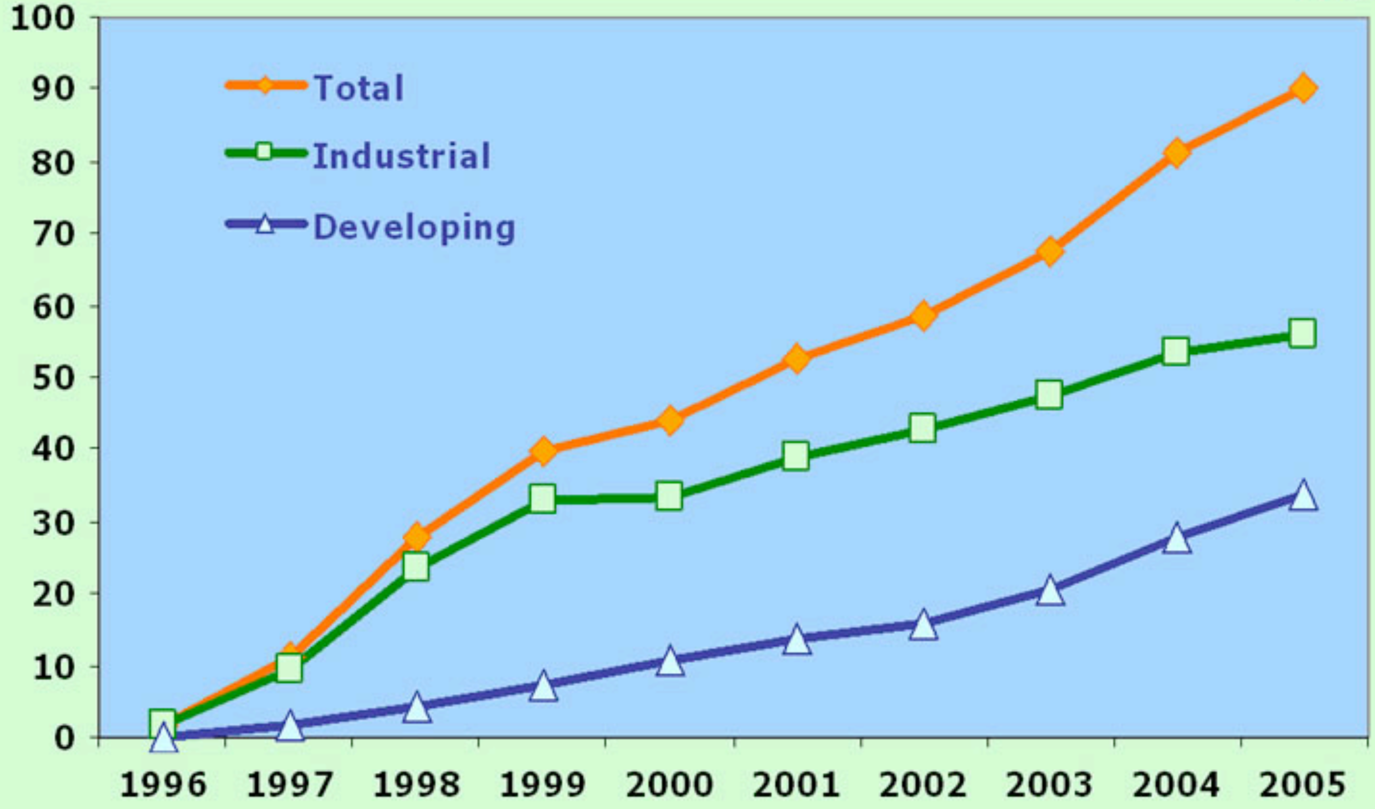
GMOs and the Environment:

What are the issues?

Angelika Hilbeck

GLOBAL AREA OF BIOTECH CROPS

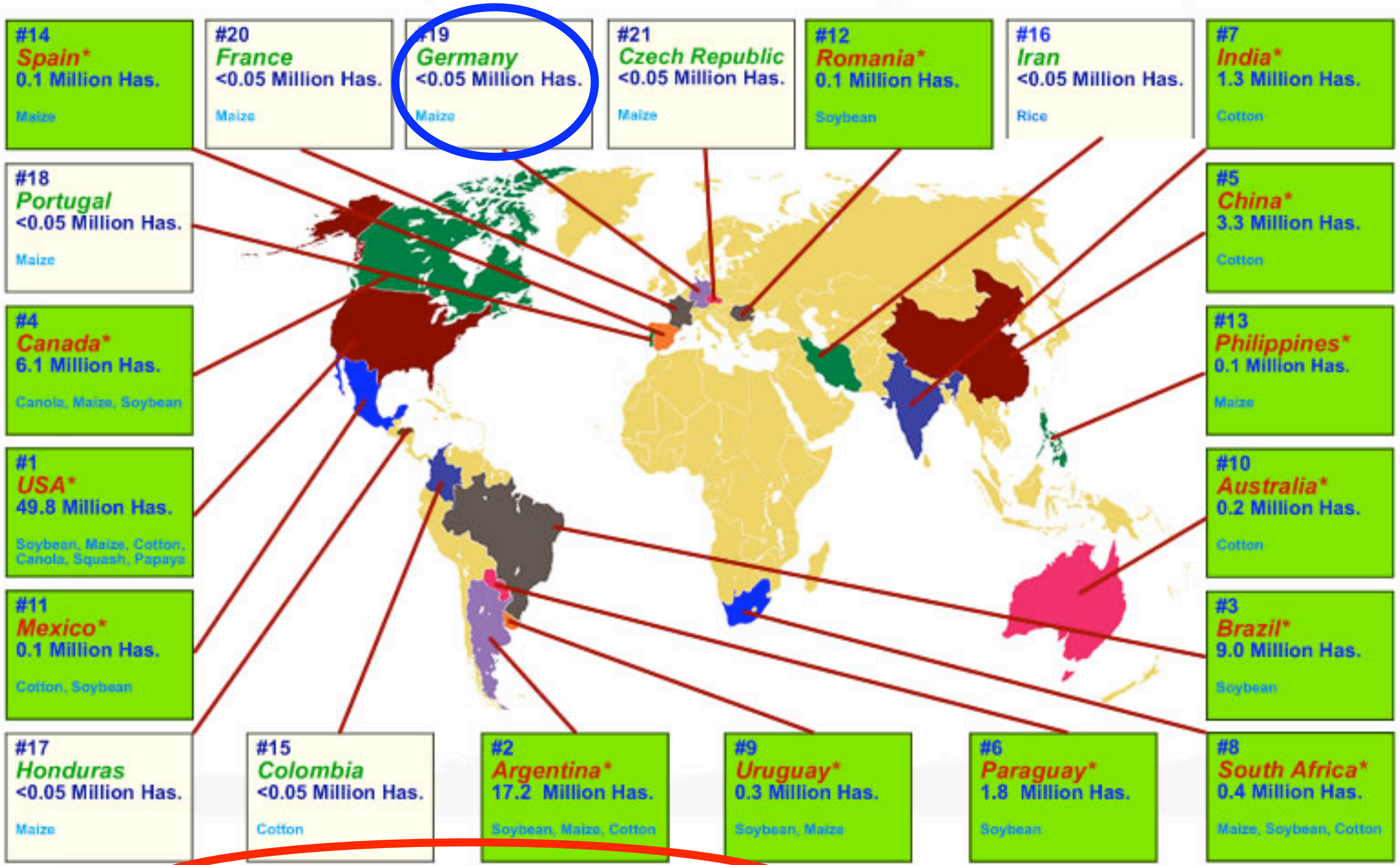
Million Hectares (1996 to 2005)



Increase of 11%, 9.0 million hectares or 22 million acres, between 2004 and 2005.

Source: Clive James, 2005

21 Biotech Crop Countries and Mega-Countries*, 2005



* 14 biotech mega-countries growing 50,000 hectares, or more, of biotech crops.

Source: Clive James, 2005

Same 3-5 countries grow same GM crops since 5-10 years!

USA	49.8 (47.6)	55%	Soya, Maize, Cotton, Oilseed rape
Argentina	17.2 (16.2)	19% 74%	Soya, Maize, Cotton
Canada	6.0 (5.4)	6% 80%	Oilseed rape, Maize
Brazil	9.0 (5.0)	10% 90%	Soya (2003 legal)
China	3.3	4% 94%	Cotton

Same 4 GM crop plants dominate since 10 years!

Soybeans	60%
Maize	23%
Cotton	11%
Oilseed rape	6%
Other	<1%

Same 2 transgenic traits dominate since 10 years!

Herbicide resistance	ca. 60%
Insect resistance	ca. 16%
Combination of both	ca. 7%
Other	<1%

BUT:

Great diversity of traits and genetically engineered organisms (plants, animals and micro-organisms) are under development

Most promising in terms of expected profits are pharmaceutical traits (enzymes, vaccines, etc.), industrial products (e.g. spider silk production in plants and animals (goats), amylopectin producing potatoes, etc.)

Why are environmental risks of GMOs discussed?

Constant influx of novel 'trans'genes and 'trans'gene combinations (traits) into the (agro-)ecosystem, and beyond, into natural and semi-natural ecosystems.

Release of self-reproducible biological organisms is potentially irreversible and adds a dimension in complexity to previous technology introductions.

What environmental risks are discussed and investigated?

How will the introduction of novel GM organisms alter and impact agriculture, biodiversity and its functions, evolutionary and ecological processes?

→ Very long term processes!

Difficult to predict!

Ecosystem services from biodiversity

- Maintain soil fertility
- Protect against soil erosion
- Maintain water quality
- Provide flood control
- Detoxify pesticides
- Natural enemies
- Source of genetic material
- Contribute to ecosystem resilience and stability

How can unwanted environmental consequences of GMOs come about?

Spread of a) transgenes and b) transgene products

Involved issues:

a) Transgenes

Unexpected pathways of spread via seeds, pollen, vegetative parts. Can be animal-driven BUT main driving factor: HUMANS and human error!!

b) Transgene products

- Input routes into ecosystem and cycling
- Metabolic products (degradation)
- Biological activity

Transgene spread as of today...

Found on Biosafety Clearinghouse website of Cartagena Protocol for Biosafety

Example I) OSR in Canada:

Late 90ies: double and triple resistant OSR plants within only 3-4 years since beginning of commercial GM OSR production

(Hall, L., Topinka, K., Huffman, J., Davis, L. & Good, A. (2000) Pollen flow between herbicide-resistant Brassica napus is the cause of multiple-resistant B.napus volunteers. Weed Science 48: 688-694)

Early 2000: Transgene spread and seed contamination in Canada progressed to a point where in most regions no GM-free OSR production possible anymore.

