SIXTH FRAMEWORK PROGRAMME
PRIORITY 5
FOOD QUALITY AND SAFETY

INTEGRATED PROJECT

FINAL ACTIVITY REPORT-Publishable Executive Summary

Project acronym: **Co-Extra**

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1 Publishable executive summary

Introduction

GMO development.

In 1983, three reports from the University of Ghent, the University of Washington, and the Monsanto Company showed that the Ti plasmid of *Agrobacterium tumefaciens* could be used to transfer foreign DNA into plant genome, thus producing the first genetically modified (GM) plants. This discovery had enormous implications for plant genetics and agriculture. In the last 20 years, plant biotechnology has grown into a multibillion-dollar international industry.

The earliest and still most important commercialized transgenic plants are corn (maize), cotton, soybean, and canola, and contained transgenes conferring tolerance to herbicides, or resistance to insects and pests.

The cultivation of GM crops is presently limited to a few countries. The United States grow 55% of GM crops, followed by Argentina (19%), Brazil (10%), Canada (7%), and China (4%). Europe cultivates almost no GM crops, except for a relatively small amount of maize in Spain.

In the next few years, this situation is likely to change dramatically. China is expected to dramatically increase its transgenic crop cultivation (currently mostly cotton). Similar increases in GMO cultivation are foreseen in India, South Africa, Australia, and even Europe.

European citizens and consumers opinions and attitudes.

Since the first arrivals in 1996 of the first shipments of GM soybean in the European harbours, European citizens and consumers have rather sceptical opinions and attitudes over the interest of GMOs. A strong movement of opposition to GMOs developed in many countries, especially in Europe, although these technologies were presented from the outset as highly promising and their advantages were often highlighted. Fostered by several highly publicised and successive food safety crises, public suspicion towards regulatory authorities, scientists and technocratic decision-making grew (Lofstedt, 2006).

However, the opinion may be changing as observed by the different Eurobarometer polls and their variations over the last years. Among important factors of rejection we can outline the focus on potential risks of GMOs and the extensive publicity given to them, coupled with the inadequacy of answers to these diverse criticisms, and a drawing up of an unfavourable risk-benefit balance (Bonny 2003, Noussair et al. 2002).

A recent study pointed out that when having freedom of choice (between GM and non-GM products) the consumers may be choosing the less expensive ones (http://www.kcl.ac.uk/consumerchoice). However, this attitude does not seem general in the UE. Mostly observed in the middle Europe this attitude cannot be generalised to the West part of the EU.

European regulation frame

Since 1990, GMO are subject to a series of European directives and regulations, for risk assessment, confined and voluntary dissemination (90/220/EC, 90/219/EC, 1139/98/CE, 49/2000/CE, 50/2000/CE some of them replaced by 98/81/EEC, 2001/18/EEC, 1829/03/EEC), detection and traceability (1830/03/EC). This GMO vertical European regulatory frame is also sustained by other specific regulations such as the 258/97/EC on “novel food and novel ingredients” or general ones as the 178/02/EEC (“food law”) regulation. After signature of the Cartagena protocol the EU released regulation (1946/03/EC) on trans-boundary movements. According to this regulation, the quality of products to be exported should be similar to the one for domestic markets.

In the late 1990s, the growing societal and political opposition contributed to a *de facto* moratorium on new market approvals of GM crops. It was adopted at a meeting of the EU Council of environmental ministers in June 1999, where five member states decided not to accept new GM crop market approvals until the existing regulatory frame was revised (Winickoff et al., 2005). Several agro-food biotechnology market applications remained subsequently blocked in the approval pipeline in the EU.

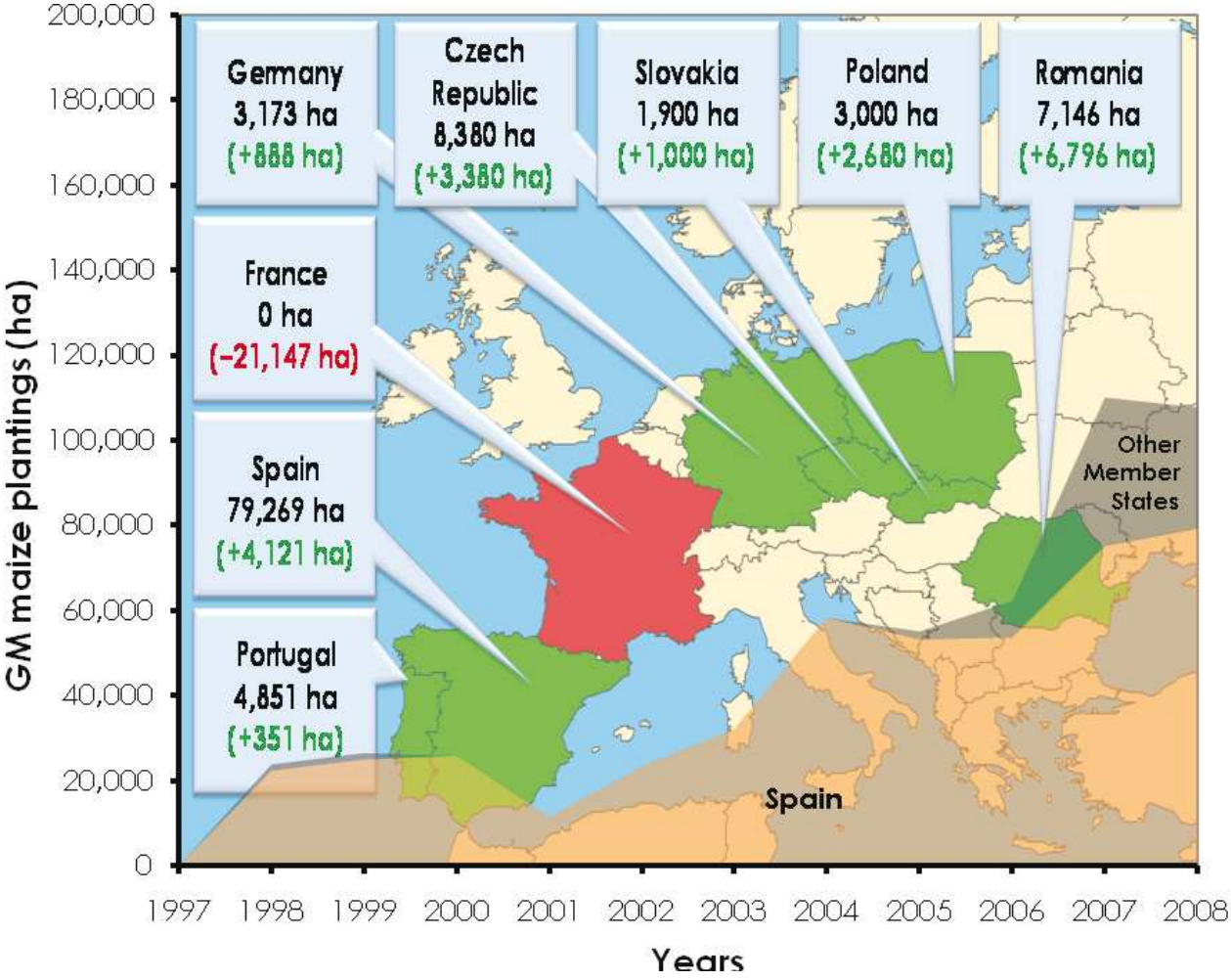
From 1999 onwards, policy-makers started to continuously revise the legal conditions under which GM crops and agro-food products were allowed to be used in the EU to slow down further erosion of public and market confidence (Devos et al., 2006). The precautionary principle, postmarket environmental monitoring and traceability were legally adopted as ways to cope with scientific uncertainties. New institutions such as the European Food Safety Authority (EFSA) were created to provide independent, objective and transparent science-based advice on the safety of agro-food biotechnology applications. Labelling and traceability of GM products became mandatory to ensure consumers' freedom of choice and the issues considered by ENGL.

While these directives and regulations are attempting to provide freedom of choice to the European consumers by an accurate labelling (258/97/EC, 2001/18/EEC and 1830/03/EEC), the producers should keep freedom of production choice through a EC coexistence recommendation (Commission Recommendation of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming).

Co-existence issues.

In parallel to the European consumers' reluctance, the adoption rate of genetically modified (GM) crops shows considerable disparities between different agricultural production regions worldwide. While the global cultivation area of GM soybean, maize, cotton and canola (oilseed rape) reached 114 million hectares in 2007, the total area cropped with GM crops in the European Union (EU) was approximately 110 thousand hectares (James, 2007).

Most approved GM crops worldwide are thus currently cultivated outside the EU, but might subsequently be imported and eventually further processed in the EU mostly for feeding purposes. Today, Bt-maize expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis* is the only GM crop to be cultivated in the EU.



Because the maintenance of different agricultural production systems is a prerequisite for providing a high degree of consumers' choice, a coexistence policy was adopted in the EU. It specifically aimed at enabling the side-by-side development of different cropping systems without excluding any agricultural option. As such, farmers would maintain their ability to make a practical choice between conventional, organic and GM crops. Since coexistence only applies to approved GM crops that were judged to be safe prior to their commercial release (Sanvido et al., 2007), safety issues fall outside the remit of coexistence (Schiemann, 2003; De Schrijver et al., 2007a).

To date there is little experience on how the new legal coexistence requirements could be implemented in the EU. Due to the heterogeneity in farm structures, crop patterns and legal environments between member states, the European Commission for coexistence. These best practices then have to be developed and implemented at national or regional levels.

However, the coexistence at the farm does not help to understand whether, how and at which price coexistence would be preserved in the remaining parts of the supply chains.

European research

The European Commission launched, in 1999 in the frame of FP5, several research programs first on "analytical traceability", i.e. on detection methods to preserve the consumers' freedom of choice by accurate labelling. Those research programs were not redundant with national programs such as the French program "*Pertinence économique et faisabilité technique des filières OGM et non OGM*" (http://www.inra.fr/genomique/communique7.html).

DMIF-GEN (http://www.dmif-gen.bats.ch/dmif-gen/body.html), QPCRGMOfood (http://www.vetinst.no/eng/Research/EU-projects/QPCRGMOFOOD) and GMOchips (http://www.bats.ch/gmochips/) programs all provided the first insights on analytical methods and the issues faced to reliably detect and quantify the GMO content in products. ENTRANSFOOD, an EC cluster, was attempting to gather information from the several programs, their coordinators meeting into this cluster, and from other parties but was mostly devoted to risk assessment.

Most of the members of those research programs were also members of ENGL (http://engl.jrc.ec.europa.eu/) which enabled all EEA enforcement laboratories to work together under the chair of the European Joint Research Center (JRC) since its official launch in 2002. They generally were also members of the CEN standardisation working group TC/34 WG 11 ensuring a good coordination between research, standardisation and enforcement abilities for developing methods to keep freedom of choice to European consumers.

After the launch in 2002 of the FP6, calls for proposals were launched in the Priority 5 (Food safety and quality) which resulted in the research projects SIGMEA (http://www.inra.fr/sigmea), Transcontainer (http://www.transcontainer.wur.nl/uk/) and Co-Extra (www.coextra.eu) with the aim of developing and implementing tools for ensuring coexistence in the European supply chains, from seeds to retailers shelves.

While SIGMEA mostly focused on fields' coexistence (Messéan et al. 2009), Trancontainer addressed biocontainment methods.

Co-Extra

Co-Extra is an FP6 (contract 007158) research program of the priority 5 (Food safety and quality) of the European Commission which started in April 2005 and finished in September 2009.

Its summary stated:

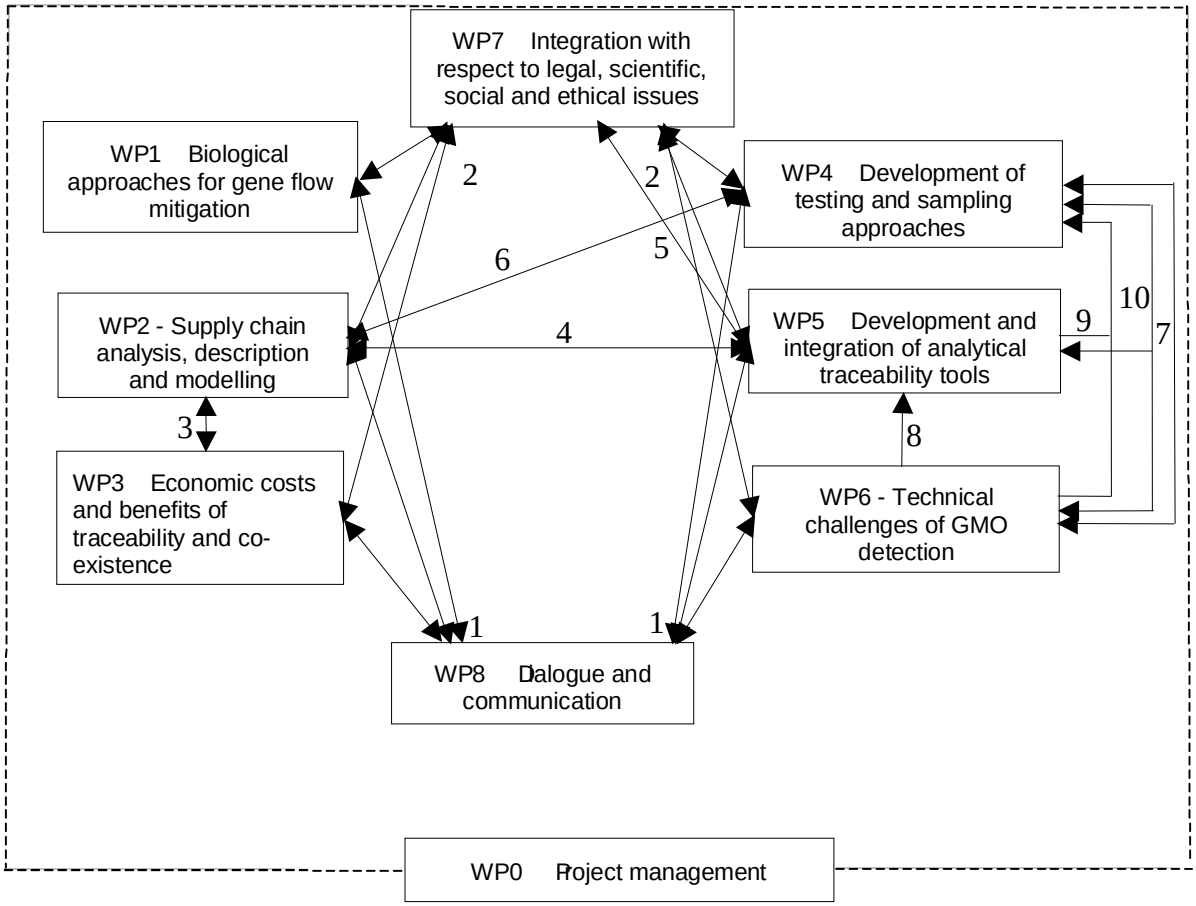
The objective of Co-Extra is to provide all the stakeholders of the food and feed chains with a central decision-support system integrating the tools, methods, models and guidelines needed to deal with the imminent arrival of large quantities of GMOs, further to the lift of the current de facto ban on GMOs in the EU. Co-Extra will study and validate biological containment methods and model supply chain organisations and provide practical tools and methods for implementing co-existence. In parallel, Co-Extra will design and integrate GMO detection tools, develop sampling plans, and elaborate new techniques to meet the challenges raised by increased demands for cost effective multiplex methods to detect as yet unapproved or unexamined GMOs and by e.g. stacked genes. Co-Extra will also study and propose the most appropriate information structure, content and flow management for ensuring reliable and cost-effective documentary traceability. All of the methods and tools that will be studied and developed will be assessed not only from the technical point of view but also with regard to economic and legal aspects. In parallel, to promote harmonisation of co-existence and traceability practices around the world, Co-Extra will survey the GMO-related legal regimes and practices that

exist in and beyond the EU. Stakeholders will be involved in the project from the start through the dialogue platform, editorial offices, focus groups, national relays, etc. Co-Extra outcomes will contribute to reinforcing consumers' confidence in labelling claims and therefore EU products at large. By helping economic stakeholders to meet consumers' requirements for reliable choices, Co-Extra will improve European competitiveness. Co-Extra outcomes will be proposed to standardisation after validation. Dissemination activities will largely benefit from the strong commitment of the European Network of GMO Laboratories.

The documentary (ISO definition of traceability) and analytical traceability studied in Co-Extra are two tools necessary for both managing the coexistence of supply chains and for controlling the results of this management.

The products to be managed originate either from the European agriculture or from imports from third countries. In several aspects this management of supply chains does not differ from systems already in place, such as waxy maize, or seeds productions. The segregation of such specialities is quite well known and controlled in the EU and several third countries, and does not impact too much European supply chains costs. The main issue in segregating GM and non-GM products lies thus in a rather low labelling threshold of 0.9% and the use of the DNA unit to measure this, as recommended by the EC.

Co-Extra was divided into 8 Work Packages, with the additional project management WP0.



WP1: This workpackage was aimed at assessing and developing whenever necessary biological tools and methods to allow producers to grow kind of crops they choose with minimised risks of admixture between GM, conventional and organic products. Leader: Dr. J. Schiemann, JKI. DE.

WP2: The objective of this workpackage was to describe and model the supply chain structures with aim to propose organization enabling co-existence throughout the feed and food chains. Leader: Dr. A. Messéan, INRA, FR.

WP3: The workpackage was assessing the internal and external costs and benefits generated by the implementation of co-existence and traceability. Leader: Dr. M. Gylling, FOI, DK.

WP4: The objective of this workpackage was to develop the appropriate control plans for the use of selected detection methods. Leader: Dr. Roberta Onori, ISS, IT.

WP5: The objective of this workpackage was to develop cost-effective and fit for purpose methods and tools for detection of GMO taxa and controls. Leader: Dr. K. Gruden, NIB, SO.

WP6: This workpackage was focused on the design new technologies to overcome the limits of current methodologies, for instance for detection of unknown GMOs and stacked genes. Leader: Dr. A. Holst-Jensen, NVI, NO.

WP7: This workpackage was aimed to integrate the project outcomes to come to the initial development of decision support tools to stakeholders and policy-makers, to define the most appropriate information structures, contents and supports to ensure reliability and cost-efficiency of documentary traceability, and to assess the reliability of the co-existence and traceability systems from selected third countries (outside the European Union). Leader: Dr. N. Alexandrova, ABI, BU.

WP8: This workpackage had to develop the stakeholders' dialogue using an internet platform (Co Extra website) and stakeholder workshops. The set up of regional offices will allow acquiring substantial knowledge on the needs and practices of the stakeholders involved in the various countries. By several means and methods relevant to the stakeholders of the different countries, the outcomes of Co-Extra will be disseminated to the different stakeholders. Links to user-friendly decision support tools for stakeholders will be provided. An editorial office as communication center is to provide a consumer oriented multi target website. Leader: Dr. K. Sinemus, Genius biotech, DE

WP0, in charge of project management was led by A. Baker and O. Mackre, INRA Transfert, FR.

All together, the 4.5 years of Co-Extra research has been performed by more than 200 scientists with their teams from 52 partners coming from 18 countries (15 European countries with Argentina, Brazil and Russia) and have attempted to provide insights into current practices and solutions to issues as well as providing solutions for unpredictable situations. For the first time, an EU research project has addressed the whole supply chain, from seeds to retailers shelves, their practices and their economic and legal environments, their requirements for taking into account both their current solutions and providing new ones. The needs of the supply chains and their impact on production of crops provided new questions on coexistence and traceability, including cost- and time-effectiveness of analytical methods and coexistence at the field level.

The practical implementation of the several observations and solutions developed by Co-Extra will have important technical, scientific, economic and legal impacts.

Main Co-Extra deliverables & results, perspectives, information dissemination & application.

For the first time a European research project on coexistence has taken on board coexistence and traceability issues so as to ensure coexistence of the whole GM and non-GM supply chains.

The first impact of this integrated approach was the confirmation by Co-Extra that the supply chains actors, downstream from the farms, are using a practical contractual threshold of ca 0.1% of GMO content (expressed in HGE units as recommended by the EC) well below the 0.9% European labelling threshold.

This confirmation of previous observations, made in 2001 during other research projects, brought about thus several changes in the planned Co-Extra activities.

This report summarises the most important results and messages from the different work-packages of Co-Extra, both in terms of scientific results and practical advice and solutions. Some issues are still pending and will need further research. Others are dependent on decisions to be taken by companies and policy makers, for instance, the European "seed threshold" and whether co-existence rules have or not to be harmonized at a European level.

This report provides a summary of Co-Extra results and does not go into details. More details can be found on the Co-Extra website (www.coextra.eu), particularly in the on-line available deliverables (http://www.coextra.eu/library/deliverables.html) and in the peer-reviewed papers published by Co-Extra partners (http://www.coextra.eu/library/publications.html).

The Co-Extra results are sometimes commented. This report also proposes perspectives of future research and presents open questions raised by Co-Extra results.

Coexistence of supply chains.

Co-existence between GM and non-GM crops

At the field level, adventitious presence of GM material in non-GM production could have several causes. The most important biological parameters are flowering biology (mainly the ability of pollen to move from one type of crop to another), the ability of the crop to make fertile crosses with related wild relatives that may grow in or around the production field, and the survival ability of its seed and other storage structures if they are left in the field.

In this context, the general objective of Co-Extra was to analyze, further develop and validate biological mitigation methods for restricting pollen-mediated gene flow during cultivation by removing or reducing the fertility of pollen as well as to identify the major drivers of pollen flow over fragmented landscapes.

Biological mitigation techniques may restrict pollen-mediated gene flow and could help to reach adventitious presence below legislated labelling thresholds and meet local or international requirements for safety and/or coexistence depending on the transgenic insert and the type of GM crop.

Thus, there is a need to verify their long-term effectiveness under various environmental and agricultural conditions and to ensure that instabilities of biological mitigation techniques are limited or even prevented.

In addition, Co-Extra focused on open research questions, like information's about the major drivers of pollen-mediated gene flow in maize over large distances and fragmented landscapes or the consequences of GM seed admixture on the non-GM harvest product in maize.

Co-Extra worked on crops for which GM varieties are already approved (maize) or might be close to authorization (rapeseed), and on crops whose authorization is expectable during the next 5 years (sunflower, tomato, tobacco).

The outcome of field trial studies depends highly on the climatic conditions during cultivation. Due to special climatic conditions in 2006 at all locations and the drought in 2007 in Bulgaria Co-Extra must repeat several field experiments or got less yearly results than foreseen.

Biocontainment measures.

Biocontainment (also called bio-confinement) measures could be used to reduce or even eliminate plants pollination through for instance pollen cloud reduction, pistil fecundation of both related crops and wild relatives.

Already existing biological containment tools like cytoplasmic male sterility (CMS) in maize and sunflower, male sterility in tomato, cleistogamy in oilseed rape and plastid transformation in tobacco were tested for their stability and reliability. Large scale studies were performed with maize and rapeseed, small scale studies with sunflower, tomato and lab experiments with tobacco. Viable pollen production, cross pollination, crop yields, and other themes relevant to bio-confinement were taken into account.

A first objective of Co-Extra was to test the stability and reliability of biological containment tools such as cytoplasmic male sterility (CMS) in maize, cleistogamy in oilseed rape and plastid transformation in tobacco.

Gene flow levels in CMS maize and cleistogamic oilseed rape were studied over generally 3 years, except some years where weather conditions or divagating animals impeded experiments, in field experiments located at different sites in Europe (UK, Germany, France, Switzerland and Bulgaria). Plastid transformation and its ability to reduce gene flow were tested only in laboratory taking tobacco as a model.

The experiments and results are developed into deliverables D1.2; D1.3; D1.4; D1.5; D1.6; D1.7; D1.8; D1.11; D1.12 and D1.14.

Maize

CMS types testing

Ring field trials were carried out in 17 different environments in Europe over two years with 22 CMS versions of modern European CMS maize hybrids to analyse the stability of the CMS trait. The CMS hybrids were grown in a randomized complete block design with five replicates, each arranged into sub-plots, each of which contained one CMS hybrid in one row, 6 m long. The single-row sub-plots, 0.80 m apart, were sown randomly inside each block at plant density of 6.25 m-2. Two rows of a commercial hybrid (Goldenso) were sown at the edges to avoid border effects. To determine whether or not viable pollen was released, self-pollination was carried out from 09:00 to 11:00h, i.e. the optimal time frame (Jarosz, 2003). Seventy five (75) plants per hybrid, per sowing date and per location were tested for male sterility.

Figure 1: Ring field trials 2005/2006.

Stable and unstable male sterility occurred in all three CMS maize types. T-cytoplasm hybrids were the most stable under a wide range of environment, while S-cytoplasm hybrids often showed partial restoration of fertility. C-cytoplasm was similar to T-cytoplasm with regard to maintaining male sterility.

The data demonstrate that stable cytoplasmic male sterility in maize may be an effective way to prevent GM pollen-mediated gene flow to adjacent fields if 100% stable T- and C-cytoplasms are used.

Plus-Hybrid system testing

Ring field experiments were carried out in 11 environments in Europe over two years to assess the advantage of the Plus-Hybrid system. Five hybrids in their CMS- and fertile forms, but also three additional pollinators were selected for their combining and pollinating abilities within the system, taking into account the CMS and Xenia effects. The right selection of CMS- and fertile counterparts will allow an additional benefit for the farmers by significantly increasing grain yield. The experimental layout consisted in pure-stand small-plot trials where the pollinators and the CMS hybrids were grown in a randomized split-plot design with 3 replications. The main plots were the pollinator blocks (Fig. x.1), consisting of 12 rows, each 21.8 m long and 0.75 m apart (0.70 m in Bulgaria). Six sub-plots, each 2 rows wide and 5 m long, were randomly distributed within the pollinator block. Two rows of pollinators were sown between the CMS hybrids to ensure an optimal pollen supply. Three border rows and rows on the lateral side (3.4 m long) were additional pollen sources and acted as a buffer zone to minimize the contamination by pollen from neighbouring pollinator blocks

Appropriate combinations of CMS hybrids and fertile pollinators used as an agricultural bio-containment system can thus lead to a significant gain in yield, as observed for the Plus-Hybrid system. Promising Plus-hybrid combinations, leading to gains in yield above 10% across all environments and reaching 15 to 20% in specific environments, were observed. Three highly responsive CMS hybrids and four generally good pollinators were identified.

Conclusion

The Co-Extra data demonstrate that stable cytoplasmic male sterility in maize is an effective way to reduce or even eliminate GM pollen-mediated gene flow to adjacent fields if stable T- and C-cytoplasms are used. One of the CMS types (Texas CMS) has a highly sensitivity to an important fungal disease. Its use might be limited to specific situations on small scales, for instance for GMO developed for non-alimentary purposes, such as pharmaceutical ones. Furthermore, appropriate combinations of CMS hybrids and fertile pollinators used as an agricultural bio-containment system can lead to a significant gain in yield.

Rapeseed

At the difference of maize, rapeseed is a more easily diffusible plant species. Co-Extra examined by field experiments the ability of a cleistogamic rapeseed line previously developed to participate to biocontainment through the lack of flower opening.

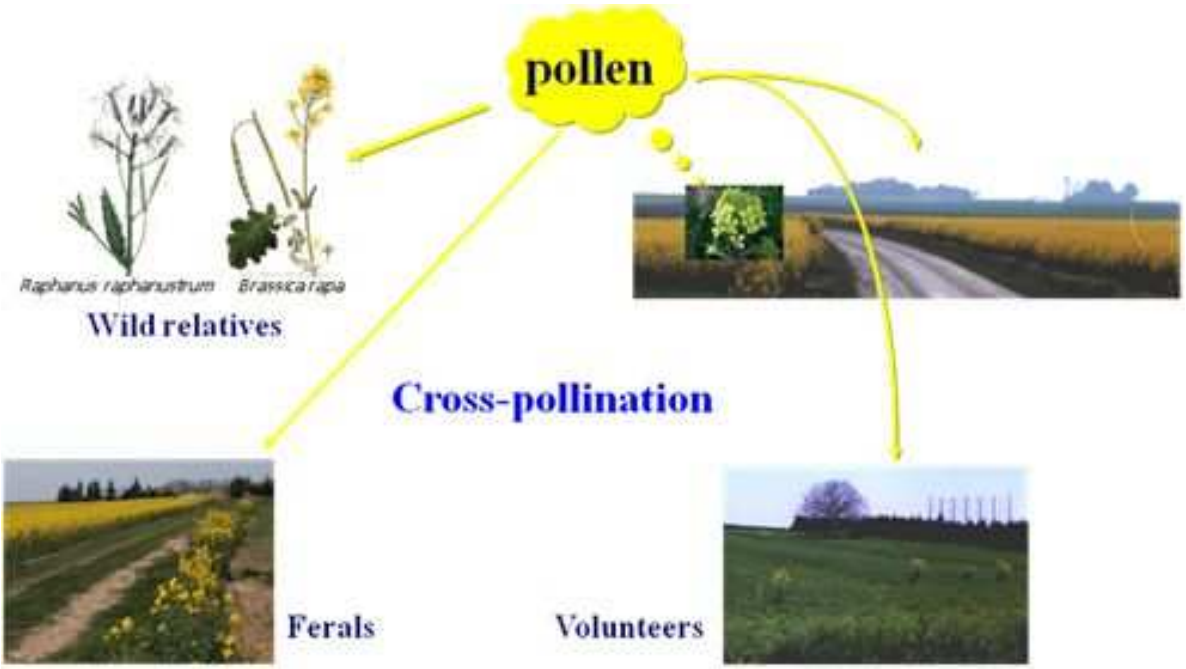


Figure 2: Rapeseed dissemination ways.

