds. Case 2: pollination patterns in the study area were predicted from pollination patterns estimated from a smaller-scale experiment (Klein *et al.*, in press). Case 3: all parameters were jointly estimated. Case 4: frequencies of cultivars were estimated independently in each of the 12 pollen clouds.

## RESULTS

We found a significant effect of the spatial distribution of fields on the sampled pollen clouds: the complete model (Case 3) achieved a significantly better fit than the model including one BPC (Case 1). This result is consistent with the high level of differentiation among pollen

clouds, measured by a  $\phi_{FT}$  of 0.214. However, this differentiation pattern was not completely explained by that complete model: the model including 12 different fertilizing pollen clouds (Case 4) reached a significantly better fit.

Using the complete model (Case 3), the exponential kernel led to a significantly worse fit than the exponential power. The exponential power and the geometric kernels achieved the same fit and both predicted significantly more long-distance pollination than those estimated in a previous and smaller experiment (comparing Cases 3 and 2, Figure 1). For all dispersal kernels, the estimates of the scale parameter gave inconsistent values.



**Figure 1.** Relative values of the estimated dispersal kernels, with distance to source, using the complete model (Case 3, Landscape scale) compared to those estimated in a previous experiment (Case 2, Small scale). Open squares hold for the exponential functions, the open circles for the exponential power functions and the crosses for the geometric function. All functions were normalized by the value they took at 50m (see Results).

When using both the exponential power and the geometric kernel in the complete model (Case 3), the BPC represented 47% of the fertilizing pollen clouds. Its composition included cultivars that were not grown as fields within the study area in 2002. It was significantly different from the distribution of cultivars grown in 1999 (year of the previous rotation) in 2001 and in 2002 (data not shown).

## CONCLUSION AND PERSPECTIVES

We investigated the frequency of long-distance pollination of oilseed rape at the landscape level. Our model was based on a pollen dispersal kernel and did not aim at separating insect versus wind pollination. Pollen dispersal kernels supposedly do not depend on the shapes, sizes and locations of the source and recipient populations, this assumption being questionable for insect-pollinated species (Cresswell & Osborne, 2004).

The inconsistency of the estimated scale parameters of all dispersal kernels could be due to the distribution of distances from sources where pollen clouds were sampled (only at least at 50 m or 300m) as shown by simulations (not shown). These simulations further showed that the shape parameter

of the dispersal kernels were correctly estimated (data not shown).

Using the experimental data, we showed that small-scale estimates of dispersal kernels largely underestimated observed pollination when extrapolated to the landscape scale and that estimated dispersal kernels predicted huge amount of long-distance pollination consistently with results of Thompson *et al.* (1999). The largely used exponential function is clearly inappropriate to model cross-pollination at the landscape scale. Furthermore, we showed that fertilizing pollen clouds above male-sterile plants were almost half composed of uncontrolled pollen sources (volunteers, feral populations and uncharacterised fields). A better characterisation of the genetic composition of both fields and feral populations should greatly improve our understanding of the contribution of pollen sources to fertilizing pollen clouds over a landscape.

The pollen movement studied here represents only the immigrant part of the total pollen received by male-fertile plants, which are mostly selfed or pollinated by neighbouring plants. However, this immigrant pollen cloud is particularly important for risk assessment because it contains genes from both neighbouring and distant fields.

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# Comparison of research results on cross-fertilization in maize

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**Abstract:** Various parameters with varying levels of relative importance have been identified to play a role in the study of cross-fertilization in maize. The present review paper's summary (Devos *et al.*, in press) addresses the parameters that should be considered when comparing research results and/or when proposing specific co-existence measures limiting cross-fertilization in maize.

## INTRODUCTION

As maize is a cross-pollinated crop relying on wind for the dispersal of its pollen, European member states will impose strict technical co-existence measures to reduce cross-fertilization between transgenic and non-transgenic maize in order not to exceed the tolerance/labelling thresholds for the adventitious and technically unavoidable presence of genetically modified (GM) material in non-GM produces. Various biological, physical and analytical parameters have been identified to play a role in the study of cross-fertilization in maize. The amount of variables and their variability may hamper the comparison between research results and make it difficult to define the appropriate length of isolation distances and/or pollen barriers. Here, we address some of the parameters that can hamper the comparison between research results. For the details of the used data sources and models we refer to the review paper (Devos *et al.*, in press).

## PARAMETERS TO CONSIDER

### Definition of isolation distance and pollen barrier

Although the terms isolation distance and pollen barrier (or buffer zone) are clearly distinct, they are regularly mixed up in literature. An isolation distance separates fields with GM and non-GM maize by a zone of open ground or a zone with low growing crops while a pollen barrier consists of plants that are sown or planted around the source or recipient field. If the outer parts of fields function as a barrier, the distance between the inner parts increases. Barriers may also introduce competing pollen (if the barrier is of the same species as the crop) and/or may serve as a physical barrier to air and consequently pollen flow. Moreover, a pollen barrier of maize has been proven to reduce cross-fertilization levels more effectively than an isolation distance of the same length. For the future, it might be advisable to match the common vocabulary to similar definitions.

### Quantification approach

Depending on the quantification approach, research data are expressed in different units. If out-crossing is spotted in hybrid progeny either by phenotypic markers (xenia), by detection of transgenic DNA and/or proteins or by the application of an appropriate selective pressure, out-crossing is expressed as a percentage of grains/kernels. Using DNA analyses for the quantification of the GM content in non-GM produce, cross-fertilization is expressed as a percentage of genomes. So far, the results of DNA quantification are not smoothly convertible to results obtained by phenotypic markers.

### Hemizyosity

Owing to the production of current GM hybrid varieties, the transgene is generally present either in the seed parent or in the pollinator: GM hybrids are hemizygous for the transgenic trait. Hence only half of the pollen produced carries the transgene. Compared to a pollen donor that is homozygous for the screened trait only half of the cross-fertilization is measured.

### Analysed plant material

The material to be analysed depends on the use of maize. In grain maize, adventitious mixing is restricted to the grain fraction of the plant: the cross-fertilization level is expressed per grain lot. In corn cob mix and in fodder maize, out-crossing (expressed as a percentage of genomes) is expected to be diluted, since vegetative parts of the maize plant are maternal tissue. In non-processed fresh sweet maize, it might be necessary to monitor per individual ear.

### Experimental design

The results of field trials will differ according to the implemented design. In different studies, small recipient plots or even individual plants have

been planted at various distances from a source in order to measure how far viable maize pollen can successfully fertilize a maize ovule. Such a design does not reflect the real agricultural situation, and is not suited to quantify the adventitious GM content of recipient fields of commercial size. Individual plants or small recipient plots are much more prone to cross-fertilization than large recipient fields, which may result in an overestimation of the out-crossing level when making extrapolations. Recent studies carried out in France, Germany, Spain and the UK mimicked worst-case commercial on-farm situations (e.g. pollen source next to or completely surrounded by a recipient field). As the probability of cross-fertilization diminishes with increasing distances, sampling was done at different positions within the recipient fields in order to calculate the average percentage of cross-fertilization over the whole field. The recommendations yet made for isolation distances and/or pollen barriers, based on discrete out-crossing levels, may therefore be too conservative and thus larger than the ones actually needed.

### CONCLUSIONS

Considering the various parameters playing a role in the study of cross-fertilization, it seems difficult to compare research results and to define the appropriate isolation distances and/or pollen barriers limiting out-crossing. However, some consistent facts and patterns are observed over trials that have been conducted under different geographical and climatic conditions, allowing making certain recommendations for co-existence measures.

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# Gene flow from herbicide-resistant GM soybean to conventional and wild soya in the centre of the origin in the Russian Far East

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**Abstract:** Soybeans are cultivated in two regions of the Russian Federation: Far East along the Chinese border, and the southern area of the European part of Russia. The Far East is the area where wild relatives of soybeans occur naturally. For this reason, we carried out research to evaluate possible natural hybridization between *G. max* and *G. soja* and conventional soybean under conditions prevailing in Khanka Lake region of the Russian Far East. The wild soy involved in the study was collected from natural populations growing adjacent to agricultural fields of cultivated soybean. Hybrid plants were not found during the two years of the study of hybridization between conventional and GM-RR soy and GM-RR and wild soy. These results are important for developing co-existence management programmes for GM soya and for determining likely gene flow rates to wild soya in the environmental conditions in the centre of origin of this agricultural plant.

Soybeans are cultivated in two regions of the Russian Federation: Far East along the Chinese border, and the southern area of the European part of Russia. The Far East is the area where wild relatives of soybeans occur naturally. Cytological, morphological, and molecular data and data on cross-pollination suggest that *Glycine soja* is the probable ancestor of *G. max*. *Glycine gracilis* is considered to be a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*. *G. gracilis* may be an intermediate in the speciation of

*G. max* from *G. soja* or it may be a hybrid between *G. soja* and *G. Max* (Skvortzow, 1927; Komarov, 1958). Thus there is a potential for gene flow from introduced genetically modified (GM) cultivars to the Far East region to these species and a potential co-existence issue with conventional or organic soya bean crops (Dymina *et al.*, 2001).

Earlier we reported that cross pollination between these closely related soy species was evaluated under field conditions and in the greenhouse (Seitova *et al.*, 2004; Dorokhov *et al.*,

2004). Several local cultivars of soybeans were successfully fertilized by pollen from wild soy. However, when GM soybean cv. "Stine 2254 RR" was used to pollinate plants of *G. soja* and *G. Max*, no herbicide-resistance plants occurred during two growing seasons. Thus, natural cross-pollination between plants of the soybean species appears to be an extremely rare event, with a frequency below the sensitivity of the experiment. Hybridization was detected in artificial pollinations between *G. max* and *G. soja* under controlled conditions in a greenhouse (Dorokhov *et al.*, 2004). On average, 3.7% of cross-pollination gave fertile F<sub>1</sub> seeds. These seeds were of variable colour and size, and differed from the seeds of their parents. Plants obtained from F<sub>1</sub> "Stine 2254 RR" x *G. soja* hybrid possessed RAPD and ISSR complex patterns typical of both parents. Transgenic DNA was detected by PCR analysis in these F<sub>1</sub> hybrids; however, the target DNA fragment was not detected in F<sub>2</sub> and F<sub>3</sub> plants. This indicates that, in the case of "RoundUp Ready" soybean, the transgenic DNA was eliminated in F<sub>2</sub> progeny of hybrids between GM and wild plants of soybean (Dorokhov *et al.*, 2004).

The implications of gene flow from crop to wild plant depend on many factors. Under selective pressure, the crop-wild hybrids may or may not have a greater adaptive advantage compared to their parents. If hybrids were more competitive, there could be an increase in their weediness and invasiveness (Chevre *et al.*, 1999). The chances of transfer of glyphosate resistance to wild soy in regions of Asia and Russian Far East are limited by other factors such as flowering asynchrony between soybean and its relatives; extent of sexual compatibility; abundance, method, and distance of pollen spread; and environmental conditions pertinent to cross-pollination. Our results indicate that transfer of genetic material from GM soybean to wild relatives or other soybeans is low. For this reason, we are now carrying out research to evaluate possible natural hybridization between *G. max* and *G. soja* and conventional soybean under prevailing conditions in Khanka Lake region of the Russian Far East. The wild soy involved in the experiment was collected from natural

populations which are established near to fields of cultivated soybean. Molecular markers have been used for studying the genetic structure of a population of wild soya at the coast of the Khanka Lake. It was determined that the population of wild soya was genetically similar all along the coast (Nedoluzko, 2005). To identify potential interspecific hybrids, we have used attributes of colour and size of seeds in the F<sub>2</sub> generation. For molecular identification of hybrids between GM soybean and wild and conventional soya and for monitoring gene flow, various molecular markers, such as RAPD and ISSR (Ignatov *et al.*, 2004) and specially developed kits (developed and manufactured by Centre "Bioengineering" RAS and Applera, USA) for border sequences of 2254 RR soya will be used.

## CONCLUSIONS

Hybrid plants were not found during the two years of the study of hybridization between conventional and GM-RR soy and GM-RR and wild soy. These results are important for developing co-existence management programmes for GM soya and for determining likely gene flow rates to wild soya in the environmental conditions in the centre of origin of this agricultural plant.

## ACKNOWLEDGEMENTS

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# GMOs and genetic contamination: tools and practices for *Brassica* and *Solanum* under Finnish conditions

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To be prepared for the potential risk of GMOs, methods need to be developed both to prevent genetic contamination in the field, as well as to monitor and verify purity and to detect the degree of contamination. Gene flow from GMOs can be detected by quantitative PCR of the transgene, but in the absence of that information molecular markers must be employed. These need to be developed for the most likely GMO crops for Finland. These marker systems can also then serve as an ideal means of measuring gene flow under Finnish conditions without having to carry out a GMO release. Our key objectives are 1) to develop cost-effective, sensitive, and robust tools for the monitoring of gene flow and contamination in turnip rape (*Brassica rapa*), oilseed rape (*Brassica napus*), and potato (*Solanum tuberosum*) 2) to use these tools to gather data on the parameters for pollen- and volunteer-mediated flow 3) to

use these data to model gene flow and make risk estimates 4) on the basis of the data and models, to prepare recommendations for co-existence under Finnish climatic and agronomic conditions. The over-wintering and regeneration abilities of dispersed potato tubers for production of volunteer plants are tested under field conditions representing the extremes found in Finland. We have developed dominant transposable-element markers specific to the most commonly sown and the newest varieties of turnip rape and oilseed rape in Finland. These markers are highly sensitive (less than 0,1% foreign DNA can be detected in a sample). Markers are used to generate data on the potential risks for gene flow and volunteers under boreal conditions at various distances. Preliminary results indicate pollen-mediated flow in *Brassica* to be similar to published findings for more southerly latitudes.





# Variation of viability and fecundation capacity of maize pollen with transport distance

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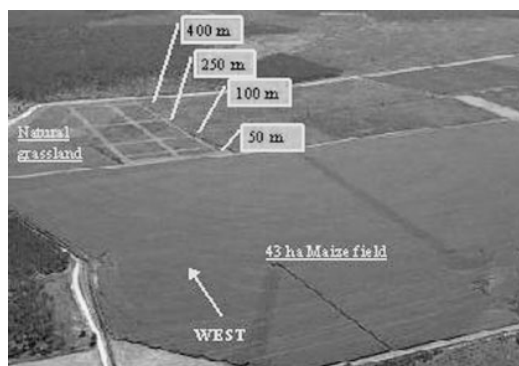
**Abstract:** Two experimental fields were set up in 2002 and 2003 with detasseled maize plants located at different distances from a pollen source (50, 100, 250 m), a 500 ha maize field in 2002 and a 25 ha field in 2003. The number of pollen grains falls down with distance and the viability decreases drastically. In good climatic conditions, pollen viability is only 17% at a 250 m distance from a source with 70% viability. The fertilization capacity is between 2 and 3 times as small as the viability rate. Experimentations were conducted in two types of artificial conditions: in the first type the plants at flowering are placed in conditions representative of two summer days, and in the second type the pollen is exposed to an air flow at different temperatures and humidity rates. At 30°C, the viability is equal to zero after 50 minutes in correlation with pollen dessication. The pollen does not lose its capacity during transport because its duration is very short, from 2 to 3 minutes, depending on wind velocity. The lightest pollen, which is the least viable, may be transported the farthest.

## CONTEXT

With the introduction of GM crops, rules of coexistence must be established. Field trials are currently the way used to estimate the isolation distances between GM and conventional maize in order to match the legal threshold of 0.9%. In the perspective of understanding and predicting these isolation distances, pollen dispersal models have been developed (Aylor *et al.*, 2003; Foudhil, 2002; Jarosz *et al.*, 2003). Beside modeling, measurements of the viability and the fertile capacity of maize pollen were performed.

## MATERIALS AND METHODS

Two experimental campaigns were conducted in 2002 and 2003 where maize plants without tassels were located at several downwind distances (50, 100, 250 m) from a 43 ha maize field (Figure 1) in 2002, and a 25 ha one in 2003. In parallel, two further experimentations were conducted under artificial conditions: in the first type the plants at flowering are placed in controlled conditions representative of two summer days, and in the second type the pollen is exposed to an air flow at different temperatures and relative humidity rates.



**Figure 1.** Picture of the experimental site in 2002. Maize plants without tassels were located at four distances downwind from a 43 ha maize field.

### Pollen Viability

Pollen viability was measured on Petri dishes (35 mm diameter) containing a nutrient “agar”. Open dishes were placed at the cob insertion height. As the number of pollen grains decreases drastically with the distance from the source, the number of dishes was larger at long distance (5 dishes at 50 m, 10 at 100 m and 20 at 250 m). The exposure duration was 150 minutes (8:00 UT to 10:30 UT) each day.

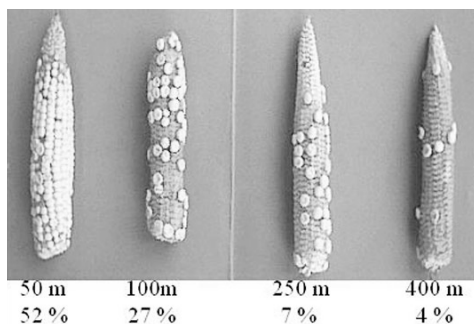
### Fecundation rate and fecundation capacity

The fecundation rate is the number of pollen grains on a cob divided by the grain potential of this cob estimated as the number of emerged silks. The fecundation capacity is the number of grains on a cob divided by the number of pollen grains trapped on the silks of this cob. This operation requires that the number of pollens be smaller than the number of silks. However, it should be noted that one silk could receive more than one pollen kernel. The fecundation capacity was measured in 2002 (on only one day with wind) and in 2003 (3 days) .

## RESULTS

### Field experiment

Pollen viability decreases with distance. Within the field the viability is variable (40% to 70%) as it is at 100 m downwind (10% to 30%) The fecundation rate decreases with distance from the source. After a day of exposure of the cobs, it can reach 10% (in 2003) and 20% (in 2002) at a distance of 50 m. At 250 m, the fecundation rate was 1 - 2% due to a small pollen deposition rate. Figure 2 shows the maximum fecundation after complete flowering.



**Figure 2.** Fecundation capacity at each downwind distance. Plants were first detasseled. Cobs were exposed during the whole flowering period of the upwind maize field.

The fecundation capacity decreases from 25% to 5% (Figure 3) with distance. It is on average 3 times as small as the viability rate. The rate of decrease for each day exposure is roughly the same for the 2 years. The climatic conditions during the 3 days in 2003 were characterized by medium temperatures (from 15°C at 7h UT to 25-30°C at 16h UT) and relative humidity between 90% at 9h UT and 45% at 18h UT. The wind speed was 2 m/s on July 4 and 5 and 3 m/s on July 9 on average. At long distance, the number of pollen grains is clearly smaller than at 50 m, which may be explained by the experiments conducted in controlled conditions.