(Lyle F. Friesen*, Alison G. Nelson and Rene C. Van Acker. (2003). Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. Agronomy Journal. Canada)

Lesson learned: GM-traits can spread within shortest time under unrestricted, side-by-side production without any coexistence rules.

Example II) Bt-Maize in Mexico – an unresolved issue...

2001:

Quist, D, & Chapela, I.H. (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. Nature 414: 541-543.

„The Mexican government has confirmed earlier reports that transgenic maize is growing within the country's borders and has apparently contaminated wild varieties, despite a national ban on the cultivation of genetically modified (GM) crops.

A government-commissioned study has shown that as many as 95 per cent of maize fields in the Mexican states of Oaxaca and Pueblo contain evidence of GM 'contamination'— the highest level yet recorded.

The announcement — made yesterday (18 April) at the biodiversity convention meeting at Den Hague, the Netherlands — is the latest twist in a heated scientific and political row over whether or not GM maize is contaminating wild strains in Mexico, the genetic home of maize."

Katie Mantell, 19 April 2002, Source: SciDev.Net

2003:

ETC Group Report and Press Release. GMO-contaminated maize land races in 9 Mexican states found.

2004:

CEC (Commission for the Environmental Cooperation of the North American Free Trade Association). Maize and biodiversity: The effects of transgenic maize in Mexico.

Lesson learned: Likely cause human-driven movement and trade of whole Bt-maize kernels to remote areas – intended for consumption but ‚tried' in plantings.

BUT 2005: No GM contamination after all?

Or: Where did the transgenes go?

S. Ortiz-García, E. Ezcurra, B. Schoel, F. Acevedo, J. Soberón, and A. A. Snow. Absence of detectable transgenes in local landraces of maize in Oaxaca, Mexico (2003-2004). PNAS

.... Next chapter to come???

Example III) StarLink maize

2000: Bt maize variety not permitted for human food (because of unresolved allergy issues) found in Taco Shells and other maize products.

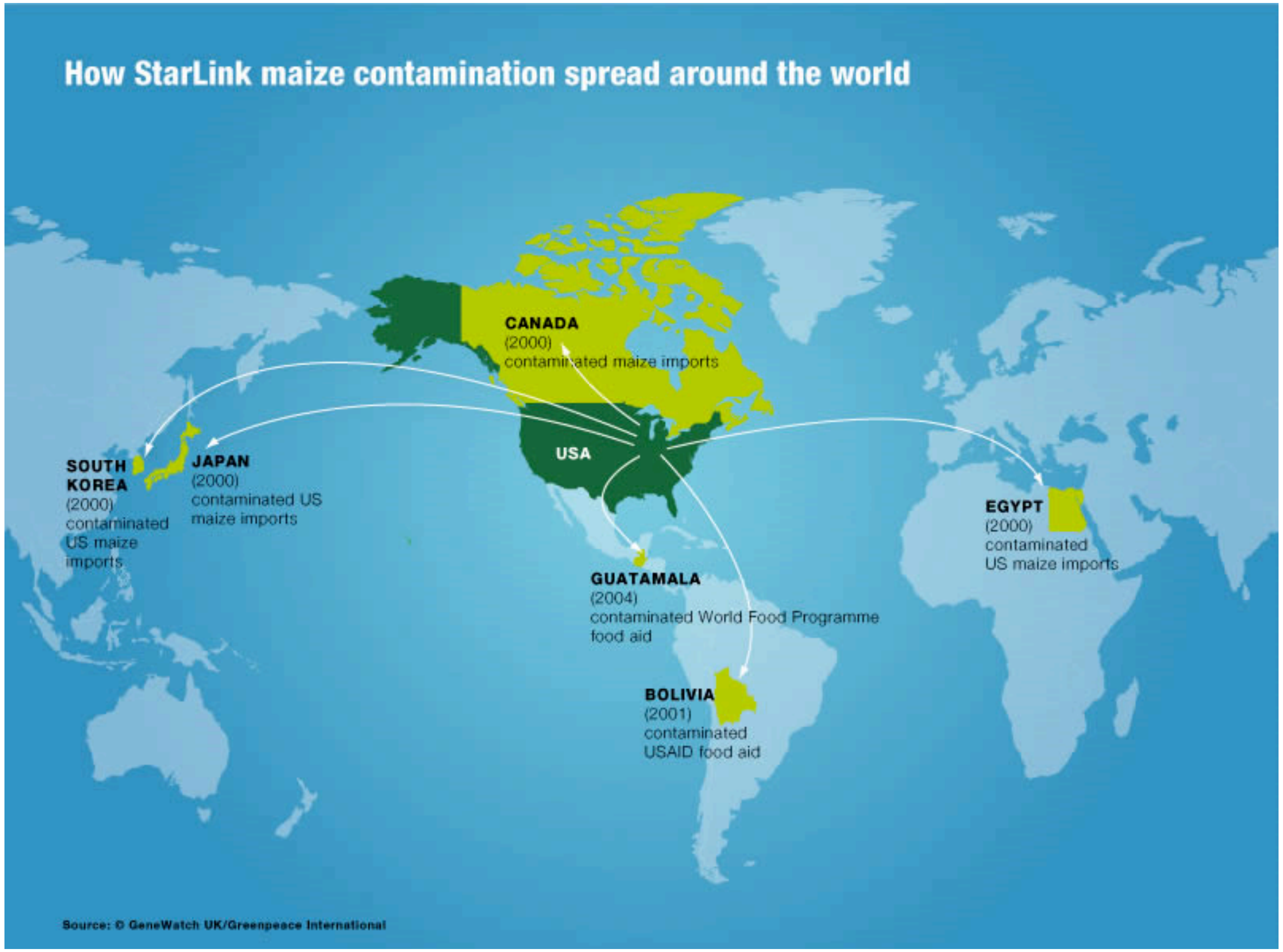
Action: Maize products and StarLink maize recalled from market

Lessons learned:

No sufficient separation of food and feed production chain

Very fast long distance spread (faster than short distance in many instances) when driven by humans through trading and transporting

But: Although only on the market for a few months ...



CONCLUSIONS - Can transgenes be kept on a leash?

(Marvier & van Acker. 2005. Frontiers in Ecology and the environment 3 (2); 99-106)

(1) the movement of transgenes beyond their intended destinations is a virtual certainty; and
(2) it is unlikely that transgenes can be retracted once they have escaped.

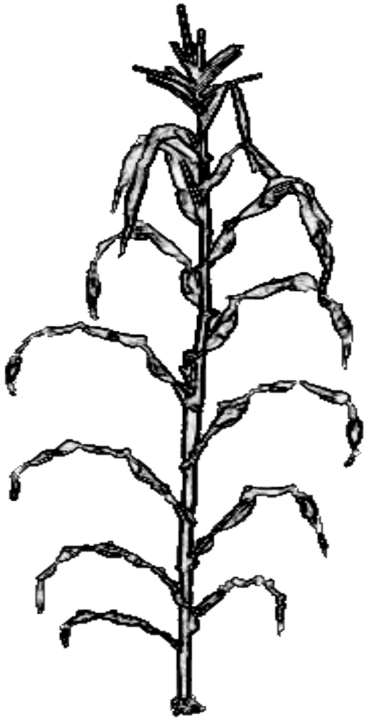
Re-examination of our risk management policies and our assumptions about containment is essential as genes coding for pharmaceutical and industrial proteins are being inserted into the second generation of GM food crops.

Even the best designed risk management can be foiled by human error, a reality that is underestimated by most GM crop-risk analyses.

Thus, our evaluation of risk should assume that whatever transgene is being examined has a good chance of escaping.

Spread and cycling of transgene products...

