<table>
<thead>
<tr>
<th>Page</th>
<th>Participant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Broder Breckling</td>
</tr>
<tr>
<td>11</td>
<td>Jeremy Sweet</td>
</tr>
<tr>
<td>15</td>
<td>Gerd Neemann</td>
</tr>
<tr>
<td>22</td>
<td>Ralf Wilhelm</td>
</tr>
<tr>
<td>29</td>
<td>Yiyang Yuan</td>
</tr>
<tr>
<td>39</td>
<td>Ulrikke Middelhof</td>
</tr>
<tr>
<td>42</td>
<td>Nengwen Xiao</td>
</tr>
<tr>
<td>56</td>
<td>Marko Bohanec</td>
</tr>
<tr>
<td>67</td>
<td>Fajun Chen</td>
</tr>
<tr>
<td>84</td>
<td>Arie Vonk</td>
</tr>
<tr>
<td>88</td>
<td>Chris Topping</td>
</tr>
<tr>
<td>94</td>
<td>Yongbo Liu</td>
</tr>
<tr>
<td>109</td>
<td>Jozsef Kiss</td>
</tr>
<tr>
<td>114</td>
<td>Paul Henning Krogh</td>
</tr>
<tr>
<td>121</td>
<td>Niels Bohse Hendriksen</td>
</tr>
<tr>
<td>125</td>
<td>List of participants</td>
</tr>
</tbody>
</table>
Research background

• General ecology
  • Interactions of plants, animals, microorganisms

• Ecological modelling
  • Structures, functions, dispersal, behaviour …

• Epistemology and systems theory
  • Self-organisation, emergent properties

• GMO:
  • Gene flow modelling on large scales
  • Monitoring concepts
Project involvement

• Monitoring (UBA)
  • Development of an integrative GMO monitoring concept

• GenEERA (BMBF)
  • Dispersal of (GM) oilseed rape in Northern Germany

• SIGMEA (EU)
  • GMO assessment - toolbox

• GeneRisk (BMBF)
  • Systemic risks, interdisciplinary integration

A basis for risk assessment: Murpy‘s Law

• What can go wrong will go wrong

A joke with a serious background: Only when all potential results have been assessed, well informed management decisions can be taken

... Risk management must be prepared to all eventualities;
... Risk assessment helps to discover them
Two risk categories

1. Risks with direct linkage of cause and effect

   - Interaction of many single events giving rise to an unintended whole

   http://de.wikipedia.org/wiki/Verkehrsstau

   http://nl.wikipedia.org/wiki/Schade

2. Systemic Risks

   - Interaction of many single events giving rise to an unintended whole

   http://eo.wikipedia.org/wiki/Diskto

Systemic Risks of GMO are less investigated

• Required:
  – An overview of different organisation levels

• Structuring of GMO risk research should follow the organisation of biosciences across different levels

This yields an emergent systematisation
GMO: Risk Dimensions

- GMO risks can occur on all levels of organisation
  - Starting at the level of molecular interaction
  - Up to large-scale structural effects
- Assessment requires concepts, methods, expertise on the various levels

GMO: Molecular risks

- Metabolomic integrity
  - New metabolites?
  - Altered quantities?
  - Gene regulation integrity
- Combinatory effects of genetic background
GMO: Risks on the landscape level

- Land use conflicts
- Are distance regulations functional in different regions?
- Example maize gene flow
% cross fertilisation
(11 well documented field studies)

[Graph showing % log cross fertilisation and log distance]

Example maize gene flow

- Large fields, low density

~6,000 Maize fields in Brandenburg

Broder Breckling
bbreckling@iuw.uni-vechta.de
12.12.2011
Simulation 40% GM maize cultivation

- Very few impurities

Brandenburg 40% GV Scenario

Labelling threshold

- Small fields, high density

~20,000 maize fields in Schleswig-Holstein

Example maize gene flow
Example maize gene flow

Schleswig-Holst. 40% GV Scenario 2

Labelling threshold

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.1

0 10 20 30 40 50 60 70

• Potentially relevant impurities

• Interface to socio-economics and regulation

• Global transport and dispersal

• Long term biodiversity implications

• Evolutionary integrity

GMO risks on cross-regional scale
Outlook on core research topics

- Molecular assessment
  - Metabolomic integrity
  - Cellular regulatory networks and epigenetics
- Populations
  - Gene flow on different scales
  - Nontarget population density changes
- Ecosystems
  - Trophic interactions (tri-, multi-…)
- Landscapes
  - Biogeographic variability, dispersal processes
  - Web-based data access facilities
- Socio-economics
  - Segregation implications of GM and other agriculture
  - Macro economic cost-benefit analysis

GMLS 2012
conference
14./15. June 2012

Survey on independent GMO risk research

Many thanks for your attention

www.gmls.eu
Research Priorities on GMPs

Jeremy Sweet

New challenges in risk assessment of Genetically Modified Plants – Establishing a research programme aiming for integrative risk assessment  Copenhagen December 2011

General Comments

- GMPs introduced into the EU should fully integrate with sustainable agriculture, integrated farming systems and integrated pest (and disease) management (IPM).
- Q. 1. What is their potential contribution to sustainable/integrated farming systems and what problems do they cause?
- Q. 2. How can this potential be achieved without adverse effects? ie: how should they be managed?
Research Priorities: Considering the main types of GM crops

- Herbicide Tolerant Crops – a systems approach
  
  Research on:
  1. Techniques to maintain botanical diversity and higher trophic level diversity at desired levels, considering also the functional value of plants and arthropods within crop rotations.
  4. Integrating weed management in whole crop rotations including with other HT and GM (eg Bt) crops in rotations.
  5. Economic analyses of systems.
  6. Developing DSS systems for managers.

- Bt crops: considering Cry1, 3, 34/35 etc....

- Research on:-
  1. Managing pest resistance development considering a wide range of strategies separately and in combination, eg: gene stacking, refugia, crop rotations, pesticides, etc....
  2. Managing Bt x HT crops considering maintenance of biodiversity and functional diversity for IPM in crop rotations.
  3. Integrated Pest (pesticide) and weed (herbicide) management in whole crop rotations including with Bt and HT crops.
  4. Developing DSS systems for managers.
Research Priorities: Considering the main types of GM crops

• Disease Resistant (DR) GMPs: Research on
  1. Durability and spectrum of GMP resistance to pathotypes in order to develop disease resistance management strategies
  2. Study a wide range of resistance management strategies separately and in combination (e.g., gene combinations, refugia, rotations with different resistances, diversification, pesticides, etc.)
  3. Study resistance expression of DR plant to non-target pathogens so as to determine interactions with other pathogens and fully integrate disease management.
  4. Integrate Disease, Pest (pesticide) and weed (herbicide) management in whole crop rotations including with Bt and HT crops

• Abiotic Stress Tolerant Plants: Research on:
  1. Methods to evaluate fitness characters in existing and new receiving environments
  2. Methods to determine ecological impacts in new receiving environments (e.g., use of surrogate plants)
  3. Management requirements, their environmental impact (e.g., resource usage) and integration with other cropping systems
  4. DSS for risk assessors/managers for assessing fitness and environmental impacts
  5. Socio-economic impacts of stress tolerant plants
Summary: Research Priorities

• **HT**: Management systems at Farm/rotational scale to achieve IPM

• **Bt**: resistance management and IPM at farm and regional scale

• **DR**: More research data on interactions with pathogens to allow development of resistance management and IPM strategies

• **Abiotic Stress tolerance**: studies of methods to determine fitness, invasiveness and environmental/agronomic impacts
1) Variability of Agricultural Landscapes
and its potential impacts on GMP cultivation
Variability of Agricultural Landscapes: Consequences for GM Crop Cultivation?

### Evaluation of composition of agricultural landscapes to ascertain importance of landscape structure (2002 - 2008)

1. Selection of 42 landscape sectors
   - Size: 9 km²
2. Mapping of fields, meadows, pastures and wild habitats
3. Mapping of composition of vegetation (vegetation relevés)

### Suggestion for ranking agricultural habitats due to their relevance for hosting crops

<table>
<thead>
<tr>
<th>Category 0</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No occurrence of volunteer crops</td>
<td>Volunteer crops rarely occurring</td>
<td>Volunteer crops occasionally occurring</td>
<td>Volunteer crops frequently occurring</td>
</tr>
<tr>
<td>Forests, Woodlands</td>
<td>Fallow ground dominated by large herbaceous plants</td>
<td>Verges of roads and paths</td>
<td>Unsealed paths and summer paths</td>
</tr>
<tr>
<td>Banks of streams running through greens or woodlands</td>
<td>Shrub- or woody edges</td>
<td>Banks of streams running through croplands</td>
<td>Wet spots or disturbed plots in cropped fields</td>
</tr>
<tr>
<td>Water bodies</td>
<td>Tree alleys</td>
<td>Disposal sites and other disturbed sites</td>
<td>Edges of headlands</td>
</tr>
<tr>
<td>Sealed surfaces</td>
<td>Intensively managed crop fields</td>
<td>Field margins of fields cropped with winter crops</td>
<td>Field margins of fields cropped with summer crops</td>
</tr>
<tr>
<td>Meadows and pastures</td>
<td>Meadows and pasture margins</td>
<td>Weed communities of fields cropped with grasses or crops for feed</td>
<td>Weed communities of fields cropped with summer crops</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grounds of settlements</td>
<td>'Fallow' croplands cultivated with cover or energy crops</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uncultivated fallow croplands</td>
</tr>
</tbody>
</table>

- Pleistocenic Lowlands
- Loess dominated Landscapes
- Basin Landscapes of Highlands
- Highlands
variability of agricultural landscapes: consequences for gm crop cultivation?

```
 Berkatal (Northern Hessian Highlands):
 - Maize Cultivation: 29 ha = 6,2 % of field area
 - Maize-Fields-No: 25 (field margins: 6,1 km)
 - Maize Field Ø: 1,2 ha (blue-green)
 - O.R. cultivation: 103 ha = 22 % of field area
 - O.R. Fields-No: 139 (field edges: 27,5 km)
 - O.R. field Ø: 0,7 ha (yellowish)
 - Field edges (total): 193,2 km
 - Path, road verges: 62,0 km

 Grebbin (NE German Lowlands):
 - Maize Cultivation: 121 ha = 17 % of field area
 - Maize Fields-No: 6 (field margins: 5,6 km)
 - Maize Field Ø: 20,2 ha (blue-green)
 - O.R. cultivation: 179 ha = 25 % of field area
 - O.R. Fields-No: 7 (field edges: 3,9 km)
 - O.R. field Ø: 25,6 ha (yellowish)
 - Field edges (total): 52,2 km
 - Path, Road verges: 35,6 km
```


Variability of Agricultural Landscapes: Consequences for GM Crop Cultivation?