The stability and allo-pollination rate of cleistogamic oilseed rape lines was tested with ring field trials in four locations in Europe using two new genetic backgrounds over two years

The two cleistogamic lines were both winter-types and homozygous at the locus controlling the cleistogamic trait, but they differ for their genetic background. Two wintertype control cultivars were used in each trial: in each trial, two treatments were applied to the four genotypes: application or no application of a growth regulator. At each site, a split-splot field layout, using a four replicate, randomized block design was carried out with minimum sown plot areas of 22.5 m^2. During the flowering period, the stability of the cleistogamy trait was assessed visually by scoring the degree of petal opening on mature flowers of the inflorescence using a three-level scale: fully opened flowers, totally closed flowers that appeared like a big yellow bud and a third class of partially opened flowers.

The experiment design for the estimation of the allo-pollination rate consisted of two plots (at least 50m x 50m), one plot sown with the cleistogamic oilseed rape, and the second plot sown with a mixture of 99% of a conventional flowering high erucic variety and 1% of the cleistogamic genotype. Trials were implanted perpendicularly to the direction of dominant winds and were isolated from exogene oilseed rape pollen source by a distance from any oilseed rape field higher than 300m. The self-fertilization rate was estimated through erucic acid content of seeds collected on cleistogamic plants (low erucic acid line), using the erucic acid content observed in seed samples coming from self- or manually cross- pollination of the cleistogamic genotype. The experimentation enabled to estimate the rate of pollination by the erucic lines simultaneously in a context of high pressure of erucic pollen and at set distances from the erucic source.

Figure 3: Field experiments for testing cleistogamic rapeseed lines.

Data on cleistogamic oilseed rape showed that some flowers were partially opened with rates varying from 0.5% to 33% principally depending on genotypes, trials (site and year) and recording dates. Even

when partially unstable, the results demonstrate that cleistogamy as a biological mitigation technique has a major potential for limiting cross-pollination due to the strong reduction of the pollen cloud. Allogamy rates of cleistogamic lines which grow up under a high pressure of allopollen varied between 4.4% and 16.2%, averaging 8.1%. The mean level of allo-pollination oilseed rape lines with open flowers amounts 30%. Accordingly, these results confirmed that cleistogamy might also be an effective tool to preserve seed purity and "identity". But further studies have to be carried out as the proportion of closed flowers is variable during the flowering period.

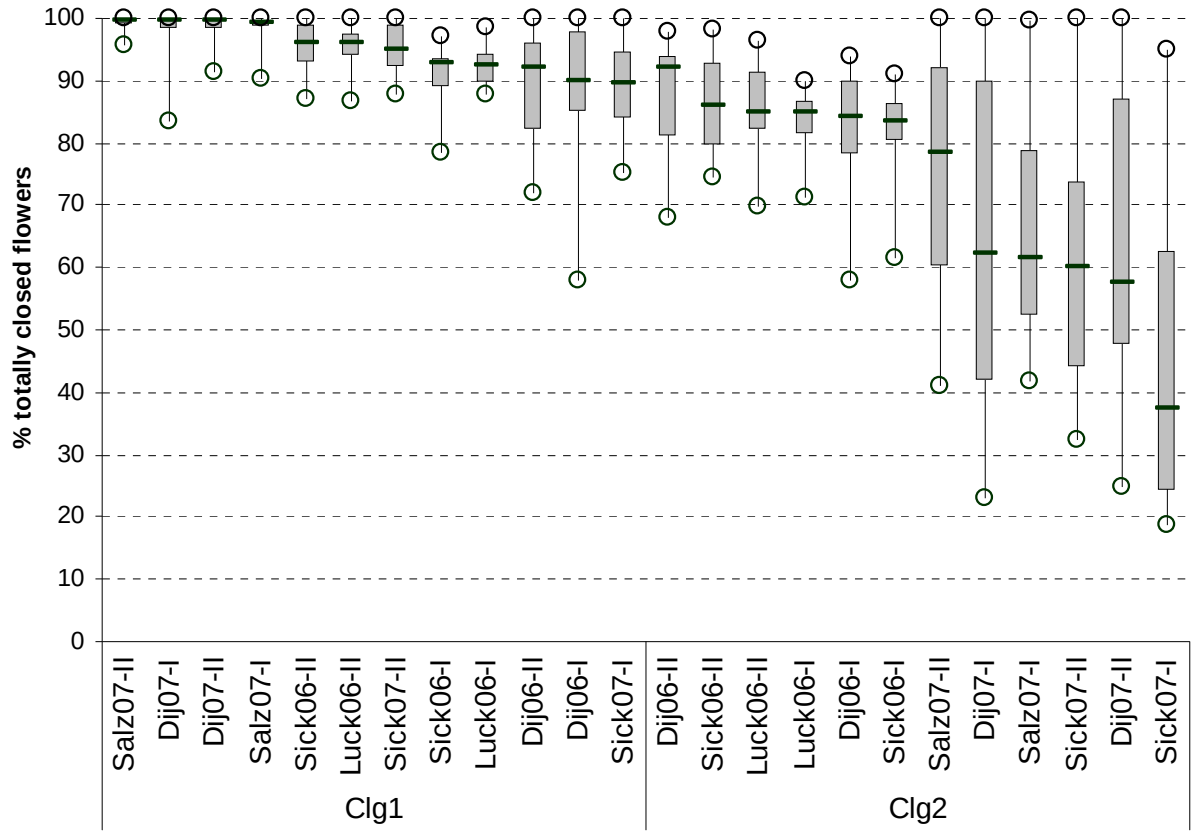


Figure 4: The proportion of closed flowers depend on the genetic background

Sunflower

In Bulgaria the pollen flow and the stability of the CMS trait of eighteen sunflower-CMS-lines were assessed in field trials in 2 locations and over 3 years. At the beginning of florescence, sunflower heads were covered with bags to prevent the pollination with foreign pollen. The assessment of the release of pollen in the bags was carried out during the flowering period. The mature sunflower heads were collected and assessed for the presence of filled seeds. The number of plants with filled seeds and the number of filled seeds in each fertile plant was assessed. The source of pollen contributing to the seed set was determined by microsatellite analysis.

Cytoplasmic male sterility in sunflower is environment dependent. The investigated CMS lines differed in regard to the stability of cytoplasmic male sterility. Four of them were fully sterile across all different environmental conditions Two CMS lines showed unstable sterility. The observed restoration of fertility in the CMS lines was most probably caused by the excessive rainfall and lower average temperatures during the period of the flowering of the CMS lines.

In conclusion, the use of CMS as a biological mitigation technique has to follow a precise evaluation of the stability of sterility of particular CMS line over various climatic conditions and multiple periods of cultivation.

Tomato

Field trials and plastic tunnel trials were conducted to assess the stability of male sterile tomato lines in Bulgaria in 2 locations and over 3 years.

Field experiments were set up in a randomized complete block design in three replicates per 10 to 16 plants. The plants were planted in three rows, the distance between the rows being 80 cm and between the plants – 35 cm. The distance between the plants included in the experiment and the other tomato plantings was 6 m or more than 15 m.

Periodically, the number of flowers and flowers that set fruit per plant was counted. After maturity, these fruits were collected and investigated for presence of seeds. If present, seeds from each plant and each fruit were individually collected for further determination of their genotype (that could be resulting from self – or cross fertilization).

In tomato two types of male sterility – sporogenious and functional – are potential tools for biological containment. On the base of the data of field experiments and molecular analyses it was found that no cross pollination by pollen originating from the sterile genotypes was detected in any of the locations.

Plastid transformation as a biocontainment tool with tobacco as a model.

Data mining was performed to generate information on the suitability of chloroplast transformation as a containment strategy. Plastid transformation is emerging as an alternative tool to genetically engineer crop plants. Stable transformation of the plastid genome was first achieved in early 1990s in tobacco by Pal Maliga and his associates. The technology of plastid transformation has many advantages over the classical nuclear transformation method that is routinely employed for developing transgenic plants. Major benefits of this technology include prevention of gene flow from the engineered crops to its weedy or wild species thus making it a safer alternative tool, and high level of transgene expression especially required to develop insect-pest resistant plants.

Protocols for plastid genetic engineering have been developed in few agriculturally important crop plants other than tobacco. This work briefly describes and summarizes the developments of the technology highlighting its potential application in crop improvement or for the production of pharmaceutical and nutraceuticals in plants. Plastids (chloroplasts) are maternally inherited in most crops. Maternal inheritance excludes plastid genes and transgenes from pollen transmission. Therefore, plastid transformation is considered a superb tool for ensuring transgene containment and improving the biosafety of transgenic plants.

An experimental system for transplastomic tobacco facilitating the stringent selection for paternal plastid transmission has been developed. Large-scale crosses were conducted by pollinating male sterile plants with pollen from homoplasmic transplastomic plants. The progeny were assayed for paternal chloroplast transmission by germinating seeds on Murashige and Skoog's medium in the presence of spectinomycin. Seedling phenotypes were analyzed by visual inspection under a stereo microscope. Green sectors were excised from cotyledons or primary leaves and regenerated on RMOP medium containing spectinomycin. To eliminate spontaneous spectinomycin-resistant mutants, tissue samples were exposed to double selection on medium containing spectinomycin and streptomycin. Whereas spontaneous resistance mutants bleach out on this medium, cells with transgenic chloroplasts remain green and continue to grow.

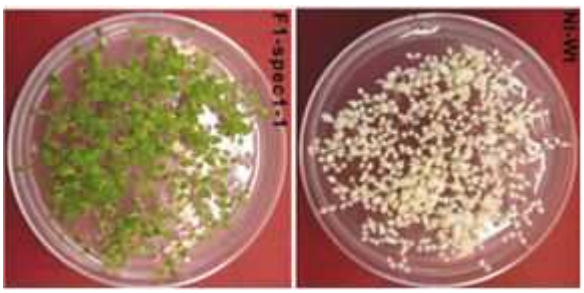


Figure 5: Laboratory experiments with transplastomic tobacco seedlings.

In this study, the strictness of maternal inheritance and the extent to which plastid transformation technology confers an increase in transgene confinement were assessed. An experimental system is described which facilitates stringent selection for occasional paternal plastid transmission. In a large screen, low-level paternal inheritance of transgenic plastids in tobacco was detected. Whereas the frequency of transmission into the cotyledons of F1 seedlings was 1.58 x 10-5 (on 100% cross-fertilization), transmission into the shoot apical meristem was significantly lower (2.86 x 10-6). These data demonstrate that plastid transformation provides an effective tool to increase the biosafety of transgenic plants. However, in cases where pollen transmission must be prevented altogether, it is suggested that stacking with other containment methods will be necessary to eliminate the residual out-crossing risk.

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Factors affecting gene flow over fragmented landscapes and large distances

The second aim of Co-Extra work on coexistence in field crops was to gain information about the major drivers of maize pollen flow over fragmented landscapes, through field experiments and modelling. Data mining was performed on the practical and technical knowledge on GM cross-pollination in maize on the basis of evidence from printed publications.

Various factors involved in maize pollen emission and pollen flow were analysed through existing data analysis and field experiments. Tools modelling velocity and pollen concentrations over heterogeneous fields were also developed to assess the cross-pollination rates between GM and conventional maize over large distances and in fragmented landscapes. Using new and previously gathered data a statistical model of pollen emission in relation to microclimate and a physical model of pollen flow based on fluid mechanics were successfully validated.

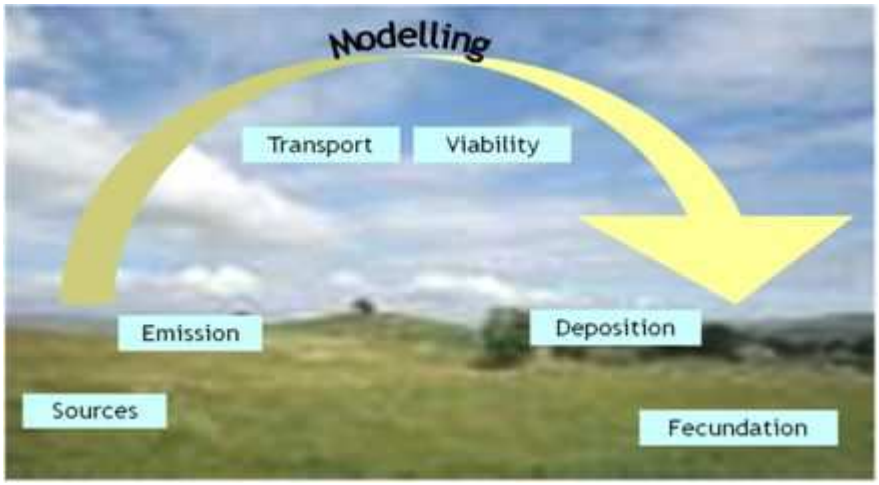


Figure 6: Pollen dissemination studies over large fragmented landscape: experiments and modelling

These experiments and results applied to single event transformations are available in D1.1 and D1.7.

Field experiments

Field trials were conducted in UK and Germany to verify the effect of different gap crops (wet vs. dry microclimatic conditions, tall vs. short gap crop) on the cross-pollination rate between two adjacent maize fields and to test the amount of cross pollination over different distances (between 10 m to 1000 m) in UK.

Tools modelling velocity and pollen concentration fields over heterogeneous canopies are required to assess the cross-pollination rates between GM and conventional crops. Laboratory experiments, modelling and theoretical work was done for collecting data about pollen emission, conservation equations for pollen concentrations and moisture as well as a deposition velocity. Based on gathered data a model of fluid mechanics was validated.

Examples of EU maize fields (maize acreage in total, the percentage of acreage on arable land, the size of maize fields and their surroundings) in various regions in four exemplary countries (Germany, France, Bulgaria and the United Kingdom) were recorded using Google Earth and digital ortho-photos

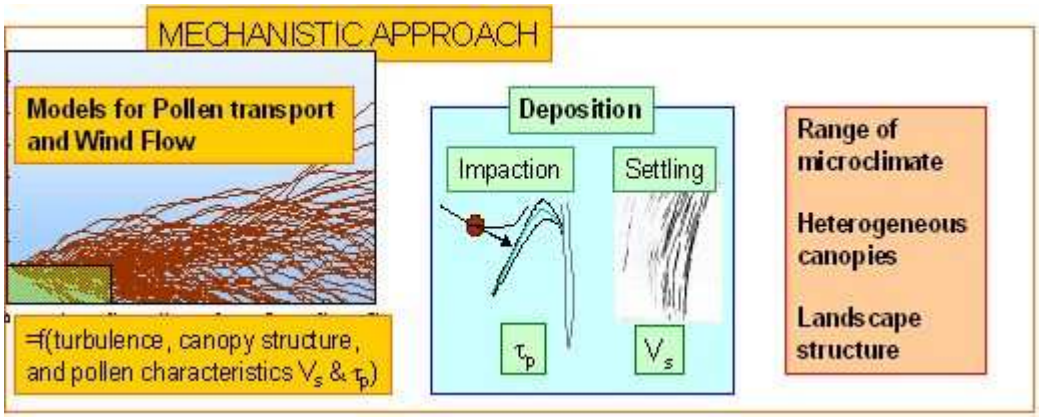


Figure 7: Mechanistic approach of pollen movement in maize

In terms of environment, it appeared that the surrounding crops of grass and cereal had no significant impact in terms of reduction of cross-pollination. The vertical pollen transport over distance was more dependent on strong and predominant wind conditions than on different thermal conditions induced by different gap crops.

The comparison of a tall sunflower crop vs. a short clover–grass crop with regard to their ability to reduce cross-pollination, when grown as a buffer between pollen donor and recipient maize plots, showed also no significant difference between the cross-pollination rates. However, topography affected cross-pollination. There was a greater reduction in pollen mediated gene flow in the more hill-scaped situations than on the flat prairie land. However, even in these instances, the cross pollination was still noted to have occurred at distances up to 1000 m.

Modelling

Published experimental and modelling studies aimed at characterizing pollen dispersal have shown that most pollen emitted by a source field deposits within a short distance from the latter, but also that the observed dispersal functions have long fat tails, making it possible for pollen to contaminate plants at rather long distances. Such possibility has been recently confirmed from (i) a series of airborne measurements of maize and oilseed rape pollen concentration and viability in the atmospheric boundary layer, (ii) chamber measurements of pollen viability in a large range of temperature and humidity conditions and (iii) observations of fecundations in isolated plots of white-kernel maize, at several km from any maize field. In order to better understand long-range dispersal of maize pollen an approach has been developed to simulate the trajectories and dehydration of pollen grains in the atmosphere at regional scale. To this purpose the non-hydrostatic meso scale Meso-NH model has been modified so as to introduce source terms for pollen emission, conservation equations for pollen concentration and moisture, and a deposition velocity. Simulations have been performed over South-West France on several days during the maize pollination period. MesoNH is run in a two-way nested configuration including three nested computational domains down to a 2-km horizontal resolution. GIS-based land-use maps are used for the surface conditions, featuring all the maize fields of the region, as previously identified from satellite data. Considering several days during which airborne measurements were performed, observed and simulated concentration profiles are found to agree well throughout the atmospheric boundary layer. The simulations allow the pollen plume to be characterized through each day and deposition maps of viable pollen to be produced. The calculated deposition rates at remote distances from the maize fields are in the same range as those observed in situ. Additional test simulations are also performed using specific land-use patterns. The results show that background fortuitous contamination is unavoidable at regional scale and provide quantification of long-distance cross-pollination.

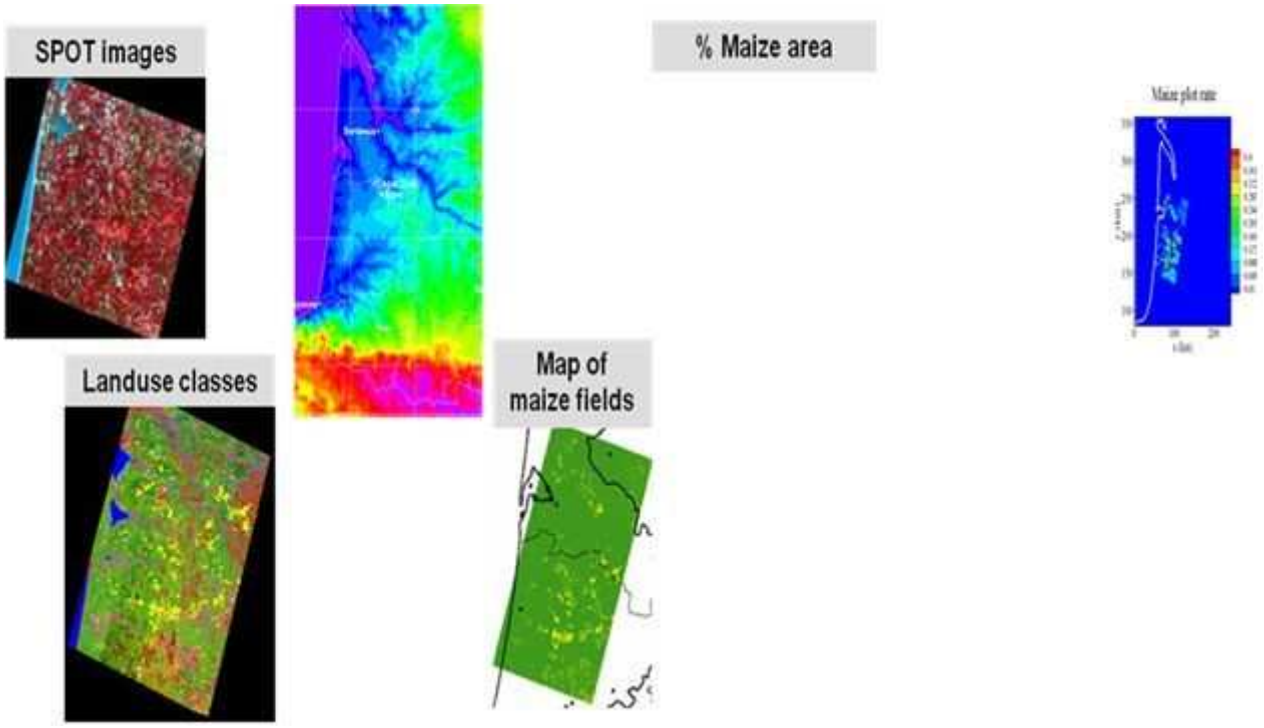


Figure 8: RS / GIS identification of maize fields.

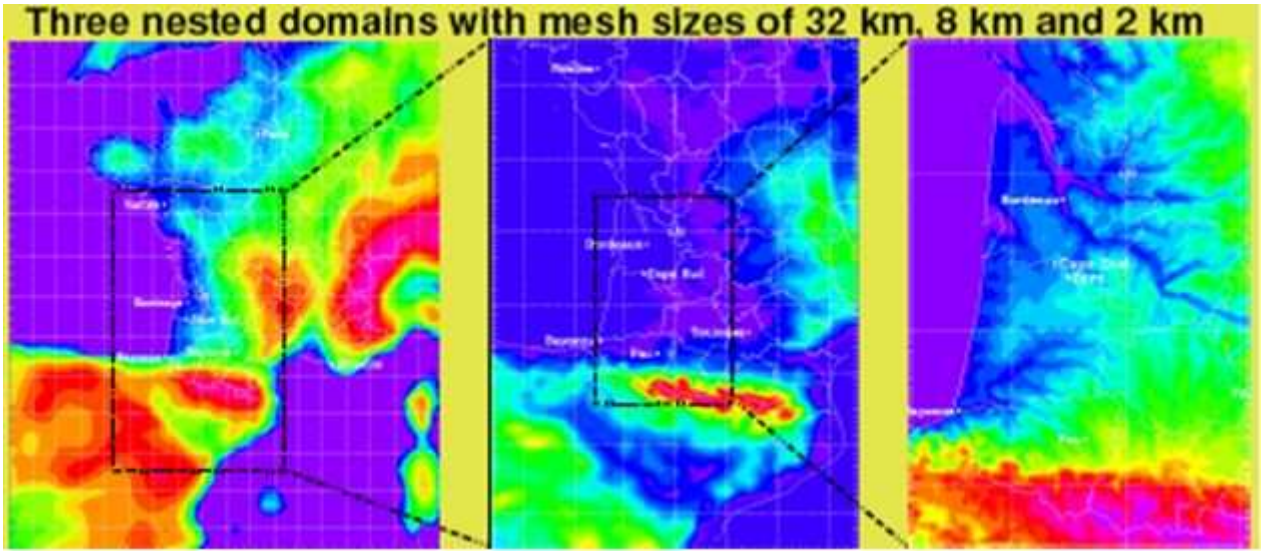


Figure 9: Mesoscale atmospheric transport model.

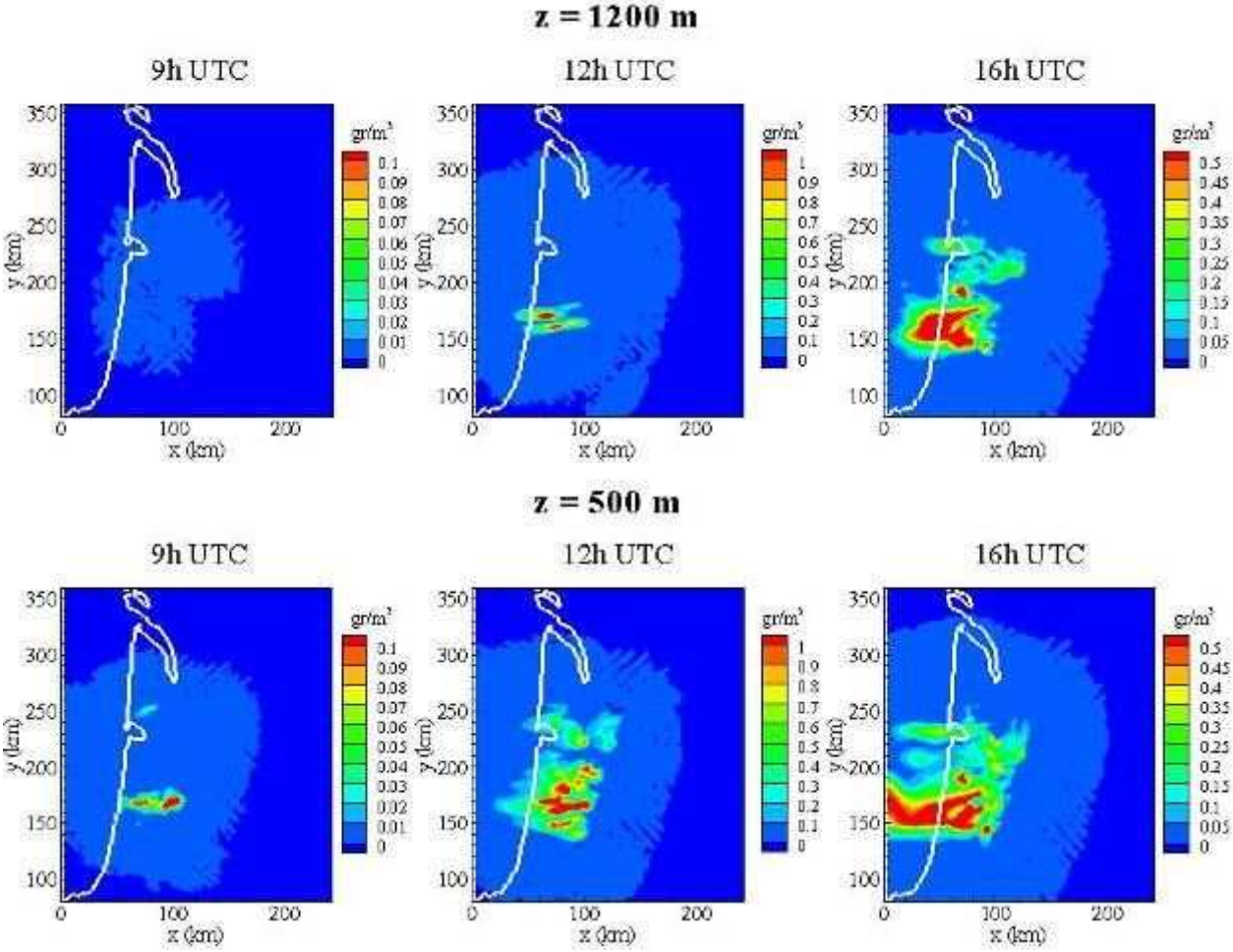
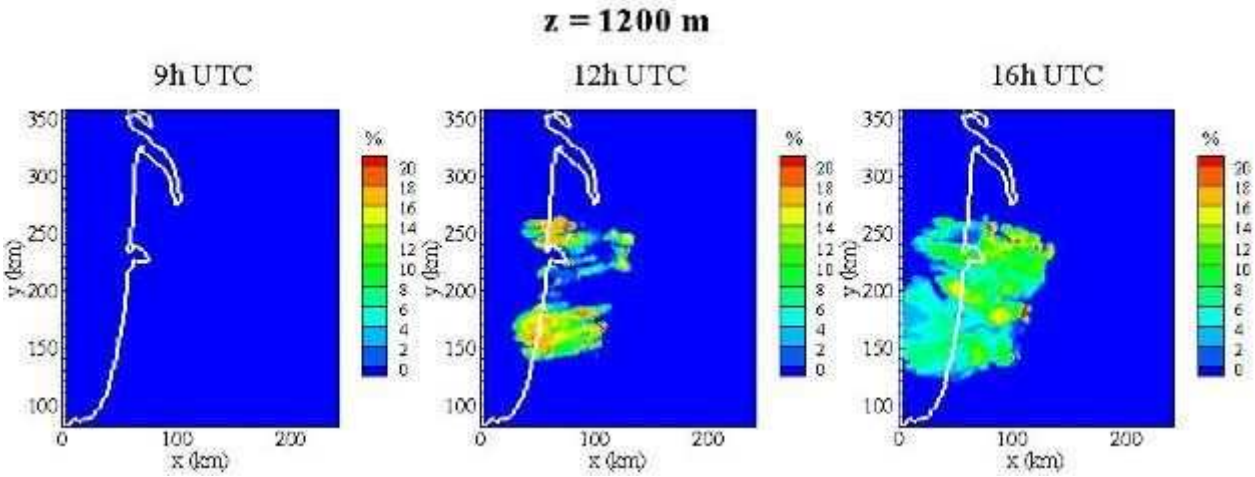


Figure 10: Regional pollen plume.