Case example: Insecticidal Bt-plants



Constitutive Bt-expression

→ all plant parts

→ most plant fluids, except perhaps phloem/xylem

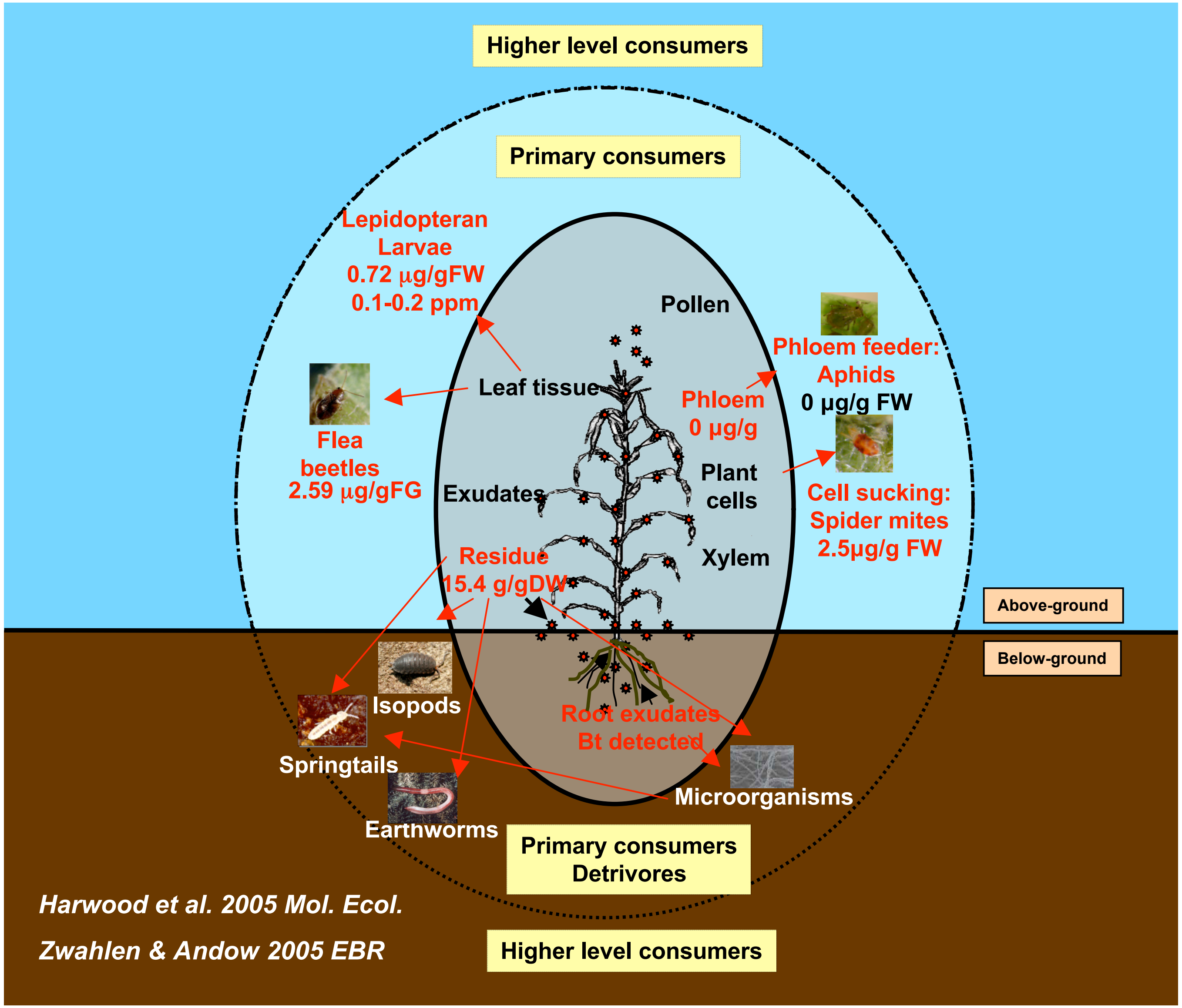
→ season-long

Molecular weights of expressed transgene product (=Bt-toxin) 65, 69 and 91 kDa

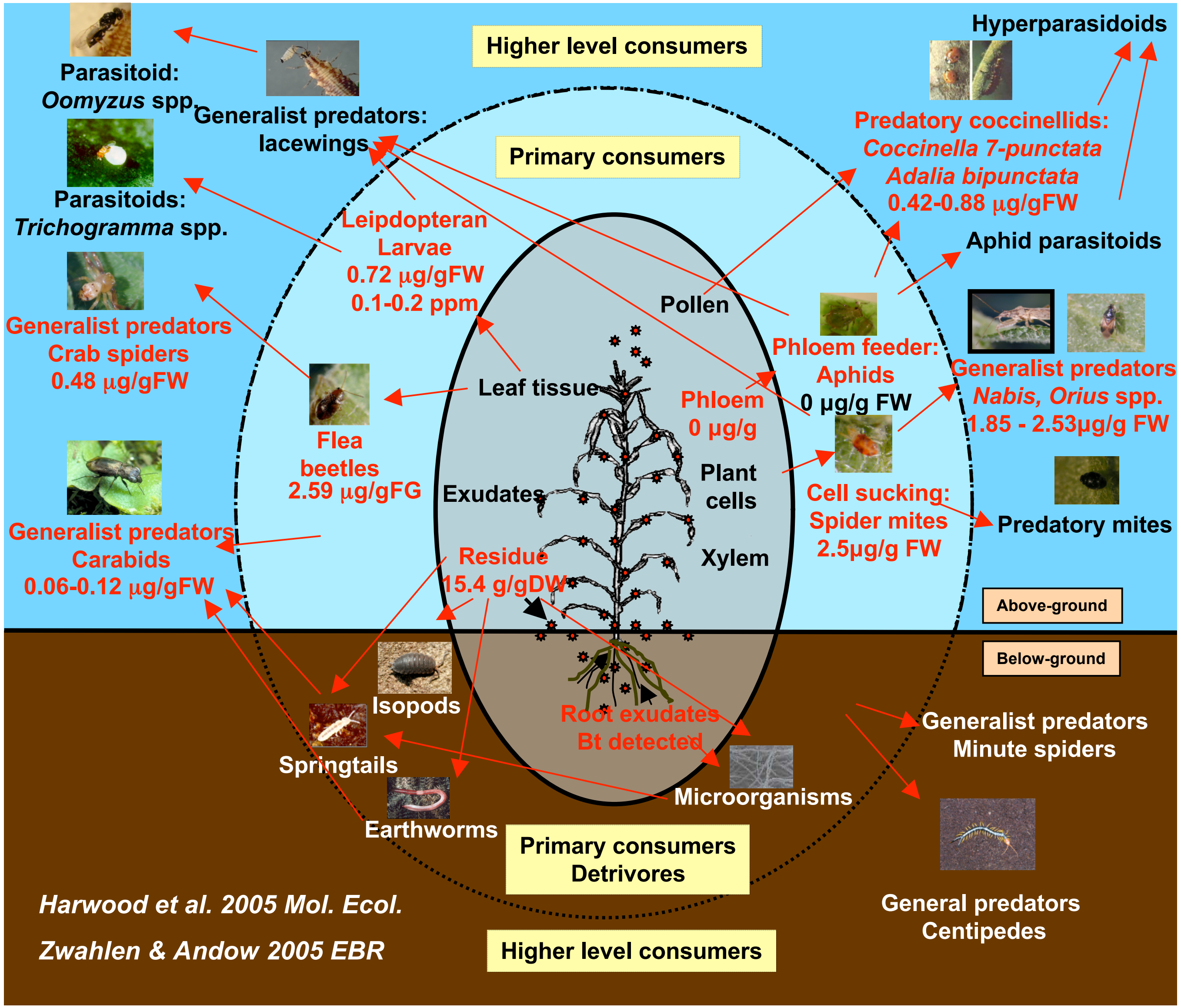
Other fragments <50, 40 kDa due to in-plant processing

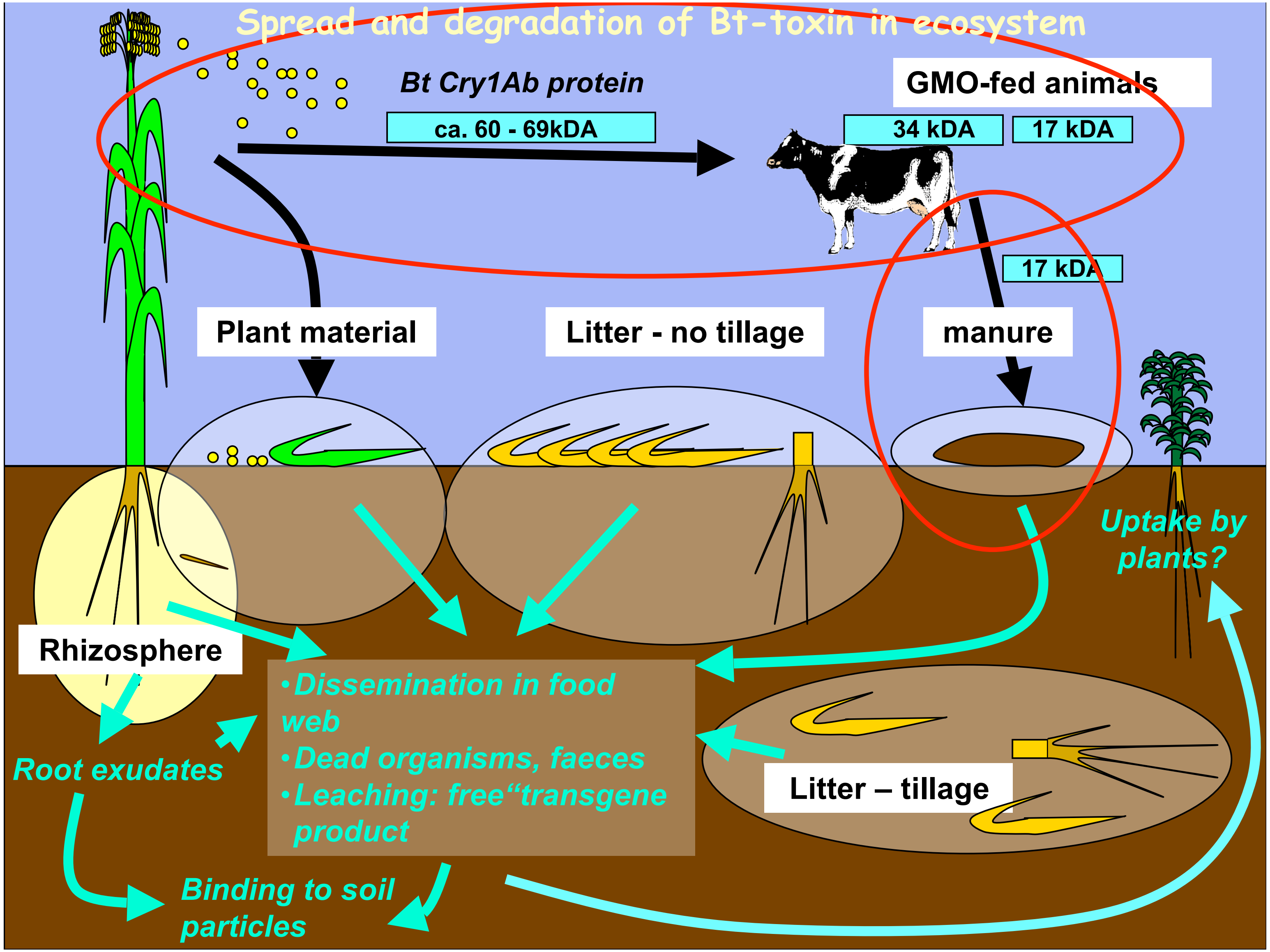
→ Unclear bioactivity

Spread of Bt-toxin in foodweb in agro-ecosystem
Exposure pathways to Bt-toxin from transgenic maize



Spread of Bt-toxin in foodweb in agro-ecosystem
Exposure pathways to Bt-toxin from transgenic maize




Spread and degradation of Bt-toxin in ecosystem
Bt Cry1Ab protein
ca. 60 - 69kDA
GMO-fed animals
34 kDA
17 kDA
17 kDA
Plant material
Litter - no tillage
manure
Uptake by plants?
Rhizosphere
•Dissemination in food web
•Dead organisms, faeces
•Leaching: free"transgene product
Root exudates
Litter – tillage
Binding to soil particles

Lessons:

Transgenes and transgenic products will spread quick and far if no stringent rules for coexistence and transport are installed & enforced.

It will be very difficult to trace them back to source and to where they *go* after few years (if not months) – important implications for safety and liability and IP issues

Longterm environmental consequences on-going ...

Evidence exists:

- Evidence for possible adverse food chain effects on ‚nontarget organisms‘, like biocontrol organisms such as lacewings and a number of other arthropods at least in laboratory trials with Bt-plants (review by Hilbeck & Schmidt in press)

for weed-associated food chains in HR crops, e.g. arthropods, farmland birds, etc. (Farm Scale Evaluations (FSE) in England)

- Contamination of genetic resources

CIMMYT investigation of own maize germplasm did not yet reveal any...

-Resistance problems

Without systematic monitoring programs, impact on biodiversity and its functions not detectable in early stages!

