11th ISBGMO
Buenos Aires | ARGENTINA 2010

11th International Symposium on the Biosafety of Genetically Modified Organisms

The role of Biosafety Research
in the decision-making process
Organized by the International Society
for Biosafety Research (ISBR)

Monday 15 November – Saturday 20 November

Centro Cultural Borges
Viamonte 525 - Galerías Pacífico - Buenos Aires Argentina

ISBR
International Society
for Biosafety Research

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International Society for Biosafety Research

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Consejo Argentino para la Información y el Desarrollo de la Biotecnología

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3.1 Organising Committees

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Moisés Burachik	Argentina
Wendy Craig	Italy
Sally McCammon	USA
Joachim Schiemann	Germany
Kristina Sinemus	Germany
Carmen Vicién	Argentina

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Carmen Vicién (Project Manager)	University of Buenos Aires
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Vanga Siva Reddy	India
María Mercedes Roca	Honduras
Clara Rubinstein	Argentina
Joachim Schiemann	Germany
Alison Snow	USA

3.2 Welcome Address

The Local Organizing Committee, the Symposium Committee and the International Society for Biosafety Research (ISBR) welcome you to the 11th International Symposium on the Biosafety of Genetically Modified Organisms in Buenos Aires, Argentina. The subject of this Symposium, "The role of biosafety research in the decision making process," foretells the importance of the activities and presentations that will take place during this meeting.
The sustained growth of agricultural biotechnology needs, among other things, science-based regulatory decisions. Scientists, regulators and other stakeholders attending this Symposium will have the opportunity to share experiences and learn about the contributions that biosafety research can make to the critical steps of decision making.
Argentina and some other Latin American countries are among the main producers of GM crops. For this reason Buenos Aires provides an appropriate venue for this Symposium where delegates can share knowledge and information gained from experiences in this region. In addition the city offers a wide array of cultural attractions. We warmly welcome you to Buenos Aires and hope you find the meeting interesting, useful and stimulating. We also wish you a pleasant and enjoyable stay in Argentina.

Patrick Rüdelsheim, President International Society for Biosafety Research (ISBR)
Jeremy Sweet, Chairman of the Symposium Committee of ISBR
Moisés Burachik, Chairman of the Local Organising Committee

3.3: Key note Speaker and Chairs Biographies

Keynote Speaker

Roger Beachy
Director, National Institute of Food and Agriculture, USDA
Chief Scientist, USDA
Washington, DC

Dr. Roger Beachy was appointed to be the first director of the National Institute of Food and Agriculture (NIFA) in October, 2009, and in January 2010 was appointed Chief Scientist of USDA. NIFA is responsible for awarding extramural funds for Research, Extension and Education for the U.S. Department of Agriculture. Prior to this appointment, he served as the founding president of the not-for-profit Donald Danforth Plant Science Center in St. Louis, Missouri. In this role, Dr. Beachy was responsible for developing and implementing the Danforth Center's strategic direction, recruiting its staff, and formulating its research programs. Dr. Beachy, a member of the National Academy of Sciences, is internationally known for his groundbreaking research on developing virus-resistant plants through biotechnology.

From 1991 to 1998, Dr. Beachy headed the Division of Plant Biology at The Scripps Research Institute, a leading biomedical research center in La Jolla, California. He was also Professor and Scripps Family Chair in Cell Biology and co-director of the International Laboratory for Tropical Agricultural Biotechnology (ILTAB) at Scripps.

Dr. Beachy was a member of the Biology Department at Washington University in St. Louis from 1978 to 1991, where he was Professor and Director of the Center for Plant Science and Biotechnology. His work at Washington University, in collaboration with Monsanto Company, led to the development of the world's first genetically modified food crop, a variety of tomato that was modified for resistance to virus disease. His technique to produce virus resistance in tomatoes has been replicated by researchers around the world and his groundbreaking work has led to the production of many types of virus-resistant plants.

Research under Dr. Beachy's direction has led to a number of issued patents and pending applications. He has edited or contributed to 50 book articles, and his work has produced more than 230 journal publications.

Dr. Beachy has received a number of honors for his research. He is a member of the U.S. National Academy of Sciences and in 2001 received the Wolf Prize in Agriculture. He is a fellow in the American Association for the Advancement of Science, the American Academy of Microbiology, the National Academy of Science, India, and the Academy of Science of St. Louis. He was elected Foreign Associate of the Third World Academy of Sciences. He was the 1991 recipient of the Bank of Delaware's Commonwealth Award for Science and Industry and the 1990 recipient of the American Phytopathological Society's Ruth Allen Award. Dr Beachy was awarded the Dennis Robert Hoagland Award from the American Society of Plant Biologists, an honorary Doctor of Science degree from Michigan State University, and the William D. Phillips Technology Advancement Award from the St. Louis County Economic Council. Dr. Beachy was named R&D Magazine's Scientist of the Year for 1999. In 2003, he was elected

Councilor for the National Academy of Sciences, and currently serves as a member of the editorial board of the Proceedings of the National Academy of Sciences.

Dr. Beachy has served on numerous boards and committees, including the board of the International Crops Research Institute for the Semi-Arid Tropics in Hyderabad, India, and the board of the NIDUS Center for Scientific Enterprise, and other voluntary boards in the St. Louis region. He is a member of a number of scientific societies, including the American Society of Plant Biologists, American Phytopathological Society, American Society for Biochemistry and Molecular Biology, and American Society for Virology. He currently serves as President of the International Association for Plant Biotechnology. He has served as consultant in plant biotechnology for several companies and frequently lectures on the applications of biotechnology in agriculture, nutrition, and human health.

Dr. Beachy holds a Ph.D. in plant pathology from Michigan State University and earned a B.A. in biology from Goshen College in Goshen, Indiana.

Session Chairs and Co-Chairs

Session 1 Co-Chairperson: Professor Dalia M. Lewi

Dalia Lewi was trained as an Agrarian engineer in the School of Agronomy, University of Buenos Aires (1988). She did her doctorate in the Faculty of Exact and Natural Sciences, University of Buenos Aires (2005). She is institutional project coordinator for maize and cotton genetic transformation at "Instituto de Genética Ewald A. Favret" (IGEAF), CICVyA, INTA (National Institute of Agriculture Technology). She is also main coordinator of Project INTA AERG 233272 "Identification and evaluation of capacities for transgenic event biosafety regulation" and Project INTA AERG 233261 "Development and adaptation of tools for transgenic transformation of crop species". She also has the position of Associate professor of Agricultural Genetics at the Agronomic Faculty, Morón University and is INTA Representative at CONABIA (National Advisory Commission on Agricultural Biotechnology) in the Agriculture Ministry.

Session 1 Co-Chairperson: Professor Moisés Burachik is also Chairman of the Local Organising Committee of ISBGMO 11. - see description above

Session 2 Chairman: Professor Alan Gray

Professor Alan Gray retired as Director of the Centre for Ecology and Hydrology, Dorset U.K. in 2003 following more than 35 years' research in plant ecology and genetics, and over 200 publications – mainly on gene flow, population ecology and genetics, and conservation genetics of natural plant populations. He has been involved in risk assessment for GMOs since 1990 undertaking research and providing independent advice to the UK Government, being a member of ACRE (the Advisory Committee on Releases to the Environment – the UK's statutory advisory committee on GMOs) from 1994 to 1999 and its Chairman from 1999 to 2003. Since retirement Alan has continued his involvement with biosafety of GM crops (e.g. on the UK GM Science Review), with science governance and peer audit, and has recently completed, with a co-author, a Flora of British Grasses.

Session 3 Chairperson: Professor Allison A. Snow

Dr. Allison A. Snow is a professor in the Department of Evolution, Ecology, & Organismal Biology at The Ohio State University, USA. Trained as a plant ecologist at the University of Massachusetts, she received postdoctoral fellowships from the National Science Foundation and the Smithsonian Institution. Her research combines molecular and ecological approaches to understand how quickly crop genes move into wild populations, and the extent to which novel transgenic traits could benefit weedy and semi-weedy relatives of sunflower, rice, sorghum, squash, switchgrass, and radish. She is the lead author of a 2005 position paper by the Ecological Society of America on environmental effects of genetically engineered organisms. A Fellow of the American Association for the Advancement of Science and the Aldo Leopold Leadership Program, she has served on the editorial boards of Ecology, Ecological Monographs, Evolution, Frontiers in Ecology, and Environmental Biosafety Research. She also has served as President of the Botanical Society of America, Treasurer of the International Society for Biosafety Research and as an adviser to the US National Academy of Sciences, the US Department of Agriculture, the World Trade Organization, and the US Presidential Commission for the Study of Bioethical Issues.

Session 4 Chairperson: Professor Margareth de Lara Capurro Guimarães

Margareth Capurro is Assistant Professor, Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil. Previously she was Postdoctoral Fellow, Department of Molecular Biology and Biochemistry, University of California, working on transgenic mosquitoes refractory for dengue transmission.

Her research includes the study of vector-parasites interactions, mosquitoes transgenesis and transgenic lines that can reduce/block dengue and malaria transmission. She collaborates with OXITEC for testing suppression of field population of *Aedes aegypti* mosquitoes transgenic lines in an endemic country. In addition she is Coordinator of Mosquitoes Control on REDE USP de Doenças negligenciadas and a Member of Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (INCT-EM), Brazil.

Session 5 Chairman: Professor Dr. Esteban Hopp

Prof. Dr. Esteban Hopp is National Scientific Coordinator of Molecular Biology, Bioinformatics and Advanced Genetics at Argentina´s National Agronomy Research Institute (INTA), Professor of Genetics at the University of Buenos Aires, and a former member of Argentine GMO Biosafety Commission (1991-2009) and prominent member of international biotechnology organizations like RedBio (FAO). Dealing with both GM and genomic plant biotechnologies and actively collaborates in national and international research networks. He pioneered South American research on transgenic plant

development, biosafety and the use of molecular markers to assist plant breeding. Author of 153 scientific publications, 3 patents of invention and director of 26 PhD thesis.

Session 6 Chairperson: Professor Nina V. Fedoroff

Photo: Douglas Mills, New York Times

Nina V. Fedoroff received her Ph.D. in Molecular Biology from the Rockefeller University, and has served on the faculties of the Carnegie Institution of Washington, the Johns Hopkins University and the Pennsylvania State University, where she was the Director of the Biotechnology Institute and the founding Director of the Huck Institutes of the Life Sciences. She is the Willaman Professor of the Life Sciences and an Evan Pugh Professor at Penn State, as well as a member of the External Faculty of the Santa Fe Institute and Distinguished Visiting Professor of the King Abdullah University of Science and Technology (KAUST) in Saudi Arabia. Fedoroff has published two books and more than 130 papers in scientific journals. Among her awards is a 2006 National Medal of Science, the highest honor awarded to US scientists. She is also a member of the U. S. National Academy of Sciences and the American Academy of Arts and Sciences. Fedoroff served as the Science and Technology Adviser to the Secretary of State and to the Administrator of the US Agency for International Development (USAID) from 2007 to 2010. She is President-elect of the American Association for the Advancement of Science (AAAS).

3.4: Information about Argentina and Buenos Aires

ABOUT ARGENTINA

• Political division: 24 Provinces and 5 main geographical regions.

• It is the 8th largest country in the world (largest countries: Russia, Canada, USA, China, Brazil, Australia & India). It is the second largest country in South America, following Brazil.

• It is the largest Spanish-speaking country.

• It is the 4th most populated country in Latin America, behind Brazil, Mexico & Colombia.

• Total country area: 3,761,274 km2 (including Antarctic territories).

• Main ethnic groups: White (85%), Mestizo (mixed Amerindian and white) (10%), Indigenous & others (5%).

• Seasons of the year:

Summer (Dec 21st – March 20th)	Temperature 80º F – 27º C
Fall (March 21st – June 20th)	Temperature 60º F – 15º C
Winter (June 21st – Sept. 20th)	Temperature 53º F – 11º C
Spring (Sept 21st – Dec 20th)	Temperature 60º F – 15º C

• "Argentina", comes from the Latin word ARGENTUM, that means 'silver', so Argentina means 'country of the silver'.


National Coat of Arms


National Flag

• Independence Day: July 9th, 1816.

• Capital city: Buenos Aires.

Buenos Aires Must

Museums:

- MALBA (modern art museum). Also worth are the
- Bellas Artes Museum (in Recoleta area) and the
- Centro Borges (center of the city, free, next door to Galerias Pacifico Shopping mall).
- On Sunday, go to San Telmo fair in the morning and to Recoleta in the afternoon (both the artisans fair & the center/museum exhibitions). You may even see Evita´s tomb next door

Shopping malls:

- Galerias Pacifico shopping malls or to
- Paseo Alcorta (full of design shops). Or walk around
- Palermo Hollywood, a Soho-like new district

Plaza de Mayo – May Square

It is the oldest square in Buenos Aires. Its location was determined in the 2nd foundation of the city, in 1580. It is surrounded by historical and government buildings, such as the Cabildo, the Casa Rosada (National Government House), the Palace of the Government of Buenos Aires, banks, and ministries.
On June 11th 1580, Spanish Juan de Garay performed the foundation ceremony of the "Ciudad de la Santísima Trinidad" and "Puerto de Santa María del Buen Ayre" in the land where nowadays Plaza de Mayo is located. The village was raised around it.
The "Pirámide de Mayo" is in the center of the square, this monument that was built in 1811 to celebrate the first anniversary of the revolution.

Obelisco – Obelisk

The "Obelisco" reminds in each of its faces a historical "porteño" fact: the first foundation of the city in 1536; the second and definitive foundation of the city, in 1580; the first time the National flag was raised in the city, in the year 1812 (in the church of San Nicolás, that was raised precisely where nowadays the "Obelisco" stands); and the

constitution of Buenos Aires as the Argentinean capital in 1880. The monument is one of the meeting points in the city for political, cultural, sports and social meetings and public acts.

Location: Plaza de la República, in the crossroad of Corrientes and 9 de Julio Avenues.

Opening: 1936

Milongas

Choose the alternative (to big tango shows): the neighborhood milongas. The best, most classic, and inexpensive way to see real tango danced by real people is at a *milonga*. Classes are often offered before the dances begin. Our picks include:

- Parakultural in Salón Canning (Address: Scalabrini Ortiz 1331; tel. 54 11 4832 6753)
- La Viruta (Address: Armenia 1366, tel. 54 11 4779 0030)
- Confitería Ideal (Address: Suipacha 384; tel. 54 11 5265 8069)

Recoleta Cementery

A labyrinth of haunting, gorgeous mausoleums belonging to the city's rich, famous and powerful families. The most famous tomb is that of Evita Perón, but many others are also interesting.

Free English tours on most Tuesdays and Thursdays at 11 a.m.
Address: Junin 1790; tel. 54 15 5614 8869. www.cementeriorecoleta.com.ar

AVENIDA CORRIENTES BY NIGHT
Eat the best Pizzas! Go to the Theatre!
And buy all sorts of Books until midnight…

"While the whole world abandons the crazy downtown area, go walking along the most emblematic street in the city, with the sun at your back."
Take in the frenetic buzz of workers hurrying home and spectators lining up outside brightly lit theaters.

Around the Obelisk, between Callao Av and Esmeralda St, Corrientes Av has the biggest theatre concentration in the entire city. Practically all the most important theatre halls from Buenos Aires are located in this stretch. Corrientes avenue is our local Broadway and many musicals and plays have been transferred here.
Also we recommend you to stop for a slice of greasy pizza and *faina* (chickpea bread) at one of the many stand-up pizza joints, such as Guerrin and then browse the endless new and used bookstores at Corrientes Avenue.

Café Tortoni

It is the oldest café in the city. The marble and wooden tables, the old pictures on its walls, the traditional menu, the waiters, and the customers, had turned it into the archetype of the bar in Buenos Aires.
A French immigrant called Touan founded it in 1858. The Tortoni was established in its current location in 1880. In the interior of the bar there are pictures, poems, and busts that tell the story of this bar. Jazz and Tango shows are performed at Tortoni Café.
Location: Avenida de Mayo 825

Caminito

Covered with paintings and sculptures, Caminito Street is one of the favorite walks of Buenos Aires. It is surrounded by typical houses from La Boca, with painted sheet walls of different colors.

Its winding route is because it follows the course of a stream that flowed until the beginning of the 20th Century.

In 1959, Caminito was officially inaugurated as an open sky museum.

There are street shows and a craftworks open fair on Saturdays and Sundays, from 10am to 7pm.

Location: between Garibaldi, Araoz de Lamadrid, del Valle Iberlucea, and Magallanes streets, at La Boca Neighborhood

SAN TELMO - Feria de Plaza Dorrego – Dorrego Fair

The Dorrego Fair is the heart of the old San Telmo neighborhood, at the southern part of the city.

The pubs from the area place their tables in the street and it is one of the places of the city with more street artists.

On Sundays there is a great antique and handcrafts fair at Plaza Dorrego, that gathers more than 10,000 visitors and has 270 sale stands. At the fair you may find "fonolas," used books, tango albums, out of stock collectible magazines, valuable antiques, époque clothes, cloth, and embroidery.

Location: it is delimited by Defensa, Humberto 1º, Bethlem and Aieta streets, at San Telmo Neighborhood

Shopping in Palermo

Specially during weekends, the neighborhood of Palermo—and its sub-neighborhoods also known as Palermo Hollywood, Palermo Soho, Palermo Chico, etc.—is abuzz with people shopping in the design and clothing stores and the small artisan market at Plaza Serrano and drinking coffee or wine at sidewalk cafés**.** Think the Village & Soho in New York. This is our local version…

Address: Serrano and Honduras Streets, at Palermo Neighborhood

Museo Carlos Gardel – Carlos Gardel Museum

Carlos Gardel was one of the most popular artists from the National and International history, and he is still a symbol for porteños. The museum is located at the last house that the singer lived in Buenos Aires.

Gardel was born in 1890 in Toulouse, France, and he arrived to Buenos Aires with his mother two years later. He died in a plane crash in Medellín, Colombia, in 1935. He was buried at Chacarita Cemetery, where his tomb can be visited. The museum depends of the Buenos Aires government; it exhibits personal articles of Gardel and samples of his work.

Location: 735 Jean Jaurés, at Abasto Neighborhood

Teatro Colón – Colon Theatre

It is one of the main lyrical theatres in the world. During the 20th Century, the most important directors, singers, and dancers from the period performed here.
In 1857 the first Teatro Colón was opened, in front of Plaza de Mayo. In 1888 it was closed and reopened in 1908. The main hall, horseshoe shaped, is considered as one of the best acoustics halls in the world. The dome is decorated by painter Raúl Soldi.
The theatre has a stable cast, a corps of ballet, orchestras, set design and costumes workshops, a library and a museum. Seating capacity is 3542, with room for 700 people standing.
Location: 618 Cerrito Av

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From Argentina: (011) 15.6395.3000
From outside Argentina: +54911.6395.3000
By e-mail:
info@bafreetour.com

Where to have the best food and drinks

Pizza

- Guerrin (Address: Corrientes, Av. 1368, tel: 4371-8141)
- Los Inmortales (Address: Av. Corrientes 1369, tel: 4373-5303)
- Las Cuartetas (Corrientes, Av. 838 , tel: 4326-0171)

Asado

- La Caballeriza (Address: Boulevard Chenault 1878, tel: (11) 4773-4035)
- Siga la Vaca (Address: Alicia Moreau de Justo 1714,
  Tel: 4315 6801)
- Las Lilas (Address: Alicia Moreau de Justo 516, Puerto Madero, tel: 4313-1336, www.laslilas.com

Pasta

- Don Chicho (Address: Plaza 1411, tel: 4556-1463)
- Marcelo (Address: Alicia Moreau de Justo, tel: 4342-8689)
- La Parolaccia Casa Tua (Address: Cerviño 3561 Esq. Salguero, tel: 4783-0200)

Argentinean Regional Food

- Divina Patagonia (Address: Balcarce 958, tel: 4300-6454)
- El San Juanino (Address: Sánchez de Bustamante 1788, tel:
  Tel: 4822-8080)
- La Querencia (Address: Junín y Juncal,
  tel: 4821-1888)

Traditional Cafes of Buenos Aires

- Café Tortoni (Address: Av. de Mayo 829)
- Café Las Violetas (Address: Av. Rivadavia 3899)
- La Biela (Address: Av. Quintana 596)

Alfajores and Dulce de Leche

- Havanna (Address: Alicia Moreau de Justo 1864)
- Balcarce (Address: Lavalle 669)

Wine Bars

- Bodega del Fin del Mundo (Address: Honduras 5663, tel: 4852-6660)
- Winery (Address: Av. del Libertador 500, tel: 4325-3400)

Pubs

- Down Town Matias (Address: Reconquista 701, tel: 4311-0327)
- Antares (Address: Armenia 1147, tel: 4833-9611)
- Kilkenny (Address: Marcelo T. de Alvear 399)
- Buller (Address: Paraguay 428, tel: 4313-0287)

Drinks

- Sucre (Address: Sucre 676, tel: 4782-9082)
- Million (Address: Parana 1048, Recoleta, tel: 4815-9925)
- Unico (Address: Honduras 5604)

GENERAL INFORMATION

Distance from the **Main International Airport "Ministro Pistarini"**, popularly known as **Ezeiza**, to Buenos Aires city is of 33 Km, so depending on the traffic it is a 30 /45 minutes drive. The following is information of some Taxi Companies posted at the official web site of the airport (www.aa2000.com.ar):

TAXIS Municipalidad de Ezeiza
(5411) 5480-0066 /9383
TAXIS Municipalidad de la Ciudad de Buenos Aires
(5411) 15 5305-2610 /2612
REMIS Manuel Tienda León / Shuttle service
(5411) 4314-3636 / 4315-5115
www.tiendaleon.com.ar
Tienda Leon has a Shuttle service that leaves from the airport every hour or every half hour to and from Down Town

REMIS Transfer Express
0800 4444 Transfer (872)
reservas@transferexpress.com.ar
REMIS Vip Cars
(54 11) 5480 4590 /4594
REMIS World Car
(54 11) 5480-1215

CAR RENTAL COMPANIES
ALAMO (NATIONAL)
Located at the airport in Arrivals area, Terminal A
(5411) 0810-999-25266
(5411) 5480-5580/5581
www.alamoargentina.com.ar

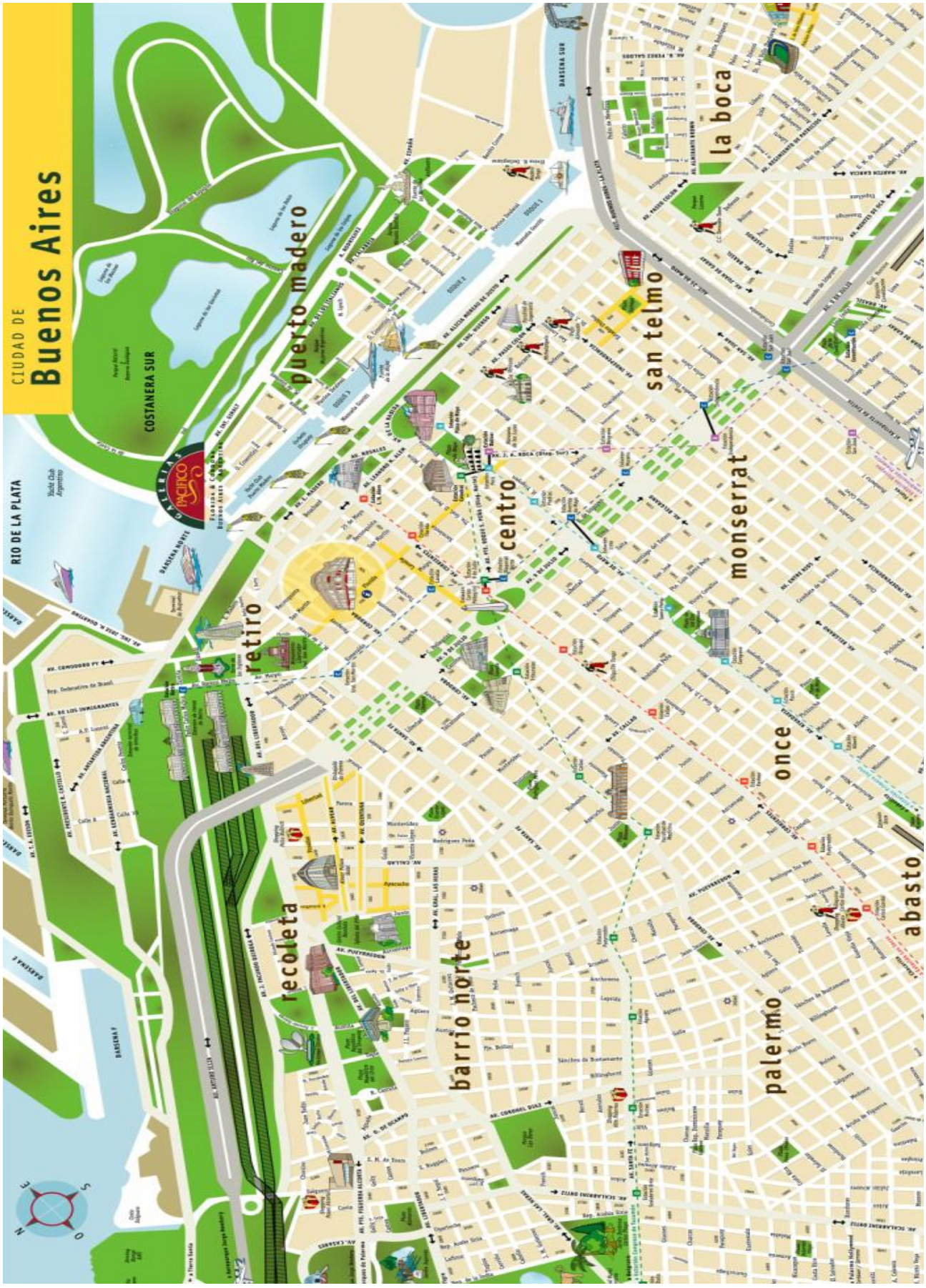
ANNIE MILLET-HERTZ
(54 11) 4480 0054
www.milletrentacar.com.ar
AVIS
(54 11) 4480 9387/ 4378 9640
www.avis.com
LOCALIZA
(54 11) 4816-2799 / 5480-5337
www.localiza.com

3.5 Venue

The Borges Cultural Center (Centro Cultural Borges)
Viamonte and San Martin, Buenos Aires