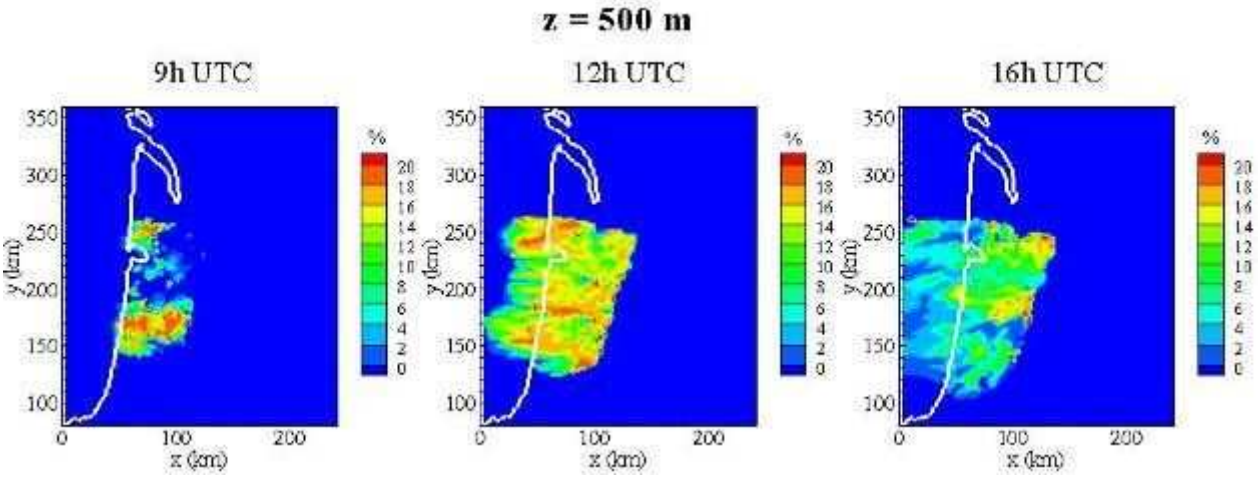


Figure 11: Regional pollen viability.

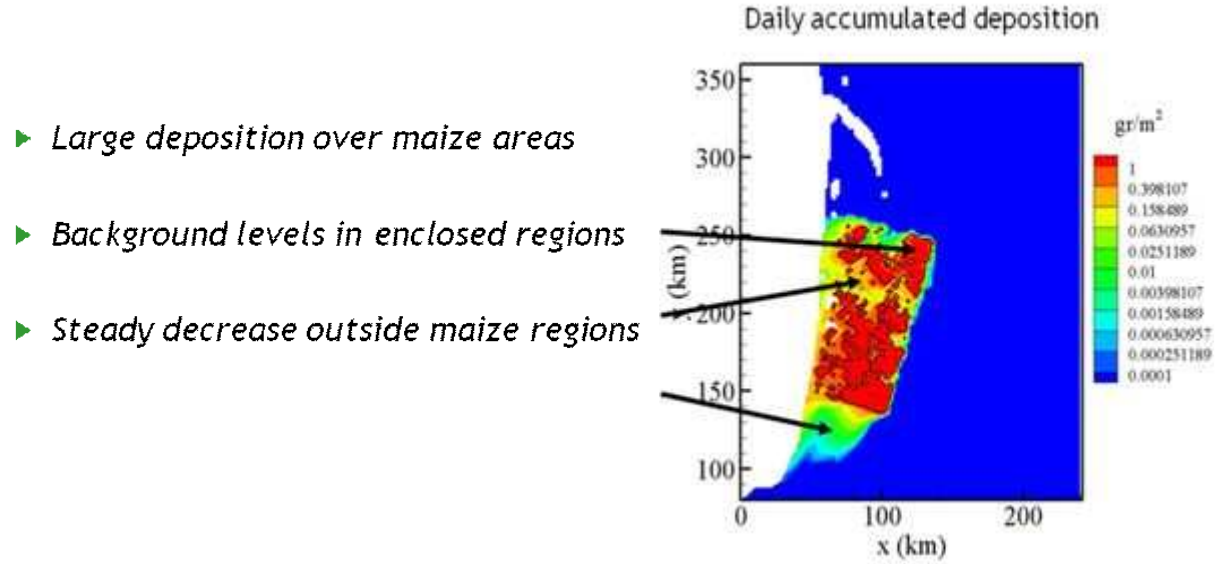


Figure 12: Regional deposition of viable pollen

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Conclusion

Technical measures can ensure that coexistence at the 0.9% labelling threshold for maize hybrids would be achievable on a long-term basis, provided there are high levels of seed purity. Co-existence for maize grain production is feasible but highly dependent on local conditions (e.g. cropping systems, landscape patterns) and on the evolution of practices (e.g. rate of adoption of GM varieties in a region and crop management). Furthermore, various possibilities can be used in different situations (e.g. time-lag of flowering vs. isolation distances) and local operators should be able to choose for themselves the best solutions depending on local constraints.

Models have been developed and were successfully validated for pollen-mediated gene flow in maize over large distances and heterogeneous landscapes. Considering several days during which airborne measurements were performed, observed and simulated concentration profiles are found to agree well throughout the atmospheric boundary layer. The simulations allow the pollen plume to be characterized through each day and deposition maps of viable pollen to be produced.

The calculated deposition rates at remote distances from the maize fields are in the same range as those observed *in situ*. The results provide evidence that background fortuitous contamination is unavoidable at regional scale.

Seeds

The third aim of Co-Extra work on crops concerned seeds. Seeds are the starting point in the supply food chain and so maize field experiments were conducted to evaluate the effect of seed admixture thresholds on out-crossing and the final level of admixture in the harvest product.

The issue of farmers using farm saved seeds and corn populations instead of hybrids was addressed in the work on legal issues.

Seeds admixtures

The main sources of adventitious presence in non-GM maize are seed impurities, GM cross-pollination, and GM kernel transfer via machinery. The average potential rates of adventitious presence occurring at various stages during farm production are relevant to the 0.9 % threshold set by the EU labelling legislation.

To evaluate the effect of different seed thresholds on the final outcrossing rate in the harvest product, ring field experiments with maize seed admixtures (1% kernel/kernel) have been conducted in four locations in Europe over two years.

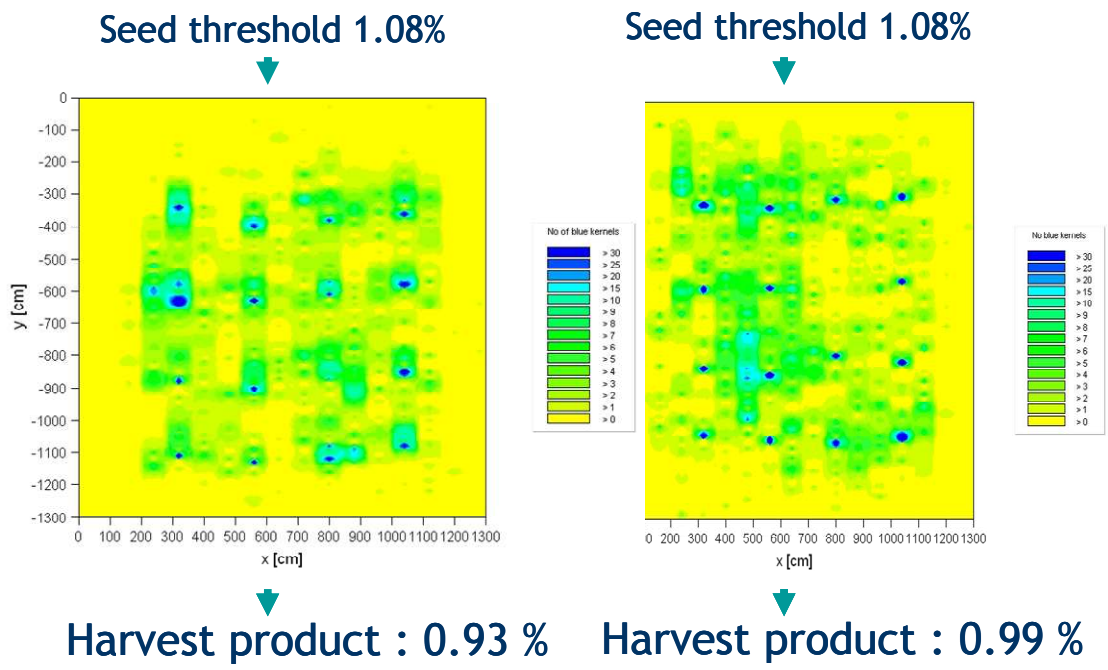

Figure 13: Consequences of seed admixture on the harvest product (% seeds)

The Co-Extra data demonstrated that the final GMO rate in the harvest product is nearly similar to that of the seed admixture for current GM varieties (but will differ with stacked GMOs) but highly dependent on local conditions (flowering coincidence, the site and climatic conditions).

The seed purity is of utmost importance for ensuring coexistence in the fields. Any seed threshold (not yet determined at the EU level), should be lower than the labelling threshold but also leave enough leeway to make it possible the coexistence at the field level.

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Non-GM seeds availability

Coexistence will be feasible and sustainable only if non-GM high yielding seeds are made available to conventional or organic farmers.

Co-Extra took as an example the evolution of availability of soybean seeds after the observation in 2007 that Argentinean farmers wishing to come back to non-GM seeds, due to GM seeds and RoundUp® herbicide prices increases, were unable to proceed due to the absence of seeds integrating the latest breeding development. We can thus foresee that the situation observed in third countries, such as the U.S.A., Brazil and Argentina, the leader countries in soybean production, may be a good draft of the EU situation after a high adoption rate of GM seeds.

A high adoption rate of GMOs will raise the question of whether non-GM varieties will go on being developed for farmer uses, i.e. whether new competitive non-GM varieties will be bred and released. This issue has become more prominent in 2009 with anecdotal information revealing that some U.S. and French farmers had also difficulties to access non-GM soybean and maize (at the requested GMO content) seeds, respectively.

In this context, the aim of Co-Extra (D3.11.3) was to provide some information on the current situation on the soybean seed market and on soybean plant breeding for the three leading producers of soybeans, and to discuss to what extent the apparent seed shortage of 2009 in the U.S. was just a short-term issue or whether it reveals a more general trend of strong decline of competitive non-GM seed breeding and supply.

These investigations on non-GM soybean seeds availability in America are of interest to the European Union because they provide new facts and perspectives on the issue of the future non-GM soybean supply in these countries. Europe depends on soybean imports and the question of the future availability of non-GM materials for feed uses, linked for instance to labelling policies, is an important source of debate. Issues like identity preservation in the supply chain have already been tackled in different works but the question of the availability of non-GM seeds for farmers in exporting countries has not been investigated yet. It may also provide insights on a future evolution in EU non-GM seeds availability despite the exercise were limited to the soybean crop.

This focus on research and development of new soybean varieties in countries with different adoption rates of GM soybeans and different economic and legal contexts also gives new information on whether, and how, the development of a GM culture, of any species, in a country might exclude the development of alternative non-GM culture of the same crop.

Co-Extra explored data from variety registers or from databases on intellectual protection rights, like certificates or patents on varieties, in the three countries; on phone or e-mail interviews with actors of the sectors considered (researchers, plant breeders, farmers, firms or non-profit organizations' employees, civil servants or journalists) in the three countries; on an Internet search on companies' public documents (like variety catalogues) and on scientific and gray literature.

Co-Extra got by this way an overview of the world soybean GM and non-GM markets. This overview describes the current non-GM soybean production and the current demand for non-GM soybean seeds in the three countries. In a second section the different types of intellectual property rights for plant breeders, the market structure in the soybean seed industry, the non-GM soybean breeding activity and the availability of non-GM seeds for farmers in each country were examined. In a third section, prospects on the future of non-GM soybean breeding activity were discussed.

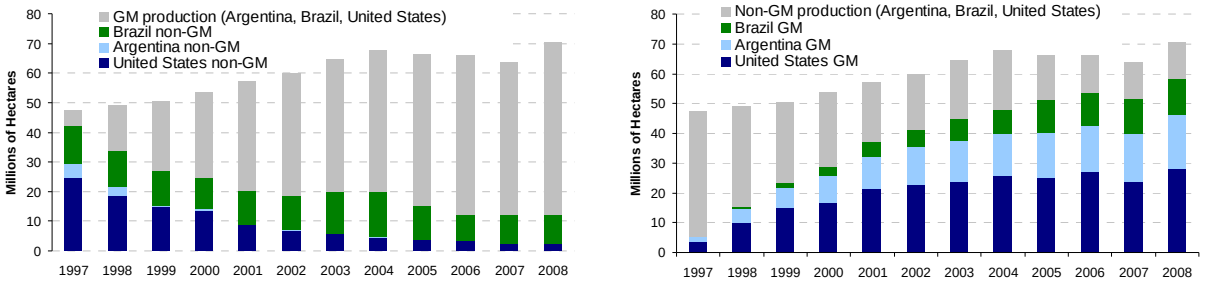


Figure 14: GM and non-GM soybean areas planted in Argentina, Brazil and the United States (Source: USDA/FAS and ISAAA, ArgenBio, CONAB, www.soystats.com)

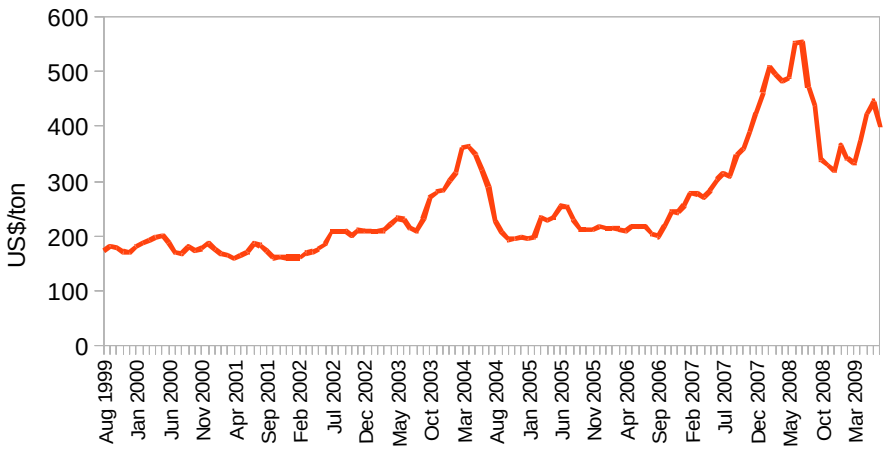


Figure 15: Monthly price of soybeans (Source: Chicago soybean futures contract, US$/ton)

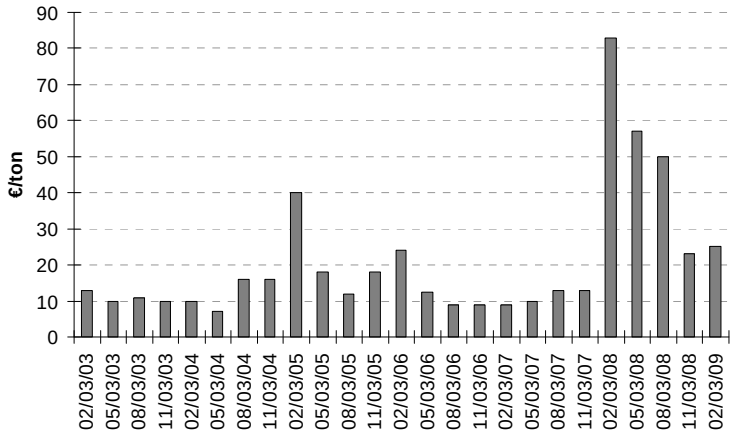


Figure 16: Premiums paid by a French poultry company for non-GM soybean from Brazil

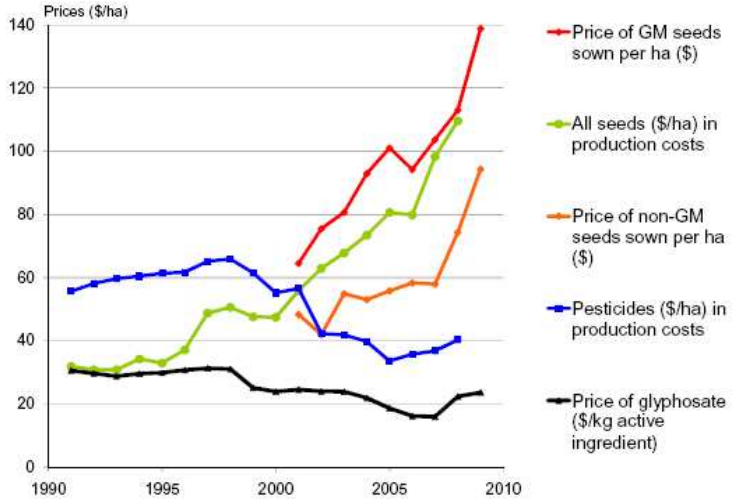


Figure 17: Trends in seed & pesticide costs in the production costs of soybean in the U.S. (Source: USDA (NASS and ERS) from Bonny, 2009).

The information gathered in this report gives a quite clear picture of the situation:

- In the U.S.A., some farmers had difficulties to get non-GM soybean seeds in 2009, but it was a short term problem, caused by an increase in the demand for these seeds that had not been forecast by seed producers and sellers, with non-GM soybeans up to about 9% of soybean plantings. It is expected to be solved next year by increases in non-GM seed production. In terms of diversity, at least 162 non-GM public or private varieties are currently available for farmers.
- In Brazil, around 45% of soybeans produced in 2009 were non-GM and the public enterprise EMBRAPA may guarantee the availability of non-GM seeds.
- In Argentina, where almost all the soybean production is genetically modified, no new non-GM seeds have been registered since 2005. Most of the few non-GM producers are using old varieties.

The main drivers that shape the non-GM breeding activity and determine the future availability of non-GM seeds are:

- The level of the demand for non-GM soybeans, and therefore, for non-GM soybean seeds, creates incentives to private producers to develop such varieties. In Argentina where almost all producers grow GM soybeans, local seed breeders, that dominate the seed market, see no need to release non-GM seeds. At the opposite, in Brazil, the European demand for non-GM soybeans provides good market opportunities for non-GM growers and seed producers. In the U.S.A., the Japanese demand for non-GM soybeans for food uses, resulting in high premiums paid to farmers, also stimulates the activity of small companies developing these varieties and controlling the whole supply chain from seed breeding to exports. Similarly, leading breeders in the U.S.A. still develop non-GM soybeans and claim that they will develop such varieties as long as a demand exists, although this may not necessarily be the case if this demand becomes very low.
- The public sector also has a strong influence on the availability of non-GM seeds. Without the breeding activities of State universities, the availability of such seeds would be limited in the U.S.A. and the foreseeable decrease of these breeding programs may seriously challenge the future availability of new competitive non-GM varieties for farmers. In Argentina, the last new non-GM varieties for general use were registered in 2005 by INTA, and this public institution does not anymore focus its activities on soybean breeding. This situation shows that when demand is low, public sector programs are necessary to guarantee that farmers have a choice between GM and non-GM seeds. In a very different context, the activity of the public company EMBRAPA still guarantees a good availability of non-GM varieties in Brazil.
- The legal framework on intellectual property rights for seeds, and particularly the right to patent new varieties, has significant consequences on the global breeding activities and may result in less competitive non-GM varieties being developed. Because seed companies may have difficulties or simply not be able to access patented varieties developed by other firms, in the next years they may have to develop varieties relying only on their own (and on the public) germplasm resources, and make non-GM varieties available only depending on the ability and will of industry leaders to breed and release them.
- More generally, mergers and acquisitions, by decreasing the number of firms breeding soybean, may reduce the number of firms breeding non-GM soybeans.
- Lastly, the techniques used by breeders to integrate GM traits in their varieties influence significantly the existence of non-GM elite lines that, if released, compete with GM varieties. The development of RR varieties, mainly done through forward breeding, has hindered the development of non-GM programs so far. But the release of new genetic traits (like RR2Y or GAT) may change this situation. It is indeed more flexible for firms developing different types of varieties, with different traits, to develop their new varieties in a non-GM background. But still, the companies that will release their own new genetic traits may chose, as Monsanto did in the 1990s, to develop all their varieties in a GM background and abandon non-GM breeding.

Further investigations would be needed at a firm scale to clarify the different incentives and strategies generated by the emergence of these new genetic events and their consequences on firms choices to breed and release, or not, non-GM varieties.

In another part of its work (D3.11.2), Co-Extra pointed out that the genetic resources, as those under the auspices of CGIAR, should be preserved. Accordingly international technical protection measures should be put in place, with indemnification, compensation systems for hosting countries and their farmers.

Gene Stacking

More and more GMO obtained by gene stacking are approved. The latest one, SmartStack®, is incorporating 8 independent traits. Due to the HGE unit used for calculating the European GMO percentage, it was of utmost importance to determine whether the stacked traits are specifically impacting the fields' outcomes and gene flow models already available.

Co-Extra investigated thus the impact of gene stacking on adventitious GM presence due to pollen flow and seed admixture as well as its translation in terms of percentage of GM-DNA in a non-GM harvest.

Co-Extra established, in the case of GM varieties bearing one to four stacked events, the relationships between the cross-pollination rate between GM and conventional fields, the percentage of GM kernels and the percentage of GM-DNA in a non-GM recipient crop harvest. In addition the relationships between the rate of seed admixture and the percentages of GM material were determined in a non-GM harvest.

Thanks to these relationships, through several examples, the number of events and the stacking structure of the emitting fields drastically impacts on the ability for a non-GM maize producer to comply with certain GM kernel or GM-DNA unit based thresholds.

Conclusion

The pollen flow expected to occur during the growth of crops is highly dependent on the crops' biology:

- The seed purity is of utmost importance for ensuring crop purity and the seed content (threshold not yet determined at the EU level), as well as being lower than the labelling threshold should also leave enough leeway for other sources of admixture during cultivation and post harvest. The better the seed purity, the lower the adventitious presence and the easier will be the management of coexistence. Lower thresholds are commonly required in other supply chains for marketing, quality or safety purposes.
- The techniques and procedures for obtaining seeds with low levels of admixture are already available since the non-Gm seeds sold in third countries cultivating GM crops also have high levels of genetic purity. As observed in other research programs such as the INRA research program held in 1999-2000, low seed admixture thresholds might increase the price of seeds, which may not impact drastically the final products prices.
- New sampling strategies need to be tested for achieving lower seeds and commodities thresholds, such as the practical threshold (ca 0.1% or lower) used in the supply chains. So far, most studies on seeds have focused on an expected seed threshold of around 0,5% and a crop labelling threshold of 0.9%. The results of the Co-Extra study for achieving a 0.1% threshold in maize commodities fields are expected soon.
- Biocontainment measures may facilitate coexistence but the practical implementation of biocontainment measures raises several issues: one of the CMS of corn types (T type) has a high sensitivity to a fungal pathogen which caused an epidemic in the 70s and severely reduced seed production. Their use might be limited to the growth of small-scaled transgenic fields, e.g. for the synthesis of non food / feed GMO such as pharmaceuticals.
- The practical implementation in farmers' fields of such interesting mixtures of CMS corn and fertile varieties requires further studies.
- Due to the effect of the definition of the DNA unit as recommended by the EC, the increasing number of stacked genes will rapidly increase the GMO content of seed of outcrossed non-GM plants, measured as HGE[1]. Accordingly, biocontainment methods may be recommended in order to minimise GMO content in seeds and crops.

Farmers using farm saved seeds should benefit from the same protection measures, such as long distance isolation, as farmers producing certified seeds.

In conclusion, according to the results of SIGMEA[2] models and the results of Co-Extra, coexistence in open pollinating crops in European agriculture, will be problematic in regions with small fields where it will be impractical to use large isolation distances. If lower crop thresholds are required (as required in many supply chains, i.e. a practical threshold of ca 0.1% for the EU 0.9% labeling threshold), and thus for seed production, then dedicated seed and GM/non-GM production areas may be required. In all cases, good information systems for farmers and policy makers will be required.

Co-Existence in the supply chains downstream farms

Supply chains management

The objective is to facilitate co-existence along the feed/food chains by characterizing the organisational schemes of supply chain product management from the field to the shelf space of the retailer, describing the different components of domestic and "imported" co-existence (fields, silos,

[1] Haploid Genome Equivalent

[2] Sustainable Introduction of Genetically Modified Crops into European Agriculture, FP6 research program.

transportation, processing, retailing), identifying driving - biological, societal, economic – factors, designing representative predictive models, identifying hot spot situations and testing various measures and scenarios of traceability and co-existence systems.

Co-Extra

- Described current food/feed supply chains and identified driving factors impacting quality and purity from seed purchase to end user delivery, either from accidental or intentional mixing,
- Designed descriptive/predictive models for admixture along the whole supply chains taking into account variability of processes,
- Identified the more sensitive points and processes as well as the “hot spot” situations,
- Tested biological, technological, organizational mitigation measures in terms of efficiency and feasibility as well as costs,
- Assessed the overall scenarios developed into Co-Extra, data integration and DSS part, in terms of efficiency, feasibility, costs and acceptability,
- Identified further research activities which would reduce risk of admixture and facilitate co-existence.

Empirical analyses

The work started with an empirical analysis of various supply chains through literature and stakeholder interviews in order to provide

- A general description of supply chains and critical points,
- An overview of current traceability and segregation systems put in place to cope with GM/non-GM segregation,
- A foresight analysis of possible strategies that stakeholders would implement to cope with future scenarios in terms of GM adoption.

The empirical analysis of supply chains through literature and stakeholder interviews was faced with two major problems:

- Supply chains have not been faced to the coexistence issue with the same degree, especially due to the fact that a few GM varieties have been authorized in Europe. The analysis of the current solutions adopted to deal with coexistence between GM and non-GM products was not always possible. Thus, we also studied existing specialties supply chain (such as waxy maize, upper standard rapeseed, erucic rapeseed, etc) to gain insight into how some stakeholders cope with the coexistence between different types of conventional products.
- Stakeholders were also quite reluctant to provide some information on their processes, their strategies. It was also difficult to get internal parameters for the simulation model. We made various assumptions in the model to cope with this uncertainty.

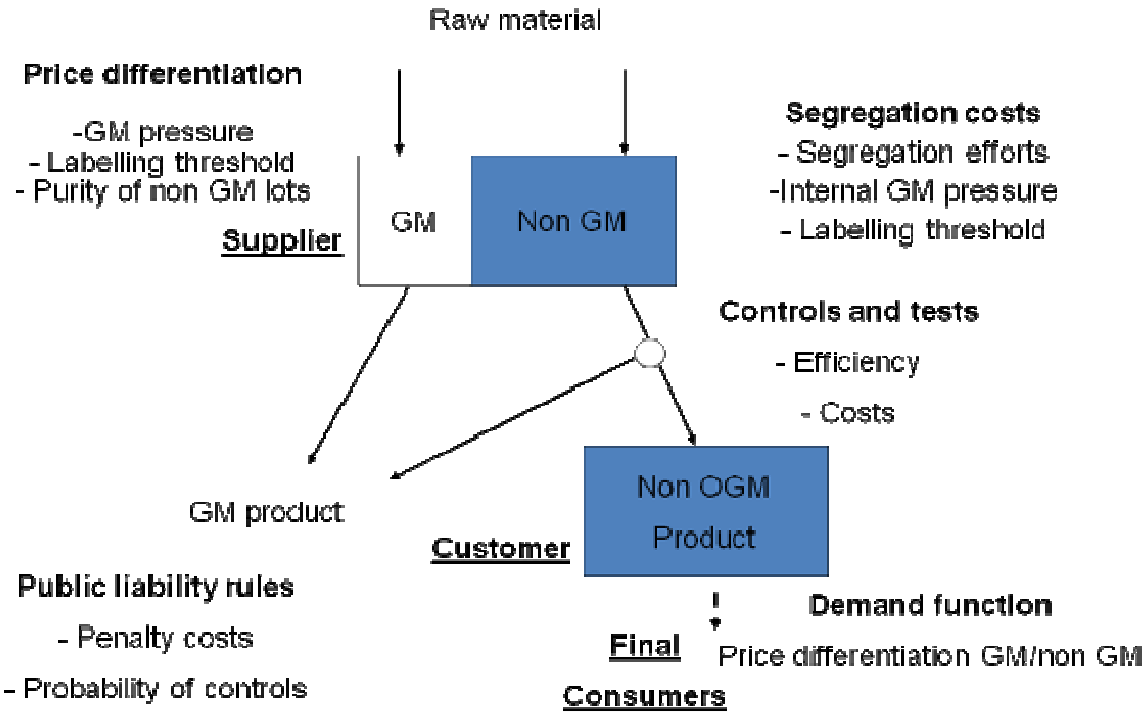


Figure 18: GM and non-GM supply chains organisation and products segregation.