- GM crops will be cultivated in agricultural landscapes being a mosaic of arable fields and non-cultivated habitats
- Linear disturbed ecosystems like field margins, road and path edges, banks of streams running through arable land represent a landscape specific network of habitats being in more or less close connection to cropped fields or to seed transportation routes
- Regionally prevailing Farming Systems determine the density of the network of field edges as well as the network of paths, roads and ditches
- The network of 'wild habitats' are of importance for the risk assessment of genetically modified plants with regard to two cases:
  - Margins, edges or banks rich in annual plant species represent habitats having the potential for hosting GM crops as well as potential wild species and favouring their dissemination *(the closer the network, the higher the risk of dissemination)*
  - Margins, edges or banks rich in perennial plant species represent habitats with the potential for hosting host plants of NTO lepidopteran species which may be affected by Bt-maize pollen expressing Cry1 expressing pollen *(the smaller the network, the lower is the risk of affecting NTOs through pollen)*
- These results indicate potential landscape specific risks; however, they are still not confirmed — a modeling approach with regard to 'hot spots' could help to identify their importance

2) Current Alterations in Field Management

a) Cultivation of Cover Crops

and its potential impacts on GMP cultivation
Impacts of Cultivation of Cover Crops:

- Increasing tendency of cultivating plants on arable fields two times per year, mainly conducted by cultivating cover crops between the cash crops

  Why?

- Benefits of Cover Crops:
  1) Reduction of fertilizer leaching:
     - Nitrate
     - Other mobile fertilizers
  2) Increasing humus content:
     - Improving soil biology (fauna, microorg.)
     - Improving soil structure
     - Increasing water availability
     - Indirect mitigation of soil degradation
  3) Soil coverage:
     - Suppression of weeds
     - Indirect mitigation of soil degradation
  4) Diversification of crop rotation

<table>
<thead>
<tr>
<th>Species</th>
<th>Scientific name</th>
<th>Seeding time</th>
<th>Winter hardiness</th>
<th>Voluntee-rism</th>
<th>N-Extraction (kg N/ha)</th>
<th>Yield (t/ha)</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian Ryegrass</td>
<td><em>Lolium multiflorum</em></td>
<td>End of July or later</td>
<td>good</td>
<td>possible</td>
<td>90 - 120</td>
<td>3,8</td>
<td>Cover cr. &amp; animal feed</td>
</tr>
<tr>
<td>Winter Rye</td>
<td><em>Secale cereale</em></td>
<td>End of September</td>
<td>very good</td>
<td>low</td>
<td>?</td>
<td>15,0</td>
<td>Cover cr. or energy resource</td>
</tr>
<tr>
<td>Turnip Rape</td>
<td><em>Brassica rapa</em></td>
<td>August – Sept.</td>
<td>good</td>
<td>low - medium</td>
<td>90 - 130</td>
<td>3,0</td>
<td>Cover cr.</td>
</tr>
<tr>
<td>Wild Mustard</td>
<td><em>Sinapis arvensis</em></td>
<td>August – Sept.</td>
<td>no</td>
<td>low</td>
<td>100 - 130</td>
<td>4,3</td>
<td>Cover cr., org. Fert.</td>
</tr>
<tr>
<td>Wild Radish</td>
<td><em>Raphanus raphanistrum</em></td>
<td>August – Sept.</td>
<td>no</td>
<td>low</td>
<td>90 - 120</td>
<td>4,0</td>
<td>Cover cr., org. Fert.</td>
</tr>
</tbody>
</table>
Consequences of Cover Crops on Cultivation, incl. GMOs:
- Increasing opportunities regarding crop rotation
- Increasing organic matter may improve abundance of soil fauna and soil microorganism

Questions:
- More rapid degradation of Bt-proteins and/or herbicides?
- Increasing application of non-selective herbicides?
- Lesser soil-related impacts through consecutive cultivation of GMOs?

Variability of Agricultural Landscapes and Alterations in Field Management - Consequences for GM Crop Cultivation?

2) Current Alterations in Field Management

b) Conservational Farming (Low or No-Tillage)
and its potential impact on GMP cultivation
Impacts of Conservational Soil Management Strategies:

- Increasing tendency of reducing soil management of arable fields, mainly by applying low or no-till farming
  
  **Why?**
  
- **Benefits of reduced soil management:**
  1. Improving soil structure
  2. Mitigation of risk of soil degradation
  3. Increasing humus stocks, especially of permanent humus components
  4. Stabilization of soil fauna and flora
  5. Improving soil water availability due to reduction of evaporation
  6. Retention of mobile fertilizers in the rooted zone

**How?**

- **Techniques of conservational soil treatment:**
  1. No till: Any residues (stubbles, straw) remain as mulch on the soil surface
  2. Using harrows or cultivators (grubbers) for mixing up the upper soil layer
  3. Wide-base tyred equipment is recommended (avoiding of soil compaction)
  4. Possibly special drilling devices are necessary
  5. Application of non-selective herbicides may be necessary

Consequences of Conservational Soil management on Cultivation, incl. GMOs:

- Increasing organic matter may improve abundance of soil fauna and soil microorganism

**Questions:**

- Increasing application of non-selective herbicides?
- More rapid degradation of Bt-proteins and/or herbicides in soils?
- Lesser soil-related impacts on consecutive cultivation of GMOs?
Assessing and managing impacts of crop biotechnologies

Ralf Wilhelm, Joachim Schiemann
JKI, SB

Institute for the Biosafety of Plant Biotechnology

Julius Kühn-Institut,
Federal Research Centre for Cultivated Plants
(founded 2008 by integrating 3 research centres)

Research centre & federal authority:
GMO act, pesticide act, acts for chemical compounds

Headquarters: Quedlinburg

15 Institutes

Budget (Stand: 01.01.2009):
Governmental: 70,5Mio. €
Funding: 5,0 Mio. €
Total: 75,5 Mio. €

Permanent staff: ~ 810  Total ~1150
Scientists: ~ 250
Research

- Plant genetics (supporting breeding)
- Quality traits
- Plant nutrition and soil science
- Plant protection and phytosanitary
- Cultivation/ crop management
- Impact assessment & Biosafety

Institute for the Biosafety of Plant Biotechnologies

- Risk assessment for plants derived from modern biotechnologies
- Coexistence measures
- Monitoring of impacts

Risk assessment, risk management and biosafety research

- Biosafety research
- Safety assessment
- Risk management
- Decision making
- Reduction of assessment burden
- Implement measures

Generate information

New technologies
- Confinement and mitigation
- Ecological profiling

Crop

Environment

GMO
New plant breeding techniques

Evaluation of new technologies, defined by the EU-Commission in 2007

<table>
<thead>
<tr>
<th>Technology</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc Finger Nuclease (ZFN) Technology</td>
<td>(restriction and manipulation of DNA, new: TALENs)</td>
</tr>
<tr>
<td>Oligonucleotide Directed Mutagenesis</td>
<td>(precisely directed point mutations, ODM)</td>
</tr>
<tr>
<td>Cisgenesis/Intragenesis</td>
<td>(genes/cDNAs of sexual compatible species)</td>
</tr>
<tr>
<td>RNA-dependent DNA methylation</td>
<td>(miRNA, RNAi and hairpin RNA for DNA methylation)</td>
</tr>
<tr>
<td>Grafting</td>
<td>(grafting on a GMO rootstock)</td>
</tr>
<tr>
<td>Reverse Breeding</td>
<td>(isolate parental genotypes by suppression of meiosis)</td>
</tr>
<tr>
<td>Agro-Infiltration</td>
<td>(transient expression by Agrobacterium transformation)</td>
</tr>
<tr>
<td>Synthetic Genomics (Biology)</td>
<td>(transplantation of synthesized genes and genomes)</td>
</tr>
</tbody>
</table>

* Lusser et al., 2011. JRC Scientific and technical report.

New plant breeding techniques

Addressed concerns
-> new risks by new methods?
-> appropriate tools for RA

Methods
gene targeting by site specific nucleases (ZFN or TALENs)
genetics of genes involved in DNA recombination

Expected output
-> evaluation of unintended effects by usage of site specific nucleases
-> principle understanding which factors drive or inhibit gene targeting

ZFN = Zinc Finger Nuclease
TALENs = Transcription Activator Like Element Nucleases
Confinement and mitigation

Expanding existing and novel confinement strategies (FP 6, FP 7)

- improve available confinement tools (cytoplasmic male sterility, cleistogamy) for current needs
- to provide “superior” tools (e.g. plastid transformation)
- New challenges – new confinement strategies ...

... Agro-Infiltration

"Spray-N-Trait": the Future Process

Growing bacteria

Field agroinfiltration

Limitations today: technical regulatory

Biomass processing

Source: NOMAD
Confinement and mitigation

**Addressed concerns**
- reduce environmental impact of biotech plants
- Coexistence
- leakiness

**Methods**
- field testing of new confinement strategies
- development/improvement of confinement strategies

**Expected output**
- evaluation of efficiencies for mitigation/confinement, management measures
- recommendations for new confinement strategies and techniques

---

Physiological - Ecological Profiling (PEP)

Holistic scope of ERA

Effect chains (ERA)
Physiological - Ecological Profiling (PEP)

Addressed concerns
-> „unintended“ effects
-> targeting of ERA and PMEM

Methods (lab to field)
-> quantitative reviewing
-> ~omics
-> modelling
-> [... field ecology]

Expected output
-> knowledge gaps in quantified impact assessment
-> ERA: „ecology and substantial equivalence“; relevant questions/limits for ~omics
-> PMEM: crop-specific pre-evaluation of causalities; targeted planning

Future research items

Broadening focus to novel trends in agriculture

1) Challenges and impact assessment of novel technologies
   -> site-specific modifications, targeted regulation
   -> method-specific RA

2) Confinement and mitigation
   -> improving/evaluation of confinement strategies
   -> strategies to reduce regulatory burden

3) Quantitative physiological-ecological profiling
   -> ERA: improve „risk modelling“, management planning
   -> Monitoring: causalities, interpretation, optimized methods
The environmental risk assessment of transgenic Bt rice by using *Folsomia candida* (Collembola: Isotomidae)

Yiyang Yuan
Institute of Zoology, Chinese Academy of Science
Supervisor: Prof. Feng Ge
2011.12.

The development of Bt crops in China

Chinese government is preparing to commercialize the transgenic Bt rice in China

A brief introduction of *Folsomia candida*

- Widely distribute throughout the world, consume fungi in soil and plant litter.
- Carry out key processes in soil ecosystems (nutrient cycling, decomposition of organic matter etc.).
- A model arthropod in both ecotoxicological and nonecotoxicological studies because of its vulnerable to the effects of soil contamination and easy of culture in the laboratory and relatively short generation times at room temperature.
Effects of Bt rice on *F. candida*

- Bt rice residues
  
  Assessing the impacts of changes in rice compounds after *Bt* gene inserted on *F. candida*.

- Bt proteins
  
  Finding out the useful genetic biomarkers of *F. candida* to Bt proteins, as well as the mechanism that how does *F. candida* cope with Bt proteins.