Gene products may have soil ecosystem effects

In a review of the effects of GM plants on **soil systems** (http://www.defra.gov.uk/environment/gm/research/epg-1-5-214.htm) 16 out of 25 peer-reviewed studies of 9 GMO crops showed effects on the soil community or soil system:

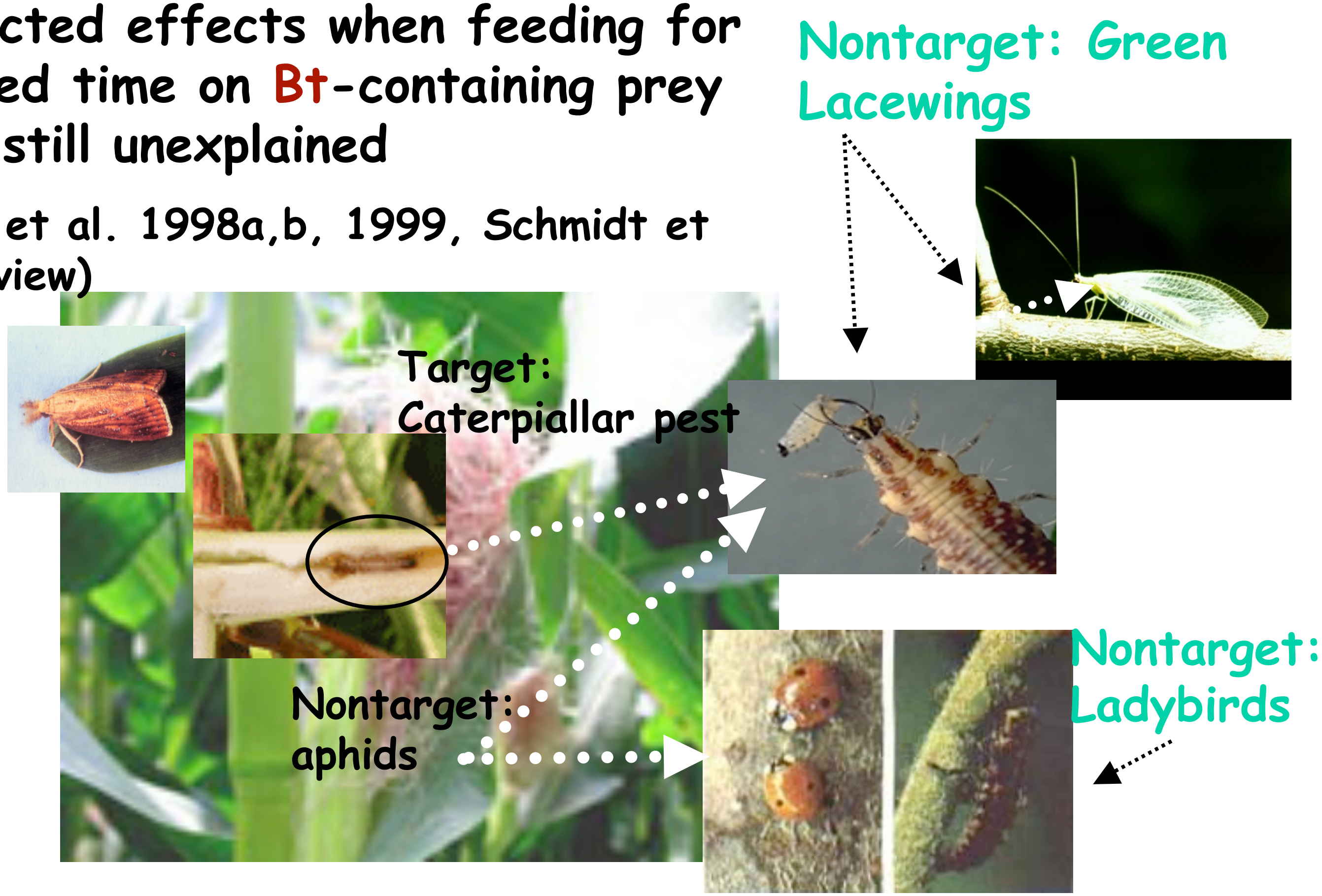
- fungal and bacterial diversity and activity;
- numbers of protozoa, nematodes and collembola;
- woodlice mortality.

The authors conclude :

- Most transgenic plants have detectable effects on the soil system
- These are mostly minor compared with differences between cultivars or those associated with weather and season.
- There is a lack of monitoring activity which is linked to a concept of damage to the system.

Unexpected effects when feeding for prolonged time on Bt-containing prey – most still unexplained

(Hilbeck et al. 1998a,b, 1999, Schmidt et al. in review)



Field validation and data not conclusive yet (series of publications on field trials with Bt-maize and Bt-cotton in the US published in Environmental Entomology 2005)

HR-crops are sold on simplicity

FSE – Farm Scale Evaluations

3-year field experiments of commercial proportions with HR-oilseed rape (OSR) (Glufosinate) and HR-maize (Glufosinate) and HR-sugar/feed beet (Glyphosate)

<u>Agricultural weeds:</u> In HR-OSR and –beet significantly lower diversity and abundance.

- In maize higher diversity BUT atrazin was used as herbicide (very persistent, banned in EU now)

<u>Associated invertebrate fauna:</u> Bees, butterflies and seed feeding carabids significantly lower densities in HR-fields

Evaluation by the competent commission in the UK - ACRE

Further consequences on higher trophic levels, e.g. on farmland birds (skylark) are likely – some invertebrate species showed such effects

Production of HR-maize under the field conditions as in the FSE (incl. atrazine application) does not lead to damaging effects – recommendation to grant permission for production

Because no systematic, coordinated, country-wide monitoring of environmental impacts exist in main production countries, we must rely on anecdotal observations by farmers, consultants, field workers, etc. in ag-systems:

- **Resistance against herbicides and weed problems (USA, Argentina)**

- **multiple-resistant HR-oilseed rape in Canada – HR-OSR volunteers are weeds in HR crops (Canada)**

- **Beginning resistance against Bt-Toxins (Australia 2005)**

Resistant weeds against Round-up increase where HR crops are grown large scale

Roundup (Glyphosate)-resistance problems with:

‚Horseweed' (*Conyza canadensis*) in HR-soybeans (Late 90ies/early 2000 in USA, Argentina, S-Africa)

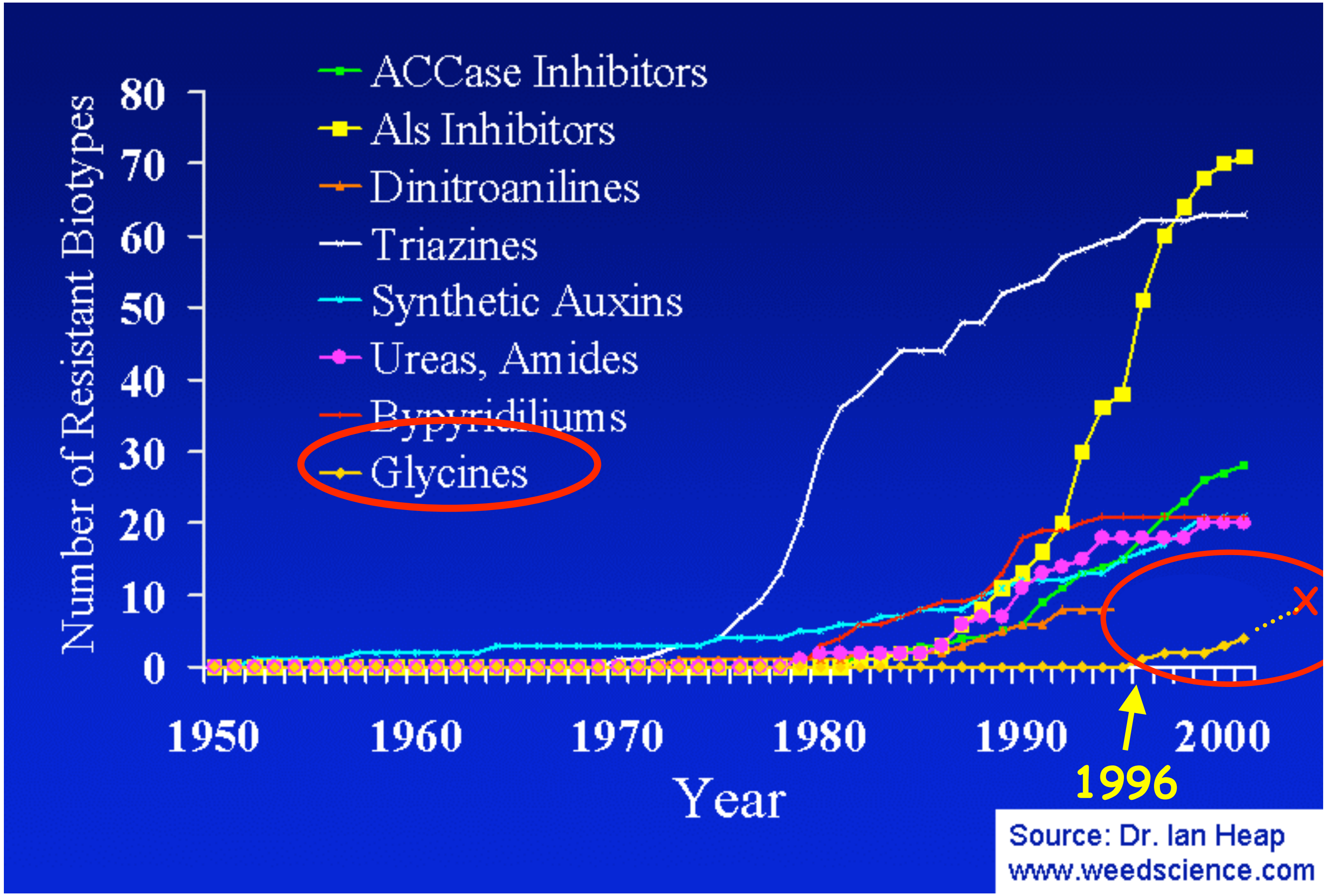
Plantago lanceolata: South Africa since 2003

Amaranthus palmeri: USA in HR-cotton since 2005

Lolium rigidum: South Africa and Australia (around 2000)

(Owen & Zelaya. 2005. Herbicide-resistant crops and weed resistance to herbicides. Pest Management Science 61: 301-311

Global resistant weed register: http://www.weedscience.org/in.asp)


ACCase Inhibitors
Als Inhibitors
Dinitroanilines
Triazines
Synthetic Auxins
Ureas, Amides
Bypyridiliums
Glycines
Number of Resistant Biotypes
0
10
20
30
40
50
60
70
80
1950
1960
1970
1980
1990
2000
Year
1996
Source: Dr. Ian Heap
www.weedscience.com

CONCLUSIONS - safe use of GM plants requires:

- **Reliable and rigorous pre-release risk assessment** and – management regimes

- Post-release monitoring programs

- Coexistence rules

GMOs and the Environment:

Impacts and how to assess them

GMOs are subject to regulation.