In addition, European GM maize is mainly used in feed production and is already segregated from maize for human food so that additional measures have not been required in most instances. Animal products derived from animals fed GM feeds are not labelled. So coexistence is currently not an issue in animal product supply chains and more generally speaking in the EU.

Third countries importing plant products into Europe, with generally very large fields, have implemented efficient traceability and product segregation of their exports.

From interviews and observations conducted with European and third countries companies involved in commodity supply chains, it is apparent that a majority of stakeholders are applying a threshold which is lower than the labelling threshold (generally from 1/3rd to 1/10th of the labelling threshold, more generally 0.1% of DNA based unit GMO content). These observations confirm those made since 2001 in other studies on GM and non-GM supply chains (such as US IP[3] systems). This practice is similar to the ones used in other supply chains management (mycotoxins, allergens, pathogens, etc.) as well as in seeds production (use of AQL[4] and LQL[5] levels). This very common practice of using a practical threshold lower than the official one (for quality or safety purposes) can be explained by the assurance required by stakeholders to protect themselves against unanticipated contamination and sampling and analytical measurement uncertainties (see analytical traceability part below). In addition, this practice is easy to implement today because European current sources of GM contamination are limited and easier to manage. It is difficult to assess what will be the behaviour of stakeholders under different future scenarios considering seed thresholds, non-GM demand, possible changed consumer preferences and increased GM crops and materials in supply chains.

[3] Identity Preservation

[4] Acceptable Quality Level

[5] Low Quality Level

The thresholds contractually used by stakeholders affect the whole supply chain management including crop and seed thresholds. The extreme is the adoption of GMO free thresholds which are being interpreted by some EU members States as a 0.1% admixture in products.

In addition to the analysis of their current strategies, Co-Extra has explored how stakeholders could manage coexistence along supply chains, when GM foods are adopted in the EU.

Modelling

A modelling approach of vertical relationship along the supply chain was carried out aiming at:

- Representing current strategies of coexistence,
- Identifying changes in context (demands, GM adoption, regulation) and mitigation measures (biological, technological, logistics) that would make it easier to handle with GM/non-GM segregation,
- Using such models or derived decision-support systems (collaboration with the Co-Extra partners working on DSS and data integration) to discuss with stakeholders;

Three kinds of modelling approach were implemented:

1. analytical model (generic model),
2. simulation model (simulation model of the coexistence between GM and non-GM along supply chain, based on the example of the starch maize supply chain and simulation model of collection of maize grain)
3. a combination of models (a model of farmers' varietal choice and a spatially explicit gene-flow model - MAPOD®).

The Co-Extra DSS[6] modules were further implemented (see below) as DEXi multi-attribute models, developed from two sources:

1. models (MAPOD® gene-flow model for the regional maize DSS module and the simulation model for the dryer and starch modules)
2. expert knowledge. The model has been developed in collaboration with Joseph Stefan Institute partner.

Generic model

The originality of this generic model is that it endogenously considers admixture risks between GM and non-GM products, and the resulting consequences in terms of product compliance with regulatory labelling threshold.

[6] Decision Support System

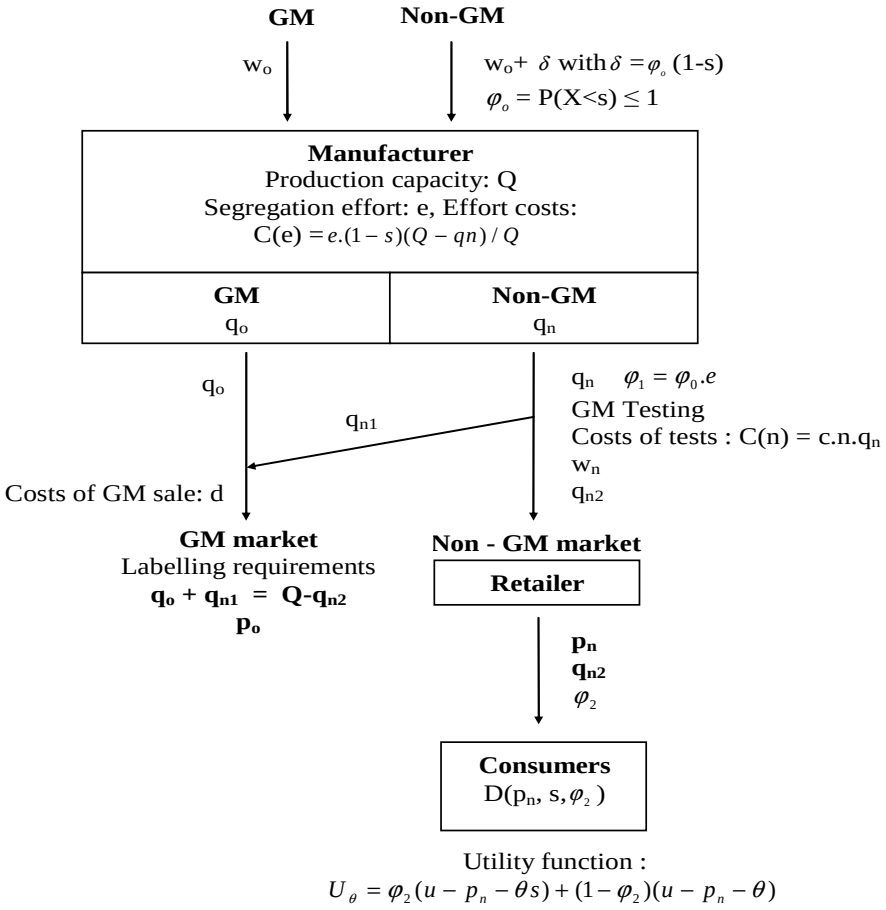


Figure 19: generic model of supply chains segregation.

The results suggest that a public constraint about private sampling strategies may be a relevant regulatory tool, which has not been considered up to now for the regulation of GM/non-GM coexistence in chains. In the setting studied with this model, (i) a regulation of private sampling strategies at the downstream levels of the chains can allow to reduce regulatory interventions at the upstream levels, and (ii) a regulation of sampling strategies can be a substitute at ex-post liability rules based on penalty costs.

Simulation model

A simulation model of the coexistence management between GM and non-GM products, based on the example of the starch maize supply chain has been developed. This model takes into account more "complex" admixture functions that the generic model described above. It allows various management scenarios.

In principle, stakeholders can use three different segregation strategies to cope with coexistence along supply chains:

- If they have dedicated factory plants (strategy 1), they can separate GM and non-GM material but this may lead to increased costs (transportation or under-utilisation of some plants if the market demand changes).
- They can also use separate production lines in the same factory plant (strategy 2), which is more flexible than dedicated plants but not always feasible (for example starch factories use single production lines);
- The temporal specialization of process lines (alternating between GM and non-GM batches) is more flexible, but requires regular cleaning of equipment or downgrading of non-GM batches

(strategy 3). Downgrading involves removing non-GM batches that do not meet a targeted threshold for GM presence, and are therefore are diverted into the GM supply chain.

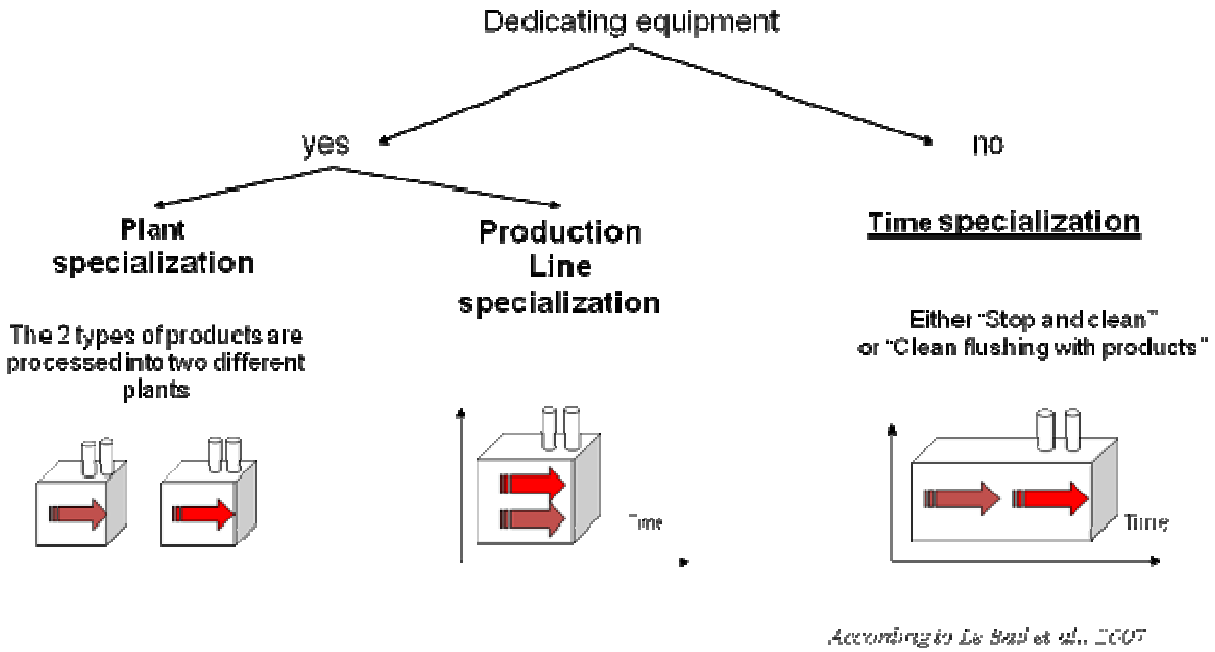


Figure 20: models of supply chains organisation for segregating GM and non-GM products

In general, segregation of GM and non-GM supply chains is technically feasible, but the organisation of the chain, from the upstream seeds and farmers to the downstream operators, plays a critical role in maintaining/improving the probability of compliance with the official EU labelling threshold level of 0.9% (with a practical threshold of ca. 1/10th of this threshold). On the contrary, upstream farm batches may comply with the threshold but, if chain management strategies are not appropriate, the level of compliance of the final product may be very low.

Models have been developed by Co-Extra to assess the effect of various variables on the GM adventitious presence in non-GM batches and the probability of compliance of non-GM batches with a given threshold, at each step of supply chain (from the field level to the end user). These models can be used with the 0.9 % labelling threshold as well as with lower thresholds such as the ca. 0.1% practical threshold used by the operators.

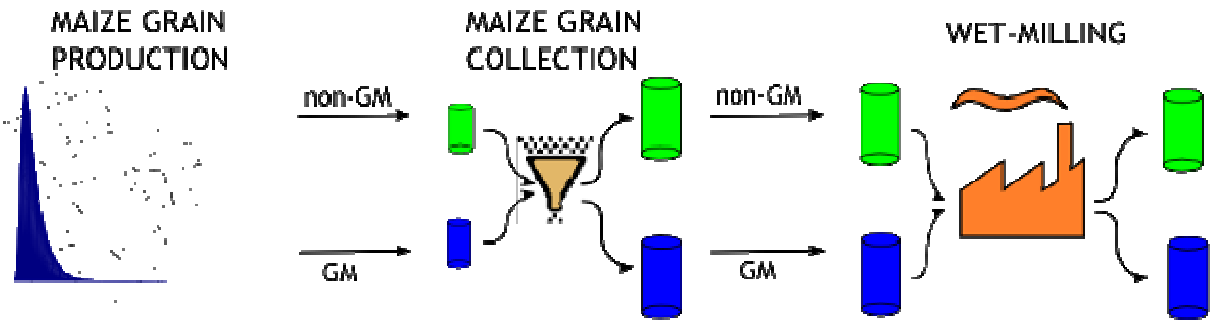


Figure 21: supply chains organisation to assess the compliance of batches with a given threshold

The supply chain simulation model (based on the example of the starch supply maize chain) can test several management scenarios and compare the various strategies (i.e. automatic downgrading

versus each batch processed subsequent to the processing of GM material is automatically put into the GM supply chain if a PCR test indicates the batch does not comply with the required threshold).

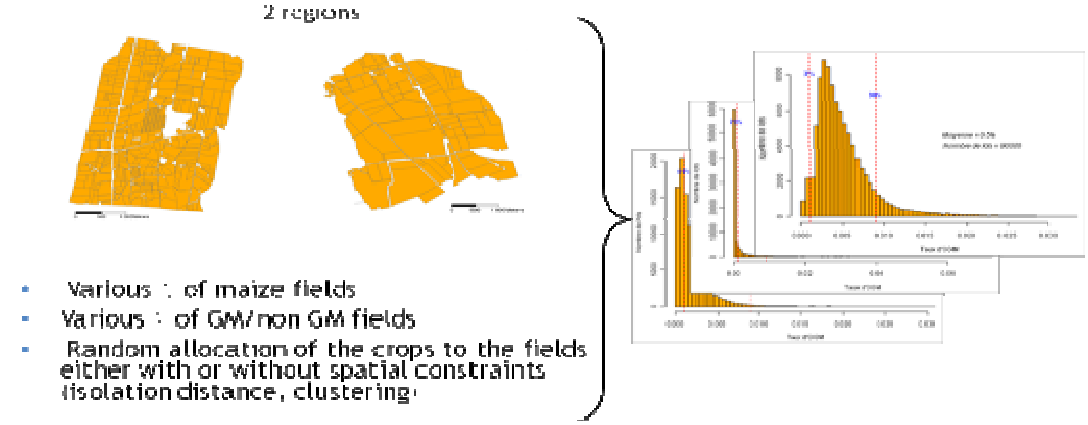


Figure 22: MAPOD based simulations: initial admixture distributions in non-GM flows

By using gene flow models, such as MAPOD, it is possible to estimate the adventitious presence of GM material in non-GM maize at the farm gate. The Co-Extra results show that this information helps in the implementation of an automatic downgrading strategy and may therefore save further PCR testing. This requires strict vertical organisation but can increase overall profitability.

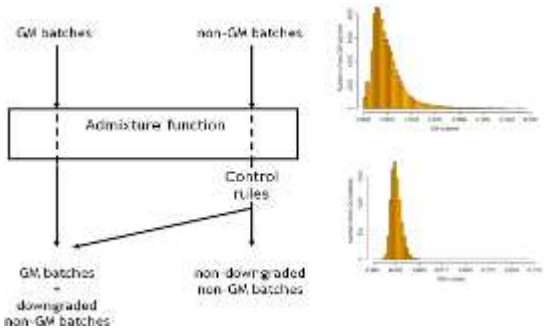


Figure 23: Physical flows at each step of the chain

- As the “non-GM” characteristic is not observable by the final consumers, public regulation is necessary to enforce the compliance of final products to the compulsory labelling threshold. This compliance can be obtained through public controls and penalties costs in case of non compliant non-GM products (ex post regulation). It can also be obtained through testing and sampling rules imposed to private stakeholders (ex ante regulation).
- When GM and non-GM materials are processed in the same production line (strategy 3), from an economic point of view there is a trade-off between the level of compliance of the final product and the number of downgraded non-GM batches. This trade-off depends upon both the relative value of the penalty cost incurred as a consequence of non-compliance (when a non-GM batch does not meet the threshold) and the non-GM price premium in the marketplace.

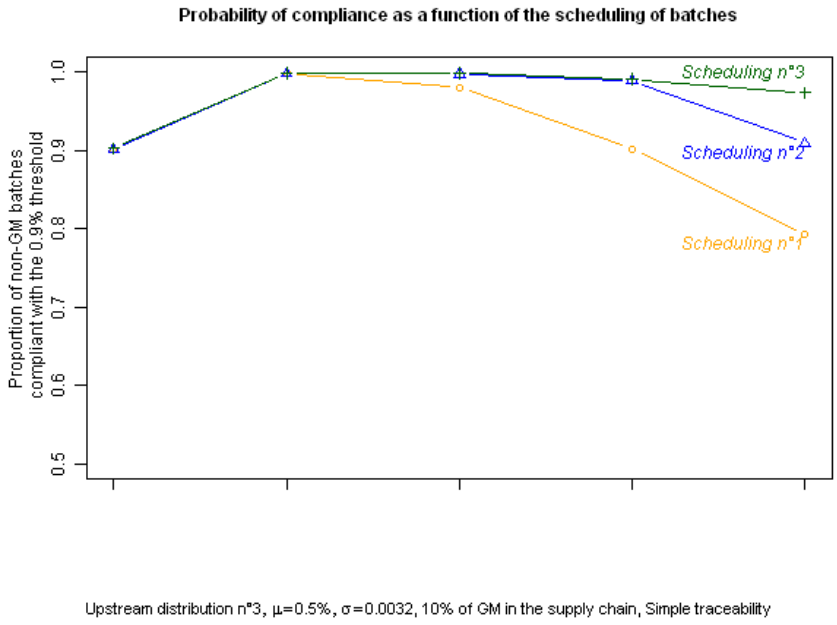


Figure 24: Probability of compliance according to the scheduling of batches (Upstream distribution 3).

	Proportion of GM in the supply chain	10%	50%
Probability of compliance of non GM batches	*Field*	90.1%	
	Collection	99.8%	
	Storage	100%	
	Starch	92.8%	63.2%

Table 1: Probability of compliance according to the GM proportion in the chain (Upstream distribution 3)

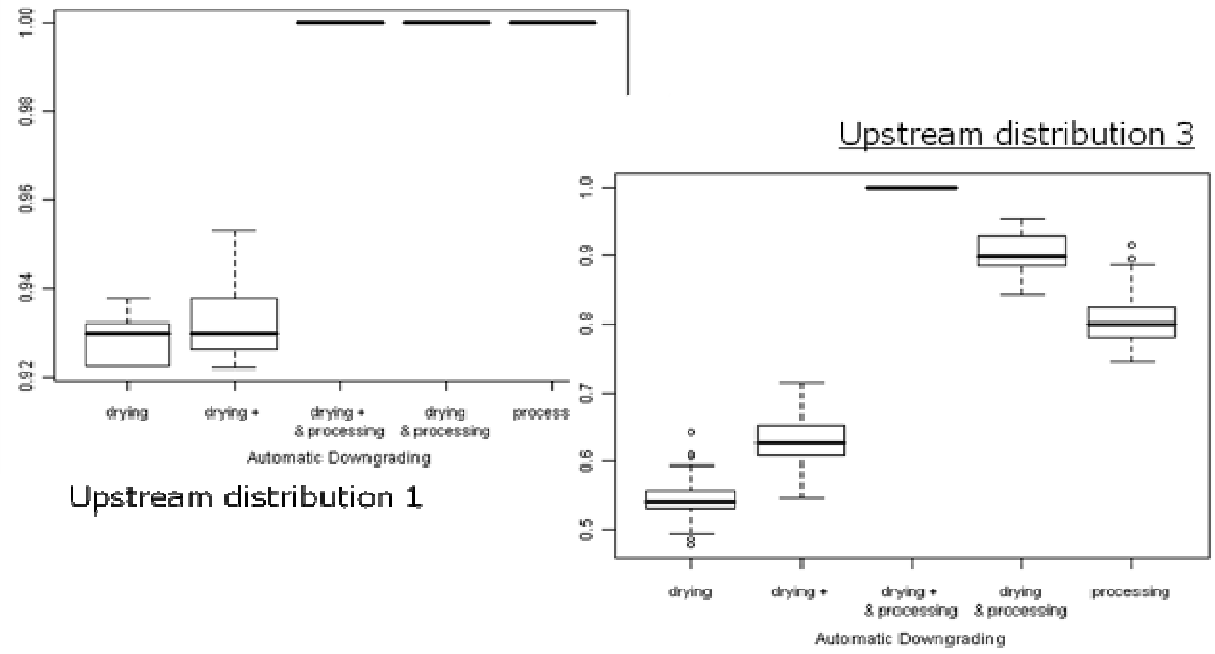


Figure 25: Probability of compliance of non GM batches according to the chain strategies

Co-existence between GM and non GM products seems difficult to implement within the same supply chains when the GM pressure is high. It is only viable from an economic point of view if there is a price differentiation between both products in the marketplace. This is not always the case, and therefore some stakeholders have stopped segregating GM and non-GM compound animal feed-stocks (because products derived from animals fed with GMO's are currently not labelled).

Conclusion

In conclusion, knowledge has been gained on the main commodity supply chain in Europe**:** soybean, maize, sugar beet, rapeseed, wheat, fresh tomato and potato supply chains. The overall supply chain organisation, physical flows, information flows, and relation between stakeholders has been described and analysed. More specifically, Co-Extra has gathered information on the current systems set up to cope with the coexistence between GM and non-GM products (or between existing specialties supply chains, such as conventional and waxy maize). Critical points of the coexistence between GM and non-GM products within these supply chains have been identified. Strategies that can be adopted to handle issues arising at these critical points have been proposed.

Documentary traceability

Documentary traceability (ISO 22005:2007) is an important pillar of the European regulation system (see for instance 178/02/EEC regulation) and is thus not new to European firms and the third countries companies working with.

Traceability is thus used in the GM and non-GM coexistence management as also required by the European directive 2001/18/EC and regulation 1830/03/EEC. It allows the cost-effective management of supply chains, by using data from rather raw materials, more easily analysable, in terms of sampling and detection procedures, provided critical points are identified along the supply chains and analytical controls are appropriately made.

An important observation is that around the world, most of the analytical controls are made on raw products while that the further compliance to EU 0.9% labelling threshold is assessed mostly by documentary traceability. Accordingly, the impact of the analytical traceability and controls measure costs should be considered as rather low on the final products prices, except in the case of direct consumption in "short" supply chains (e.g. sweet corn).

The concept of “co-existence” is always directly related to the concept of “segregation”, which is the shape that the organization of the supply chains essentially takes to make coexistence possible. The term “coexistence” is linked with different meanings, which are sometimes confused in several studies. The first one concerns the links between co-existence and segregation and competition strategies. The second one is mostly linked to the problem of co-existence and segregation in relation with differentiation trends and GM events multiplication.

The Co-Extra work on documentary traceability shows the existence of three typical forms of organization systems for the supply chains in the case of non-GM products importations:

- The first one is a long and “containerised” supply system, which can be observed in Argentina and Brazil, using the ocean transport (generally called “hard IP”).
- The second system is a long bulk supply system, also using sea transport. This system, used in Argentina and Brazil to guarantee the European importers with the grains type, is an IP system of segregation.
- The third system is an intra-European system.

Since the enforcement of the Regulations 178/2002 and 1830/2003, traceability and labelling are required for GM food and feed products in Europe. In Argentina and Brazil traceability of GM food and feed is optional and not officially required, labelling is officially required in Brazil. The quality systems and the certification are a voluntary action of a part of the companies or cooperatives, most of which are attempting to export their products, directly or by the intermediate of grain traders such as ADM, Bunge, Cargill and Dreyfus companies.

The experience on co-existence and traceability, gathered in the Co-Extra Project is of particular relevance to the stakeholders and entrepreneurs, willing to implement new supply chain and quality system. However, these observations have little application for co-existence between farmers, due to the quite larger size of numerous farms in those exporting countries.

Economy of supply chains

As reported about the studies on the management of supply chains, the interaction of Co-Extra partners with the companies has been rather difficult and thus the retrieval of quantitative data has been almost impossible.

The objectives of this work have been:

- A description of value chain structures in existing food and feed commodity systems in the EU and third countries exporting to the EU,
- An identification of cost and benefits related to coexistence and traceability in selected food/feed supply/processing chains,
- A description of the EU consumers attitudes to GM labelling and GM food products,
- An estimation of coexistence costs and benefits for selected food and feed products in the EU,
- An assessment of the economy wide impact of EU consumers reaction to the EU labelling and traceability requirements.

The supply/processing chain analyses are based on collected empirical data from interviews with a large number of companies together with data from literature and expert opinions. The interviews were performed using a common interview guide developed jointly by the partners also working on supply chains management (see above). Five supply chains in 7 countries were analysed: maize, wheat, soybean, rapeseed and for processed products frozen pizza, chocolate and compound feed.

Country / Commodity crop	Germany FW, IVV	Denmark FOI	Poland SGGW/WAU	Switzerland FiBL	Belgium Hogent
Rapeseed	Oil	Oil	Oil	Oil	---
Wheat	Starch	Flour, feed	---	---	---
Sugar beet	Sugar	Sugar	---	---	---
Maize	Starch	Silage, feed	---	Silage, feed	---
Soy	---	Feed	---	---	Feed

Table 2: supply chains studied for economic purposes.

Germany FW, IVV	Frozen pizza Chocolate (bar)
Denmark, FOI	Compound feed
Belgium, Hogent	

Table3: supply chains of processed compounds studied for economic purposes

A descriptive universal adaptable microeconomic simulation model has been used for calculating co-existence and traceability costs in all stages of the analysed value chains. The calculation methodology covered the supply chain from seed to end-use food/feed product and was based on the results of the Co-Extra work on supply chains management. The quantitative and qualitative data were gathered from stakeholders' interviews and existing published data sources. Some partial assumptions on meaning ful data had to be used to bridge data gaps. The cost types are calculated at each level of the value chain and resulting costs are transferred to next level via increased commodity prices for non-GM products. Individual case-sensitive factors were used in the calculation (GMO pressure and acreage, production volumes, yields, capacities and product prices, etc.).

Generally speaking, the cost-reduction impact of general European directives and regulations, such as the 178/02, making mandatory the implementation of traceability in European supply chains, is not properly estimated by the companies. Moreover, the positive impact of already-implemented traceability and controls, due to both the general, or GMO specific directives and regulations, on e.g. companies' image, decreases of market withdrawals or recalls, welfare, or development of markets niches, impact of GMO and non-GMO supply chains organisation on products management related to safety issues (e.g. management of products containing allergens or mycotoxins), is also not properly estimated. On several occasions, the use of analytical controls was over-estimated since low-cost documentary traceability is mostly used. Several third countries have already put in place efficient segregation strategies of GM and non-GM products, in order to gain new markets, which can be used for any value-added supply chains.

This situation may be due to either a lack of analytical analyses of the impact of these different legislations frames or to a willingness of companies to disclose such results, maybe for concurrence related issues, or both.

We can translate this lack of accurate data as a lack of companies' willingness to carefully carry out cost-benefit analyses on coexistence in order to get argument for additional costs increasing companies' profits.

Coexistence of GM and non-GM supply chains is possible only if all stakeholders can valorise their production. This is particularly important for animals-derived products which are not labelled,

according to whether that animal was fed with GM or non-GM products. Accordingly co-existence can be insured in the EU only if GMO-free labelling is possible, including animals fed with non-GM products.

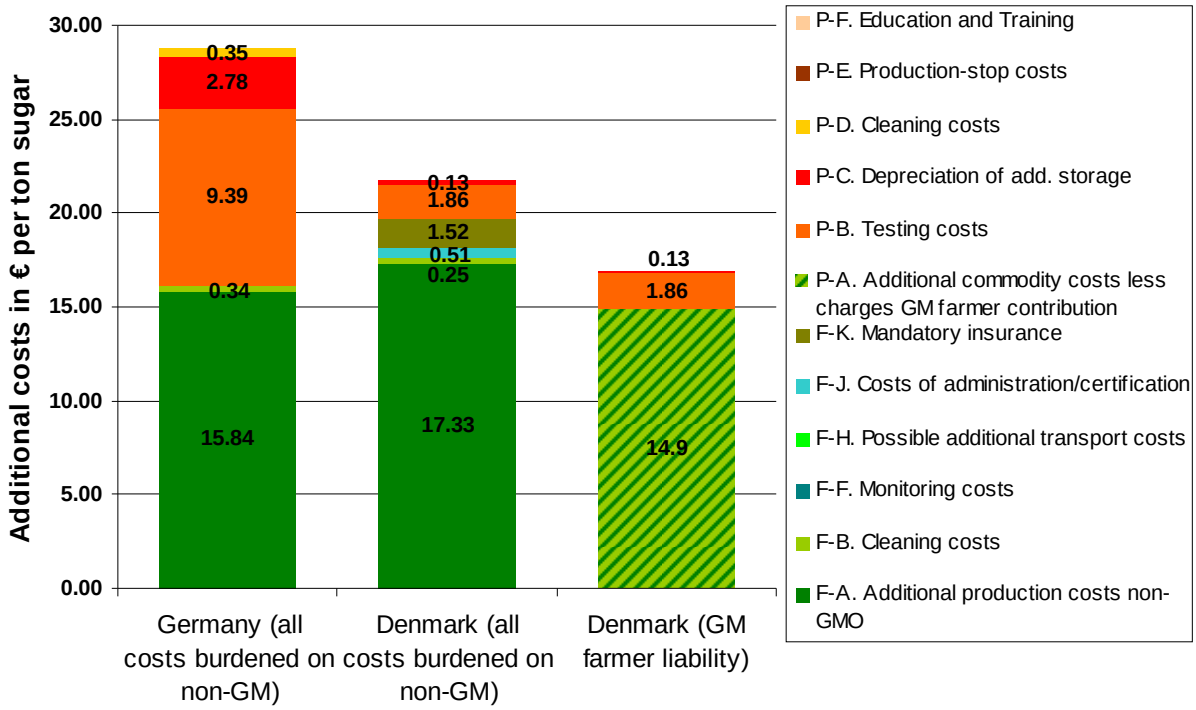


Figure 26: cross national comparison of allocation of additional co-existence costs in Germany and Denmark.

