

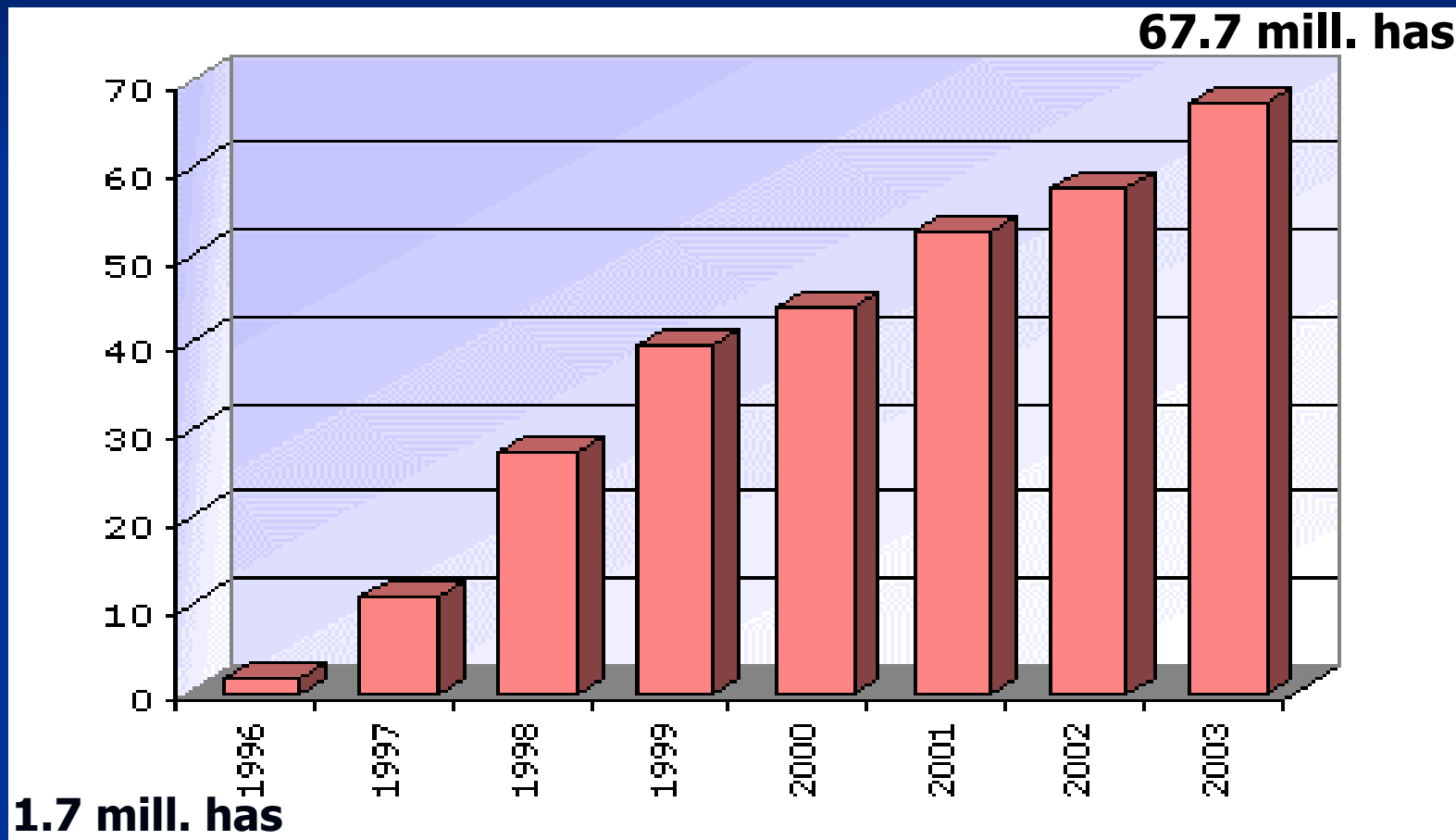
Detection and quantitation of GMO in official food and feed control