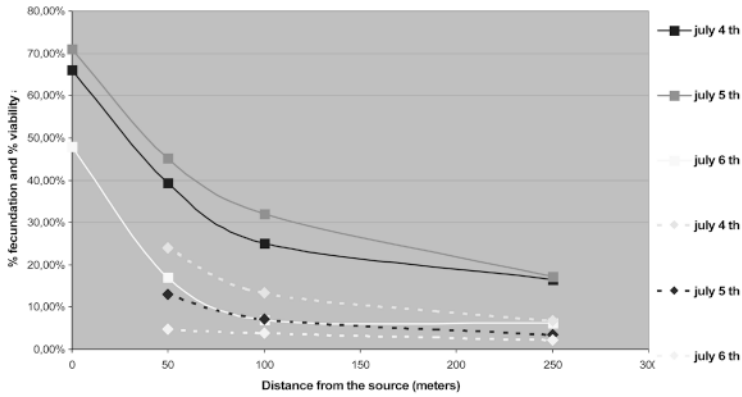


Figure 3. Pollen viability and fecundation capacity as a function of distance downwind from the maize field.

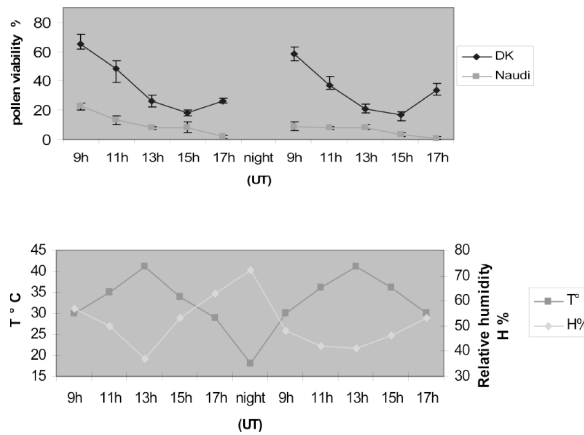
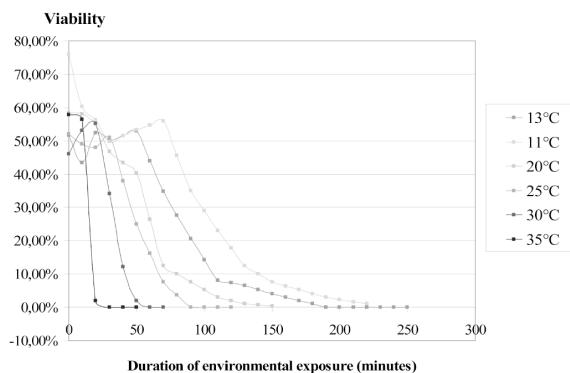


Figure 4. Top graph: Pollen viability of two maize varieties (DK and NAUDI). Bottom graph: temperature and relative humidity experienced by the plants.

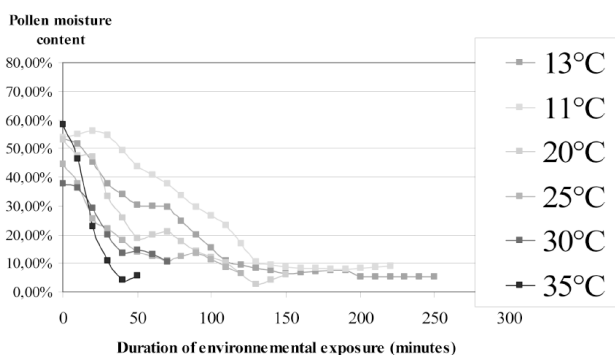
**Artificial conditions**

Figure 4 shows the variation in pollen viability for two varieties during two consecutive simulated summer days. Only fresh pollen was collected for measurements by shedding the anthers of 3 plants. The viability decreases quickly during the day and increases again at night. When the air temperature is above 26°C the viability seems to decrease. Pollen from the Naudi cultivar seems to recover less at nights than pollen from the DK variety.

Fresh pollen collected from shedding anthers of two cultivars was exposed to a controlled air flow at different temperatures and a constant relative humidity of 50%, until the complete loss of viability (Figure 5). The loss of viability is very rapid at hot temperatures. The pollen can survive only 20 minutes at 35°C, 100 minutes at 25°C and 200 minutes at 13°C. Measured pollen moisture content indicates that viability is correlated with humidity: the most viable pollen grains are the most humid. (Figure 6).



**Figure 5.** Pollen viability as a function of time for varying temperature at constant humidity.



**Figure 6.** Pollen humidity as a function of time for varying temperature at constant air humidity.

## DISCUSSION

Figure 5 indicates that the time of flight of pollen during its transport from the source field to the target field (1-2 minutes for 250 m at 2 m/s) cannot explain the loss in viability observed in the field (Figure 3). One hypothesis to explain this loss of viability with distance is the possibility for pollen to segregate according to its weight. Indeed, Jarosz *et al.* (2003) demonstrated that the settling speed of pollen depends on its humidity. The driest pollen falls down slower than the

most humid grains by a factor of about 2. Others experiments (not published) show that close to the source (1 m downwind) the viability of the pollen trapped at 2 m height is smaller than the viability of the pollen trapped at 0.5 m height.

## CONCLUSIONS

These experimentations show not only that the pollen quantity decreases with increasing distance from the source, but also that the viability and fecundation capacity both decrease with distance

and over time. However these experimentations also prove that the decrease in pollen viability with distance cannot be due to the transport by the wind itself. Other mechanisms should be considered to explain this behavior. One hypothesis is that the least viable pollen may fly longer due to a smaller settling speed. More work is needed to clarify this issue. Mechanistic models could be used to test several hypothesis such as the segregation of pollen according to the settling speed of the grains.

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# Estimation of demographic parameters for feral populations of oilseed rape

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**Abstract:** Assessing the environmental risks associated to the introduction of transgenic oilseed rape requires a good knowledge of the ability of this species to disperse and to persist outside fields. We therefore performed a 3-year survey of feral oilseed rape populations at the landscape scale and thus collected counting data of plants within successive developmental stages in about 100 populations. Using the framework of branching processes, we developed quasi-likelihood methods to estimate survival rates of plants and to assess the effects of external factors on these survival rates. Getting relevant estimates of demographic traits will allow assessment of the invasion risks linked to transgenic feral populations and will guide the choice of adapted management strategies to limit gene escape via these populations.

## INTRODUCTION

Transgene escape is favoured when a cultivated species can persist outside fields as feral populations, which is the case of winter oilseed rape. The transfer of transgenes to feral populations could induce two main risks. First, transgenic feral plants could invade semi-natural habitats and thereby modify the natural balance of these habitats. Secondly, feral-to-crop gene flow could lead to contamination of harvested seed and economic impacts. Risk assessment

thus requires a detailed knowledge of the determinants of feral population dynamics and accurate estimates of demographic parameters, specific to the habitats of feral oilseed rape (e.g. roadside verges), are necessary to improve the realism of existing models (Colbach *et al.*, 2001; Garnier & Lecomte, in press). We therefore used field data collected during a 3-year survey of oilseed rape feral populations to estimate demographic traits and to highlight the effects of environmental factors on plant survival during the flowering period.

## METHODS

The feral plants had not been marked individually during the field survey so that the field data set did not allow the use of classical capture-mark-recapture methods to estimate demographic parameters. Data were available for numerous populations (about 100 each year), where numbers of plants were recorded in successive stages: rosettes **R**, bolting plants **D** and **E**, flowering plants **F**, plants with pods **G** and plants with ripen pods **G<sub>s</sub>**. Mowing and herbicide spraying performed on feral plants were also recorded. Data were collected monthly within each population, from January 2001 to June 2003. We used the framework of branching processes to perform statistical analyzes on these counting data (Hall & Heyde, 1980). Since likelihood methods are not convenient in the case of multitype branching processes, i.e. when several stages coexist, we used other estimation methods such as quasi-likelihood methods. The principle used was the following: consider that the number of plants in stage **F** on month  $t$ ,  $F_t$ , is explained by the numbers of plants in stages **D** and **F** on month  $t-1$ ,  $D_{t-1}$  and  $E_{t-1}$ . The conditional expectation of  $F_t$  is then:  $E(F_t | D_{t-1}, E_{t-1}) = p_{DF} D_{t-1} + p_{EF} E_{t-1}$ , where  $p_{DF}$  and  $p_{EF}$  are transition probabilities from stage **D** to stage **F** and from stage **E** to **F**. The conditional variance of  $F_t$  was written as the sum of two binomial variances:  $\text{Var}(F_t | D_{t-1}, E_{t-1}) = p_{DF}(1-p_{DF}) D_{t-1} + p_{EF}(1-p_{EF}) E_{t-1}$ , weighted by different possible types of over-dispersal. The conditional expectation and variance of  $F_t$  were then used to estimate the probabilities  $p_{DF}$  and  $p_{EF}$  by quasi-likelihood methods. This method was then applied for all demographic transitions occurring during the flowering period to estimate the transition probabilities. The large set of surveyed populations provided replications to test the effect of various external factors on plant survival. Within a stepwise approach, we first analyzed the global survival of plants  $p_{RG5}$ , from rosettes (**R**) in spring to mature plants (**G<sub>s</sub>**) in summer. Supplementary data (i.e. mowing and herbicide spraying events) gathered monthly during this period was then used to explain the variability observed and to test the effect of external factors on  $p_{RG5}$ .

## PERSPECTIVES

Accurate estimates of demographic parameters will allow quantitative predictions of the dynamics of feral populations and therefore assessment of the risk of colonization of semi-natural habitats by transgenic feral populations. Moreover, highlighting the determinants of feral plant survival will help with the selection of adapted strategies to control the spread of feral populations and therefore to manage the potential risks induced by these populations. More generally, information about demographic traits will allow quantification of the general processes involved in the establishment and persistence of feral crop plants outside fields.

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# Using a spatial and stage-structured invasion model to assess the spread of feral populations of transgenic oilseed rape

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**Abstract:** The risk of roadside verge invasion by transgenic plants is favoured when a cultivated species can persist outside fields as feral populations. We chose oilseed rape as a model species to evaluate the spread of genetically modified herbicide tolerant (GMHT) feral populations under selection pressure (herbicide spraying) in the medium term. We developed a stepwise invasion model that combines stage-structured dynamics and seed dispersal within an integro-differential equation. Modelling choices were made to conform to our intention to obtain methodological insight about the necessity to integrate long-distance seed dispersal in models of gene flow among oilseed rape. We thus assumed that roadside verges are a one-dimensional and uniformly suitable habitat and that events of dispersal and demography are deterministic. We performed elasticity analyses of population growth rate and invasion speed to highlight the determinants of population demography and spread. Rare events of long-distance dispersal controlled population spread. The risk of road verge invasion by feral populations of GMHT oilseed rape under selection pressure is thus real and does exist, since it was proved experimentally that oilseed rape can be dispersed by vehicles.

## INTRODUCTION

Genetically modified traits could modify the fitness of plants and accentuate the weediness of transgenic cultivated species escaped from crops, particularly if these species can persist outside fields as feral populations, which is the case of winter oilseed rape. Two main types of risks are induced by feral populations of GMHT oilseed rape. First, from a demographic point of view, GMHT feral populations could invade semi-natural habitats

sprayed by herbicide and thus modify the natural balance of communities on roadside verges, with consequent environmental impact. Second, from a genetic point of view, these populations might behave both as pollen sources and pollen sinks, and thus reinforce gene dispersal among oilseed rape fields (Colbach *et al.*, 2001). Here, we assess the invasion of semi-natural habitats (roadside verges) by feral populations of GMHT oilseed rape, using a modelling approach and focussing on demographic and dispersal aspects.

## METHODS

The model couples stage-structured dynamics of a feral oilseed rape population and stage-dependent dispersal (Garnier & Lecomte, in press). We take account of short-distance dispersal (pod popping) and long-distance dispersal (via vehicle wheels and agricultural engines). This invasion model is based on an integro-differential equation (Neubert & Caswell, 2000) that describes the evolution of plant density  $\mathbf{n}(x,t)$  at each spatial location  $x$ :

$$\mathbf{n}(x,t+1) = \int_{-\infty}^{+\infty} [\mathbf{A}_{\mathbf{n}(y,t)} \circ \mathbf{K}(x-y)] \mathbf{n}(y,t) dy$$

where  $\mathbf{K}$  is the dispersal kernel matrix and  $\mathbf{A}$  is the matrix of transitions in the life-cycle. A mixture dispersal kernel is used to combine long-distance dispersal (via vehicle wheels) and short-distance dispersal (via pod popping). The habitat of feral oilseed rape in roadside verges was assumed uniformly suitable. The population is initiated at  $t=0$  by a batch of seeds located on  $x=0$  on the roadside verge and population expansion occurs from this origin. During following years, seed immigration from nearby crops or seed losses by transport are neglected, since seed immigration would induce spatial demographic heterogeneity that cannot be considered under these assumptions.

This deterministic model provides analytic computation of the growth rate  $\lambda$  and the asymptotic invasion speed  $c^*$  of the population and allows elasticity analyses of  $c^*$  and  $\lambda$ .

## RESULTS AND DISCUSSION

Results show that patterns of elasticities are the same for population growth rate  $\lambda$  and invasion speed  $c^*$ . Hence the transitions that influence  $\lambda$  most have also a large impact on the value of  $c^*$ , as described in Neubert & Caswell (2000). The highest values of elasticities are observed for the transitions producing mature plants and seeds. As dispersal and reproduction are closely linked

in the case of oilseed rape, both reproducing and dispersing stages were expected to play a key-role in population growth and spread.

The invasion speed  $c^*$  computed in the absence of long-distance dispersal was  $c^*=1.6$  m/yr. When an extremely small fraction of seeds dispersed over a long distance, the invasion speed increased to values ranging from 3.2 m/yr to 45.3 m/yr, i.e. from a two-fold increase to a 28-fold increase. For small values of long-distance dispersal probability ( $p_{LDD} < 10^{-6}$ ),  $c^*$  depended mainly on mean distance of long-distance dispersal  $b_{LDD}$ . The speed  $c^*$  increased with both  $p_{LDD}$  and  $b_{LDD}$  for larger values of  $p_{LDD}$ . These results show that long-distance dispersal controls population spread, even though the large majority of the seed pool produced by the populations does not disperse far from the source. What really matters is not the frequency of events of long-distance dispersal but their very existence, since invasion speed is quite independent of long-distance dispersal probability if the latter is small but non zero.

## PERSPECTIVES

Results highlighted the critical impact of long-distance dispersal on population spread along road verges. Long-distance dispersal events should thus be integrated in landscape-scale models of gene flow for oilseed rape otherwise they would underestimate feral population spread. This modelling approach showed that the risk of colonisation of roadside verges by feral GMHT oilseed rape under selection pressure does exist. These feral populations could therefore increase the risk of contaminations (via pollen or seeds) between GM and non-GM crops in a context of crop coexistence. Limiting feral oilseed rape dispersal should be quite difficult, since its expansion mainly relies on a few seeds dispersed at long distance. Experimental studies should thus concentrate on collecting quantitative data about seed dispersal by vehicles or by verge mowers.

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# Public GMO location registers for supporting national co-existence measures

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**Abstract:** According to Article 31 (3b) of Directive 2001/18/EC, Member States shall establish public registers for recording the location of GMOs grown under part C (placing on the market). The primary intention is the monitoring of potential adverse environmental effects of such GMOs.

This register (GMO location register) was established in February 2005 in Germany by the Federal Office for Consumer Protection and Food Safety (BVL; see BVL 2005). The register records the geographical location and reference of the GMO cultivation site(s) in form of exact cadastral data, and thus can be used for co-existence measures by informing farmers about GMO cultivation in their neighbourhood. The foreseen site(s) shall be notified at least three months ahead of cultivation. The BVL publishes the information on the respective locations, the name, and the characteristics of the genetically modified organism in the Internet. Furthermore, farmers or other relevant stakeholders can apply for more detailed person-related data of GMO users in case of potential conflicts with co-existence measures e.g. isolation distances. We report the first year experience with the location register in respect to (i) data base development (ii) notification statistics, and (iii) public interest.

## INTRODUCTION

According to Article 31 (3b) of Directive 2001/18/EC (EC 2001) Member States shall establish public registers for recording the location of GMOs grown under part C (placing on the market). The primary intention is the monitoring of potential adverse environmental effects of such GMOs. In Germany the location register was implemented in Germany's Genetic Modification Act in February 2005 (BRD 2005). A second aim

of the register foresees measures to enable co-existence of GM and Non-GM crop cultivation.

## LEGAL DATA REQUIREMENTS

Farmers are since 2005 committed to notify any GM cultivation site to the public register in Germany. The notification should take place at maximum nine and at minimum three months ahead of cropping in a specific form to the BVL.

Necessary data comprise the geographical location with exact grid references (exact cadastral data), the name and the characteristics of the genetically modified organism and personal data of the farm manager. Changes in crop growing (time period, area) have to be notified in due time as well. All information except personal data is open to the public by internet. Third party farm managers or other stakeholders can apply for releasing single personal data in case they can accredit to be affected by the GMO cropping. The decision for release of personal data is taken by the BVL based on scientific principles e.g. if a farmer cultivates the same crop species within a reasonable distance to the GM field.

## DATA BASE

The data base is provided through an Oracle data base application server using Java J2EE Server applications. Beside data administration, several additional services are available such as (i) selected internet presence of the data, (ii) secured online access for other federal authorities to sensitive personal data, (iii) service online data input for farm managers, (iv) simple statistical data analysis, (v) flexible data structure in case of changes in National or EU regulations, and (vi) interface-provider to other databases and Geographic Information Systems (GIS). In any case the data base must meet legal requirements of confidentiality.

## NOTIFICATION STATISTICS FOR 2005

The main GM crop notified in 2005 was MON810 maize (99% of all notifications). During February, 117 fields were notified by 44 farm managers comprising a cropping area of about 1000 ha. From this starting point a large number of these notifications were withdrawn until June 2005. Some estimated reasons for that were (i) changes of cropping plans on farm or in scientific research programs, (ii) economic reasons, (iii) availability of seeds, (iv) public protest, (v) negotiation with neighbours, or (vi) changes in the seed registration

procedure. This list of reasons is most likely not complete. However, 60 fields of 34 farm managers succeeded to start cultivation of a GM crop comprising an area of about 350 ha. On 16 of the 60 fields the size was reduced in comparison to the original notification. The remaining 57 GM fields in the register were withdrawn. Farm managers did not officially report any field vandalism directly to the GMO location register or the BVL.

## PUBLIC INTEREST

The location register created a lot of public awareness in 2005. Newspaper and broadcasting stations informed mainly about number and size of GM fields. Some Non Governmental Organizations (NGO) linked the location register with own geographical maps and internet information how to reach GM fields. Although four stakeholders applied in total, only one applicant received access to personal data. Vandalism in GM fields was occasionally reported in newspapers but seemed to be limited to less than 5 locations during the growing season 2005.