Many nations have national legislation.

Internationally binding legislations:

Cartagena Protocol

...has put forward rules for Risk Assessment

Requirements by Cartagena Protocol

Annex III - Risk Assessment

Objective: ...*identify and evaluate the potential adverse effects* ...
General principles: *Risks .... should be considered in the context of ... the likely potential receiving environment.*
Methodology: 8a - f. *An identification of any novel genotypic and phenotypic characteristics* associated with the LMO that may have *adverse effects on biological diversity in the potential receiving environment.*
b. ..*likelihood of these adverse effects* being *realized, ... exposure...*
c. ..evaluation of consequences should these adverse effects be realized.
e. ..identification of strategies to manage these risks.
f. ..uncertainty (gaps of knowledge)..
Points to consider
(g) *Receiving environment. Information on the location, geographical, climatic and ecological characteristics,* ...
9 d – *Insert/s and /or characteristics of modification.*

Possible Environmental Impacts

I) ‚Trans'gene Flow & Consequences resulting from unwanted and uncontrolled spread of novel transgenes and their traits in the ecosystem (conservation of GM-free gene pools or communities of organisms)

II) Biodiversity & Nontarget Effects resulting from:

- transgene products (z.B. insecticidal toxins) and their target and non-target effects (e.g. food chains/web)

- unintended pleiotropic and epigenetic changes (e.g. altered secondary metabolic products) on biodiversity of flora and associated fauna (e.g. food chains/webs)

III) Resistance development (agronomic problem)

Possible Adverse Effects

I) In agro-ecosystem (generally associated with biodiversity services)

- Development of secondary pests
- Development of resistent pests or weeds (‚super weeds)
- Damaging of naturally-occurring biocontrol organisms
- Impact on soil organisms involved in re-cycling of soil nutrients and soil fertility
- Decline of endangered/protected species of farmland
- Threating of GM-free production reducing future choices

Possible Adverse Effects

II) In natural ecosystems (via gene flow)

→ Decline of biodiversity (loss of species, potential invasiveness of GMOs, etc.)

→ Loss/decline of ecological functions/processes (re-cycling of chemical and biochemical compounds, trophic relationships/population regulation)

→ Shifts in species spectra (incl. loss of species)

→ Damaging of protected/endangered species (nature conservation)

III) To the protection/conservation of genetic resources (seeds, gene pool, organisms (= particular gene combinations), their use (value) and way of production (centers of diversity)

Current testing follows the pesticide paradigm: Ecotoxicological Testing

Strategy: Expose single species (standard set) to single chemicals in a hierarchical tiered system.

-Tests commence with simple inexpensive range-finding tests on single species

- Measure acute toxicological response to a chemical stressor

- Proceed to more expensive higher tiered tests (incl. some chronic toxicity tests), only if first tier experiments yield results of concern.

Standard non-target organisms tested according ‚pesticide paradigm'

Water fleas (*Daphnia magna*) – acute, 48 hrs static renewal with pollen
Springtail (*Folsomia candida*) – chronic, 28 days, yeast + test material
Earthworm (*Eisenia foetida*) – 14 days, soil + test material
Honey bee (*Apis mellifera*) – acute, 45 minutes, undigested pollen + water

Predatory/parasitoids insects
Hippodamia convergens - adults tested, bitrophic
Nasonia vitripennis – adults tested; pupal parasitoid of house flies, minor ecological relevance, bitrophic
Chrysoperla carnea – larvae, bitrophic, coated meal-moth eggs, ca. 1 week

Testmaterial used:
- Lyophilized leaf protein as dietary test material
- Microbially produced, activated Bt-toxin

Test duration:
- short time, acute

Test endpoints: toxicological parameters

Differences between pesticides and GM insecticidal plants

Pesticides:
- Release controlled by applicator: timing, point location, etc.
- Degradation begins immediately after application
- Mode of action typically acute, immediate also for nontargets

GM Bt-Plants
- Release continuous and in all plant parts
- Tissue-specific production coupled to plant physiology
- Mode of action not immediate (takes 2 days or longer before target dies)
- Sublethal, chronic effects more important for nontargets.

→ Induces very different dynamics/types of non-target effects

Implications for GM Plants

- Current transgenic insecticidal Bt-plants express highly bioactive toxins in high concentrations in all plants part (some including pollen) throughout the entire season.

- GM product expression coupled to plant physiology and metabolism, with concentrations varying depending on tissue, plant age, location and season.

Conclusion: GM plants and their novel transgene products resemble plants rather than chemicals!

‚Scientifically sound' nontarget effect testing should account for that!

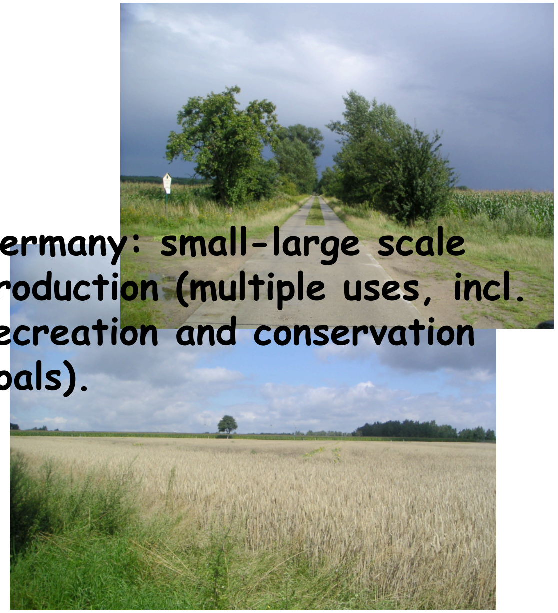
An improved ecological approach for environmental risk assessment is necessary!!!

Country-specific CONTEXT frames the assessment process

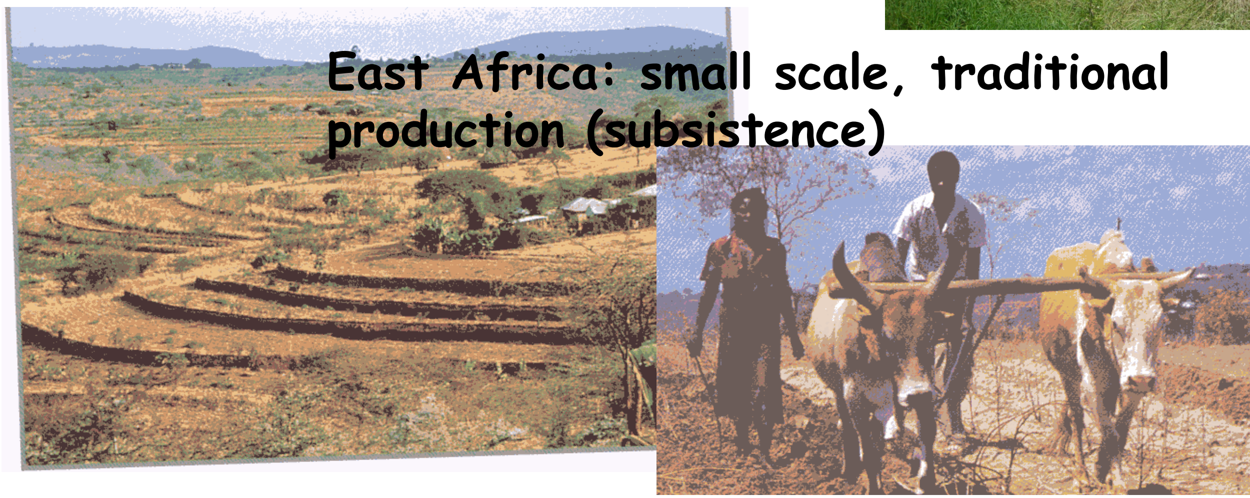
-

1 size does NOT fit all!!!

Brazil: large scale industrial production (yield/cash)



Germany: small-large scale production (multiple uses, incl. recreation and conservation goals).



East Africa: small scale, traditional production (subsistence)

Requirements by Cartagena Protocol

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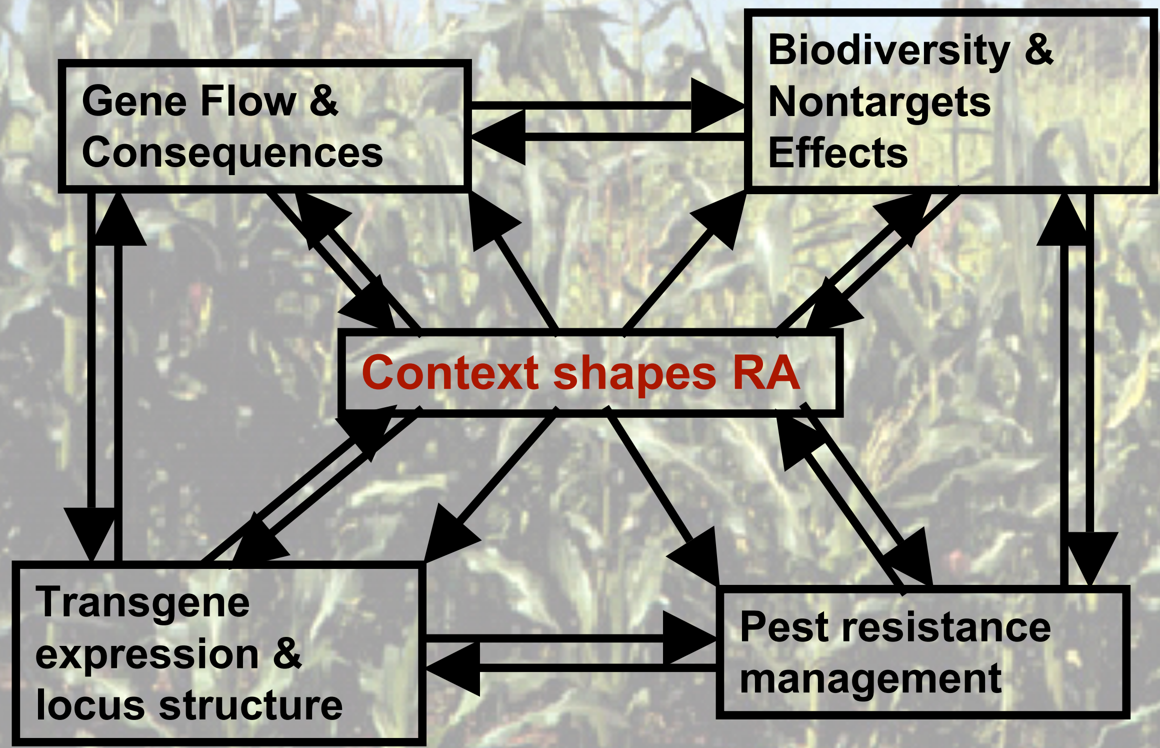
Framing Ecological Risk Assessment



Scientific sections

- Problem formulation and options assessment (PFOA)
- Transgene expression and locus structure (TELS)
- Gene flow and its consequences
- Biodiversity & non-target impacts
- Resistance evolution and management

I. Problem Formulation and Options Assessment (PFOA)

A framework developed for:

- **identifying critical unmet societal need** aimed to be satisfied by GMO (*Problem formulation*)
- Systematically structured **comparison** with other possible **alternative options** (*Options assessment*)

Question-driven stakeholder process that is transparent, equitable and accountable

Findings can be used to **characterize** the ecological, agricultural, socio-economic and cultural **receiving environment**

Problem Formulation and Options Assessment Process

Step 1: Problem Formulation

Whose problem is it? What underlying aspects are involved that require change?