Sven Pecoraro

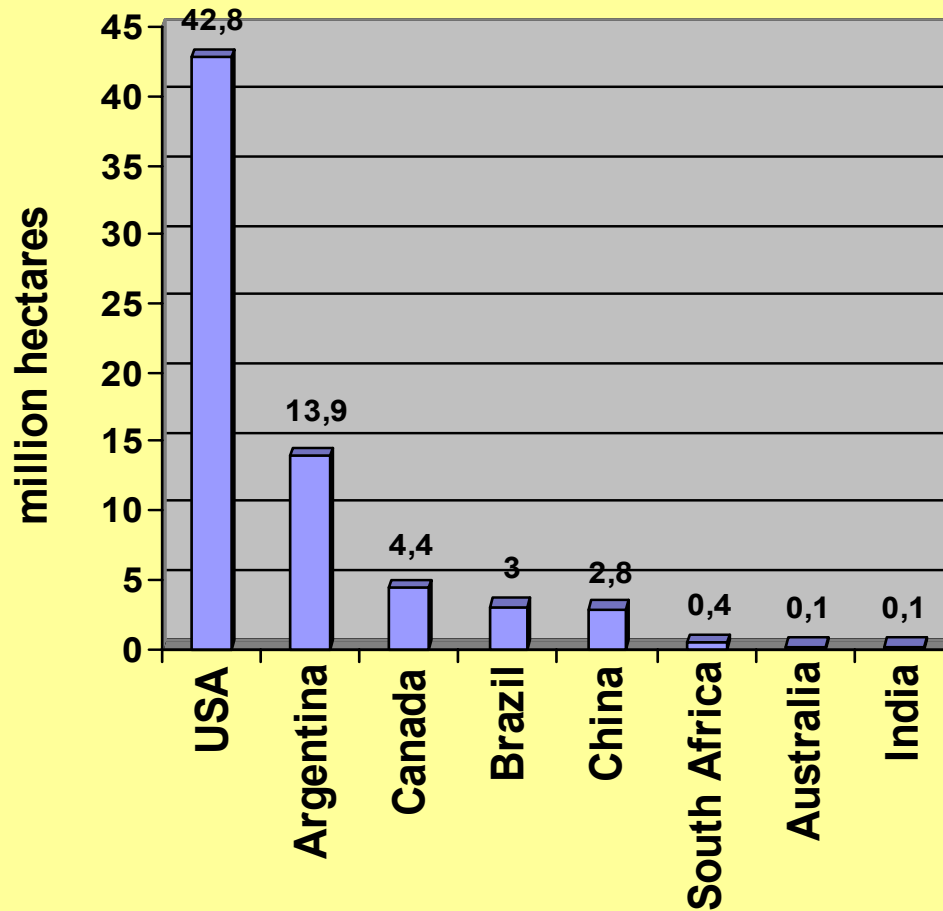


Bavarian Health and Food Safety Authority (LGL)

Worldwide acreage of genetically modified crops 1996-2003 in Mio. hectares:



Acreage of genetically modified crops per country in Mio. hectares, 2003 :

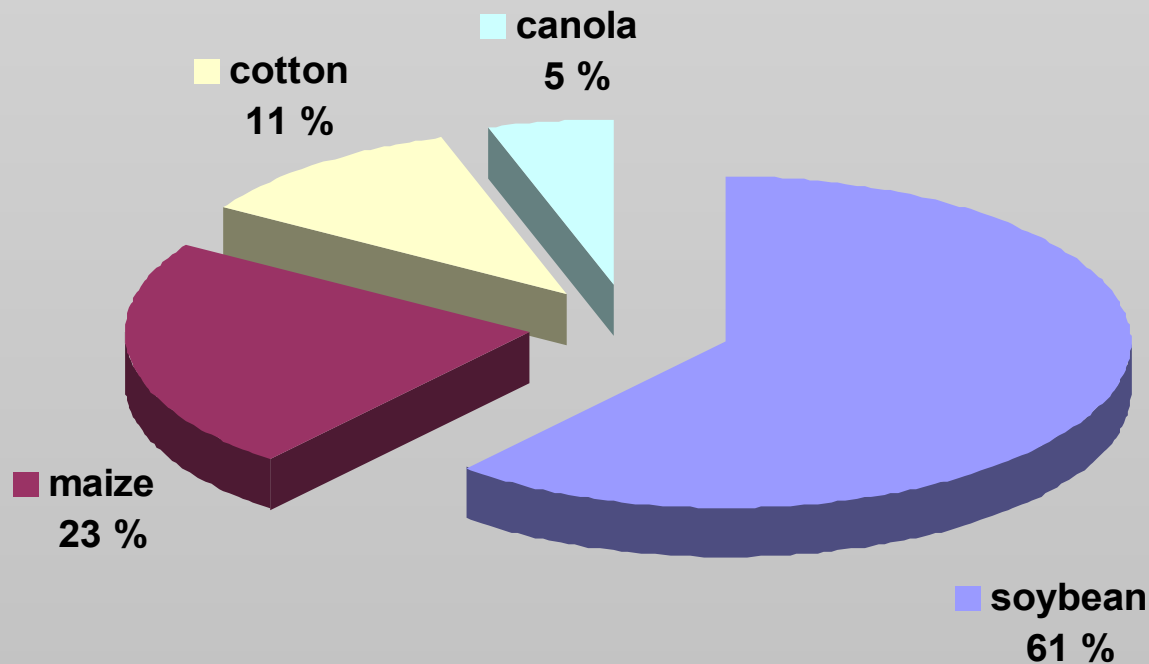


2003:

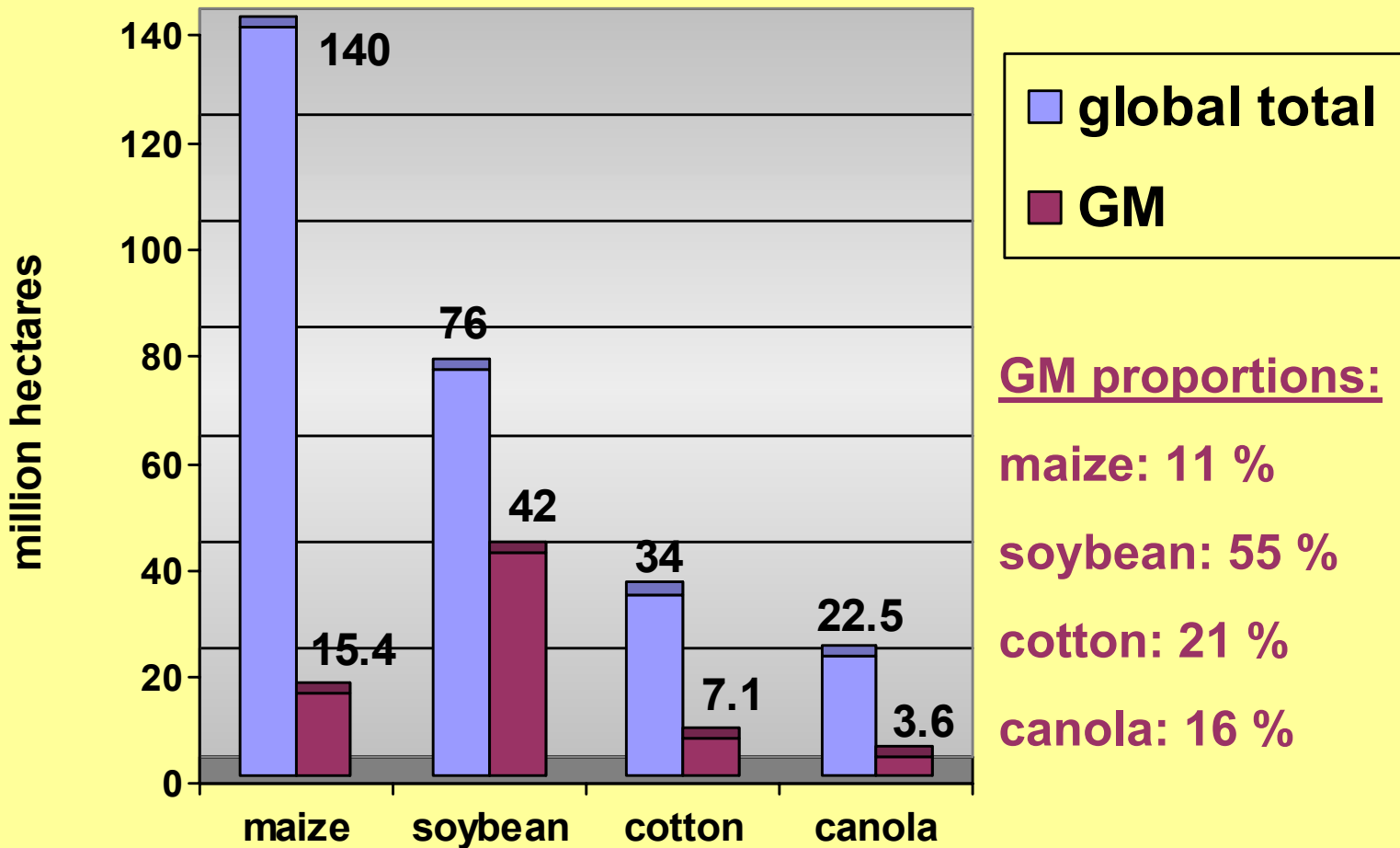
**18 countries
were growing
GM crops**

GM crops that are worldwide extensively grown:

Total GM area 2003: 67.7 mill. has
4 plants cover almost 100 % of the global GM crop acreage



Proportions of grown GM crops related to conventional crops, 2003:



What other GM plants are on the world market ? (I)

plant	charakteristic trait
adzuki bean	insect resistance
broccoli	herbicide tolerance (phosphinocithrin acetyltransferase)
canola	modified seed fatty acid content (high levels of lauric acid), herbicide resistance (glufosinat-ammonium; glyphosate, Roundup™)
cantaloupe	delayed fruit ripening due to low ethylene production
carnation	altered flower colour
cauliflower	herbicide tolerance (phosphinocithrin acetyltransferase)
chicory	herbicide tolerance (phosphinocithrin acetyltransferase)
chrysanthemum	viroid resistance
corn	insect resistance, herbicide tolerance (phosphinocithrin acetyltransferase)
cotton	herbicide tolerance, (5-enoylpyrovylshikimate-3-phosphate synthase)
cucumber	fungus resistance
flax	tolerance to triasilfuron, metsulfuron-methyl

What other GM plants are on the world market ? (II)

plant	characteristic trait
melon	virus resistance
papaya	resistance to Papaya Ring Spot Virus
petunia	altered flower colour
potato	insect resistance, resistance to Potato Virus Y
rice	herbicide tolerance, (5-enoylpyrovylshikimate-3-phosphate synthase), altered glutelin content, virus resistance (Rice Stripe Virus), reduced allergenicity (antisense albumin),
soybean	herbicide resistance (glyphosate, Roundup™); increased production of oleic acid in seed (desaturase)
squash	virus resistance (CMV, ZYMV, WMV2)
sugarbeet	herbicide resistance (glufosinat-ammonium; glyphosate, Roundup™)
sweet pepper	virus resistance (Cucumber Mosaic Virus, CMV)
tabacco	Production of low level of nicotine
tomato	delayed ripening, virus resistance (Cucumber Mosaic Virus, CMV)
torenia	altered flower colour

Requirements to put a GMO on the European market

General aspects:

- production, trading, cultivating of a GMO is **forbidden unless it is explicitly allowed**
- **authorisation** procedure required,

including

- **risk assessment** (European Food Safety Authority, EFSA)

New European legislation on GMO (I)

From **April 2004** on: New EU regulations on GM food and feed expand existing regulations

- **(EG) Nr. 1829/2003**

regulation of the European Parliament and the Council **on genetically modified food and feed**

→ lays down Community procedures for the authorisation and supervision of genetically modified food and feed

→ lays down provisions for the labelling of GM food and feed

New European legislation on GMO (II)

- (EG) Nr. 1830/2003

regulation of the European Parliament and the Council concerning the **traceability and labelling of GMOs and the traceability of food and feed products produced from genetically modified organisms** and amending Directive 2001/18/EC

→ framework for the traceability of products consisting of or containing GMOs, and food and feed produced from GMOs; facilitating accurate labelling, monitoring the effects on the environment, withdrawal of a product if necessary

New European legislation on GMO (III)

- (EG) Nr. 65/2004

regulation concerning a system for the development and assignment of **unique identifiers for GMOs**

➤ Broad prescription for **authorisation, traceability** and **labelling** of GMOs

New European legislation on GMO (III)

- **Liability of labelling any GMO and GM food/feed product** regardless of the feasibility to analytically detect GM DNA / protein in the product
- **Feed is treated as food** in terms of authorisation, traceability and labelling
- **Threshold 0.9 %** related to the individually considered ingredient; provision: the presence is **adventitious or technically unavoidable** (approved GMO)
- The **authorisation** granted is **valid 10 years** throughout the community and is renewable

New European legislation on GMO (IV)

- **Community register** will be available with information about genetically modified food and feed
- System for the **tracability** and **monitoring** of a GMO
- **Unique identifier** for each GMO
- ! Applicants will provide **methods for the detection, sampling and identification of the transformation event** to the European Food Safety Authority, EFSA
European Network of GMO Laboratories, ENGL:
validation of detection methods, evaluation of data
- ! Applicants will provide **samples** of their food/feed and their control samples

What has to be labeled ? (I)

food or feed that

- is a GMO (e.g. tomato, corncob, potato)

What has to be labeled ? (I)

food or feed that

- is a GMO (e.g. tomato, corncob, potato)
- that contains or consist of a GMO (e.g. yoghurt with GM *Lactobacilli*)

What has to be labeled ? (I)

food or feed that

- is a GMO (e.g. tomato, corncob, potato)
- that contains or consist of a GMO (e.g. yoghurt with GM *Lactobacilli*)
- that is produced from or contains ingredients produced from a GMO (e.g. oil from GM canola)

What has to be labeled ? (II)

- **food**
- **food ingredients**
- **food additives**
- **feed**
- **feed additives**
- **flavouring**

What has to be labeled ? (III)

GMO type	Example	present	future
GMO plant	chicory	yes	yes
GMO seed	maize kernel, canola seed	yes	yes
GMO food	maize, soybean, tomato	yes	yes
Food produced from a GMO	maize meal	yes	yes
	refined oil from canola, maize, soybean	no	yes
	glucose sirup	no	yes
food from animals fed with GM feed	meat, eggs, milk	no	no
food produced with enzymes from GMOs	cheese (chymosine), bread (amylase)	no	no
additives or flavouring from GMO	highly pure lecithine in chocolate	no	yes
GMO feed	maize	no	yes
feed produced from GMOs	maize gluten, soybean shred	no	yes
feed additives produced from GMOs	vitamine B2 (riboflavine)	no	yes

Defined phrasing of labelling:

'genetically modified'

or

'produced from genetically modified (name of ingredient)'

shall appear in the list of ingredients
immediately following the ingredient concerned

Tasks of the official food and feed control

- **Control of labelling** of food and feed by **qualitative and quantitative PCR analysis** for approved GMOs (e.g. maize, soybean)
- control of producer's documents for traceability of GMOs (certificates, IP systems, PCR results etc.)
- also: check for non approved GMOs

2004: detection of non approved (Europe) GM papaya from Hawaii, USA

Approved GM Food in the European Union:

- **1 soybean:** GTS 40-3-2; Roundup Ready™
Soybean; herbicide tolerance
- **5 maize:** Bt 176, Bt 11, MON 810, T 25, MON 809
herbicide tolerance, insect resistance
- **7 canola:** e.g. MS1xRF1, Topas 19/2,
herbicide tolerance
- **2 cotton:** oil from cotton seed line 1445 and 531
herbicide tolerance, insect resistance

herbicide tolerance:

- gluphosinate, BASTA™, Liberty Link™:
→ tolerance: bar gene from *Streptomyces spec.*, enzyme acetylates gluphosinate
- glyphosate, Roundup™:
→ tolerance: epsps gene from plant/MOs, enzyme with reduced affinity to glyphosate

insect resistance:

Various protein toxins from *Bacillus thuringiensis* which protect against insects like the *corn borer*

Basic strategies for GMO detection I :

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1.) **screening**: testing for a genetic element that is common in GMOs: e.g.