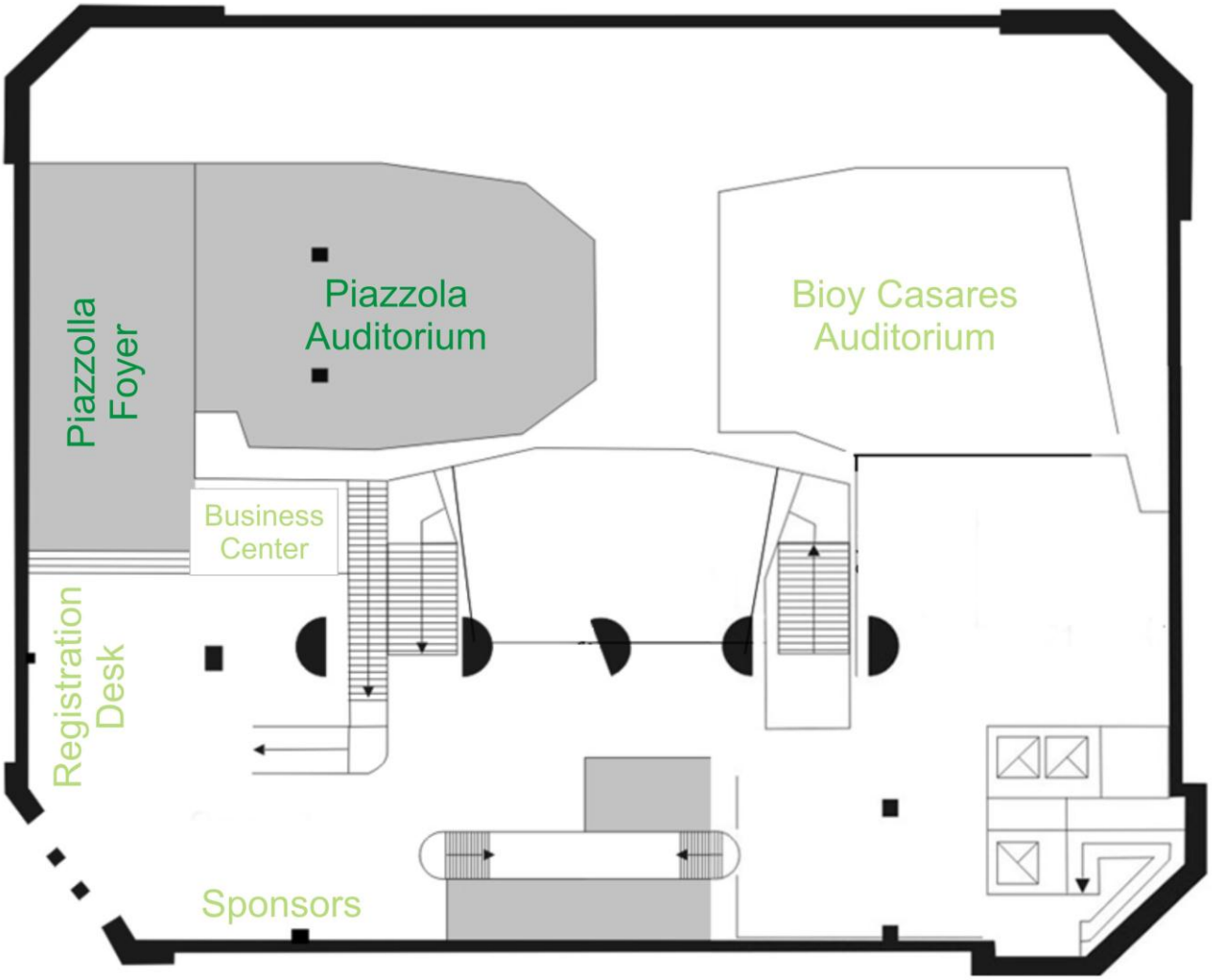
The Borges Cultural Center is an important cultural undertaking created by the Foundation for the Arts (Fundación para las Artes) a non-profit organization. The center was established in October 1995. Occupying over 10,000 square meters, the Borges Cultural Center is located within **Galerias Pacifico** — a prestigious building dating back to the end of the 19th century and considered a historical national monument in Argentina.
The goal of the Borges Cultural Center is to support and promote cultural and artistic expression, advance education in its areas of interest, and to promote Argentina's historical, cultural, and artistic heritage both domestically and abroad. Visitors can enjoy a wide array of cultural activities, such as art exhibits, music, dance, films, theatre, literature, and various educational programs. Official Site: http://www.ccborges.org.ar/

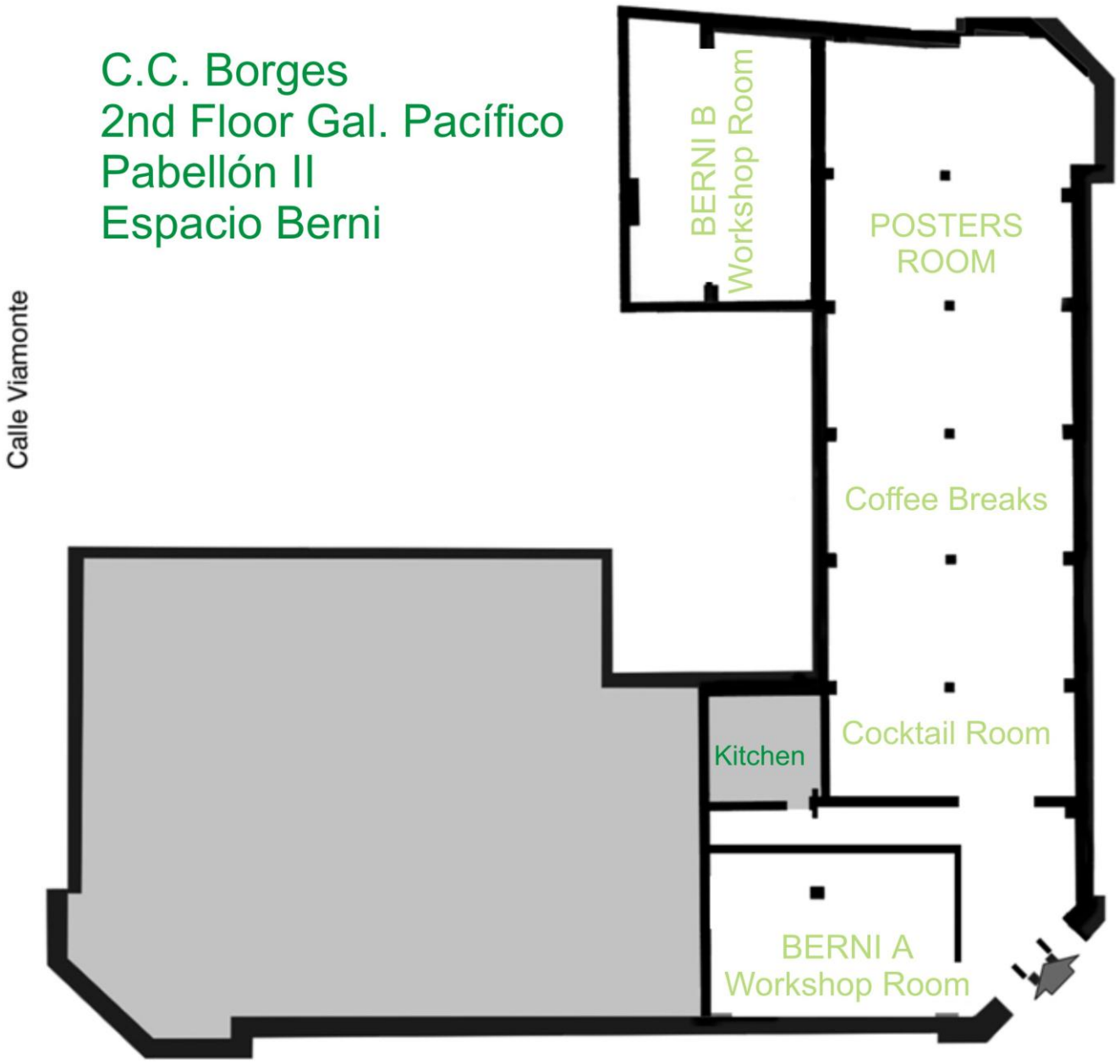
General Services at Galerías Pacífico
Information Desk: Ground Floor – Florida side. Telephone: 5555-5110/5118. E-mail: informes@galeriaspacifico.com.ar.
Schedule: Sundays and Mondays from 10 a.m. to 9 p.m. Tuesdays to Thursdays from 10 a.m. to 10.30 p.m. Fridays and Saturdays from 10 a.m. to 11:30 p.m.
Public Telephones: On all the floors of the mall, in corridors that precede the restrooms and service stairs.
Guided tours in MP3: Get to know the history of Galerías Pacífico, the building, its murals and its artists, together with our guides. A stroll not to be missed. Duration: 20 minutes. From Mondays to Fridays at 11:30 a.m. and 4:30 p.m. at the Central Dome. Ask for the headphones at the Information Desk of the Ground Floor. Languages: Spanish, English, Portuguese and French. Information desk and reservations: (54 11) 5555-5110.
Wi-Fi Area: High speed wireless internet connection in the food court of Galerías Pacífico.
Taxi service: Ask for "Radio Taxi Premium" from their booths located on the Ground Floor, San Martín entrance and Córdoba entrance.
Pharmacy: Schedule: from 10 a.m. to 9 p.m. every day. Store number 137, Lower Level. Telephone (54 11) 5555-5137/39. E-mail: galerias@vantage.com.ar / www.vantage.com.ar
Travel Agency: Lower Level – Viamonte side. Telephone: 5246-2011. e-mail: galeriaspacifico@lesamis.com.ar / www.lesamis.com.ar
Bank: HSBC: Lower Level – Viamonte side – Food Court. Schedule Mondays to Fridays from 10 a.m. to 4 p.m. Telephone: (54 11) 4312-4806 ext.104
Cashpoint/ATM: Located on the Ground Floor, Viamonte entrance and on the Lower Level, fountain sector.
Currency exchange office: Metropolis: Lower Level, Fountain side. Schedule: from Mondays to Sundays from 10 a.m.m to 9:30 p.m. Telephone: (54 11) 5555-5162.



See this map at **http://www.progaleriaspacifico.com.ar/download/mapa_galeriaspacifico.jpg**

C.C. Borges 2nd Floor Gal. Pacífico

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Pabellón II
Espacio Berni
Calle Viamonte
BERNI B
Workshop Room
POSTERS
ROOM
Coffee Breaks
Cocktail Room
Kitchen
BERNI A
Workshop Room

3.6 Social Events

MONDAY

19.00-21.30	**Welcome Reception**	
19.00 - 19.30	Registration	Foyer Piazzola auditorium
19.30 - 19.45	Welcome Address	Piazzola auditorium
19.45 - 20.15	Choir (1)	Piazzola auditorium
20.15 - 21.00	Cocktail	Berni Room

WEDNESDAY

20 - 00 hs	**Symposium Dinner**
19.15-19.30	Depart from Conference Center
20.30-21.30	Dinner at Café de los Angelitos (2)
22.00-23.30	Tango Show
23.30	Back to Conference Center

(1) Chorus of INTA Group
Direction and Arrangements: Fernando Martorell

Chorus Blog: http://corodelgrupointa.blogspot.com

Resume

The Chorus of INTA Group, was created on April 11th, 2006 by the initiative of the Board of INTA (National Institute of Agricultural Technology), in order to give to the employees, collaborators, family and friends of the Institutions that are part of INTA Group, a meeting space where the music and the choir, are an excellent pretext to share a nice and fun moment of personal growing, in a musical and human way.

The Institutions that are part of INTA Group, are as follow:
* INTA (Instituto Nacional de Tecnología Agropecuaria – National Institute of Agricultural Technology)
* INTEA (Innovaciones Tecnológicas Agropecuarias - Agricultural Technological Innovations)
* FUNDACIÓN ARGENINTA.

Since its creation, the direction of the Chorus and the vocal and choir arrangements have been in charge of Fernando Martorell.

Their repertoire consists of popular music songs from Argentina and Latin American.
Nowadays it is formed by 27 members.
Amongst their most important concerts we can mention:

* Fundación Pléroma.
* Fundación Mosen Sol.

* Auditorio San Rafael.
* Teatro Avenida.
* Teatro del Globo (Buenos Aires).
* Fundación Argeninta.
* INTA Castelar
* Paseo La Plaza.
* Círculo Militar de Buenos Aires
* Auditorio AMIA
* Universidad del Salvador.
* Centro Cultural Borges
* Teatro LyF

FERNANDO MARTORELL RESUME

In the year 1993 he obtained the title of MUSIC EDUCATION TEACHER, at the Municipal Music Conservatory "Manuel de Falla".

In December of 1999 he obtained the title of MUSIC EDUCATION PROFESSOR, at the same Superior Institute of Music.

He studied singing, choir direction, composition and harmony with Daniel Di Pace Professor, vocal technique, relaxation and voice impost with Oscar Ruiz and Leticia Caramelli, and choir direction with Antonio Russo.

He did various perfectioning courses specifically in the music area, as well as in what regards to the teaching didactics.

Since the year 1990 he works as Director and Arranger of various chorus and vocal groups, having participated in 600 concerts with the various groups that he directed.

Since its creation in the year 2003, he is an active member of ADICORA (Asociacion de Directores de Coro de la Republica Argentina – Chorus Association Directors of the Argentine Republic), affiliated at the office located at the city of Buenos Aires.

In the year 2007 he founded the Argentinean Choir Network.

MEMBERS OF THE CHOIR OF INTA GROUP

DIRECTION AND ARRANGEMENTS: Fernando Martorell

SOPRANOS:
Selva Gómez
Amelia Grego
Dalia Lewi
María Cecilia Pacheco
Marcela Prack
Tania Sandoval

Antonieta Torregiani
Liliana Torres
Rosa Violante

ALTOS:
Silvia Arisnabarreta
Marta Bryner
Gladys Capalbo
Sabina Carrera
María José Eyherabide
Melisa García
María Rosa Gentile
Ana Henriksen
Adriana Marchionno
Graciela Menotti
Victoria Rivero

TENORES:
Ariel Arbiser
Matías Kon Calderón
Gastón Landaburo
Marta Sozzi

BASSES:
Alberto Corti Murtagh
Enrique Ruvinsky
Alejandro Subatín

(2) About Café de Los Angelitos

Café de los Angelitos is a vivid witness of more than 100 years of Buenos Aires history. During the past century, through its doors have past many political and artistic environment personalities and over its tables have been written many pages of the history that made of Buenos Aires the City of Tango.

Café de los Angelitos offers an Internacional Level Tango Show composed by 21 artists, 7 dance couples a band and 2 singers

Symposium Tour

Excursion and field visits (information at the Registration Desk)

3.7: Programme

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Monday November 15th

08:00-18:00 General Registration

09:00-18:30 Workshops
Piazzola and Bioy Casares Auditoriums, Berni A and Berni B

19:00-21:30 Welcome reception and cocktail
Piazzola Auditorium and Berni Room

WORKSHOPS AND TRAINING WORKSHOPS PROGRAMME

	09:00-13:00	**1.1 Biosafety Capacity Building: Lessons learned, challenges and future directions** *Organisers: Wendy Craig, Marianela Araya Quesada (ICGEB)*
	09:00-13:00	**1.2 Practical Uncertainty Analysis for Environmental Risk Assessment** *Organisers: Paul Keese (OGT Australia), Janet Gough / Libby Harrison (ERMA-NZ)*
	09:00-13:00	**1.2 Practical Uncertainty Analysis for Environmental Risk Assessment** *Organisers: Paul Keese (OGT Australia), Janet Gough / Libby Harrison (ERMA-NZ)*
	09:00-13:00	**1.3 Ecological studies of arthropod species for regulatory non-target risk assessment of GM crops** *Organisers: Franz Bigler (Agroscope ART, CH) and Monica Garcia-Alonso (Syngenta, UK)*
	09:00-16:00	**1.4 Training Workshop for Latin America: Management of Confined Field trials (in Spanish)** *Coordinators: Carmen Vicién (University of Buenos Aires), Silvana Zampierin (Argentine Seed Association), Robert Potter (Consultant, Canada)*
	14:00-17:00	**2.1 OECD Workshop: Environmental Risk Assessment, Harmonisation and Decision-Making** *Coordinator: Sally McCammon (APHIS, USDA)*
	14:00-17:00	**2.2 "Best practice" experiences in communicating biosafety research topics to the broader society** *Coordinators: Kristina Sinemus (Germany) and Gabriela Levitus (ArgenBio)*
	16:00-18:00	**2.3 Training Workshop: Stewardship of GM Crops (CLI)** *Organisers: Deb Carstoiu and Denise Dewar (Crop Life International)*
BERNI ROOM	11:00-11:30 / 16:00-16:30	**Coffee breaks**
	13:00-14:00	**Lunch break (free time)**

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Tuesday November 16th

All sessions will be at Piazzola Auditorium

09:00-09:15	**Welcome address** *Moisés Burachik (Chair, Local Organizing Committee) - Patrick Rüdelsheim (President, ISBR)*
09:15-13:00	**Session 1. Biosafety Research Challenges and Experiences in Latin America** *Chairs: Dalia Lewi, Moisés Burachik - Coordinators: Carlos Borroto Nordelo, María Mercedes Roca, Leda Mendonça*
	1.1 Current status of Biosafety Issues for GM crops in Latin American countries: the Argentinean perspective - *Carmen Vicién, University of Buenos Aires, Argentina*
	1.2 Argentina: 20 years experience, challenges and learnings *Moisés Burachik, Biotechnology Directorate, Ministry of Agriculture, Livestock and Fisheries, Argentina*
	1.3. The development of the Biosafety Regulatory Framework in Honduras: a case study for Central America - *María Mercedes Roca, Zamorano University, Honduras*
11:00-11:30	**Coffee break and Poster Sessions 1 & 2**
	1.4 Challenges of GM crops in centers of origin: GM corn in Mexico *Ariel Alvarez Morales, Intersecretarial Commission for Biosafety and Genetic Modified Organisms, Mexico*
	1.5 Gene Flow analysis for Environmental Safety in the Neotropics: rice, an introduced species with wild/weedy compatible relatives *Zaida Lentini* and Luisa Fernanda Fory*** **ICESI University, **International Center for Tropical Agriculture (CIAT), Colombia*
	1.6 Roundup Ready® soybean (GTS 40-3-2) environmental post-market monitoring *Geraldo Berger, Monsanto Company, Brazil*
13:00-14:30	**Lunch break**
14:30-18:30	**Session 2. Problem Formulation – improving the quality of an Environmental Risk Assessment** *Chair: Alan Gray - Coordinators: Morven McLean, Monica Garcia, Raymond Layton, Alan Gray*
	2.1 Problem Formulation: the first step towards the end goal of a useful risk assessment *Raymond Layton, Pioneer Hi-Bred, USA*
	2.2 Protection goals - where's the harm? *Alan Gray, Center for Ecology & Hydrology, UK*
	2.3 The Grass is always Greener: New Zealand as the receiving environment *Libby Harrison, Environmental Risk Management Authority, New Zealand*
	2.4 Comparing Apples and Oranges? The challenges of developing appropriate comparators for environmental risk assessment *Chris Pollock, Aberystwyth University, UK*
	2.5 Assessing the ecological risks from combining insect-control traits: the example of VipCot *Alan Raybould, Syngenta, UK*
16:00-16:30	**Coffee break and Poster Sessions 1 & 2**
	2.6 Problem Formulation for NUE Sorghum: first steps in assessing the Environmental Risk of an open pollination crop engineered with a yield improvement trait *German Serino, Advanta Seeds, Argentina*
	2.7 Using Problem Formulation in Environmental Risk Assessments: practical methods for the assessment of unintended effects *Monica Garcia-Alonso Syngenta, UK*
	2.8 Problem Formulation to assess gene flow from herbicide-tolerant transgenic rice to weedy rice (Oryza sativa) - *Griselda Arrieta-Espinoza, Costa Rica University*
	2.9 Design considerations for laboratory non-target studies used to support Environmental Risk Assessment - *Joerg Romeis, DEA Agroscope Reckenholz-Tänikon Research Station, Switzerland*
	Panel Discussion: Perspectives on Problem Formulation *Panelists: Monica Garcia-Alonso, Libby Harrison, Chris Pollock, Joerg Romeis, German Serino*
18:30-19:30	**ISBR Members Meeting (Piazzola Auditorium) - Poster Sessions 1 & 2**

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Wednesday November 17th

All sessions will be at Piazzola Auditorium

09:00-09:45	**Keynote Address** *Roger Beachy, NIFA, USDA*
09:45-13:00	**Session 3. Biosafety considerations for crops for non-food/feed uses, biofuels and energy crops** *Chair: Allison Snow - Coordinators: Allison Snow, Joachim Schiemann*
	3.1 Status and regulation of non-food/feed crops in Europe *Inge Broer Rostock University, Germany*
	3.2 Status and regulation of non-food/feed crops in the USA *Elisabeth Hood, Arkansas State University, USA*
	3.3 Recent advances in biological confinement technologies *Joachim Schiemann and Alexandra Hüsken JKI, Germany*
11:00-11:30	**Coffee break and Poster Sessions 3 & 4**
	3.4 The Benefits and Risks of next generation of microalgal biofuel production systems *Richard Sayre, Donald Danforth Plant Science Center, USA*
	3.5 Comparison of a weedy relative of sugarcane in two environments highlights traits leading to increased invasiveness *Bonnett GD*, Olivares-Villegas JJ*, Letondor C*, Saltonstall K*** **CSIRO Plant Industry and CRC for Sugar Industry Innovation through Biotechnology, Australia* ***Smithsonian Tropical Research Institute, Panama*
	3.6 Ecological Assessment of transgenic grasses: baseline studies of native and improved switchgrass for biofuel *Allison Snow*, Amy Campbell*, Emily Heaton**, Maria Miriti** **Ohio State University, USA **Iowa State University, USA*
13:00 -13:15	**Guest Lecture** **Biosafety research under the EU Framework** *Jens Hoegle, Research Programme Officer, European Commission, Belgium*
13:15-14:30	**Lunch break**
14:30-17:30	**Session 4. GM insect developments and biosafety** *Chair: Margareth Capurro - Coordinator: Camilla Beech*
	4.1 GM Insects in Agriculture: GM fruit flies and pink Bollworm (first Environmental Impact Statement) *Robert Rose, USDA, USA*
	4.2 Confined large scale field trial of GM Aedes aegypti for dengue control in Mexico *Janine Ramsey, National Institute of Public Health, Mexico*
	4.3 A risk analytical approach under conditions of limited knowledge and uncertainty to support biosafety and decision making *Paul De Barro, CSIRO Ecosystem Science, EcoSciences Precinct, Australia*
16:00-16:30	**Coffee break and Poster Sessions 3 & 4**
	4.4 MosqGuide: Best Practice for deployment of genetic vector control methods against mosquito vectors in disease endemic countries *John Mumford, Imperial College London, UK*
	Panel Discussion - *Summary by Chair*
17:30-18:30	**Poster Sessions 3 & 4**

19:30	**Depart from Conference Center**
20:15	**Symposium Dinner and Show. Café de los Angelitos, Rivadavia Av 2100**
24:00	**Transportation back to the Conference Center will be provided**

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Thursday November 18th

All sessions will be at Piazzola Auditorium

09:00-13:00	**Session 5. Biosafety aspects of GM-based agronomic traits protecting against yield reduction due to abiotic stress** *Chair: Esteban Hopp - Coordinators Danny Hooftman, Rosie Hails*
	5.1 Back to the Future: old tools to meet new challenges for regulators from abiotic stress tolerant GM crops *Joe Smith, OGT, Australia*
	5.2 Considerations for Risk Assessment: how special are genetically modified abiotic stress tolerant crops? *Greet Smets, Patrick Rüdelsheim, Perseus BVBA, Belgium*
	5.3 Biosafety Assessment and field evaluation of transgenic forages for enhanced tolerance of biotic and abiotic stresses *German Spagenberg, La Trobe University and Victorian AgriBiosciences Centre, Australia*
	5.4 A Case Study for yield and stress traits: the challenges and success encountered in the regulatory strategy employed for drought tolerant corn, MON 87460 *Bernard Sammons, Hugo Campos, Tom Nickson, Joy Whitsel, Michael Horak, and William Reeves. Monsanto Co., USA*
	5.5 A genetic analysis of the introgression process from crops to wild relatives under abiotic stress conditions: the case of lettuce *Brigitte Uwimana, Clemens van de Wiel, René Smulders and Richard Visser. Plant Breeding, Wageningen University, The Netherlands*
11:00-11:30	**Coffee break and Poster Sessions 5 & 6**
	5.6 What can we learn from crops that have already evolved invasiveness or weediness about crop gene introgression and its biosafety impacts - *Norman Ellstrand, University of California, Riverside, USA*
	5.7 An overview of methods for measuring enhanced fitness and invasiveness, their environmental consequences and how they can be applied in Environmental Risk Assessment - *Danny Hooftman, NERC-Centre for Ecology and Hydrology, UK*
	5.8 Developing a framework to assess potential changes in fitness of GM plants: do stress tolerant plants need a new paradigm? - *Hails, RS (CEH, Oxford) and Sweet, JB (SEC, Cambridge), UK*
13:00-14:30	**Lunch break**
14:30-18:00	**Session 6. New applications of biotechnologies and their associated risk assessment issues** *Chair: Nina Fedoroff - Coordinators: Sally McCammon, Phil MacDonald*
	6.1 Overview of new technologies and their applications *Nina Fedoroff, Huck Institutes of the Life Sciences, Penn State University, USA*
	6.2 Precise genome modification in plants: EXZACT technology and outcomes *Vipula Shukla, Dow Agrosciences, USA*
	6.3 The use of RNAi for crop improvement: benefits and potential issues *Adriana Fusaro, University of Sydney, Australia*
	6.4 Synthetic Genomics: science and governance - *Michele S. Garfinkel, J. Craig Venter Institute, USA*
	6.5 Effects of transgene insertions and their detection using genomics tools *Brian Miki, Agriculture and Agri-Food Canada, Canada*
16:00-16:30	**Coffee break and Poster Sessions 5 & 6**
16:30-18:00	**Panel Discussion with the speakers and** *Hennie Groenewald, South Africa Biosafety, South Africa* *Paul Keese, Office of the Gene Technology Regulator, Australia* *Jonathan Latham, Bioscience Resource Project, USA* *Jaimie Schnell, Canadian Food Inspection Agency, Canada*
18:00-18:30	**Closing Session** *Chair: Jeremy Sweet - Presentation by the New President of ISBR, Bao-Rong Lu - Announcement and Presentation of the 12th ISBGMO - Acknowledgments*
18:30-19:30	**Poster Sessions 5 & 6**

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Friday November 19th

All sessions will be at Piazzola Auditorium

09:00-19:30 Workshops
Piazzola and Bioy Casares Auditoriums, Berni A and Berni B

WORKSHOPS AND TRAINING WORKSHOPS PROGRAMME

	09:00-13:00	**3.1 Testing and deployment of GM mosquitoes: what is different?** *Organiser: Camilla Beech (Oxitec, UK)*
	09:00-17:00	**3.2 Training Workshop: Problem Formulation: Putting it into practice (ICGEB)** *Organisers: Wendy Craig, Marianela Araya Quesada, Alan Gray*
	09:00-17:00	**3.3 Design considerations for laboratory studies on non-target arthropods for risk assessment of GM plants (IOBC/WPRS & CERA/ILSI RF)** *Organisers: Jörg Romeis (Switzerland) and Morven McLean (USA)*
	14:00-18:00	**4.1 Support Systems for Risk Assessment of GM crops (FURARN)** *Organisers: Sylvia Burssens (IPBO Gent, Be), Kathy Messens (HOGENT, Be) , Dulce de Oliveira (IPBO, Be), George T. Tzotzos (UNIDO, Austria), Ine Pertry (IPBO Gent, Be), Jeremy Sweet (Cambridge, UK)*
	17:30-19:30	**4.2 Establishing an International Network for Competence in Experimental Field Releases of GMOs** *Organiser: Joachim Schiemann (Germany)*
BERNI ROOM	11:00-11:30 / 16:00-16:30	**Coffee breaks**
	13:00-14:00	**Lunch break (free time)**

Saturday November 20th

08:00-16:00 Excursion and field visits (information at the Registration Desk)

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3.8: Oral Presentations

SESSION 1
Biosafety Research Challenges and Experiences in Latin America

1.1 Current status of biosafety issues for GM crops in Latin American countries: the Argentinian perspective

Carmen Vicién,
Faculty of Agronomy - University of Buenos Aires, Argentina.
e-mail: cvicien@agro.uba.ar

An overview of different experiences regarding biosafety for GM crops in Latin American countries is presented. The aim is to consider cases (crops and issues) in which research has enabled decision making as well as others where measures or research are already needed.
The discussion on a number of biosafety challenges is introduced in terms of research requirements and capacities, as well as on the status of risk analysis for current and future developments. It is also reinforced the idea of effective communication with decision-making referents in order to precise questions and make focus on issues to be solved.
In some cases there is a need to consider information on biodiversity and conservation strategies, mostly because some species have scarce commercial use, although their potential for several uses is high; being traditional varieties with practically no conventional breeding.
There are also situations in which regulatory decisions regarding commercial crops have been delayed, pending on the definition of parameters for environmental risk assessment, including scopes, constraints and protection goals.
Some countries have undertaken post-release monitoring initiatives in order to identify the occurrence of adverse effects of GM crops due to their large-scale cultivation; such effects are being flagged for further investigation tending to make use of existing monitoring initiatives.
Studies on gene flow are mostly focused in covering gaps from previous research related to baselines of biological information, out-crossing and gene introgression or effects on fitness. The approach is to continue with previous studies accomplished by different national research institutions, with the inclusion of LA cooperative actions, by means of exchange of information and methodologies already developed, and the use of models known at international level.
With relation to the study of potential effects on non-target organisms, emphasis is being given to considerate different biological indicators, with a centre in the functional aspects of the organisms. Protocols are defined on the basis of functionality, making use of existing data bases, consults to experts and, when necessary, development of specific studies. Available tools (taxonomies, protocols, data bases) are being shared, taking into account gaps on the relevant information.
Some countries of the region have yet to establish how to assess the safety of stacked event products, which represent a significant part of the material to be evaluated by regulatory agencies. In that sense a general need is to focus in solving biosafety issues in novel developments, reducing complexity and introducing precision in regulatory processes for those that are familiar, without affecting completeness of risk evaluations.