### Table 1

The three Bt-rice varieties, their concentration of Bt-protein and their corresponding near-isogenic non-Bt rice varieties used in the experiment for diet and addition to soil.

<table>
<thead>
<tr>
<th>Bt – rice variety</th>
<th>Bt protein (μg/g)*</th>
<th>Bt proteins</th>
<th>Near-isogenic variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huahui1</td>
<td>0.80±0.07 Ab</td>
<td>Stacked</td>
<td>Minghui63</td>
</tr>
<tr>
<td>BtShanyou63</td>
<td>0.84±0.04 Ba</td>
<td>Stacked</td>
<td>Shanyou63</td>
</tr>
<tr>
<td>Kemingdiao</td>
<td>1.75±0.08 Aa</td>
<td>Cry1Ab</td>
<td>Xuishui</td>
</tr>
</tbody>
</table>

*μg/g dry maize leaves (mean ± S.E). Means with the same uppercase letters are not significantly different between three *Bt* varieties for each organ (ANOVA, P<0.05). Means with the same lowercase letters are not significantly different between three organs for each *Bt* varieties (ANOVA, P<0.05). N.D.: None detected.
The effects of Bt rice on antioxidant enzyme activities of *F. candida*

Fig. 1. The number of juveniles reared on the rice and yeast treatments. Yeast + Bt proteins: individuals fed with yeast mixed with two pure Bt proteins (final concentration of Bt proteins was 852.81 ± 365.20 ug/g, Cry1Ab: Cry1Ac = 1:1). Each value represents the average (± SE) of five replicates. Different uppercase letters indicate significant differences across non-Bt-rice near-isogenic variety treatments (LSD test; d.f.=2, 12). *: significant differences at P<0.05 between Bt-rice varieties and their near-isogenic lines (d.f.=1, 8).

Fig. 2. The number of surviving adults reared on the rice and yeast treatments. Yeast + Bt proteins: individuals fed with yeast mixed with two pure Bt proteins (final concentration of Bt proteins was 852.81 ± 365.20 ug/g, Cry1Ab: Cry1Ac = 1:1). Each value represents the average (± SE) of five replicates. Different uppercase letters indicate significant differences across non-Bt-rice near-isogenic variety treatments (LSD test; d.f.=2, 12). *: significant difference at P<0.05 between Bt-rice varieties treatments and their isolines treatments (d.f.=1, 8).

Fig. 3. The activities of some antioxidant enzymes in *F. candida* can be affected by feeding with certain Bt rice varieties.

**The activities of some antioxidant enzymes in *F. candida* can be affected by feeding with certain Bt rice varieties.**
The direct and indirect effects of Bt rice on *F. candida*’s survival, reproduction, and growth

Comparisons between different rice organs of each variety

**Rice feeding experiment**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Root Survival</th>
<th>Stem Survival</th>
<th>Leaf Survival</th>
<th>Yeast Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minghuai3</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Hualai1</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Xiaohui</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Keningdao</td>
<td>a</td>
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<tr>
<td>Shanyou63</td>
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<td>a</td>
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<tr>
<td>Bihanyou53</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Yeast</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

**Artificial soil experiment**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Root Survival</th>
<th>Stem Survival</th>
<th>Leaf Survival</th>
<th>Yeast Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minghuai3</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Hualai1</td>
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<td>Yeast</td>
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Fig 4 Comparisons of the mean values of *F. candida*’s survival, reproduction and growth (final body length) between populations fed with rice roots, stems and leaves of each variety. Vertical bars are standard errors. Means with the same letters have no significant differences between root, stem and leaf treatments (uppercase letters: P<0.01; lowercase letters: P>0.05)

Comparisons between different non-Bt rice varieties of each organ

**Rice feeding experiment**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Root Survival</th>
<th>Stem Survival</th>
<th>Leaf Survival</th>
<th>Yeast Survival</th>
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<td>Yeast</td>
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**Artificial soil experiment**

<table>
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<th>Variety</th>
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<th>Stem Survival</th>
<th>Leaf Survival</th>
<th>Yeast Survival</th>
</tr>
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<tbody>
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<td>Xiaohui</td>
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<td>Yeast</td>
<td>a</td>
<td>a</td>
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</table>

Fig 5 Comparisons of the mean values of *F. candida* survival, reproduction and growth between populations reared in artificial soil mixed with 3 non-GM rice varieties and stems of each variety. Vertical bars are standard errors. Significant differences between transgenic varieties and their near-isogenic varieties treatments *: P<0.05; **: P<0.01

Fig 6 Comparison of the mean values of *F. candida*’s survival, reproduction and growth between populations fed the three non-GM varieties. Vertical bars are standard errors. Means with the same letters are not significantly different for the three non-GM varieties treatments (uppercase letters: P<0.01; lowercase letters: P>0.05)

Fig 7 Comparisons of the mean values of *F. candida*’s survival, reproduction and growth between populations reared in artificial soil mixed with 3 non-GM rice varieties. Vertical bars are standard errors. Means with the same letters are not significantly different between the three non-GM varieties (uppercase letters: P<0.01; lowercase letters: P>0.05)
**Comparisons between Bt rice varieties and their near-isogenic lines of each organ**

**Rice feeding experiment**

- **Number of surviving adults**
  - [Graph showing the number of surviving adults for different rice varieties and treatments.]

- **Number of juveniles**
  - [Graph showing the number of juveniles for different rice varieties and treatments.]

- **Body length (mm)**
  - [Graph showing the body length for different rice varieties and treatments.]

**Artificial soil experiment**

- **Number of surviving adults**
  - [Graph showing the number of surviving adults for different rice varieties and treatments.]

- **Number of juveniles**
  - [Graph showing the number of juveniles for different rice varieties and treatments.]

- **Body length (mm)**
  - [Graph showing the body length for different rice varieties and treatments.]

**Fig 8** Comparisons of the mean values of *F. candida*’s survival, reproduction and growth between populations fed with transgenic varieties and their near-isogenic varieties. Vertical bars are standard errors. Significant differences between transgenic varieties and their near-isogenic varieties treatments: *: P<0.05; **: P<0.01 A: root; B: stem; C: leaf.

**Fig 9** Comparisons of the mean values of *F. candida*’s survival, reproduction and growth between populations reared in artificial soil which mixed with transgenic varieties and their near-isogenic varieties. Vertical bars are standard errors. Significant differences between transgenic varieties and their near-isogenic varieties treatments: *: P<0.05; **: P<0.01 A: root; B: stem.
Conclusions

- The direct and indirect effects of Bt rice on *F. candida* are very different, and these mainly depend on the differences of organs, varieties.

Bt rice had no toxicity to *F. candida* directly and indirectly

- The differences between Bt rice varieties and their near-isogenic lines are similar with between different rice organs or varieties.

---

Effects of Bt proteins on *F. candida*

---

[Bar charts showing the effects of Cry1Ab+1Ac on yeast and yeast mixed with Cry1Ab+1Ac.]
Results of effects of Bt proteins(Cry1Ab:Cry1Ac=1:1) on
gene expression of *F. candida* by using microarray.

A total of 27 differentially expressed genes were found

<table>
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<th>ori_ID</th>
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<th>Denominator(s+s0)</th>
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A significant mechanism that F. candida (non-target organism) cope with Bt proteins is exist.

The research is continuing…

### Conclusions

- No negative effect of Bt proteins was found on collembolan F. candida.
- A significant mechanism that F. candida (non-target organism) cope with Bt proteins is exist.

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The future research items

- The indirect effects of Bt crops on non-target organisms (NTOs) should be evaluated, especially mediated by the biological chain.
- Find out the differences between plant compositions of Bt varieties and their near-isogenic lines, especially the differential plant compositions that may affect the NTOs.
- More useful physiological and molecular biomarkers should be explored.
- Mechanism of NTOs response to Bt protein should be studied.

Thank you for your attention!
Background:

Biologist, thesis in agricultural sciences

Topics:

Project „Ecosystem research in the Bornhöved Lakes Region“

Middelhoff U, Breckling B 2005: From single fine roots to a black alder forest ecosystem: how system behaviour emerges from single component activities. Ecological Modelling 186, 489-501
Project: Generic Methods of Investigation and Extrapolation of Oilseed rape Dispersal (*Brassica napus* L.) (GenEEERA)


Middelhoff U et al. 2011: An integrative methodology to predict dispersal of genetically modified genotypes in oilseed rape at landscape-level - a study for the region of Schleswig-Holstein, Germany. Ecological Indicators 11: 1000–1007

- Model based analyses of the spread of GMO: assessment for potential impacts on nature conservation areas in Northern Germany
- Ecological Area Sampling (EAS): concept for a national monitoring of genetically modified organisms
- Information system for the monitoring of the environmental impacts of genetically modified organisms (ismo)

- European enforcement project deliberate release of genetically modified organisms (EEP-DR)
Regulatory clarification:

Interplay between the GMO regulation (Directive 2001/18/EC) and pesticide regulation (Regulation (EC) No. 1107/2009)

Future research should:

enable regulators to link management options for different uses of herbicides (HR) and biocide (e.g. Bt) strategies to national biodiversity strategies

supply criteria, methodology and approaches for meaningful experiments (e.g. DR and stress tolerance)

and monitoring (design, baseline, power)
Ecological risk assessment of transgenic cotton on no-target soil organisms

Nengwen Xiao
xiaonw@163.com

Chinese Research Academy of Environmental Sciences (CRAES)

2011.12.12

About CRAES

- Submitted to State Environmental Protection Administration of China (SEPA),
- CRAES carries out innovative basic scientific research on environmental protection, taking national strategy of sustainable development.
- Ecology protection: conservation of ecosystem, biodiversity, biosafety
Bt cotton and Bt + CpTI cotton

- **Bt transgenic cotton** acreages have increased rapidly in recent years around the world. In 2010, Global area reached 148 million hectares (Bt cotton occupied 15.5 million ha) and 3.5 million ha in China (James, 2010).

- **Cowpea trypsin inhibitor** (CpTI) is a serine protease inhibitor. It almost inhibited the major agricultural pests, including most of the lepidoptera insect pests, and some Coleoptera, is a very broad spectrum insecticide. CpTI has become the most widely used insecticidal protein except the Bt toxin.

- **Bt + CpTI cotton**: Transgenic cotton expressing both the Bt protein (Cry1Ac) and the CpTI (cowpea trypsin inhibitor protein,) was developed and commercialized in 2001 (Guo et al., 1999).
Safety evaluation of GMOs to soil environmental

Soil organism
- earthworm,
- nematodes,
- acarid,
- springtail,
- protozoan

Bioaccumulation
Degradation
Cascading effect

Content

1. Effect of transgenic cotton on earthworm

2. Effect of CpTI+Bt cotton on nematodes community

3. Future research
1. Effect transgenic cotton on earthworm

Earthworms for soil environmental monitoring

- A very important non-target terrestrial soil organisms of Toxic substances, five species of eco-toxicological tests (EU).