- **35S promoter**: from Cauliflower Mosaic Virus

- **T-nos terminator**: from *Agrobacterium tumefaciens*

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- 2.) **construct** specific test: detecting sequences within a GM construct; e.g. sequence of a junction between connected genes

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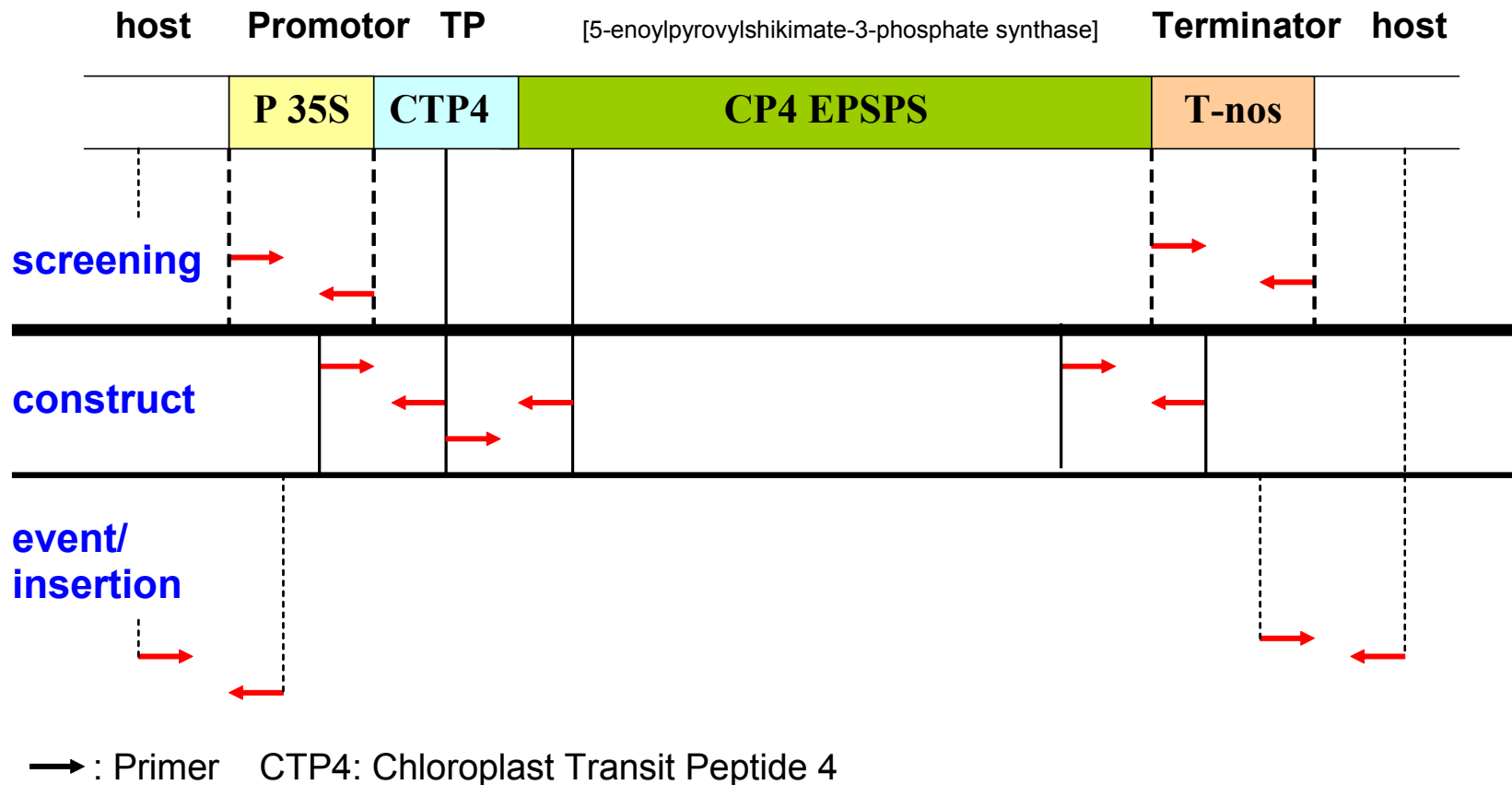
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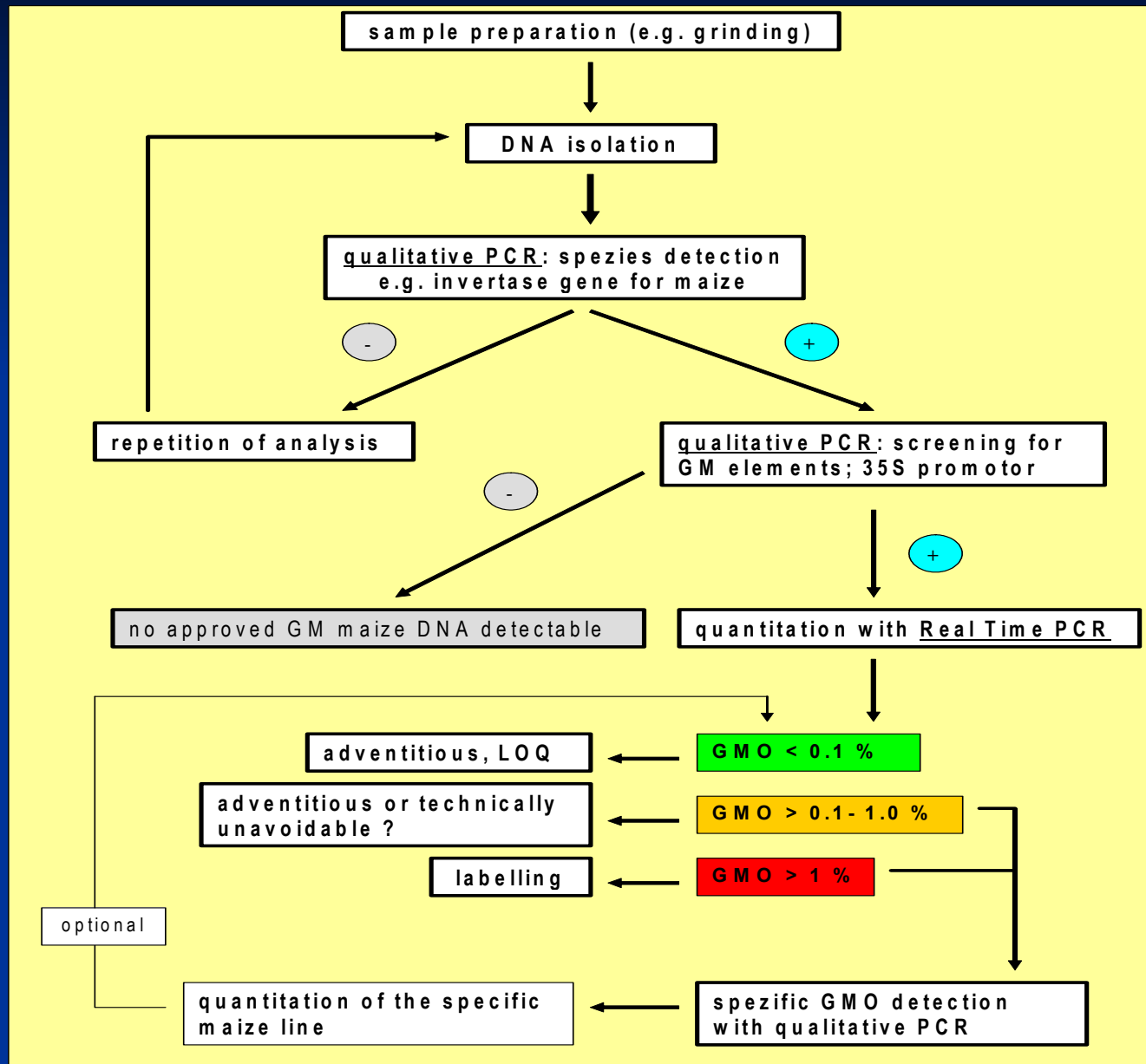
3.) **event** specific test: detecting specific sequences of the insertion site of the GM construct into the host genome = unique to a certain GM plant