Step 2: Prioritization and Scale of Problem

Is it a core problem? Do the people recognize the problem as important? How extensive is the problem? How many people are affected? How severe is the problem (local intensity)?

Step 3: Problem Statement

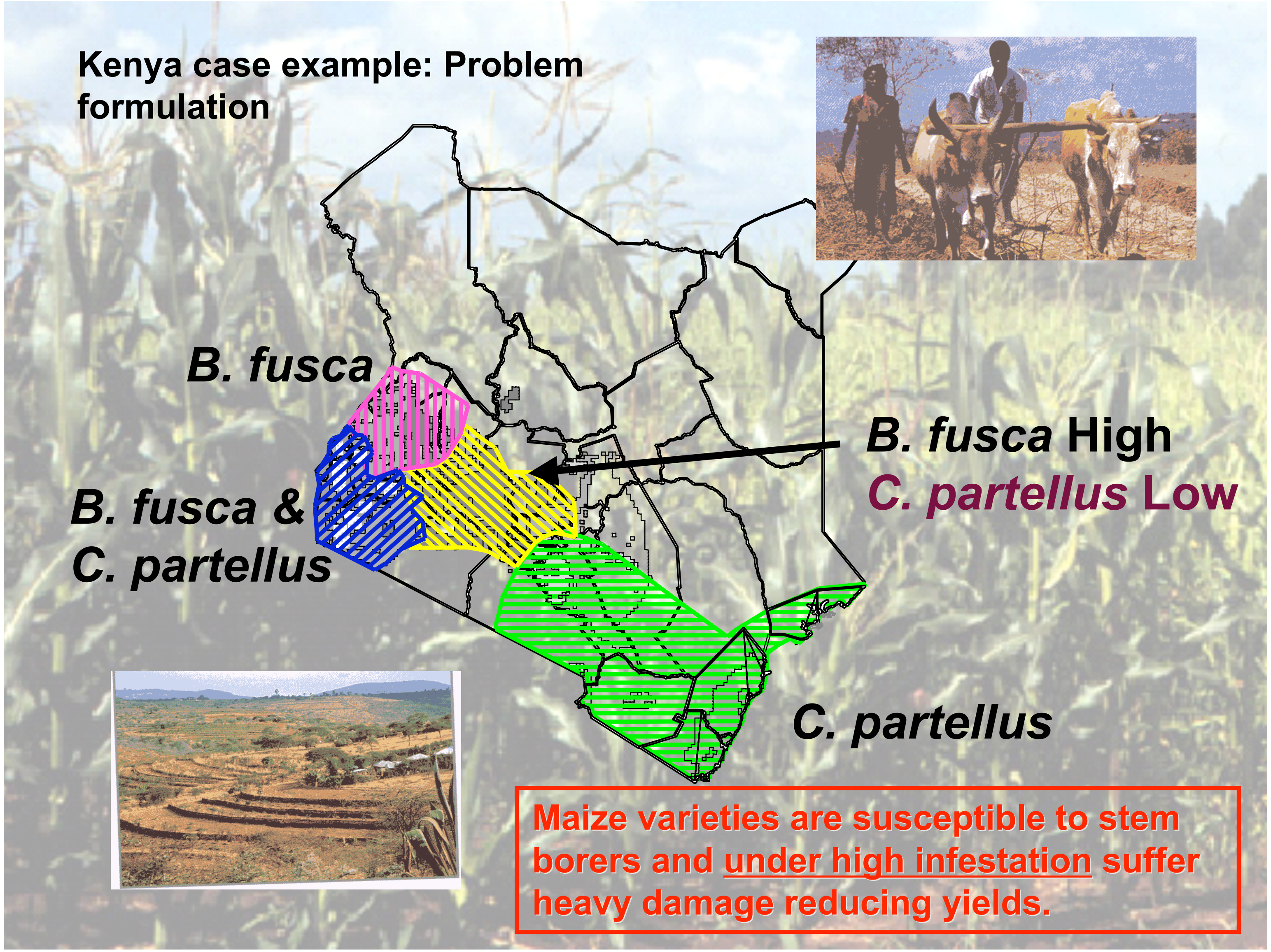
Step 4: Solution Options

Comparison, competition of best solution option

Step 5: Attributes for Solving Problem

Step 6: Changes required and anticipated for a solution option

Step 7: Impact to the System


Kenya case example: Problem formulation
B. fusca
B. fusca &
C. partellus
B. fusca High
C. partellus Low
C. partellus
Maize varieties are susceptible to stem borers and under high infestation suffer heavy damage reducing yields.

II. Transgene Expression & Locus Structure (TELS)

A framework developed to examine structure of transgenes as integrated in the GM crop and novel phenotypic and genotypic characteristics

Step 1: Transgene locus structure through complete sequencing of transgene and flanking regions is essential (number of transgene loci, location in genome, number of copies at each locus, marker genes, open reading frames, etc.) (9d)

Step 2: Methods to determine transgene expression patterns over crop development (9d)

Step 3: Transgene transmission from generation to next (9d)

PFOA shaping Requirements for Molecular Characterization of GM Crop (transgene expression and locus structure)

• Yield potential will depend on efficacy of insecticidal GM crops against various pest species

→ transgene expression and control efficacy

• In what range of crop production systems GM crop is likely to be used?

→ species-specific efficacy

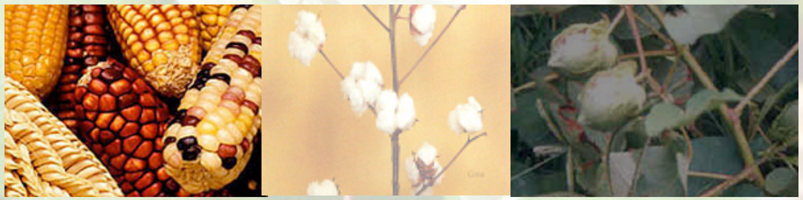
III. Gene Flow and its consequences

Stepwise approach:

1. Identify **recipients of gene flow** (wild and crop relatives) and their geographic distributions in your country
2. **Likelihood** of spread of transgenes - **recipients at greatest risks**
3. **Consequences of gene flow - possible adverse effects** for identified possible recipients

→ **biodiversity** (flora and associated fauna)

Identification of risks of greatest concern in your country, develop experiments to clarify these specific risks early on.



PFOA shaping Risk Assessment of Gene Flow

- **Clarification of farmer‘s breeding practice, seed exchange and seed recycling**

How does it work, criteria, effectiveness, rules for seed exchange?

- **Value of landraces, purity of other crops**

Lack of barriers to gene flow will lead to quick spread modulated by perceived problem (farmer‘s selection)

→ **Will determine speed and direction of transgene spread**

V. Resistance Evolution and Management

Step 1 Identification of species at risk - non-target and target species can evolve resistance

Step 2 Dose and dominance. Technical concepts that determine whether resistance is dominant or recessive

Step 3 Assessing degree of risk - mating behavior, alternative host plants, mobility of species, fitness costs of resistance, etc.

Step 4 Management strategies. Typically refuges are necessary but size and location determine their effectiveness

PFOA shaping Pest Resistance Management Strategies

Relevant issues addressed:

• Crop production area, main GM crop user group and efficacy of GM crop for given pest problems – resistance management strategy (high dose/refugia vs. low doses, seed mixtures)?

• Host preference and non-preference of different species of the pest - quantity and quality of refugia present (presence of alternative hosts)?

• Susceptibility of various pest species and biotypes – resistance development?

PFOA shaping Risk Assessment of Biodiversity and Nontarget Effects

- **Identifies aspects of biodiversity that need to be addressed**

Landraces, wild/weedy relatives, region-specific associated fauna (species of conservation concern and cultural significance)