According to the results of the analysed food supply chains, and particularly due to companies assertions, only additional costs can thus be expected by organising co-existence between GM and non-GM products in the value chain from production of farm crops up to the production/processing levels of the single supply chains and by maintaining mandatory (or voluntary) thresholds and regulations. Depending on factors like crop requirements, farming, storage and elevating systems, processing strategies, monitoring managements etc, the total additional costs of co-existence and product segregation, for some systems, might increase to 13% of the total product turnover at the gates of rapeseed oil mills or starch industry processing wheat and maize.

Country	Germany	Denmark	Poland	Switzerland
Joint preconditions	■ 50 % regional GM adoption rate ■ Accepted non-GM price premium from the farm/elevator level ■ Products for human consumption and food industry ■ Processing strategy: **temporal specialisation**			
Individual assumptions	■ Processing capacity 600.000 tons rapeseed per year ■ Share GM commodity: 30 % ■ Price premium non-GM rapeseed: 4.6 % ■ Restriction to input testing ■ Strategy requirements: Additional storage capacities, flushing and production stop, personnel education	■ Processing capacity 90.000 tons rapeseed per year ■ Share GM commodity: 20 % ■ Price premium non-GM rapeseed: 11 % ■ Restriction to input testing ■ Strategy requirements: additional storage capacities and production stop costs due to changeover	■ Processing capacity 400.000 tons rapeseed per year ■ Share GM commodity: 50 % ■ Price premium non-GM rapeseed: 6.1 % ■ Restriction to input testing ■ Strategy requirements: cleaning, personnel education, auditing (all efforts here economical insignificant)	■ Processing capacity c.a. 150.000 tons rapeseed per year ■ Share GM commodity: 20 % ■ Price premium non-GM rapeseed: 17.4 % (incl. IP) ■ Restriction to output testing ■ Strategy requirements: cleaning/flushing efforts
Total additional costs	■ **74.10 € per ton** ■ **8.3 % of turnover**	■ **83.16 € per ton** ■ **8.3 % of turnover**	■ **21.6 € per ton** ■ **3.6 % of turnover**	■ **106.98 € per ton** ■ **5.3 % of turnover**

Table 4: cross national comparison of costs of co-existence and traceability of rapeseed oil production at the Crusher level.

Finally, an assessment was made on the economic impact of European legislative frame. The economy wide implications of EU coexistence regulations were assessed by a CGE (Computable General Equilibrium) model methodology based on the multi-region multi-sector GTAP (Global Trade Analysis Project) model. The model covers world trade flows. The most recent version (version 7) of the GTAP database has been aggregated into 5 regions and 20 sectors and employed for the study.

However, for most value chains the question of co-existence is a theoretical one at the moment. The implementation and permanent running of co-existence and segregation systems in the food industry can decrease the additional costs due to savings e.g. in the testing requirements of raw materials or routine procedures during the documentation process.

The segregation, traceability and labelling systems for maintaining the GMO threshold below 0.9% hardly provides currently any significant additional benefits for producer, retailer or consumer (as this would be the case e.g. in organic production, fair traded products etc.). Thus it is possible that no actor of the value chain may be willing to pay the incurred costs of co-existence measures occurring along the line of the supply chain.

Consumers opinions and propensity to pay for non-GM products

Consumers propensity to pay non-GM products

The description of consumers' attitude towards GM labelling and the calculation of propensity to pay (ptp) for non GM food are based on data from on street interviews in 5 countries (Denmark, Germany, Spain, United Kingdom and Poland; 1,614 interviews done by a professional market research institute) employing random sampling of households using income and region as stratification variables. The results obtained from a questionnaire with choice experiments (stated choice experiments) provided the consumers' propensity to pay (PTP) estimation by using a conditional multinomial logit model.

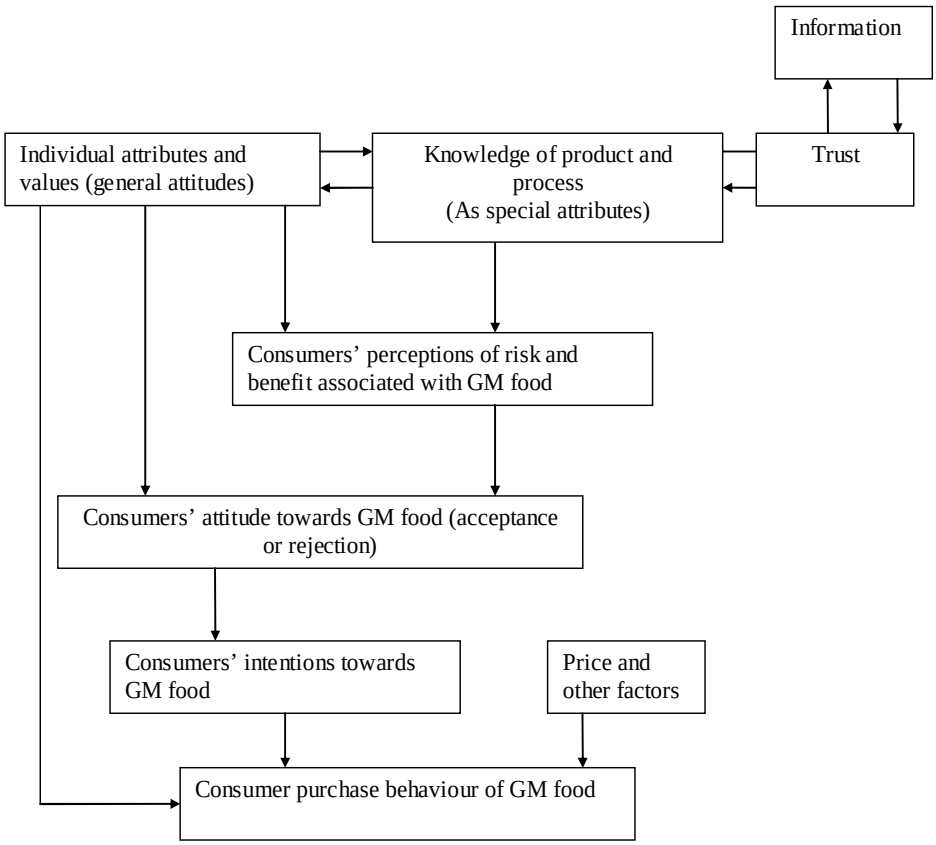


Figure 27: Literature Review: The theoretical model of analysis.

From the literature review, it can be also concluded that:

- Most consumers relate GM food to a negative impact on their personally utility, and have not "ptp" for such products.
- Moreover it was also seen that labelling associated with GM food and the type of genes associated with the modification is very important in food choice. As well as individual-specific characteristics (age, gender…).

Based on this result and on:

- Lancaster consumer theory: goods are selected by consumers, based on their characteristics which are the source of consumers utility (Lancaster, 1966),
- Random utility theory: Individuals will choose, among a set of alternatives, the good that generates the highest utility (MacFadden, 1974).

Accordingly, Co-Extra concluded that choice experiments is the best methodology for analysing consumers intentions towards GM food.

The choice experiment framework can be described as:

- A good can always be characterized by its characteristics or attributes.
- Individuals select among alternative options the good that generates the highest utility, where each option is characterised by a number of attributes with different levels.
- Following (Louviere et al., 2000) the probability of an individual *q* choosing a particular alternative *i* out of the set of alternatives *J* can be calculated as:

$$P_i = \frac{1}{\sum_{j=1}^{J} \exp-(V_i - V_j)}; j = 1,...i,...,J \quad i \neq j$$

- Which lead to use the conditional logit choice or conditional multinomial logit (MNL) model.
- Maximum likelihood is usually used to estimate the population parameters from the observed sample
- Socio-demographic characteristics (SDC) have been included in the analysis by interaction with the attribute levels.

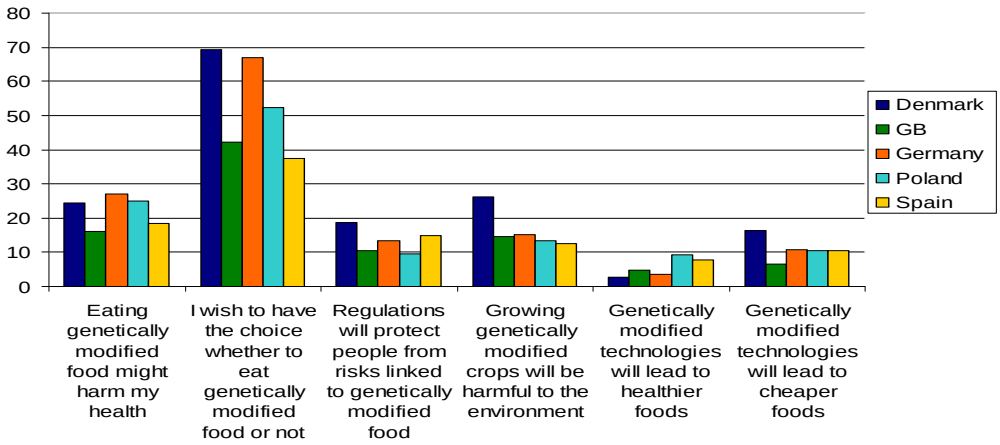


Figure 28: Measuring consumer preferences, a choice model application. To try to measure attitudes to GM technology, respondents were given a number of statements expressing a range of views on the GM issue (strongly agree).

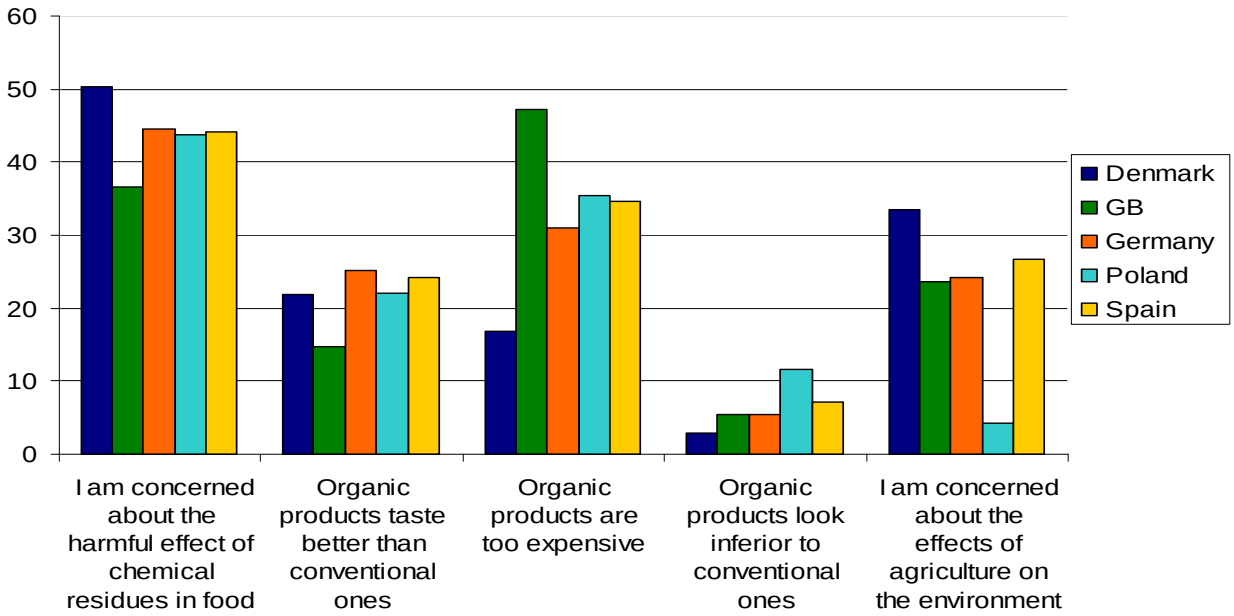


Figure 29: Measuring consumer preferences, a choice model application. Respondents were asked attitudinal questions about organic products and farming methods.

Since European consumers, of the countries studied, rarely accept genetic modifications in food products, they are unwilling to pay extra money for product differentiation in the sense of a labelled food product that contains GM materials below the labelling threshold of 0.9%. Besides farmers and seeds companies' production- and crop-related benefits by genetically modified crop varieties like pesticide resistances, anticipated higher yields or increased contents of substances, the benefits for the consumer are quite vague, intangible and hardly convincing. As shown in the consumer surveys in

the countries analysed, the putative health or environmental benefits of GM crops are mainly unknown, uncertain and the consumers sees no reason for spending more money on these products.

- More consumers in Denmark, Germany and Poland thought eating GM foods might harm them than did those in GB and Spain. Relatively few consumers, in each study country, agreed strongly with the statement that GM technologies will lead to healthier food and to cheaper food.
- Apart from Spain, consumers in the four other study countries required '*compensation*' in order for them to choose GM food products. Furthermore, the level of '*compensation*' has to be higher when GM technology is associated with environmental benefits, than when it is associated with health benefits.

The Co-Extra results of consumers' propensity to pay for non-GM products should be usefully compared to those obtained in the consumers' survey carried out under the coordination of the King's College[7].

Stakeholder opinions and attitudes on coexistence of GMOs with conventional and organic supply chains

Analysis of stakeholder opinions and attitudes towards coexistence and traceability issues

Part of this project was the organisation of interaction with relevant stakeholders' representatives affected by the issue of co-existence. The goal was to map the opinions and attitudes of relevant stakeholders with regard to co-existence and to create interaction between stakeholders as such. The stakeholder interaction is threefold:

- Interaction with a group of stakeholders on a European level through a Stakeholder Advisory Board.
- Regional stakeholder workshops in seven European countries, which were conducted by the national relays.

  The goal of the workshops was to bring relevant regional stakeholders together and organize an active interaction among stakeholders on the topic of co-existence. To achieve a deeper interaction among stakeholders break-out sessions where organized in which a particular case was discussed in more detail, and depending on the number of participants, different groups were formed representing a particular part of the supply chain.

  Co-Extra was particularly interested in answering the following questions:

  - What role does the local policy context mean for the discussion about co-existence?
  - Do stakeholders in different countries come up with the same items / opinions. What are the commonalities?
  - What items / opinions are unique for a certain region and why? So what are the differences between countries?
  - Is there a lot of disagreement between stakeholders in a certain region, or not?

  To enable the identification of these commonalities and differences also a common reporting framework was set up for the local partners to use. The regional offices have organised the workshops. Workshops were all one-day workshops, except for the Netherlands workshop which lasted only half a day. Participation to the workshop was upon invitation.

- An online stakeholder questionnaire, which was implemented on Co-Extra's website (D8.3a)

[7] http://www.kcl.ac.uk/schools/biohealth/research/nutritional/consumerchoice.

The questionnaire was set up using mostly closed questions, which where divided into a number of different categories. The first part of the questionnaire was intended to get a good identification of the respondents, where they amongst others had to identify from which country they were, and to what category of stakeholder they belonged. The closed questions were statements to which respondents could react using a 5-point scale:

Definitely agree – tend to agree – don't know – tend to disagree – definitely disagree.

The draft of the questionnaire was sent to the members of the Co-Extra Stakeholder Advisory Board and to all regional partners involved in this part of the Co-Extra project. The comments received were taken up to finalize the questionnaire. The questionnaire was translated into seven different European languages with the help of different Co-Extra partners. The final questionnaire was made available online on a secure part of the Co-Extra server.

For organizing response to the questionnaire an e-mail address database was set up using the input of different regional partners. It proved to be impossible to generate a complete database of individual relevant contacts for the questionnaire. Generating an address database with relevant individual contacts for all EU member states would have been a project in itself. This is why it was decided to let the questionnaire have an open access, with a link on the homepage of the Co-Extra website. Nevertheless an e-mailing was done using the addresses generates (over a 1,000 addresses), and in the e-mail message addressees were asked to forward the e-mail to persons within their network that potentially would also be interested in the topic of co-existence. The seven regional offices within the Co-Extra project that also organised the regional workshops have also sent an e-mail message to their network of stakeholder contacts to ask them to fill in the questionnaire.

From a methodological point of view this way of working opened up the questionnaire to attempts by certain categories of stakeholders among to influence the outcome of the questionnaire through mobilizing their network to fill in the questionnaire. Co-Extra partners were very much aware of this risk and have therefore decided not to present any overall opinions and attitudes, but only compare opinions and attitudes between different stakeholder categories. The questionnaire had, unfortunately, only 444 respondents. As a result of the used methodology, the relatively low number of respondents, and the fact that in some categories of stakeholders only limited response was given, the outcomes of the questionnaire are only of a qualitative nature.

Among a broad spectrum of attitudes and information needs of stakeholders the following are the most dominant:

- There is an overwhelming wish to have the GM labelling thresholds for seeds regulated. This is over different countries and different stakeholders. Without these thresholds it is difficult to set practical co-existence measures.
- There is a general conviction and concern about the costs that co-existence regimes will entail in practice. Most stakeholders are of the opinion that co-existence measures will entail costs – as any regulation will entail costs – but there is difference of opinion on how significant these costs will be.
- There is a concern about the practicalities of sampling and testing strategies. Guidance may be necessary here, and perhaps also a discussion on whether testing is necessary in all situations, or that in many situations sampling will do, followed by testing if a problem has arisen.
- A common concern on how to deal with unauthorized events. Nobody would like to be confronted with an unauthorized event – especially one that is not authorized anywhere in the world – and there are questions on whether it is possible to prevent contamination with such events at all times.
- Especially from the side of the NGOs and organic farmers: a discussion on the legal meaning of the concepts of 'adventitious' and 'technically unavoidable'. There is general recognition of the fact that the 0.9% is a labelling threshold. But there is difference of opinion on what the consequences of these concepts are for the design of co-existence measures. What should practical co-existence measures be aiming at?

- Most stakeholders are not supporters of a hybrid regulatory model with coexistence rules both on the European and the country level, but some may stress the need for flexibility, especially on the practical level.
- Many stakeholders recommend to monitoring the development of practical co-existence measures and compensation schemes in the different EU member states, with an eye on harmonization and the prevention of competitive advantages and disadvantages for particular farmers.
- Farmers are inclined to see co-existence regulatory frameworks as yet another set of requirements that will increase the amount of paperwork that they have to do. They are not in favour of having to be certified or licensed to be able to grow GM crops.
- The questionnaire also showed that although co-existence is an economic and choice issue, some stakeholders perceive, present or use it as an environmental or social issue, especially those stakeholders having a more negative opinion about GMOs.

Traceability and controls in supply chains

By traceability Co-Extra understand both the analytical traceability, carried out by analytical methods, and documentary traceability according to its usual standardized meaning (ISO 22005:2007).

The results described below strongly benefited from the involvement of the JRC[8] (IRMM and IHCP institutes) and of numerous ENGL[9] members as Co-Extra partners.

During the process of focus groups with stakeholders, the question of how to deal with "botanical impurities" was raised both in feed and food supply chains. A report (D4.11) was established summarizing all our technical knowledge and European legislation. There is unfortunately no easily applicable technical alternative to the microscopic counting of representative sub-samples. Accordingly, the current practices of adding non-GM products (e.g. soybean into maize commodities) of such botanical impurities would continue, even though rather expensive. This document raises several issues the EC and Competent Authorities should consider for both analytical and economic issues particularly in the light of Asynchronous approvals, LLP (Low Level Presence) and economic impact faced by European importers.

Efficient and cost effective sampling and testing approaches are needed in order to implement co-existence and traceability, stakeholders need first reliable sampling procedures to obtain representative samples secondly validated methods with suitable reference materials, and finally novel methods due to the increase of the number of GM crops.

Sampling and methods validation

The use of GMO is subjected to legal constraints, either within a "deregulation" system (e.g. USA) or an authorizing (e.g. EU) framework. To assess compliance with national and international requirements there is a continuous and increasing need for reliable and cost- and time-effective analytical methods in all areas of analysis.

The reliability of a method is first determined by the validation process, which is the procedure providing evidence of suitability of an analytical method for its intended purpose. All laboratories in charge of GMO detection are working under a quality system within an accreditation scheme for which the compliance of the laboratories' measurement uncertainties (repeatability and reproducibility) with those obtained in validated method is mandatory. Accordingly, the validation of analytical methods and the implementation of the validation process have been key goals within the Co-Extra project.

Additionally training activities for harmonisation in the field of detection and traceability, targeting non-EU countries, were carried out

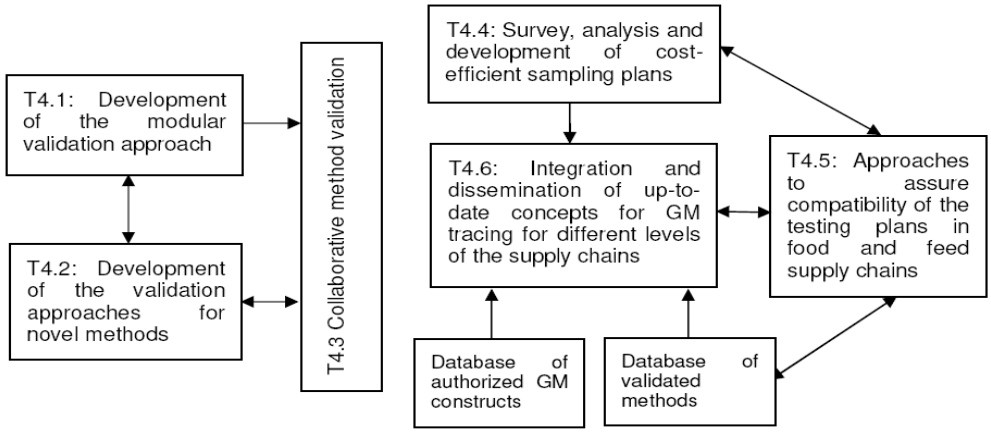


Figure 30: sampling and methods validation working scheme used

[8] Joint Research Center of the European Commission (Geel [Belgium] and Ispra [Italy])

[9] European Network of GMO Laboratories.

Validation

At the start of the project only a limited number of properly validated methods for GM testing were available, and procedures of validating the detection methods were defined only in the case of the established simplex PCR-based methods and were not directly applicable to the validation of cost-effective methods, such as multiplex PCR or micro-arrays.

Generally, the ISO/IUPAC/AOAC model for the method validation has been applied in the validation of GM detection methods following a global approach in which the whole process from the product to the final measurement outcome is to be validated as a whole.

The modular approach for method validation, proposed by Holst-Jensen and Berdal (2004), describes the analytical procedure as a series of successive steps, which could be validated separately. This approach has been considered as a good basis for developing a cost-effective validation process by the stakeholder and for its further flexible implementation in routine laboratories. In addition the ISO/CEN standards and CODEX guideline are already orientating towards this modular approach. However, the modular approach for validation needed to be tested through experiments in practice and the criteria for the definition of the quantity and quality of the output of the different analytical steps must be laid down.

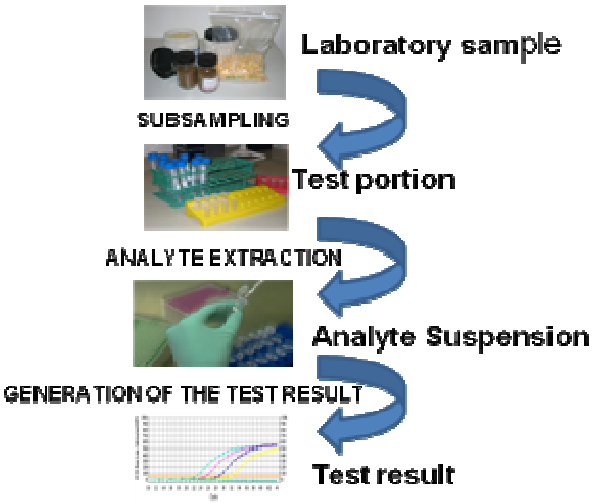


Figure 31: schematic flowsheet representation of the DNA-based analytical procedure for GMO analysis (based on Holst-Jensen and Berdal, 2004).

The methods used for GMO detection identification and quantification should be validated in order to have confidence in their performance. Four Analytical methods were validated according to the ISO 5725 standard and the ENGL criteria

The fuzzy-logic based principle was extensively illustrated in the context of method validation with both single and multiplex PCR (i.e. GMO chip DualChip®) and run via dedicated tools (AMPE software). AMPE is an Expert System for analytical validation and for the integration of performance criteria into a measure of the "global quality" of a method, an essential tool for data processing harmonisation.

Sampling and control plan

Sampling represents the initial step and in most cases the major crucial step of the analytical chain particularly when targets or analytes are not homogeneously distributed as for GMOs (see e.g. the Kelda project[10]). The analysis of samples not representative of the lots to be analyzed for compliance could get to wrong decision and then to waste of cost and efforts. Development of sampling methods has been an important goal within the Co-Extra project.

[10] http://bgmo.jrc.ec.europa.eu/home/sampling_KeLDA.htm

Different sampling approaches and concepts have been developed for various kinds of matrices (seed, grains, feed, food) in the past years, and have been published as European or International Standards or Guidelines of international or national organizations (ISTA, GIPSA). However, these sampling plans do not address the GMOs specifically, and sampling plans are not available for all the products to be tested. In practice, sampling for control purposes is rarely performed by taking representative samples but by taking only a low number of “random samples”, even if the analysis of samples not representative of the lots to be analyzed for compliance could get to wrong decision and then to waste of cost and efforts. In addition, research results (i.e. the JRC-led projects KESTE and KELDA) showed that the current sampling approaches and statistical models may not be the most appropriate ones if the GM distribution is heterogeneous.

For these purposes, sampling approaches holistically throughout the whole supply chain were developed, to identify the most appropriate points for sampling and testing in the supply chain. Stakeholders were also involved in the project through dedicated workshops and questionnaire and dissemination activities.

Dedicated software tools to support sampling and sub-sampling plans aimed at GM detection through the food and feed chain were developed: SISSI a novel approach to estimate the optimal sample size in experimental data collection and OPACSA (OPtimal ACceptance Sampling by Attributes) a new statistical optimisation software including a cost function to find the cheapest and most reliable mode of analysis by sub-sampling. It has to be outlined that the EC recommendation for sampling is also based on such sub-sampling strategy and thus could be adapted for using the OPACSA cost function and optimisation.

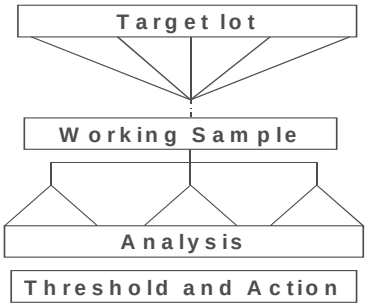
In certain cases of co-existence it is also important to determine, before harvesting in the field, the level of adventitious presence of GMOs in a non-GMO field. Based on the predictions of spatial variability of out-crossing rate, different sampling schemes were developed and validated. After an initial work focusing on the 0.9% labelling threshold, new work has been started for a 0.1% level and results will be soon available.

General control plans should be undertaken where several analytes could be sampled, with low-cost sampling methodologies. In this regard, the current sampling methodologies for mycotoxins (the more heterogeneously distributed analyte in a lot) could fulfil the requisite of a representative sampling also for GMOs and derived products. An important experimental work is currently under way to test this assumption.

Models have been developed by Co-Extra to assess the effect of various variables on the GM adventitious presence in non-GM batches and the probability of compliance of non-GM batches with a given threshold, at each step of supply chain (from the field level to the end user).

The examination of several data sets of results of the measurement of the GMOs quantity in flour by PCR-based methods collected through inter-laboratory studies showed that the use of the log-normal transformation is necessary to correctly estimate measurement uncertainty of the whole detection process. Uncertainty Profiles built from estimates of measurement uncertainty generally give a range of 50 to 200% of assigned concentrations for materials that contain at least 1% GMO. This range of 50 to 200% is consistent with European Network of GMO Laboratories and the EU Community Reference Laboratory (ENGL and CRL) validation criteria and can be used as a fitness for purpose criterion for measurement methods. The effect of this on the enforcement of EU labelling regulations is that, in general, analytical results need to be less than 0.45% to ensure companies to compliance and greater than 1.8% to demonstrate non-compliance with a labelling threshold of 0.9%. These results on inter-laboratories reproducibility explain the observation made in Co-Extra that companies involved in the food and feed supply chains are using a contractual practical thresholds around 0.1% for complying with the European labelling threshold for GMOs, which is set at 0.9%.