## CONCLUDING REMARKS

The register received broad attention in the public. The location register is not only of importance for environmental GM crop monitoring purposes, but also substantial to inform the public about cultivation of GM crops and thus for co-existence measures. The register could in future be linked with geographical information system (GIS) data. It is questionable at this time whether the necessary costs and legal requirements are available in the next few years. It is also still under discussion whether the register may enhance GM field vandalism or "mobbing" of farm managers. Newspapers reported mobbing in some cases, which started a political discussion to reduce public access to exact location data. However, the location register seems to be a useful and widely accepted general tool for co-existence measures and environmental monitoring despite the above mentioned problems and critics.

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# Common sunflower (*Helianthus annuus* var. *annuus*) – potential threat to coexistence of sunflower crops in Central Europe

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**Abstract:** Common sunflower (*Helianthus annuus* var. *annuus*) has been found in the Czech Republic since the 1960's. This species exists as a ruderal on railways and at river ports. It has been reported twice on arable land but its presence was only temporary. In 2004, we have found a dense population of sunflower on arable land. The infested crop was sunflower with common sunflower plants dispersed mainly in the field margins, however some plants were in the mainpart of the field. Common sunflower was also growing in ruderal habitats near the field.

We predict the following consequences of common sunflower spread through the country: it could become a problem weed in sunflower crops due to the non-availability of a selective herbicide; as a weed, this species could infest other crops, which currently suffer from volunteer sunflower occurrence, such as maize, beet or soybeans. Gene flow can occur between this weedy form and the crop, and this must be considered during the risk assessment of transgenic sunflowers prior to their introduction into European agricultural systems.

## INTRODUCTION

The risk of gene flow between transgenic and non-transgenic crops increases when volunteer/feral individuals occur or when hybridising wild or weedy relatives are present. Such a species can act as a reservoir of genes and a «green bridge» for gene flow and the gene flow may be difficult to control because these species may be difficult to manage. In Europe, this problem is fairly well understood for oilseed rape or sugar beet. A similar situation can be found in sunflower in North America, where common (wild) sunflower is native. But this species is also now present in Central Europe, which presents the possibility for

gene transmission between the crop and the wild form of this species. It also presents the possibility of transgene escape if genetically modified (GM) sunflower is grown in this region.

## OCCURRENCE OF COMMON SUNFLOWER

Genus *Helianthus* is native to the North American continent. It is represented by both annual and perennial species. Common sunflower (*Helianthus annuus* var. *annuus*) is widespread through most of United States (Snow *et al.*, 1998). Recently common sunflower was introduced to many parts of the world where it occurs as a

weed. In Europe, common sunflower has been reported in Switzerland, Germany, Belgium, Finland, Sweden, Russia, Czech Republic, and Slovak Republic – but mostly only temporarily (Jehlík 1998).

In the Czech Republic common sunflower has been reported since the 1960's. This species exists as a ruderal on railways and at river ports. It has been reported twice on arable land but its presence was only temporary. In 2004, we have found a dense population of sunflower on arable land (Velká Ves near Kolín). The infested crop was sunflower with common sunflower plants dispersed mainly in the field margins, however some plants were in the main part of the field. Common sunflower was also growing in a ruderal habitat near the field. (Holec *et al*, 2004). Two hundred and fifty to three hundred flowering individuals were found in the same location in mid September 2005, mostly growing as weeds in sunflower crop, but also in neighbouring ruderal plant communities and a few plants were found in a maize field. Common sunflower plants were producing in an average of 50 (5 – 126) inflorescences (seed heads) but only 10 – 15% of them were able to produce viable seeds (achenes). The majority of inflorescences were still flowering at time of crop harvest which limited potential seed production. Late flowering and seed ripening could be used in common sunflower management – growing early sunflower cultivars together with early crop desiccation can decrease common sunflower seed inputs into the soil seed bank.

In 2005, we also found a sunflower crop field (Tursko near Prague) infested with common sunflower plants (30 – 40 individuals randomly distributed through the field) growing within the crop rows suggesting that impure seed had been used.

## RISKS FOR CO-EXISTENCE

Common sunflower is self-incompatible and thus hybridises with sunflower crops when it occurs in crop stands in low numbers, it therefore presents a high risk for hybrid offspring

production. Thus, common sunflower can receive crop genes, including transgenes and act as a transgene reservoir. In addition common sunflower can occur as a weed in other crops which can break the temporal isolation between succeeding sunflower. In addition, common sunflowers which occurring in field margins and other crops will outcross with sunflower crops, increasing the spread of genes and production of hybrid offspring production.

## CONCLUSIONS

In Central Europe, common sunflower is still a rare adventitious species. It can act as a weed in crop stands of sunflower, where it can not be managed by herbicides. It can also occur in other crops such as maize, beet or soybean. Gene flow between the weedy form of sunflower and sunflower crops is likely, and this must be considered during the risk assessment of potential transgenic sunflower introductions into the European agricultural systems.

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# Effect of wind direction on field-to-field cross-pollination of wind-pollinated GM crops

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**Abstract:** Using an original mathematical model and (a) records of wind direction and speed from 27 weather stations across Europe and (b) the relative intensity of pollen release from a source GM field and pollen receptivity in a sink non-GM field over the flowering period, we estimate the relative cross-pollination levels according to the relative orientation of neighbouring GM and non-GM fields. We consider the important commercial wind-pollinated crops: oilseed rape, maize, sugar beet and rice, all of which pose a medium to high risk to confinement except for rice.

The high cost and effort of field trials prohibits testing transgene confinement in all landscapes, and so theoretical models can provide valuable predictions. Cross-pollination rates and the concentration of airborne pollen are known to vary with compass direction from the source field. Here, we use observations of the frequency of wind directions to predict the relative level of cross-pollination. We find that the relative cross-pollination level can vary greatly according to the relative orientation of neighbouring GM and non-GM fields. At a given site and orientation from a GM field, we predict how the relative cross-pollination rate varies from year to year. Here, we propose methods to predict the likely range in levels of cross-pollination based on the limited data from field studies that is typically available. In addition, we suggest how the relative cross-pollination rate may be modelled as a function of the time lag between peak flowering in adjacent fields. This could be used to reduce the expected cross-pollination rate to an acceptable level.



# Experimental study of maize pollen emission under field conditions

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**Abstract:** Experiments measuring maize pollen emission at field scale were carried out in 2004 and 2005 under various conditions of cultivar, sowing date, site and environment. The experimental results could help in the formulation of a semi-empirical model which simulates the source term of models of pollen dispersal at field or landscape scale.

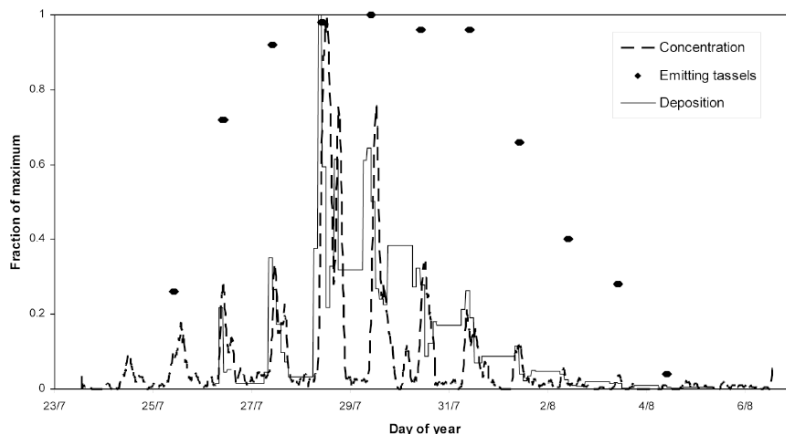
## INTRODUCTION

To quantify gene flow resulting from pollen dispersal in maize, both physical and statistical approaches have been used (Lavigne *et al.*, 1998; Jarosz *et al.*, 2004). Although there is still some difficulty in predicting distances of potential genetic pollution, particularly when distances are long, another key issue today is our ability to characterize the pollen source behaviour. It is well known that pollen emission from male flowers follows a nycthemeral cycle but climatic factors such as time since sunrise, relative humidity and turbulence may significantly modify the temporal pattern of pollen production under non limiting environmental conditions. Such biophysical knowledge of the climate-pollen emission relationship is of major importance in providing

forcing variables of physical models used to estimate pollen dispersal at various distances from the source. This paper discusses experimental results of maize pollen emission under various weather, cultivar and sowing date conditions.

## MATERIAL AND METHODS

During the early summer of 2004 and 2005, measurements of pollen concentration and deposition were made on pollinating maize crops. During the full pollination period, mean concentration measurements were made every two hours at the male flower level using a Burkardt trap. Estimates of pollen deposition were obtained using containers placed within the pollen source at 50 cm above ground level. Pollen deposition



**Figure 1.** Pollen emission dynamics characterized by the fraction of emitting tassels, the air-borne pollen concentration (measured using a Burkard trap) and the deposition at the bottom of the crop (measured in the middle of a 2.4 m circle with plants removed).

sensors were handled manually in 2004 and mechanically in 2005. Micrometeorological variables were measured including global radiation, air temperature and relative humidity, air turbulence above the canopy, rainfall and wetness duration above the crop. Following preliminary results (Jarosz *et al.*, 2003) on maize pollen emission obtained in a large study of pollen dispersal downwind from a pollen source, experiments devoted more specifically to pollen emission were carried out in France on 2 sites (Grignon near Paris and Pau in the South-West of France) with 2 or 3 different sowing dates.

## RESULTS AND DISCUSSION

Using a number of similar data sets, various types of statistical models were tested to simulate daily maximal pollen concentration and variation of adimensional pollen concentration during the day on sunny days. The combination of simple analytical models such as logistic or Gaussian seems to provide a consistent approach for estimating the potential emission at any time during pollination.

## PERSPECTIVES

Our intent is to develop a model to estimate the response of pollen emission to environmental limiting conditions. This model would be helpful in estimating the source term of models of pollen dispersal at short or long distances. One model under consideration is semi-empirical and based on the threshold concept using functions such as daily maximal emission and relative emission governed by environmental factors.

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# Oilseed rape in Danish settings: varietal purity and volunteer populations

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**Abstract:** The present study is part of the SIGMEA activities under EU-framework 6. The study aims at collecting information to be used in forecasting the adventitious presence of transgenes in oilseed rape cultivated in a Danish scenario. Purity of 14 certified seed lots were analysed by ISSR markers and subsequent assignment tests, and the results indicated that some certified seed lots were not as pure as anticipated from the seed certification procedures. Using the same set of DNA markers and software, volunteers were identified in 3 fields of winter oilseed rape. Here, 6-32% of the plants collected at random were assigned to other varieties than the one cultivated in that year. For volunteer oilseed rape populations collected in other crops, assignments based on the DNA markers showed that different seed sources had produced the plants.

## INTRODUCTION AND APPROACH

### Varietal purity

New co-existence regulations are to some extent based on knowledge obtained from propagation of conventional varieties, as it is believed that present control measures for seed propagation will ensure a high degree of seed purity, and thus limit future adventitious presence of GM.

However, today's varietal purity is almost solely based on morphological markers that are likely to underestimate the degree of adventitious presence. Therefore, in order to evaluate the purity of present day certified seeds, seed lots from 14 different oilseed rape varieties were analysed by 54 ISSR markers. The 14 varieties were cultivated in the period 1985-2004, and represented some of the most commonly grown varieties during that period. Individuals from the varieties were reallocated to variety using the AFLPop assignment software.

## Volunteer populations

Adventitious presence is also influenced by contribution from volunteer plants. In order to cope with these volunteer populations, knowledge about the origin of the populations is needed. We analysed the genetic composition of 4 volunteer and 1 feral population(s) as well as individuals from 3 oilseed rape fields. Information was collected from the farmers about which oilseed rape varieties were previously grown at the sites, and when this cultivation took place. Farmers were also interviewed about the cultivation practice at the sites. The plants from the volunteer populations or oilseed rape fields were analysed by the same 54 ISSR markers as the 14 certified seed lots. Subsequently the plants from the individual populations were assigned to the reference set of certified seed lots using AFLPop. In this way it was possible to see which frequency of the volunteers that derived from the present or the previous oilseed rape varieties grown at the site, and to detect volunteers that derived from other sources.

## MATERIALS AND METHODS

The reference varieties were Artus, Aviso, Bristol, Canberra, Cannon, Capitol, Ceres, Contact, Elan, Express, Falcon, Global, Karola and Labrador. The 3 winter oilseed rape fields plus 5 volunteer/feral populations were collected from different sites in Zealand. DNA extraction was carried out according to Doyle & Doyle (1990), and ISSR analysis was performed according to Johannessen *et al.* (2005) and carried out on 30 individuals from the volunteer/feral populations, 48 individuals from the oilseed rape fields and 40-50 individuals from the reference varieties (~ certified seed lots). The anchored primers 888 (Charters *et al.*, 1996) and 834 (Ma *et al.*, 2003) were used.

The assignment of plants was performed using AFLPop version 1.1 (Duchesne & Bernatchez, 2002) that can also be applied on ISSR markers. A certain genotype was only allocated to a

population when it was  $10^2$  times more likely that it belonged to this population. Allocation to population "none" (unknown variety/population) was also possible.

## RESULTS AND DISCUSSION

The results show that 6 of the certified seed lot were pure, for 4 other seed lots a few plants (2-8%) allocated to other varieties among the reference set or to "none". The remaining 4 seed lots had a high frequency of "none" allocation possibly reflecting the close relationship between these varieties. So in conclusion, some of certified seed lots were not as pure as anticipated from the current seed propagation procedures, as the presence of other varieties found was well above the allowed adventitious presence (< 0,3% for food and < 1% feed; < 10% in hybrid varieties due to incomplete restoration of fertility). There were no obvious correlations between % adventitious presence and age of varieties or breeding method (i.e. dihaploid or hybrid variety). The analysis for volunteers in the oilseed rape fields also hints to a much larger frequency of volunteers (6-32%) than previously assumed, and this adventitious presence could not be explained alone by impurities in the certified seeds. Not unexpectedly, the analysis of volunteer populations in other crops pointed to previous cultivation as the most probable source of volunteers, however, other sources also contributed to the volunteer populations.

The surprisingly high frequencies of adventitious presence that we found in certified seeds and oilseed rape fields urge cautious cultivation of GM oilseed rape. Our findings are being re-examined in another more comprehensive study.



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# Applicability of the isolation distances in Italian farming systems

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**Abstract:** The Italian law on co-existence foresees the respect of the isolation distances between GM and non GM cultivations of the same species. Considering the reduced size of most Italian farms, application of the law seems unfeasible. We have simulated isolation zones around a squared GM maize cultivated field. The following results emerged from our analysis: the area of "isolation zone", 25 m wide, should be equivalent to 50% of a 3 hectares field. For a typical Italian farm, an isolation distance of 200 m is not applicable; observing isolation distance of 200 m, less than 4.6% of total Italian farms have the minimum area necessary to cultivate almost 1 hectare of GM maize. Considering these results, it's practically impossible for the majority of Italian farmers to cultivate GM maize. Moreover, the isolation distances hypothesized would not be enough to avoid transgenic contamination.

## INTRODUCTION

The Italian law on co-existence (January 28 2005, n°5 Law) foresees the bind for the farmer that intends to cultivate GM varieties to respect the isolation distances from the conventional cultivations of the same species, that will be defined in the regional co-existence plans. Therefore, without an agreement between bordering farmers, it will be necessary to create an opportune "isolation zone" (or "buffer zone") around the GM cultivated field. In case someone intends to cultivate GM varieties, such a

requirement would be a problem for most Italian farms, seen their reduced size.

In fact, 64,3% of approximately 2.5 millions farms, use an equal or smaller than 2 hectares cultivated area (CA), while only 4.6% has more than 20 hectares. The average CA of a single farm is approximately 5 hectares (ISTAT, 2000).

With this feature we intend to highlight the difficulties, or the impossibility, to apply the hypothetical isolation distances for the maize co-existence in the Italian farming systems context.

## APPROACH AND METHODS

In our simulation we have assumed as a hypothesis that the farm's CA is set up just by a squared field and that without an agreement between neighbours, the GM maize farmers are forced to achieve the isolation zones prescribed by law inside their own field.

In the maize case, the necessary isolation distances, to contain the transgenic contamination under 0.9% threshold, according to those in discussion and/or already adopted in some European countries (Germany, Denmark and Spain) and also on the basis of available literature (Brookes *et al.*, 2004; Ingram, 2000), they can be hypothesized, for Italy, from few meters (20-30 m) to hundreds of them (100-200-300 m).

Considering what we've said above, we have assumed several isolation distances (25, 100 and 200 m) around hypothetical square form fields, which represents the whole farm's CA, all of it, with GM maize.

Regarding the distance of 25 m we have tried to understand how the surface share assigned to the isolation zone varies as the dimensions of CA varies too.

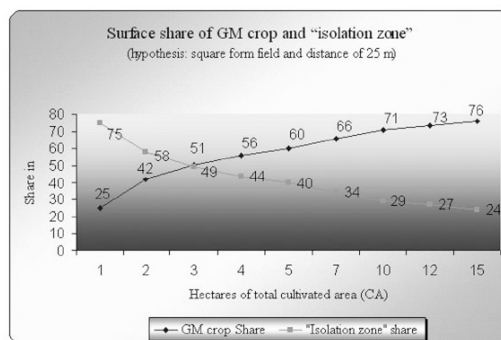
Moreover, for each one of the three mentioned distances, we've tried to highlight (1) which surface share in a standard average Italian farm would be assigned to the isolation zones, (2) how many farmers are in conditions to cultivate at least one hectare of GM maize.

## RESULTS AND DISCUSSION

In general, the percentage share of the surface occupied by the isolation zone increases as the isolation distance established by the law increases too, as the total cultivated field surface decreases, and when it goes from a squared to a narrow and long shape.

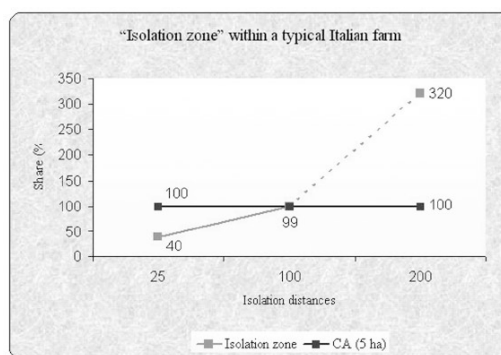
In particular, the following results emerged from our analysis:

The area of "isolation zone", 25 m wide, should be equivalent to 24% of a 15 hectare field, to 50% of a 3 hectare field and up to 75% of a 1 hectare field (Figure 1).