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1.2 Argentina: 20-years Experience, Challenges, Learnings

Moisés Burachik
Biotechnology Directorate, Ministry of Agriculture, Livestock and Fisheries, Argentina
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Argentina started assessing genetically modified (GM) crops in 1991. The first approval of a GM crop, glyphosate tolerant soybean, was in 1996, what coincided with the global commercialization of biotech crops. Only six countries were using this technology at the time. Since then, the area planted here with GM crops has grown from the initial 370,000 ha to more than fifty times that number, placing Argentina as the third largest grower of biotech crops in the world. Argentina represents 16% of the global biotech crop area.
The circumstances that led Argentina to this early adoption were very particular and local, but the structure upon which Argentina based its regulatory model may be replicated. Human resources were crucial for the Argentine model. The crops and the traits as well as the environmental and economic benefits they would provide to farmers and to Argentina as a commodity exporter were very important, but without the human resources committed to design and implement clear, science-based regulations, these factors on their own would not have produced these results.
Argentina's regulatory framework is scientific and rational. Both scientists and researchers from the public and private sector worked together to design it. The Competent Authority, the Secretary of Agriculture, Livestock and Fisheries, is advised by multidisciplinary bodies whose members belong to different institutions of the public and private sector. This balance of the public and the private, the academics and the research scientists provided a very productive, balanced combination of science, societal interests and field experience.
As scientific advances are opening new avenues for biotechnology applications, biosafety research is an essential input in the decision making process. The analysis of research advances and gaps is crucial for further deployment of agricultural biotechnology. Field-, trait- and commercial-related questions must be analyzed from a biological and practical perspective to aid regulators and researchers identify relevant issues.
The experience accumulated has showed Argentina the pathway to deal with the new challenges brought by these forthcoming advances. On the practical side is decision making, which requires biosafety research, the implementation of sound, science-based regulations and a strategic view on sustainability. On a more theoretical perspective, this rich 20-year period on GM crops regulation has taught Argentina the importance of clear rules, proficiency and up-dating as the basis for sound, scientific criteria on regulatory issues.

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1.3. The development of the Biosafety Regulatory Framework in Honduras: a case study for Central America

Maria Mercedes Roca [1]; Carlos Almendares[2]

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Honduras is a small nation in Central America, struggling to cope with the many social, economic and environmental challenges faced by development countries, a globalized economy and other threats like climate change. Like most of Mesoamerica, the Honduran staple diet consists principally of maize and beans and Honduras is considered a center for diversification for maize. Maize is also widely used for animal feed. According to the National Institute of Statistics (INE), maize production in the country occupies more than 40% of arable land of a total of 1.8 million Ha, but average yields are very poor at 1.53 Ton/Ha. The demand far exceeds the supply and Honduras needs to import approximately 900,000 tons of maize, or about 47% to meet this demand (INE, 2008). With this context in mind, and with an agenda to increase agricultural productivity in the country, the Honduran Government developed in 1998 a Regulatory Framework for the Biosafety of Biotechnology, with an emphasis on transgenic crops (in Spanish, Reglamento de Bioseguridad con Enfasis en Plantas Transgénicas). This framework rests under the Honduran Fitosanitary Law developed in 1994. At the same time, a National Committee for Biotechnology and Biosafety (NCBB), consisting of professionals with expertise in competent areas, was established to evaluate applications for importation and cultivation of genetically modified (GM) crops and advice the Honduran Government, through the Ministry of Agriculture. In 1998 the NCBBB undertook an extensive revision of the publicly available, existing documentation regarding the biosafety of GM maize. The focus was on sound scientific data and on learning from the experiences of countries that had de-regulated GM maize. In 2002, after extensive revision of the published literature and evaluations undertaken in the country, the first authorization for commercial imports and cultivation were granted for the events MON-810 (Bt) and NK603 (RR) and later for stack events. By 2010, the CNBB has evaluated 16 applications for genetically modified banana, maize, soy and rice. Of these, 2 authorizations have been granted for commercial cultivation in an area of 15,700 Ha to date, 1 for semi-commercial cultivation under controlled conditions and 12 were approved for experimental purposes. This paper explores the factors that allowed the Government of Honduras, through the Ministry of Agriculture and the advice of the CNBB, to develop a biosafety regulatory framework that allows the importation and cultivation of GM crops in Honduras. It also explores some of the factors surrounding the equivalent processes in other countries in the region.

1.4 The release into the environment of genetically modified organisms in a center of origin and diversity: the case of maize in Mexico

Ariel Alvarez Morales
Secretario Ejecutivo de la Comisión Intersecretarial de Bioseguridad de los Organismos Genéticamente Modificados, México

Mexico is one of the 17 recognized megadiverse countries of the world, and it is the center of origin and/or genetic diversity for such important crop species as maize, beans, squash, chili peppers, vanilla, tomatoes, cotton and avocado among many others. However, Mexico is not self sufficient in its requirements for food and, in the case of maize, although the country produces all the white maize required to feed its population, still needs to import between 6 and 10 million tons of maize, mainly yellow maize, to fulfill the requirements of the food and feed industry.

Therefore, Mexico has recognized the need to increase food productivity and has adopted a policy to allow the different available technologies to contribute to this end. One of these technologies being the genetic modification of crops through recombinant DNA. However, the ultimate decision on this technology has yet to be taken as the history of its application and acceptance has been rather difficult.

In 1988, Mexico allowed its first genetically modified organism (GMO) field test, and since then has gone through different phases in the way the government has dealt with the GMO issue, and also how other stakeholders have reacted.

In my opinion it is possible to identify at least three distinctive phases:

a) From 1988 to 1997. Field trials with GMOs were conducted under an incipient legal framework and both government regulators and proponents were learning from their own experience as well as that being developed around the world. Opponents to the technology increase their activity steadily mainly basing their claims in the opposition to use GMOs by the European Union. During this period industry, and national and international research centers conduct field trials with crops such as cotton, maize, tomato, potato, tobacco, soybean and alfalfa among others.

b) From 1998 to 2005. A non-official moratorium was established for the field testing of GM maize and field trials from research centers decreases until, by 2005, the government received no petition from public institutions at all. Large multinationals continued to test and commercialize GM cotton, and there were also few other species that were tested such as soybean. Mexico ratifies the Cartagena Protocol on Biosafety and publishes its Law on the Biosafety of Genetically Modified Organisms which includes the Intersecretarial Commission of Biosafety of Genetically Modified Organisms (CIBIOGEM). Although in principle the moratorium to conduct field tests with maize was revoked in 2005 by the government receiving petitions to conduct field trials with maize, none is reviewed positively in this period.

c) From 2006 to 2010. The first permits for field trials with maize were granted by the competent authority but the first ones were revoked on legal grounds after a legal recourse was presented by NGOs which, besides stopping the field trials, resulted in sanctions against two public officials of the Ministry of Agriculture. Nevertheless, in the following years work was done to structure a robust legal framework, and finally at the end of 2009, 22 permits were granted for the experimental release of GM maize in the Northern States of Mexico. These are all properly conducted and the government is at the moment evaluating the results presented by the applicants.

The next phase will have to resolve very important issues:

1. If maize is allowed to be released in a commercial scale, can gene flow to the landraces be avoided?
2. What could be the consequences on the genetic diversity of the landraces if gene flow occurs?
3. Are there IP consequences of gene flow from commercial GM maize to the landraces?

The short answer to the first issue is no. Most of the people with knowledge about the maize agricultural system in México would very much doubt that implementing measures to stop gene flow with be extremely difficult and probably not cost-effective. The Biosafety Law requires that centers of origin and current genetic diversity be identified for those species for which Mexico is center of origin, and in these areas no GMO of the same species being protected can be released. These centers of origin would be an easier target for monitoring and ensuring that they remain GMO-free, rather than implementing a national program to monitor these occurrences.
However, in order to accept the previous proposal, one needs to have an answer for the second issue. It is rather difficult to accept the idea that gene flow from GM maize to the landraces could destroy or even diminish biodiversity, so far there is no a plausible scientific explanation to support this concern. Furthermore, experience tell us that after more than 50 years of growing hybrid corn alongside the landraces, there has been no decrease in the number of recognized landraces, although the same issues were raised as concerns when hybrids were introduced in Mexico. Nevertheless, we must proceed with caution and find the way to solve this issue on the bases of scientific and technical knowledge, gained through experimentation and careful monitoring of properly set field releases.
With respect to the third question, an agreement must be reached between authorities and developers of the technology to clearly define cases where there is the intention to appropriate and profit from the technology illegally, from those cases were transgenes are present in a crop and these are being conserved not with the intention to use the technology but through the normal agricultural practices such as saving seed from one season to the next, and finally, there should be an explicit recognition of owners of the land races to maintain these free of transgenes if they so wish.

1.5. Gene Flow Analysis for Environmental Safety in the Neotropics: Rice, an introduced species with wild/weedy compatible relatives

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While several studies are in progress in Asian and temperate rice regions to understand the gene flow/ introgression dynamics from cultivated rice into the wild/weedy complex, baseline information in tropical America is needed since gene flow rates and introgression are significantly affected by genotype X environmental interactions. Rice (*Oryza sativa*, AA genome) is an introduced domesticated species from Asia that has become one of the most important staple grains for human consumption in tropical America in recent decades. The rice genus, *Oryza* has a pan-tropical distribution. Four wild *Oryza* species (*Oryza glumaepatula*, *Oryza grandiglumis, O. alta* and *O. latifolia*) have been recorded in tropical America. Weedy rice (commonly known as red rice) is sympatric with the rice crop. In the Neotropics, the weedy rice complex is broadly diverse and maybe composed by various *Oryza* species that yet have not been fully identified. Weedy rice appears to be the main candidate for gene flow and introgression from cultivated rice, since it is compatible and usually intermingled with the rice crop, it may also serve as the introgression bridge from the crop to the wild *Oryza* species. The main goal of this research is to generate baseline genetic information using rice as a model for the development of guidelines on the safe introduction and use of novel agriculture traits (biotechnology derived or not native from the place of introduction), while reducing the likelihood of potential environmental impact on native biodiversity in the Neotropics.

1.6 Roundup Ready® soybean (GTS 40-3-2) environmental post-market monitoring

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The Roundup Ready® soybean (event GTS 40-3-2) was evaluated by the National Biosafety Technical Committee (CTNBio) and was approved by the Communicate # 54, published on the Federal Register in October 1st/1998. Besides the approval, the Communicate # 54 also requested the conduction of a Post-Market Monitoring (PMM) by Monsanto do Brasil Ltda. during five years in commercial fields located in representative soybean growing areas in Brazil, to compare the Roundup Ready® System (using Roundup® and conventional herbicides) with the conventional system for weed control, assessing environmental indicators.

After years of legal injunctions, the Roundup Ready® soybean was finally approved for commercialization by the Law 11.105 legalized in March 24/2005, and the PMM was installed in the crop season 2005/2006, which was the first year of certified Roundup Ready® soybean seeds available to the Brazilian growers. Previously, preliminary activities of monitoring were conducted using conventional varieties, to define locations and to test the methods and logistical aspects. In the following four crop seasons, evaluation and sampling activities were conducted in commercial fields, with the last crop season conducted in 2009/2010. Commercial fields located in representative soybean areas in Brazil were monitored.

Activities and environmental indicators were monitored, among them: weeds community diversity, resistance development to glyphosate, gene flow, glyphosate residues in grains and soil, physical and chemical soil properties. Important agro-environmental processes and non-target organisms were evaluated as well, as soils kinetics and enzymatic activity, microorganisms' diversity, nitrogen biologic fixation, soil and aerial insects, including the activities off-seasons conducted in the areas.

To conduct the laboratory analyses, researchers from universities and companies with recognized personnel and facilities capacities were involved. To assure the results quality, standard procedures for each sampling and evaluation were elaborated, with analytical methodology determined by the researchers.

After each season, a final annual report was prepared and submitted to CTNBio, containing all the consolidated reports, signed by the researchers and with the respective statistical analysis. Based on these results and on visits to the monitoring fields, a Technical Opinion was signed by the members of the Scientific Council, which is composed by recognized professors and doctors, two of them members of the Brazilian Academy of Science. Following detached comments from Technical Opinions of the Scientific Council:

"The consolidated reports of the first year of the environmental monitoring of Roundup Ready® soybean, related to the 2005 off-season and 2005/2006 crop season, are consistent, complete and well developed. Within each area, in general, there were no consistent significant differences related to treatment effects. Occasional differences among treatments did not follow defined patterns and can be attributed to random effects. Significant variations occurred among the monitoring areas, an expected behavior in function of the different edaphoclimatic conditions."

“Field works were well conducted, as showed by the data variation coefficients and observed in the Scientific Council members' visit to the fields. Considering the nature of the job, as remarkable points are detached the sampling quality, transport logistics, and sampling conservation, as well the method adjustment, all of them fundamental to the results obtained. The results, although preliminary, show similarities in the environmental quality indicators manifestation when compared the three management systems evaluated."

In conclusion, the results obtained during the five years of PMM for the Roundup Ready® soybean showed that there are no significant differences among treatments, confirming the CTNBio conclusion in the Communicate # 54, which states that "there are no evidences of environmental, animal or human health risks related to the use of the genetically modified soybean."

SESSION 2
Problem Formulation – improving the quality of an environmental risk assessment

2.1 Problem Formulation: The First Step towards the End Goal of a Useful Risk Assessment

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Keywords: environmental risk assessment, problem formulation, genetically modified, crop, biodiversity

Problem formulation is the first step towards producing a useful risk assessment. A useful risk assessment is one that provides sufficient high quality information and analysis so that risk managers can make decisions. Problem formulation helps assure that the risk assessment will be easily understood and make efficient use of resources. There are two distinct phases in problem formulation: (1) establishing context and (2) formulation of an assessment plan.
Establishing the assessment context has three steps: (1) understanding any protection goals and environmental policies in order to fulfill the needs of the assessment end users, (2) summarizing what is already known about stressor characteristics, and (3) defining the environment of interest. Not establishing the context for a risk assessment leads to poor resource utilization and may produce something that is not useful for risk managers.
The first step in establishing the context for the risk assessment is to understand the needs of the end user. Risk assessments used for regulatory purposes are conducted within a regulatory context – meaning they are designed to meet the needs of risk managers that make regulatory decisions. Risk managers are external to the risk assessment process and supply two critical contextual items: management goals and the definition of "harm." Setting management goals and defining "harm" generally have societal or legislative drivers. For example, society may seek to protect charismatic species while classifying other species as pests. Or government policies may restrict certain activities (planting of GM crops) and at the same time allow other activities (planting of crop varieties developed using other types of methods) to occur without restrictions. Acceptable harm is defined by society or regulatory policy. In some geographies the definitions of harm are relatively straightforward, such as clear limits established to limit effects on endangered species. Other definitions of harm are less clear. For example, a regulatory policy might indicate that "harm" might occur when populations of a specific insect show a slight, but statistically significant, reduction in a field planted with a GM-variety as compared to a non-GM variety. But the same regulatory agency may not define as "harm" the large scale changes in population levels in the same species that occur as the GM-crop is rotated to another crop. Setting explicit management goals and defining "harm" provide the risk assessor with a clear set of criteria to ensure the assessment will meet the needs of the end user.
Risk is a combination of hazard (toxicity) and exposure. So the first step in establishing context is to gather all available information about the hazard (e.g., mode of action, the spectrum of activity) for the stressor as well as the expected exposure (e.g., temporal/spatial intensity, frequency). The available knowledge, or the identification of gaps in knowledge, helps form a foundation for developing an effective plan for conducting the risk assessment. For example, the risk assessment plan for a novel insecticidal protein with an entirely new mode of action in a new crop for which there are few hazard or exposure data available is very different than a risk assessment plan for an insecticidal protein which is already used in a variety of crops worldwide and for which significant information exists about activity spectrum across a range of species and environments. A formal assessment of what is known, and what is not known (knowledge gaps and critical areas of uncertainty) also helps in understanding which portions of the assessment will likely be the most critical. This assessment focuses resources and efforts on the area(s) most needed to reduce uncertainties in the overall risk assessment.

The third step is to define the environmental context for the stressor. In general, risk assessments are conducted on a case by case basis – meaning that they are usually environment specific and a risk assessment conducted for one environment may not be applicable to another environment without additional work. For example, the environmental context for a research trial that is conducted in a glasshouse is very different than the environmental context for full scale unrestricted cultivation. Likewise, there are differences in the environmental context for small scale field research trials as compared to import of grain that is processed within a few kilometres of a port facility. Explicit definition of the environmental context aids in designing the current risk assessment, but also helps in determining the potential applicability of the risk assessment to other cases.

Once the risk management, knowledge, and environmental contexts are understood, the problem formulation process can move to the assessment planning phase. This phase takes the information developed during the context establishment phase and sets out a series of actions that are needed to address critical areas of uncertainty: (1) development of a conceptual model, (2) generation of testable risk hypotheses, and (3) determination of an analysis plan.

A conceptual model is a description of the potential hazards, exposures, and interactions that contribute to potential risk. The model can be developed using text, graphics (flow chart), or a combination of both. Model development is useful for several reasons. First, it serves as a structure to make sure that all critical contributors to risk are taken into account. Second, it often can be used to identify potential key information gaps and research needs. Third, it frequently serves as an effective communication tool among those who are working on the assessment, as well as with those who will later need to understand and utilize the results of the assessment. If a conceptual model of the problem, including hazard, exposure, and linkages, cannot be developed, this may indicate that the problem is not well understood and more time is needed to examine the stressor and the overall context of the assessment.

Information from the context establishment phase and the conceptual model is useful in developing hypotheses that can be tested. Hypotheses can be developed for both the hazard and exposure sides of the risk equation. If hazard is negligible, then detailed exposure studies are probably not needed. If exposure is negligible, then detailed hazard studies are probably not needed. Studies designed to address specific hazard or exposure hypotheses have a high likelihood of providing useful results to inform the risk assessment; observational or anecdotal studies may be interesting but are less likely to provide useful information. The process of developing hypotheses defines such things as (1) the organisms of interest, (2) the appropriate methods and measures of exposure, (3) the appropriate endpoints (what should be measured during any tests that will be conducted), and (4) the statistical criteria to be used in accepting or rejecting the hypotheses. An inability to construct well defined, testable hypotheses may indicate a gap in the information developed during the context phase or in the conceptual model. Development of testable hypotheses is an integral part of a useful risk assessment.

The last action step in the assessment planning phase is to develop an analysis plan. Not all information or tests are of equal value. Focusing resources on those hypotheses that are most critical to the risk assessment saves resources, not only for the risk assessor, but also for the risk managers who must review and utilize the results of the assessment. A structured plan can be developed to guide the studies that will be conducted. This plan is based on the context, the conceptual model, and the potential testable hypotheses. A tiered system of analyses can be used in selecting which studies to conduct. The tiered system begins with high dose or “worst-case” studies to identify areas of critical uncertainty in the assessment. If high-dose laboratory based assays do not indicate significant hazard, then it is unlikely that conducting field studies for that taxon will provide additional useful information for the risk assessment. If the high-dose laboratory studies indicate a potential for hazard, then additional work (laboratory, greenhouse, or field) may be required to understand if the observed effect might actually occur within the environmental context identified in the first phase of problem formulation. The benefit of using a tiered process is that potential problem areas can be quickly identified and resources can be directed towards areas of greatest uncertainty. The analysis plan provides a clearly defined and understandable process to

efficiently conduct studies, interpret results, and, if necessary, conduct additional studies to reduce critical areas of uncertainty within the risk assessment.
Problem formulation is an important first step in producing a risk assessment that will be useful to the end user – generally a risk manager. Key ingredients to effective problem formulation include establishing the context for the assessment and developing a plan containing hypotheses that is based on a conceptual model. Effective problem formulation then results in a risk assessment that is goal-driven, readily understood, and makes efficient use of resources.

2.2 Protection Goals – Where's the Harm?

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The assessment of potential risk to the environment from the cultivation of GM crops is, quite properly, regarded as a scientific activity. It is carried out in almost all countries by regulators and experts with science training and backgrounds, whose skills in assessing risk are clearly based on their understanding of a range of biological, and especially ecological, processes. That uncertainties remain once an assessment of the potential risks of growing particular crops has been made is often said to arise from deficiencies in our understanding of these processes – the so-called 'deficit' model which broadly states that if we knew more about the biology and ecology of the plant and its pests or its wild relatives or the ecosystem-level impact of growing the crop, then we would make a better or more accurate risk assessment. In this presentation I suggest that one of the outcomes of applying Problem Formulation to Environmental Risk Assessment (ERA) for GM crops is to emphasise that actually most of the residual uncertainty stems from a lack of clarity about protection and management goals, about what it is that we need to protect and what exactly would constitute harm to those goals?
Problem Formulation is the initial phase of ERA in which risk hypotheses are created and plans devised to test them, and has the critically important function of detecting and gauging potential risks and focusing research effort and resources on key areas of uncertainty. Operationally, it can be divided into four headline questions covering four overlapping 'stages' in the process:
What do we not want to see harmed? (or What must be protected?) At this stage, we attempt to identify assessment endpoints which we derive from current protection or management goals. Ideally, this should include an explicit statement about the degree to which the assessment endpoints must be protected.
Can we envision a way in which it could be harmed? Here we trace the potential pathways to harm by linking the release or cultivation of the crop to the entity we do not want to see harmed. This stage could involve the development of a conceptual model and is hugely dependent on the skills and experience of ecologists and other biologists involved in the ERA.
How can we assess whether it (or they) is likely to be harmed? This is the stage at which we formulate risk hypotheses based on the understanding gained at Stage 2 and devise an analysis plan. This will include researching the relevant data and deciding whether further information or specifically targetted experiments are needed.
What needs to be done to comply with regulatory requirements? Finally the problem formulation is placed in the context of specific international, national, or local policies and regulations. Again, ideally this should include explicit statements about what is 'acceptable' harm and provide the risk assessor with guidance on any thresholds which might be tested at later stages of the ERA.
Of these four stages, the middle two are clearly recognisable as the domain of the risk assessor in that scientific procedures are used to develop and test conceptual models and to devise and test risk hypotheses. Whilst science is important at the first and last stages of problem formulation, it is equally clear that these stages depend largely

on societal concerns as expressed through public policy in the form of protection or management goals - goals which are not the outcome of scientific enquiry but of policy decisions.

On a global scale, the most generic protection goals with respect to GM crops are provided by the Cartagena Protocol (SCBD 2000), Annex III of which declares that "...the objective of risk assessment... is to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment..." These important but broadly stated goals include three terms: 'biological diversity' (biodiversity), 'sustainable use' (sustainability), and 'adverse effects' (harm) which can be defined more easily than they can be evaluated in practice (and have, in any case, entered the lexicon both of policy makers and environmentalists). In particular, the detection and evaluation of potential adverse effects (harm) presupposes that agreement can be reached about whether an effect or change is harmful or not and that there may be an accepted metric or method enabling harm to be measured. Although they provide important statements of policy, such broadly stated goals do not help us to decide what changes constitute harm and more particularly 'unacceptable' harm, or indeed what data may be needed for an ERA.

In deriving assessment endpoints from these goals, and from national and local protection goals based on them (e.g., the Biodiversity, Habitat and Species Action Plans of the UK), regulators are therefore driven to cast their definitions of adverse effects at the level of a directional change in the entity of value – *reduced numbers or populations* of a protected species, *reduced abundance* of insects that provide services such as natural pest protection or pollination, *increased establishment* of undesirable organisms such as weeds, and so on. Rarely (never?) has it been possible to go beyond such directional change to agree on actual measurement endpoints – trigger or threshold values that would unequivocally signify 'harm.'

As well as rarely considering the magnitude of directional changes which may result from the cultivation of GM crops, ERAs have explicitly ignored both potential mitigation effects and the interaction between local effects and the wider landscape and environment. Even in the case of arguably the largest example of a project designed to inform ERA, i.e., the UK's Farm Scale Evaluations of the effects of growing herbicide tolerant oilseed rape, sugar beet, and forage maize, where a general reduction in the abundance of weeds and insects occurred in fields of the first two species, the reduction alone was widely interpreted as being 'harmful.' This huge experiment was unable to consider the extent to which the reduction in insect abundance would impact the actual species of concern (farmland birds such as the skylark), especially in a scenario of mixed herbicide-tolerant and non-tolerant crops or in situations where habitat creation might offset the impact of reduced infield insect abundance.