Within the project a framework for the analysis of control plans, defined as a test procedure combined with a sample acceptance limit, has been developed in order to enable stakeholders to make objective choices about the effort that should be put into sampling and testing in order to make objective choices of sampling and testing strategies. The main factors that can affect the reliability are the GMO heterogeneous distribution in the lot and the effect of analytical uncertainty.



Sampling scheme

- Primary samples
- Mixing
- Laboratory sample

Analytical method performance

Analytical method

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Development and Integration of Analytical Traceability tools

The number of GM crops worldwide is increasing continuously. Reliable and efficient methods for GMO detection are essential for establishing an efficient system for traceability as well as for monitoring different aspects of GMO coexistence with conventional and organic crops. In the next few years it is expected that a large expansion of GM crops will occur, both approved for use in EU as well as in other parts of the world.

The EC Regulation 1829/2003 requires that petitioners of new GMOs provide sequence information characterising the GMO, methods to detect and quantify the GMO, and appropriate reference materials, before authorisation may be given. This substantially facilitates the work of detection labs as CRL-GMFF laboratory is collaboratively validating methods and publishing the validation results (Davison and Bertheau, 2007). However all currently available methods were validated using TaqMan® real-time PCR chemistry and limited type of apparatus, that may confer a monopoly and thus increase the price of analytical traceability. It is thus important to determine the commutability between the several PCR chemistries and apparatus combination as well as to look for alternative DNA amplification methods.

The current legal frame resulted in the establishment of the CRL-GMFF[11] which is responsible for the validation of specific quantitative identification methods provided by the notifiers in connection with applications for authorisation of new GMOs. The validation studies are performed in collaboration with

[11] Community Reference Laboratory, Joint Research Center, Ispra, Italy

the ENGL[12]. Unfortunately, the current mandate of the CRL is restricted and does not cover validation of other methods applied by the routine laboratories such as screening and multiplex methods needed to decrease the analytical costs and timespan.

The analytical, enforcement and private, laboratories are mostly using screening methods in the first steps of the analytical process to first determine whether GMO may be or not present in the analyzed samples. Development of screening methods, and the related donor organisms control methods, however, is not covered by GMO-CRL mandate and it represents an important burden to analytical laboratories. On the other hand the mere existence of large numbers of GMOs might increase the costs of analytical traceability to a level economically unacceptable for the consumers. As for any quantitative detection area (pathogens, food and feed microbes, mycotoxins or allergens producing organisms), limits of detection, quantification, determination of outliers, accuracy of measurements, etc. are issues whose improvement will benefit to both analysts and stakeholders.

On coexistence and traceability viewpoint in supply chains, stakeholders may be looking for onsite and rapid, even though not quantitative, methods like protein based method (thus using antibodies for detecting the GMO analyte) and or high throughput non-destructive methods for e.g. shipment unloading.

Therefore, there is an urgent need for the improvement of existing methods (e.g. real-time PCR) and the investigation of new methods for the analytical system to become more cost- efficient, which was the main objective of this Co-Extra activities part. Additionally, suitable and reliable calibrants have to be defined for each method. Several non-PCR based approaches were evaluated for their performance in comparison to available PCR detection methods. The methods have also been assessed/adapted for use in on site (fields and silos) detection, which is of primary importance both for coexistence studies as well as for different stakeholders (like farmers, cooperatives, companies and inspection services).

Most of the analytical controls are made on raw and low processed products while documentary traceability is more predominant for the remainder of the supply chains. The analytical traceability may impact the costs and time required to control products for GM presence; - development costs, lack of appropriate methods, the need for methods to be validated and established for routine use, discrepancies between laboratories applying different methods, different implementation of the methods in accredited laboratories and unclear communication between stakeholders in relation to test reports and interpretation.

Thus, several improvements towards more efficient and effective analytical traceability were made in the frame of Co-Extra.

DNA based detection methods.

- **DNA extraction.** In DNA based detection methods Co-Extra has addressed the procedures for DNA extraction from highly processed matrices, mainly oil and lecithin samples, matrices with both low DNA content and numerous DNA amplification enzymes inhibitors. Different protocols were tested in combination with real-time PCR, as a method not often used in DNA detection step, to assess the yield and the quality of extracted DNA. The protocols were changed in iterating process of improvements (D5.7, Doveri and Lee, 2007).
- **Real-time PCR.** Most of the work on the DNA detection methods was focused on real-time PCR as this is currently the method of choice in routine GMO detection laboratories. Several new assays were developed for detection of elements common in the newly released GMOs and for detection of donor organisms of different sequences introduced into GMOs. In view of cost improvements, most of the assays were combined to form a duplex system. Also new taxon reference assays were developed to enable quantitative analysis of new GM crops on the market or to improve the existing detection system:

    - duplex real-time PCR assay for detection and quantification of wheat and barley (Rønning et al., 2006)
    - duplex and simplex real-time PCR methods for detection and quantification of tomato, eggplant, potato and pepper (Chaouachi et al., 2008)

[12] European Network of GMO Laboratories, http://engl.jrc.ec.europa.eu/

- **Methods performance.** Different parameters, like limit of detection, specificity, repeatability, were checked to verify the performance of the method (D5.10, Ronning et al, 2006, Chaouachi et al, 2008, Pansiot et al, submitted, Van den Bulcke et al., in preparation).

  Limits of real-time PCR multiplexing were tackled. Adjustments in the design of probes (choice of reporters and quenchers) and machine setup (choice of fluorescence sources as well as choice of excitation filters) were performed to show that a successful pentaplex (5plex) real-time PCR can be designed (D5.10).

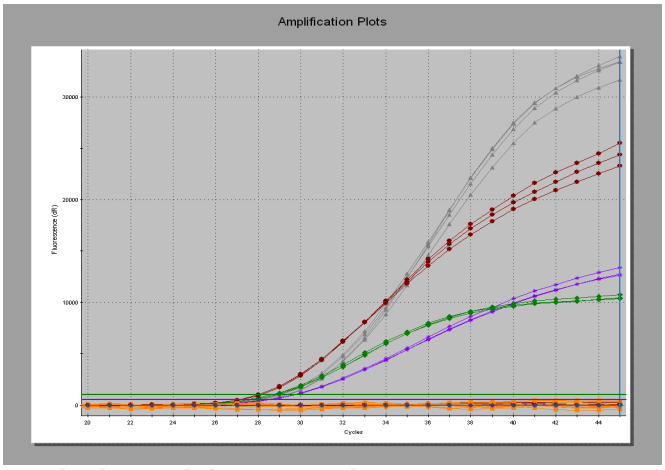

Figure 32: example of quantitative real-time pentaplex PCR.

- **Onsite detection methods.** Some real-time PCR devices are portable and therefore enable on-site analysis of samples. Co-Extra tested and adapted one duplex real-time PCR assay also for its applicability on field. A procedure for in field DNA extraction was adapted in parallel (D5.10, Allnutt et al, in preparation).

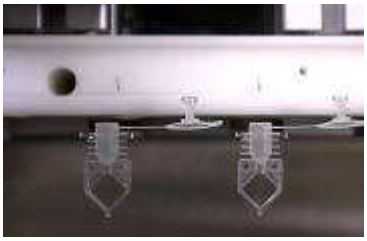

Figure 33: Portable PCR devices enable GMO detection on the field or at the customs.

- **PCR in combination with alternative detection system.** PCR amplification strategy was combined also with capillary electrophoresis to allow higher multiplexing options then in real-time PCR. First a multiplex PCR is preformed in the presence of labelled primers. In the next step the products are separated and identified based on characteristic size and colour in capillary electrophoresis. Different parameters of performance were checked with each assay. One pentaplex assay and one hexaplex were shown to have appropriate characteristics to be applicable in routine qualitative analysis therefore this approach can outperform real-time PCR multiplexing (Nadal et al, 2006, Nadal et al, 2009).

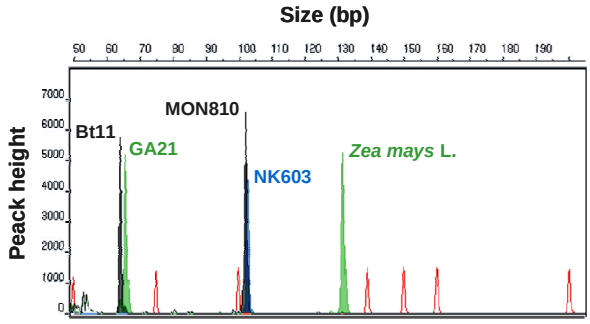

Figure 34: example of results using fluorescent capillary electrophoresis. PCR amplified targets are identified by size and colour.

- **Alternative systems for real-time PCR.** Availability of reference materials is a major bottleneck in the development of an integral traceability system. At the moment certified reference materials are available for EU-authorised GMO events. These are used for quality control and for the calibration of measurement systems. The main drawback is the dependence on GMO patent holders concerning the provision of suitable raw material for the production and certification of reference materials. In an inter-laboratories comparative study, the suitability of gDNA (genomic DNA), pDNA (plasmidic DNA) and WGA-DNA (whole genome amplified DNA) for the calibration of GMO quantification methods was evaluated, together with the stability aspects using real-time PCR measurements (D5.1, D5.8, van den Bulke et al., Trapmann et al., papers in preparation).

  Real-time PCR is the technology chosen by most routine GMO detection laboratories. However, practically all validated real-time PCR methods for GM testing apply TaqMan® probes and most protocols are adjusted for use with ABI apparatus. Several real-time PCR chemistries were compared and evaluated in the project: LNA, MGB, Molecular beacon and CPT probes, Plexor and Lux systems of labelled primers and nonspecific DNA intercalating dye SybrGreen. Those were the chemistries that at the time being have been shown most interesting for different GMO related application or had the potential to outperform TaqMan (D5.9, Andersen et al, 2006, La Paz et al, 2007, Buh-Gašparič et al, 2008). Similar comparison of performance of different real-time PCR devices on the market was performed. For this study WP partners that had at least two devices of different type in the lab. Altogether, 5 labs participated in the study and the following devices were included: Applied Biosystems AB7700 and AB7900, Cepheid Smartcycler II, BioRad Chromo4, Corbett Research Rotorgene 3000 and Roche Light Cycler. Analysis was organised as a ring trial, with all labs using the same cycling and analysis parameters on the same set of samples (D5.9, Allnutt submitted).

- **Automation of GMO detection.** The potential cost reduction and increase in accuracy in GMO detection was tackled in several points. Among those attempts, different pipetting automation and DNA extraction devices have been tested. Simple DNA extraction automation of pre-existing methods so far carried out manually is mostly not reasonable, as e. g. automation of centrifugation steps or extraction steps with organic solvents is extremely complex and costly. Consequently a new magnetic bead based DNA extraction method for KingFisher® Flex from Thermo Scientific was established which can combine automation with low cost for instrumentation and which has got the potential for general applicability, e.g. to be suitable for many different sample types. Automation of PCR setup and performing reactions in 384 well format can be another potential solution reducing the ‘hands-on-time’ and material cost per PCR analysis, but downsizing reaction volumes may cause trade-offs in performance. Two devices were included in testings; Hamilton Microlab Star Liquid Handling Station and PerkinElmer Multiprobe II, to verify reproducibility as well as robustness in order to be relevant in routine testing practice (D5.12).

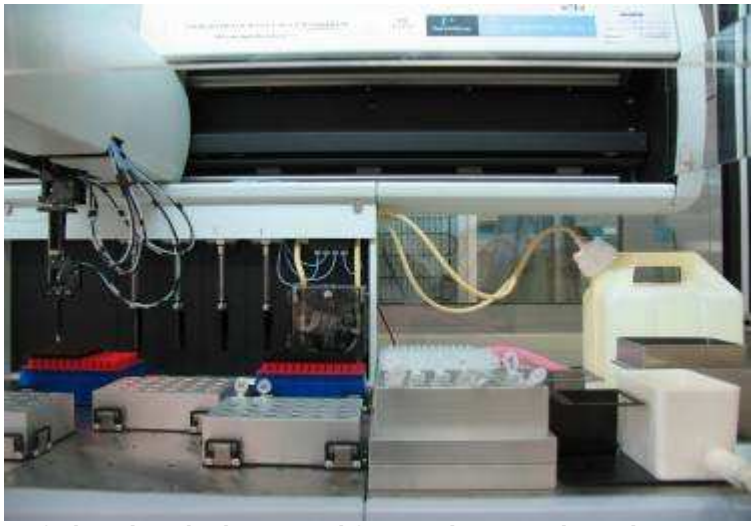

Figure 35: Example of pipetting device tested for use in GMO detection

- **Alternatives to PCR.** Some non-PCR amplification methods avoid the exponential amplification of errors associated with exponential PCR amplification methods. Therefore, non-PCR real-time amplification methods were explored as a quantitative alternative to real-time PCR. Additionally, there is potential in improved cost efficiency of GMO detection with some of the system as there is no need for use of expensive devices. Loop-mediated-amplification (LAMP) was tested first in combination with agarose gel detection of products (D5.11, Lee et al., 2009, Lee et al., in preparation). Further on, Lumora's proprietary BART technology was combined with LAMP to provide a bioluminescent output in real-time during amplification (D5.11, Kiddle et al, in preparation). Another alternative isothermal amplification strategy included in testings was RDC (Reaction Deplacement Chimeric). NAIMA, transcription based isothermal amplification strategy (D5.11, Morisset et al., 2008), was developed in collaboration with WP6 for multiplex detection of product on microarrays. Also real-time analysis of NAIMA product amplification was designed in the presence of SYBR®Green I (D5.11, Dobnik et al, in preparation).

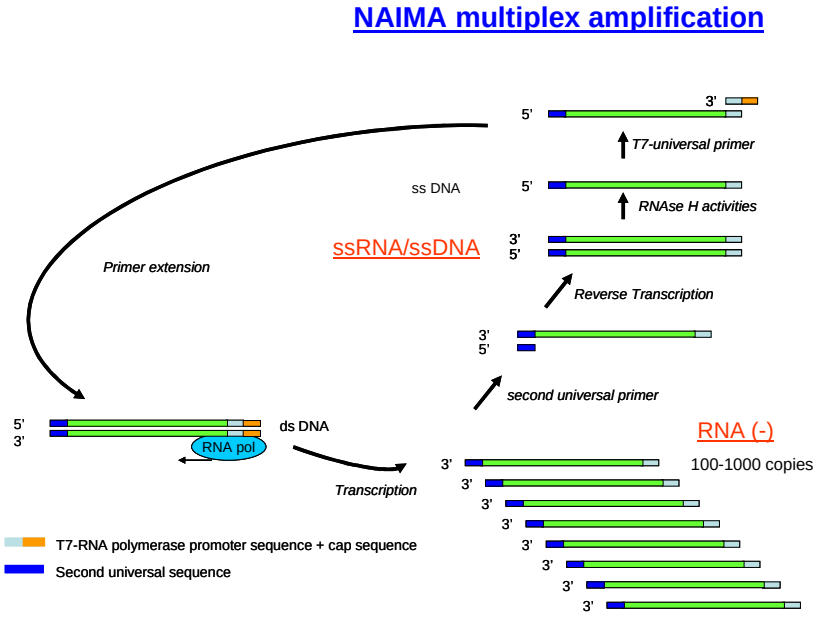

Figure 36: Example of NAIMA amplification strategy, an isothermal amplification mediated by action of RNA polymerase, RNase and reverse transcriptase that was developed within Co-Extra.

Non-DNA based detection methods.

Protein based detection methods are widely accepted by different stakeholders as a mean of fast and non-expensive initial screening of large number of samples. One of the weak points of protein based testing is however relatively low sensitivity, thus making it unreliable for certain specific uses, like with complex or processed materials. Within the project we have produced novel monoclonal antibodies for detection of CryIAb protein. Their sensitivity was first tested in ELISA format and further on they were integrated into lateral flow devices (D5.11, Allnutt et al, in preparation).

Several reports indicated that GM crops can be identified also by some physical means. In the project potential of near-infrared (NIR) hyperspectral technique for the detection and quantification of GMO was tested. This technique is especially interesting for accurate analysis of expensive materials (like seeds) as it is non-invasive and fast. Special emphasise was put on the analysis of spectra to provide reliable qualitative results. Roundup Ready soybean kernels and barley lines were used as a model (D5.11).

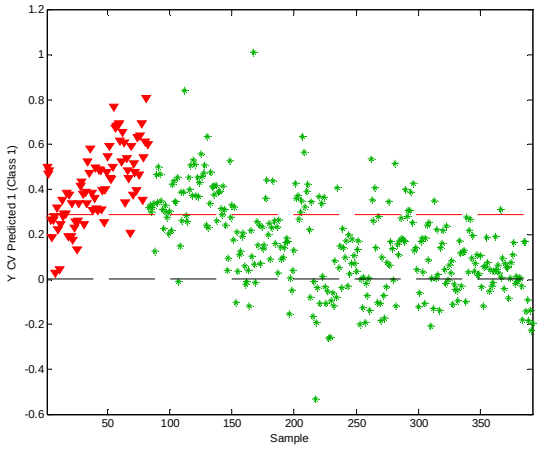

Figure 37: example of single kernel NIR spectrum analysis using statistical tools. GM kernels are shown in red, while non GM kernels are shown in yellow.

- **Improving reliability and limits of quantification of detection methods.** Taxa reference assays are equally important for reliable quantitative analysis as the event specific assays. But while there are strict criteria set for evaluation of event specific assays by CRL-JRC, there is no recommendation set for testing of taxa reference assays. Reliability of maize reference assays was checked by statistical analyses of real-time PCR results in parallel to Conformation Sensitive Capillary Electrophoresis (CSCE) followed by fragment sequencing for direct confirmation of differences in nucleotide sequences (D5.13, Papazova et al., 2009, Ghedira et al, 2009). Comparative analysis was performed also for existing oilseed rape, potato and rice reference assays, all taxa for which several assays were developed and for which the problems with specificity were already reported. Evaluation was performed in view of global genetic pool, for each crop independently (D5.13).

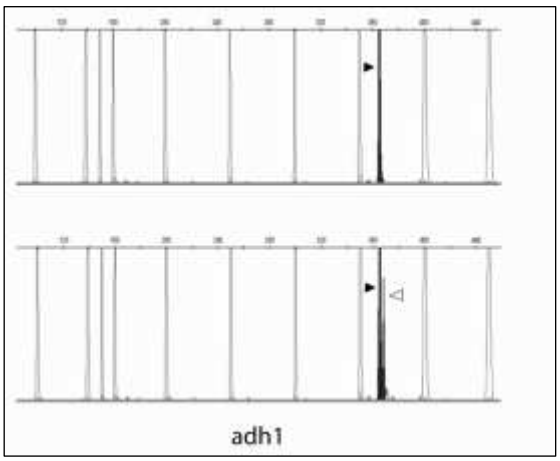

Figure 38: Example of heteroduplex (indication of SNP in the genome) found in Adh1 (NK603) amplicon target sequence. The major peaks corresponding to the heteroduplexes are indicated with a black arrow, the minor peaks indicating the presence of heteroduplexes are indicated with a transparent arrow.

Several statistical approaches were also developed or applied within WP5 to extend the scope of GMO detection. One successful example is the use of most-probable-number statistics to improve limit of quantification in GMO analysis for samples that contain limited amounts of DNA. Here instead of exact quantification of targets number per reaction, number of targets is calculated from the ratio between positive and negative results of amplification in a series of low copy number sample dilutions reactions (D5.6, D5.10, Berdal et al.,). It has been previously reported that some substances can interfere with PCR amplification thus affecting also the final quantitative result. Statistical analyses were also applied to provide practical tools for reliable quantification in GMO detection (D5.4, Cankar et al, 2006).

- **Decision support system for evaluation of methods developed for GMO detection.** With increasing number of GMOs on the market numerous analytical methods are becoming available to enable efficient enforcement of analytical traceability. Analysts are facing a complex situation as different methods can perform similar tasks. A qualitative multi-attribute model for evaluation of DNA extraction and DNA detection methods was developed together with computer scientists in WP7. Methods are being evaluated based on their performance; applicability and practicability (see report of WP7 for more details and D7.12, D7.15).

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- Van den Bulcke M., et al. A theoretical introduction to "COSYPS": an integrated Decision Support System for GMO screening and detection (submitted July 2009)
- Van den Bulcke M., et al. Plasmids as qualitative and quantitative calibrants in GMO detection: state of the art, benefits, limits and future (in preparation).
- Van den Bulcke M., et al. Use of pJANUSä-02-001 as Calibrator Plasmid for GTS 40-3-2 (Ropundup Ready Soybean) Detection: An Inter-Laboratory Trial Assesment (in preparation).

Technical challenges in GMO detection

The initial objectives for this Co-Extra activities part are listed below, followed by an explanatory statement of the work performed in relation to each specific objective. Relevant peer-review publications are given in square brackets.

1. Methods that can be used to simultaneously search for and detect several different objects, e.g. all maize GMOs are often referred to as multiplex methods, in contrast to simplex methods that can only be used to detect one object, e.g. single GMO maize. The first primary objective of WP6 was to develop multiplex screening methods that may be used to determine if samples contain GMO derived materials or not, to identify the GMO from which the material is derived and to identify the species from which ingredients in the sample are derived.

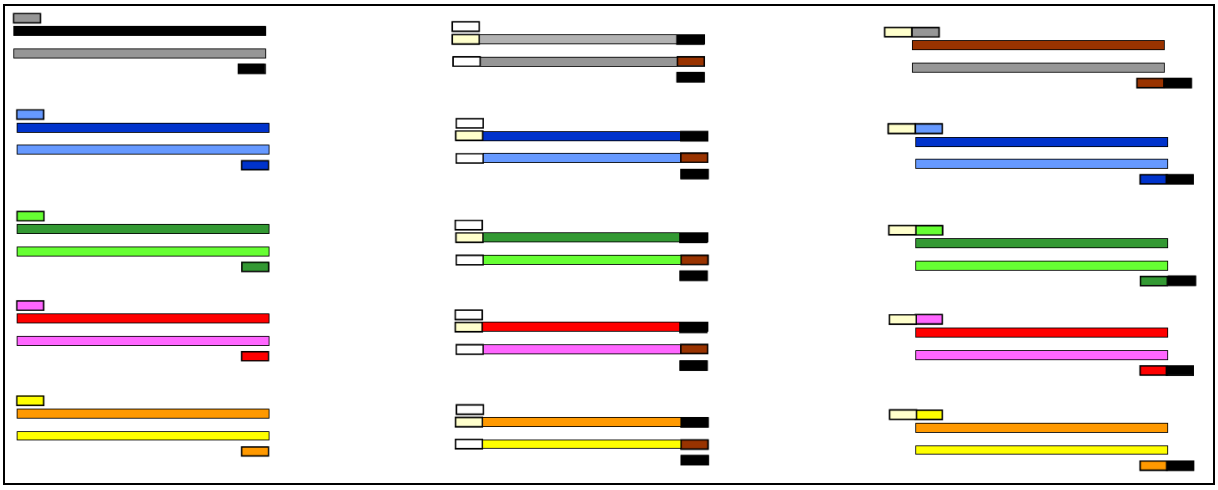

Figure 39: Alternatives for multiplexing of amplification reactions. Left, combinations of five individual simplex assays into a pentaplex assay (N targets and N primer pairs). Middle, five targets with shared flanking sequences that can host primer sites can be amplified with a single primer pair (N targets, 1 primer pair). Right, five individual simplex assays can be converted to a multiplex assay by use of tailed primers in the first cycle and universal primers in the following amplification cycles (N targets and N primer pairs with tailed primers for 1 primer pair).

A broad range of multiplex screening methods were developed and/or tested in the initial phase of the project [1, 3, 4, 8, 15, 16, 17, 21]. In connection with deliverable D6.8 which summarised the performance characteristics, resource requirements and developmental stage of all the methods developed in relation to this and some additional objectives, it was decided that a limited number of the methods should be tested in transfer laboratories to assess their performance, fitness for purpose, robustness, etc. One method, i.e. the DualChip® GMOchip [17] (Fig. 40) developed by EAT was already collaborative trial validated in WP4 [12]. The outcomes of the transfer tests are reported in deliverable D6.9. Experimental and theoretical specificity testing of primers and probes to be used in the detection methods is important, but may also be difficult. One of the obstacles has been the absence of tools that compare results for individual primers and probes when

performing theoretical specificity testing against publicly available sequence databases. UniquePrimer [18] is an online tool that was developed in relation to other Co-Extra detection parts and this tool facilitates such testing.

The work is reported in documents D6.1, D6.2, D6.3, D6.7, D6.8 and D6.9

2. GMOs that have not been authorised for use as food or feed in the European Union are by definition unauthorised. GMOs that are not authorised may be more or less well known. For example a GMO that is pending authorisation where EFSA has already conducted a risk assessment, is well known. A GMO that has been modified with novel genetic elements, i.e. elements not used in authorised and well known GMOs, may be classified as unknown. Development of methods to determine if samples contain unauthorised and in particular unknown GMOs was the second primary objective of this activities part. These methods should also facilitate successive characterisation and tracing of the unauthorised and unknown GMOs.

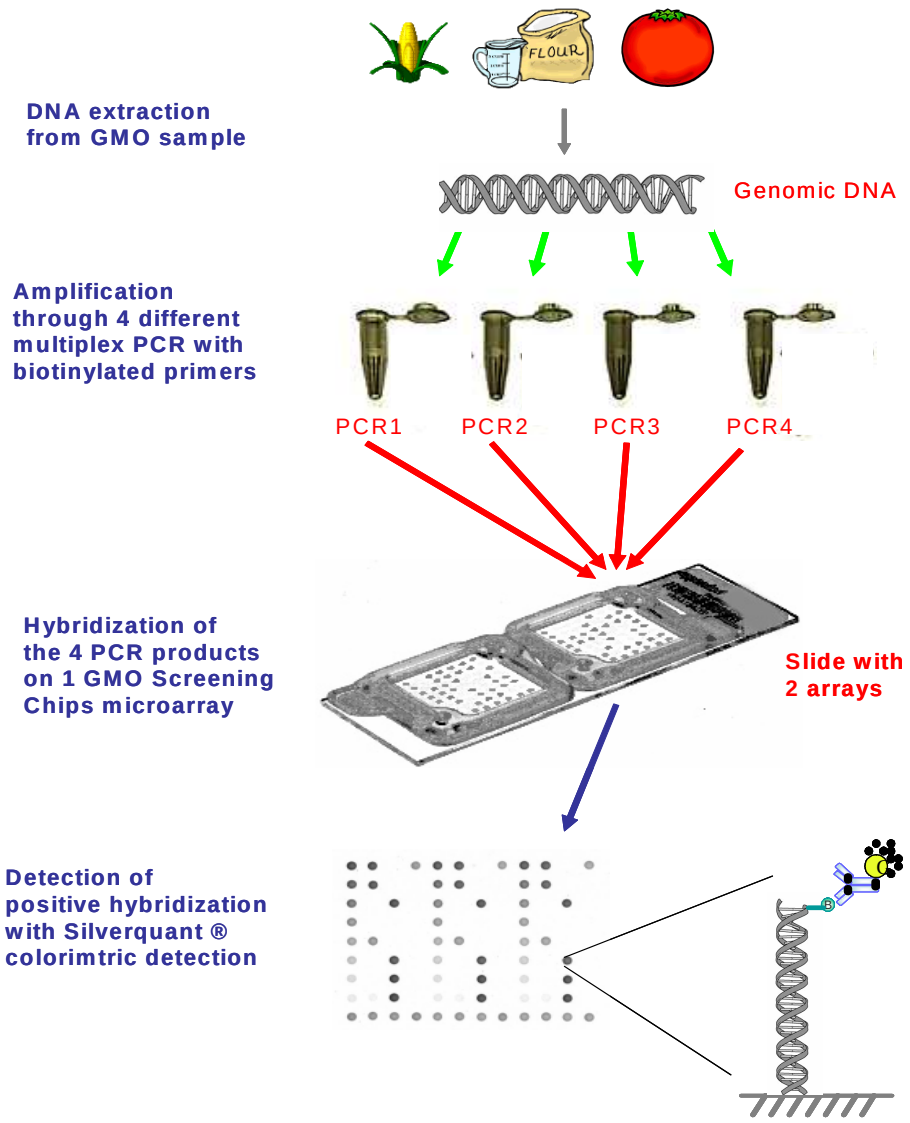

Figure 40: The DualChip® GMOchip methodology.

Various methods developed in WP6 apply the "matrix approach" (see Fig. 41 below) which can also be used to screen for possible presence of unauthorised GMOs by observations of inexplicable analytical results. The principle of using anchor-PCR fingerprint profiles to detect and identify GMOs (see Fig. 41 below), followed by sequencing of the anchor-PCR products for confirmation of suspected presence of unauthorised GMO was demonstrated. A quantitative differential PCR approach was also developed [6]. This approach is relying on the use of two or more quantitative real-time PCR methods applied on the same sample, where the target quantities should be equivalent in the presence of only authorised GMOs, and where non-equivalent quantities indicate the presence of unauthorised GMO. Advanced assays for characterisation of unknown GMOs by high density microarray analysis [5, 23] or subtractional transcriptome sequencing [20] were also developed. These assays exploit the rapid growth in publicly available sequence data while at the same time making almost no prior assumptions about the type of genetic modification that may have taken place. While the matrix approach, anchor PCR and quantitative differential PCR approach make at least some prior assumptions, and therefore may be unable to detect truly unknown GMOs, the microarray hybridisation and transcriptome sequencing approaches can also be used to detect and initiate characterisation of truly unknown GMOs.