**Figure 1.** Effect of cultivated area (CA) on surface share distribution between GM crop and "isolation zone".

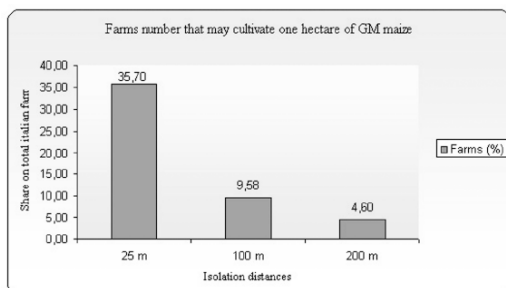
Taking into account a typical Italian farm, with an average CA of 5 hectares, and distances of 25 m and 100 m, the "isolation zone" should be equivalent to 40%, for the first distance, and to 99%, for the second distance, of the total CA. In the latter case, an isolation distance of 200 m is not applicable (Figure 2).



**Figure 2.** Effect of distance on share of the "isolation zone" within a typical Italian farm of 5 hectares.

To cultivate 1 hectare of GM maize, observing isolation distance of 25 m, 100 m and 200 m, it is necessary to have fields of, respectively, 2.25 hectares, 9 hectares and 25 hectares. In addition, considering the distribution of the farms per CA, respectively, less than 35.7%, about 9.58% and

less than 4.6% of total Italian farms have the minimum area necessary to cultivate almost 1 hectare of GM maize (Figure 3).



**Figure 3.** Effect of isolation distance on farms number with minimum cultivated area (CA) necessary to cultivate one hectare of GM maize.

## PERSPECTIVES

Under such conditions, respecting the isolation distances already suggested or adopted in some European countries or proposed at national level, it becomes practically impossible for the majority of Italian farmers to cultivate GM maize. Moreover, the isolation distances hypothesized, although suitable for to meet 0.9% threshold, would not be enough to avoid transgenic contamination (Brunet, 2003; Luna, 2001).

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# Gene flow between wheat cultivars: *T. aestivum* and *T. turgidum* outcrossing in an individual recipient plant basis under field conditions

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**Abstract:** A two-year field study was conducted to assess the potential outcrossing under natural conditions between two bread wheat cultivars (*Triticum aestivum* L.) and between bread and durum wheat (*Triticum turgidum* L. var. *durum*).

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is the world's most important food crop species. It is predominantly a self-pollinating crop that has variable outcrossing rates depending on genotypes and environmental conditions (Waines & Hegde, 2003). Transgenic herbicide-resistant wheat varieties are being field-tested and probably in few years transgenic certified wheat cultivars will be commercially available. GM wheat cultivars will be grown

next to non-GM wheat, thus, data on potential outcrossing events are useful for assessing the risks of future genetic pollution, with attendant safety and economic implications. Bread wheat can also coexist in the field with the second major cultivated wheat species, the durum wheat *Triticum turgidum* var. *durum*, usually grown for pasta. There are few data on the literature about the outcrossing rates between these species under field conditions. That information is necessary for the risk assessment study in the case of coexistence.

## MATERIALS AND METHODS

The experiment was carried out on the INIA experimental station in a field trial surrounded by pines and isolated from wheat fields during 2003 and 2004.

### Intraspecific gene flow

Five *T. aestivum* cv Deganit dark colour-grained wheat 1 x 1 m plots, with 100 plants sown in four rows (25 plants per row) and spaced 25 cm were used as pollinator blocks. One *T. aestivum* cv Pavon white colour-grained wheat was sown inside each block. Plants were allowed to flower freely and seed formed on Pavon recipient plants were collected. F<sub>1</sub> grains were sown and plants were grown to maturity in the greenhouse. Cross-pollination was evaluated by the dark colour of F<sub>2</sub>-grains derived from F<sub>1</sub> plants.

### Interspecific gene flow

The same experimental design as described for the intraspecific gene flow was used. One *T. turgidum* cv Nita was sown into each block. Cross-pollination from Deganit to Nita was evaluated by the formation of F<sub>1</sub> shrivelled grains. Hybridity of the plants was confirmed on the basis of their chromosome number.

## RESULTS AND DISCUSSION

### Intraspecific gene flow

In *T. aestivum* cv Deganit, dark grain colour is inherited as a semi-dominant trait. F<sub>1</sub> hybrid grains obtained in previous hand crosses between Pavon and Deganit not allowed distinguish the dark colour of the Deganit parental wheat, but this trait was clearly expressed in the F<sub>2</sub>-grain derived from F<sub>1</sub> plants. Gene flow rates were calculated as the percentage of plants that give dark-grained progenies related to number of grains sown. The rates of gene flow in an individual plant basis, with an average of

2.15% in 2003 and 0.57% in 2004, were into the rates obtained by Martin (1990) in Arkansas (EEUU) and Hucl (1996) in Saskatoon (Canada) under similar agroclimatic conditions and with greater pollen sources. Differences among blocks from 0 to 4.5% of hybrid grains were detected.

### Interspecific gene flow

Hybrids between Nita and Deganit were easily identified by the shrunken endosperm of the pentaploid grains. Outcrossing values obtained were lower than for the intraspecific gene flow and also higher in 2003 (average of 0.28%) compared with 2004 (0.19%). These values are in the order of the average rates (0.08-0.41%) obtained by Matus-Cádiz *et al.* (2004) in durum wheat plants growth at 0.2 m of a 50 x 50 pollinator block.

Low temperatures and high rainfall in 2004 are probably responsible for low gene flow rates as has been previously described by Loureiro *et al.* (2005).

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# Perspectives for co-existence in Slovenia

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**Abstract:** Farmers should have the possibility to choose between conventional, organic and GM crop production while respecting all the regulations. In our study we tried to illustrate problems facing introduction of GM plants to Slovenia, using the potential economic loss, which could occur due to the unintended presence of GM admixture in the non GM crop, taking into account that farmers are not able to market the product, and to describe perspectives for co-existence. Looking at the cropping structure in Slovenia and assessing the availability of GM cultivars, we could foresee interest of farmers for growing GM corn, potatoes and possibly oil seed rape and sugar beets. Due to expected extension of GM plant production in to the Slovenian crop production, there is a need to construct the scientific and technological basis for GM production, and to organize Slovenian farming in a way, that will enable the co-existence between conventional, organic and GM production.

After the first commercialisation of a transgenic plant in 1994, large production of transgenic crops started in 1996. The introduction of GM crops in the EU has raised questions concerning gene dispersal and co-existence with non-GM-farming. Biotech production in the world grows with an annual rate of 20%. More than half of the world soybean production and approximately 30% of the corn production is based on biotech agriculture. Biotech products dramatically decreased use of pesticides and significantly increased crop yields. Recent experiences in the USA and also in the EU, in particular in Spain, prove that co-existence in agriculture is possible.

Slovenia is the only EU member without any field tests of GM crops. In the future, quoting official statements, Slovenia will begin with

selective and cautious introduction of GM crops since the production quantities are unimportant for the EU market, and due to the fact, that Slovenian agriculture seeks an opportunity in the so-called bio-agriculture and eco-tourism. Both niche markets are in conflict with the GM crop production. In spite of negative public opinion on biotech farming, Slovenia cannot be an isolated island of organic production in the EU. Presence of GM plants above the allowed threshold abides farmer to label the product. That would affect the marketability of the product, since there are couple of regulations in place not allowing any adventitious presence of GMO in the product (Regulation on organic production and manufacturing and Regulations on integrated production). Farmers as well receive subsidies for growing crops under these regulations.

Maize is the most important field crop in Slovenia. In the year 2004 it was grown on 73.041 ha or on more than 40% of all fields. Approximately two thirds are intended for grain production (45.966 ha), and one third is for whole plant silage production (27.045 ha).

Present range of maize production is a reflection of specialization and concentration in agriculture. In the case of Slovenia, field crop production supports well-formed animal husbandry with a comprehensive part of voluminous and concentrated feed. The most part of maize is used directly on farms for animal feeding and only a minor part of maize appears on the market for human consumption (3.000 tons). Intensity of production is on high level and we can expect interest of producers to use GM varieties of maize.

Potato is as well an important field crop, which has a long tradition in Slovenia. Due to problems with diseases and pests we can expect interest of producers to use resistant genetically modified varieties.

Slovenia is characterised by large geographical diversity which results in the distribution and size of farmland, which is in most parts characterized by a very small size of less than two ha.

Due to expected move of GM plant production in to the Slovenian farming, there is a need to construct the scientific and technological basis for GM production and to organize the agricultural production in a way, that will enable the co-existence between conventional, organic and GM production. At the same time farmers would have possibility to choose between different kinds of production, obeying law and decrees. The results are to improve competitiveness of Slovenian farmers and economic operators by increasing the confidence of consumers with regard to Slovenian agricultural products.

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# Quantification of pollen gene flow in large maize fields by using a kernel colour trait

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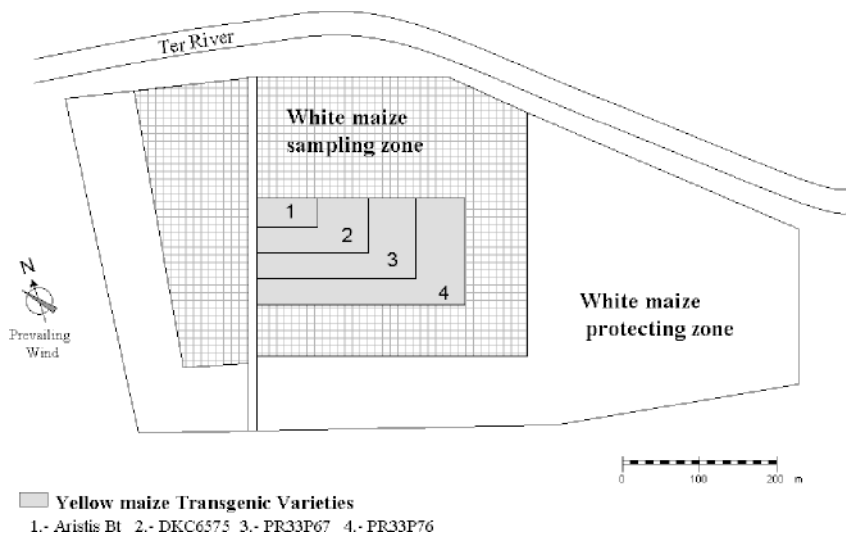
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**Abstract:** A field trial was conducted aimed at determining the gene flow in large fields and the influence of the size of the field in cross-fertilization. A nucleus of 4 ha with four different Bt commercial hybrids of maize (yellow kernels) was surrounded by conventional maize (white kernels) to fill a total area of 27 ha. Cross-pollination was detected by counting the yellow kernels in the white cobs. In zones adjacent to transgenic nucleus, the white maize rows behave as buffer zone and at 10-15 m distance the mean of the samples had a rate of cross-pollination lower than 0.9%. However, when a path of 10 m broad separated transgenic nucleus from the white maize, the rate of cross-pollination was much higher. These results suggest that a separation distance without anything that disturbs pollen flow, has a very few effect in preventing cross-pollination. Analyses by SSR markers to determine the influence of the size of the field in cross-pollination are still in progress.

## INTRODUCTION

Gene flow quantification in large fields requires analysing a great number of samples and, depending on the method used, this could be a very expensive and time consuming process. The use of maize varieties with a different trait such as kernels colour has been

shown to be a useful tool in these studies (Klein *et al.*, 2003; Ma *et al.*, 2004). Here we present results obtained in a field where Bt maize cultivars with yellow kernels and conventional maize cultivar with white kernels have been used. This trial aimed at determining the gene flow in large fields and the influence of the size of the field in cross-fertilization.



## MATERIAL AND METHODS

The field trial was conducted in Girona (Spain) during the growing season of 2004. Four ha of the centre of the field (Figure 1) were sown with four different *Bt* commercial hybrids of maize occupying different areas [Aristis Bt (0.25 ha); DKC6575 (0.75 ha); PR33 P67 (1.25 ha) and PR33P76 (1.75 ha)]. Conventional maize hybrid PR32Y52 was planted in the surroundings to fill a total area of 27 ha. *Bt* hybrids have the kernel endosperm yellow whereas PR32Y52 had a white endosperm. White or yellow characters are in homozygous stage and cross-fertilization between *Bt* and white maize was easily observable by visual counting. An automated weather station was placed in the middle of transgenic fields during flowering period. Coincidence of flowering period among the hybrids used was determined by using pollen traps.

At maturity a systematic sampling (three cobs/sample) was conducted to determine the extent of cross-fertilization in the whole field. Moreover, in order to determine from what *Bt* hybrid pollen comes from, other samples to be analysed by SSR were taken at different distances from the zone of the field placed in the prevalent wind direction.

## RESULTS AND DISCUSSION

Coincidence of flowering among all varieties was very high with the exception of Aristis Bt that started flowering three days earlier but then, two days of raining favoured its flowering synchronization with the other varieties.

More than 700 samples (3 cobs/sample) were taken to quantify the gene flow in the white field. As expected, the gene flow was higher in the prevalent wind direction. Gene flow strongly decreased with the distance to transgenic nucleus in such a way that in the zones of the field where white maize was sown adjacent to transgenic nucleus, the white maize rows behave as buffer zone and at 10-15 m distance the mean of the samples had a rate of cross-pollination lower than 0.9% (less than 18 yellow grains/sample). However, in the zone of the trial where a path of 10 m broad separated transgenic nucleus from the white maize, the rate of cross-pollination found in the cobs from plants placed near the path was much higher. These results suggest that a separation distance without anything that disturbs pollen flow, has a very few effect in preventing cross-pollination.

Analyses by SSR markers to determine the influence of the size of the field in cross-pollination are still in progress.

## ACKNOWLEDGMENTS

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# Quantifying a crop's potential for gene flow: an Irish perspective

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**Abstract:** A gene flow index (GFI) has been established to quantify a baseline gene flow data set for Ireland's primary crops. The GFI incorporates four strands of crop-mediated gene flow into a format that permits the calculation of a crop's potential for gene flow. We propose that the attained indices will highlight those crops that require additional measures in order to minimise gene flow in accordance with anticipated co-existence guidelines.

## INTRODUCTION

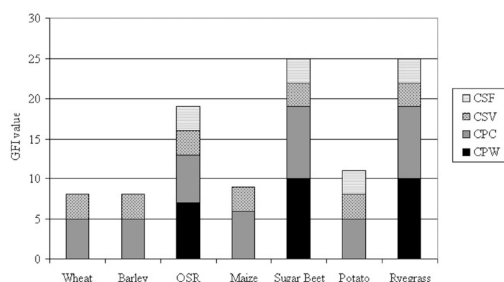
The issue of gene flow is of particular importance when considered in the context of co-existence. The provision of a numerical index that quantifies a crop's potential for gene flow could facilitate both scientists and policy makers in quantifying the risk a GM crop poses to effective coexistence. We have expanded upon previous GFI systems (de Vries et al., 1992) to include seed-mediated gene flow, the role of feral populations and pollen-mediated crop-to-crop gene flow. Our numerical scale combines four strands of analysis to assess the potential for gene flow from each described crop.

## APPROACH

Applied to wheat, barley, potato, sugar beet and perennial ryegrass, oilseed rape and maize, calculated GFI values pertain to the propensity of each crop to form viable hybrid / volunteer / feral individuals. Composed of four strands [crop pollen-to-wild relative (CPW); crop pollen-to-crop (CPC); crop seed-to-volunteer (CSV) and crop seed-to-feral (CSF)], each strand contains a series of sequential questions designed to provide a 'yes/no' answer, which in turn equates to a relevant score (Flannery et al, 2005).

## RESULTS AND DISCUSSION

Both ryegrass and sugar beet exhibited high gene flow potential (GFI=25/27). The potential for pollen and seed-mediated gene flow in potato (GFI=11/27) relates to combined tuber and true potato seed (TPS) production. Wheat (GFI=8/27) and barley (GFI=8/27) recorded low indices, with gene flow potential for maize (GFI=9/27) limited to pollen-mediated crop-to-crop and seed-mediated crop-to-volunteer. Oilseed rape confirmed its ability to disperse its genetic material with a GFI=19 (Figure 1).



**Figure 1.** Graphical representation of combined pollen and seed-mediated gene flow for wheat, barley, oilseed rape (OSR), maize, sugar beet, potato and ryegrass.

GFI values attained from strands CSF, CSV, CPC and CPW (see text)

This research has established a baseline gene flow data set for Ireland's crops through the provision of a novel numerical index. It is intended that the model will complement existing systems models (e.g. Colbach *et al.*, 2003) by generating a value which can be used for comparison to the outcomes of more mechanistic models.

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# The Plus-Hybrid system as a method of transgenic pollen flow containment in maize

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**Abstract:** The Plus-Hybrid system, the growth of a transgenic cytoplasmic male sterile (CMS) hybrid and a second unrelated hybrid as pollen donor, has been proposed as a method for transgenic pollen flow containment in maize. Moreover, Plus-Hybrids increase the grain yield, attributable to the combination of the CMS and the Xenia effects, improving the acceptance of this containment strategy. At present, we aim at defining optimal hybrid combinations leading to a higher expression of the Plus-Hybrid effect, and to set the optimum ratio of transgenic CMS mother hybrid and pollen donor hybrid. Furthermore, the stability of the cytoplasmic male sterility will be investigated.

## INTRODUCTION

The co-existence between genetically modified (GM) and conventional or organic maize is one of the main interrogations around the commercial introduction of GM maize. Under suitable atmospheric conditions, viable GM maize pollen may travel some distance and fertilize non-GM crops. Agronomic measures such as spatial isolation and border rows alone cannot reliably prevent the dispersal of transgenic pollen.

Through the Plus-Hybrid system, i.e. the growth of CMS and therefore male sterile, transgenic hybrids and non-transgenic hybrids

as pollen donor, the release of transgenic pollen may be avoided. An additional positive effect of growing Plus-Hybrids may be the increase in yield, triggered by the combination of the CMS and Xenia effects.

## APPROACH

The growth of transgenic CMS maize hybrids in combination with a lower proportion of non-transgenic and unrelated hybrids serving as pollen donor, results in the CMS GM plants releasing no pollen or no viable pollen, so that the transgenes cannot escape from the GM maize field (Feil *et al*,

2003; Feil & Stamp, 2002). 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 (Stamp *et al.*, 2000). Second, pollination of the CMS hybrids by genetically distinct pollen donor hybrids (non-isogenic pollination) can bring about additional grain yield benefits through Xenia (Weingartner *et al.*, 2004; Weingartner *et al.*, 2002). 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 (Schnable & Wise, 1998), 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.

In our present and future field experiments, we want to determine which ratio of transgenic CMS hybrid and non-transgenic pollen donor hybrid would ensure an optimal pollination and therefore a full seed-set.

Also, we aim at determining the optimal hybrid combinations capable of inducing the Plus-Hybrid effect on yield. For this purpose, modern commercial hybrids from the Swiss breeding company DSP (Delley Seeds and Plants AG) and other European breeding companies in their CMS and fertile versions will be tested for their male and female combining ability within the system, taking into account CMS and Xenia effects.

These field tests will be accompanied by a fingerprinting analysis of the hybrids. The pedigree information of the hybrids, in combination with the data obtained in the field, should help us to develop a prediction tool for the Plus-Hybrid effect and to develop Plus-Hybrid prototypes.