Basic strategies for GMO detection II :

herbicide tolerance (glyphosate; Roundup™)



Detection of GM maize in food/feed

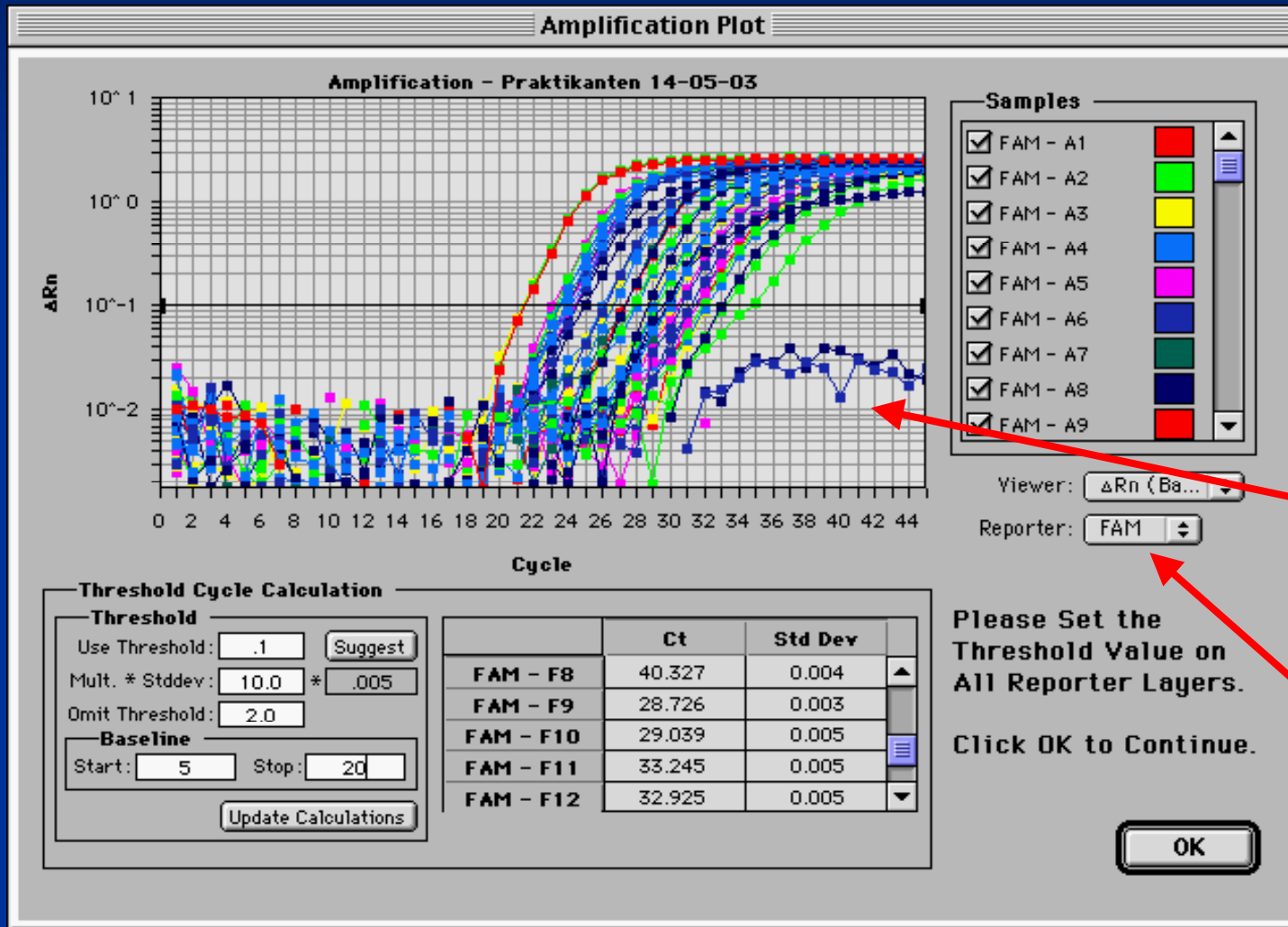


Problems with qualitative and quantitative PCR in food/feed

- Highly processed material
- Degraded DNA
- No DNA detectable (e.g. oil from canola)
- PCR inhibiting substances

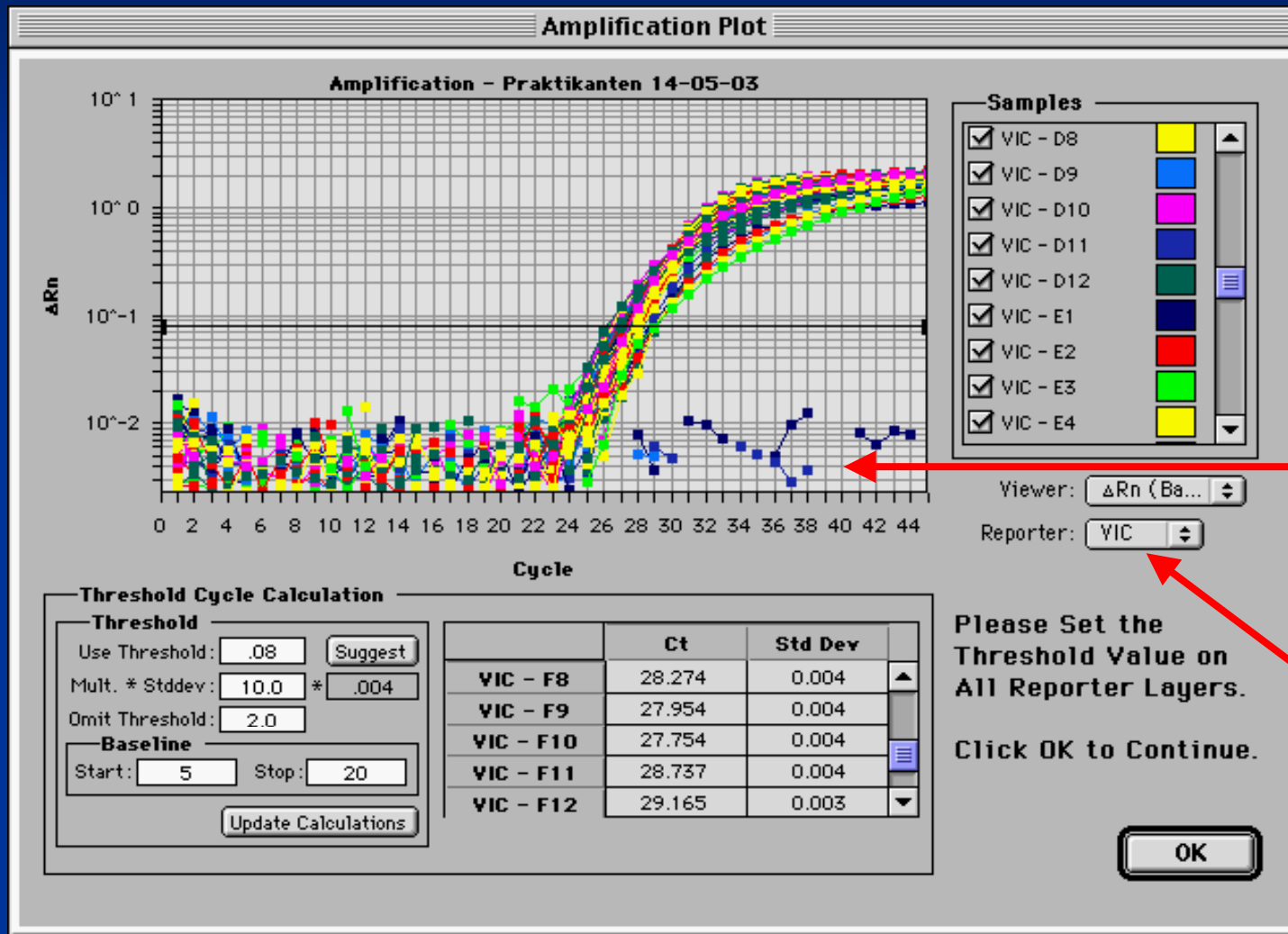
Inhibition of qPCR (I)