The work is reported into documents D6.1, D6.3, D6.6, D6.7, D6.8 and D6.9.

Analytical module/ GMO event	Screen A	Screen B	Screen C	Screen D	Screen E	Screen F	Screen G	Screen H
GMO_One	+	+	+	-	-	+	-	-
GMO_Two	+	+	-	-	-	+	-	-
GMO_Three	+	+	-	+	-	-	+	-
GMO_Four	+	-	-	+	-	-	+	-
GMO_Five	-	+	+	-	-	-	-	+
GMO_Six	-	-	-	-	+	-	-	+
GMO_Seven	+	+	-	-	+	-	-	-
GMO_Eight	-	-	+	-	-	+	-	-
GMO_Nine	-	-	-	+	-	-	-	-
GMO_Ten	-	-	-	-	-	-	+	-
Sample_1	+	+	+	+	-	+	-	+
Sample_2	-	+	+	-	-	+	-	+
Sample_3	-	+	-	-	+	-	+	-

Sample_1	Sample_2	Sample_3
Perfect	Part_missing	Mostly_missing
Perfect	Part_missing	Mostly_missing
Part_missing	Mostly_missing	Mostly_missing
Part_missing	Negative	Mostly_missing
Perfect	Perfect	Mostly_missing
Part_missing	Part_missing	Partly_missing
Part_missing	Part_missing	Partly_missing
Perfect	Perfect	Negative
Perfect	Negative	Negative
Negative	Negative	Perfect
A,B,C,D,F,H/none		
	B,C,F,H/none	
		G/B,E

Figure 41: The matrix approach, schematically illustrated. Upper left, the matrix, i.e. a tabulation of the response by various GMOs (GMO_One, etc.) to tests performed with analytical modules (Screen A, etc.) based on evidence from analyses of reference materials. Lower left, examples of results from analyses of three samples. Upper right, matching the results for each sample against the matrix, GMO by GMO indicates "Perfect match" if the pattern revealed by a particular GMO is reproduced completely with the sample; "Partly missing" if only some parts of the GMO specific pattern is reproduced, "Mostly missing" if less than half of the GMO specific pattern is reproduced, and "Negative" it no part of the GMO specific pattern is reproduced. Lower right: For each sample the observed pattern is analysed with respect to the match against the GMOs. If all the results can be explained with single or specific combinations of GMO specific patterns, then none of the results are inexplicable and there is no indication of presence of unauthorised GMO (samples 1 and 2). If there are results that cannot be explained , e.g. because in all authorised GMOs the detected targets are combined with targets not observed in the sample, then there is a strong indication of the presence of unauthorised GMO (sample 3; inexplicable elements in red).

2a

A Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	150	-	69	75	262	-
T2	-	320	150	-	-	-
T3	164	98	-	-	-	-
T4	79	330	-	-	460	-
T5	91	-	-	-	150	-
…	-	-	-	-	-	-

B Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	-	-	-	-	-	-
T2	-	-	-	-	-	-
T3	-	-	-	-	-	-
T4	-	-	-	-	-	-
T5	-	-	-	-	-	-
…	-	-	-	-	-	-

C Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	150 76	-	69	75 69	262 366	-
T2	-	320 169	150	-	-	-
T3	164 321	98 199	-	-	-	-
T4	79 360	330 360	-	-	460 220	-
T5	91	-	-	-	150 220	-
…	-	-	-	-	-	-

D Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	350	-	69	-	-	-
T2	-	250	150	-	-	-
T3	240	110	-	-	-	-
T4	130	360	-	-	-	-
T5	230	-	-	-	-	-
…	-	-	-	-	-	-

E Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	-	320	69	67	310	-
T2	-	-	150	-	-	-
T3	59	190	-	-	-	-
T4	-	-	-	250	-	-
T5	-	420	-	-	-	-
…	-	-	-	-	-	-

F Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	-	96	-	-	120	-
T2	-	150	-	-	-	-
T3	-	230	-	96	-	-
T4	-	260	-	-	300	-
T5	-	-	-	-	190	-
…	-	-	-	-	-	-

2b

X Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	150	-	69	75	262	-
T2	-	320	150	-	-	-
T3	164	98	-	-	-	-
T4	79	330	-	-	460	-
T5	91	-	-	-	150	-
…	-	-	-	-	-	-

Y Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	150 350	-	69	75	262	-
T2	-	320 250	150	-	-	-
T3	164 240	98 110	-	-	-	-
T4	79 130	330 360	-	-	460	-
T5	91 230	-	-	-	150	-
…	-	-	-	-	-	-

Z Anchor primer

Tetracutter	A1	A2	A3	A4	A5	…
T1	230	-	69	130	-	-
T2	-	40	150	-	360	-
T3	350	150	-	-	-	-
T4	150	320	-	-	-	-
T5	300	-	-	-	-	-
…	-	-	-	-	-	-

Figure 42: The anchor PCR approach produces a "fingerprint" profile for each GMO. By comparing the profiles of a sample (X, Y or Z) with the profiles produced by reference materials (A-F) is it possible to determine if a sample contains unauthorised GMOs, including GMOs with a previously unknown profile. In the example shown does sample X match perfectly with reference A, sample Y matches the combined presence of A and D, while the profile observed in Z is unique and may represent an unauthorised/unknown GMO.

3. The third primary objective was to develop multiplex quantitative methods to assess if a sample exceeds legally defined thresholds or not, including identification of samples that should be subject to refined quantitative analysis such as single-event real-time quantitative PCRs.

Initially five approaches were explored and developed. With time, one of the approaches (MQDA) turned out to be inferior and further work on the approach was stopped. The other four approaches; NAIMA (NASBA implemented microarray analysis; Fig. 43) [13, 14, 26], quantitative two-stage multiplex PCR [7], one-step semi-quantitative PCR with limitators and multiplex ligation-mediated PCR [25] were developed further and the NAIMA approach was transfer tested in

another laboratory than the developing lab. The work is described in documents D6.1, D6.2, D6.3, D6.7, D6.8 and D6.9

4. The fourth primary objective of WP6 was development of guidelines and recommendations for design of rational GMO testing schemes on the basis of multiplex methods. In other words, giving guidance to users of analytical methods, in particular multiplex methods, on how they may use their methods in the most rational way.

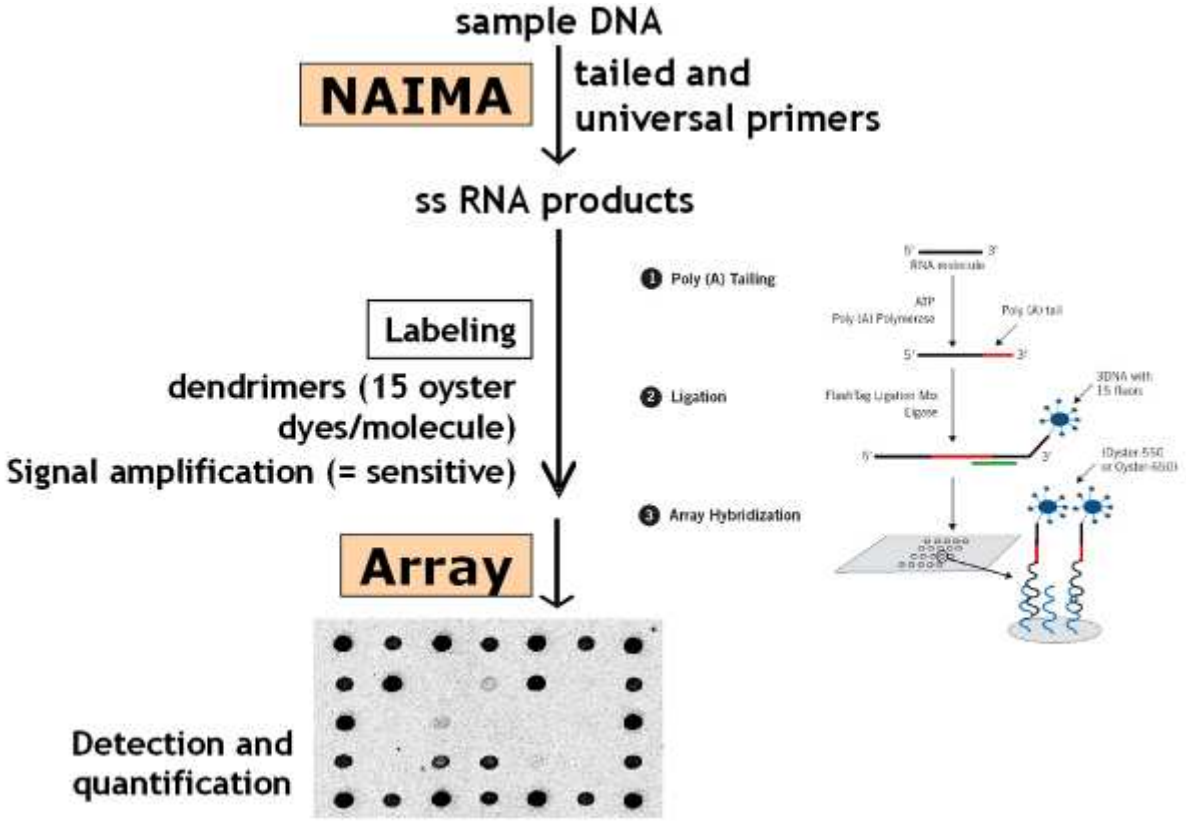

Figure 43: The NAIMA approach in combination with microarray detection of amplified nucleic acids.

The concerned Co-Extra partners through the WP leader interacted closely with the Co-Extra partners responsible for development of the Co-Extra Decision Support System (DSS). Contributions were specifically linked with the DSS modules on analytical methods, unauthorised GMOs and database. The main guidance to users of analytical methods is therefore presented in the DSS, several peer reviewed scientific publications [1, 2, 3, 5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 23, 25, 26] and book chapters. Relevant guidance is therefore also provided in these scientific publications as well as the WP deliverable reports D6.4, D6.5, D6.6, D6.8, D6.9 and D6.10.

5. Gene stacked GMOs, i.e. GMOs with more than one novel trait gene (Fig. 44) have increased their market share dramatically over the last few years. The fifth primary objective of WP6 was to assess the possibility to identify target analytes for commercial gene stacked events (cGS), and to develop corresponding methods for the detection of the cGS.

An early preliminary [2] and later a more comprehensive review of this topic [11] were prepared and published. Analytical methods were only partly developed, as the general conclusion in the review report [11] was that no cGS associated specific target analytes exist and that detection is only feasible on the basis of single item (e.g. seed, plant) analysis or by application of statistics in compartmentalised materials with LOQ < GMO concentration << 100%. In reality this means that

detection of cGS is only feasible in exceptional cases. The review paper [11] discusses the consequences of this in relation to current EU regulations. The related deliverable is D6.4.

6. The predominant technology for nucleic acid based GMO detection is the polymerase chain reaction (PCR) technology. The sixth primary objective of this activities part was to assess if alternatives to PCR technologies for comprehensive GMO testing are available and fit for purpose. Such alternatives would include both target (nucleic acid) and signal amplification methods. And, if available and fit, Co-Extra proposed and developed corresponding methods (alternative to PCR).

Several alternatives to PCR were explored as integrated parts of methods [5, 10, 13, 14, 19, 20, 23, 26]. These alternatives include direct hybridisation of labelled genomic DNA to microarrays [5, 23], multiple displacement whole genome amplification (MD-WGA) [5, 10, 23], NASBA [13, 14, 26], use of immobilised PNA probes and immuno-PCR [19] and transcript sequencing [20]. Two topical reviews were also prepared; one is the deliverable D6.7 report, the other a peer-review publication [13].

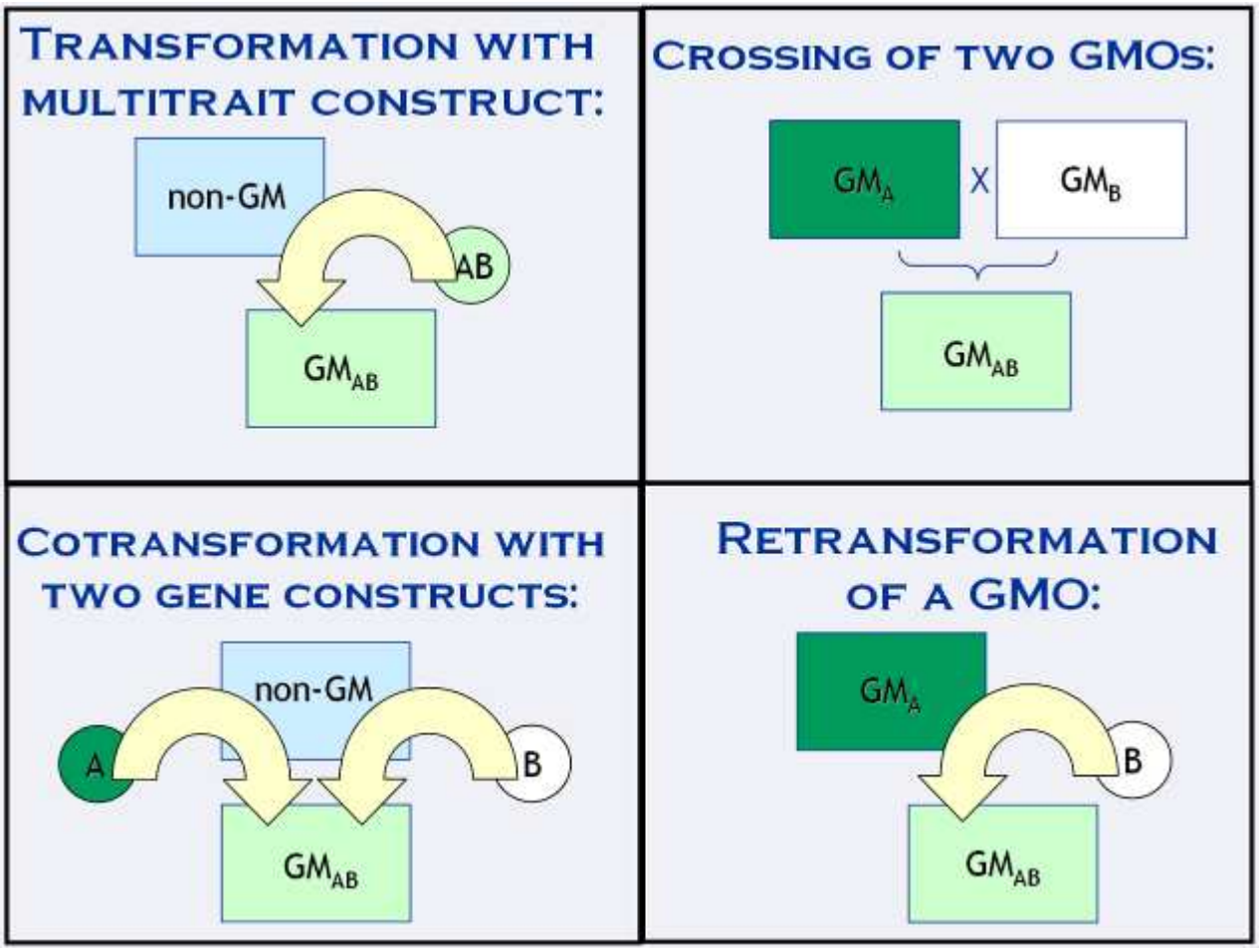

Figure 44: Four different routes to creation of a gene stacked GMO.

7. The detectability of the analyte, i.e. the protein or nucleic acid that the analyst is trying to detect is usually sensitive to a broad range of factors. The most important factors are the quantity and purity of the analyte. However, it has also been raised as a concern that for example two DNA sequences may respond differently to material processing, purification

methods, etc. Such differential response is often referred to as bias, and the impact could be severe on the accuracy of e.g. quantitative measurements of GMO concentration. The seventh primary objective of this activities part was therefore to assess on a range of target analytes if particular bias in stability can be observed relative to:

- Analyte and analyte structure (e.g. DNA vs. protein, AT:GC bias in DNA, domains in protein folding structure)
- Type of locus (coding/non-coding, mobile element, functional domain)
- Organism (e.g. plants and their endo- and epiphytes and -parasites [e.g. particular bacteria, virus' and fungi], and naturally occurring donors of genes targeted by GMO detection methods, but also plants with different nutritional composition [protein-, starch- or fat-rich)
- Tissue (e.g. fruits vs. leaves, seeds, etc.)
- Product of processing (e.g. dry milled vs. wet milled, heated vs. air dried, etc.)
- Analyte extraction method (e.g. CTAB vs. SDS or guanidine based DNA extraction methods)
- Specific analytical detection method (e.g. PCR vs. low temperature hybridisation or isothermal amplification)

The work was divided into two main directions: pre-harvest and post-harvest bias or instability. The pre-harvest studies considered multiple cultivars of the GM plants, and examined sequence motifs involved in the function and detection of the transgene, and house-keeping genes for detection, to see if allelic variance was observed and how that would eventually affect detection. Two peer review publications report on the results of these studies [22, 24]. The post-harvest studies considered sequence specific (size, structure) effects related to processing of materials (heat, acid, UV, etc.) and to choice of DNA extraction method. Parts of the post-harvest results are closely related to issues on validation of the modular approach and modular validation of DNA extraction modules and PCR modules in WP4. Good communication with WP4 was therefore crucial throughout the project period to avoid overlap and ensure optimal integration of activities. Peer review publications are foreseen but not yet submitted, reporting on the post-harvest bias studies. This work is described into deliverables D6.5 and D6.10.

8. The eighth primary objective was to contribute to development of guidelines on how bias may be dealt with by the analytical laboratories, both in relation to setting up rational testing schemes, validate methods and define the domains of application of the analytical methods.

The results of the studies indicate that bias is only exceptionally a problem with PCR, provided that the size of the amplicons (the length of the DNA sequence motif detected with the methods) is similar and short (typically < 100 basepairs). Within the limitations indicated, the general guidance points towards the use of bias testing only in the exceptional cases. However, several examples of bias were observed, primarily in connection with heavy processing such as heating, use of acids (low pH) over extended time, etc. This is explained primarily by the primary structure (the DNA sequence) of the amplicon. Tools developed in sampling and validation activities part may also be applied to test (optionally) for bias in connection with DNA extraction, specific PCR methods, or other factors.

9. The ninth primary objective of this Co-Extra activities part was to communicate with stakeholders to identify and incorporate their needs in the planning of the research activities, and to disseminate achievements that stakeholders may implement.

Throughout the project duration time, Co-Extra was very attentive to the public debate, relating to issues on traceability, detection, identification and quantification of GMOs. The Co-Extra partners have also been very active in dissemination of results, among others in attempts to trigger responses from stakeholders. We would particularly point to the peer review publications [2, 9 and 11].

The title of this "Technical challenges of GMO detection" activities part illustrates what the Co-Extra partners have been up against; - the technical challenges remaining for reliable GMO detection. The tasks included development of multiplex methods (qualitative and quantitative), methods for detection of unknown GMOs, solutions to cope with the stacked GMOs, alternative technologies that do not rely

on the polymerase chain reaction (PCR) and thus may overcome some of the undesired aspects of PCR, and solutions to cope with possible bias in analytical measurements. Multiplexing is expected to reduce costs and workload, since fewer analysts need to be performed. While multiplexing is a challenge commonly faced by molecular analysts, with a wide range of proposed approaches, it still has not found a very good, reliable and universally applicable solution. The GMOs also pose an additional challenge in this respect, that multiple targets are present simultaneously but in highly variable concentrations, and that they all need to be correctly identified and quantified. The majority of multiplex assays are qualitative, simply because it is much easier to develop a method that will reliably produce a yes-no response than a method that is robust to all sorts of interference between reagents and other components in the analytical environment. Authorised GMOs and GMOs approved in other (non-EU) jurisdictions are described in sufficient detail in publicly available documents to allow method developers to design targeted methods. These GMOs are therefore "known". Some GMOs have accidentally been introduced without approval in any jurisdiction, e.g. Bt10 and E32 maize, LL601 and Bt63 rice. However, these GMOs all contain one of more of the same introduced genetic elements that we find in the known GMOs, and therefore may be detectable with some of the targeted detection methods. Thus, these GMOs are unauthorised but only partially unknown. Unknown GMOs are by definition unknown, i.e. the introduced genetic elements are all novel and no targeted detection method can be used to detect these GMOs. Detection of unknown GMOs is therefore particularly challenging. Gene stacking can be achieved by at least four different routes (Fig. 44), but only exceptionally will there be a physical linkage between the introduced genetic constructs. It means that the targets for analytical methods may segregate independently or semi independently. For the analyst this means that it may be impossible to assess if detection of molecular markers for two different GMOs is caused by simultaneous presence of the two GMOs in the sample, or if the two molecular markers have been stacked in a single GMO. Co-Extra partners therefore wanted to assess if this problem could be overcome, and how. Finally, it has been claimed in several publications that bias is a problem in connection with quantitative GMO analyses, but no causative explanation has been proposed. The WP6 partners therefore wanted to assess if, when and why bias may be a problem. Answers to when and why could then offer clues to possible solutions to improve the reliability of quantitative GMO analyses.

The matrix approach used by several partners as a basis for the multiplex methods is described in **Fig. 41**. This approach can be applied to detection of both of authorised and unauthorised GMOs. The matrix approach exploits pre-existing knowledge about the response of individual GMOs to particular testing methods. The response of the sample to the testing methods is then compared to the pre-existing knowledge (pattern) and match-mismatch scores provide a basis for determining which GMOs the sample contain.

Multiplex amplification can be achieved in three different ways, see **Fig. 39**. Notably, multiplexing may concern only the reagents for detection, e.g. with multiple primer sets, or it may concern only the targets, e.g. with universal primers amplifying a particular gene found in multiple taxa, with allelic variation. A sample may contain only one target, but it is not known to the analyst which target. Then it may be appropriate to use a PCR based detection method with a single "universal" primer pair. A positive signal may or may not be further analysed by use of e.g. array hybridisation or size determination by electrophoresis [1, 3]. If multiple targets can also be present in the sample then some degree of interference between the targets may be a problem. More advanced assays introduce multiple primers (and probes) but may work well for samples with only one target. Once multiple primer pairs and probes are used and multiple targets may be present in the sample simultaneously but in highly variable concentrations (e.g. 0.02, 0.7 and 25%, respectively), then it may be very difficult to optimise the assay for reliable performance under all realistic scenarios.

A common strategy for the multiplex assays is the separation of the amplification and the detection steps. A broad range of alternative amplification strategies including PCR, ligation mediated PCR, NASBA, multiple-displacement whole genome amplification (MD-WGA) and signal amplification were explored. Detection was either done using capillary electrophoresis and identification by size and colour (**Fig. 45**), by array hybridisation (**Fig. 46**) or by DNA sequencing.

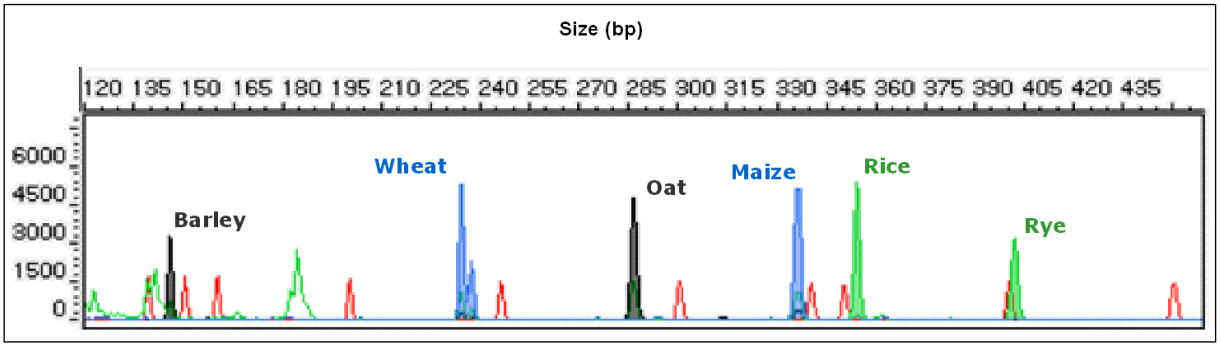

Figure 45: Example of detection and identification of amplification products by size and colour using capillary gel electrophoresis. Here six cereal species are detected and identified by black, blue and green peaks. Horizontal axis corresponds to product size in base pairs, while the vertical axis corresponds to signal intensity for each peak in the sample.

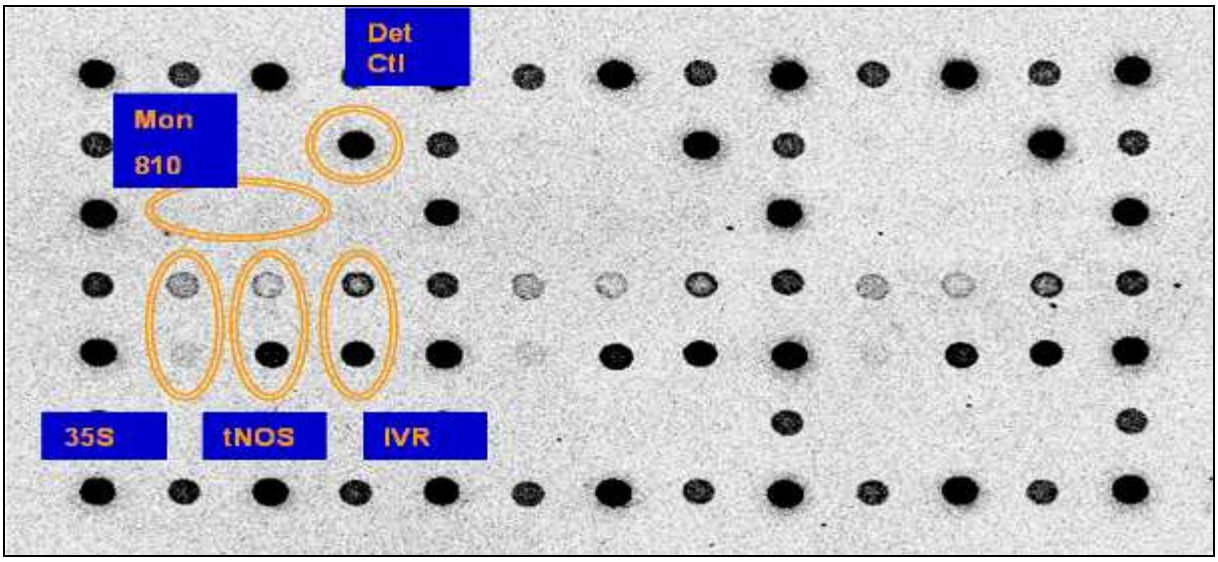

Figure 46: Example of detection and identification of amplification products by array hybridisation. Here the results for four particular targets are shown. Probes for each target are spotted in duplicate on each subarray, and each sub-array is spotted in triplicate. Three of the specific targets produce positive signals, while one is negative. A detection control is also included.

Direct hybridisation of genomic DNA to high density microarrays was employed by one partner in relation to detection and characterisation of unknown GMOs. The same partner also explored high throughput transcriptome sequencing in combination with DNA subtraction approaches for detection and characterisation of unknown GMOs.

Differential quantitative PCR was applied to assess if samples are likely to contain inexplicable concentrations of particular (screening) targets, as a means of determining if samples contain unauthorised GMOs. This technique is the first to have been developed which may directly be used by routine laboratories using routine detection methods and a few lines statistical package. Its inter-laboratory validation is still underway.

Extensive literature reviews were used in connection with most tasks, as preparation of reviews and position documents was considered important.

Stability of the several targets used for assessing the GMO content is an issue for both companies and enforcement laboratories. For solving that issue, particular genes and genetic construct elements in multiple cultivars of transgenes were sequenced for pre-harvest analyte instability bias assessment. For post-harvest bias studies, samples and extracted DNAs were subjected to presumed DNA degrading treatments and successively extracted DNA was analysed using quantitative real-time PCR.

Different products were also subjected to different DNA extraction modules and different real-time PCR modules to identify if any systematic bias pattern could be revealed.

Moreover the validation of methods for detecting for instance stacked genes and unapproved GMO is a challenge per se. Stacked genes were generally not available as Certified Reference Material at the beginning of the Co-Extra and despite time and budget reallocations their detection validation was not performed. In a similar way, detection of unapproved GMO was carried out on model mixes of GMOs and mostly validated by the expertise of the partners and ENGL members in performing such detection.