A further important aspect of the Plus-Hybrid system as a method of transgenic pollen flow containment is the stability of the cytoplasmic male

sterility. The lack of a reversion to male fertility of the CMS, triggered either by environmental factors or by the presence of fertility restorer genes in the breeding populations (Schnable & Wise, 1998) is an important consideration. This aspect will as well be analyzed in the frame of our investigations.

## PERSPECTIVES

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 and where male sterility systems can be incorporated.

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# Volunteer wheat seed fecundity: contributions to a mechanistic agronomic model

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**Abstract:** A mechanistic model was developed to assess the degree of admixture of glyphosate resistant (GR) wheat volunteers in western Canadian cropping rotations. Field trials were conducted to investigate the effect of pre-seeding and post-seeding herbicide applications and crop competition on volunteer wheat fecundity and density in canola and pea crops. GR volunteer wheat fecundity (seed production plant<sup>-1</sup>) was greater than wheat grown as a crop, in the absence of herbicides. GR volunteer wheat fecundity was reduced as herbicide rates increase; pre-seeding herbicide application had a greater effect on volunteer densities, and in-crop herbicides had a greater effect on fecundity. The data derived from these field trials were used to develop a wheat fecundity submodel to more accurately predict seedbank longevity and the degree of admixture in crops.

## INTRODUCTION

Glyphosate resistant (GR) wheat was used to model the significance of crop volunteers to seed admixture within western Canadian crop rotations. Volunteer fecundity influences seed bank longevity and thus admixture of seeds in subsequent crops. Volunteers may be less fecund than crops due to less favorable

microsites. Volunteer fecundity is influenced by both crop competition and herbicides applied prior to and post seeding. Field trials to assess the contribution of these factors on volunteer wheat fecundity were conducted. A mechanistic population model similar to that described by Hansen *et al.* (2002) was developed to predict the influence of agronomic parameters on seed admixture.

## METHODS AND MATERIALS

Field trials were conducted in 2004 and 2005 near Edmonton, Alberta, Canada to quantify the fecundity of volunteer wheat within pea and canola crops. GR volunteers were seeded prior to the crop. Herbicide treatments were applied pre-seeding and post-seeding at four rates in a factorial, replicated design. Quizalofop/glyphosate was applied prior to seeding and glufosinate or imazamox/imazethapyr applied in canola or peas, respectively. Surviving GR volunteer wheat plants were hand harvested and density, spikes plant<sup>-1</sup>, seeds head<sup>-1</sup>, and 1000 kernel weight assessed. Plots were harvested and GR wheat admixture assessed.

A mechanistic model was developed based on the annual lifecycle of volunteer wheat. Submodels included seedbank viability and emergence, herbicide selection, outcrossing, volunteer wheat fecundity, and harvest losses. Data from these field trials were applied to the fecundity submodel to predict seed admixture.

## RESULTS AND CONCLUSION

Pure stands of four spring wheat cultivars in Canada averaged 104 seeds plant<sup>-1</sup> with a seed kernel weight of 31 mg (Wang *et al.* 2002). Preliminary data from field trials suggests volunteer wheat plants produce 154 seeds plant<sup>-1</sup> with an average seed weight of 28 mg in the absence of herbicide controls. Pre-seeding herbicide application at the highest rate reduced the volunteer fecundity by 45% and individual seed weight by 13%. In-crop herbicide applications had a greater influence on volunteer fecundity, reducing the seeds plant<sup>-1</sup> by 59% and individual seed weights by 34%. GR volunteer plant densities were reduced 94 and 85% by pre-seed and in-crop herbicide applications respectively. Combining high rates of pre- and post-seeding herbicides resulted in no remaining GR wheat volunteers in 15 of 16 instances.

The mechanistic model predicts that reductions in volunteer wheat fecundity decreased seed bank replenishment, plant densities in subsequent years, admixture in harvested seed, and the GR wheat re-planted in farm saved seed. The model approximates volunteer densities derived from agronomic field trials in which volunteer GR wheat populations were virtually eliminated two years following GR wheat production (Harker *et al.*, 2004). Volunteer seed fecundity is a key component to accurately model GR wheat admixture.

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# Weed beet as a co-existence issue for GM sugarbeet

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**Abstract:** Populations of weed beets expanded into central European sugar beet production areas in 1970's, creating a serious problem. Sugar beets can cross with wild relatives, and so it may be necessary to monitor wild and weed populations for transgene escape. The highest frequency of dispersal is via seeds that persist in the soil for many years. In our studies the proportion of surviving seeds decreased with time and two years after burial only 12.5% of seeds were able to germinate.

## INTRODUCTION

Hybridization between closely related species can be a way of transgene flow directly into wild populations; thereby crop plants with weed/wild relatives are of particular concern. Crop traits may escape from cultivation to wild relatives and persist for many years as seed in soil seed bank. Sugar beet (*Beta vulgaris* L.) has sexually compatible wild relatives with which can hybridize under favorable circumstances. The life cycle of cultivated beet is biennial, inflorescence and seeds are developed during

the second year. A few individuals can, however, bolt and produce seeds during the first year. In Germany, Britain, Denmark and France, there are populations of annual weed beets, which can become problematic dispersal sources if GM outcrossing occurs. Bartsch *et al.* (1999) showed that transfer of the transgene from sugar beet to *Beta maritima* is possible. Vigouroux *et al.* (1999) reported that hybridization between bolting GM sugarbeet and weed beet occurred under field conditions. Studies have shown that GM wild/weed beet hybrids are likely to survive as non GM weed beets. At present, no GM beets are approved for commercially growing in the EU.

## MATERIAL AND METHODS

Data on seed establishment were recorded. Samples of 100 weed beet glomerules, harvested from four weed beet populations which have been collected in sugar beet fields, were mixed with chernozem soil and put into nylon bags. Every month during the next 2 years, four bags were excavated and used for germination test (20 °C, 16 hrs day/8 hrs night regimes, light intensity  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The seed persistence study was carried out using non-transgenic seeds.

## RESULTS AND DISCUSSION

Observations of seed production performed at the end of vegetation have shown high variance between localities as well as within populations, especially, in the plant height and number of fruits. On average, each plant can produce 500 up to 5000 glomerules, each contained from  $2.3 \pm 1.2$  to  $3.4 \pm 1.3$  viable fruits. Longden (1982) found that intact bolters left to grow to maturity produce in average 1000-1919 seeds per plant. Many of them survive so that the weed beet problem progressively becomes worse with each rotation. Weed beet seeds appear to be able to remain viable in soil for at least 7 years (Gunn, 1982). Ploughing and burying of seed prolongs the life of the seed. In Duvel's burial experiment, sugar beet seed buried in 20 cm and 1 m gave 8.35 and 9% germination respectively after 6 years and less than 1% after 21 years (Toole, 1946). In our experiments (Figure 1) weed beet lost viability with time, two years after burial only 12.5% of seeds were able to germinate. The starting low value of germination indicated influence of dormancy and residual dormancy may have reduced the values at later dates.

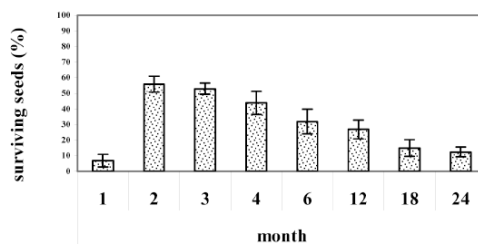


Figure 1. Changes in weed beet seed survival with time after burial.

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## ACKNOWLEDGEMENT

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# Simulation of gene flow using GENESYS under Danish conditions for oilseed rape co-existence

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**Abstract:** Co-existence of GM and non-GM winter oilseed rape varieties was studied under Danish farming conditions, using simulations carried out with the GENESYS model. GENESYS quantifies the effects of cropping systems on gene flow among crop plants and volunteers in time and space. Results from two scenarios are presented.

## INTRODUCTION

In a Report from the Danish Working Group on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops (Tolstrup *et al.*, 2003), measures of agricultural practice for ensuring compliance with requested threshold values for co-existence of GM and non-GM crops have been suggested for the major Danish crops. These measures include distance between GM and non-GM varieties of a given crop, growing intervals between GM and non-GM varieties and field size and shape. A simulation study of gene flow from winter oilseed rape under Danish growing conditions was carried out in connection with this report using the simulation model GENESYS developed by Colbach *et al.* (2001a,b). Here, these Danish

results are presented and reconsidered using a revised version of GENESYS.

## APPROACH

GENESYS was developed for modelling gene flow *via* pollen, seed and volunteers from GM winter oilseed rape to non-GM winter oilseed rape in France with the aim of studying the influence of characteristics of a regional cropping system. The input variables of the model are (1) the regional field pattern including uncultivated areas; (2) the crop rotation for each field, (3) the cultivation techniques of each crop (e.g. tillage, sowing date and density, use of herbicides, harvest date); and (4) oilseed rape variety characteristics, comprising GM (e.g. GM herbicide tolerance)

vs. non-GM varieties and crop production vs. hybrid seed production. The model is based on the annual oilseed rape life cycle (seed bank, seedlings, adults, flowers, seed production) for crop plants and volunteers and depends on crops and cultivation practices. During flowering and seed production, pollen and seeds are dispersed between fields, depending on rape flowering dates, seed loss rates, as well as field sizes, shapes and distances.

For the present simulations, an existing field pattern from the County of Viborg in 1998 was used together with different typical Danish crop rotations and management strategies for conventional and organic farming as established by the Oilseed Rape Task Group contributing to the Danish Report on Coexistence. Other input variables were also made to vary comprising harvest seed loss, relative competitive abilities of GM and non-GM varieties and random dispersal of seeds during harvest and transport. The analysed output variable was the proportion of GM seeds in non-GM oilseed rape harvests.

## RESULTS AND DISCUSSION

Using the Danish agricultural practice for simulations of the relative effects of different management strategies (e.g. cropping interval) on the adventitious presence of GM seeds, similar results was obtained as those by F. Angevin, N. Colbach, J.M. Meinard and C. Roturier (Chapter 3 in Bock *et al.*, 2002) studying gene flow under French and German farming conditions. Comparing absolute values may be misleading because these vary considerably from farm to farm, depending on the farming system and the surrounding area. Results from two scenarios will be shown: 1) gene flow from a GM oilseed rape field to the surrounding agricultural area and 2) gene flow to a non-GM oilseed rape field located in an area where all other oilseed rape fields were cultivated with GM varieties. The results emphasised the importance of the spatial pattern, size and shape of fields combined with the actual crop rotations.

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# The impact of post-harvest management upon reliability of GMO screening

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**Abstract:** We analysed soybean kernels produced from certified GMO free seeds in a field in Czech Republic using lectin, 35S CaMV, T-NOS, EPSH synthase and CaMV specific primers. Samples taken directly from the field were tested negative for 35S CaMV and T-NOS. Samples analysed after transportation and namely after storage in a silo were found positive for all target sequences apart from EPSH synthase. Presence of CaMV virus was detected being probably caused by contaminations during post harvest management. We indicated that just the use of 35S CaMV and T-NOS sequences for GMO screening may be insufficient for GMO detection.

## INTRODUCTION

Genetically modified organisms have become a part of the food chain. In EC all GMOs and derived products are subjected to strict regulations. All approved GMOs and derived products have to be labelled. Companies very often run their internal audits and ask for laboratory analysis of arbitrary chosen lots. Analysis are not cheap, so companies often ask for the use of screening methods only (Vollenhofer *et al.*, 1999). Among GM crop Roundup Ready soybean is widely used. Soya and derived products are used mainly as animal feed. Soya is grown to some extent in Europe and also in southern parts of the Czech Republic.

PCR based methods are in place to detect GMOs. Most of approved GMOs were developed using 35S CaMV promotor from cauliflower mosaic virus and NOS terminator (T-NOS) derived from *A. tumefaciens*. These elements are used for GMO screening (Vollenhofer *et al.*, 1999) and in some cases as well for transgene quantification (TaqMan GMO 35S Soya Detection Kit, TaqMan GMO 35S Maize Detection Kit, Applied Biosystems). We analysed possible contamination of soybean harvest from plants raised in south Moravia (Czech Republic) from certified GMO free seeds produced in Canada. Food products containing soybean were identified as GMO positive by a laboratory elsewhere.

## MATERIAL AND METHODS

DNA was isolated from grounded soybeans according to the protocol prEN ISO 21571:2002. The presence of GM elements were detected by PCR: 35S CaMV, T-NOS terminator, EPSH synthase (Vollenhofer et al., 1999), CaMV (Wolf et al. 1999) Detection limit of the reactions 5 copies was estimated with the expected confidentiality 98%. TaqMan GMO 35S Soya Detection Kit, Applied Biosystems on ABI 7700 instrument was used for quantification. For comparison quantification was done according to Pietsch & Weiblinger 2000 as well.

## RESULTS AND DISCUSSION

Samples harvested directly from the field, taken from a transport van, storage silo and pre-processing purification were available. Already visual inspection of the samples showed up increasing number of impurities after transportation and namely after the silo off-loading. Several other plant species or their parts were found in the lot including *Brassica napus* L.

Samples taken directly during the harvest were tested negative for 35S CaMV, T-NOS terminator, EPSH synthase and thus were identified as GM free. However, samples analysed after transportation and storage were found positive for 35S CaMV, T-NOS terminator regardless the impurities were manually set aside and kernels were washed by sterile water. Transgene specific (Roundup-Ready, EPSH) tests were negative. Presence of other CaMV ORF was detected by PCR in 35S CaMV positive samples. CaMV virus presence was thus confirmed. The presence 35S CaMV sequence was quantified by real-time PCR. Samples taken after transportation and storage contain more than 5% 35S CaMV sequence, by manufacturer pre-purified samples contain 1.5% of 35S CaMV sequence. After manual purification content of 35S CaMV sequence decreased to 1.4% (transport), 1.7% (silo) and 1.1% (pre-processing). After kernel washing content of 35S CaMV sequence dropped down to 0.35% (transport),

0.75% (silo) and 1.1% (pre-processing). Presence of construct specific sequence (EPSH) was not found by real-time PCR. Thus we believed that CaMV titer was measured in fact.

It is apparent, that screening methods and quantification based on 35S CaMV is inadequate for GMO detection as it was shown by other authors as well (Anonym, 2000). As a footnote, companies should improve their manufacturing practices. Development of cost effective method allowing multiple gene identification is required. DNA arrays technology offers such possibility.

## ACKNOWLEDGEMENT

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# Monitoring the stability of the transgenic sequences – application of new approach

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**Abstract:** Stability of transgene sequences is a crucial prerequisite for application of the DNA based methods that are used for detection, identification and quantification of GM crops.

A new technique for mutation screening based on the heteroduplex formation is tested and optimized for the model species *Arabidopsis thaliana*. The optimized procedure was then used to screen target sequences in some commercial transgenic events. The suitability of this new approach for high throughput screening for single nucleotide polymorphisms (SNPs) and its applicability for molecular monitoring of transgene events are described.

## INTRODUCTION

The introduction of biotech crops into the European crop system is a fact. Since the new regulatory framework entered into force in the EU (April 2004), new transgenic crops are being approved for use in food, feed, and import and processing. The growth and cultivation of the crops is always associated with exposure to stress conditions, which are potential sources of genetic instability.

The stability of the nucleotide sequences is becoming very important in recent times, as there is a high demand for monitoring the stability of the

transgenic sequences of the commercial crops. In the context of the EU requirements for labelling of GM derived foods, the stability of the transgenic insert and the junction regions appears to be an important issue. This is a crucial factor regarding the application of event specific assays.

The development of high throughput and sensitive approaches for mutation scanning has been accelerated during the last years and improved systems are becoming available. Recently Applied Biosystems developed a new polymer that is able to distinguish the wild type and mutation molecules based on their heteroduplex mobility differences ([www.appliedbiosystems.com](http://www.appliedbiosystems.com)). We tested the

sensitivity and the throughput of this method on the *Arabidopsis thaliana* model plant. Further, we continued with the application of this technology for monitoring the stability of the transgenic and endogene sequences of some commercial transgenic events.

## APPROACH AND METHODOLOGY

A test fragment of 447 bp containing 1 base pair substitution A>C between Col and C24 *A.thaliana* ecotypes was used for optimization of the technique.

The pattern of two event specific fragments and two endogenes is investigated: Mon810 – the p-35S/plant border region and SSIIb maize endogene; Soya GTS 40-3-2 – p-35S/plant border and the soya specific endogene lectin. The Heteroduplex Mobility Analysis (HMA) is performed according to the producer's description and using ABI3130 *Avant* Genetic Analyzer (ABI).

## RESULTS AND DISCUSSION

The HMA is tested in *Arabidopsis* DNA pools and the results indicate that one homozygous mutation can be detected in a pool of 10 plants, which makes this technique very sensitive. Other heteroduplex based techniques can detect 1 mutation in 8 (McCallum *et al.* 2000).

Based on the optimized procedure we initiated study on stability of the transgenic junctions in commercial events and the species specific endogenes used in quantitative GMO analysis. We investigated the patterns generated from the P-35S/plant border of RoundupReady soya event GTS40-3-2 and maize event Mon810 together with SSIIb and lectin in order to set them up as a reference patterns for mutation scanning.

## CONCLUSIONS AND PROSPECTIVE

HMA gives opportunity to detect mutation in known sequences in a sensitive and high throughput manner. The methodology is easy to perform for limited time and the patterns are highly reproducible.

The method has the potential to be applied for monitoring purposes. Based on the database of the validated methods for GMO detection and quantification the patterns can be generated for every event specific assay. A similar database can be created for endogene sequences as well.

# GM seed quantification in terms of thresholds compliance

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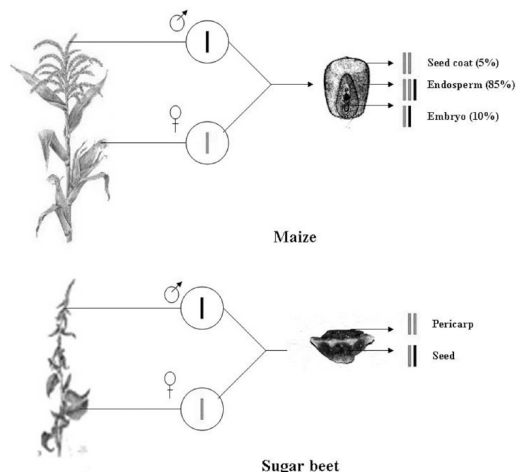
**Abstract:** The European Commission proposes the establishment of thresholds for adventitious and technically unavoidable presence of GMOs in conventional seed lots. These thresholds vary between 0.3 and 0.7% depending on the reproductive system of the plant and the likelihood of its presence in the final product in order to comply with the threshold for food/feed.