- Ecotoxicology method of System environmental safety about earthworm was development
  - Acute toxicity test method (ISO 11268-1; OECD 207)
  - Subchronic toxicity test (ISO11268-2; OECD 222)
  - Field ecotoxicity test method (ISO 11268-3)

- Biomarkers to monitor soil pollution.
  - Enzyme, lysosomal neutral red time, heat shock protein, metallothionein, and ultrastructural changes, DNA damage, macromolecular adduct
① Effect Bt toxin Cry1Ac on *Eisenia fetida* in artificial soil

- **Bt toxin:** Cry1Ac, 16000 IU.mg⁻¹.
- **Earthworm:** *Eisenia fetida*, 300-400 mg.
- **Test soil:** dry cow dung 1:1 with an artificial soil mix. Artificial soil test according to OECD soil preparation Rule 207.
- **Four concentration gradient of Bt protein:** 10, 1, 0.1, 0 mg Bt toxin Kg⁻¹ soil.

② Effect of CpTI+Bt cotton on earthworm

*Amynthas hupeiensis* at laboratory experiment

- **Material**
  - **cotton:**
    - Transgenic *Bt+CpTI* cotton (SGK321)
    - parent isogenic variety (ShiYuan 321)
  - **earthworm**
    - *Amynthas hupeiensis* (Michaelsen, 1895)
  - Earthworm fed with SGK321 and SY321 cotton leaf
Effect of CpTI+Bt cotton on A. hupeiensis at field test

- PVC pipe Φ7.5cm,
- Cotton: SGK321 and SY321
- Drying cotton leaf was added Each tube
- Sampled every 2 weeks, a total of 4 times.

Effect

- Survival rates
- Weight change
- Nutrients changes
  - 1) protein content; 2) the total amino acids;
- Physiological and biochemical effects of earthworms
  - Digestive enzymes (Amylase, Trypsinase)
  - Antioxidant enzymes (POD, SOD, CAT)
  - Detoxification enzymes (Carboxylesterase, acetylcholinesterase)
Effect of Bt toxin on *E. fetida*

Weight changed rates of *E. fetida* cultivated with different concentration Bt toxin

![Graph showing weight changed rates over days for different concentrations of Bt toxin]

Protein content and AchE, CAT and cellulose activity of *E. fetida*

<table>
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<th>Concentration (mg. kg⁻¹)</th>
<th>Duration (d)</th>
<th>Protein content (mg. g⁻¹)</th>
<th>AchE activity (nmol mg⁻¹ min⁻¹)</th>
<th>CAT activity (µmol mg⁻¹ min⁻¹)</th>
<th>Cellulose activity (nmol mg⁻¹ min⁻¹)</th>
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<td>13.48 ± 0.35 a</td>
<td>5.92 ± 0.63 a</td>
<td>63.22 ± 5.05 a</td>
<td>47.80 ± 3.88 a</td>
<td>166.59 ± 41.61 a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>14.00 ± 2.21 a</td>
<td>3.57 ± 0.36 a</td>
<td>69.35 ± 3.47 a</td>
<td>56.85 ± 0.93 a</td>
<td>348.36 ± 4.82 a</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>15.02 ± 2.19 a</td>
<td>4.01 ± 1.01 a</td>
<td>85.80 ± 3.10 a</td>
<td>55.23 ± 1.75 a</td>
<td>153.34 ± 20.80 a</td>
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<tr>
<td>1</td>
<td>2</td>
<td>13.62 ± 1.04 a</td>
<td>6.30 ± 0.94 a</td>
<td>76.38 ± 5.13 a</td>
<td>55.22 ± 7.04 a</td>
<td>246.03 ± 73.18 a</td>
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<td>13.29 ± 2.06 a</td>
<td>4.98 ± 0.67 a</td>
<td>80.41 ± 5.29 a</td>
<td>54.90 ± 11.29 a</td>
<td>172.13 ± 52.67 a</td>
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<tr>
<td></td>
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<td>14.61 ± 2.53 a</td>
<td>3.50 ± 0.76 a</td>
<td>83.02 ± 3.78 a</td>
<td>66.93 ± 0.91 a</td>
<td>225.21 ± 42.27 a</td>
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<tr>
<td>0.1</td>
<td>2</td>
<td>13.00 ± 2.40 a</td>
<td>6.15 ± 1.21 a</td>
<td>88.75 ± 3.79 a</td>
<td>58.19 ± 7.68 a</td>
<td>265.82 ± 101.87 a</td>
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<td>3.56 ± 0.07 a</td>
<td>89.38 ± 6.06 a</td>
<td>54.46 ± 5.02 a</td>
<td>262.84 ± 25.81 a</td>
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<td>80.55 ± 1.92 a</td>
<td>68.70 ± 6.51 a</td>
<td>323.80 ± 72.80 a</td>
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<tr>
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<td>2</td>
<td>13.64 ± 2.24 a</td>
<td>5.96 ± 0.44 a</td>
<td>89.69 ± 4.90 a</td>
<td>62.81 ± 6.96 a</td>
<td>287.17 ± 196.82 a</td>
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<td>5.23 ± 3.28 a</td>
<td>91.45 ± 4.01 a</td>
<td>64.33 ± 3.72 a</td>
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<td>15.11 ± 1.20 a</td>
<td>4.77 ± 1.09 a</td>
<td>86.26 ± 2.96 a</td>
<td>73.79 ± 1.92 a</td>
<td>159.54 ± 13.89 a</td>
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</table>
### AVONA results on the biochemical activity of *E. fetida* exposed to Bt toxin

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<tr>
<th>Biochemical measurements</th>
<th>Bt Concentration</th>
<th>Duration</th>
<th>Concentration × Duration</th>
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<tr>
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<td>df</td>
<td>F</td>
<td>P</td>
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<tr>
<td>protein content</td>
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<tr>
<td>cellulase activity</td>
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<td>1.155</td>
<td>0.347</td>
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<tr>
<td>catalase activity</td>
<td>3</td>
<td>1.583</td>
<td>0.219</td>
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<tr>
<td>AchE activity</td>
<td>3</td>
<td>1.939</td>
<td>0.150</td>
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<tr>
<td>GST activity</td>
<td>3</td>
<td>0.924</td>
<td>0.452</td>
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</tbody>
</table>

**Effect of CpTI+Bt cotton on *A. hupeiensis***

- **Amylase activity**
- **Trypsinase**
- **POD activity**
- **SOD activity**
- **Acetylcholinesterase**
- **Carboxylesterase**

Bt toxin and CpTI+Bt cotton has not ecological risk to earthworm.
2. Effect of CpTI+Bt cotton on nematodes community

- Nematodes presence across many trophic levels in soils, they are vitally important in soil environments and ecosystem processes (Barker and Koenning, 1998; Ingham et al., 1986).
- Nematodes has been widely used in evaluation soil environmental quality or environmental disturbance.

**Sampling Methods:**
- 100 cm$^3$ ring knife to take 0-5 cm soil samples, soil samples 100 cm$^3$,
- 2 cotton variety, Four replicates of each variety, each in three samples,
- **Sampling:** 2009.07, 2009.08, 2009.09, 2009.10; 2010.05, 06, 07, 08, 09, 10, 11
The richness, functional group and c-p values of nematodes of different cotton field

### SY321: 39 genera
### SGK321: 34 genera

<table>
<thead>
<tr>
<th>Genera</th>
<th>Seedling type (FT)</th>
<th>c-p value</th>
<th>Number of nematodes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pseudoleonema</strong></td>
<td>Ba^2</td>
<td>1^2</td>
<td>84.1^3 209.4^4</td>
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<tr>
<td><strong>Rhodolaimus</strong></td>
<td>Ba^2</td>
<td>1^2</td>
<td>67.9^2 72.6^1</td>
</tr>
<tr>
<td><strong>Diplocyrtus</strong></td>
<td>Ba^2</td>
<td>1^2</td>
<td>99.6^2 114.7^4</td>
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<tr>
<td><strong>Rhodolaimus</strong></td>
<td>Ba^2</td>
<td>1^2</td>
<td>0.4^2 0.8^1</td>
</tr>
<tr>
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<td>Ba^2</td>
<td>1^2</td>
<td>1.5^2 0.5^1</td>
</tr>
<tr>
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<td>0.0^2 0.3^1</td>
</tr>
<tr>
<td><strong>Flesus</strong></td>
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<td>2^2</td>
<td>3.8^2 8.8^1</td>
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<tr>
<td><strong>Monokolinium</strong></td>
<td>Ba^2</td>
<td>1^2</td>
<td>138.2^2 99.4^1</td>
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<td><strong>Protomitridae</strong></td>
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<td>1^2</td>
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<td>2.0^2 1.3^1</td>
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<td>0.3^2 4.6^1</td>
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<tr>
<td><strong>Wolinema</strong></td>
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<td><strong>Eucytheridus</strong></td>
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<td>2^2</td>
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<tr>
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<td>Ba^2</td>
<td>2^2</td>
<td>718.8^2 689.2^1</td>
</tr>
<tr>
<td><strong>Monokolinium</strong></td>
<td>Ba^2</td>
<td>1^2</td>
<td>0.3^2 1.7^1</td>
</tr>
<tr>
<td><strong>Prionoleonema</strong></td>
<td>Ba^2</td>
<td>3^2</td>
<td>2.2^2 3.4^1</td>
</tr>
<tr>
<td><strong>Aphelenchus</strong></td>
<td>Ba^2</td>
<td>4^2</td>
<td>0.1^2 0.6^1</td>
</tr>
<tr>
<td><strong>Pseudoleonema</strong></td>
<td>Fa^2</td>
<td>2^2</td>
<td>2.1^2 0.9^1</td>
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<tr>
<td><strong>Aphelenchus</strong></td>
<td>Fa^2</td>
<td>1^2</td>
<td>121.1^2 64.7^1</td>
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<td><strong>Nematodirus</strong></td>
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<td>2^2</td>
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<tr>
<td><strong>Acantholaimus</strong></td>
<td>Fa^2</td>
<td>4^2</td>
<td>0.9^2 1.5^1</td>
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<td>2^2</td>
<td>0.7^2 0.1^1</td>
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<td><strong>Heteroderma</strong></td>
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<td>1.7^2 0.1^1</td>
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<td><strong>Dorylaimus</strong></td>
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<td>4^2</td>
<td>2.6^2 4.2^1</td>
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<td><strong>Aporcelaimus</strong></td>
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<td>5^2</td>
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<td>0.1^2 0.0^1</td>
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<td><strong>Eubungia</strong></td>
<td>O^2</td>
<td>4^2</td>
<td>37.3^2 17.9^1</td>
</tr>
</tbody>
</table>

2) Effect on the abundance and diversity of soil nematode

Abundance of nematodes

Number of genera

H'

Simpson Index
Conclusion

- No ecological risk was observed CpTI + Bt cotton on soil nematodes and earthworms with present evidence.
3. Future research

1. Risk assessment about other GM Crops to earthworms and nematodes

- Bt rice
- Transgenic corn
- Transgenic oilseed rape

- Earthworm
- Nematodes population
- Other soil organisms: Beetle, acarid
2. GMOs effect on soil structure and fertility

- Effect of GM crop on soil **microbial community** diversity and ecological functions.