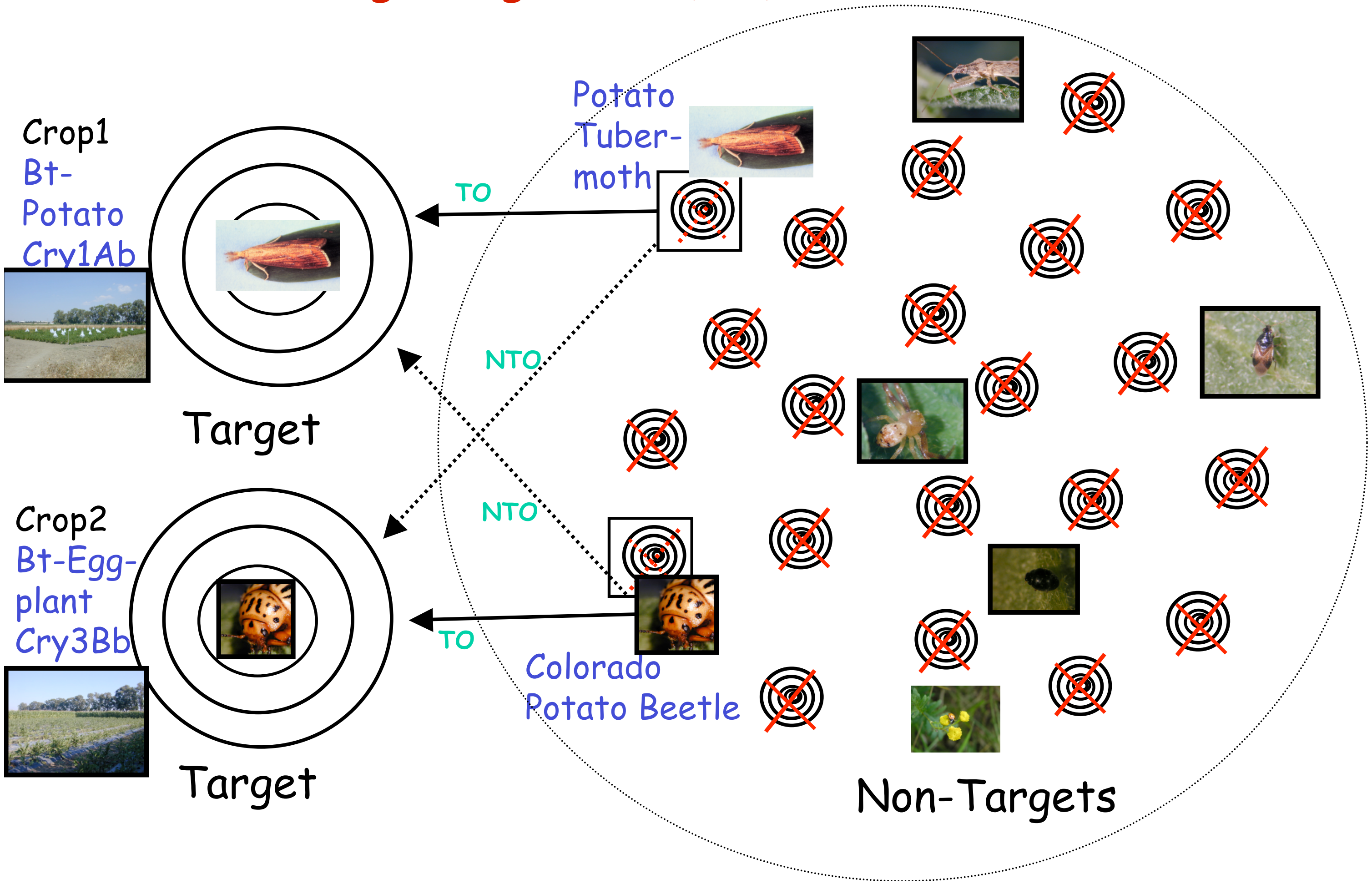
Interference with other control options or management systems that may also target other more important problems

***Kenya: Striga* spp., pathogens (e.g. *Aspergillus flavus*), post-harvest pests, etc.**

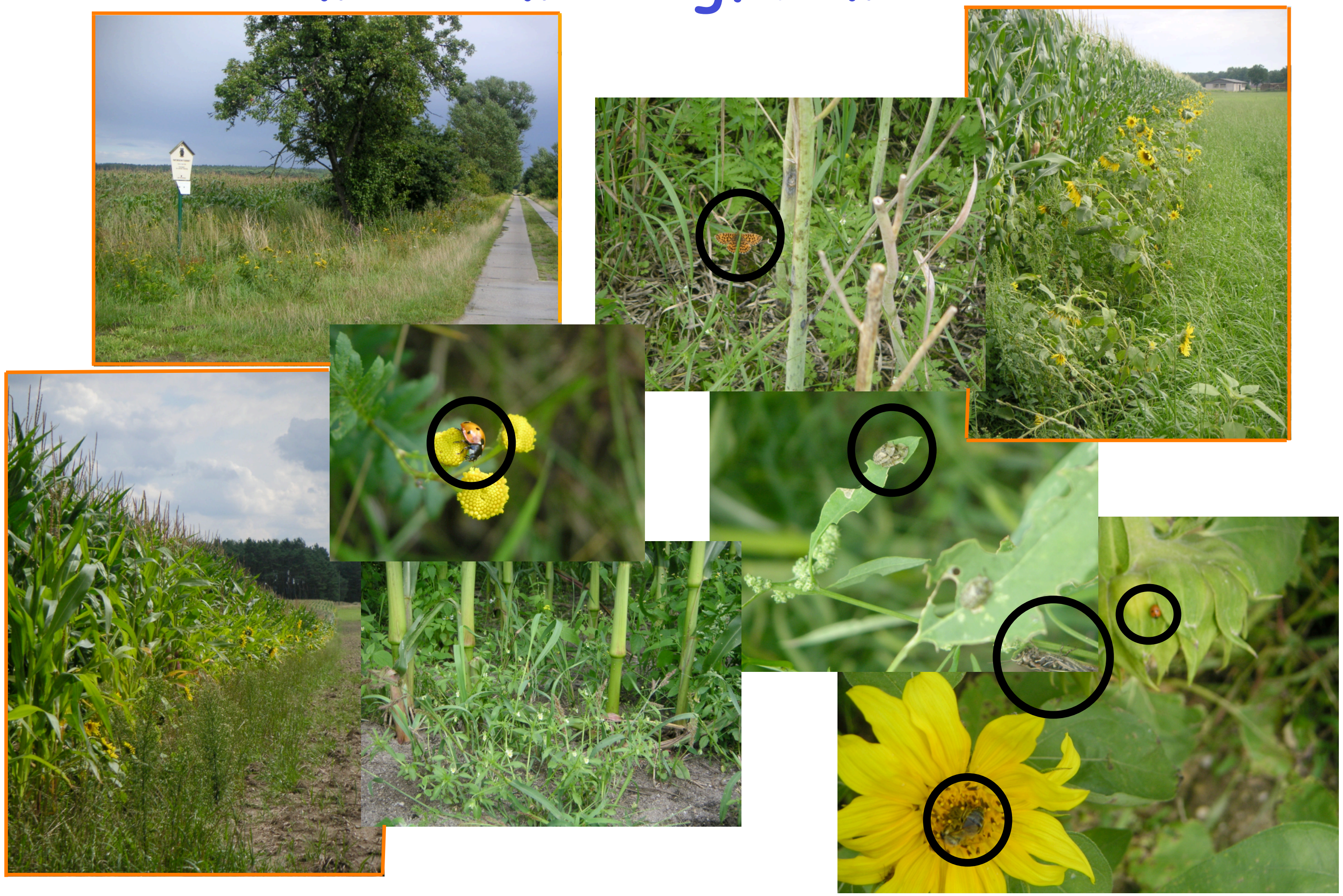
What are NON-Target organisms (NTO)?

What are Target organisms (TO)?

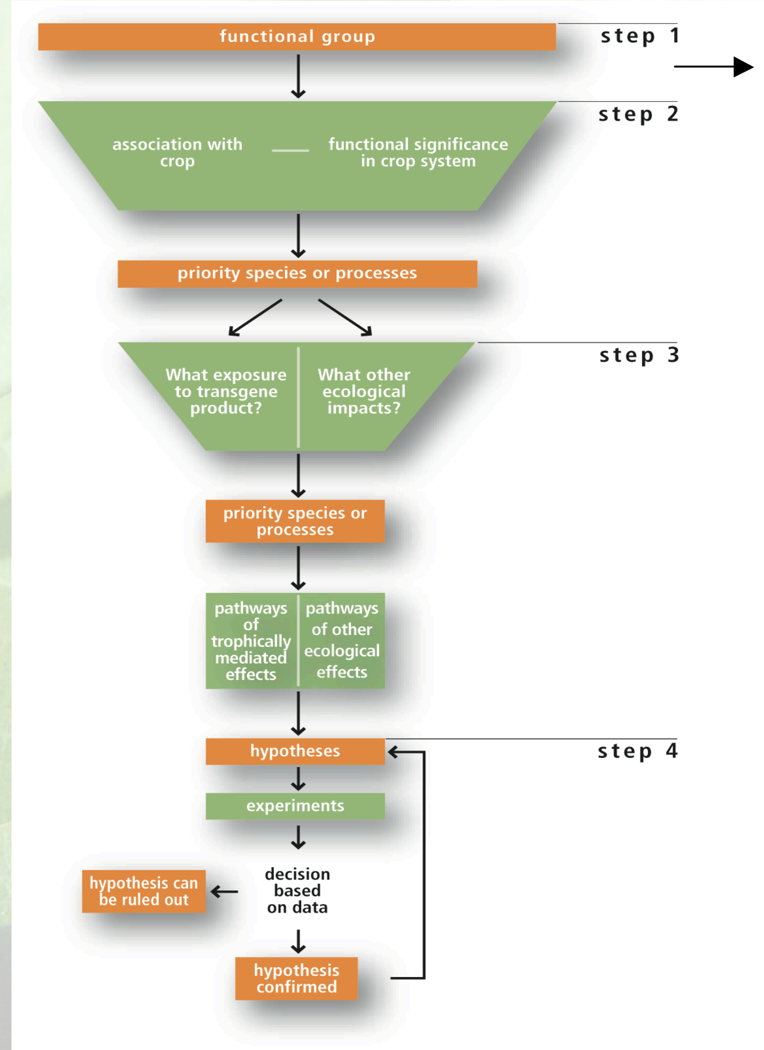
Non-trivial!!



How do you select appropriate species and test them in a meaningful manner???



The Funnel Framework



Step 1 = 1st reduction

Case-specific important functional groups are identified

Assessment to be done for each <u>case</u>

Selection of functions guided by:

a) Crop biology and agronomy

b) Novel type of trait (e.g. IR, HR) – intended effect

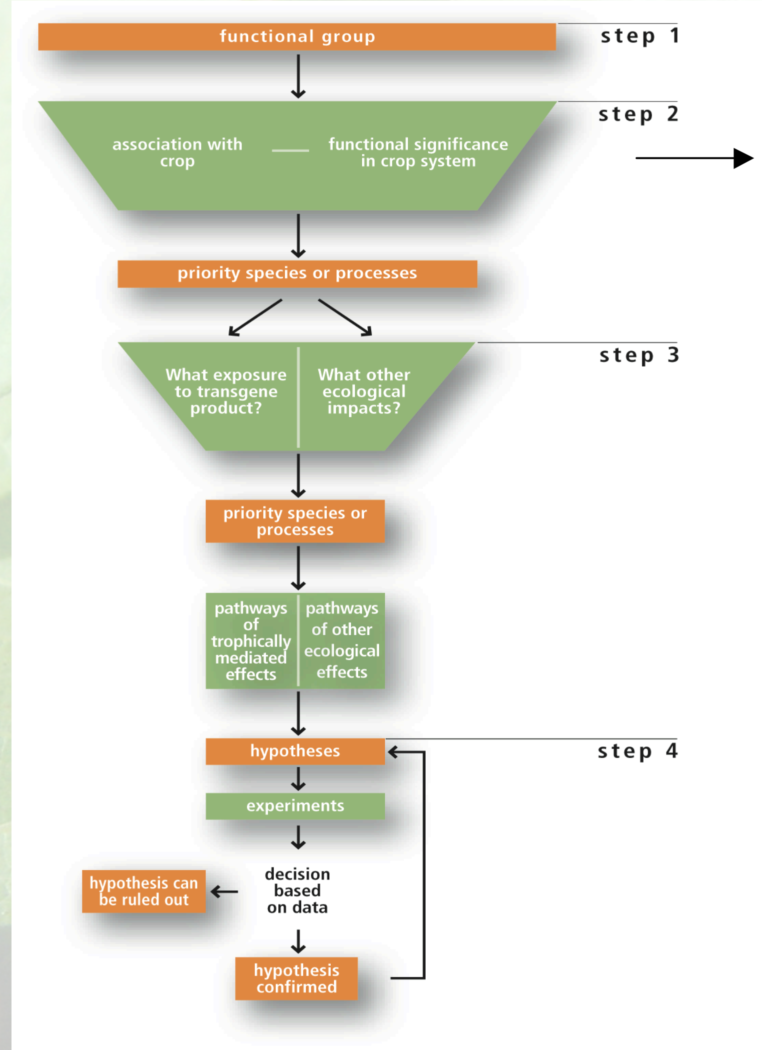
c) Receiving environment, farming practices – intended use

DISCUSSION ON:

DEFINITION OF A CASE?