GM seed contamination levels are used to be expressed on a seed to seed basis. Moreover this is the measurement unit widely used in practice. Recently, the European Commission recommended that the GM content in food/feed and seeds should be expressed as a number of haploid transgenic genomes related to the total genome number (Recommendation 2004/787/EC). The genome number does not precisely correspond to the seed number, especially in some cases like maize and sugar beet. The relation between number of seeds and number of genomes is investigated. The importance of this in regard to the implementation of the threshold is also featured.

## INTRODUCTION

In the frame of the development of traceability and labelling system for GMOs and GM derived products, the EC previously proposed thresholds for adventitious and technically unavoidable presence of GMO in seed samples. These thresholds varied between 0.3 and 0.7% depending on the reproductive system of the plant and the likelihood of its presence in the final product.

Quantification of seeds by seeds producers is very often performed on seed basis where the GMO contaminations are measured and expressed as a number of transgenic seeds to the total number of seeds. The application of the DNA base quantitative techniques (Real Time PCR) requires quantification based on number of DNA molecules. The commutability of these two measurement units is doubted especially in cases where the male and female genomes are not equally represented in the seed (Fig.1).



**Figure 1.** Inheritance of the male and female genomes in the maize and sugar beet seeds. The maize seed contains diploid seed coat fully maternally inherited, triploid endosperm with 1/3 male genomes and 2/3 female genomes and the diploid embryo with equal distribution of male and female genomes. The sugar beet “seed” in practice is a fruit, made up from fully maternally inherited pericarp and the seed embedded inside.

Here we describe the relation between number of seeds and number of genomes for maize and sugar beet. The importance of this in regard to the implementation of the genome number as a measurement units is discussed.

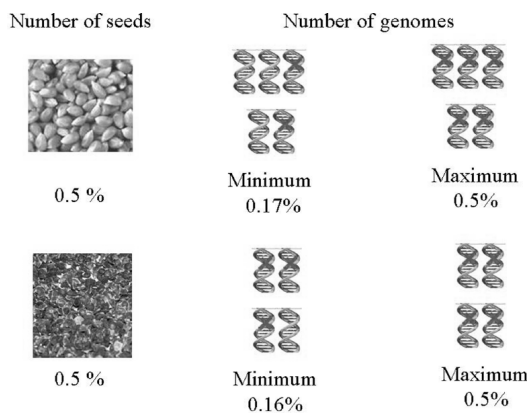
**APPROACH**

The relation number of seeds- number of genomes in homo and heterozygous maize and sugar beet seeds is established based on the DNA amounts and copy number in the respective seed compartments (Papazova et al. 2005a, 2005b). Based on this the quantification on seed and genome basis is compared for model based on the sample containing transgenic seed at the threshold level (0.5%).

**RESULTS AND DISCUSSION**

The seed of maize and the sugar beet contain different structures with irregular distribution of

male and female genomes (Fig.1). This means that depending on the inheritance of sequence of interest the heterozygous seeds will differ in their transgenic content (Papazova et al. 2005a). This unequivocal relation makes difficult the conversion number of seeds-number of genomes. For instance, the genome copies in maize seed sample containing 0.5% (seed/seed) GM seeds can vary between 0.17% and 0.5% in genome copies corresponding to the presence of only heterozygous paternal seeds and homozygous transgenic seeds (Papazova et al. 2005b). For the sugar beet seeds these values vary between 0.16% and 0.5% (Papazova, unpublished) (Fig.2).

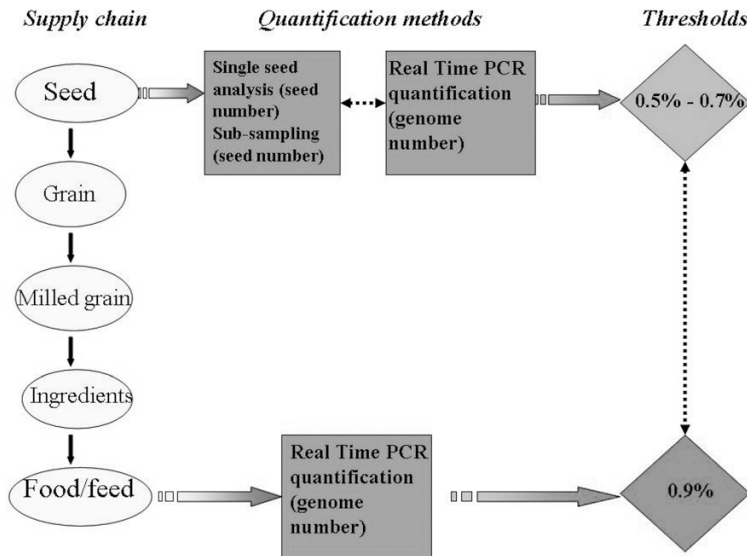


**Figure 2.** Relation between the number of seeds and number of genomes illustrated for seed sample containing 0.5% transgenic seeds.

**CONCLUSIONS**

Measuring the GM contaminations on a seed, or kernel basis is still a widely used practice. Apparently, the relation number of seeds-number of genomes is ambiguous which can make difficult the interpretation of the results obtained with different quantification approaches (fig.3).

However, the GM contaminations are recommended to be expressed in genome copies for food and feed products and for seeds as well (787/2004). The proposed seed thresholds for seeds are defined also on seed basis. How these thresholds for every crop comply with the 0.9% threshold for food and feed is a question that has to be answered.



**Figure 3.** Methods for quantification used at the different steps of the supply chain where the threshold requirements have to be fulfilled. The relation number of seeds-number of genomes is not equivocal due to the genetic inheritance of the target sequences. This adds uncertainty at 2 points (dashed line): compliance within different methods for quantification and the compliance between the threshold for seeds and food/feed.

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# Czech farming systems and farmers opinion in relation to the introduction of GM crops

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**Abstract:** Farming systems and agronomic practices influencing the coexistence of GM and non-GM crops in the Czech Republic were analyzed on the basis of questionnaires distributed in 100 farms covering 165,000 ha. GM crops of highest farmers' interest are herbicide resistant oil-seed rape and maize. For the first time, the farmers would like to grow these varieties on 45% of total crop acreage. They may see more benefits than risks consequent to the introduction of GM crops. The crop of highest economical importance is oil-seed rape which covers 12% of arable land. Unfortunately, in the case of oil-seed rape coexistence, 45% of fields can not be isolated sufficiently to achieve recommended separation distances and 54% of farmers can not store the GM-production separately after the harvest.

## INTRODUCTION

Growing of GM-crops introduces new farming systems risks of gene escape. Ecological consequences of gene-flow between wild plants, weeds, and crops have been extensively studied and documented. At the present time many studies are taking place in different agroecosystems across Europe to generate information on outcrossing, seed persistence in soil, volunteers, etc. This information is essential for modeling of spatial and temporal behavior of transgenes in the landscape. Farm conditions and agricultural practices are one of the important variables in these models because they can be vary according to different natural

patterns in the various geographical regions. An example of farm conditions influencing the introduction of GM crops in the Czech Republic is given in this paper.

## MATERIALS AND METHODS

A questionnaire was created and distributed among farmers belonging to the Union of Oil-seed Rape Growers. This group represents around 50% of rape acreage in CZ. The questionnaire consisted of 3 parts: (i). General information about the farm (location, size, cropping system, etc.), (ii). Practices used in current production system of oil-seed rape, sugar beet, and maize and anticipated

problems with respect to the introduction of GM varieties, (iii). Farmer's knowledge of genetic modifications and personal opinion on introduction of GM crops (expected benefits and risks). About 270 questionnaires were collected and 100 of them covering 165,000 ha have been evaluated to date and provide the information presented in this paper.

## RESULTS AND DISCUSSION

Large farms are typical in Czech agriculture. The average size of farms observed was 1647 ha; 90% of the total observed acreage consisted of farms of more than 1000ha. This size distribution observed in the study illustrates the typical situation, that one farm (e.g. a cooperative) manages the fields in more community units (villages). However, small farms can be closely located between fields of the larger farm units. This situation complicates the application of effective separation distances to reduce cross pollination between GM and non-GM fields, which is most effective when used in large field situation and where returns to farmers are sufficient to cover the costs of sowing decisions based on co-existence. Effective communication between farmers is also essential to ensure that necessary and protective measures are carried out on both sides: by GM and also by non-GM growers.

The elevation above sea level of farms within the survey ranged from 220 – 650m, which influenced the cropping systems and farming practices. All of the Union of Oil-seed Rape Growers farms grow rape, which is an important crop in CZ with 12% share of arable land. Conventional soil tillage is commonly used for rape; reduced tillage is applied on 23% of total rape acreage, in particular in the regions lower than 350m above sea level. Hybrid varieties are sown on 27% of the crop area.

Control of broad-leaved weeds is carried out predominantly with pre-emergence products (71% of area), using two herbicides. Additionally, post-emergent graminicides were used on 74% of rape area against volunteer cereals and on 11% of

the area against *Agropyron repens*. GM varieties can markedly simplify weed control and, by a reduction in herbicide volumes and elimination of products requiring soil incorporation can deliver both environment and cost benefits.

Owing to the dominance of rape in cropping systems, the isolation of GM fields from non-GM fields can present problems. The farmers estimate that an isolation distance of 500m (as currently for seed production) can be achieved in only 45% of rape fields. Another problem identified was the crop segregation at and after harvest. 54% of farmers cannot separate harvested products, 24% of farmers can separate only one lot and 22% can separate more than two lots.

The first GM-crop grown in the Czech Republic is Bt-maize. Injury by the pest *Ostrinia nubilalis* in conventional maize was mentioned 61% of farmers; In 11% of cases, the crop damage was economically serious. Pre-emergent weed control was used on 54% of the maize area, 16% was treated as split application pre- and post-emergence. The introduction of herbicide-tolerant maize hybrids would substitute help reduce the use of the triazine and chloracetamide soil herbicides.

The highest number of herbicide treatments was mentioned in sugar beet. On average, sugar beet farmers used 3.3 applications per field. Weed beets represent a serious problem in CZ. In this survey 15 – 70% of the fields were infested by weed beet. 60% of farmers consider the weediness with weed beet as constant, and 40% as an increasing problem. The problem is probably caused by high acreage of beet on individual farms (101ha/farm), eliminating hand-weeding and hoeing as an option in these large fields. With introduction of GM varieties, attention must be given to seed purity (contamination by transgene weed beet) and prevention of seed bank renewal.

The survey showed that 44% of farmers have insufficient information about the types and properties of current GM crops. Farmer attitudes to GMOs was classified on a scale of 1-5, with

1 =positive to 5 =negative. An average of 2.5 was found, which was less favorable than our expectation. Maize and oil-seed rape are the first GM-crops, which the farmers anticipate growing, on average on 45% of fields for the first time. Benefits expected by farmers are: saving of pesticides (71% of farmers), simplification of technology (69%), reduced costs (59%). 45% of farmers could not specify possible risks of GM crops. Of those that were mentioned, health (12%), and ecological (6%) risks were highest. 13% of farmers believe that there are no risks associated with the technology.

## **ACKNOWLEDGEMENT**

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# GMO quantification: an approach to measurement uncertainty (MU) assessment

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**Abstract:** Measurement uncertainty (MU) of analytical results forms the most important parameter in validation of analytical methods and quality control in laboratories. Presented is an approach for evaluating MU of quantitative real-time PCR data for GMOs. The study includes the setting up of a cause and effect or 'fishbone' diagram which forms the central idea of the traditional bottom-up approach. It bases on the following steps: specifying the measurand, identifying all sources of uncertainty, quantifying standard uncertainties and calculating combined and expanded uncertainties. This approach was however combined with the top-down strategy, using data from intralaboratory validation studies and internal quality control (IQC) measures. In this way, four standard uncertainties were identified and quantified: precision, bias, the uncertainty associated with reference materials (RMs) and sampling and sample preparation error. Mean relative MU values of 100% and 50% were obtained for GMO concentration levels of 0.1% and 5% respectively, in soybean and maize flour.

## INTRODUCTION

Today, analytics and bioanalytics, including the application of quantitative GMO analysis, require long-term monitoring of the quality of their measurements. Quality forms the key issue of the ISO/IEC 17025 (1999) standard, prescribing general requirements for the competence of testing and calibration laboratories. Fundamental principles of ISO/IEC 17025 are that traceability along the analytical procedure should be demonstrated, validated methods should be used and results should be reported together with their MU. Different approaches exist for estimating the MU of analytical measurements. The most

well-known approaches are (1) the "bottom-up", "error-budget" or "component-by-component" approach (ISO-GUM, 1995; Ellison *et al.*, 2000) and (2) the "top-down" methodology described by the Analytical Methods Committee (1995) and others, e.g. Maroto *et al.* (1999). While the first is based on identifying, quantifying and combining all individual contributions to uncertainty, the second approach uses data which are available from method performance studies. An adapted approach for determining the MU of quantitative real-time PCR data for GMOs is presented. Data available from internal validation studies and principles for IQC were used in an "adapted error-budget" method, based on the ISO-GUM and Eurachem/CITAC-QUAM guidelines.

## METHODS AND MATERIALS

Ten different PCR assays were selected, specific for the soybean specific lectin (*Le1*) gene, the maize specific sucrose synthase (*SSIb*) gene, promoter-35S and terminator-*nos*, and construct-specific sequences for transgenic soybean GTS 40-3-2 (Roundup Ready, Monsanto) and maize events Bt11 (YieldGard, Novartis), Bt176 (Maximizer, Ciba Seeds), GA21 (Roundup Ready, Monsanto), MON 810 (YieldGard, Monsanto) and T25 (AgrEvo). Absolute number of copies were determined with real-time PCR by using multiple target plasmid (MTP) DNA calibrators containing 20, 125, 1500, 20000 and 250000 copies of each respective fragment (Kuribara *et al.*, 2002). By combining a transgene PCR with a plant PCR, ten methods for relative GMO quantification are obtained. For each method, the step-by-step MU procedure as described in the ISO-GUM guide was applied.

## RESULTS AND DISCUSSION

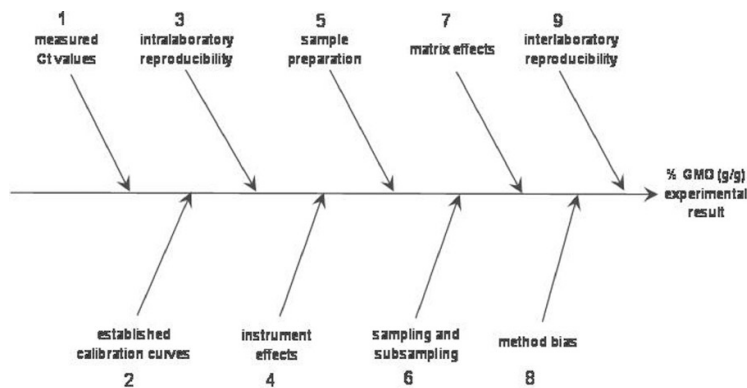
MU assessment started with specifying the measurand and the mathematical relationship between the measurand and the parameters upon which it depends. A cause and effect or "fishbone" diagram was then set up (Figure 1), recording all sources of uncertainty and their mutual relationships.

By combining different error sources and using repeatability, intralaboratory reproducibility and trueness data available for the different relative methods, standard uncertainties ( $u$ ) could be quantified. Four significant uncertainty contributions were defined: precision ( $u_1$ ), bias ( $u_2$ ), uncertainty of CRMs ( $u_3$ ), and the error associated with sampling and subsampling ( $u_4$ ). Application of the law of propagation delivered the combined uncertainty ( $u_c$ ), which was finally multiplied by a factor two to calculate the expanded uncertainty ( $U$ ). Data are shown for four methods in Table 1.

Expanded uncertainties were calculated for the 0.1% as well as 5% concentration level and taking into account the effects of pre-PCR steps such as sampling, subsampling and sample preparation. Absolute MU values of 0.103% and 2.56% were obtained for the 0.1% and 5% level respectively, corresponding with relative MU values of 100% and 50% respectively.

## CONCLUSIONS AND PERSPECTIVES

Based on a combination of both the bottom-up and the top-down approaches for MU, we tried to identify all possible sources of uncertainty which could contribute to the overall MU on the final % GMO result. As sampling and subsampling errors were also included, we believe that our MU



**Figure 1.** Cause and effect diagram for relative real-time PCR quantification of GMOs based on two absolute genome copy number estimations. Only main sources of uncertainty are shown here (derived from full diagram by Taverniers, 2005).

Relative PCR method	Sample (% GMO)	u-1	u-2	u-3	u-4	u-c	U (= 1.96*u-c)
p35S/lectin	0.1	0,0139	0,03	0,0242	0,0321	0,0521	<b>0,102</b>
	5	0,3201	0,69	0,2605	0,97	1,2599	<b>2,47</b>
tNOS/lectin	0.1	0,0153	0,04	0,0242	0,0341	0,0599	<b>0,117</b>
	5	0,462	0,67	0,2605	0,897	1,2389	<b>2,43</b>
RRS/lectin	0.1	0,0086	0,04	0,0242	0,0272	0,0548	<b>0,107</b>
	5	0,1068	0,94	0,2605	1,017	1,4132	<b>2,77</b>
Bt176/SSIIb	0.1	0,021	0,01	0,029	0,021	0,0427	<b>0,084</b>

**Table 1.** Standard uncertainties  $u$ , combined uncertainties  $u_c$  and expanded uncertainties  $U (= 1.96 * u_c)$  for four relative GMO quantification methods and two different concentration levels of GMO. Uncertainties  $u_1$  and  $u_2$  are calculated from the relative standard deviations ( $RSD_{R_i}$ ) and % bias values on ten replicate measurements performed in-house. Uncertainties  $u_3$  are read from the certificates of the used CRMs (CRM-412R-1 and CRM-412R-5 for RRS and CRM-411-1 for Bt176). Factor 4 uncertainties cover sampling, subsampling and sample preparation errors and are set at 30% and 20% for 0.1% and 5% GMO levels respectively. All data are given as absolute values, i.e. as a % of GMO.

calculations are realistic. An additional consideration however is that, for instance, matrix effects, method bias and interlaboratory effects have not been accounted for. It is important to estimate analytical result uncertainties separately for each measurand level, and to take into account these MU values when interpreting and reporting quantitative results, in the frame of regulatory compliance and decision making. Finally, it is worth to mention that the GUM approach that formed the basis for this study is also presented by ISO as the approach to follow for any new method of GMO analysis (ISO/DTS 21098, 2004).

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# Setting up of a decision support system and a general control plan for GMO analysis

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**Abstract:** We present a practical implementation plan for the analysis of GMOs in seeds, food and feed products. By looking to the analytical question and the particular sample, a case-by-case approach is followed, based on a decision support system. In this schematic structure of analysis, real-time PCR is presented as a common technique for screening, identification as well as quantification of transgene events. Included in the control plan is anchor PCR, raised as a tool for more detailed analysis of “ambiguities” or “irregularities” which may be observed in a sample. The overall picture is a general but comprehensive approach to GMO analysis, in which also fingerprinting analysis can be integrated.