- Soil **physical and chemical properties**, soil **fertility** of transgenic crop (Organic matter, total nitrogen, phosphorus, potassium, cation exchange capacity).

3. Detection and monitoring methods of GMOs

- Simple, accurate and fast detection methods for foreign genes

  - Rapid-tests strip to detect CpTI protein of transgenic cotton
  - Test kit to detect CpTI protein of transgenic cotton using particle-enhanced turbidimetric immunoassay
Thanks!
Decision Support and Qualitative Multi-Attribute Modelling

Marko Bohanec
Jožef Stefan Institute, Department of Knowledge Technologies
Ljubljana, Slovenia

Decision Support Research at IJS

http://kt.ijs.si/MarkoBohanec/dss.html

Decision Support
supporting people in complex decision-making processes

Decision Analysis

Multi-Attribute Modelling

Qualitative Multi-Attribute Modelling

• Tools
• Applications
EU Projects on GMO

ECOGEN 2003-2006 http://www.ecogen.dk/
Soil ecological and economic evaluation of genetically modified crops

Sustainable introduction of genetically modified crops into European agriculture

Co-existence and traceability of GM and non-GM supply chains

ECOGEN and SIGMEA Models
Evaluating cropping systems in terms of ecology, coexistence and economy

multi-attribute model
“Grignon” Model: Ecology Part


“Grignon” Model: Economy Part
Soil Quality Model: Structure

Soil diversity
- Bacterial diversity
- Macro-fauna
- Endogeic richness
- Anecic richness
- Epigeic richness

Leaching
- Decomposition
- Soil functionality
- Plant growth
- Plant growth

Soil function
- Activity
- Mineralization
- Bacterial activity
- Fungi

Soil quality
- Activity
- Endogeic richness
- Anecic richness
- Epigeic richness

Detritivore biomass
- Worms biomass
- Protozoa biomass
- Nematodes biomass
- Enchytraeid biomass
- Detritivore mesofauna
- Bacteria/Fungi biomass


ESQI: ECOGEN Soil Quality Indicator

Web Service: http://kt.ijs.si/MarkoBohanec/ESQI/ESQI.php
GMO and Conventional Maize Co-Existence

Problem:
Can GM maize be grown in coexistence with plants on other fields?

Criterion:
Genetical interference (Adventitious Presence)
Typical target AP: 0.9%

Factors:
pollen flow, volunteers, feral plants, mixing during harvesting, transport, storage and processing, human error, accidents, ...

SMAC Advisor Architecture

1. SMAC Advisor Wizard User Interface
2. Co-Existence Multi-Attribute DEXi Model
3. MAPOD Simulation Results
SMAC Advisor: Co-Existence of GM and non-GM Maize

SITUATION

- Own GM field and its context
- Neighbouring non-GM fields

Expert-created concepts

Simple rules

Data: MAPOD simulations

Expert + Data

SMAC Advisor: Decision Support Tool
Unapproved GM Detection
Based Only on Product Traceability Data

Unapproved GM Detection
Based Only on Product Traceability Data

**Table: Attribute Description**

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGM</td>
<td>Detection of Unapproved GM using Traceability Data only</td>
</tr>
<tr>
<td>GeographicalOrigin</td>
<td>UGM risk related to the geographical origin of the product</td>
</tr>
<tr>
<td>EU</td>
<td>Does the product originate in an EU country?</td>
</tr>
<tr>
<td>GM_Region</td>
<td>Does the product originate in a region of large GMO production?</td>
</tr>
<tr>
<td>SystemsUsed</td>
<td>UGM risk due to used traceability systems</td>
</tr>
<tr>
<td>TraceabilitySystemInPlace</td>
<td>Is a traceability system in place?</td>
</tr>
<tr>
<td>IP_GMO</td>
<td>Are IP systems for GMO being used?</td>
</tr>
<tr>
<td>IP_Other</td>
<td>Are other IP systems being used?</td>
</tr>
<tr>
<td>AnalCtrl_Systems</td>
<td>Are there systems used that include analytical control?</td>
</tr>
<tr>
<td>PrivateContracts</td>
<td>Are there any private contracts?</td>
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<tr>
<td>Logistics</td>
<td>UGM risk originating in logistics</td>
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<tr>
<td>Log_Complexity</td>
<td>Number of interactions in the supply path</td>
</tr>
<tr>
<td>Interactions</td>
<td>Number of companies involved in logistics</td>
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<tr>
<td>Log_Storage</td>
<td>UGM risk due to storage used</td>
</tr>
<tr>
<td>Harbour</td>
<td>Has the product been shipped through harbor(s)?</td>
</tr>
<tr>
<td>Silo</td>
<td>Has the product been stored in silos?</td>
</tr>
<tr>
<td>MethodsUsed</td>
<td>UGM risks based on the appropriateness of used methods and available results</td>
</tr>
<tr>
<td>AppropriateMethods</td>
<td>Have appropriate methods been used?</td>
</tr>
<tr>
<td>AppropriateSampling</td>
<td>Have appropriate sampling methods been used?</td>
</tr>
<tr>
<td>AppropriateAnalysis</td>
<td>Have appropriate analysis methods been used?</td>
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<td>AnalyticalResults</td>
<td>Risks according to analytical results</td>
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<td>Results</td>
<td>Analytical results, if available</td>
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<td>Certificate</td>
<td>Regulation-based certificate with relation to GMO’s under emergency measure</td>
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**Evaluation results**

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<th>Bulk Maize</th>
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<td>GEO</td>
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<tr>
<td>EU</td>
<td>high</td>
</tr>
<tr>
<td>GM_Region</td>
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<td>SystemsUsed</td>
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<tr>
<td>IP_Other</td>
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<td>AnalCtrl_Systems</td>
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<tr>
<td>PrivateContracts</td>
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<td>Logistics</td>
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<td>Interactions</td>
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<td>Harbour</td>
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<tr>
<td>Certificate</td>
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GM Presence Due to Transportation
Based Only on Product Traceability Data


GM Presence Due to Transportation
Based Only on Product Traceability Data

Attribute Description

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Description</th>
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</thead>
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<tr>
<td>GM_Presence</td>
<td>Transportation Module: Assessment of GM presence due to transportation</td>
</tr>
<tr>
<td>TraceabilityData</td>
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</tr>
<tr>
<td>Products</td>
<td>Risk due to product characteristics</td>
</tr>
<tr>
<td>CropRisk</td>
<td>Crop/product type</td>
</tr>
<tr>
<td>CropSpecies</td>
<td>Crop species</td>
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<tr>
<td>ProcessingLevel</td>
<td>Processing level</td>
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<tr>
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</tr>
<tr>
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<td>Number of countries involved in storage</td>
</tr>
<tr>
<td>CountriesAtRisk</td>
<td>Are there countries at risk involved?</td>
</tr>
<tr>
<td>CoexistenceMeasures</td>
<td>Are coexistence measures in place in countries?</td>
</tr>
<tr>
<td>Transportation</td>
<td>Risk due to transportation route</td>
</tr>
<tr>
<td>Storage</td>
<td>Risk due to storage</td>
</tr>
<tr>
<td>DedicatedSilos</td>
<td>Dedicated silos used for non-GMO?</td>
</tr>
<tr>
<td>Carriers</td>
<td>Risk due to carriers</td>
</tr>
<tr>
<td>NumberOfHarbours</td>
<td>Number of harbours involved</td>
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<td>Dedicated carriers used for non-GMO?</td>
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<td>Analytical data available about unintended admixture</td>
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<td>Is analytical data available?</td>
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<td>Analytical data, if available</td>
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Data Mining and Decision Support

Conclusion

- Expertise in developing multi-attribute evaluation models and decision support systems related to GMO and risk assessment

Qualitative modeling:
- “Umbrella” models: combining expertise from different domains
- Facilitates communication between experts from different areas
- Provides foundation for development of decision support tools and systems
- The approach has pros and cons

Lessons learned:
- Development of models only from data (statistical analysis, data mining) is often inadequate and
  must be combined with expert-based model development to achieve operational results.

Future challenges:
- Further improvement of decision modeling methods and tools, e.g.:
  – relational models
  – dynamic models (models with cycles)
  – combining qualitative and quantitative evaluation
- Applications of these methods to IRA of GMO
  – from research prototypes to DSS regularly used in practice
Current Projects and Proposals

Project proposals:
- GMO-CIS: Development of a comprehensive information system (CIS) on issues of GMO coexistence and traceability for stakeholders (GMO-CIS) [failed]

Current projects:
- Relational models for decision support: Integrating predictive and evaluation models: Problem of managing the risk of pesticide leaching, by combining/integrating the output of existing models for hazard assessment and exposure models both across space and time
- Using relational decision trees to model out-crossing rates in a multi-field setting: Predict the gene flow from GM to non-GM maize fields under real multi-field crop management practices at a regional scale
New challenges in risk assessment of GM Plants
– Establishing a research program aiming for integrative risk assessment
(Copenhagen workshop December 12-13 2011)

Research on the ecological risk assessment of transgenic Bt crops against insect pests under global climate change

Reporter: Dr. Fajun Chen & Prof. Megha N Parajulee

Tel: 806-786-8940; E-mail: fajunchen@njau.edu.cn

NAU Profile
http://www.njau.edu.cn

Nanjing Agricultural University (NAU) is a multi-disciplinary national key university under the direct jurisdiction of the Ministry of Education. It is a pioneer of modern agricultural education, research and extension, and one of the first agricultural institutions in to offer four-year bachelor programs. There are 16 colleges in the university offering 59 Bachelor programs, 106 Master programs, and 65 PhD programs, as well 6 professional Master programs. The university also houses 13 postdoctoral stations.

The university maintains close cooperation and exchanges with over 100 universities and research institutes around the world, including 42 universities with which it has institutional agreements.
Entomology research at NAU

- Insect Population Ecology
  - [http://eco.njau.edu.cn/](http://eco.njau.edu.cn/)
- Insect Molecular Ecology and Evolution
  - [http://mee.njau.edu.cn](http://mee.njau.edu.cn)
- Insect Chemical Ecology
- Insect Pest Biocontrol
- Insect Physiology and Molecular Biology
  - [http://ento.njau.edu.cn](http://ento.njau.edu.cn)
- Insect Taxonomy
- Molecular Toxicology

The College of Plant Protection

Entomology research at NAU
Insect Population Ecology

Radar Monitoring

Prof. Zhai Baoping

Climate Change

Associate Prof. Fajun Chen

Assistant Prof. Hu Gao

GIS Model

Prof. Zhai Baoping

Associate Prof. Liu Xiangdong

Molecular Ecology

Fajun Chen
Introduction to the Transgenic Bt Crops adopted worldwide and in China

Transgenic *Bacillus thuringiensis* (Bt) crops has been commercially adopted worldwide after 1987, and excellent against target lepidopteran pests (James, 2008).