In this talk I suggest that, as long as this situation remains and ERAs are based on poorly characterised protection goals and undefined thresholds for 'harm,' and continue to ignore wider effects and agricultural changes (and even possible benefits), they are likely to become increasingly irrelevant in helping to address the environmental challenges of future food production. ERA for GM crops cannot remain a 'special case' if we are to produce more food from the same land area whilst reducing the impact on the environment (coined "sustainable intensification" in Godfray *et al.* (2010)

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2.3 The Grass is always Greener: New Zealand as the Receiving Environment

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Keywords: receiving environment, New Zealand, environmental risk assessment, genetically modified

The receiving environment in New Zealand is greater than just the ecosystem or agricultural production system that new organisms are likely to come into contact with upon release. It includes all of New Zealand, and its society and culture. This broad definition stems from the devastating impact of introducing exotic species into New Zealand in the past. Our cultural history and current societal views have also shaped the current regulations for releasing new or modified organisms. As a result, New Zealand is very cautious about introducing anything new into the environment and our risk assessment requires extensive information on a wide range of potential adverse effects.

New Zealand is a small country that consists of three main islands plus a number of smaller offshore islands. It evolved in total isolation from any other land mass for 80 million years. Land mammals never established and many unique endemic species evolved as a result—they are flightless, large, and ancient. Unfortunately, the very qualities that make New Zealand plants and animals unique also make them very vulnerable to introduced predators, competitors, and a rapidly changing habitat. Hundreds of plant and animal species were introduced without knowing the impact they would have on this vulnerable environment. In a very short time, many species became extinct and over 2000 remain threatened.

New Zealand was the last major land mass to be populated. The first people to arrive were Polynesians from the tropical Pacific. They arrived 800-900 years ago and are the ancestors of New Zealand's indigenous people—the Māori. The first Europeans arrived less than 300 years ago. At first settlement was slow, but increased rapidly from the mid 1800s. During the same period, the Māori population declined significantly from war and introduced diseases. Today, New Zealand has a multicultural population of approximately 4.3 million, of which 68% are European and only 15% are Māori.

An important moment in New Zealand's history was the signing of a treaty between Māori tribes and Europeans in 1840. The Treaty is New Zealand's founding document and has shaped many aspects of New Zealand society. It acknowledges Māori existence, their prior occupation of the land, and that their culture and traditions will continue and be respected. Initially, the intent of the Treaty was not recognised by the European settlers. Māori culture and traditions declined along with the population. Today, honouring the principles of the Treaty is an important part of government legislation, and many Māori traditions have become part of contemporary New Zealand life.

New Zealand's primary industry is pastoral farming. We share our land with 39 million sheep and 10 million cattle. Meat, wool, dairy, and other agricultural products account for half of New Zealand's export income. Apples, kiwifruit, and grapes are also important horticultural products. New Zealand is free of most of the significant agricultural pests and diseases found elsewhere, making us attractive to international markets.

New Zealanders feel a strong connection to the environment and outdoor activities are a big part of our culture. For years, we have been branding New Zealand as "clean and green" both at home and overseas. Tourism is a significant industry and New Zealand is promoted internationally as "100% pure". Most tourists come to experience the New Zealand landscape and outdoor life.

New Zealand has an active Green political party which is very anti genetic modification and has had considerable influence over governments in recent years. There are several vocal lobby groups who believe all genetic modifications are risky, unnatural, and unwanted in New Zealand. The Green party and the anti GM lobby groups are frequently in the media claiming that the release—or even a field trial—of GM plants or animals will destroy

New Zealand's "100% pure reputation". They strongly oppose all applications to field test GMOs and have taken legal action in an attempt to overturn decisions on several occasions.

Genetically modified organisms are regulated in New Zealand under the Hazardous Substances and New Organisms (HSNO) Act. It is *environmental* legislation and its purpose is to protect the environment and people by preventing or managing the adverse effects of any new or genetically modified organism. It was formed in response to our history of unwanted introductions and the continuing decline in our native species and natural habitats. It recognises the importance of Māori culture and specifies that the principles of the Treaty must be taken into account.

All GMOs are considered to be new organisms. The risks of releasing GMOs into the New Zealand environment are assessed using the same criteria as the risks of releasing **any** new species of plant, animal, or microbe. Even if an unmodified version of the organism already exists in New Zealand, the genetically modified version has to undergo a full risk assessment as if it were a new species being introduced.

A GMO will not be approved for release if there is *any* possibility that it might displace native species, damage natural habitats or adversely affect human health and safety. An application to release—or even field trial—a GMO must provide extensive information on the risks and benefits to the environment, to the economy, and to society. The applicant will have to undertake consultation with Māori and ensure that their culture, traditions, and their relationship with the environment will not be adversely affected by the release (or field trial) of the modified organism. The application will be open to public submission and a hearing will be held if requested.

Since the HSNO Act came into force in 1998 there have been only 19 field tests or outdoor containment applications approved for GMOs. The applications were for crops such as onions and brassicas, and for producing biopharmaceuticals from the milk of modified cows. There have been no applications submitted for the release of genetically modified crops. But that may change in the future.

Researchers are investigating genetically modified clover that could reduce the amount of methane gas produced by sheep and cattle. Approximately half of New Zealand's greenhouse gas emissions come from the agriculture sector, of which methane accounts for about two thirds. Reducing animal methane emissions would have significant environmental benefits to New Zealand. There may also be additional benefits to livestock health through increased nutrients and reduced bloat, and a reduction in nitrogen waste.

When the research was recently publicized, it created an interesting—but not unexpected—mixture of reactions. The popular media reported on the revolutionary breakthrough to solving our greenhouse gas problems, while the extreme anti-GM groups continued to claim it will destroy our "clean, green" reputation. However, a Greenpeace spokesperson was reported as being "relatively relaxed" about the research because the modification does not involve genes from another species and will benefit the environment.

So will it be good for New Zealand or will it be it bad? As regulators, we will need to consider this question within the context of all New Zealand as the receiving environment. Hypothetically, the benefits we will need to assess are:

- Benefits to the environment through:
  - Reduction in green house gas emissions
  - Reduction in nitrogen runoff into rivers
- Improvements to animal welfare by:
  - Increased nutrient value
  - Reduction in bloat
- Economic benefits to the agricultural sector:
  - Reduced costs associated with poor animal health
  - Increase in productivity

Clover is already widespread throughout New Zealand, so we will need to address what additional risks a genetically modified clover may create. Public submissions are likely to claim that:

- GM-clovers will damage our 100% pure brand—which could adversely affect tourism;
- losing our GM free status could adversely affect New Zealand's exports;
- other industries or groups may be affected, e.g. organic farmers may not be able to feed their cattle on non-GM clover; and
- cultural concerns about maintaining guardianship over the life-force of valued species.

And then there will be the 'yuck factor'—a generalized society view that it is unnatural, unwanted, and will destroy our clean, green image.

The difficulty will be that all these things are almost impossible to assess. They are either perceived effects, or intangible and difficult to measure. Are the benefits to the environment greater than the belief there will be a reduction in cultural guardianship?

It is likely the modified clover will only make a small reduction in methane emissions from cattle, and it will be difficult to determine how much of a contribution it makes to reducing green house emissions overall. Similarly benefits relating to improved animal health and productivity may also be insignificant and difficult to measure.

There is no evidence to support the strongly held perception that New Zealand's "clean, green" image increases tourism and premiums for our agricultural products. New Zealand is not as clean, green and 100% pure as it is commonly portrayed. The human impact from urbanization and intensive farming is the same as in other developed countries. Yet the tourists still come to look at the landscape and our milk is still exported. In all likelihood, releasing a GMO will have no effect on tourism or our exports.

The extensive and broad information requirements of the HSNO Act are often cited as the reason no GMOs have been released. Others claim it is because New Zealanders want to remain GE free. However, the most likely reason is that there is no demand to release a GMO in New Zealand at present. We do not grow canola, soy, maize, or cotton on a large scale, and we do not have many of the pest problems associated with those plants found in other countries.

In the end, New Zealand's acceptance of genetic modification will be market led. Currently, there is no demand but this is likely to change in the next 10-15 years. New Zealand may come under pressure from international markets to produce GM crops and reduce its carbon footprint. Soon, the agricultural sector will be subjected to a carbon emissions trading scheme and farmers will be looking for ways to offset the extra financial cost.

ERMA New Zealand is the administrator of ***environmental*** legislation. It is our role to protect the environment, the health and safety of people, and Māori culture and traditions from the adverse effects of introducing new plants and animals. New Zealanders learned the hard way how important that is and as a result we now have a very extensive risk assessment process. The New Zealand regulatory environment has changed from allowing almost anything into the country, to stopping almost everything.

But it is also our responsibility to not inhibit innovation and economic growth. Genetic modification is becoming more acceptable worldwide and the demand for GM in New Zealand may be increasing. When the time comes to assess an application to release a GMO, New Zealanders need to be confident that we can manage the risks and maximize the benefits. This is why we have such a broad interpretation of the receiving environment. We need to maintain open and transparent communication between regulators, researchers and the public so that the people of New Zealand and our international markets can trust us to make the right decisions.

2.4 Comparing Apples and Oranges? The challenges of developing appropriate comparators for environmental risk assessment.

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Keywords : GM Crops, wider biodiversity, biological significance, harm, ERA, baseline changes.

Introduction

The basis of European legislation on the cultivation of GM crops (and on the release of other GM organisms into the environment) is to use evidence presented by the applicant to assess the capacity of the release to cause harm to human health and to the environment. In terms of harm to the environment, both direct and indirect effects can be considered. This is particularly significant in the case of herbicide-resistant crops, where altered weed-control strategies are likely to impinge on the "natural" food chains within and around farmer's fields. The farm-scale trials of GMHT crops (FSEs) carried out in the UK between 2000 and 2005 were designed to assess the magnitude and significance of these indirect effects under four cropping systems and thus to provide information that could underpin subsequent risk assessments. In this review, I will briefly summarise the findings and put them in context with other studies on the impact of novel agricultural practices on wider biodiversity. This issue is particularly acute for countries like the UK where the overwhelming majority of the landscape is managed, and where agricultural production is in competition for light, water and nutrients with the on-farm ecosystems that make up the "natural" environment. This means that the nature of comparisons made as part of the environmental risk assessment (ERA) are of particular significance.

A summary of the key results from the FSEs.

Fuller details of methodologies, design, statistical approaches and results for various groups of organisms are described in Firbank et al, 2005 and references therein. The null hypothesis that was tested was that there would be no difference between GMHT and conventional crops in terms of abundance of weeds, weed seeds and invertebrates (i.e. the main lower-level components of on-farm food chains). In all four cases (spring- and winter oil seed rape (OSR), sugar beet and forage maize) this hypothesis was falsified with significant differences between treatments. These differences were entirely attributable to the herbicide regime used rather than to the genetically modified crop itself. However, the differences between different crops were as great or greater than the differences between GMHT and conventional within the same crop. In addition, GMHT forage maize was shown to increase biodiversity (mainly associated with the time of application of broad spectrum herbicides) whereas there were negative effects for the other crops. The results were deemed statistically valid under peer review and the trials were extensive enough to be representative of UK farming.

The regulatory implications of the FSEs

Under the current EU regulatory system, the FSE's clearly demonstrated "harm" to wider biodiversity in the case of spring-sown GMHT OSR and sugar beet. The results for winter-sown OSR were less clear cut, and no harmful effects were detected in the case of GMHT maize. No applications to cultivate GMHT OSR have been received since these results were published, so it is not wholly clear how companies will address the challenges of managing wider biodiversity, but ACRE considered the regulatory implications (ACRE, 2007). ACRE expressed a number of concerns regarding the application of the results of the FSEs to future applications to cultivate GM crops in general and GMHT crops in particular. These are outlined, extended and discussed below.

- <u>The nature and scale of the comparisons vs. the scale of other changes not covered by regulation</u>

The FSEs were a large set of experiments with substantial replication and encompassing a wide suite of measurements. Any future studies on HT crops could restrict the number of measurements (concentrating mainly upon weed and weed seed abundance) and to some extent reduce the number of replicates without sacrificing statistical power. Nevertheless such studies will take time and cost a lot of money (>6M US$ for the FSEs). This has to be set against the changes in wider biodiversity associated with unregulated changes in agronomy such as the

shift from spring to winter cereals and from hay to silage. Both of these had profound effects on the abundance of weeds and weed seeds needed to support the non-farmed ("wild" or "natural") food chains. The broad impact of modern agriculture on biodiversity is discussed by Edwards and Hillbeck (2001). If the decline in wider biodiversity continues, then comparisons between GM and non-GM carried out at one time may not be relevant to similar comparisons carried out at a different time.

- The biological significance of negative effects

In the current EU regulations, there is no quantitative definition of harm. The FSEs defined biological significance as a statistically valid change in abundance in any component of >50% (positive or negative). This was in line with approaches taken in wildlife surveys etc. This figure is far larger than changes defined as significant in other elements of ERAs, raising wider issues about precision and significance. Furthermore, biological significance at the landscape scale is highly dependent upon the relative extent of cultivation of the various cultivars and farming practices being compared. This does not, to my knowledge, form part of an ERA. A facile conclusion from the FSE results would be that it would be significantly better for UK biodiversity to replace conventional forage maize with GMHT rape wherever possible!

- The potential role of mitigation

The way in which the EU regulatory framework is currently interpreted gives little scope for mitigation. The BRIGHT trials on GMHT sugar beet (Sweet et al, 2004) showed that alterations in agronomic practice could maintain the cultivation advantages of HT whilst preserving much of the in-field biodiversity. Also field margin management is routinely used in conventional "conservation" tillage to offset impact "in-field". Given the likely gradual introduction of GMHT within Europe, it would seem sensible to allow the ERA to indicate potential negative effects against appropriate comparators and then to describe a range of approaches to mitigation, the success of which could be established by monitoring after release.

- The value of the trait

ACRE (2007) pointed out that other regulatory systems permitted applicants to balance negative impacts with the positive benefits that might accrue from cultivation of a novel variety. This comparison seems to me to be key to an effective, evidence-based regulatory system and is part of a growing movement to define the full costs and benefits of all agricultural systems (Pretty, 2008).

Conclusions

An effective ERA must assess the impact of introducing a novel trait measured against a suitable comparator. For field studies on whole systems, this comparator should continue to reflect current best practice, but more cognisance should be given to biological significance, to broader (landscape-scale) land use patterns and the changes therein, to the wider opportunities for mitigation at such scales, and to the "value" of the novel trait being introduced. I do not believe that the current EU regulatory system does this.

Disclaimer

The views presented in this abstract and in the resulting presentation are my own and do not represent either the UK government position or the formal opinions of ACRE.

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2.5 Assessing the Ecological Risks from Combining Insect-Control Traits: The Example of VipCot.

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Keywords: non-target, transgenic, insect-resistant, risk assessment, VipCot, cotton, *Heliothis virescens*, *Helicoverpa zea*

Non-target organism risk assessments for the cultivation of transgenic insect-resistant crops are based on tests for potentially harmful unintended changes in the crop that may result from transformation, and evaluations of whether the insecticidal proteins will have harmful effects on nontarget organisms at exposures that will result from cultivation of the transgenic crop (Romeis et al., 2008). The heart of the risk assessment for the insecticidal proteins is a series of laboratory effects tests that expose representative surrogate species to purified protein at concentrations in excess of predicted worst-case exposures in the field. If no adverse effects of the proteins are seen in the laboratory studies, risks to nontarget organisms from cultivation of the insect-resistant transgenic plants are negligible.

Transgenic crops containing combinations of insecticidal traits are being produced by conventional breeding (e.g., Halpin, 2005). Although such crops may contain traits that have been approved for cultivation, combinations of approved traits ("stacks") often require additional regulatory approvals before they may be cultivated; for example, all combinations of approved traits are regarded as new transgenic crops in the European Union (De Schrijver et al., 2007), and crops that combine two or more pesticidal traits require registration by the United States Environmental Protection Agency (Taverniers et al., 2008).

Non-target organism risk assessments for regulatory approvals of transgenic crops with combined insect control traits may be based on a simple premise: the risk assessments for single traits are applicable to those traits in combination, provided there are no interactions among the traits that increase risks to non-target organisms. Increased risk from cultivation of crops containing combined traits could result from higher exposure of non-target organisms to one or more of the insect control proteins, or from increased toxicity of one or more of the proteins. It follows that a non-target organism risk assessment for a transgenic crop in which two or more well-characterised insect control traits are combined could comprise tests of two hypotheses:

1. The concentrations of insect control proteins in the stack are not consistently greater than in the separate transgenic events comprising the stack.
2. There are no interactions among the proteins that increase their toxicity in combination above that predicted from their toxicities alone.

This paper will illustrate these concepts using the example of VipCot cotton.

VipCot cotton was produced by combining transgenic Events COT67B and COT102 through conventional breeding. Event COT67B produces a full-length (FL) Cry1Ab and Event COT102 produces Vip3Aa19; FLCry1Ab and Vip3Aa19 are insect control proteins derived from *Bacillus thuringiensis* and both target lepidopterous pests of cotton. Worst-case estimated exposures of non-target organisms to FLCry1Ab and Vip3Aa19 that would result from

cultivation of Event COT67B cotton and Event COT102 cotton, respectively, are below the no observable adverse effect concentrations for these proteins. Production of FLCry1Ab and Vip3Aa19 during cultivation of cotton varieties derived from Event COT67B or from Event COT102 therefore poses low risk to non-target organisms.

The dry-weight concentrations of FLCry1Ab in COT67B cotton, of Vip3Aa19 in COT102 cotton, and of both proteins in VipCot cotton were measured by ELISA from glasshouse-grown material. Leaves were analysed at four developmental stages, and squares, roots, flowers, pollen, bolls, seeds, and whole plants at a single developmental stage. There were no statistically significant differences in FLCry1Ab concentration between COT67B cotton and VipCot cotton in any tissue. There were no statistically significant differences in Vip3Aa19 concentration between COT102 cotton and VipCot cotton in squares, flowers, pollen, bolls, seeds, or whole plants. In leaves and roots, there were statistically significant differences in Vip3Aa19 concentration between COT102 cotton and VipCot cotton; however, the differences were relatively small (16 – 38%), and were not consistent in direction. The hypothesis that FLCry1Ab and Vip3Aa19 are not consistently higher in VipCot cotton than in its component events was corroborated; therefore, exposure of non-target organisms to FLCry1Ab and Vip3Aa19 from the cultivation of VipCot cotton is likely to be no greater than from cultivation of the component events.

The hypothesis that there are no interactions between FLCry1Ab and Vip3Aa19 that increase their toxicity in combination above that predicted from their toxicities alone was tested using bioassays with tobacco budworm (*Heliothis virescens*) and cotton bollworm (*Helicoverpa zea*). The experimental design was the same for both assays. First, the sensitivity of insects to a range of concentrations of FLCry1Ab and Vip3Aa19 separately was measured. The data were used to predict the response to mixtures of FLCry1Ab and Vip3Aa19 and compared with the observed response to mixtures: a trend of higher mortality than predicted would indicate synergism; a trend of lower mortality than predicted would indicate antagonism.

Vip3Aa19 and FLCry1Ab have different modes of action (Lee et al., 2003; 2006), and therefore the predicted effect of the mixture was calculated using a model called independent joint action. Under this model, if component A alone kills x% of a sample, and component B kills y%, a mixture of A + B is predicted to kill $x + y - xy/100$ (Colby, 1967). In several bioassays, there was no trend for an excess or deficit of mortality over predictions for either tobacco budworm or cotton bollworm over a range of concentrations and ratios of FLCry1Ab and Vip3Aa19. The data corroborated the hypothesis of no synergism between FLCry1Ab and Vip3Aa19.

Corroboration of the hypotheses of no increased concentration or toxicity of FLCry1Ab and Vip3Aa19 in VipCot compared with COT67B cotton and COT102 cotton, respectively, supports the conclusion that the risk to non-target organisms from the cultivation of VipCot cotton is the additive risk from cultivation of the component events. As cultivation of COT67B cotton and COT102 cotton exposes non-target organisms to FLCry1Ab and Vip3Aa19 at concentrations well below those that have adverse effects, production of those proteins during cultivation of VipCot cotton also poses low risk to non-target organisms.

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2.6 Sorghum Modified to Improve Nitrogen Use Efficiency

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Keywords: sorghum, problem formulation, nitrogen use efficiency

Problem formulation, or hazard identification, is the first step in environmental risk assessment (ERA) (USEPA, 1998; Hill, 2005) and aims at explicitly stating possibly relevant problems and establishing approaches for analysis. This case study describes an application of the problem formulation methodology (Wolt et al., 2010; Nickson, 2008) for the adoption of nitrogen use efficient sorghum genetically engineered with a barley Alanine aminotransferase (AlaAT) gene. Much of the basis for this work has been put together in a discussion panel during the 2009 Workshop on Environmental Risk Assessment of Genetically Modified Organisms organized in Buenos Aires, Argentina, by CERA, ILSI Argentina, and Argenbio.

Problem Context

Nitrogen fertilization enables farmers to achieve higher yields and is therefore likely to continue to increase as global population and food requirements grow. Nitrogen may represent a significant portion of farming input costs, significantly impacting profitability. Nitrogen fertilizer applications have exceeded 100 million Tons during 2009-10, and are expected to grow approximately 2% yearly for the next five years (Heffer and Prud'homme, 2010). While nitrogen fertilizers are effective in driving crop yield improvements, they carry an adverse impact on the environment because more than one-half of the nitrogen applied by growers is not used by the plants, whereas the excess nitrogen leaches into the air, soil, and water as pollution. The unabsorbed nitrogen fertilizer is volatilized as N_2O, which accounts for an estimated one-third of the greenhouse gases produced by agriculture, and as nitrogen runoff into the water, resulting in massive algae blooms that upon death decompose and deplete the water of oxygen, therefore leading to death of fish species by hypoxia. The Nitrogen Use Efficiency (NUE) trait aims at reaching yields equivalent to conventional varieties with significantly less nitrogen fertilizer, implying significant savings in farming inputs and environmental benefits. The NUE technology used in the GM events presented here is based upon the expression of a barley AlaAT gene developed by Arcadia Biosciences. Canola and rice field trials carried out over five growing seasons have demonstrated significant yield improvements of GM plants expressing AlaAT over control varieties using less nitrogen fertilizer (Good et al., 2007; Shrawat et al., 2008; Beatty et al., 2009). Barley and wheat greenhouse efficacy trials have demonstrated that these crops may also improve their NUE via AlaAT genetic engineering (Okamato et al., 2010).

The *Sorghum* genus (*Poaceae*) diverged from sugarcane (n=8, 10), its closest relative among cultivated plants, nearly 5 million years ago as its genome complement was established at (n=10). Before domestication, sorghum species were spread throughout Africa, India, Southeast Asia, Oceania, and the Pacific islands. Anthropological

evidence suggests that hunter-gatherers nearly 10,000 years ago consumed the predecessor to today's cultivated sorghum, then a grass from the steppes and savannas of sub-Saharan Africa. Both sugarcane and sorghum are more recent domesticates than the other major grass crops and have captured comparatively little of the genetic potential of their corresponding taxa, until they weredomesticated nearby today's Ethiopia, ca. 4000–3000 BC. Initial domestication of sorghum presumably focused on converting wild types with small, shattering (dehiscent) seed to improved types with larger, nonshattering seed. Numerous varieties of sorghum were created through the practice of disruptive selection, whereby selection for more than one level of a particular character within a population occurs as a result of a balance between farmer selection for cultivated traits and natural selection for wild characteristics, generating both improved sorghum types, wild types and intermediate types.

Sorghum (*Sorghum bicolor* (L.) Moench, (2n=2x=20) is usually grown as an annual crop for food, feed, and/or energy. It has a remarkably high yield potential, exceeding 10 Tons per hectare, attributed to its extremely efficient energy conversion based on C4 carbon metabolism. Sorghum is frequently grown as a "self-insurance crop" because its characteristic drought tolerance enables profitable operations when other crops suffer yield penalties because of insufficient water. Also because of this, sorghum is the choice crop in marginal areas with lower precipitation regimes and lower farming inputs. Sorghum's energy conversion efficiency, capacity to store energy as grain, sugar, and biomass, and adaptability to marginal areas –without necessarily displacing food crops- make it one of the most promising crops from a bioenergy perspective.

The sorghum crop propagates sexually. The presence of rhizomes, which appear in some of the species of the *Sorghum* genus, is the primary morphological feature that distinguishes johnsongrass, a related weed, from sorghum (Celarier, 1958). The sorghum flower is a panicle that has a central rachis with short or long primary, secondary, and sometimes tertiary branches bearing groups of spikelets. The length and closeness of the panicle branches determines panicle shape, which varies from densely packed conical or oval to spreading and lax. The seed or grain is a free caryopsis 4 to 8 mm in diameter, partially covered by glumes. Sorghum is predominantly self-pollinating and shows little inbreeding depression, but natural outcrossing does occur (McGuire 2004; Reddy, 1997; Pederson et al., 1998). Outcrossing rates are higher at the top of the panicle, where flowering initiates, than at middle or lower sections of the panicle Sorghum pollen is anemophily, normally viable for 3-6 h in the anther and 10-20 min outside, and requires light to germinate (Artschwager and McGuire, 1949). There is a recent report indicating the presence of bees on sorghum flowers, but no confirmation of their involvement in pollination has been claimed as of today (Schmidt and Bothma, 2005). A significant volume of reports describe the crossing ability, spontaneous hybridization, and fitness of sorghum and its related wild species (Dillon et al., 2007; Arriola and Ellstrand, 2002). Outcrossing rates range between 0.1 and 30% (Doggett, 1988; Ellstrand and Foster, 1983), but more typically between 1 and 10% for typical commercial hybrids bearing compact heads. Wild subspecies or varieties of sorghum with open, grass-like panicles such as sudangrass [*S. bicolor* subsp. *drummondii* (Steud.) de Wet ex Davidse] may display 30-60% outcrossing rates (Maunder and Sharp, 1963).

The history of the sorghums evidences a continuous gene flow process between sorghum and its feral congeners. Varieties from Africa, India and China have been crossed to crop sorghum in breeding efforts (Reddy et al., 2006). Genetic evidence supports that interspecific hybridization between African crop sorghum and Asian *Sorghum* species occurred during the crop migration to India and China (Rooney and Smith, 2000). Remarkably, landrace *Sorghum halepense* (johnsongrass) is reported to be an allopolyploid resulting from interspecific hybridization between African *S. bicolor* and the Asian *S. propinquum* (Paterson et al., 1995). Crosses between johnsongrass and crop sorghums may yield viable and fertile hybrids (Hadley, 1953; Sangduen and Hanna, 1984), and continuous, unintended hybridization between crop sorghum and wild populations, including johnsongrass, frequently contaminate breeding plots. In contradistinction, crop-specific alleles have been detected in progeny of johnsongrass planted as far as 100 m from cultivated sorghum (Arriola and Ellstrand, 1996), with reported outcrossing frequencies of 0-12% varying according to the distance between johnsongrass and the crop. Other studies also show genetic and/or morphological evidence for the continuous process of crop-to-wild gene flow in

sorghum (Doggett, 1991; Harlan, 1992; Aldrich and Doebley, 1992; Aldrich et al., 1992; Morrell et al., 2005). This process is also profuse: wild sorghums with diverse morphotypes have been reported in many of the sorghum-growing regions of Africa, often as a crop–wild–weed complex including indistinct races of *S. bicolour* (de Wet 1978; Ejeta and Grenier, 2005). Arriola and Ellstrand (1997) have compared fitness-related parameters of johnsongrass-sorghum hybrids with nonhybrid johnsongrass under agricultural conditions, demonstrating that crop-weed hybrids are as fit as their nonhybrid parental types.