## INTRODUCTION

Labelling and traceability requirements for GMOs are provided in the new regulations (EC) 1829/2003 and 1830/2003. Traceability is the ability to trace GM crops and their derived food or feed products at all stages of agricultural production, food or feed processing and distribution (Auer, 2003). Principally aiming at keeping GMO and non-GMO products separated, traceability provides consumer's choice. However, separating and tracking GM crops through the food chain is difficult. The regular mixing of non-GMO- and GMO-derived material during harvest, transport, etc., is considered unavoidable and for

this reason, regulatory thresholds for adventitious presence of GMO traces have been introduced.

The maintenance of threshold levels (0.9 % for authorized GMOs in the EU) and the traceability provisions bring about the need for adequate GMO testing. A practical implementation plan for controlling the presence of GMOs in food and feed is presented. Our approach is product-based, makes use of a general scheme of decision trees, and integrates GMO screening, identification and quantification. Fingerprinting techniques, like anchor PCR, are included as a useful tool for analysis of irregularities such as unauthorized GMOs.

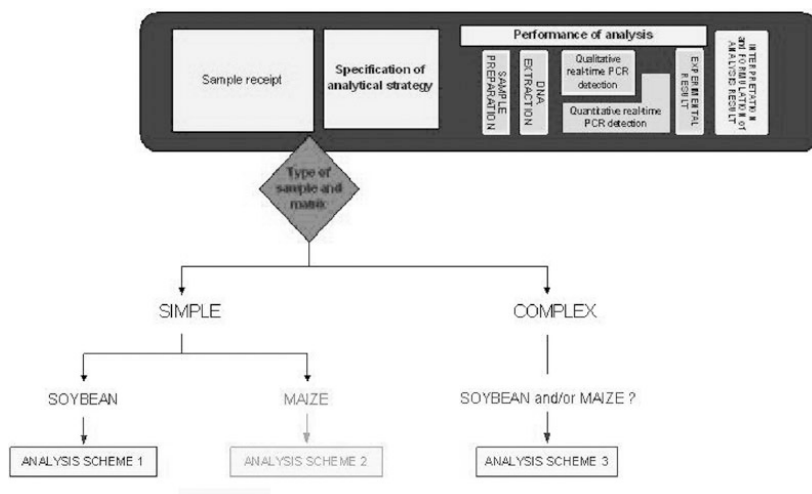
## RESULTS AND DISCUSSION

Presented is the practical course of a GMO analysis, starting from the laboratory sample and finishing with reporting the result (Figure 1). First, depending on the composition and the characteristics of the sample, a different strategy for GMO analysis is followed. Second, within each analysis scheme, we look at the specific question asked. Other schemes of analysis, applicable for other species or other matrices can be added later on to this tree.

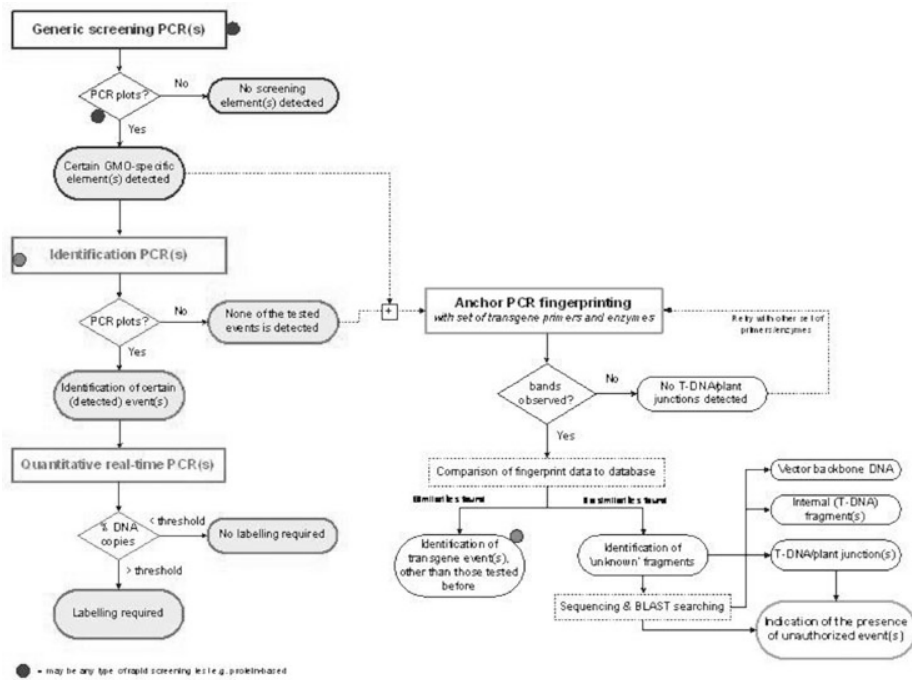
Three types of GMO tracking are needed: (1) a rapid screening assay for the presence of GMOs, (2) identification tests to unambiguously determine which events are present, and (3) quantitative methods to measure the precise amounts of GM ingredients in the sample (Auer, 2003). We integrated the three conditions in one and the same technique, which is real-time PCR. Upon positive signals in the generic screening PCR(s), identification of a number of events can be performed. Other techniques could also be used such as ELISA or lateral flow strip for generic screening. The principles of this integrated approach are shown in Figure 2.

## CONCLUSIONS

Horizontal implementation of GMO traceability and labelling laws is encouraged by using common analytical methods or techniques. We propose to integrate generic screening, identification, quantification and fingerprinting of GMOs into one strategy for routine analysis. Such a control plan is usable in a certain environment and at a certain moment, allowing to make a statement on the presence and/or content of a number of authorized events. The open and unbound format of our decision support system allows for extension at any time.



**Figure 1.** Decision tree for specification of the strategy of analysis. General scheme of the detection strategy and classification of matrices and analysis schemes (Taverniers, 2005).



**Figure 2.** Integrated approach to in-depth GMO analysis, combining generic screening, PCR for event-specific identification, real-time PCR for quantification and anchor PCR fingerprinting for generating extra information on the transgene status of the sample, including the presence of other (authorized) events than those tested in step 2, as well as the possible presence of non-authorized, unknown events (Taverniers, 2005).

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# Ten years of coexistence across the globe

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**Abstract:** In 2005, farmers planted crops enhanced with genetically modified (GM) traits for the 10th season in a row. Over this ten year period, coexistence between GM and conventional or organic crops has been successfully addressed in many countries. On-farm experience in North America and 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. This poster will present some of the concrete solutions that ensure a well-functioning coexistence throughout various regions in the world.

## COEXISTENCE IS A REALITY AND RELIES ON GOOD AGRICULTURAL PRACTICES (GAP)

Coexistence is based on the premise that farmers should be free to cultivate the crops of their choice, including GM. Since 1996, GM crops have been grown on over 400 million hectares, a >47-fold increase in 10 years (Runge & Ryan, 2003). During this period, on-farm experience worldwide has proven that coexistence between GM, conventional and organic growing systems can be obtained with the help of simple, practical solutions that often do not require government intervention or complicated legislation.

### Coexistence in North America

In North America, coexistence is working and allows farmers to grow products for the domestic food/feed sectors as well as for export

to markets that accept GM or require non-GM products. There is no national legislation to cover coexistence. Suppliers of GM seed provide farmers with “Technology Use Guides” or “Crops Stewardship Guides”. These contain advice on Good Agricultural Practices that allow growers of GM and non-GM crops to meet their market needs.

In the case of GM maize for example, advice may include minimizing cross-pollination by identifying prevailing winds, implementing buffer rows and barriers, harvesting the outer strips of the adjacent conventional maize field separately, holding discussions with neighbours about planting intentions, and/or speaking to grain buyers to ensure that contractual requirements are identified. The American Seed Trade Association (ASTA) has created a web-based information site to help maize growers locate grain handling facilities willing to purchase and handle biotech maize (<http://asta.farmprogress.com/>).

Examination of trends in the planting of GM and conventional or organic crops suggests that the growth of the GM crop area has not impeded the development of these sectors in North America (Brookes, 2004).

### Coexistence in Spain

Insect-protected *Bacillus thuringiensis* (Bt) maize has been grown in Spain since 1998 with no negative consequences on the global maize market of the country. To date, there is no national legislation covering coexistence, although a document is now in preparation in line with recent EU recommendations (Commission Recommendation 2003/556/EC provides guidelines for the development of national strategies and best practices that, where necessary, can be applied to keep products from non-GM fields below the labelling threshold. Since 2003, coexistence in Europe is subject to Regulation (EC) no. 1830/2003 that sets a labelling threshold of 0.9% in the case of the unintentional or technically unavoidable presence of GM in non-GM crops or products).

As in North America, growers are advised by suppliers on practices to minimize cross-pollination into neighbouring non-GM maize fields, where this is necessary (e.g. intense machinery cleaning, planting buffers between GM and non-GM fields). Traceability and proper channelling have also been key elements in the system.

### AN ADDITIONAL TOOL FOR EUROPE: GRAIN MATCHING

In 2005, Bt maize was planted in four countries additionally to Spain (Portugal, the Czech Republic, Germany and France). Coexistence is being addressed in various ways in each of these countries. As in Spain and North America, the main element is having in place workable and fair science-based Good Agricultural Practices. In the unexpected case where traces > 0.9% were to occur despite adherence to GAP, practical systems such as "grain matching" are being tested in countries such as Germany. Working in collaboration with grain elevators, the GM

farmer offers to match his neighbour's grain with an equivalent amount of standard conventional grain, available from his own or another farm for example. Grain matching can be reduced to a paper exercise at the level of the grain elevator.

### CONCLUSION

GM crops have been growing alongside conventional and organic crops for 10 years now, with no confirmed cases of economic damage to the non-GM neighbours. Where necessary, practical measures based on Good Agricultural Practices have successfully been implemented in various countries to ensure coexistence of the various agricultural systems. In Europe, plantings have expanded in 2005 to five countries. Additionally to GAP, a simple proposal involving grain matching is under evaluation to answer the needs of various growers and markets.

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# Evaluation of Protein Strip Tests as tool for the Detection of Genetically Modified Crops in co-existence strategies on the Farm

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**Abstract:** The determination of the presence of genetically modified (GM) plant products by the detection of expressed Genetically Engineered (GE) proteins using lateral flow Protein Strip Tests (PST) has been evaluated. Five major GE proteins (CP4-EPSPS, CryIAb, Cry9C, the PAT/*pat* and PAT/*bar* protein) could be detected at low levels in different matrices (including seeds and leaf tissue) in Roundup Ready soy and maize, Bt maize and hybrid SeedLink oilseed rape. Results of PST and RT-PCR analysis of GM feed products were comparable. The use of PST as a "Farm GM-monitoring tool" is proposed.

## INTRODUCTION

The detection of the presence of GMO and GM-derived products along the food/feed chain is critical in the realization and success of any co-existence strategy. Protein Strip Tests (PSTs) are lateral flow immunotests capable of demonstrating the presence of GE proteins in extracts of GM materials, such as seeds (Stave, 2002). Here, an evaluation of the use of PSTs for general application in the detection of GM material on the farm is presented.

## MATERIALS & METHODS

The tested materials and matrices were obtained from the Technology Provider Companies or from the Institute of Reference Materials and Methods (IRMM, JRC-Geel, Belgium). All analyses have been performed following the manufacturer procedures (*in casu* Envirologix, Neogene, or Strategic Diagnostics). The following kits have been used: CP4-EPSPS (Neogene); CP4-EPSPS,

CryIAb, Cry9C, PAT/*pat* (Envirologix); CP4-EPSPS, PAT/*bar* (Strategic Diagnostics).

Grinding of the matrices was performed using either a blender, either by crushing the material with a plastic stick. Homogenized matrix was extracted with neutral pH tap water (1/1.5 to 5 w/v). After vortexing (20-30 sec), the insoluble material was allowed to settle for 5-10 min. If necessary, the homogenate was cleared by centrifugation at 10.000 x g for 5 min. and analysed by PST.

The detection sensitivity by PST of the GE proteins present in seeds and leaf tissue derived from different GM events was determined by v/v dilution of extracts from seed powders of known % of one particular GM event.

The use of PST in quantitative GM detection was evaluated by «Accuscan» analysis of PST positive signals of a dilution series of GTS 40-3-2 soy seed powders.

## RESULTS AND DISCUSSION

The presence of the GE proteins (CP4-EPSPS, CryIAb, Cry9C, PAT/*pat*, and PAT/*bar*) in seeds and leaves from GM plants and in IRMM certified reference materials was tested by a v/v dilution approach (see table 1). The detection sensitivity of the PST in matrices is high (> 1:1000) for the CP4-EPSPS protein (GTS-40-3-2 soy and NK603 maize), the Cry9C protein (CBH 351 maize) and the PAT/*bar* protein (CBH351 maize and MS8/RF3 oilseed rape). The PAT/*pat* protein was only detectable in Bt 11 maize leaf tissue but not in seeds. The CryIAb protein was detectable in Bt 11 maize seeds only (1:200), but not in leaves from both Bt11 and MON810 maize. In none of the IRMM CRMs, any CryIAb or PAT protein was detectable. All control non-GM matrices were negative for all PST (data not shown).

Quantitative PST analysis has been evaluated using an «Accuscan» analysis of a v/v dilution series from extracts of 100% GTS 40-3-2 soy seed powder and of 1% GTS 40-3-2 IRMM CRM. Preliminary results demonstrate that a quantitative approach may be possible.

Homogenized powder (5 g. samples) of several bovine, poultry or pork feed products was analysed by CP4-EPSPS PST (Neogene & Envirologix). In all feed samples tested, only trace amounts CP4-EPSPS protein could be detected. Quantitative PCR confirmed the PST data. An impact on feed product labelling requirements is discussed.

In conclusion, PST can be used at several stages of on-farm use of GM plant products (seed, crop plants, and feed). PSTs have a number of considerable advantages to other GM detection methods (e.g. PCR): the tests are simple, fast, cheap, and can be virtually performed on any location for almost all types of matrices. PST can in several cases be an interesting alternative to more sophisticated GM detection technologies such as PCR.

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**Table 1.** Detectable GE protein levels in GM seed and leaf material by PST: the dilution approach (v/v)

Trait Crop/Matrix (Event)	CP4	CryIAb	Cry9C	PAT/ <i>pat</i>	PAT/ <i>bar</i>
Soy/IRMM (GTS-40-3-2)	1:10000	na	na	na	na
Maize/IRMM (Bt 176)	na	nd	na	na	nd
Maize/IRMM (Bt 11)	na	nd	na	nd	na
Maize/IRMM (MON 810)	na	nd	na	na	na
Maize/IRMM (NK 603)	1:1000	na	na	na	na
Maize/IRMM (GA21)	nd	na	na	na	na
Soy/Seed (GTS-40-3-2)	1:10000	na	na	na	na
Maize/Seed (Bt 11)	na	1:200	na	nd	na
Maize/Seed (CBH 351)	na	na	1:1000	na	1:5000
Maize/Seed (MON810)	na	nd	na	na	na
Maize/Seed (NK 603)	1:1000	na	na	na	na
Maize/Seed (GA21)	nd	na	na	na	na
Maize/Leaf (Bt 11)	na	1:100	na	1:2	na
Maize/Leaf (CBH 351)	na	na	1:1000	na	1:10000
Maize/Leaf (MON810)	na	1:10	na	na	na
Oilseed rape/Leaf (MS8/RF3)	na	na	na	na	1:5000

note: 'nd': not detectable; 'na': not applicable



# Test of coexistence under German field conditions - results from the “Erprobungsanbau” 2004 with Bt-maize

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**Abstract:** In the 2004 pre-commercial plantings (“Erprobungsanbau”), genetically modified (GM) and conventional varieties of maize were cultivated under commercial conditions in seven German federal states. The study focussed on the question of whether and to what extent cultivation of GM maize on fields of 0.3 to 23.0 ha leads to the presence of GM DNA in the harvest of neighbouring conventional maize fields. This paper presents results for silage and grain maize, as well as crushed husks and cobs. The highest levels of GM DNA (average 1.1% for silage maize and 1.0% for grain maize) were found in the 0 to 10 m conventional maize strips immediately bordering the GM maize fields. With increasing distance, values quickly decreased. The results show that, as of 20 m from the GM fields, all values were below the labelling threshold established by Regulation (EC) No. 1829/2003. No differences were found between silage and grain maize, although for silage maize the complete plant including the GM-free plant parts are harvested.

## INTRODUCTION

In agriculture, each individual farm owner makes his or her own decisions with regard to cultivation and distribution. Farmers should be free to decide for or against a certain method of cultivation, whether it is based on conventional, GM or organic systems. The most important requirement for the coexistence of different systems is a reliable legal framework that can be applied under real life conditions. The

objective of the 2004 pre-commercial plantings (“Erprobungsanbau”) was to gather practical experience in order to assist in the development of workable coexistence regulations for Germany. For this purpose, both GM and conventional maize were cultivated under commercial conditions. The GM maize (hereafter called Bt- maize) contained a gene originally isolated from the bacterium *Bacillus thuringiensis* conferring tolerance to the European corn borer, an insect pest also found in Germany. The conventional maize varieties were the corresponding near-isogenic varieties.

## MATERIALS AND METHODS

The objective of the pre-commercial plantings was to determine the content of GM DNA in conventional maize grown in the vicinity of Bt-maize. Bt-maize was cultivated on plots of 0.3 to 23.0 ha situated within fields of conventional maize (for details see Weber *et al.*, 2005). The sites were located on 30 commercial farms throughout Germany, from Mecklenburg-Pomerania in the North to Baden-Wurtemberg and Bavaria in the South. Varying farm structures and climatic conditions were taken into account so as to mimic real life situations. Sowing and harvesting were performed with the techniques specific to each farm involved. At two locations, the study had to be interrupted shortly after sowing due to a high frit fly infestation and uneven germination, respectively. Table 1 shows the distribution of the harvested locations across the seven federal states in Germany.

Sowing, growth of the plants and harvesting were monitored by independent experts. Pollination time, pollen shed for Bt-maize, and silk emergence for the conventional maize were also recorded. Information on wind velocity and direction during the time of blossoming was collected from the nearest meteorological service stations.

For the determination of GM levels (% of GM DNA), samples were taken in the conventional maize fields in strips at distances of 0-10, 20-30 and 50-60 m from the GM field in all wind

directions. Three samples of approximately 7 kg fresh matter (silage maize) and 2 kg (kernel maize) were taken from each strip. The three samples were dried, ground to a particle size of less than 4 mm and pooled to form a collective sample. Each pooled sample was split into five sub-samples; two were analysed for their GM level, the others served as backup samples.

The GM level in all harvested samples was quantified based on a "MON810"-specific approach (Hernandez *et al.*, 2003). The analyses were carried out by quantitative PCR in two laboratories, both certified according to ISO 17025.