In China, the damage caused by cotton bollworm, *Helicoverpa armigera* has been greatly alleviated in recent years due, in part, to the adoption of Bt cotton after 1997 (Men et al., 2003; Wu et al., 2008).

On 27 November 2009, transgenic Bt rice, expressing fused *Cry1Ab* and *Cry1Ac*, were approved by China, and is expected to become commercially available in the near 2–3 years (James 2009).

How about the pest problems faced by Bt rice prior to commercial usage in field and especially under global climate change?
Our Research on Transgenic Bt Crops

Bt cotton vs. non-Bt cotton (from 1998)
- **Restorer line:**
  - Gk-12 \((\text{Cry1Abc})\) vs. Simian-3 (the parental line)
- **Restorer line:**
  - 338 \((\text{Cry1Abc})\) vs. GP5415 (the parental line)

Bt rice vs. non-Bt rice (from 2004)
- **Restorer line:**
  - KMD \((\text{Cry1Ab})\) vs. XSD (the parental line)
- **Restorer line:**
  - HH1 \((\text{Cry1Ab}+\text{Cry1Ac})\) vs. MH63 (the parental line)
- **Hybrid line:**
  - Bt-SY63 \((\text{Cry1Ab}+\text{Cry1Ac})\) vs. SY63 (the parental line)

Our Research on Transgenic Bt Crops

Bt cotton vs. non-Bt cotton
- **Target insect pest:**
  - Cotton bollworm, *Helicoverpa amigerna*
- **Non-target insect pest:**
  - Cotton aphid, *Aphis gossypii*
  - Cotton whitefly, *Bemisia tabaci*

Bt rice vs. non-Bt rice
- **Target insect pest:**
  - Stem borer, *Chilo suppressalis*
- **Non-target insect pest:**
  - Rice planthoppers, *Nilaparvata lugens, Laodelphax striatellus, Sogatella furcifera*
Problems Faced by Transgenic Bt Crops in Field and under Global Climate Change

In Field:
- Target-insect pests occurring
- Non-target insect pests arising
- Time-spatial dynamics of Bt toxins expressing

Under global climate change:
- As global warming and CO₂ arising, significant decreases in Bt-toxin content were found, which may reducing target-resistance against lepidopteron pest?
- As global warming and CO₂ arising, significant increases in second defense chemicals’ content were found, which may increasing target-/no-target resistance against insect pests?

Three topics

- Topics 1#: Dilution Effect Test
  - How Does Bt-toxin Content of Bt Cotton (Rice) Relative to the Increased Biomass under Elevated CO₂

- Topics 2#: Target-insect pests’ occurrence
  - Impacts of Elevated CO₂ on the Target Resistance of the Bt Cotton (rice)

- Topics 3#: Non-target-insect pest arising
  - Impacts of Elevated CO₂ on the Occurrence of non-Target insect pests of the Bt Cotton (rice)
Research item #1

How Does Bt-toxin Content of Bt Cotton (Rice) Relative to the Increased Biomass under Elevated CO₂ (Dilution Effect Test)

Open Top Chamber (OTC)

Plant tissues’ biomass of Bt cotton and Bt rice grown under ambient and elevated CO₂

Significant increases in plant tissues’ biomass were found in most plant tissues of Bt cotton and Bt rice in elevated CO₂ relative to ambient CO₂.
Plant tissues’ Bt-toxin content of Bt cotton (rice) grown under ambient and elevated CO₂

Significant decreases in Bt-toxin content were found in most plant tissues of Bt cotton and Bt rice in elevated CO₂ relative to ambient CO₂

Many significant decreases were found for elevated CO₂ relative to ambient CO₂

While some significant increases were also shown, here!
### Dilution Effect Test --- Bt cotton

**Speculation on the significant decreases in Bt-toxin content of Bt cotton grown in ambient and elevated CO₂**

<table>
<thead>
<tr>
<th>Measured parameters</th>
<th>Bt toxin (ng/mg)</th>
<th>Biomass (g per plant)</th>
<th>Bt toxin (mg per plant)</th>
<th>Significance level</th>
<th>Dilution effect</th>
</tr>
</thead>
</table>

#### 45 days after sowing

<table>
<thead>
<tr>
<th>Component</th>
<th>Bt toxin</th>
<th>Biomass</th>
<th>Bt toxin</th>
<th>Significance level</th>
<th>Dilution effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>- (***))</td>
<td>+ (**)</td>
<td>- (')</td>
<td>&lt; (***))</td>
<td>?</td>
</tr>
<tr>
<td>Petiole</td>
<td>- (***))</td>
<td>+ (**)</td>
<td>- (ns)</td>
<td>&lt; (***))</td>
<td>&lt;</td>
</tr>
<tr>
<td>Shoot</td>
<td>- (')</td>
<td>+ (**)</td>
<td>+ (')</td>
<td>&lt; (')</td>
<td>&lt;</td>
</tr>
<tr>
<td>Root</td>
<td>- (***))</td>
<td>+ (ns)</td>
<td>- (ns)</td>
<td>&lt; (ns)</td>
<td>&lt;</td>
</tr>
<tr>
<td>Total plant</td>
<td>- (&quot;')</td>
<td>+ (&quot;')</td>
<td>- (')</td>
<td>&lt; (&quot;&quot;)</td>
<td>&lt;</td>
</tr>
</tbody>
</table>

#### 90 days after sowing

<table>
<thead>
<tr>
<th>Component</th>
<th>Bt toxin</th>
<th>Biomass</th>
<th>Bt toxin</th>
<th>Significance level</th>
<th>Dilution effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>- (***))</td>
<td>+ (ns)</td>
<td>- (')</td>
<td>&lt; (***))</td>
<td>?</td>
</tr>
<tr>
<td>Petiole</td>
<td>- (')</td>
<td>+ (ns)</td>
<td>- (ns)</td>
<td>&lt; (')</td>
<td>&lt;</td>
</tr>
<tr>
<td>Square</td>
<td>- (')</td>
<td>+ (ns)</td>
<td>+ (ns)</td>
<td>&lt; (')</td>
<td>&lt;</td>
</tr>
<tr>
<td>Boll</td>
<td>- (')</td>
<td>+ (ns)</td>
<td>+ (ns)</td>
<td>&lt; (')</td>
<td>?</td>
</tr>
<tr>
<td>Shoot</td>
<td>- (ns)</td>
<td>+ (')</td>
<td>+ (ns)</td>
<td>&lt; (ns)</td>
<td>&lt;</td>
</tr>
<tr>
<td>Root</td>
<td>- (***))</td>
<td>+ (ns)</td>
<td>- (ns)</td>
<td>&lt; (ns)</td>
<td>&lt;</td>
</tr>
<tr>
<td>Total plant</td>
<td>- (&quot;')</td>
<td>+ (&quot;')</td>
<td>- (')</td>
<td>&lt; (&quot;&quot;)</td>
<td>&lt;</td>
</tr>
</tbody>
</table>

### Dilution Effect Test --- Bt rice

**Speculation on the significant decreases in Bt-toxin content of Bt rice grown in ambient and elevated CO₂**

<table>
<thead>
<tr>
<th>Measured parameters</th>
<th>Bt toxin (ng/mg)</th>
<th>Biomass (g per plant)</th>
<th>Bt toxin (mg per plant)</th>
<th>Significance level</th>
<th>Dilution effect</th>
</tr>
</thead>
</table>

#### 50 days after sowing

<table>
<thead>
<tr>
<th>Component</th>
<th>Bt toxin</th>
<th>Biomass</th>
<th>Bt toxin</th>
<th>Significance level</th>
<th>Dilution effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>- ('')</td>
<td>+ (')</td>
<td>+ (')</td>
<td>&gt; (')</td>
<td>✔</td>
</tr>
<tr>
<td>Aboveground tissues</td>
<td>- ('')</td>
<td>+ (')</td>
<td>+ (')</td>
<td>&gt; (')</td>
<td>✔</td>
</tr>
<tr>
<td>Total stem</td>
<td>- ('')</td>
<td>+ (')</td>
<td>+ (')</td>
<td>&gt; (')</td>
<td>✔</td>
</tr>
</tbody>
</table>

#### 100 days after sowing

<table>
<thead>
<tr>
<th>Component</th>
<th>Bt toxin</th>
<th>Biomass</th>
<th>Bt toxin</th>
<th>Significance level</th>
<th>Dilution effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flag leaf</td>
<td>- ('')</td>
<td>+ (ns)</td>
<td>- (ns)</td>
<td>&gt; ('')</td>
<td>✔</td>
</tr>
<tr>
<td>Flag-leaf sheath</td>
<td>- (ns)</td>
<td>+ (ns)</td>
<td>+ (ns)</td>
<td>&gt; (ns)</td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>- ('')</td>
<td>+ (ns)</td>
<td>- (ns)</td>
<td>&gt; ('')</td>
<td>✔</td>
</tr>
<tr>
<td>Leaf sheath</td>
<td>- ('')</td>
<td>+ (ns)</td>
<td>+ (ns)</td>
<td>&gt; ('')</td>
<td>✔</td>
</tr>
<tr>
<td>Shoot</td>
<td>- ('')</td>
<td>+ (ns)</td>
<td>- (ns)</td>
<td>&gt; ('')</td>
<td>✔</td>
</tr>
<tr>
<td>Root</td>
<td>- (ns)</td>
<td>+ (')</td>
<td>+ (')</td>
<td>&gt; (')</td>
<td>✔</td>
</tr>
<tr>
<td>Total stem</td>
<td>- ('')</td>
<td>+ (ns)</td>
<td>- (ns)</td>
<td>&gt; (')</td>
<td>✔</td>
</tr>
</tbody>
</table>
SUMMARY

1. It is ascertained that the dramatic increases in biomass significantly dilute Bt-toxin content in the below tissues:
   1. The 45-DAS petiole and shoot, and the 90-DAS square of Bt cotton
   2. The 50-DAS tissues and the 100-DAS leaf sheath of Bt rice

2. While significant decreases of Bt-toxin content in the 90-DAS leaf and boll of Bt cotton weren’t resulted by the dilution effect, which may be caused by the adverse effects of elevated CO₂ on the Bt-gene expression.

3. In the other tissues, the significant decreases in Bt-toxin content were partially resulted by the dilution effect of elevated CO₂.