In Argentina, sorghum is used as grain and forage for cattle, as well as a rotation crop to maintain production and soil structural stability. Grain sorghum is for the most part grown under no-till farming schemes and is sown from mid-September throughout November. Fertilization practices have increased during the last decade where urea is applied along with atrazine. Harvesting operations take place between February and April. Sorghum is used as a predecessor for a soybean crop for its contribution to the increase in organic matter in soils degraded after intensive use.

The predominant weed related to Sorghum in Argentina is *Sorghum halepense* (sorgo de alepo, pasto ruso, or johnsongrass, in English). The introduction of glyphosate-tolerant soybean in Argentina and its wide adoption since 1996 have allowed grain sorghum cultivation in areas where it was previously impossible because of heavy and aggressive presence of johnsongrass. Shattercane, (*Sorghum bicolor* (L.) Moench), a noxious weed derived from wild and/or cultivated sorghum varieties, however, is not present in Argentina.

Sorghum is considerably affected by insects. Soil insects sporadically cause significant damage when infestations occur during germination and early seedling stages, which are normally associated to the presence of weeds during the previous fallow. Scarab beetles and wireworms head the soil insect list, followed by army cutworms, lesser cornstalk borers, and the spotted maize beetles. Other insects affect sorghum during its growth and development, most importantly sorghum midges, the main sorghum pest in Argentina, which attacks the crop during flowering and occasionally leading to the loss of the full crop when not controlled in time. Greenbug, an aphid, may produce significant crop damage in the case of early infestations by killing seedlings. The stalk borer may also cause important losses, particularly in the case of late sowing dates. Minor insect pests include the green stinkbug and the bollworm.

The Argentine regulatory framework for Genetically Modified Crops aims at protecting a variety of environmental values, among the most important of which are the environmental functionalities, the sustainability of the productive system, and the biodiversity, and non-environmental values such as the protection of the national exports (Burachik, 2009). Considering the crop and trait background described above, it becomes evident that the main problem of concern for NUE sorghum derives from the outcrossing of genetically modified sorghum with locally present, johnsongrass weeds. In this particular case, the scope of the environmental risk assessment would initially be limited to semi-natural habitats in Argentina where johnsongrass grows and affects the sustainability of the agricultural system.

A critical challenge of problem formulation is to identify an observable, measurable property that adequately reflects a more broadly stated concern. To this end, assessment endpoints, i.e., valued ecological entities that can be defined such that a risk to them can be identified and their attributes can be protected, are defined. The principal assessment endpoint of more nitrogen use efficient sorghums is the relative abundance of johnsongrass weeds in agricultural fields and nearby areas that act as weed reservoir.

Problem definition

From an environmental risk perspective, therefore, the problem may be clearly defined from this case study: Will the introduction of NUE sorghum derive into a more competitive hybrid via crossing of the engineered sorghum with its johnsongrass wild relative? The problem in Argentina is restricted to johnsongrass, as opposed to other countries where other weeds that are inter-fertile with sorghum, i.e., shattercane, coexist with sorghum.

A potential exposure pathway of johnsongrass to NUE sorghum, a sine qua non prerequisite for damage to occur, may happen from contact in the field of sorghum pollen with johnsongrass' receptive stigma, or oppositely, of johnsongrass pollen with sorghum's stigma. Dispersal of hybrids may then happen most significantly mechanically via harvesting equipment or forage transport. Other non-GM sorghums may be also exposed by direct contact with GM sorghum, or indirectly via contact with hybrid johnsongrass that may bear NUE genes.

Exposure of johnsongrass to NUE sorghum and subsequent hybridization may lead to potential hazards such as increased weediness within the field of the sorghum/johnsongrass hybrids with respect to non-AlaAT bearing hybrids or to non-hybrid johnsongrass. Moreover, increased weediness near the field, particularly in areas that may serve as weed reservoirs, is also considered a possibility, because these areas, influenced by nitrogen runoff, may provide an adequate niche for weeds that are more efficient in using nitrogen. Additional hazards include NUE gene flow from the GM sorghum to non GM sorghum.

Gathering information may help limit the hybrid NUE sorghum/johnsongrass problem. Potential information sources include Advanta's sorghum breeders (Sr. Breeder Vicente Trucillo and Jr. Breeder Pedro Pardo), who will provide data regarding sorghum varieties and distribution in Argentina. As referred to above, a good deal of reports and manuals deal with the physiology and reproduction of sorghum. These reports serve as a good source of information on sorghum outcrossing and biology. Scientists at Advanta and Arcadia Biosciences (the NUE trait developers) may also provide specific information on the NUE trait inheritability, physiology, and biochemistry.

It is instrumental, from a problem formulation standpoint, to formulate risk hypotheses, i.e., tentative explanations taken to be true for the purpose of argument or investigation that may in turn develop into scientific hypotheses: a specific, testable postulate that will be part of the analytical phase of the ERA. A conceptual model may be helpful in formulating risk hypotheses[4]. The conceptual model involves an NUE gene that may be transferred to johnsongrass, yielding a fertile offspring that preserves the weedy characteristics. In the offspring, it is a requirement that the gene continues to function, and that it also confers a competitive advantage to the hybrid, and that this advantage is translated into an increase in weed populations. The following hypotheses may be tested to contradict this model: 1, the gene does not move from sorghum to the weed; 2, the gene is not functional in the weed where it was transferred; 3, the gene does not confer advantage to the hybrid; and, finally, 4, the gene cannot move to other sorghums.

Methodologies that may be used to test the above hypotheses may include making crosses in the greenhouse and the laboratory (under strictly contained conditions to ensure no transgene escape to the environment), study the johnsongrass/NUE sorghum hybrids for presence of the barley AlaAT gene and function, taking weediness-related measures: biomass, phenotype, vigor, flowering, rhizome size, and others, under different nitrogen conditions. It is key that the NUE-bearing hybrid population is generated from a wide range of johnsongrass genotypes displaying a range of NUE responses, such that phenotypic differences in the hybrids is weighted in contrast to weed NUE in different genotypes. This helps define if the NUE hybrid sorghum/johnsongrass performance may fall within the natural range of variability of johnsongrass.

Conclusions

The case study presented here draws specific attention to outcrossing of the subject crop, sorghum, with wild, weedy relatives. Different reports deal with measurements of outcrossing frequencies between sorghums, all of them supporting the claim that gene flow does occur between sorghum and johnsongrass and other wild relatives. The question mark derived from the problem formulation process in this case study lies on the contribution of the barley AlaAT gene to the weediness of johnsongrass. Environmental Risk Assessment frameworks include subsequent steps that: assess exposure, i.e., "how likely is it to happen"; assess consequences, i.e., "would it be a problem"; characterize risk, i.e., "what is the risk"; and identify mitigation options, i.e., strategies to manage risks2. These instances of the Environmental Risk Assessment are essential to enable NUE and other GM technologies to safely add significant new value to agricultural systems.

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2.7 Using Problem Formulation in Environmental Risk Assessments: Practical Methods for the Assessment of Unintended Effects

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The methodology to be used for the environmental risk assessment of genetically modified (GM) crops has been a major subject of debate for many years. However, after much research and many scientific discussions, a conceptual framework based on a tiered approach is emerging and gaining acceptance amongst risk assessors, regulators, and the scientific community. The first step within this framework is "Problem formulation". It is at this

step that the purpose of the risk assessment is clearly outlined, all data available is gathered, hypotheses are formulated and a first risk characterization is conducted. In some cases, risk can be satisfactorily characterized at this step as sufficient data is available and no further analysis is needed. In other cases, the conclusion is that additional data is needed to proceed with the risk assessment; here problem formulation offers a useful methodology to establish what additional data is needed and to outline a plan that allows obtaining these data in a logical and pragmatic way. Therefore, problem formulation is an essential step for a well designed and logical environmental risk assessment process.

Environmental risk assessments are usually focused on the evaluation of the probability that a harmful effect may occur due to exposure to a known stressor. At the problem formulation step, assessors collect all data available on the stressor(s) (mode of action, known toxicity, previous uses…) and establish potential exposure pathways. There is often a misconception that data used in problem formulation are data generated exclusively for this purpose. In reality, data used in the problem formulation step are data previously available, like data obtained during development, data from scientific publications, and data typically generated for all commercial applications. Indeed, problem formulation is the step at which the risk assessor establishes what is already known, whether sufficient information is available to characterize risk, and if not, what additional data has to be generated. When further data is considered necessary, a well conducted problem formulation allows the preparation of a clear testing plan, where studies are conducted to address specific questions with clear testing hypotheses and relevant endpoints.

The information generated for commercial applications in most countries usually includes data on the receiving crop, on the genes intended for insertion, their sources, the proteins they express, molecular characterization data on the resulting GM crop, expression data, compositional analysis, agronomic characterization, toxic and allergenic potential, *etc.* Applicants must comply with these data requirements in order to have their applications considered by regulatory authorities. Thus, most applicants strive to generate these data and comply with the requirements so timely approvals can be obtained without undue delay. This means that from the start there is a large amount of data available to the risk assessor at the problem formulation step that allows the focused planning of the environmental risk assessment.

Currently, one of the concerns about the environmental safety of GM crops is that the genetic modification introduced may result in unintended effects that could lead to adverse effects to the environment that may not be immediately apparent during the pre-commercial assessment, but may manifest themselves after the crop is widely commercialised. Some regulatory systems now request applicants to conduct a risk assessment that includes an evaluation of the probability that these unintended, unidentified effects may occur. Obviously, this represents a major challenge because it becomes unclear what is exactly under assessment. However, there are practical and pragmatic ways in which risk assessors using the current risk assessment methodology, including problem formulation, can conduct such assessments. This presentation discusses ways in which this could be achieved.

It is widely accepted that traditionally cultivated crops have a history of safe use and familiarity for consumers or animals and the environment; this is the basis of the concept of familiarity used in many regulatory systems. The risk assessment for a new GM crop therefore could start with a comparison of the GM crop with its non-GM conventional counterpart, conducting what is known as a “Comparative assessment”. Using problem formulation, gathering the data generated for the standard regulatory package for commercial applications, assessors can establish what is different in the GM crop compared with its conventional counterpart. This assessment offers three possible outcomes: (1) intended differences are identified, (2) unintended differences are identified and (3) no differences are identified (which here we will also refer to as “unintended, unidentified”). Thus, the outcome of this comparative assessment allows the risk assessor to establish what is different in the GM plant compared with known conventional counterparts that are already considered safe. Differences between the GM plant and its non-GM counterpart or comparator do sometimes occur, often at random, given the large number of comparisons

made. However, these differences do not necessarily indicate adverse effects. The risk assessor's first task after identifying a difference is to establish whether this difference is biologically relevant or not, and the probability that this difference will lead to an adverse effect in the field. If this is the case, then the risk assessment is focused on these identified biologically relevant differences. For the purpose of the risk assessment, any differences considered biologically relevant, are assessed in the same way, regardless of whether these differences were intended or unintended. Where no biologically relevant differences are identified, no further data is generated under this risk assessment framework.

Some regulatory systems, like the one in the EU, account for the fact that the environmental risk assessment is based on the assessment of potential environmental effects due to identified characteristics of the GM crop that may lead to adverse effects and establish additional requirements to address unintended, unidentified effects (i.e., monitoring), but these apply once the GM crop is commercialized and the methodology to address those is currently under discussion but not yet fully established.

In summary, an assessment of potential unintended, unidentified effects can be conducted using problem formulation. As expected in most risk assessment frameworks, this approach would not offer absolute answers and no certainty of whether unintended, unidentified effects will occur and cause harm or will not occur; uncertainty is intrinsic to risk assessment. However, this methodology provides a way to conduct an assessment of potential unintended, unidentified effects without embarking on large data gathering efforts where it is unclear what effects are being evaluated and which are unlikely to yield useful information for the risk assessment.

2.8 Assessment of Gene Flow from Herbicide-Tolerant Transgenic Rice to Weedy Rice (*Oryza sativa*): Inputs to Problem Formulation

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Rice (*Oryza sativa* L.) is the most important cereal worldwide as it currently supplies calories for more than half of the population. However, its production is severely constrained by weeds, diseases, and pests. Weedy rice, also known as red or feral rice, along with other species such as *Echinochloa* spp., *Leptochloa* spp., and several sedges and broadleaf weeds substantially reduce rice yields.

Weedy rice is present in practically all the rice-growing regions and agroecosystems in more than 50 countries (Valverde, 2005) and affects rice grain and seed production, and quality of harvested rice for industrial processing (Arrieta-Espinoza, et al., 2005). Weedy rice constitutes a complex of *Oryza* biotypes associated and evolved as a conspecific crop mimic that is harvested and resown with the rice crop (Stewart, et al., 2005). Several characteristics render weedy rice one of the most important weeds in rice production in Latin America (Madsen, et al., 2002; Valverde, 2005). This complex includes plants setting red-pericarp seeds, widely known as red rice (Olofsdotter, et al., 2000), although some morphotypes have white pericarp. Plants are frequently taller than green revolution rice cultivars, tiller earlier, and produce more tillers and panicles per plant allowing them to compete efficiently with the crop for nutrients, water, and light. In addition, seed dormancy favours weedy rice maintenance in the soil banks for long periods, while seed shattering allows dissemination within fields (Diarra, et al., 1985; Noldin, et al., 1999).

The physiological and genetic similarities between weedy and cultivated rice make its selective removal with herbicides very difficult; hence heavily infested fields are often abandoned (Kwon, et al., 1991; Montealegre and Vargas, 1992). However, the productivity constraints caused by this weed could be overcome through the use of herbicide resistant rice (HRR) cultivars.

Glufosinate- and glyphosate-resistant rice varieties have been developed by genetic engineering and imidazolinone-resistant (IMI) rice produced by mutation breeding and commercialized as Clearfield® rice. Herbicide resistant varieties allow a selective, broad-spectrum, post-emergence weed control in rice. In the case of transgenic cultivars field experiments would be require to gather scientific evidence to elucidate some of the main issues regarding hybridization rates between a transgenic cultivar and the weedy rice as an assessment endpoint. In this context, the risk hypothesis of this study was the estimation of the maximum gene flow under experimental field conditions from a transgenic glufosinate-resistant herbicide variety to weedy rice. In addition, the effect of genetic identity, sympatry, flowering overlap, and the performance of hybrids on gene flow was studied.
Baseline information was generated by performing a comprehensive morphological (Arrieta-Espinoza et al*.,* 2005) and molecular characterization (Trejos, 2006) of the Costa Rican weedy rice complex and its weedy rice life cycle (Sánchez-Olguín, et al*.,* 2007). In the morphological study, 21 distinct morphotypes were identified and the morphometric relations of weedy rice with commercial rice varieties, landraces, and wild species [*O. glumaepatula*, *O. grandiglumis*, *O. latifolia* (Costa Rica), *O. rufipogon* (Asia) and *O. glaberrima* (Africa)] were defined by comparing 27 *O. sativa* traits. As a result, the majority of weedy morphotypes grouped with the commercial rice varieties (*sativa-like*) and others were more closely related to *O. rufipogon* (*rufipogon-like*). A third group exhibited intermediate characters between *O. sativa* and *O. rufipogon.* It is very important to point out that none of the weedy rice grouped with the wild species (Arrieta-Espinoza, et al., 2005). The molecular characterization using microsatellites showed that weedy rice is highly diverse. However, it confirmed the association of most of the weedy rice to cultivated varieties subspecies *indica* and *japonica*. But no relation was found with the wild species *O. glumaepatula, O. Rufipogon,* and *O. glaberrima* (Trejos, 2006). In both studies, weedy rice was separated from the Costa Rican native wild species *O. glumaepatula*, probably as a result of the geographical isolation of the wild species (its habitat is restricted to the Northen zone of the country), the absence of flowering overlap with cultivated rice (Zamora-Meléndez, et al*.,* 2003) and the reproductive barriers between *O. sativa* and *O. glumaepatula* species (Quesada *et al*., 2003; Trejos, 2006). The phenological study showed variation in vegetative and reproductive phase among weedy rice morphotypes as well as different anthesis overlapping spans with rice varieties. The description of weedy rice life cycle demonstrated that 70% of weedy rice morphotypes overlapped in flowering to some extent with commercial rice variety Setesa-9, while 50% overlapped in flowering with CR-5272 and CR-4338 varieties. Just one morphotype overlapped with CR-1821 variety (Sánchez-Olguín, et al*.,* 2007).
The previous data allow the selection of a diverse and representative sample of 58 weedy rice accessions belonging to six weedy rice morphotypes to perform a field study to determine hybridization rates from a locally-developed glufosinate-resistant *indica* rice (*O. sativa*) to the Costa Rican weedy rice complex (Sánchez, et al., 2009).
The field trial applied a methodology mimicking crop-weed growing patterns and herbicide resistance was used a selectable marker for hybrids. The main result of this study indicated that anthesis overlap, individual identity of the accessions, and morphotype affected hybridization rates between weedy and cultivated rice. However, the weedy-rice infestation level (density) had no effect on hybridization rate. A higher hybridization rate (0.3%) was obtained with long anthesis overlapping weedy rice (10-14 days) than with short anthesis overlapping plants (4-9 days) in which 0.1% of hybridization was determined. The hybridization rates varied among accessions; in 11 out of 58 no glufosinate-resistant hybrids were detected. In 21 of the accessions evaluated, rates from 0.01% to 0.09% where observed, while in another 21 accessions out of 58, rates between 0.1 a 0.9% were recorded. The highest rate of 2.3% corresponded to 5 accessions (Sánchez, et al., 2009). Morphological traits of the hybrids were evaluated and related to the parental lines in a second field trial. The weedy-transgenic line hybrids show a high variation in morphology. Positive heterosis was observed in productivity traits such as plant height, culm number, flag leaf length and width. However, negative heterosis was observed for seed set in all the F1 hybrids types. In the case of seed shattering, a decrease of the condition was observed as 33% of the hybrid plants showed easy seed shattering.

The research described a possible exposure scenario of gene flow from the glufosinate-resistant *indica* rice transgenic to weedy rice. The data obtained show the consequences of this scenario that are essential for an environmental risk assessment (ERA). The relatedness of weedy rice to cultivated rice as well as its genetic diversity affects gene flow. *Sativa*-like weedy rice exhibits the highest hybridization rates, but the effect of particular individuals (accession) is also a key aspect. The reproductive biology of the individuals is also significant because 11 out of 58 accessions did not hybridize with the transgenic line, but are compatible through manual crosses, showing the complexity of the process. The data obtained in this research stresses the requirement of a strict crop management program, if transgenic rice is deployed, to avoid gene flow. Regulatory authorities could request the integration of weed management to guard improved transgenic rice, including control of volunteer rice, related rice species, and hybrids through crop rotation and use of herbicides with different mode of action. Furthermore, the significance of developing molecular containment mechanisms or transgenic mitigation systems could contribute to the deployment of transgenic rice and to reduce the eventual gene flow and introgression of novel transgenic traits from cultivated rice to weedy rice populations.

2.9 Design Considerations for Laboratory Non-Target Studies Used to Support Environmental Risk Assessment

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Keywords: environmental risk assessment, insect-resistant transgenic plants, laboratory study design, non-target effects, tiered risk assessment

Non-target risk assessment of GE crops

Genetically engineered (GE) plants, and food and feed products derived from them, are strictly regulated by governments internationally. Environmental risk assessment of GE plants is designed to answer very specific questions about the potential risks of introducing such plants into the environment. Problem formulation directs the scope of risk assessment and defines explicit expressions of the environmental entities that are to be protected (termed assessment endpoints) against a potential stressor. A typical assessment endpoint that concerns non-target arthropods (NTAs) is ‘‘beneficial arthropod abundance.’’ Problem formulation further generates testable scientific hypotheses and endpoints to measure (termed measurement endpoints) that are relevant for decision-making and are subsequently addressed in the analytical phase of the risk assessment (Raybould 2006; Wolt et al. 2010). Problem formulation should culminate in a conceptual model delineating how harm can occur by a particular stressor on the assessment endpoint, leading to an analysis plan that is consistent with the risk hypotheses and should establish the relationship between the stressor and the ecological impacts of concern. A

typical risk hypothesis related to NTA effects of an arthropod-resistant GE plant is: "The expressed protein is not toxic to NTAs at the concentration present in the field" (Raybould 2007; Romeis et al. 2008). The risk hypotheses are then typically addressed following a tiered framework that is conceptually similar to that used to assess the environmental impact of conventional chemical plant protection products (Hill and Sendashonga 2003; Garcia-Alonso et al. 2006; Rose 2007; Romeis et al. 2008).

Based on the risk hypotheses, early-tier laboratory experiments are conducted under worst-case exposure conditions where species representative of NTAs present in the receiving environment that are likely to be exposed to the arthropod-active protein are exposed to concentrations of the protein in excess of exposure in the field. This increases the likelihood of detecting adverse effects on NTAs, if present. If no adverse effects are seen under these worst-case exposure conditions, the risk can be characterized as being acceptable and there may be no need to conduct any further testing because of the minimal probability of adverse effects in the field where NTAs are exposed to much lower concentrations of the arthropod-active protein. Early-tier testing thus allows elimination from further consideration risks that are negligible, and allows assessors to focus resources to address more significant risks or uncertainties.

The applicability of the tiered risk assessment framework to GE plants has become evident from the experience with GE crops expressing Cry proteins derived from *Bacillus thuringiensis*; a recent meta-analysis of published studies on non-target effects of such *Bt* crops has confirmed that laboratory studies "…predicted effects that were on average either more conservative than or consistent with effects measured in the field" (Duan et al. 2010).

Laboratory study design considerations

Good study design is critical for early-tier laboratory studies because it contributes to the robustness of, and confidence in, environmental risk assessments for GE plants (Romeis et al. submitted). Good study design seeks to minimize the probability of erroneous results: false negatives – the failure to detect adverse effects of substances that are potentially harmful in the field, and false positives – the detection of adverse effects when the substance is unlikely to be harmful in the field. Erroneous results may arise if the conduct of the test introduces bias, or exposes the test NTAs to conditions that are significantly different from those under which the test is known to be reliable.

Early-tier laboratory studies should accurately determine the effects on surrogate non-target arthropods of known concentrations of the test substance. In most cases, the test substance will be a purified protein produced in microbial expression systems, or, alternatively, GE plant tissue.

Unbiased and reliable test systems reduce the probability of false positives and negatives by a combination of several test protocol design criteria:

a. The test substance must be well characterized and described. This includes the source and purity of the arthropod-active protein, and its stability and homogeneity in the carrier through which it is provided to the test organism.
b. The test substances must be biochemically and functionally equivalent to the protein or other active ingredient produced in the GE crop.
c. The bioactivity of the test substances, as provided to the test organisms, must be established (e.g., in sensitive insect bioassays).
d. Test organisms should be exposed to high concentrations of the test substance relative to predicted exposures in the field (if possible) or dose-response studies should be performed.
e. Exposure of the test organisms to the test substance should be confirmed by, for example, use of a positive control and diet analysis to measure the concentration of the test substance.
f. Endpoints should be measured that are likely to indicate the possibility of adverse effects on the abundance of NTAs or other assessment endpoints. Risk assessors should agree on how to interpret and use these data in the risk assessment. Determination of the measurement endpoint(s) should consider the

knowledge about the impact of the arthropod-active protein on the target organisms, knowledge about the biology of the selected NTA species and life-stages, and the availability of reliable test protocols.

g. A sufficient number of replicates need to be included in the study so that effects can be detected with a certain statistical power.

h. Negative control treatments must be included to assess the suitability of the test system, the organisms (e.g., health) and the test conditions, and to evaluate potential effects of the matrix or formulation in which the test substance is delivered. Test results from assays with unacceptable high negative control mortality should be discarded.

Conclusions

Confidence in a conclusion of no adverse effect on a surrogate species, and confidence in extrapolating that conclusion to other species, depends upon the ability of the laboratory study to detect such effects. Thus emphasis is placed on attributes that reduce the likelihood of false negatives (criteria b-g above). Adhering to the principles and recommendations outlined in Romeis et al. (submitted) should increase confidence in the results of early-tier laboratory studies, and thereby reduce data requirements for stressors that pose low risk. The guidance provided should facilitate the reproduction of a study, peer review of such tests by others in the scientific community, and in general benefit regulatory authorities by enhancing the quality of information generated for use in risk assessments. High confidence in the results of early-tier laboratory studies is a precondition for the acceptance of data across regulatory jurisdictions and should encourage agencies to share useful information and thus avoid redundant testing.

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SESSION 3
Biosafety considerations for crops for non-food/feed uses, biofuels and energy crops

3.1 Status and regulation of non-food/feed crops in Europe

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GM plants used for non-food or non-feed purposes are considered to be the third wave of GMOs coming to the market. These plants are either used for energy delivery or produce new compounds such as plant made pharmaceuticals (PMP) or plant made industrials (PMI). While the energetic use mainly focuses on increased biomass of crop plants, PMI use the huge potential plants have to perform cheap biomass for the additional production of compounds like starch, cellulose, PHAs, and protein-based biomaterial. Genetic engineering of plants for the production of novel polymers and platform chemicals can help to alleviate the demands for limited resources and potentially provide a platform to produce valuable compounds in bulk quantities. Recent advances in enhancing the production of novel compounds in transgenic plants include multigene transformation and the direction of biosynthetic pathways to specific intracellular compartments. It now appears feasible to produce interesting proteins such as spider silk or collagen, novel carbohydrates, and biopolymers that could replace petroleum-based plastics using transgenic plants. Direct production of novel compounds in biomass crops, with the aim to produce bioenergy as a co-product, provides a promising way to improve economics of transgenic plants as biofactories (Börnke and Broer 2010).