## RESULTS

Table 2 shows the results for silage and grain maize, as well as crushed husks and cobs. One location in Brandenburg was not included in the evaluation, since the DNA analysis showed that errors occurred during sowing and harvesting. Since there was no main wind direction during pollination, average values for all directions were used. In the 0 to 10 m strips, GM levels exceeded 0.9% in six out of eighteen cases for silage maize and in four out of eight cases for grain maize. This result was expected, based on results from other studies (Brookes *et al.*, 2004). In the 20 to 30 m and 50 to 60 m strips, none of the 27 locations evaluated showed levels higher than 0.9%. If individual results are analysed separately according to their position

**Table 1.** Number of harvested locations in seven federal states of Germany

Federal state	Silage maize	Grain maize	Crushed husks and cobs
Bavaria	8	2	-
Brandenburg	3	-	-
Baden-Wurtemberg	1	-	-
Macklenburg-Pomerania	2	-	-
Saxonia	3	1	-
Saxonia-Anhalt	1	5	1
Thuringia	1	-	-
Total	19	8	1

Table 2. % of GM DNA in the harvest of conventional maize fields bordering GM maize fields

Site (coded)	Bt-maize (ha)	Distance		
		0 –10 m	20 –30 m	50 –60 m
<b>Silage maize</b>				
1.01	3.0	0.19%	0.06%	0.00%
1.02	1.9	3.74%	0.23%	0.04%
1.04	1.3	0.64%	0.15%	0.11%
1.05	0.4	0.02%	0.01%	0.00%
1.06	0.4	0.14%	0.08%	-
1.07	0.3	0.26%	0.08%	0.03%
1.09	7.0	0.63%	0.07%	0.03%
1.10	1.0	0.23%	0.02%	0.02%
2.01	9.0	0.82%	0.19%	0.15%
2.02	23.0	0.65%	0.64%	0.16%
3.01	1.0	0.20%	0.13%	0.01%
4.01	2.3	3.30%	0.59%	0.21%
4.02	4.0	0.72%	0.48%	0.29%
6.01	4.9	2.12%	0.32%	0.11%
6.02	6.5	2.77%	0.32%	0.10%
6.04	3.0	0.60%	0.29%	0.19%
7.01	1.1	0.94%	0.30%	0.29%
8.01	15.7	2.66%	0.27%	0.25%
<b>Grain maize</b>				
1.03	1.8	1.86%	0.69%	0.36%
1.08	2.9	1.61%	0.26%	0.18%
6.03	18.3	0.63%	0.32%	0.07%
7.02	8.5	1.23%	0.32%	0.11%
7.05	8.5	1.00%	0.58%	-
7.06	5.0	0.21%	0.09%	0.02%
7.07	5.0	0.80%	0.28%	0.05%
7.08	5.0	0.52%	0.08%	0.05%
<b>Crushed husks and cobs</b>				
7.04	6.2	2.81%	0.36%	0.07%

and distance from the Bt maize fields, single samples (4% of all analysed samples) exceeded the 0.9% threshold also at 20 to 30 m. This is however of low relevance under commercial field conditions since a larger field is harvested and the GM level in the final material will be well below threshold values. Average GM levels were generally not higher for grain maize than for silage maize. Since only grains and not the rest of the plant are affected by outcrossing

from Bt plants, one would have expected to find, at a constant pollination rate, higher GMO concentrations in grain maize. Possible explanations for this lack of difference are that there was considerable variation between the sites and that the total amount of DNA is lower in the plant than in the grains, so that the ratio of DNA from the plant (without grains) compared to total DNA is therefore not the same as for dry mass.

## CONCLUSION

The results of the pre-commercial plantings show that for silage maize and grain maize, solutions can be found that allow the coexistence of production with genetically modified and conventional maize. Where needed, specific actions such as including buffer rows of 20 m can be implemented to avoid the presence of GM material in the neighbouring conventional fields.

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# Stability of the sterility trait in maize CMS hybrids

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**Abstract:** The growth of mixtures of transgenic CMS (cytoplasmic male sterile) and conventional maize hybrids as pollen donors has been proposed as a method for transgenic pollen flow containment. This system has the potential to contribute to coexistence between GM and non-GM crops, given that the cytoplasmic male sterility does not permit any reversion to male fertility of the CMS plants, due either to environmental factors (temperature, photoperiod, water availability) or to the presence of fertility restorer genes in the genetic pool. The environmental factors which may lead to partial or complete restoration of male fertility in CMS hybrids are being investigated, and a protocol for the molecular identification of the major fertility restorer genes will be developed.

## INTRODUCTION

In 1933, Rhoades described for the first time the phenomenon of CMS in Peruvian maize. Breeders understood rapidly that cytoplasmic male sterile can be used to perform crosses without the need of manual detasseling, and can therefore save time and money. Hundreds of CMS sources have been identified since then. The identified CMS sources could be gathered in three main types: CMS-T (Rogers & Edwardson, 1952), CMS-S (Jenkins, 1950), and CMS-C (Beckett, 1971). These CMS types are defined according to the specific nuclear fertility restorer genes (*Rf* genes) capable

of countermanding the CMS trait and restoring pollen fertility (Schnable & Wise, 1998; Buchert, 1961).

For the commercial adoption of CMS as a method of biological containment of transgenic pollen flow (Feil *et al.*, 2003; Feil and Stamp, 2002), it is important that *Rf* genes are absent from the genetic pool, which may otherwise lead to transgenic pollen production during the breeding process or in the commercial field. It is known as well that the CMS-S and CMS-C types of sterility present different degrees of male fertility restoration depending on the environment.

## APPROACH

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 at mapping 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.

A further major part of our studies will be to determine to which extent different environmental factors can trigger restoration of male fertility. Modern maize hybrids with different CMS types will be sown under different climatic conditions in different regions in Switzerland and France and at different sowing dates. Controlled self-pollinations and *in vitro* pollen germination tests will be conducted in all cases to test eventual pollen formation and viability.

## PERSPECTIVES

A final aim will be to provide an estimate of the risk of outcrossing of transgenic pollen when growing transgenic CMS maize hybrids.

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# Flowering synchrony and gene flow between cropped and volunteer spring wheat in western Canada

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**Abstract:** The potential introduction of genetically-engineered (GE) wheat necessitates investigation into the flowering phenology and synchrony between cropped and volunteer wheat. Although wheat typically flowers for only three to five days, ontogenetic synchronization typical of most determinant plant species could result in a high degree of flowering synchrony and potentially contribute to transgene movement. We examined the flowering synchrony of volunteer and cropped wheat at various volunteer wheat densities and emergence times, as well crop densities and heights. The results of this study will both determine if flowering synchrony between cropped and volunteer wheat exists and will provide an indication of the importance of flowering synchrony in potentially facilitating transgene movement between cropped and volunteer wheat.

## INTRODUCTION

Wheat was the most prevalent crop produced in Canada in 2004, grown on nearly 10 million hectares (FAO, 2005). Consequently, volunteer wheat is an abundant weed in western Canada, persisting on at least 18% of western Canadian fields (Leeson *et al.*, 2005). The potential for transgene movement in wheat occurs at both temporal (volunteer populations) and spatial scales (pollen-mediated gene flow and seed

movement). In order to predict the potential movement of transgenes, studies must be designed to examine the various factors that conspire to facilitate movement of the transgene (Lefol *et al.*, 1996; Waines & Hegde, 2003). An important prerequisite for transgene movement is that both species must flower synchronously. If GE wheat were grown commercially in western Canada, the large scale production of wheat in this region would undoubtedly result in a considerable portion of GE wheat cultivars containing non-GE

volunteers within, or grown alongside non-GE cultivars. In these cases, the only effective barrier to gene flow will be flowering asynchrony between cropped and volunteer wheat. Flowering behavior and synchrony therefore will be fundamental to gene flow and transgene movement. Simard and Légère (2004) recently demonstrated that flowering synchrony was a major determinant of gene flow between canola (*Brassica napus* L.) and wild radish (*Raphanus raphanistrum* L.). However, in contrast to canola which can flower for several weeks, wheat crops tend to flower for only three to five days (Hucl, 1996) and volunteer wheat emergence generally occurs early in the growing season (DeCorby & Van Acker, 2004). Therefore, flowering between the two may be largely asynchronous. Because no information exists regarding the synchrony of flowering between wheat crops and volunteers, our objective was to examine flowering phenology and synchrony between volunteer and cropped wheat as a function of various factors.

## APPROACH

To address our objective, two separate experiments were initiated at two sites near Winnipeg, Manitoba, Canada, in 2005. The first experiment examined the flowering phenology and synchrony of volunteer and cropped wheat at various volunteer densities and emergence times while the second examined various crop densities and heights. Both experiments utilized a split-plot design. Main plots in the first experiment consisted of various volunteer wheat densities (10 – 80 plants m<sup>-2</sup>) and sub-plots were composed of relative times of volunteer emergence (50 GDD before to 50 GDD after crop emergence). In the second experiment main plots were crop densities (75 – 600 plants m<sup>-2</sup>) and sub-plots were crop cultivars of different heights (two tall stature, two semi-dwarfs). Volunteer wheat in the second experiment was seeded parallel to the crop at 30 plants m<sup>-2</sup>. Flowering was rated daily based on the Zadok's Scale (Zadok's *et al.*, 1974) to determine days to first flower, days to 5%, 50%, and 95% flowering, and days to final flower with plant height at flowering as recorded.

This provided a number of estimates for both the volunteers and crop including mean days to 5, 50, and 95% flowering, mean days to first and last flower, length of flowering period, and flowering overlap. Data analysis was conducted using a combination of regression analyses and analyses of variance.

## RESULTS AND DISCUSSION

Flowering synchrony was highly dependent on volunteer wheat time of emergence relative to the crop. Volunteers emerging earlier than 75 GDD before the crop flowered synchronously with it for only 3-4 days and had generally completed 40-60% of flowering at the initiation of crop flowering. Volunteers emerging between 60 GDD before and 60 GDD after the crop exhibited the greatest degree of flowering synchrony with the crop (90-100% synchrony), while those emerging more than 70 GDD after the crop generally exhibited little flowering overlap (0-10% synchrony). Thus there appears to be a 50 GDD window on either side of crop emergence in which volunteer emergence results in synchronous flowering with the crop. When volunteers and crop were seeded at the same time, flowering synchrony also varied with crop cultivar and density. Flowering in the variety Oslo overlapped with volunteer flowering for only 2 days with 75% of flowering in Oslo completed when synchronous flowering commenced. In contrast, the varieties Vista, Amazon, and Prodigy flowered synchronously with volunteers for 5 days, presenting a large window for gene flow to occur. Crop density affected flowering synchrony to a lesser degree than cultivar. Among 6 days of flowering overlap, 80% and 75% flowering synchrony with volunteers was observed in crop densities of 300 and 600 plants m<sup>-2</sup>, respectively, whereas 90 to 100% synchrony was observed in crop densities of 150 and 75 plants m<sup>-2</sup>. Seed has been harvested from these studies and will be used measure actual levels of gene flow (using the HT trait in the volunteer variety) and to assess the importance of these factors (as well as flowering synchrony) in facilitating intraspecific gene movement in wheat.



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# Progress on GM/ non GM crop co-existence plans for the UK

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**Abstract:** The poster explains the basic principles and the progress made to date on developing government proposals for co-existence of GM/ non-GM and organic crop cultivation.

## INTRODUCTION

England, Scotland, Wales and Northern Ireland are each responsible for measures within their own region. They are working closely together but there will be some variations between administrations. The plans for England are the most developed so those are the ones described here.

## APPROACH

The government set out its policy on GMOs in a statement to Parliament in March 2004. This included some basic principles for co-existence. These are that GM farmers should bear the main

responsibility for taking action to avoid cross-contamination; that such contamination must be limited to within the EU's 0.9% labelling threshold; and that measures should have statutory backing. It also announced that the Department for Environment, Food and Rural Affairs (Defra) would develop detailed proposals for public consultation. The development of detailed proposals has been informed by the European Commission Guidelines, a report on co-existence and liability from an independent government advisory body, and from evidence provided by research, particularly that gained from the farm scale evaluation trials held in the UK. In addition, Defra held a number of workshops with a wide range of stakeholders to discuss different aspects of co-existence.

## **CO-EXISTENCE MEASURES**

We have commissioned research on separation distances for maize and oilseed rape from the National Institute of Agricultural Botany (NIAB), using data on cross-pollination from the farm scale evaluations. This will inform our decisions on proposed separation distances and will be published at the same time as our proposals. We have also considered the role of seed impurities, volunteers and seed transfer via machinery in transferring a GM presence.

We will propose which measures should be statutory and which could be covered in a code of practice.

## **RELATED ISSUES**

The government has said that it will consider whether there should be specific measures for organic farming and will provide guidance to farmers who wish to establish voluntary GM-free zones. The consultation proposals will also discuss whether there is a need for a public register of where GM crops are grown. We will consult on options for compensating non-GM farmers who suffer financially because their crops are contaminated above the labelling threshold. The government has made it clear that any such compensation will be funded by the GM sector.

The government is committed to having co-existence measures in place before commercial cropping takes place. This is not expected before 2008 at the earliest.

# GMO and non GMO co-existence implications in Poland as a New Member State of EU

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**Abstract:** Growing concerns are observable over the coexistence between genetically modified and non-modified organisms in Poland. However, Poland already has in place a legislation and control system that regulates issues connected with GMO use, from scientific experiments, production, through import, distribution and extending to the placement of products containing GMO on the market. Despite the existence of GMO regulatory framework in Poland there are still issues that need particular attention such as trade, where deficiencies in proper labeling of GMO food products were noted. Poles, in general, are in favor of scientific research using genetic engineering in food production and processing. However, they are afraid that GMO in food products might have a negative impact on the environment and human health.

## INTRODUCTION

Growing concerns are observable in Poland over the coexistence between genetically modified organisms (GMO) and non-modified organisms (non-GM). This is because of significant stipulations of many stakeholders. So far GMO use is very restricted in Poland. According to official data provided by responsible authorities, currently there is no GMO cultivation in Poland (Simonides, 2004). However, since September 2004 the European Commission permitted 17 varieties of GMO maize MON810 to be grown in the European Union. Polish authorities asked for a two-year temporary prohibition, backing up this claim by the need to strengthen the existing law on GMO plant cultivation (Press

release, 2005). There are no specific measures on coexistence in Poland as a result it is difficult to communicate aspects related to coexistence to various stakeholders. Proposal of amendment to existing law on genetically modified organism referring to coexistence was made in 2004 but the amendment has not been adopted. There is much opposition to the introduction of GMO crops at the local and regional level. As a result, 11 provinces have already announced that they aim for a total ban of GMO crops (GMO free zones, 2005). The authorities of Malopolska province, with one of the highest shares of area subject to organic production in Poland, emphasize that such a ban is the only option to ensure the further development of organic farming.

## GMO IN POLISH LEGISLATION

In accordance with the Convention on Biological Diversity, Poland has developed the project of National Strategy for Conservation and Sustainable Use of Biological Diversity. The operational goals of the Strategy cover implementation of a biosafety system, including a new law on GMO. At present there are two basic regulations that refer to GMO issues in Poland: The legal act of 22 June 2001 on genetically modified organisms and legal act of 11 May 2001 on health conditions of food and nutrition. As a result of the harmonization process with "acquis communautaire", on 14 October 2004 the Council of Ministers approved and sent to the Parliament the proposed amendment to the law on genetically modified organisms. The proposal sets new rules, among others, for closed use of genetically modified microorganisms and genetically modified organisms as well as their introduction to the market. Also proposed is a joint monitoring system of GMO use. As a result of these changes in the regulatory framework of GMO, once again the responsibilities and tasks of different state bodies have been re-defined. As a result, currently in Poland there are eight different authorities responsible for observance of existing legal regulations in the scope of GMO. In reference to the GMO control system, on 10 March 2003 the Minister of Environment - the governmental administrative authority competent for GMO, indicated three laboratories that are empowered to undertake testing and provide opinions on GMO. These three laboratories will form a part of the European Network of GMO Laboratories.

## MARKET CONTROL

Competence in the scope of market control was divided between four institutions: Inspection of the Trade Quality of Food Products (raw material, processing, wholesale), State Sanitary Inspection (identification of GMO in food products), Trade Inspection (retail trade), State Veterinary Inspection (control of products of

animal origin). In 2004, Inspection of the Trade Quality of Food Products (IJHARS) carried out controls, which aimed to check out the conditions of transport, storage, documentation as well as labelling of products that might contain GMO in Poland (The report on, 2004). Two main products were selected, soya and maize. The results of the controls show that 99% of products from soya were labelled as GMO free that what was confirmed by appropriate certificates (Solae Europe, Cerestar, Gene Scan, Solbar). In the case of maize, 84% of products traded as GMO free had the required certificates. Only 1% of all controlled products did not have any information about GMO on their labels. Nonetheless, 61% of checked products were labelled incorrectly. Among the samples that were analyzed in depth, 3.77% contained over 0.9% GMO, including two samples declared as GMO free. Compared to the results of a similar control carried out in 2003, the number of tested samples in 2004 rose by 45% and accordingly, the share of products containing GMO (over 0.9%) decreased by 35%. Nevertheless, in 2004 the knowledge of traders about GMO legislation and their responsibilities in this respect increased in comparison to the 2003 controls. Comparable to the previous year not all controlled entities had any system or procedures in place that could ensure traceability and correct identification of GMO products.

## PUBLIC PERCEPTION OF GMO

Surveys on the public perception of biotechnology were conducted in Poland, similar to Eurobarometer in the EU, in 1996, 1999, 2001 and 2003. In the 2003 survey on a representative sample of Poles (1007 respondents above 15 years old) 74% respondents declared that they heard about GMO (Janik-Janiec *et al.*, 2003). However, the majority admitted that they are not sufficiently informed about this issue. More than 50% of Poles are in favour of scientific research using the biotechnology and genetic engineering in production and processing of food. However, 58% of respondents are afraid that the GMO

in food products might have negative impact on environment and human health. Compared with the 2000 survey, there is decreased support for research on GMO in food (by 18%) and an increase of GMO related threats to health and environment (by 7%). The respondents were very much concerned about the regulatory framework of GMO and 83% of them expected that all issues related to GMO should be strictly regulated by the law and supervised by the government. Almost 75% of Poles believe that new legislative measures concerning GMO should be consulted with civil society. Compared to the previous surveys these results clearly indicate that less Poles support GMO in food products and they have higher expectations concerning the scope of regulatory framework and labelling of GMO products.

## CONCLUSIONS

Although Poland has legislation that regulates the supply chain of GMO products, which is currently being developed and harmonized with EU acquis, it is not respected, especially with regard to trade. Also, the control system still requires development. Moreover, consumers are not sufficiently informed about advantages and disadvantages of biotechnology and GMO products and the scope of national and EU legislation, of which they have very high expectations. Consumers expect solid information and a transparent system on GMO use. The coexistence on the farm level does not exist in Poland yet, as officially, there are no GMO plantations. However, the economic aspects of coexistence are becoming more and more relevant in Poland. There is also an urgent need to discuss the conditions and measures required to ensure coexistence between various farming systems.

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