Research item #2

Impacts of Elevated CO₂ on the Target Resistance of the Bt Cotton (rice)
(Target-insect pests’ occurrence)
Larval survival rate (A) and pupal weight (B) of cotton bollworm, *Helicoverpa armigera* fed on Bt cotton grown in ambient and elevated CO₂

(SSP - susceptible colony; RST – resistant colony)

Larval survival rate (A) and pupal weight (B) of stem borer, *Chilo suppressalis* fed on Bt rice grown in ambient and elevated CO₂
SUMMARY

- CO\(_2\) level significantly affected the fitness and performance of cotton bollworm and stem borer
  - Significant decreases in larval survival rate and pupal weight were found for *H. armigera* RST colony and *C. suppressalis* in elevated CO\(_2\) (cf ambient CO\(_2\)).
- The larval survival rate of the resistant colony (RST) was significantly higher than that of the susceptible colony (SSP) for cotton bollworm at same CO\(_2\) level
- Significant decreases were also indicated in pupal weight of the RST colony relative to that of the SSP colony in elevated CO\(_2\).

CONCLUSIONS

- Although significant decreases in Bt-toxin content are shown in elevated CO\(_2\), the efficiency of Bt cotton (rice) against target lepidopterans is still higher compared with ambient CO\(_2\).
- Here, maintenance of the Bt efficacy at dramatically reduced level of actual Bt-toxin is not to the target efficiency from Bt transgenes, it may be attributed to:
  1. **Plant-Chemistry Defense** - the discernible increases in plant allocation to inherent secondary defenses owing to elevated CO\(_2\)
  2. **Plant-Physical Resistance** - changes in leaf microstructure due to elevated CO\(_2\)
- So there are still potentially dramatically adverse consequences of higher future CO\(_2\) levels for the Bt technology
Research item #3

Impacts of Elevated CO₂ on the non-Target Resistance of the Bt Cotton (rice) (non-target-insect pest arising)

Experiment Design & Treatments

Effects of high T, elevated CO₂, and their combination impacts on the occurrence of three planthoppers fed on transgenic Bt rice

- **CO₂ level**
  - Ambient CO₂
  - Elevated CO₂

- **Temperature level**
  - High T
  - Low T

- **Transgenic treatment**
  - Cry1Ab纯合基因型（恢复系）
  - 克螟稻（KMD）
  - 珍本秀水XSD
  - Cry1Ab+Cry1Ac融合基因型（恢复系）
  - 华恢BH1 + 珍本明恢MH63

- **Planthopper species**
  - Brown planthopper (BPH)
  - Small brown planthopper (SBPH)
  - White-backed planthopper (WBPH)
  - Sogatella furcifera
**Light-trap**

**White backed planthopper**  
*WBPH*  
*Sogatella furcifera*

**Small brown planthopper**  
*SBPH*  
*Laodelphax striatellus*

**Brown planthopper**  
*BPH*  
*Nilaparvata lugens*
Effects of temperature levels on population abundances of white backed planthopper, *S. furcifera*, fed on transgenic Bt rice with dual *Cry1Ab+Cry1Ac* genes

Different lowercase letters indicated significant difference between the treatments of high temperature and low temperature by one-way Repeated ANOVA at d.f.=24, with F=8.93 and P=0.04<0.05

Effects of CO₂ levels on population abundances of white backed planthopper, *S. furcifera*, fed on transgenic Bt rice with pure *Cry1Ab* genes

Different lowercase letters indicated significant difference between the treatments of high temperature and low temperature by one-way Repeated ANOVA at d.f.=24, with F=8.93 and P=0.04<0.05
Conclusions

➤ Under the Global Climate Change (especially high temperature and elevated CO₂), transgenes of Bt rice can significantly affect the population patterns of rice planthoppers occurring.

- For the dual Cry1Ab+Cry1Ac Bt rice, global warming, especially under elevated CO₂, appears to be for a factor accelerating the outbreaks of WBPH relative to BPH and SBPH.
- For the pure Cry1Ab Bt rice, elevated CO₂, especially under global warming, appears to be for a factor reducing the occurrence of WBPH relative to BPH and SBPH

➤ Compared with pure transgene, dual transgenes may be faced much more adverse effects of global climate change especially on non-target resistance.

Future Research

➤ Transgene Diversity: Single traits as well as multi-stacked traits should be used simultaneously in transgenic Bt crops fields to take advantage of high transgenic crops/cultivars diversity for optimal performance against not only target-insect pests, but also non-target pests.

➤ Necessary tactics and measures should be taken to improve the Bt-toxin expression against target lepidopterans under elevated CO₂.
  ➤ De-methylation of Bt-transgene
  ➤ Chemical-induced regulation of Bt crops
  ➤ N-fertilizer management in Bt crops’ field
Thanks [谢谢] Thanks
Research in ERA of GM-plants

Research Background & Suggestion for Future Research

J. Arie Vonk

Biology (MSc) University of Groningen
Ecology (PhD) Radboud University Nijmegen
Researcher
  Ecotoxicology National Institute for Public
  Soil ecology Health and Environment (RIVM)
  Nematodes & Wageningen University (WUR)
  Risk assessment
Research in ERA of GM-plants

Current project

- Ecology Regarding Genetically modified Organisms (ERGO; funded by NWO)
  - What are the effects of GM crops on non-target soil organisms and soil functioning?
  - Nematode DNA Barcode-Based tool (Nematology, WUR)
  - Links between changes in nematode community and soil functioning
  - Identification of response window (NOR, baseline, reference) under different agricultural practices
  - Guidance for ERA of GM-crops using nematode tool

- Exploration lab/greenhouse conditions
  - plant species and cultivar effects
  - toxicity of plant metabolites on nematode species

- Exploration field conditions
  - plant species
  - plant species and cultivar effects

- Testing GM crops
  - plant species, cultivar and GM-effects

- Focus on ecosystems
Research in ERA of GM-plants

Suggestion for Future Research (1)

- **In situ** tools to determine GM-induced effects
  - Link between measured group of organisms and effects at ecosystem level
  - Quantitative
  - Development of response window

Suggestion for Future Research (2)

- Inclusion of Management
  - GM-crops developed to change current practices
    - e.g. resistance against pests => reduced spraying
  - Realistic comparison of potential negative and positive effects
  - Identify relative impact of GM relative to other agricultural practices
Research in ERA of GM-plants

Suggestion for Future Research (3)

- Life Cycle Assessment (LCA)
  - e.g. GM-crops developed for specific industrial processes
    - Comparison whole process of growing and production
  - e.g. drought resistant GM-crops
    - Land use and irrigation effects
- Inventory of required information

Thank you
An alternative modelling approach to regulatory risk assessment (primarily populations and NTOs)

Chris Topping
Dept. BioScience
Aarhus University, Denmark

Large-scale sources of variation in evaluation of risk

+ DYNAMICS associated with management, climate, and ecology of NTOs can lead to feedback loops further exacerbating these problems.

These factors would normally be considered to be stochasticity or explained by general terms.

But there could be an alternative...
Simulation modelling has progressed a lot in the last few years.

Integration of drivers and actors
Built as an extensible framework for current system understanding

With ALMaSS you get dynamic landscapes these dynamics allow new feedback potential not present in static models but definitely present in the real world.

This provides some idea of the dynamics, even for only two of many concurrent factors:

Vegetation Types

Vegetation Biomass
Simulated RAs can be simple, in this case evaluating the spatial dynamic aspects of recovery.

Insecticide application to central zone of a beetle simulation (80% mortality on contact)
No drift one application per year for 10 years

...or more complex e.g. this PLRA – Voles and an endocrine disruptor which induces epigenetic changes.

NRTB = population size relative to baseline
Increasing the assumed toxicity of an endocrine disruptor did not increase population impacts proportionally
Other managements treated orchard locations amount of alternative habitat all had impacts as large as those caused by toxicological parameters.

Fig. 3. NOEL scenario: NOEL was halved five times from 50 to 1.5625 mg/kg bw. The 30-year period with pesticide application is illustrated by the pesticide phase and the recovery period refers to the last 60 years of the simulation after ended pesticide treatment.
and the link to GMPs...

- Simulations for pesticide risk assessment indicate that the environmental context is critical to the result.
- Thus, simple approaches like TERs or Hazard Quotients do not capture the system responses.
- My proposal is therefore that for GM crops, environmental impact assessments should not follow the pesticide protocol, but more fully consider the environmental context into which the GMP is used.

Building comprehensive RA models

- These systems are complex, and we do not know everything about them...
- ...but, that does not mean that we should not use our best knowledge to predict impacts.
- Therefore what we need is a method of development that takes into account:
  - ‘fat-tails’ and avoiding the sin of omission (type II error)
  - the extent of our knowledge;
  - the needs of the stakeholders (e.g. EFSA/Industry/EC);
  - an assessment of how well the model fulfils its aims.
BUILDING COMPREHENSIVE RA MODELS

• This process will of course take more time and effort than developing simple models.
• The aim is to model the system not the individual case.
• For use in regulatory assessment we need to consider different landscapes and farming systems, and maintain these as the real world changes.
• However, the result should be durable and extendable, able to cope with future needs without huge investment.

Assessing different landscape and farming systems

<table>
<thead>
<tr>
<th>Landscape Element Type</th>
<th>Bjerringbro</th>
<th>Herning (B)</th>
<th>Præstø (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bushes/scrub</td>
<td>1.5</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Fields (rotation)</td>
<td>52.1</td>
<td>70.5</td>
<td>66.1</td>
</tr>
<tr>
<td>Heath</td>
<td>0.0</td>
<td>3.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Linear features (excl. hedgebanks)</td>
<td>1.8</td>
<td>3.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Hedgebank</td>
<td>0.1</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Permanent Pasture</td>
<td>8.2</td>
<td>1.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Unmanaged grassland</td>
<td>4.1</td>
<td>2.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Urban</td>
<td>9.0</td>
<td>4.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Water</td>
<td>1.7</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Wetland</td>
<td>1.1</td>
<td>2.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Woodland</td>
<td>19.0</td>
<td>8.6</td>
<td>19.6</td>
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<tr>
<td>Woodland plantation</td>
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<td>0.6</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Here impacts of farming system can be compared across landscapes.

Positive scores indicate a positive density and/or distribution response to a farm management/landscape combination.
Risk assessment of transgenic crops: gene flow and its ecological consequences

Yongbo LIU

Chinese Research Academy of Environmental Sciences

Research interests
My interests mainly focus on gene flow, biosafety of transgenic crops; morphology, evolution, conservation, restoration, and management; sustainability of bioenergy crops...