Plants are also considered to be a promising production platform for vaccines and many different immunogens have been expressed in higher plants. The most important advantages of plant-derived vaccines include low production costs, capability for large-scale processing, convenient storage of the material, the absence of human or animal pathogens, and reduced downstream processing. However, 25 years after the first antigen expression in plants only one plant-derived antigen has received regulatory approval by the USDA. The main problems with plant-derived vaccines seem to be low yields, and vaccines of insufficient and inconsistent quality (Mikschofsky et al. 2009)

The production of biopolymers and pharmaceuticals has proven to be feasible and might contribute to a sustainable agriculture. Nevertheless, application still lies in the fare future. Before transgenic plants can be cultivated and disposed, they undergo an authorization procedure based on a safety assessment to guarantee there safe usage. The EFSA GMO Panel considers that for GM plants used for non-food or non-feed purposes the comparative approach is valid, but will need to be applied carefully. For these plants, the assessment of the potential impact of the differences identified in the comparative analysis is particularly important with regard to accidental intake by humans, livestock and wildlife animals, the exposure of farmers and workers handling the GM plants, and the exposure of passers and of people living in the vicinity. (EFSA 2009)

EFSA sets the focus of the evaluation for human and animal safety on the risks resulting from oral exposure through accidental intake (through inadvertent entry in the food and feed chain via admixture or gene flow or through accidental consumption in the field) of the GM plants/plant parts used for non-food or non-feed purposes by humans and animals. The EFSA scientific opinion states that the risk assessment for plants used for non-food or non-feed purposes has to take into account the confinement measures when applied. To allow for a quantitative risk assessment, this is to be integrated in a two-step risk assessment. In a first step, risks for human and animal health and the environment of the GMO need to be assessed based on an exposure assessment without the consideration of the confinement measures and in a second step, confinement measures as proposed and applied by the applicant should be taken into account (EFSA 2009).

According to EFSA, the use of GM plants for non-food or non-feed purposes, for example the production of novel compounds, expands the role of crop plants. The target products could have adverse effects when in contact with humans, animals or the environment, or when consumed by humans or animals. Where new potential GM plant risks are identified, the plants are likely to require more specific risk management conditions, such as methods of production stewardship, defined confinement measures, safety thresholds and inspections (EFSA 2009).
In addition the EFSA GMO panel addresses the influence of external factors such as abiotic and biotic conditions on the effectiveness of confinement measures. The applicant therefore should provide data that allow the assessment of confinement measures under all environmental conditions envisaged taking worst-case scenarios into account. In this regard it may be necessary and useful for the applicant to narrow the geographical area in which he seeks permission for the product (EFSA 2009). The EFSA asks applicants to apply methods reducing accidental intake, preventing gene flow into the environment, enforcing monitoring and emergency measures for restricting gene flow. In addition, the stability of the new compound in the environment and its bio-activity has to be assessed. In special cases confinement should be considered to prevent or reduce herbivory and leakage through drainage or sewage.
Envisioning these high demands concerning the risk assessment of PMI and PMP, a consortium of scientists and small sized companies (BioOK) developed new methods or modified existing ones to assess the impact of PMI and PMP on soil, NTOs and the consumer.
The intention of the BioOK risk assessment system is to establish a structured and sequential approach to conduct the risk assessment for a transgenic crop. Since the fact, that a plant produces a novel compound is not per se a reason to assume that its impact on environment or consumer is increased, BioOK at first theoretically identifies all possible risks that are based on a scientifically founded hypothesis of cause and effects. Subsequently, standardized methods specifically developed for the analysis of transgenic plants are used to analyze the potential risks. These methods fulfill the following criteria: 1. They target a specified problem 2. They identify and simulate the exposure, 3. They focus on the analysis of the potential cause of hazard 4. They simulate a worst case scenario, 5. They are linked to thresholds and indicators to deliver solid data for the decision whether a difference between the transgenic plant and its near isogenic line causes a hazard or not. Potatoes expressing each one of two different antigens as PMP or a biopolymer as PMI were used as model plants and compared to the near isogeninc lines and the transgenic control. Six different potato varieties are used to gain baseline data and to identify indicators and thresholds that can be used for a standardized risk assessment.
To raise the efficiency of the assessment effort, BioOK adapted existing methods so far used for non-genetically modified organisms and developed new ones if no adequate methods were available. *In vitro* systems and *cell culture* methods in the laboratory or greenhouse were established in order to substitute extensive feeding studies or field trials whenever possible. Nevertheless, event specific field experiments have to be carried out to investigate environmental effects e.g. on non-target organisms or the variability of transgene expression.
Finally, the compilation of the novel and traditional methods resulted in a comprehensive, modular system for the complete risk assessment of GMOs – from the molecular characterization up to the environmental risk assessment and post-market monitoring.

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3.2 Status and regulation of non-food/feed crops in the USA

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Today, modern agriculture faces the grand challenge of meeting increasing demand for food, feed, and biofuels/bioproducts that can be produced in an environmentally sustainable manner while maintaining quality and safety and coping with a changing climate. To address these often competing challenges, we need new and diverse strategies that can be implemented in a timely manner. During the last 25 years, biotechnology has allowed the production of genetically improved varieties that have resulted in increased yields without sacrificing stewardship, quality, or safety. Regrettably, crop developers encounter significant barriers to commercialization of genetically engineered (GE) crops as the high cost of regulatory approval for GE crops coupled with the predicted market value or limited resources of the crop developer impairs the possibility of recovering the cost of development, in a manner similar to that encountered by orphan drug developers. The studies needed to achieve "substantial equivalence" for a GE crop require a significant amount of data, which can be expensive, time consuming, and may be duplicative of previous studies. In 2003, the cost of meeting regulatory requirements was estimated at $20 to $30 million US (McElroy, 2003). More recently, the cost of bringing a new GE seed to market was calculated to be $100 million US (Economist, 2009). This cost prohibitive regulatory burden has limited the commercialization of genetically engineered crops to a few multinational biotechnology companies and a few crops with a market value high enough to cover the cost of deregulation.

United States Department of Agriculture (USDA) funding programs have invested several million dollars over the last few years to develop crops with biotechnology-derived traits. However, almost none have been brought to market. Commercialization of agricultural biotechnology requires significant effort in several sectors:

- Scientific capability—can you create your crop/trait combination to yield your desired result?
- Intellectual property—can you protect your invention/crop?
- Market realities—can you market your product to a consumer? and
- Regulations—can you scale the mountain of government regulations?

These questions apply to all traits whether they are for food/feed uses, or non-food, biofuel types of applications. Some of the regulatory barriers that face crop developers in commercializing biotechnology-derived crops include:

1. Understanding the complex regulatory framework;
2. Preparing a road map to follow for achieving non-regulated status from APHIS, achieving registration at EPA, or for voluntary consultation with the FDA;
3. Defining normal ranges to establish a definition of "substantial equivalence" (OECD 1993) to which the transgenic crop is to be compared;
4. Collecting relevant and comprehensive data sets;
5. Controlling costs associated with deregulation;
6. Standardization of data collection and analysis.

Non-feed applications of biotechnology include such products as pharmaceuticals, vaccines and industrial products. The first non-food products sold from transgenic plants were proteins from corn, Avidin (A8706; 1999) and Trypsin (Trypzean T3568; 2001) through the Sigma Chemical Co. catalog. The first pharmaceutical to be sold is glucocerebrosidase (USP#5,929,304) manufactured in cultured carrot cells by Pfizer Pharmaceutical Co. This product is currently being sold in France and will likely be marketed in the US in the next few months. Although these are great first products to demonstrate the power of the system, none of these products has been through the deregulation process. The corn products are grown under permit in small volumes and the carrot cell products are manufactured in controlled tank culture without environmental release.

The most notable non-food product going through the US regulatory system currently is amylase in corn (Syngenta Seeds, Inc.). This product is designed to lower the cost of producing sugars from corn starch to enhance ethanol production. It has taken a number of years to progress through the APHIS regulatory system. Part of the hold-up has been intense negative response from the public. Considering the low hazard of the enzyme as well as its ubiquitous presence in non-GE organisms, the response is surprising.
This presentation will review the current regulations from agencies that govern aspects of plant made non-food/non-feed products and the issues they present. Some new technologies that can be applied to resolution of these issues will all be discussed.

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3.3 Recent advances in biological confinement technologies

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In its Scientific Opinion on Guidance for the risk assessment of genetically modified plants used for non-food or non-feed purposes (http://www.efsa.europa.eu/en/scdocs/doc/1164.pdf) the EFSA (European Food Safety Authority) Panel on Genetically Modified Organisms states that a comparative approach is advocated for these plants but will need to be applied carefully. Consumption is not expected, but accidental oral, dermal, ocular and inhalatory exposure is possible. The existing guidance on the environmental risk assessment of GM plants for food/feed purposes (http://www.efsa.europa.eu/en/scdocs/doc/99.pdf) is adequate but additional emphasis should be given to issues such as gene transfer and the exposure of non-target organisms, particularly wildlife feeding on these plants. The Opinion further describes the importance of risk management systems, such as post-market environmental monitoring, standard production protocols/stewardship, or confinement strategies to reduce exposure to plants used for non-food or non-feed purposes. The risk assessment for these plants has to take into account the confinement measures when applied. To allow for a quantitative risk assessment, a two-step risk assessment is recommended:

- in a first step, risks for human and animal health and the environment need to be assessed based on an exposure assessment without considering the confinement measures
- in a second step, confinement measures as proposed and applied by the applicant should be taken into account.

To assess the reliability of confinement, the GMO Panel proposes to consider the following:

- The effectiveness of confinement measures may be influenced by external factors such as abiotic and biotic conditions. The applicant therefore should provide data that allow the assessment of confinement measures under all environmental conditions envisaged taking worst-case scenarios into account.
- Applicants should describe for each GM product the details and rationale for the proposed physical and biological confinement strategy, where applicable. The proposal should specify the methodology used and its effectiveness in reducing accidental intake or preventing gene flow into the environment.
- Regarding non-food or non-feed GM plants that produce bio-active substances that are stable, or that persist for a long term in the environment, it should be considered whether the confinement should also prevent or reduce herbivory and leakage through drainage or sewage.

The European Commission has been funding several research projects aiming to develop or establish – including the assessment of their stability and reliability – biological confinement measures. A main strategic objective of

Transcontainer (Developing efficient and stable biological containment systems for genetically modified plants) was to assess the economic, environment and consumer impact of implementing biological confinement strategies in Europe. The main confinement tools were chloroplast transformation, controllable flowering, and controllable fertility.
A general objective of Co-Extra (GM and non-GM supply chains: their co-existence and traceability) was to analyze, further develop and validate biological confinement methods for restricting pollen-mediated gene flow during cultivation. Already existing biological containment tools like cytoplasmic male sterility (CMS) in maize and sunflower, male sterility in tomato, cleistogamy in oilseed rape and plastid transformation in tobacco were tested for their stability and reliability. Large scale studies over 3 years were performed with maize and rapeseed, small scale studies with sunflower, tomato and lab experiments with tobacco. Viable pollen production, cross pollination, crop yields, and other themes relevant to bio-confinement were taken into account. Stable and unstable male sterility occurred in all three CMS maize types. T-cytoplasm hybrids were the most stable under a wide range of environment, while S-cytoplasm hybrids often showed partial restoration of fertility. C-cytoplasm was similar to T-cytoplasm with regard to maintaining male sterility. The data demonstrate that stable cytoplasmic male sterility in maize may be an effective way to prevent GM pollen-mediated gene flow to adjacent fields if 100% stable T- and C-cytoplasms are used. Appropriate combinations of CMS hybrids and fertile pollinators used as an agricultural bio-confinement system can lead to a significant gain in yield, as observed for the Plus-Hybrid system.
The stability and allo-pollination rate of cleistogamic oilseed rape lines was tested with ring field trials in four locations in Europe using two new genetic backgrounds over two years. The data demonstrate that cleistogamy provides a potential biological confinement tool for oilseed rape. Flowers of cleistogamous lines are mostly closed, resulting in a strong reduction of the pollen cloud.
This presentation will review the recent advances in biological confinement technologies with a focus on data gained in the EU-funded projects described above and ongoing projects funded by the German government. Some new technologies like suicide systems for algae and bacteria will be discussed as well.

3.4 The Benefits and Risks of Next Generation Microalgal Biofuel Production Systems.

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One of the more environmentally sustainable ways to produce energy and capture carbon dioxide is the conversion of solar energy into biomass. The first-generation biofuels (alcohol and diesel) were produced from only a few crop systems including, sugar cane (sugar), maize (starch), and soy (oil). These biofuel systems were often not very efficient. Typically, only a fraction (<4%) of the solar energy captured in biomass was harvested as fuel. Inefficiencies in feedstock processing further reduced the recoverable energy and reduced net carbon capture. Extensive land area was also required to produce fuels from first-generation biofuel crops. Second-generation biofuel systems utilizing cellulose and hemicellulosics are now being developed. Conversion of cellulosics to sugars using advanced enzyme catalysts promises to increase the available carbon resources for fuel production and reduce the land area required for biofuel production. Third generation biofuel systems will offer additional advantages of improved productivity, reduced impact on agriculture and the environment and greater carbon capture. Algae have the greatest biomass yield potential of any current biomass production systems and are rapidly being developed as the nest generation biofuel systems. Algae are capable of producing 2-10 times more fuel per acre than any terrestrial crop system and with reduced environmental impact. In addition, algal production facilities can efficiently capture carbon dioxide from point sources, utilize nutrients and water from

municipal sewage treatment plants and feedlots and utilize carbohydrates from other biomass crops for efficient oil production with reduced greenhouse gas emissions compared to fermentation facilities (Sayre, 2010).
It is anticipated that advanced molecular engineering strategies will be used to develop improved microalgal strains with enhanced biomass and feedstock production capabilities as well as to express molecules used as feedstocks for "green" plastics and animal feed. Additional modifications include; incorporation of traits that allow the algae to tolerate better the environmental growth conditions encountered in cultivation systems (open ponds and closed photobioreactors). Crop protection including, controlling weedy algae, bacteria, viruses, and grazers is also a major target for genetic modification. Historically, environmental disturbances due to invasive algal blooms are typically transient and in most cases nutrient dependent. Since microalgae can be globally dispersed via aerosols considerations must be given to control measures to "contain" GMO algae. To address the possibility that genetically modified algae may have adverse environmental impacts various strategies are being developed to contain transgenic algae. Some of these strategies include; using algal strains that lack known sexual cycles to limit gene transfer to wild populations; engineering "terminator" genes into transgenic algae that will kill algae that escape production facilities, and the use of algal mutants that can only grow facultatively in controlled production facilities and not in the wild. At present, the regulatory framework for managing GMO algae is in development in the US. Assessing and managing the potential benefits and risks of GMO algae should be a critical part of policy development to ensure the greatest sustainable environmental benefits.

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3.5 Comparison of a weedy relative of sugarcane in two environments highlights traits leading to increased invasiveness

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A key issue that is addressed in regulation of GM crops is the risk of increased weediness. Most crops have closely related species that are both sexually compatible and considered weeds (Ellstrand et al., 1999). Consequently there is interest in understanding not only whether introduced traits may increase the weediness of crop plants, but also what would happen to the relative invasiveness if they were passed to wild relatives.
To better define the factors that promote weediness we have studied *S. spontaneum*, which is both a progenitor of modern sugarcane cultivars and an undomesticated, wild relative, in two environments. In the first environment, northern Australia, the species has been reported recently to occur in several locations (Bonnett et al., 2008) and whilst it has the potential to spread, it is significantly less invasive than in the second environment, Central Panama (Hooper et al., 2005) where it is the dominant species after rainforest has been cleared.
The sexual reproductive biology of *S. spontaneum* was studied in these environments to determine the timing of flowering and seed production, and what level of viable seed is produced. These measurements were taken weekly over multiple sites in Panama and monthly over 3 consecutive years in northern Australia.
The proportion of viable seed was measured by germination under optimal conditions of temperature (36^{o}C) and moisture. Separate experiments have demonstrated that the proportion of seed that germinates at temperatures below 30^{o}C is drastically reduced and that interruption of available moisture after germination has commenced, even for only a day or two, leads to much reduced germination for commercial sugarcane cultivars.
The results show that in Panama, flowering and consequently seed production occurs in the middle of the wet season when daily maximums are above 30^{o}C. There is a reasonably high level of viable seed produced (30% over

at least a six week period). Consequently, conditions are suited to successful germination and high potential for establishment and recruitment of new plants, as during the wet season is rains on most days.
By contrast in northern Australia, flowering and seed production is confined to the dry season when average temperatures are several degrees below 30°C and rainfall is infrequent. Consequently the viable seed that is produced is presented with very unfavourable conditions for germination. Reports of seed from commercial sugarcane germinating in these areas are very infrequent and localised.
These comparative studies have demonstrated differences in the key genotype and environmental interactions of *S. spontaneum* growing in Panama and northern Australia. The study has important implications for the assessment of the weediness potential of new GM traits. It is apparent that unintentional consequences of altering flowering time or response of seed germination to temperature and moisture could be undesirable as they could potentially lead to increased invasiveness and weediness.

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3.6 Ecological assessment of transgenic grasses: baseline studies of native and improved switchgrass for biofuel

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Perennial biofuel crops are being developed to reduce fossil fuel consumption and greenhouse gas emissions, while also promoting new markets for farmers. In 2005, the US Energy Policy Act authorized incentives to ensure production of 1 billion gallons of cellulosic ethanol annually by 2015, followed by the US Renewable Energy Initiative in 2006. Federal agencies have provided strong incentives for public and private investment in research on cellulosic biofuels, and vast areas of the US are being considered for cultivation of switchgrass (*Panicum virgatum*).
The objective of this presentation is to describe several experimental approaches for comparing the potential invasiveness of non-domesticated, perennial grasses with new transgenic traits, using switchgrass as a case study. Switchgrass (*Panicum virgatum*) is a US native C4 perennial grass that is cultivated for forage, soil conservation, landscaping, and prairie restoration, with planned rapid expansion to millions of acres to meet the demand for cellulosic biomass. Transgenic traits that have been examined in field trials include increased biomass, drought tolerance, increased nitrogen use efficiency, herbicide tolerance, and reduced lignin content. Current cultivars may not be invasive, but certain transgenic traits and massive increases in propagule pressure may lead to weed problems.
To address existing knowledge gaps, we are conducting research to document patterns of gene flow, population dynamics, and the relative competitive ability of cultivars vs. wild biotypes and crop-wild hybrids in a variety of locations and environmental conditions. Fitness traits of switchgrass biotypes will be measured in both cropping and non-cropping (marginal lands) ecosystems in Ohio and Iowa. We will use seed addition experiments and demographic matrix models to investigate how further cultivar improvement is likely to affect growth rates of feral

populations relative to wild ones. Life Table Response Experiments will allow us to quantify differences in population trajectories among wild vs. cultivar biotypes and determine the effects of local conditions on their potential for invasiveness.

To date, we have compared native and cultivated switchgrass varieties in a common garden experiment in Ohio for three growing seasons. Flowering times of these biotypes overlap extensively, indicating that crop-to-wild gene flow is possible. Wild plants were significantly shorter than the four cultivars tested, and produced fewer flowering shoots than two of the four cultivars. Our research will build on these preliminary comparisons to investigate the effects of competition on seedling recruitment and clonal competition in both marginal and non-marginal agronomic conditions in Ohio and Iowa. Ecological information about the potential for transgenic switchgrass to become weedy is urgently needed by USDA's Biotechnology Regulatory Services. Also, a basic understanding of pollen- and seed-mediated gene flow will help with designing requirements for field trials in which strict confinement is mandated.

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SESSION 4
GM insect developments and biosafety

4.1 Genetically modified insect regulatory decisions in the USA and how they were made

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Keywords
GMO, LMO, regulation, environmental assessment, EA, environmental impact statement, EIS, risk assessment, genetically modified insects, regulatory burden

TREATIES, LEGISLATION, AND REGULATION

The USA is not a signatory agent to the Cartagena Protocol on Biosafety (CPB) and uses existing legislation and agencies to regulate genetically modified organisms (GMO/LMO) under the Coordinated Framework for Regulation of Biotechnology (1986). The CPB is presently ratified by about 160 nations and affirms the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development. The precautionary approach or principle is followed to a lesser degree in the USA, and performance benefits of genetically modified mosquitoes (GMM) are an important consideration. The CPB is the most significant internationally ratified treaty for enacting LMO national legislation by its signatory countries. In the USA, LMOs are regulated, including risk assessment, by the US EPA for plants expressing pesticidal properties, by USDA, APHIS for LM plants under plant pest law, by USDA, Veterinary Services for livestock pests, and by Dept. Health and Human Services (DHHS), FDA for LM vertebrates that express properties affecting host biology or physiology analogous to drugs. Threatened and endangered species impact assessment is required under laws administered by the US Department of Interior. Federal regulatory decisions regarding LMO open release into the environment in the USA are subject to either Environmental Assessment (EA) or Environmental Impact Statements (EIS) under the National Environmental Policy Act (NEPA), except for the EPA registration process, which is equivalent. Although there is overlap of agency regulation, this system has proven to be effective and productive for nearly two decades and resulted in the first programmatic risk assessment and approved use of GM insects for use in sterile insect technique (SIT) crop protection. Importation and intrastate movement of LMOs that have not been previously regulated require permits and contained or confined testing to prevent unregulated LMO spread in the environment and transfer of genetic constructs into sexually compatible native species.

REGULATION INCLUDING RISK ASSESSMENT

Two Environmental Assessments (EA) and one Environmental Impact Statement (EIS) (2008) have been conducted for use of GM Mediterranean, Mexican, and Oriental fruit flies and the pink bollworm of cotton. The EAs and the EIS involved risk assessments. The EIS risk assessment addressed factors including potential hazards, short-term presence in the environment, phenotype of the modified organisms compared to the unmodified organism, biological fitness factors, and attributes and quantification of horizontal gene transfer. This EIS was an international precedent because it was the first EIS done on any LMO in the USA or elsewhere under comparable environmental laws of other countries. The Record of Decision (ROD) for the final EIS on Use of Genetically Engineered Fruit Flies and Pink Bollworm authorized the development and use of these genetically engineered insects in SIT for USDA/state cooperative plant pest eradication and control programs. The two EAs and EIS were published for public comment in the US Federal Register and many comments were received and published in the EIS Appendix E, which were considered in the decision-making process and final public documents. A description of the EA and EIS processes used in the USA is in Rose (2009). An application for a permit to test GMM *Aedes albopictus* and *aegypti* dengue vector mosquitoes has been submitted for contained testing in Florida and is

pending approval, which may involve conducting one or more EAs. The GMMs are intended for use in programs analogous to SIT. Final program implementation may also require an EIS comparable to that done previously for the cotton bollworm and fruit flies.

EA and/or EIS environmental documentation required in the Federal decision making process must provide for alternatives so that different approaches may be considered besides the preferred or proposed alternative with respective risk analyses. The ROD concluded that the alternative that involves integration of genetically engineered insects into programs is also the environmentally preferred alternative considering that the other alternatives can involve insecticide usage.

Risk assessment regulatory burden

Regulatory officials must be make aware that funding resources for permits, licenses, or registrations of many GM insects will be much less than for commercial transgenic crops because these insects will probably be developed by university affiliates or public agencies due to little commercial profit incentive, regardless their potential for the public good, which will limit ability to pay for complex risk assessment data development, registrations, permits, and long-term monitoring. In consideration of the significant costs that are incurred in the development of risk assessment data, regulatory agencies must define science-based, case-by-case specific requirements with a degree of practical parsimony rather than rely on the precautionary approach that can require data to address all theoretical risks.

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4.2 Confined Large Scale Field Trial of GM Aedes aegypti for Dengue Control in Mexico

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The advent of genetically modified mosquitoes (GMM) and their potential use to control vector-borne disease (VBD) transmission, represent a specific departure from the paradigm surrounding plant or seed transgenics. There are numerable issues which characterize this difference, and the present work will discuss some of the more visible of these, including the programmatic, technical, those pertaining to risk assessment, biosafety, and ethical, social and cultural considerations.

The most obvious of these differences is that control of VBDs is carried out by government institutions, and transgenic vectors would be licensed, purchased and applied by and for public health programs (PHP), and therefore without “apparent” corporate or special interests. However, a key feature of VBD in PHPs, at least in most of the developing world, is their lack of outreach, community dialogue, sector consultation or self-evaluation. Communities, decision makers, even technicians are largely unaware of the rationale behind VBD program strategies (even about the VBDs), and how, why, or what methods are chosen and applied. Hence, what could or

should be unbiased, equitable and evidence-based rationale, for strategy or product selection and use, will probably be plagued by the same problems currently observed in most VBD programs (i.e. currently for insecticides): lack of evidence- or technical-based decision making, or efficacy evaluation. Current decisions are in fact largely based on marketing and/or politically correct appearances despite lack of cost-efficacy (i.e. space fogging when more than 90% of households shut doors and windows when activities are conducted, or spraying of highly ecotoxic and ineffective insecticides from planes), or the program officer´s personal bias.

Risk assessment and biosafety aspects of any GMM will depend on the individual product, whether it is directed at vector or virus/parasite population replacement, and therefore uses a gene-drive system, or whether it targets mosquito population reduction, using a variety of strategies which reduce vector fitness, such as sterilization. The first transgenic *Aedes aegypti* strain in confined large cage trial in Mexico, OX3604C, has a novel transformation which mimics sterilization using a dominant lethal, requiring continuous release of male mosquitos to mate with wild-type females, and loss of remaining transgene through outcrossing after several generations. Neither the mosquitos nor the transgene persist in nature. Risk could result from nuisance biting from populations of female mosquitos released at the same time as males, which is avoided given that females of the OX3604C cannot fly or reproduce, and hence only male (non-biting) mosquitos would be released to the environment. Another key factor in regards to biodiversity is that *Aedes aegypti* is an isolated and does not interbreed with other species within (i.e. *Ae albopictus*) or outside the genus (Culiciidae). The species is highly selected for human domestic habitats (breeding and resting sites) and the females rely and prefer only a human blood source. Assessment of the risks to the environment (biodiversity) and human health, of controlled testing are important to balance in the face of increasing classic and hemorrhagic dengue cases in Mexico and the world.

The scientific and technical requirements to test a GMM in semi-field conditions is a complex endeavor, which is not just an extension of the genetic engineering in the laboratory, or the preliminary test in security insectaries under laboratory conditions. The concept of regulatory considerations usually conjure an image of government burocracy, and solid rationale to protect environment, biodiversity and human well-being, such as the regulatory procedures now quite well developed in Mexico by CIBIOGEM (www.cibiogem.gob.mx). However, it is clear that true authorization for research must be based on a wide and thorough consent from society. Without the authorization from all levels of society and within multiple community perspectives, the solutions offered and their acceptability will be less than sustainable, and eventually ineffective. Most government VBD control programs the world over are plagued and ineffective precisely by this omission.

There are many levels of research when studying novel biotechnology, from the confined through to operationalization and or scale-up. Society delegates to government officials and even to academicians certain attributes to develop information and solutions for all aspects of social structures. But this does not imply that society divests itself of the knowledge, that one ignores the urgent need to find sustainable solutions, that one discourages public debate, or avoids general consultation. Society cannot willingly participate in what it does not know and/or understand. Hence, it is important to position the topic of VBD control, and in this case dengue, within the society, for social dialogue. The current confined testing of OX3604C considered essential a proactive community engagement program, at all levels of society, and studies are currently focused on evaluating what information and knowledge is required to constitute effective authorization.

Given an integral and transparent approach to testing and potentially adopting new technology such as GMM, research conducted under controlled conditions with standardized procedures, can resolve unanswered questions and advance our knowledge regarding the potential of GM technologies to tip the balance for all strategies currently used to revert the increasing morbidity and mortality due to dengue in the world.