IDENTIFICATION OF '*POTENTIALLY ADVERSELY AFFECTED FUNCTIONS'*

The Funnel Framework



Step 2 = 2nd reduction

All relevant species and ecological processes associated with the crop ranked on:

- How closely associated with crop?

- If adversely affected, how significant would the effect be?

Retained species taken to 3)

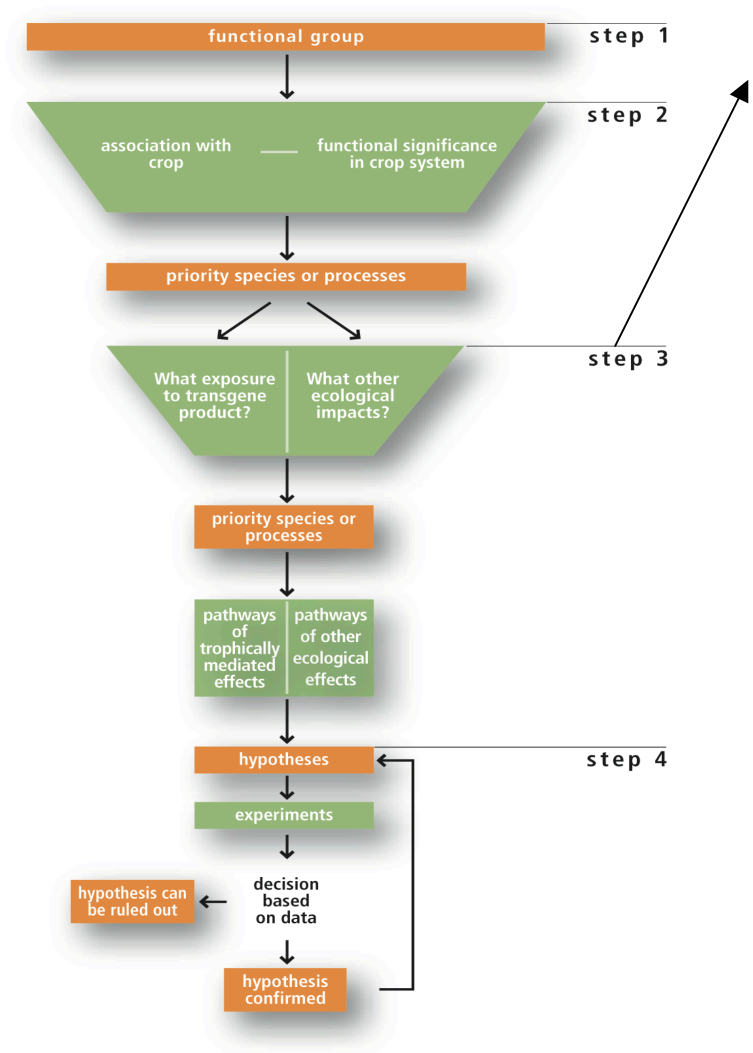
Tools: Ranking matrices

Outcome:

A reduced list of species most closely associated with crop

IDENTIFICATION OF POTENTIALLY 'ADVERSELY AFFECTED FUNCTIONAL SPECIES/ ECOL. PROCESSES'

The Funnel Framework



Step 3 = 3rd reduction

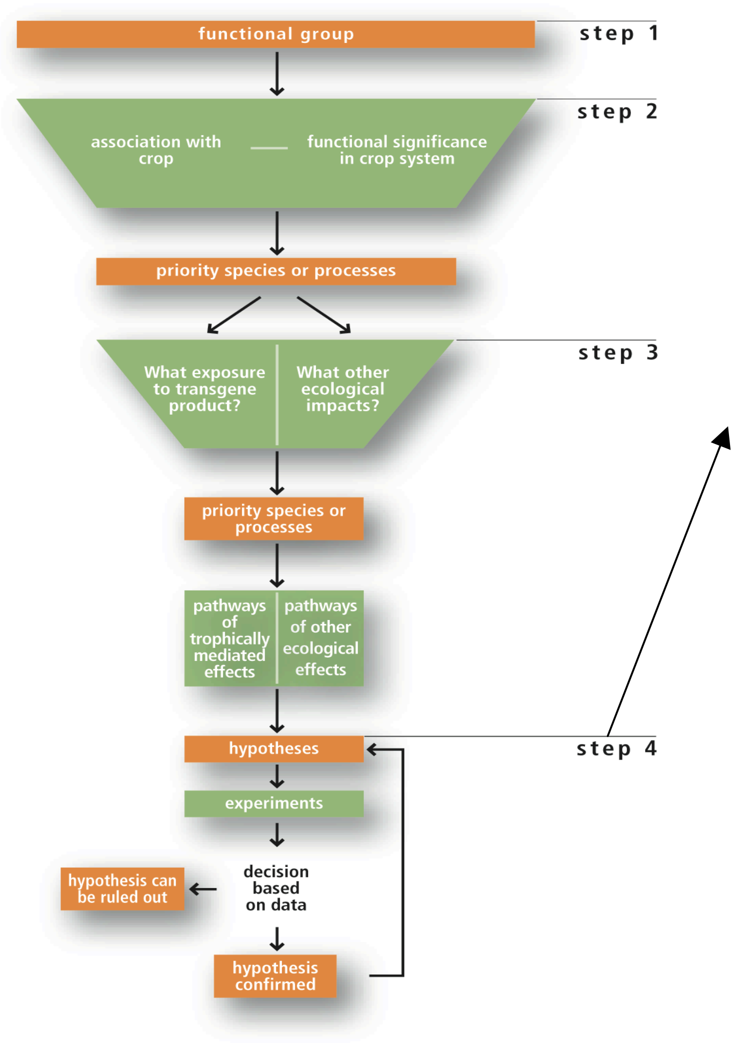
How likely is contact between retained species/processes and transgene product?

Other ecological impacts, e.g. caused by changes in plant compounds and crop management practices? E.g. HR-plants

Identify and prioritize knowledge gaps and uncertainty

LIKELIHOOD ASSESSMENT FOR EXPOSURE

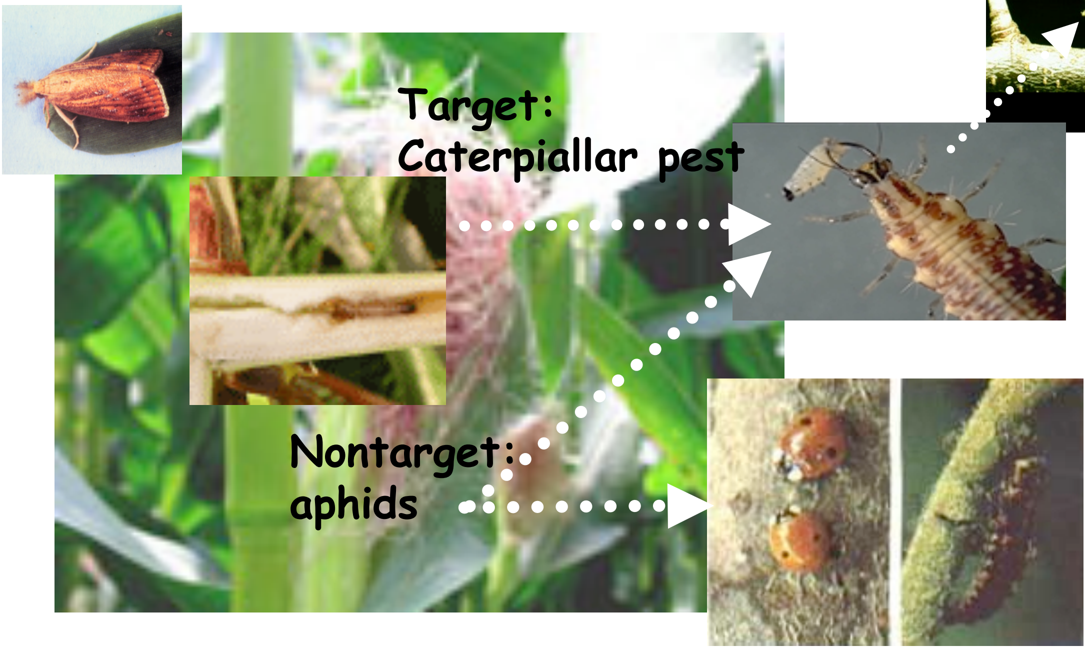
The Funnel Framework



ADVERSELY AFFECTED SPECIES/PROCESSES X LIKELIHOOD OF EXPOSURE = POSSIBLE ADVERSE EFFECTS (POSSIBLE RISK OR HAZARD)

Step 4 = develop adverse effects scenarios and formulate <u>as testable research hypothesis</u>

Effects of pesticidal transgene products, e.g. Bt-toxins (other in development) causing foodchain effects on important biocontrol organisms



Example: Could we have identified this adverse effect scenario?

„The Curse of the Maracuja" – **Frankfurter Allgemeine Sonntags zeitung (FAZ Sonntag) 30 July 2006**

Maracuja production increasing worldwide

Main producer = Brazil,

Main producing area in Brazil = North eastern or tropical regions of Brazil (= small scale, resource poor farmers)

Caatinga (shrub-like forest lands) = cut down for increasing Maracuja production

Maracuja has a particular biology, in particular **pollination biology**!!
Only certain bees can fertilize the flower = **Carpenter bees**!!

Carpenter bees live in woods! Drill holes in wood for nesting etc.
→ Need Caatinga!!

PLUS: worldwide **pollinator crisis** due to overuse of insecticides in industrial ag-production – in particular in Brazil!! HANDpollination!!