Education
- Ph.D., Life Science, Université de Bourgogne (Dijon) / Institut National de la Recherche Agronomique (INRA), Dijon, France; 2007-2010
- Ph.D., Ecology, Institute of Botany, the Chinese Academy of Sciences, Beijing; 2004 - 2011
- B.Sc., Horticulture, Human Agricultural University, Human; 2000 - 2004

Professional Activities
Peer-reviewer for Natural Hazards
Journal of Vegetation Science

http://ybliu.weebly.com
• Context
• Major works
• Future focus of RA
### GM Approvals in China (ISAAA, 2010)

<table>
<thead>
<tr>
<th>Species</th>
<th>Varieties</th>
<th>Transgenic traits</th>
<th>Food</th>
<th>Feed</th>
<th>Import</th>
<th>Planting</th>
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<td>6</td>
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<td>2004</td>
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<td>2</td>
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<td>2009</td>
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### Gene flow

1. Seeds
2. Pollens

GM crop
Wild relatives
GM plants
Volunteers

Yongbo Liu
Gene flow

Effects of hybridization
• High vegetative growth
• Low seed production
• Intermediate morphology

Effects of transgenes
• New characteristics
• Increase or decrease fitness

CONTENT

• Context
• Major works
• Future focus of RA
Major works

1. Hybridization and segregation
2. Fitness effects of transgenes
3. Ancient introgression

Materials

- Transgenic oilseed rape (*Brassica napus*)
- Wild brown mustard (*B. juncea*)
- Wild radish (*Raphanus raphanistrum*)
1. Hybridization and segregation

Could transgene stably inherit to descendants?
Does the parent morphology impact the fitness of progeny?
### 1. Hybridization and segregation

<table>
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<tr>
<th>Plant type</th>
<th>No. of plants</th>
<th>Resistant</th>
<th>Susceptible</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
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<td>$F_2$</td>
<td>25</td>
<td>21</td>
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<td>$BC_1N$</td>
<td>62</td>
<td>27</td>
<td>35</td>
<td>1:1</td>
<td>1.03 NS</td>
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#### % of $B. napus$ plant type

<table>
<thead>
<tr>
<th>Plant type</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>$BC_1NS$</td>
<td>39%</td>
<td>b</td>
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<td>$BC_1NS^*$</td>
<td>86%</td>
<td>a</td>
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<tr>
<td>$BC_1NR$</td>
<td>73%</td>
<td>a</td>
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</table>

#### % of large flower

<table>
<thead>
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<th>Plant type</th>
<th>%</th>
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<tr>
<td>$BC_1NS$</td>
<td>52%</td>
<td>b</td>
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<tr>
<td>$BC_1NS^*$</td>
<td>64%</td>
<td>ab</td>
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<tr>
<td>$BC_1NR$</td>
<td>74%</td>
<td>a</td>
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</table>
1. Hybridization and segregation

1. Segregation ratio of herbicide-resistant gene was consistent with Mendelian ratio
2. Backcross progeny with morphology-crops is more easily to establish as volunteers and feral plants in field
3. Monitoring backcross with *B. napus* pollens is difficult


2. Fitness effects of transgenes

Are transgenic plants superior competitors?
Does the presence of transgenic plants impact the production of susceptible plants?
2. Fitness effects of transgenes

Whether population production increases with increasing proportion of resistant plants?
Could resistant plants replace susceptible plants?

2. Fitness effects of transgenes

Simulation of insect-resistance
With wild brown mustard (B. juncea)
2.1 Simulation of insect-resistance

2. Fitness effects of transgenes

Backcross progeny of Bt-transgenic canola with wild *B. juncea*
2. Fitness effects of transgenes

Seed number per plant

No insects

Seed number per plant

With insects

% of trBC2

% of trBC2

Seed number per plant

0 2000 4000 6000 8000 10000

0 2000 4000 6000 8000 10000

25% 50% 75% 100%

0% 25% 50% 75% 100%

4000 6000 8000 10000

2000 4000 6000 8000 10000

50% 25% 0% 75% 100%

▲ trBC2

▲ ntrBC2

3. Ancient introgression

Does the long-term existence of crop alleles in wild relatives affect the morphological and life history traits?
3. Ancient introgression

Silique characteristics

- Diameter of the largest and smallest article
- Diameter of the largest and smallest constriction
- Length of articles
- Silique length

Flower characteristics

- Petal width
- Petal length

Brassica napus
3. Ancient introgression

DFA analysis

CONTENT

- Context
- Major works
- Future focus of RA
Future focus

Invade potential of trangenic crops
Population effects
Long-term effects
Remote sensing monitoring

Future focus

Farming practice
Crop multi-culture
Agriculture biodiversity
Insect- and herbicide resistant genes
Oilseed rape, rice, cotton ...
Thank you for your attention!
Establishing a research programme aiming for integrative risk assessment?:

R&D Experiences and Proposals

J. Kiss
Plant Protection Institute,
St. István Univ., Gödöllő, Hungary

„New challenges in risk assessment of Genetically Modified Plants,”
Copenhagen, 12-13 December 2011
Background and prior activities:
Effect of Bt-transgene (MON810) on Non-Target arthropods in maize

Effect on HT maize on field botanical and arthropod diversity
Background and prior activities:
Spatio-temporal approach for managing pests: WCR (*Diabrotica v.v.*)
(population growth, dispersal, modelling
Landscape composition and natural enemies

Background and prior activities:
System approach for developing IPM: Maize, Maize based Systems
Future research items:
Integrative ERA 1:

• All the things what has been presented by Jeremy
  +
• Representative cropping/farming systems?
• Crops (events, traits, combinations) likely to be present,
• ERA in spatial and temporal context:
  – rotation system over years
  – crops on spatial scale
• Develope scenarios (and use for)
• Developing models for risk managers

Future research items:
Integrative ERA 2:

• IPM as approach, frame and baseline,
• Risks, benefits across years and fields
• Regulating mechanisms in cropping systems,
• Indicators and parameters in systems
• PMEM part of ERA in systems,
• PMEM and IPM indicators?
Future research items:

Integrative ERA 3:

- Socio-economic components of ERA,
- Stakeholder involvement
- Farmers involvement
- Knowledge and capacity development

Thank you for the invitation
Outline

› What I have learned about GM effects on the soil ecology
› Need for an *integrated* risk assessment
› Suggested elements of an integrated assessment of farming practices
Observations of soil fauna in the lab and in GMO fields

- Generally speaking there are no effects of GM-crops on soil organisms compared to near-isogenic crops under field conditions.
- A toxic effect of Cry proteins observed for nematodes but at levels far above normal field soil concentrations (Marroquin et al. 2000; Höss et al. 2008, 2011).
- Thus, non-target organisms may be susceptible to Cry proteins, although they are taxonomically very distant to target organisms.


Unintended effects hardly observed for soil fauna

- Variety effects are often observed.
- Substantial equivalence and definition of 'limits of concern' need to be defined.
- The collembolan, *F. candida*, fed rice leaves display stress response, but it is a detrivore and not a herbivore.
- There is always a specific response to a specific variety by a collembolan.

What do species do in ecosystems?  
Lawton 1994

› **Redundancy:**
   Will species loss change the ecosystem structure and functioning

› **Ecosystem engineers**

Proposed research questions:
- Do *Lumbricus terrestris* and *Aporrectodea longa* affect water percolation in the same manner?
- Which species are actually crucial for ecosystem persistence of functionality?


---

**Earthworms increase soil infiltration**

Figure 1. Volume of soil leachates collected from field plots with decreased (open bars) or increased (shaded bars) earthworm population. Values are means SE per earthworm enclosure on each of 11 sampling dates (n =12)

Staining earthworm macropores to detect water infiltration potential

Biopore functioning
Extracting individual earthworm species from visible burrows

Recording biopores
Integrative ERA / accounting for complexity

› The farming system is the real environmentally operative unit consisting of
  › Cropping sequence
  › Fertilisation
  › Tillage
  › Pest management
  › Spatial distribution of natural - and managed farmland

Integrating what?

› All benefits and costs of management of ecosystems
  › Everything that has a benefit or could harm the sustainability of an ecosystem

› Harm with respect to:

› Ecosystems provides the services:
  › Carbon sequestration
  › Green-house gases
  › Water flooding
From biodiversity to economic evaluation

Field measurements
John Rytter, AU

Soil physics
Jack Faber, ALTERRA

Biopores
P.H. Krogh, AU

Hydrological modelling
Jon Finch, CEH

Translation of ecological information into ecosystem services
Katarina Hedlund, Lund

Valuation of ecosystem services
Mette Termansen, AU
Unai Pascual, 

Paul Henning Krogh
Bacillus thuringiensis and cry-toksins

- Spore and crystal germination
- Spore and crystal multiplication
- Spore germination and vegetative multiplication
- Sporulation and crystal formation
- Toxin activity
Research activities

• Natural occurrence of *B. thuringiensis* and Cry
• *B. thuringiensis* as a pest control agent
• The cry mode of action
• Effects of Cry on target – and non-target
• The fate of Cry – effects on microbial communities and processes
• Exchange of Cry-genes

Advicement

• At national level for 20 years
• Internationally (EU, OECD, UN)
• EFSA
• GMPs and Microbial pest control agents
Future research directions –
at the small level

- Interactions between cry and the intestinal microorganisms
- Effects on target – and non-target
- Importance of ”microbial quality” for effects in food-chains
- HOPE – risk assessment based on established knowledge e.g. cry mode of action

Effects of GMPs on soil ecosystems
in general

- Studies mostly restricted to direct effects by the GMP
- Notably lab-studies and small-scale field studies
- No-effects – or small transitory effects
- Studies concentrated about microbial genetical diversity, functional diversity, effects on non-target soil invertebrates, decomposition and to a lesser extent on nutrient cycling
Future directions

- From small scale to larger scale – field - landscape
- Include (in)direct effects of the GMP on agricultural practice
- Emphasis on key processes and nutrient cycling
- Effects on diversity on higher levels (e.g. landscape)
## List of participants

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
</table>
| Broder Breckling    | Landscape ecology  
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Germany                      |
| Jeremy Sweet        | Sweet Environmental Consultants  
Cambridge, United Kingdom        |
| Gerd Neemann        | Office for landscape ecology and environmental studies  
Göttingen Germany        |
| Ralf Wilhelm        | Julius Kühn-Institut (JKI)  
Federal Research Centre for Cultivated Plants  
Institute for Biosafety of Genetically Modified Plants  
JKI-SG, Quedlinburg, Germany |
| Yiyang Yuan         | State Key Laboratory of Integrated Management of Pest  
and Rodents  
Institute of Zoology  
Chinese Academy of Sciences  
Beijing                                    |
| Ulrikke Middelhof   | Federal Office of Consumer Protection and Food Safety  
(BVL) – Department 4 Genetic Engineering, Germany |
| Nengwen Xiao        | Chinese Research Academy of Environmental Sciences  
Beijing                                    |
| Marko Bohanec       | Jožef Stefan Institute, Department of Knowledge Technologies, Ljubljana, Slovenia |
| Fajun Chen          | Environmental Entomology  
Insect-information Ecology Group  
Department of Entomology, Nanjing Agricultural University (NAU), China |
| Arie Vonk           | Department of Soil Quality  
Wageningen University  
The Netherlands                             |
| Chris Topping       | Department of Bioscience  
Science and Technology  
Aarhus University  
Denmark                                    |
| Yongbo Liu          | Chinese Research Academy of Environmental Science  
Beijing                                    |
| Joszef Kiss         | Plant Protection Institute  
Szent Istvan University  
Gödöllő, Hungary                       |