4.3 A risk analytical approach under conditions of limited knowledge and uncertainty to support biosafety and decision making

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Risk analyses of novel activities are usually faced with a lack of data or precedent upon which to calculate risk. A case in point is GM insects, and the proposal to release these raises particular regulatory challenges that must be addressed. In these cases expert judgement and opinion can be invaluable in assessing risk and is often the only source of information, but obtaining this has the potential to introduce issues which need to be anticipated and addressed. The reliance on expert testimony leaves the risk analysis process open to framing, context dependence and motivational bias. In addition, expert or stakeholder solicitation has often been found susceptible to a range of cognitive biases such as the format of the question(s), past experience, overconfidence, motivational bias, lack of independence, and cultural, political or philosophical context. This is especially the case with expert testimony, as such testimony can only reflect upon the knowledge invested in the experts, which is seldom all encompassing. As a consequence, it is difficult for any one individual to assess all potential hazards which may lead to some being overlooked or undervalued, particularly those where available knowledge and information is lacking. Despite this, there are a range of methods that can be utilized to improve any estimates provided by experts. Here we illustrate a risk analytical approach for GM insects which incorporates the processes of problem definition, hazard identification, fault tree analysis, community and technical elicitation methods, construction of Bayesian Belief Nets and methods to reduce uncertainty and attain consensus risk estimates, to assess a range of experts' perceptions of risk and areas where additional research and data could help resolve uncertainty. A key finding is how experts can be managed to improve the quality of the information that they provide to ensure that the risk analysis is as rigorous as possible.

4.4 Development pathways for release of genetically modified mosquitoes: experience from sterile insect technique, biological control and pollination

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The development of genetically modified mosquitoes (GMM) involves the incorporation of new genetic technologies to develop a vector control or disease management programme, aimed at reducing diseases such as malaria or dengue. GMM technologies include some directed at reducing populations of the vectors, and others that would replace the natural population with a population of vectors with genetic traits that reduce the transmission of disease without necessarily reducing the population of the vector species (James *et al*., 2010). These technologies have some similarities with existing methods of area-wide applications in which insects are released to achieve pest control or pollination. Control based on genetically induced sterility involves continual release of mass-reared insects to achieve eradication or suppression, which is similar to the conventional sterile insect technique (SIT) (often radiation induced) and augmentative biological control or pollination. Population replacement relies on the natural spread of a new population, which has some similarity to classical biological control (based on self-sustaining populations of introduced organisms). The development pathways for GMM technologies, such as inherited lethality or gene-drive transmission inhibition, are likely to fit into these pre-existing models, but with some additional issues that result from the novelty of genetic manipulation as a tool in insect control. There may also be further issues specific to vector control in public health which distinguish them

from agricultural applications, depending on whether the strategy is based on vector population reduction or replacement with individuals that cannot transmit disease.

GMM technologies may be viewed as products in some cases, for example they may be treated as pesticide alternatives, and they may be bought by the million as are sterile fruit flies (Quinlan *et al*., 2008). They could also be considered as a component of a service in some cases, for example pollination services are often bought on the basis of the proportion of fruit setting. There are precedents for both in biological control, in which augmentative release predators or parasites are sold by the box in much the same way as a conventional pesticide. In some cases they must be registered in a manner similar to pesticides, with documented safety and efficacy. In other cases, they are sold as a service, where a provider undertakes to deliver a specified level of performance and then monitors the need for biological control or pollination inputs and releases enough insects to continually meet the specification.

An important feature of GMM is that there is a significant intellectual property component of the product, whereas biocontrol and pollination products are naturally occurring. However, in many cases there may also be intellectual property inputs in the rearing and delivery mechanisms, for instance aerial release equipment for SIT or specialised hive boxes or shelters for pollinators or natural enemies.

In classical biological control, a decision for release by a regulator may be unconditional, if there is evidence of very high specificity to the target host. Decisions to introduce new organisms have some issues in common with GM insect introductions, horizontal gene flow through hybridisation, for example. In some cases where there are perceived risks, biological control agents may be given conditional release approval. This imposes specific requirements on a releasing agency to monitor and control or eliminate populations in the event of problems arising, and a requirement on the regulator to monitor capacity and performance by the agency, and to establish rules for decisions on implementing control measures. Conditional release would depend on the technical and logistical capacity to contain or eliminate a population once it is established in the wild. The option of conditional release may as a result demand that release agencies are sufficiently large and locally accountable to be held to these requirements, which are relatively open-ended. There is potential uncertainty for the regulating agency in the resources that may need to be dedicated to the case, depending on how the release progresses.

The product or service delivery options for a range of GMM are described and compared to experiences with SIT and various forms of biological control and pollination.

The scale of release of many insects in conventional control and pollination is massive. Billions of sterile fruit flies are released per week and for non-sterile uses, billions of pollinators and millions of predators and parasites are transported across national borders in Europe every year. The numbers involved have prompted at least one risk assessment, to determine if imported bumble bees (as pollinators) posed a genetic or other risk to the natural populations in the United Kingdom.

Much of the attention focussed on GMM has concerned appropriate risk assessment methods, but where risks from the technology are judged to be acceptable then deployment decisions will need cost benefit analyses and business plans which are similar to those used in other forms of area-wide control (Mumford, 2005; Quinlan *et al*., 2008).

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SESSION 5
Biosafety aspects of GM-based agronomic traits protecting against yield reduction due to abiotic stress

5.1 Back to the Future: Old tools to meet new challenges for regulators from abiotic stress tolerant GM crops.

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Background

The world's growing population, together with climate change, land degradation and declining soil fertility, places increasing pressures on agricultural efficiencies, both on existing cropping areas as well as ever more marginal land. Abiotic stress tolerant GM crops are one of several biotechnology approaches with potential to achieve those desired gains in productivity.

However, these types of GM crops offer regulators new challenges, including:

- traits that are traditionally associated with increased weediness
- the potential to stack different abiotic stress tolerance traits, together with traits from the current generation of insect resistant and herbicide tolerant GM crops
- the potential for different abiotic stress tolerance traits and mechanisms of action to be applied to a wide diversity of different species.

Australian regulatory experience

The Australian Gene Technology Regulator is responsible for authorising the release of GMOs into the Australian environment. The risk assessment tool used to inform regulatory decision-making for GM crops has been applied to wide range of organisms and traits, for both laboratory testing and environmental release. After nine years of use this model continues to work well with all types of GMO, including abiotic stress tolerant GM plants.

Field trials of abiotic stress tolerant GM plants approved in Australia include drought tolerant cotton, sugarcane, wheat and barley; waterlogging tolerant cotton; and nitrogen use efficient sugarcane, wheat and barley.

Risk assessments done by the Gene Technology Regulator did not identify any substantiative risks associated with these small scale releases, including risks to people and other organisms due to toxicity, or from gene transfer to other organisms. The main issue of interest with any proposed commercial release of these types of GM plant would be the potential for increased weediness.

Re-examining the risk assessment toolkit

The Australian environment includes large agricultural tracts that are subject to stresses from drought, salinity and low nitrogen levels. There have been considerable efforts in non-GM plant breeding to achieve tolerance to these abiotic stresses. In addition, there is extensive experience with introduced plants that have been a problem: more than 1200 major weeds have been identified. Knowledge from this experience has been an important cornerstone in developing robust weed risk assessment methodologies.

Australia has nationally established schemes for both border and post-border weed risk assessments of non-GM plants. These two methodologies are also applicable to weed risk assessments of GM crops. In particular, the national post-border scheme addresses the potential spread and persistence of a plant, adverse impacts and potential distribution to estimate relative risk in different land use types. It has been adapted to compare the weed risk of the non-GM parent organism with the GM plant, including sexually compatible relatives that may be recipients of the introduced genetic material. The information required and methodologies for a weed risk

assessment are the same for GM and non-GM plants and applies equally well to plants modified for tolerance to abiotic stress.

Conclusion

The quest to increase crop yields and reduce inputs on agricultural land that is ever more marginal is reflected by the growing number of applications to release GM plants with abiotic stress tolerance. Regulatory scrutiny of these GM crops has so far identified the potential of increased weediness as the primary risk that warrants most consideration. The Australian regulatory experience suggests that the standard environmental risk assessment model, incorporating weed risk assessment tools for non-GM plants, remain sufficient to meet the challenges from this new generation of GM crops.

5.2 Considerations for risk assessment: how special are genetically modified abiotic stress tolerant crops?

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Introduction

Some authors suggest that there might be specific issues associated with the introduction of crop plant which has been genetically modified (GM) to be tolerant to certain stresses. Yet, it is rarely clear on what grounds such suggestions are made and to what extend these issues are specific or different for such GM plants. We report on a systematic review of different information sources (scientific literature, patents, regulatory databases, regulatory files, risk assessments, opinions and guidance documents) conducted as part of a research project commissioned by the Dutch Advisory Board COGEM.

Background

Abiotic stress may come as drought, cold, heat, salinity and nutrient shortage. The application of GM technology has significantly broadened the possibilities to improve the abiotic stress tolerance of crops and the first applications, drought tolerant maize & frost tolerant eucalypt, are close to commercialisation. An inventory of advanced techniques indicated that, although very diverse strategies are pursued to obtain tolerant plants, some general observations could be made:

- Many strategies function by using or by influencing plant-own mechanisms.
- It is incorrect to talk in general terms of abiotic stress tolerance. Usually the protection mechanisms are specific for one or a combination of few well-defined stress factors.
- In some cases “cross-talk”, a phenomenon most likely related to signalling pathways sharing common components, between different stress responses needs to be considered and can lead to a complex phenotype.
- In order to avoid yield penalty, specific promoters are selected to limit effect in complex plant processes.
- Enhanced strategies act in addition to and/or in combination with existing plant response mechanisms.

Subsequently, it was questioned if an abiotic stress tolerance new trait would pose specific challenges for the conducting an environmental risk assessment (ERA). Like other genetically modified organisms, GM plants with an improved abiotic stress tolerance will be the subject of an ERA. In this report we focus on those aspects that are identified as new and/or different when compared to GM plants which have already been commercialized.

Environmental Risk Assessment Issues

In plant characterization, an essential part of problem formulation, we identified some issues that may require an adaptation of the existing evaluation concepts:

- In the first GM plants a sequence encoding a protein/enzyme that would perform a certain function was introduced. While some stress tolerance strategies follow the same approach, also other mode of actions, *e.g.* based on transcription factors, chaperon functions or gene silencing will be developed and will require new ways to characterize the modification.
- Traits can be more complex, in particular in cases where "crosstalk" is documented. While the trait may be complex, not all effects will be of agronomic or ecological relevance. Once the full scope of the effects is understood, the ERA can be conducted taking into account all documented changes.
- Product characterization is largely based on the comparative assessment. In the case of stress tolerance special care is required in identifying receiving environments (as these may be broadened based on the trait), for comparators and baselines (as these plants will by definition behave differently under stress exposure), for interaction with other stress factors and for variation that may be caused by controlled expression.

While recognizing that an ERA is conducted on a case-by-case basis, the following aspects have been highlighted as requiring specific attention:

- An abiotic stress tolerance creates an expectation of more vigorous plant. However, the competitive advantage for the modified plant may actually be very limited or not-existing. It must also be considered that tolerance traits usually come with a fitness cost, therefore requiring tightly controlled promoters. Most authors agree that changing a single characteristic is usually insufficient to turn a crop into a weed. The more general question on how many (and what kind of traits) need to be combined before a crop plant might be losing its domesticated nature remains to be answered.
- Some authors have addressed interspecific gene flow between stress tolerant GM crops and related species. While some conditions and characteristics may promote introgression, the effect of an introgressed trait will vary.
- Due to "cross-talk", some abiotic stress response mechanisms are shared with biotic stress signalling pathways. However, these responses are a reaction of the plant to the stress factor and are not targeted in a true sense against the stressor, biotic or abiotic.
- Many of the strategies that are deployed are derived from plants and/or are conserved in various plants that have a history of safe use. Some stress metabolites, *e.g.* phytoalexins, and stress proteins, *e.g.* chitinase used to improve biotic resistance, may be anti-nutritional and allergenic respectively. In addition, stress responses or metabolic pathways that are native to plant can produce undesired compounds.

Conclusions

This review concludes that while some issues may be specific for abiotic stress tolerance traits, they will be addressed by the existing ERA approaches.

5.3. Biosafety Assessment and Field Evaluation of Transgenic Forages for Enhanced Tolerance of Biotic and Abiotic Stresses

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Case studies on biosafety assessments and field evaluations of transgenic white clover, perennial and Italian ryegrass plants expressing candidate genes for tolerance of biotic and abiotic stresses, quality and yield enhancement are described.

White clover (*Trifolium repens* L.) is the major forage legume species sown in temperate dairy pastures in Australia, and a key pasture legume in temperate climates throughout the world. However, viruses such as alfalfa mosaic virus (AMV) costs Australian farmers approximately $AU 110 million annually with AMV incidence of up to 35 per cent in white clover dairy pastures. We have developed, selected, molecularly bred and field evaluated generations of transgenic AMV resistant white clover since 1998 to generate virus-resistant white clover synthetic varieties. Field testing and biosafety assessments of transgenic white clover were conducted first under the non-statutory Genetic Manipulation Advisory Committee (PR64, PR64X) and later licensed through the federally regulated *Gene Technology Act* 2000 (DIR 047/2003 and DIR089/2008) regimes.

White clover is an obligate cross-pollinated species; hence gene flow from transgenic to non-transgenic commercial or wild populations is possible. Understanding the dynamics of gene flow is therefore an important consideration for the commercial release of transgenic white clover. Biosafety research on AMV resistant transgenic white clover was undertaken in preparation for potential commercial release, including characterisation of transgene integration and expression; allergenicity assessment of the transgene products; levels and composition of nutrients and natural toxicants; comprehensive metabolic profiling for substantial equivalence assessment as well as gene flow studies in white clover under field conditions. GMO tracking and tracing methodologies were also developed for transgene detection in fresh white clover herbage and white clover derivatives, including dried herbage, hay, silage, seeds, pollen and honey.

Furthermore, transgenic white clover plants expressing a chimeric isopentenyl transferase gene for delayed leaf senescence to enhance herbage yield and quality as well as to improve drought tolerance were generated and field evaluated. Outcomes from extensive field evaluation of these transgenic white clover plants for enhanced herbage yield and yield stability, and associated biosafety research, including gene flow modelling using phenotypically distinct non-transgenic white clover genotypes, will be presented and discussed.

Perennial ryegrass (*Lolium perenne*) and Italian ryegrass (*L. multiflorum*) are the key forage grasses of world's temperate pastoral agriculture. We have developed, selected, molecularly bred and field evaluated transgenic perennial ryegrass plants (DIR 082/2007) with re-programmed photosynthetic cells for fructan biosynthesis to enhance biomass yield and quality, as well as transgenic Italian ryegrass plants for down-regulation of the main pollen allergens *Lolp 1* and *Lolp2*. Biosafety research on the transgenic ryegrass plants was undertaken, including characterisation of transgene integration and expression; assessment of pollen hypo-allergenicity; evaluation of herbage quality nutritional composition; as well as gene flow studies in perennial ryegrass under field conditions. Experimental designs for field trials with these transgenic forages and outcomes from extensive field evaluation of transgenic ryegrass plants for enhanced herbage yield and yield stability, and associated biosafety research, including gene flow modelling using genotypically distinct non-transgenic ryegrass genotypes, will be presented and discussed.

5.4 A Case Study for Yield and Stress Traits: the Challenges and Success Encountered in the Regulatory Strategy Employed fFor Drought Tolerant Corn, MON 87460.

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Drought stress is the most frequently encountered abiotic stress in global corn production. Therefore, improving crop tolerance to drought stress, as measured by reduced yield loss under stressful field conditions is an area of great interest to developers of more sustainable agricultural systems since it is a key component of the ability to produce the increasing food, feed, and fibers needs of humankind. Even though plant breeding efforts have increased drought tolerance over the years, an increased rate of genetic gain to abiotic stress tolerance is needed. Monsanto Company has developed drought tolerant corn MON 87460 that provides a yield benefit when yield is limited by water availability. Environmental risk assessment studies with MON 87460 were designed and initiated in 2006 in multiple locations, geographies and countries.

Review of the plant characterization and risk assessment data is currently in progress by Regulatory Agencies in several countries around the world. The scientific approach adopted for the phenotypic characterization of MON87460 and challenges associated with executing this plan will be presented. Particular emphasis will be given to specific hypotheses that were tested and how research trials were designed to provide data needed. Data will be presented from field trials conducted in the 2006 or 2007 growing seasons established in either the USA or Chile. This included sites that were well-watered only (i.e., no drought stress) and sites that had predefined well-watered and water-limited treatments (i.e., no stress and stress treatments). Data were used to assess pest potential (altered weediness characteristics and adverse environmental impact) and gene efficacy. All studies included MON 87460, which expresses the drought tolerant trait, and a conventional control that did not express the drought tolerant gene, but that had a similar genetic background. In the agronomic/phenotypic field trials, commercial corn hybrids were included to provide plants characteristics data that is common to corn and familiar to farmers.

The challenges that resulted from site selection and precise stress imposition requirements for predefined drought treatments will be discussed. Because untimely rainfall or water mismanagement can and does occur in the field, obtaining quality efficacy data is a significant challenge in the commercial development of MON 87460. In addition to agronomic/phenotypic data, studies designed to assess for altered tolerance to abiotic stressors (cold, salt, and heat), ability to survive outside of cultivation, and mode of action assessments as related to efficacy will be discussed. Data supports the conclusion that MON 87460 is not altered in weediness potential, does not have enhanced tolerance to abiotic stressors, and is not able to survive in areas not managed for agricultural production. As expected, MON 87460 can provide a yield benefit under predefined drought stress treatments. Under well-watered conditions, no consistent differences were found between MON 87460 and the control. Based on the data and information generated to date, it is concluded that MON 87460 is not likely to pose an increase in plant pest potential or to have an adverse environmental impact compared to conventional corn.

5.5 A genetic analysis of the introgression process from crops to wild relatives under abiotic stress conditions: the case of lettuce

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Keywords: GM environmental risk assessment, introgression, crop-wild hybrids, fitness, abiotic stress, quantitative trait loci

Introduction

One of the risks of the introduction of genetically modified crops into the environment is the introgression of transgenes from a crop to its wild relative (Tiedje *et al.* 1989, Snow *et al.* 2005). The probability of introgression will depend on the possibility of gene exchange between crop and wild species as well as on the specific effects of a transgene on the fitness of its carriers. Gene exchange appears feasible for many crop-wild combinations worldwide (reviewed by Ellstrand *et al.* 1999 and Ellstrand 2003). The outcome of crop-wild gene exchange will depend on the performance of hybrids under natural conditions (Hails and Morley 2005). First generation hybrids between crops and wild relatives in natural populations initially have an enormous linkage drag of crop genes into the recipient hybrid, with many crop genes that are likely to affect the fitness of the hybrids (Hails and Morley 2005). The net effect may be negative, for instance if crop genes reduce a plant's competitive ability under natural conditions, or positive, for instance if hybrids inherit additively positive traits from the crop or are more vigorous than both parents (Burke and Arnold 2001).

The performance of later generations of the hybrids (progeny obtained by selfing or by backcrossing to wild plants) will depend on the natural conditions under which the plants are growing (the environment), the genetic make-up of the plants, and the interaction between these two. The genetic make-up is the specific combination of wild and crop genomic blocks, which means for transgenes that it is not only the fitness effect of the transferred (trans)gene itself which counts, but also that of its surroundings in the crop genomic block. If crop genes that enhance the fitness of crop-wild hybrids are present near the transgene's insertion site, then the transgene may 'hitchhike' into the wild population (Stewart *et al.* 2003). In this case, transgenes with no apparent or even a mildly deleterious effect on plant fitness may increase in frequency due to their linkage with enhanced fitness genes. On the other hand, the spread of a transgene will be slowed down or may even be prevented if it is incorporated in a genomic region that incurs a fitness disadvantage or even sterility.

Under natural conditions, the hybrids will be subject to stress factors, both biotic (insects and diseases) and abiotic (drought, salinity, heat, cold, etc.). Introgression of biotic stress resistance genes has been tackled by various studies, e.g., for Bt-resistance (Mason *et al.* 2003, Vacher *et al.* 2004), but so far, abiotic stress has received little attention. The study of the alleviation of abiotic stress based on genetic modification has been focussing on model crops such as rice and Arabidopsis (Xiao *et al.* 2007, Klähn *et al.* 2009) and it will not be long before the successful genes are transferred to existing lines and released as cultivars (Zhang *et al.* 2000). For instance, a GM drought-tolerant maize is expected to be commercialized in the USA by 2012 (GMO Compass).

Therefore, we have initiated a study in which we follow the genetic process of introgression from crops to wild relatives using lettuce crop-wild hybrids growing under abiotic stress conditions as a model system. The two major factors determining the fate of the hybrids (their genetic make-up and the environment) are addressed by studying multiple generations of hybrids (F1S1, BC1 and BC2) under the major abiotic stress factors drought, salinity and nutrient deficiency.

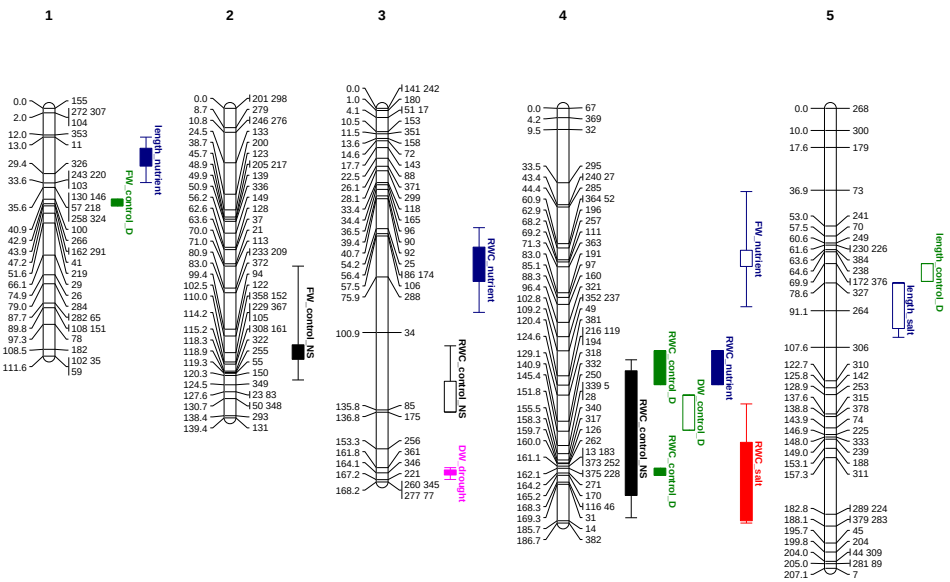
We present here the first results of this study, concerning the detection of QTLs for these abiotic stresses in the BC1 populations grown under controlled conditions (in a greenhouse). Follow-up research will concern later generations of backcrosses to the wild parent, and the interaction with the environment.

Materials and methods

We used a cross of one of the most abundant genotypes of *Lactuca serriola* in North West Europe (the "cont83" genotype in Van de Wiel *et al*. 2010) with *L. sativa*, cultivar Dynamite (see Hooftman *et al*. 2005), and created three hybrid populations, F1S1 (selfed F1), BC1 (F1 backcrossed to *L. serriola*) and BC2 (BC1 backcrossed to *L. serriola*). For this study, the BC1 populations was analyzed under greenhouse conditions. One hundred BC1 lines were grown under drought stress, salt (100 mM) stress, nutrient deficiency, or no stress (optimal conditions as control), each treatment applied separately. Under each treatment, each line was replicated 12 times. The plants were harvested at the rosette stage and biomass-related traits (shoot length, fresh weight, dry weight, absolute relative content) were measured. In parallel, the BC1 plants were genotyped with a custom-made 384 SNP marker array (an Illumina Golden gate assay, carried out in collaboration with R Michelmore and Leah McHale, UCDavis). A genetic linkage map was constructed using JoinMap 4 and QTL analysis was done using MapQTL 6.

Results and discussion

We constructed a genetic map based on 347 SNP markers scored successfully (Figure 1). Based on this map, a QTL analysis was performed on the phenotypic scores under drought, salinity and nutrient stress of the BC1 population and under controlled conditions in a greenhouse (Figure 1). The analysis produced 22 significant QTLs which were associated with the traits under stress conditions and 10 QTLs under control conditions. Out of the 22 stress-related QTLs, 15 were positively inherited from the crop, showing that lettuce crop-wild hybrids could receive traits from the crop that may be advantageous for their fitness under abiotic conditions. The QTLs were distributed across all lettuce linkage groups. However, for some linkage groups (LG 4, 7 and 9), QTLs were concentrated in specific genomic regions. These QTLs could be of pleiotropic nature (one QTL governing more than one trait) or just QTL hotspots. Whichever the case, such genomic regions could be of interest because they may enable us to identify chromosomal areas relevant for containment of transgenes. On one hand, a region with beneficial QTLs could be avoided as the transgene would have more chance of persistence through genetic hitchhiking. On the other hand, a region with non-beneficial or deleterious QTLs for the hybrids would be ideal for optimum containment of the transgene. However, at the present stage it is still too early to draw such conclusions as the analysis of the data for the later generations is still in progress, and the results need to be assessed under realistic field conditions as well.