Negative reaction might be false negative



Inhibition of qPCR (II)

Internal positive control (IPC) detects PCR inhibition



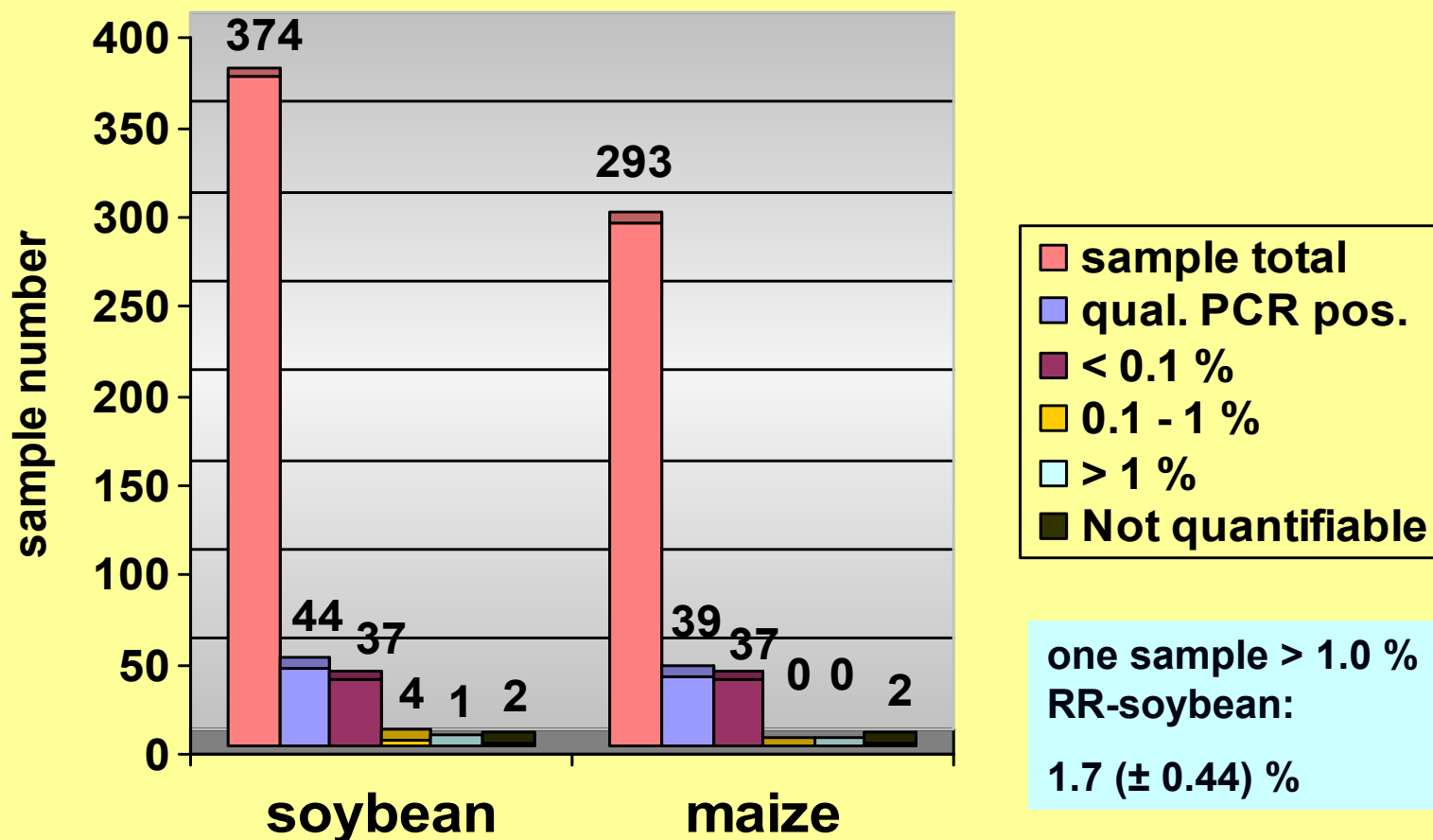
IPC:
inhibition

VIC dye

GMO qPCR results in food in Bavaria

2003:

656 samples analysed containing soy and/or maize



GMO qPCR results in feed in Bavaria 2003:

	sample total	qualitative PCR		qPCR
		non GMO	GMO	
maize	15	12	3	< 0,1 %
soy	20	6	14	4 samples: 1,3 - 12 % 3 samples: 22 - 30 % 1 sample: 60 % 6 samples: 80 -100 %

Thank you