Finally, the specific methodologies and approaches explored and developed are technically complicated and for the vast majority cannot be illustrated briefly here. Several are, however, illustrated by the partners in the related papers and deliverables.

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Conclusion

Stakeholders involved in coexistence and traceability implementation (of any supply chains) need first reliable and “fit for purpose” sampling procedures to obtain representative samples as the analysis of samples not representative of the lots to be analysed for compliance could get to wrong decision and then to waste of cost and efforts.

The proposed sampling methodologies in the field are of practical application for growers who wish to know in advance the risk of adventitious presence of GMO in non GMO production. The produced software tools dedicated to support sampling and sub-sampling and the mathematical framework developed within Co-Extra, are of high practical applicability to optimise the implementation of coexistence and traceability.

Avoiding monopolistic position and providing the ability to analysts to choose the best fit for purpose method, both in terms of accuracy and cost-effectiveness is an important step toward lowering the impact of analytical traceability onto the final costs to which consumers are faced. This is true even though the companies' assertions of using numerous analytical controls are not observed by Co-Extra, which in counterpart observed an important use of documentary traceability beside analytical controls on raw materials and at some critical points of the supply chains.

Our first attempt has been to constantly update the information toward the ENGL laboratories by both formal speeches and direct information exchanges. Although not easily visible this part of our results use is of primary importance for accurate application of EU regulation and consumers protection. As several Co-Extra partners are also members ENGL, it can be expected that Co-Extra will not be lost after completion of Co-Extra.

This expertise on GMO is again applicable to several other detection areas such as food and feed microbiology, plants and animals pathogens, mycotoxins and allergens producing organisms as it was previously the case when the GMO detection laboratories were in the FP5 developing PCR based detection methods to be applied to the whole supply chains while formalizing and standardizing such GMO detection.

The ability to detect unapproved GMO is of utmost importance not only on an analytical point of view or on a putative safety viewpoint but also in the arenas of international discussions such as Codex Alimentarius and OECD. Of course, the use of such methods which costs may drastically vary cannot be used for all developed methodologies on a routine basis.

But the most costly detection approach to detect completely unknown GMOs might be used for safety concerns about the presence of a harming organism. This part is no more of the relevance of GMO detection laboratories but of bio-defence, but should have been pointed out.

Position documents on unknown GMOs, gene stacking and analyte instability bias have received broad attention among others inside the Commission services, the Community Reference Laboratory and the European Network of GMO Laboratories, but also among other stakeholders from industry and producers. It may also have some European legal follow-up for instance on the issue of botanical impurities *versus* botanical impurities and LLP.

For a decade the GMO detection area was facing the challenge to find or train molecular biologists able to understand statistics, reproducibility, accuracy etc. For the new decade we can expect from the current development of qualitative methods a new challenge: training molecular biologists using quantitative PCR black boxes into people mastering qualitative methods with sub-sampling strategy, development and use of an increased number of strategies with decision trees and DSS modules, etc.

Finally, the expertise gained for detecting unapproved GMOs dedicated to food and feed uses should of utmost importance for detecting GMO developed for non-alimentary, putatively harmful, purposes.

Legal and policy issues

Co-Extra was attempting to address the issues of stakeholders not only from a technical or economic point of view but also from a legal point of view, taking regard not only of generally applicable regulations governing GMO approval and use, but also of contractual modifications thereof.

GMO as an object of legal expertise and of regulation

The legal framework affecting coexistence and traceability was analyzed from various perspectives. European, non-European and international approaches to regulating biotechnology in the food and feed supply chain were compared, including contractual duties and possible liability issues that may arise (D.7.3). Complications arise in particular in international settings with differing national systems, and such problems are aggravated by the fact that market participants may develop overlapping contractual regimes deviating further, even though it may be easier for vertically integrated companies. It shows the unifying effect of EU laws on a side and of private standards on the other side (D.7.28).

The coexistence project is a new modality of government of techniques (D7.21); it is particularly important concerning new technologies which until now have been managed only in reference to

potential or proven risks. This has meant that it tends to prevent the involuntary spread of technology causing the elimination of other technologies. This concept involves the perception of co-existence as an objective of *technological pluralism*, which if accepted globally, would allow the reconciliation of *knowledge society* and risk society by the promotion of a mechanism insuring public confidence in a sustainable way. As the judge's role vis-à-vis science is growing, courts endorse a more disputed role of "arbitrator of good scientific reports", which raises deep stakes that need to be correctly understood. D.7.21

This government of techniques' modality could be linked to an objective of technological pluralism such as the "energy mix", which could be further useful, regarding nanotechnologies for example. The project itself is difficult to carry out; it is even harder to find the proper rules to make it sustainable.

As risk decisions are more and more submitted to courts (national, European and international), it is of utmost importance to have a clear vision of what is required by the judges in terms of risk assessment. (D7.22). As the judge's role vis-à-vis science is growing, courts endorse a more disputed role of "arbitrator of good scientific reports", which raises deep stakes that need to be correctly understood. Co-Extra shows how European authorities have reached this solution aiming at ending the crisis generated by the public's distrust regarding GMOs food and feed. A Co-Extra study analysed the three government modalities that have been tried out to this day: the "Law of the Alliance" which designates a supple regulation conceived by experts, industry and administration; "Law as seen by the Rulers", represented by the 90/220 directive, based on risks assessment without managing farm-produced products' supply chains; the "Law as seen by the ruled", implemented by the 2003 (1829/03 and 1830/03) regulatory package.

The survey of legal, technical and political issues arising from co-existence and traceability in third countries identified some examples of workable systems and best practices that EU Member States may use when implementing co-existence and traceability rules. (D.7.16., D. 7.18). The analyses clearly showed large diversity in the extent to which third countries are considering introducing or in fact implementing co-existence measures, i.e. to maintain three supply chains. For countries candidate to EU integration, especially, a workable and reliable EU model would be highly appreciated (D.7.16., D. 7.18).

It is finally proved that coexistence is a "more in depth" form of traditional freedom of commerce and industry; it lies on a paradox: to insure all a certain freedom, it is necessary to impose strong constraints and a certain mutual tolerance.

Accordingly, Co-Extra is considering important:

- To officialise the technological pluralism as a global project allowing the reconciliation of knowledge society and risk society by the promotion of a mechanism insuring public confidence.
- To conceive rules so that this pluralism be sustainable.
- The coexistence strategies must from now on be thought of from the supply chain level and not only from field coexistence (present regulation).
- It is essential to insure a better distribution of supply chains' segregation costs by establishing a main principle; those introducing a new technology will take in charge the costs of segregation from the field to the consumer (Neighbourhood disturbances theory).
- It is important to quickly solve the question of various types of unknown or unauthorised events.
- Concerning seeds, it is important to quickly solve the matters of 1) the question of the adventious presence threshold 2) the farmer's right to use « farm saved seeds »-when these seeds have an increased probability of unwanted GMOs in some species. 3) the question of whether GM cultivation will affect the availability of genetic resources and investment in conventional plant breeding .
- As science has become more important in decision-making, there is more focus on the scientific basis of decisions, particularly in relation to environmental or health issues. What is the quality of the scientific reports on which the disputed decision rests? Does the present state of scientific knowledge justify this decision? Have all relevant scientific data been taken into account? Wasn't the previous scientific assessment too abbreviated?

The several Co-Extra deliverables give elements in order to better understand and manage these new and decisive aspects of risk decision-making.

Liability and redress issues

The legal framework affecting coexistence and traceability was analyzed from various perspectives. European, non-European and international approaches to regulating biotechnology in the food and feed supply chain were compared, including contractual duties and possible liability issues that may arise. Complications arise in particular in international settings with differing national systems, and such problems are aggravated by the fact that market participants may develop overlapping contractual regimes deviating further, even though it may be easier for vertically integrated companies. It shows the unifying effect of EU laws on a side and of private standards on the other side.

While it is still unclear how losses caused to third parties will be resolved, particularly in cross-border cases, the solutions offered by each country's laws are strongly influenced by its political attitude towards GM farming in general, and may amount to a de facto obstacle thereto.

The survey of legal, technical and political issues arising from co-existence and traceability in third countries identified some examples of workable systems and best practices that EU Member States may use when implementing co-existence and traceability rules.

The analyses clearly showed large diversity in the extent to which third countries are considering introducing or in fact implementing co-existence measures, i.e. to maintain three supply chains. For candidate countries especially, a workable and reliable EU model would be highly appreciated.

Legal Approach to the Cost-Benefit Assessment of the GMO and non-GMO Channels Coexistence

As limits of the exercise, it is important to note that the following analysis is valid only in the case of coexistence of conventional or organic productions with authorized GMOs after well organized scientific assessments. The likelihood of unexpected damage resulting from a wrong evaluation will certainly not be taken into consideration, as such is not the aim of the legislation on coexistence. The only way it relates to risk issues is by allowing individual or collective reversibility of the GMO choice.

The issue consists in appraising the advantages and disadvantages of what has become a compulsory coexistence, as opposed to what would happen without such compulsion. Therefore, it does not cover the cost-profit analysis of the GMO versus the non-GMO channel.

This study aims at exploring the advantages and disadvantages of coexistence of the GMO and non-GMO channels from a legal point of view. In other words, it is concerned with comparing the situations of different operators when coexistence of both procedures is organized following a voluntary basis, as in Europe (laws and regulations which, when combined to contractual patterns, set obligations regarding the thresholds of fortuitous presence in the productions, traceability, labelling, cultural rules, etc.) in comparison with the same operators' situation when no legal obligation is imposed, as in the United States or Brazil.

Three preliminary observations were made.

- The first one concerned the limits of the technique of cost-benefit assessment.
- The second observation deled with the allocation of coexistence charges.
- The third observation stemmed from sheer modesty.

The report studied from an original viewpoint the cost benefits of GM and non-GM supply chains by studying the contestability of cost-benefit assessment in the observed economic model. The cost-benefits analysis was then made on the seeds "segment" (companies), examining the advantages and drawbacks for farmers. Downstream the situation of agro-industry operators and consumers were analysed. Afterwards, the cost-benefits analysis continued from a public authority's viewpoint. All these cost-benefits analyses, viewed from a legal point of view, were then replaced in the context of international trade and of the "globalised word" as well as in the society as a whole.

Conclusion

Generally speaking the activities, the activities on legal and contractual obligations have been completing an important work on liability and redress, funded by the EC and published in 2006, at the farms level. The work will be of particular importance for insurances and re-assurances companies, national compensation funds and cross-boarders disputes.

They showed that national and regional legislative frames can be upset by private stewardship. However new questions araised from the sustainability viewpoint of coexistence as society

characteristic which should be legally recognised. This work also pointed out those cost-benefits analyses can be examined as a whole societal project. This may impact for instance the determination of who will bear the putative costs' burdens for segregating GM and non-GM products.

Co-Extra data integration

Co-Extra has numerous products which need to be made more readily available to stakeholders and to testing laboratories. Accordingly a large part of Co-Extra work was dedicated to the integration of data into a tool that is more easily usable by stakeholders. This work was focused onto a user-friendly Decision Support System (DSS).

The outcomes of Co-Extra inform a whole range of stakeholders: farmers, EU policy makers, importers, transporters, feed/food producers, retailers, consumers, analytical laboratories, users of test reports from analytical laboratories, operators and managers of official control with science-based, ready to use information.

The Co-Extra Decision Support System integrates some results of the Co-Extra project (such as collected data, scientific findings, obtained knowledge and expertise, formulated recommendations, developed methods and models, etc.) in a way that is potentially useful for different types of stakeholders.

The DSS provides data and advice for various decision questions that occur in supply chains involving GMOs, for instance:

- Will my (intermediary) product, given a current set of used procedures and materials, contain GMOs below a specified threshold level?
- Is there any possibility that my (intermediary) product contains unapproved GMOs?
- Which methods perform best or can be used for a given analytical or sampling purpose?
- What are the costs associated with maintaining GMO content below specified thresholds?

Co-Extra was using the approach of model-based DSS. In collaboration between experts and decision analysts, Co-Extra created qualitative models that:

- capture and represent expert knowledge in the form of hierarchically structured variables and decision rules,
- are able to assess and evaluate decision alternatives, and
- provide decision-analytical tools to analyze these alternatives (for instance, finding the advantages and disadvantages of alternatives, and analyzing the effects of changes by "what-if" and sensitivity analysis).

Currently, eight models have been implemented:

- Analytical Models: two models aimed at the assessment of analytical methods, including DNA extraction and DNA analysis methods;
- Sampling Model: assessment of sampling plans;
- Unapproved GM Model: assessing the risk of contamination with unauthorized GMO varieties based on traceability data about the product (for instance, type of product, country of origin, type and mode of transportation);
- Transportation Model: assessment of potential GM presence due to transportation based on product traceability data;
- Dryer and Starch Models: assessing the effect of control parameters (such as using different strategies for handling GM and non-GM batches) to the collection and processing of maize.
- Regional Model: on unintended admixture in regional with maize crops.

All together these modules are currently pre-validated by Co-Extra partners. A second step of validation should be started as soon as possible with ENGL members and some stakeholders before any release.

Dialogue and communication

The genesis of the communication measures in the framework of the Co-Extra project was formed by public controversy on whether an agricultural system and the entire food and feed supply chain with and without GMO products can co-exist.

In countries such as Germany, Spain, France and United Kingdom, field trials have been conducted to evaluate appropriate cropping measures, such as isolation distances between GMO and non-GMO fields. A European research program SIGMEA was specifically dedicated to these issues.

Nevertheless, great disagreement remained between political parties and professional guilds with regard to the management of co-existence. It was foreseeable that public discussion on co-existence would become more intensive. Consumers feared the end of non-GMO agriculture and freedom of choice.

Among others, a reason for this situation was the lack of available information on actual research results as well as of exchange of opinions and attitudes among relevant stakeholders. To date, science-based information on the co-existence of GMO- and non-GMO agriculture and supply chains within the Community hardly has reached its target groups, i.e. the European consumer and the open public. Public perception of these facts therefore is rather selective: 'risk' and 'limited choice' concerns evoke a much larger reaction than science-based explanations and rules, which intensifies a feeling of uncertainty in many consumers. Information must be better adapted to demand – and this means to fit the expectations, needs, interests, and knowledge backgrounds of consumers and relevant stakeholders.

Closing this gap would help the political system to devise practical and harmonised European rules for co-existence and would support consumers and other stakeholders to make informed decisions. Also missing were EU-wide regional stakeholder platforms that focus on the co-existence of supply chains and that could help to reach an understanding among the various standpoints.

Therefore, the main task of Co-Extra was to inform stakeholders about recent developments on coexistence and traceability issues in EU member states and to present the research results of the Co-Extra project on the website.

Communication work via Co-Extra's website:

Communicating and interacting with the public about research is of vital importance. Science that is communicated poorly remains unrecognised. Participants in EU-funded projects are encouraged by the European Commission to increase and enhance communication on science and research by paying particular attention to the "public communication" dimension of their work.

Disseminating and facilitating access to science-based information has been therefore one of the major objectives of Co-Extra, a European-funded project addressing the co-existence of genetically modified organisms and non-genetically modified organisms supply chains in Europe as well as their traceability. To this end, a dynamic and interactive website (http://www.coextra.eu) has been developed as the core element of the Co-Extra external communication strategy. This website has been designed to be attractive and accessible to a large audience in a very simple and practical manner. It builds upon practical experience gained in the development of other websites related to biotechnology and genetically modified organisms.

The website was developed by taking into account that "the public" is not a homogeneous population but rather encompasses numerous sub-groups. Each group constitutes a distinct audience that seeks information with an appropriate level of detail to answer questions and address concerns. Accordingly, The Co-Extra website has been structured to allow 3 main readership levels:

- Level 1, corresponding to the most accessible pages and providing general and popularised information (such as news and reportages);
- Level 2, offering information for non-specialists about the dozens of research projects within Co-Extra;
- Level 3, providing for the more expert readers the detailed scientific data from the running projects including the most recent results, reports and publications, and the list of partners/institutions involved.

Another important aspect of the website is that it supplies background information on progress on the implementation of coexistence and traceability measures in various European countries ("country sections"). This part of the website is available in several European languages in order to overcome potential barriers to users by allowing access to local information in their native language.

Last but not least, the website also provides various permanent tools that allow multidirectional interaction with its visitors (electronic newsletter, online discussion forum).

Content is displayed using a web-based platform, based on a sophisticated Content Management System. In order to maintain consistent management policy in content edition, an Editorial Office (responsible for the public information layer) and an Editorial Board (responsible for the review and endorsement of certain types of documents prior to publication) have been established.

In order to make the communication activities more attractive, multimedia tools have been integrated on Co-Extra's website. This includes video interviews on the main outcomes of the research project as well as video reportages on main events of Co-Extra (final conference, stakeholder days in York, UK and Buenos Aires,

Argentina). Additionally, interactive cartoon animations (Adobe flash-based) demonstrate the principal topics, issues and research approaches

Networking and organizing collection and dissemination of information on coexistence and traceability topics and issues

National relays were the national partners responsible for the organisation of stakeholder networks in their countries, i.e. to retrieve relevant information from their countries and to disseminate information on Co-Extra's outcomes and activities to national stakeholders and authorities. These relays then reported to the editorial office, which published relevant information on Co-Extra's website.

Another task was the analysis of stakeholder opinions and attitudes towards coexistence and traceability issues in order to streamline and dovetail the research project with expectations and needs of stakeholders. The results of these activities were directly integrated into the work agenda and items of the several other parts of Co-Extra.

The results on consumers and stakeholders opinions and attitudes are integrated in a part above.

Conclusion and perspectives

Co-Extra is the largest EC funded project on co-existence and traceability of GM and non-GM supply chains.

The Co-Extra project addressed for the first time the whole issue of coexistence of GM and non-GM supply chains by examining the practices of the supply chains from seed production to retailers' shelves with practical implementation tools such as documentary and analytical tools for supporting coexistence of supply chains. This coordinated and fruitful way of working was possible only due to the size of the Co-Extra Integrated project. In this way, the launch of such large research projects should be continued and small, fragmented research projects should be avoided where possible.

It shows that GM and non-GM supply chains coexistence, even at the farm level, cannot be addressed by studying its different components separately. Coexistence issues have to be addressed by multidisciplinary teams.

Co-Extra underlined several basic economic and legal facts. Co-Existence cannot exist without an economic valorisation of the whole supply chain which could imply, for instance, labelling of animals fed with and/or without GMOs. Co-existence also cannot exist without a sustainable availability of low-cost non-GM seeds integrating the latest genetic improvements.

More specifically, if Co-Extra focused on coexistence of GMO and non-GMO supply chains, its results can apply to most of supply chains with quality and/or safety requirements. Generally speaking, the methods, strategies, tools, models developed in Co-Extra for GM and non-GM supply chains co-existence and traceability will be used in the management of numerous other supply chains, value added and niche markets, and for detecting and excluding harmful products such as allergens and mycotoxin producing organisms or pathogens.

Traceability (on both analytical and documentary viewpoints) is a major segregation tool, for coexistence. Traceability has been studied on a regulatory viewpoint and also for its economic and social function: allowing trust to be established between actors and about activities presenting risks for admixture. We showed that, at the intersection of knowledge and risk, legal systems are trying to establish confidence in a society that links the two.

Co-Extra conducted experimental work, pollen flow models, and economic analyses to provide information for optimising segregation strategies upstream and downstream. The project released numerous technical and legal results aimed at optimising coexistence and traceability procedures and costs.

Co-Extra also developed new strategies for detecting stacked or unapproved GMOs. The EU unapproved GMO (UGM), be them resulting from asynchronous approvals or worldwide unapproved, remains an issue which should be globally considered. Detection methods have been developed which are applicable by routine or research laboratories, depending on the UGM status, safety reasons and costs to be engaged according to Competent Authorities' decisions. However, developing detection methods for detecting UGM are not of the remits of the CRL-GMFF. Co-Extra showed that such developments for detecting as a whole UGM is manageable. It thus seems important to modify the mandate of CRL-GMFF to let it develop and validate generic methods for detecting UGM, i.e. screening and construct specific GMO as well as taxa including donor organism detection methods. The corresponding strategies, Decision Support System and any tool able to harmonise reporting and decision making should be also included in the new remits.

Co-Extra was the first attempt to take into account the several stakeholders' practices, from seeds to shelves, through consumer surveys, companies' interviews and stakeholder focus groups, for developing practical solutions. Co-Extra explored the current practices in the EU and third countries, as well as the traders' practices, the bottlenecks and then proposed solutions. Co-Extra described the processes, developed models and strategies and tested several ones.

Co-Extra confirmed that stakeholders are generally using a practical contractual threshold of ca. 0.1%, well below the 0.9% European labelling threshold. That means that the co-existence between open pollinated crops is only possible by using either large distance of isolation or production of GM and non-GM products in dedicated areas, as determined by the models developed in EC-funded SIGMEA project. The technical and legal definitions of such dedicated production areas remain open. Biocontainment methods can be used to reduce isolation distances, but will require their commercial availability when proved to be stable and effective.

The current increase of GMOs with stacked genes is, due to the DNA unit used for traceability along the supply chains, rapidly decreasing the coexistence possibilities in fields. What will directly again increase the necessary large isolation distances, and / or increase the distances between dedicated production areas.

Several general societal questions can be raised from such facts or from basic questions reminded by Co-Extra, for instance should the sustainability of non-GM seeds only be market-driven or should public institutes be involved in non-GM varieties production? The same may apply to the availability of biocontainment methods, which may appear necessary, probably in a stacked way, for increasing the security of field co-existence but are all owned by companies and will probably not be easily made available to farmers, except in a few cases such as containment of small scale fields devoted to non-food production such as pharmaceuticals. Other general societal questions or proposals have been made by Co-Extra such as extending liability without fault and compensation schemes, already in place for farmers in some Member States, to the whole supply chains thus downstream from the farms. This proposal of Co-Extra to policy makers may allow a smoother development of non-GM supply chains by sharing any additional costs of non-GM supply chains between GM and non-GM supply chains thus avoiding specific consumers paying the presumable additional costs. Developing a societal, instead of a purely economical response to what appears to be a societal question, through the reluctance of European consumers to consume GMO, is one of the solutions explored by Co-Extra.

The legal studies such as cross-border disputes, liability and redress issues, coexistence as a societal model for sustainability are providing numerous insights into current questions but are also opening up new fields of research.

Similarly to these previous research programs, Co-Extra results can be expected to impact national and European legal frameworks as well as stakeholders' attitudes and practices, (including consumers). In this way, the communication and dialogue work of Co-Extra should continue.

Co-Extra particularly showed that the operators are using stewardship, for instance as sampling methods, involving practical contractual thresholds of GMO content, for instance through GAFTA agreement, independently from national legislations. This practical threshold of ca 0.1% has a huge impact on fields' outcomes content and future European seed thresholds for fortuitous and technically unavoidable content of approved GMO. The rationale of those contractual thresholds is due to both sampling and measurement uncertainties as well as pollen flow on very long distance, as observed for maize. As already said above, according to the models developed by SIGMEA, coexistence appears feasible only by using large isolation distances or dedicated production (GM or non-GM) areas.

Biocontainment measures may help to increase the flexibility of coexistence at the field level. The stacking of biocontainment systems is required, particularly when considering the development of non-food/ non feed GMO cropping, the newcomers in our game. However, the accuracy of those biocontainment systems might need to be assessed again or in some instances still to be developed. Plastid transformation may also have significant impact on the implementation of labelling or other thresholds relying on analytical methods, thus potentially creating conflicts of interests. The schedule of European coexistence implementation raises questions on the interests of working on methods which might be available after several years and their degrees of acceptance by farmers depending on the technology and "natural" or not source (e.g. CMS *versus* GURT technologies). In a market driven by seeds and varieties with numerous patents, it is indeed questionable whether the development of such "societal tools", as regards coexistence, should not be mostly driven by public research probably after some legal actions.

Furthermore, work should continue for instance on how to sustainably continue to provide non-GM seeds. Co-Extra underlined that coexistence is only feasible if non-GM seeds are available whilst a large concentration of seed producers have emerged over the last decade but offering a smaller and smaller supply of non-GM varieties. The observed withdrawal of public-funded research from the development of seed varieties raises again the question of the sustainability of coexistence.

All together, there are numerous economic and legal questions raised by coexistence, which are more the remit of the European Competent Authorities than of scientists.

The putative costs of coexistence measures have to be quantitatively and accurately measured and their distribution assessed to impede unfair charging to some supply chains and consequently to consumers. Cost-benefits of supply chains coexistence and traceability should be better assessed by taking into account the application of European general directives and regulations impacting their coexistence and traceability. Generally speaking, the socio-economic aspects of coexistence, from seeds to shelves, need to be better assessed.

The detection methods for analytically tracing GMO are more and more accurate and able to provide reliable information to end-users and consumers. Due to the development of numerous multiplexed detection techniques and the important use of documentary traceability by the operators, the economic impact is not estimated to increase final costs.

Thus this GMO based study is providing information of value for developing safer and better food and feed supply chains.

This can also be compared with the development of PCR methods for managing supply chains and standardising of PCR testing methods in 1999. Co-Extra adds value to the FP5 research programs QPCRGMOFOOD and GMOchips, by providing information of value for national and EU legislative frameworks and also for supply chains management.

Due to the large number of issues embraced by Co-Extra, a Decision Support System has been developed to integrate data and facilitate use of several tools by stakeholders from companies to laboratory analysts. Full validation of the DSS remains to be carried out after the current on-going pre-validation.

However, some issues, such as (i) how to deal with "botanical impurities" in routine analyses (in relation with LLP issues), (ii) how to technically, economically and legally manage coexistence in the fields with large isolation distance or dedicated productions areas are still pending and thus should be further researched from scientific, technical, economic and legal viewpoints. For instance, studies about large distance dissemination of viable pollen flows on fragmented landscape should be continued.

It is thus recommended:

- That such large integrated research work on supply chains coexistence should be continued as all coexistence issues are interrelated and cannot be addressed separately. It should thus continue to enable all concerned parties to work together, i.e. from agronomists to lawyers and controls' analysts.
- Such integrated work on coexistence and traceability should embrace more global issues, not only EU related ones. Global joint research and networking has to be done.
- More generally speaking, coexistence and traceability of supply chains, from seeds to shelves, with less specific focus on the GM aspect should be studied. This has already been done for instance in some other EU research project such as TRACE but should address very different issues and better harmonisation should be sought.
- That Coexistence at the field level integrates more into its research environment biotech and seeds area structures and strategies and their impacts on availability of usable tools, such as biocontainment tools.
- To study more in depth the dispersal of viable pollen on large distance over fragmented landscape for several cropping plants, thus not restricted to the currently approved GM species (maize) and the corresponding models.
- To retrieve in a GIS based (web interfaced) central repository system, preferably operated by the European JRC, all coexistence data, resulting from for instance cropping in Spain.
- To prepare a central repository GIS based (web interfaced) central repository system, preferably operated by the European JRC for Post-Market Environmental Monitoring for both Case Specific Monitoring and General Surveillance able to integrate data and increase transparency towards citizens. Monitoring might be partly funded by applicants, on the model of participation to fees to GMO detection methods validation and data provided by existing networks of Competent Authorities, scientific studies and citizens' networks which recruitment and training should be carried out in cooperation with biodiversity general studies.
- To study from a scientific, technical, economic and legal point of view:
  - The ability to have either large isolation distance between GM and non-GM crops, or to develop dedicated production areas, for countries they will follow this possibility;
  - The ability to sustainably maintain sources of non-GM seeds integrating the latest genetic improvements, when considering companies' strategies, breeding schemes according to plants' biology and patents owned by biotech and seeds companies;
  - The ability to use in acceptable way farmers and society, to stack different biocontainment methods, for both Food & Feed dedicated GMOs and GMO developed for non-food purposes. It shall be kept in mind that all the biocontainment methods are the property of companies, be there classical ones as CMS or new ones as plastids transformation which almost all methods are patented.

It is clear that it is necessary to rapidly determine future research, but probably mostly expertise from the fields for rapid implementation of coexistence from seeds to shelves. However, before launching new research or expertise actions, it is highly probable that Competent Authorities will take several decisions, such as determining the GMO fortuitous presence level(s) in seeds. The preferred hypothesis of dedicated production areas *versus* coexistence using large isolation distances should be preliminary decided. Public tools to facilitate non-GM seed provision and maybe biocontainment publicly available should be considered before any additional work on coexistence is done. It means that is necessary that decisions and harmonisation of coexistence rules should be taken and proposed to Member States in order to restrict to a minimum the expertise or research fields to be further launched. Several decisions taken in advance by European CA should pave the way to coexistence in a rapid, time- as well as in a cost-efficient way. Keeping too many large possibilities clearly hamper the ability to rapidly find solutions to coexistence issues.

Contractors involved

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Project logo

Reference to the project public website

The public website is available at: http://www.coextra.eu