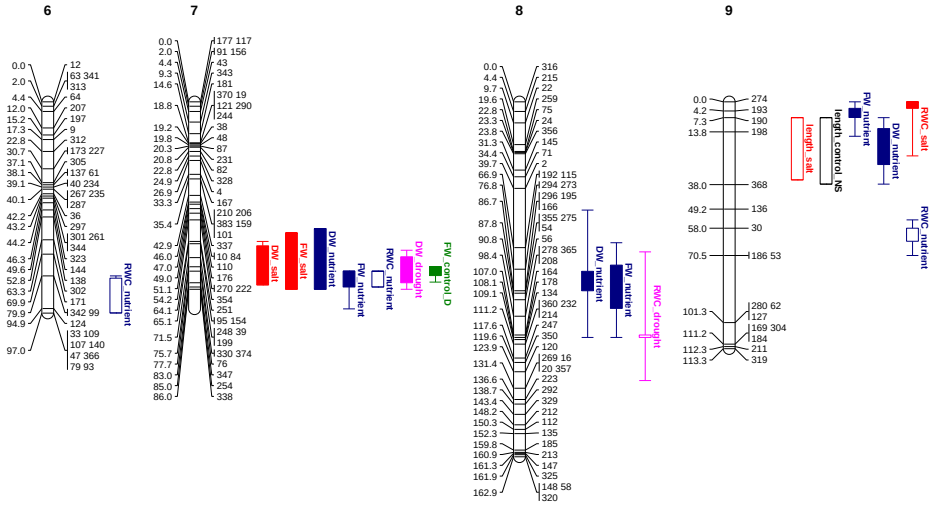


Figure 1: QTLs for drought, salinity and nutrient deficiency tolerance on the BC1 linkage map. FW: fresh weight, DW: dry weight, RWC: relative water content. Purple: drought conditions, green: control for the drought experiment, blue: nutrient deficiency conditions, red: salt (100 mM) conditions, black: control for salt and nutrient deficiency experiment. Solid boxes: QTLs from *L. sativa*, open boxes: QTLs from *L. serriola.*

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5.6 The introgression of abiotic stress tolerant alleles and the evolution of increased weediness or invasiveness: What we already know

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Questions regarding the biosafety of transgenic crops often concern the evolution of increased weediness or the evolution of increased invasiveness. Thus, biosafety research complements research in these two relatively undeveloped fields. The science of the evolution of invasiveness in plants is essentially in its infancy, and the science of weed evolution has had very limited attention for little more than a half century.
Theoretically, the introgression of an advantageous allele from one taxon to another should result in a selective sweep in the recipient population. Thus, all other things being equal, if an immigrant stress tolerance allele (abiotic or biotic, transgenic or not) confers a fitness benefit relative to the standing local variation, it should spread rapidly. Although studies documenting adaptive introgression are rapidly increasing (Arnold 2006, 2009), the superior fitness of an allele and its subsequent adaptive spread through a preexisting population does not necessarily translate into increased weediness or invasiveness.
Given that many crops, related weeds, and their wild relatives have occasionally swapped alleles for millennia (deWet and Harlan 1975, Jarvis and Hodgkin 1999, Ellstrand 2003), what do we already know about crop allele introgression and the evolution of more problematic plants? Surprisingly little, especially with regard to the role of abiotic stress tolerant alleles in such evolution. Here I consider three different sets of case studies of what we do know to draw some preliminary conclusions of the biosafety impacts of abiotic stress tolerant alleles.
First, I examine the evidence for what may well be the “worst case” scenarios. I consider those systems involving radical evolutionary change to invasiveness due to putative introgression of a trait for tolerance to abiotic stress. In particular, the worst invasive of natural ecosystems in the United Kingdom, *Rhododendron ponticum* (a Eurasian native) appears to have evolved invasiveness via introgressed cold tolerance after hybridization with the North American *R. catawbiense* (Milne and Abbott 2001).
Next, I use a recent review of the best documented cases of problematic weeds and invasives known to have evolved from crop ancestors (Ellstrand et al. 2010) to consider the relative importance of abiotic tolerance traits in tipping the evolutionary balance from crop to weed or invasive. Of 13 cases, ten involve evolution of weediness, two involve evolution of weediness and invasiveness, and one involves evolution of invasiveness. Six involve hybridization between a crop and a wild relative, an equal number involve the direct evolution of a novel problematic lineage, and one case involves hybridization between two well-differentiated crop lineages. The

majority of the novel problematic plants appear to have evolved invasiveness or weediness due to changes in aspects of their reproductive biology, such as changes in habit or the evolution of shattering. Only one appears to involve resistance to an abiotic stress.
Finally, I examine the ample experience already gained from field released crops that bear a transgene for abiotic stress, namely those crops that are engineered to tolerate herbicides. I focus on cases in which transgenic herbicide resistance has persisted for years in relatively unmanaged ecosystems: (1) wild plants descended from spontaneous transgenic canola x wild birdrape hybrids in Canada, (2) wild plants descended from spontaneous transgenic creeping bentgrass x wild bentgrass hybrids in the United States, and (3) persistent feral transgenic canola populations on roadsides in Japan, United States, and Canada (reviewed in Ellstrand 2011).
I conclude by considering what lessons can be gleaned from these studies in terms of biosafety assessment of transgenic based tolerance to abiotic stress. Not surprisingly, those lessons reveal that abiotic tolerance traits appear to be no more and no less of concern than prior transgenic traits and support continued use of the "case-by-case" approach.

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5.7 An overview of methods for measuring enhanced fitness and invasiveness, their environmental consequences and how they can be applied in Environmental Risk Assessment

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In this talk I will highlight various experiments and modelling efforts for testing the possibilities for escape, persistence and invasiveness of transgenes. I do not pretend to give a definitive answer on the question which methods should be used for conducting Environmental Risk Assessment (ERA) for abiotic stress transgenes. Though as an awareness session, I would like to overview several fitness interactions between environment and plants causing potential invasiveness that have been experimentally studied and modelled over the last 10 years.

Various overview studies have been published providing wider results and questions, which can provide further guidance (Ellstrand 2003; Andow & Zwahlen 2006; Chapman & Burke 2006; Chandler & Dunwell 2008). A compilation of such studies will provide a checklist of identified potential hazards which could be falsified using experimental data and model based explorations in time and space. It is such approach which is promoted in the proposed new EFSA guidance document on ERA of genetically modified plants (EFSA 2010).

The framework I am using here is based on this guidance, leading through a hierarchical set of questions which I group into the following: (i) indications within biological information of the species itself and it wild relatives for invasiveness (ii) potential persistence of the transgene in plants under agricultural conditions; (iii) potential persistence of the transgene outside the agricultural environment in ferals and (iv) potential persistence of the transgene outside the agricultural environment in closely related species.

Using biological information: asking whether a GM plant can grow and reproduce under current conditions and whether it can hybridize with sympatric plants (relatives or native ferals). This asks for basic but full life-cycle information about the species, reproduction and survival ability, both above and below ground (Hails & Morley 2005). Certainly the outcrossing pathway has been well described for many species (Ellstrand 2003; Armstrong *et al.* 2005). Hence the ability crops and wild relatives to hybridize is now generally accepted for most major crops and has been found even over long distances up to 21 km (Watrud *et al.* 2004; Reichman *et al.* 2006). However, this still could mean that de ERA for invasiveness could not potentially stop here: in the EU, corn has no wild relatives, which could make a difference in ERA in comparison to e.g., Northern America.

Testing for persistence of the transgene in plants under agricultural conditions, i.e., volunteers and gene flow to other crops. Studies such as Warwick *et al.* (2008) and D'Hertefeldt *et al.* (2008) found transgenic plants to persist for several generations in agricultural fields. Simulation models as Genesys (Colbach *et al.* 2001) show that in systems including various crops, planted in rotations, such volunteers could persist and potentially spread through a whole area within 10s of years (see also Claessen *et al.* 2005; Hooftman 2006). Even when volunteer populations do not become weedy, they could act as a genetic bridge between different cultivars and towards wild relatives (Hall *et al.* 2000; Reagon & Snow 2006). Transgenes have been found to persist for several years in and around agricultural fields through storage in volunteers and feral crop plants (Pessel *et al.* 2001; Pivard *et al.* 2008), potentially presenting such a hazard on a larger temporal scale. Appropriate estimation of the risks involved will vary much dependent on the quality of the life-cycle data available, in which it is important to gather all life-cycle data from repeated single experiments under non-optimal conditions (Hails & Morley 2005). Next to these simulation approaches, a wide range of population models has been developed over the last years, both matrix and integral models, which can be very useful for modelling population persistence: some examples are Bullock (1999); Thompson *et al.* (2003); Hall *et al.* (2006); Allainguillaume *et al.* (2006); Hooftman *et al.* (2007) and Damgaard & Kjaer (2009). The main question for volunteers to answer seems whether and how much populations decrease in the other crops in the years between growing the GM crop of interest.

Persistence of the transgene outside the agricultural environment. For feral populations, second generation transgenes, like biotic stress tolerance, could differ fundamentally from first generation transgenes. The stress situation likely exists outside the field as well, whereas e.g., an herbicide tolerance is less likely to provide an advantage unless the herbicide is present. However, the non-agricultural environments are likely much more diverse than within agricultural fields, making controlled-environment studies (greenhouse and lab) less applicable. Field-based studies seem necessary here to a range of conditions to test for environment x genotype interactions (Mercer *et al.* 2006; Ridley & Ellstrand 2009). Garnier & Lecomte (2006a; 2006b) developed a further set of matrix approaches that can be used, next to the ones mentioned above.

Potential persistence of the transgene outside the agricultural environment in closely related species. Few studies have shown a direct relationship of transgenes with fitness in early hybrid generations (e.g., Snow *et al.* 2003). More likely, the initial persistence of the transgene depends strongly on processes surrounding plant fitness, which are transgene related. However, certainly with transgenes for tolerance for abiotic stress, the transgene is likely to

be part of a, limited, package of genes which could provide a fitness advantage over time. So any test for differences between transgenic and non-transgenic races likely has to follow the same methods as most studies done on non-GM crops. Fitness analyses and the long-term persistence of such hybrids has been studied to a large extent in species such as *Lactuca* (Hooftman *et al.*, 2005; 2007), *Brassica* (Allainguillaume *et al.* 2006; Jorgensen *et al.* 2009), *Helianthus* (Reagon & Snow 2006; Rieseberg *et al.* 2003; 2007) and *Raphanus* (Snow *et al.* 2001; 2010; Campbell *et al.* 2006; 2009; Ridley & Ellstrand 2009). A wide variety of population models as mentioned above is available for analyses. However, fitness analyses are far from straightforward in hybrids in terms of experiments. Recent studies by Campbell *et al.* (2009) and Hooftman *et al.* (2010) have shown that experiments should take multiple generations under selective conditions, since sorting among genotypes could lead to better performance than assumed on early generation hybrids only. This can have clear effects on the population structure and the total presence of the transgene in populations, certainly under a relative invariable selection pressure as an adverse abiotic stress will provide (Hooftman *et al.* 2008). Also the competive ability of hybrids compared to the parental species might be altered (Hauser *et al.* 2003; Vacher *et al.* 2004) or even differ under varying levels of stress (Mercer *et al.* 2007). Recently genetic hitchhiking and position of transgenes on the genome have gained increasing attention (see Uwimana *et al.* in this session). Furthermore, recent evidence in *Brassica* points to large scale difference in introgression rate and fitness of hybrids between mitochondrial and nuclear DNA fragments (Allainguillaume *et al.* 2009).

In conclusion, all the experimental evidence on both transgenic and non-transgenic crop systems indicates that an integrative approach asking a hierarchical range of ecological questions is needed. Assuming the phenotypic change, caused by the transgene in the crop, will be similar in plants after escape is often proved plainly wrong. Many methods exist to get a better understanding. Abiotic stress presents certainly new challenges in environments and interactions to deal with, since it could provide substantial niche shifts of plant species. Regulators, industry and other stakeholders will have to decide how to acknowledge those interactions between environments, the transgene and plant biology in general. In this we will have to realize that GM plants might present other risks than their conventional counterparts or present new hazards not provided by such counterparts.

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5.8 Developing a framework to assess potential changes in fitness of GM plants: do stress tolerant plants need a new paradigm?

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Introduction

Some environmental concerns about GM plants relate to the potential persistence or invasiveness of the plant itself, or of its sexually compatible relatives, as a result of vertical gene flow within either agricultural or other production systems, or semi-natural and natural habitats. The potential adverse effects are of two main types. First, enhanced fitness of the GM plant or of transgenic (introgressed) relatives within production systems may make them more persistent, exacerbating weed problems that may need to be controlled by more complex weed control strategies, which themselves might cause environmental harm. Fitness is defined as the number of seeds (or propagules) produced per seed sown, and includes the whole life cycle of the plant (Crawley et al., 1993). In some studies, only components of fitness are measured – frequently this is fecundity (Snow et al.,1999). If other vital rates are unchanged (which is an assumption that should be substantiated), an increase in fecundity will lead to an increase in fitness. Fitness will vary depending upon the environmental context (including anthropogenic

influences like mowing), particularly upon the presence of inter and intra-specific competitors, herbivores and pathogens, and the abiotic conditions. The variation in fitness according to biotic and abiotic conditions is often referred to as the 'genotype by environment interaction'. It is therefore appropriate that an appropriate range of environmental conditions are considered.

Second, enhanced fitness of transgenic feral plants, or of transgenic (introgressed) wild relatives in semi-natural or natural habitats may reduce the diversity/abundance of valued flora and fauna. For instance, native plant species may be displaced, which in turn might affect species that use those plants as food, shelter, etc. Alternatively, and depending on which plant and which transgenes are involved, gene flow to wild relatives may decrease the fitness of hybrid offspring. If rates of gene flow are high, this may cause wild relatives to decline locally, or to become extinct (*e.g.* swarm effect, outbreeding depression).

Stress tolerant plants may exhibit enhanced fitness under certain conditions. For example, low temperature tolerance may result in the GM plant withstanding lower temperatures during its dormant or perennating stages than existing cultivars. Within the existing range of the crop species, the consequences might be that the modified plants can survive colder winter temperature than the conventional or get off to a better start in the post-winter warming (e.g. as volunteers). In addition, it may be that these plants can be grown beyond the original climatic range of conventional cultivars. We suggest that these hypotheses can be tested within the same framework as other GM plants. We present here a staged framework which specifies data requirements of differing levels, depending upon the GM traits under consideration, and discuss how stress tolerant plants fit in to this framework.

Problem formulation

Problem formulation should focus on the potential of a GM plant to be more persistent or invasive than conventional counterparts, and on the potential for gene flow to sexually compatible relatives whose hybrid offspring may become more weedy or invasive, or may suffer from outbreeding depression. To cover all relevant receiving environments of the GM plant and its sexually compatible relatives, problem formulation should address not only the conditions of the production system under which the GM plant will be grown, but also relevant semi-natural and natural habitats. It should also consider viable GM plant seeds or propagules spilled during import, transportation, storage, handling and processing that can lead to feral plants that colonize and invade ruderal, semi-natural and natural habitats.

The staged approach

The purpose of the staged approach is to ensure that relevant case-specific information is supplied to test hypotheses formulated in the problem formulation process, and that information requirements remain proportionate to the potential risk. We present ten questions broken down into four stages, which outline the issues to be addressed to estimate the likelihood of occurrence of adverse effects in ruderal, semi-natural and natural environments. Whether information is required for all stages or only for specific stages will depend upon the trait(s), plant species, the intended use, receiving environments under consideration, and the conclusions drawn from lower stages.

Information required for testing the hypotheses formulated in the problem formulation process can be species-, trait- or event-specific.

Species-specific background information is always required at the outset, describing the biology of the parental species including reproductive biology, survival, dispersal and cultivation characteristics in different environments. In addition, sexual compatibility with other cultivated or wild plants occurring in the EU, and the biology and ecology of these relatives should also be considered.

- **Stage 1** consists of providing *event-specific* information that enables the GM plant to be characterised, identifying intended and potential unintended differences between it and conventional counterparts. Information provided should be used to establish whether (i) the GM plant can grow, reproduce and

overwinter under EU conditions, and if so (ii) how its growth, reproduction and overwintering characteristics compare to its conventional counterpart. It is possible that GM traits may move to wild relatives through hybridisation within one growing season, even if the GM plant is unable to overwinter – consequently, it is important that the hybridisation potential described in the background information is considered before concluding on stage 1 information requirements. It should thus be considered whether sexual compatibility with any wild relatives is altered since this may result in differences in the rate of gene flow and the establishment of transgenes in other species.

Trait-specific information would be appropriate to address questions of changed fitness in stages 2 to 4, provided that potential unintended effects, resulting from the transformation process, have been shown not to alter the fitness of the GM plant compared to its conventional counterpart in stage 1.

- For plants that can either reproduce or overwinter in the EU, **stage 2** should explore whether the GM trait will enhance the potential for the GM plant to contribute to volunteer populations and persist in production systems, and if so, assess the potential environmental consequences. Stage 2 will also establish whether the GM plant will be capable of forming feral populations outside production systems, or whether the transgene can be transmitted to wild relatives independently of the existence of volunteers or ferals. Together these considerations allow an assessment of whether the transgene is likely to remain confined to production systems.
- If feral populations are likely and/or if hybridisation is plausible, then **stage 3** requires information to establish if GM traits will alter the fitness of feral plants, or of transgenic (introgressed) wild relatives. Since feral plants, or transgenic (introgressed) wild relatives may exhibit fitness differences across a wider range of environmental settings, stage 3 also consists of providing information that enables assessing the ability of these plants to occupy larger ecological niches than their conventional counterparts. It is possible that certain GM traits (for example abiotic stress tolerance) may enable the GM plant to expand its geographical range, and to grow in new areas close to wild relatives from which it was previously isolated, so the potential for this should be considered.
- Finally, if altered fitness or the ability to occupy new niches are demonstrated, **stage 4** information is needed to establish whether this will allow populations to increase and invade new communities or, alternatively if this will lead to populations of wild relatives to decline or become extinct. In both cases, the potential environmental consequences should be assessed.

The challenges posed by abiotic stress tolerant plants

Assessing the consequences of abiotic stress tolerance such as drought, salt or cold tolerance, poses challenges, as these factors are not easily manipulated in the field. In such cases the role of experiments in controlled environment chambers or possibly glasshouses may be particularly important. If substantial differences are found, the comparison may move to field trials – but whether differences are in fact found in the field depends on the run of weather. Temperatures cold enough to cause a difference in fitness for cold tolerant plants might only occur once every ten years or more and might not be experienced during the field trial. Nevertheless, a quantification of difference in controlled environments should be enough to estimate the likely behaviour in receiving environments, and then to include the possibility of weather-related survival in monitoring plans. The fitness parameters in all comparisons remain the same – germination, survival, growth, fecundity.

However, the results from controlled environments might also suggest that the modified plant would be able to survive and grow outside the existing range of the crop species – and indeed the purpose of the modification might be to extend the range of the crop. If this was the case, then locations outside the current range would be potential receiving environments for the modified crop. Here one challenge is that there is no comparator against normal practice. Modified and conventional varieties could be grown in a field trial outside the range, simply to demonstrate that the modified variety lives and conventional varieties die or at least suffer badly. To determine

the conditions under which the plants could potentially become invasive would require manipulative experiments, estimates of fitness, and an assessment of the consequences as described under stages 3 & 4. The principle difference is that the benchmark is whether fitness is greater than or less than 1 (and the circumstances under which this is the case) rather than a comparison with another genotype.

Conclusions

We have presented a framework for rigorously addressing issues around the persistence and invasiveness of GM plants, and considered how this applies to abiotic stress tolerant plants. Our conclusions are: a) the fitness parameters would be the same in all comparisons or selected from the same overarching set of fitness parameters; (b) growth chamber experiments in controlled conditions will be important for comparing modified and conventional plants to assess the impacts of the abiotic stress on life history parameters; (c) field trials over several years within the existing range of the crop species might not show any difference, depending on the weather; (d) field trials outside the current range, in regions where the factor (temperature, salinity) is likely to be effective, should indicate that the range of the crop can be increased and whether persistence and invasiveness in these environments were likely to result from growing the modified plant in the new range. This, however, will involve an absolute assessment rather than a relative comparison.

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SESSION 6
New applications of biotechnologies and their associated risk assessment issues

6.1. The Potential and the Problems of New Plant Biotechnologies

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Meeting the food, feed, fiber and fuel needs of a still-growing global population in a changing climate is one of the great challenges of the 21st century. The increasing use of science and technology in agriculture has made it possible to keep up with demand as the population expanded from roughly a billion two centuries ago to its present more than 7 billion. While the amount of land suitable for cultivation of the predominant food, feed and fiber crops has expanded only modestly over the past half century, agricultural intensification has continued to increase its productivity. However, the initial effects of climate change, a still-expanding human population of growing affluence, overexploitation of ocean fisheries and the new and growing demand for biofuels will require new approaches. Increasing awareness of the deleterious effects on natural ecosystems of excess nutrients from our current agricultural practices further demands greater attention to nutrient cycling within agricultural systems. Genetic modification of plants, animals and microorganisms can make substantial contributions to further intensification of agriculture, while reducing its adverse environmental impacts. However, regulatory barriers and ongoing public concerns present prohibitive impediments to the broader use of biological approaches.

6.2 Precise Genome Modification in Plants: EXZACT Technology and Outcomes

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EXZACT Precision Technology is a novel, versatile and effective toolkit for precise genome modification in plants. Using EXZACT, specific genes can be added, deleted or edited. EXZACT provides a means for acceleration of trait development and a powerful research tool for new gene/trait discovery.
EXZACT is based on the use of proprietary, designed enzymes known as zinc-finger nucleases (ZFNs). ZFNs are a fusion of a zinc finger-based DNA recognition domain and an endonuclease domain. The zinc finger domain can be engineered to recognize novel DNA sequences with high sequence-specificity. ZFNs induce a targeted double-strand break in the DNA of living cells, thereby invoking the cell's naturally occurring DNA repair mechanisms, including non-homologous end-joining and homology-directed repair. These repair mechanisms are highly conserved across eukaryotic systems including plant species. Non-homologous end-joining repair of double-stranded breaks leads to small insertions or deletions of DNA sequence at the cleavage site; because the break is induced at a precise, pre-specified sequence, this repair is a method for targeted disruption of native genes. Homology-directed repair uses a "donor" DNA as the template for repairing a break; donor molecules may contain novel transgenes, edits of the native gene, or a combination of sequences. By providing the cell with a donor DNA molecule that contains segments of sequence homology to the cleavage site, it is possible to drive highly efficient and precise insertion of that donor into the break.
The prerequisites for genome modification using EXZACT are DNA sequence and genome annotation. With the advent of new genome-sequencing technologies, this information is rapidly becoming available for multiple plant species including crops and vegetables. ZFNs can be utilized in any plant system that is amenable to DNA delivery, including crops, vegetables, turf and ornamentals. This broad flexibility, combined with robust tools and the

targeting precision of EXZACT technology, establishes a new paradigm in plant genetic manipulation for basic science and agricultural applications. Several examples of how this technology has been used will be provided.
The targeted approach of EXZACT Precision Technology represents a major departure from conventional methods in agricultural biotechnology. Current approaches to improving agricultural productivity rely on traditional or mutation breeding, or introduction of novel genes into the genomes of crop species by transformation. These approaches depend on sequence modifications at random locations in the genome (either naturally-occurring or induced) to create populations of individual plants that must then be screened by either forward- or reverse-genetics approaches to find the desired genetic changes. Because many plant genomes (corn is an example) are large, complex, highly redundant and/or polyploid, the size of any such population must be very large. In contrast, using EXZACT ZFNs, modifications such as deletions, insertions, edits or even gene excision can be efficiently induced at pre-specified loci or sequences in a plant genome and subsequently recovered very rapidly and easily using simple molecular screens. This allows investigators, for the first time, to routinely create changes in genes of interest. Targeted gene addition allows the investigator to identify a specific position, or locus, into which the transgene will be inserted. This approach mitigates the effect of random integration and reduces the number of transgenics that must be recovered and analyzed.
Looking ahead, agriculturally important traits that are limiting the global food supply, such as yield and drought resistance, are complex and require modification of multiple genes or even engineering of entire pathways. Engineering of such multigenic traits will depend on combining endogenous gene modifications and multiple transgenes. With new genome sequences being revealed, more potentially important genes and genetic elements are available for functional testing. Using EXZACT in research, genetic modifications can be generated with intent and assessed for impact, leading to the identification of new pathways, elements and control mechanisms. This will undoubtedly lead to discovery of novel genes and genetic elements that impact agronomic performance in ways we haven't predicted and new "modes-of-action" for engineering of traits. Furthermore, using ZFPs it is now feasible to generate multigenic transgenic traits at a single, pre-specified locus through stacking of multiple transgenes that will behave as a single genetic entity during introgression into several backgrounds. In combination with targeted modifications of endogenous genes, including editing of gene sequence to alter functionality or targeted gene disruption, the EXZACT approach makes it practical, for the first time, to engineer multigenic complex traits in a variety of plant species including commodity crops, vegetables, ornamentals, trees and biomass crops.

6.3 The use of RNAi for crop improvement: benefits and potential issues.

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Keywords: RNAi, gene silencing, crop improvement, off-target effects, GMO

Introduction

During the past decade, RNA interference (RNAi), also known as RNA silencing, has turned into a major area of interest in molecular biology and biomedical research, and for plant biologists, it has become a powerful tool for gene function studies and crop improvement. RNAi is a sequence-specific RNA degradation process triggered by double-stranded RNA (dsRNA), which leads to the suppression (or silencing) of gene expression. RNAi was initially discovered in plants as a defense mechanism against viruses (Waterhouse et al., 1998), but we now know that it is a conserved silencing mechanism present in all eukaryotes, including protozoa, plants and animals.
Different organisms have at least one RNaseIII-type endonuclease called Dicer, which processes endogenous non-coding, partially-complementary stem-loop transcripts into ~21-nt small RNAs (sRNAs) called micro RNAs (miRNAs). The miRNAs are then bound by effector proteins called Argonautes (AGOs) and will guide the sequence-specific

cleavage or translational inhibition of target messenger RNAs (mRNAs) involved in development and other processes. In plants, expansion of the gene families involved in RNA silencing enabled the evolution of other silencing pathways in addition to the miRNA pathway, with distinct, although related, roles in the defence against foreign nucleic acids like viruses, transposable elements and transgenes. In the model plant *Arabidopsis thaliana* there are 4 Dicer-like (DCL1 to DCL4) and 10 AGO (AGO1 to AGO10) genes, giving rise to at least five distinct silencing pathways (Margis et al., 2006; reviewed by Eamens et al., 2008). In the RNAi pathway, perfectly complementary dsRNA generated from exogenous invading sequences, like viruses, or from endogenous inverted-repeat (hairpin) transgenes can be degraded by DCL4, DCL2 and DCL3, giving rise to 21-, 22- and 24-nucleotide sRNAs, respectively, called short interfering RNAs (siRNAs) (Fusaro *et al.*, 2006). These siRNAs are then loaded into AGO in the RNA-induced silencing complex (RISC), guiding the cleavage of complementary sequences in invading viral RNAs or endogenous target mRNAs, which leads to the degradation of these RNAs.

The use of RNAi for crop improvements

Although large collections of sequence-indexed insertion and chemical mutants are available for Arabidopsis and rice that provide loss-of-function alleles for most annotated genes, this is not the case for the majority of important crops. RNA silencing becomes, then, a convenient approach for gene function studies and improvement of agronomic traits in species for which exhaustive mutant collections are not yet available.

The most widely used strategy for producing dsRNA in plants, and consequently triggering targeted RNAi, is the expression of both sense and antisense sequences, separated by an intron, under the same promoter. Upon transcription, these sequences form a hairpin RNA (hpRNA) molecule that triggers RNA silencing (Smith et al., 2000). This finding provided the design of hpRNA constructs (Wesley et al., 2001; http://www.pi.csiro.au/rnai/) that are now widely used for silencing genes in plants. More recently, the use of artificial miRNAs has also proven to be an efficient way of triggering RNA silencing in plants (Schwab et al., 2006).

Several examples of the use of this technology for commercial applications in plants can now be described (reviewed by Mansoor et al., 2006 and Eamens et al., 2008), with one of the first successful outcomes being the production of plants with viral resistance (reviewed by Fuchs and Gonsalves, 2007). Transgenic papaya with resistance to *Papaya ringspot virus* and transgenic potatoes resistant to *Potato leafroll virus* and PVY were among the first commercial releases and several others followed. Another example of plant protection against pest or pathogen attack conferred by RNAi is the transgenic maize resistant to western corn rootworm, where a hpRNA against a subunit of the midgut enzyme vacuolar ATPase gave protection against the insect's infestation at a level that was comparable to that provided by the *Bacillus thuringiensis* (Bt) toxin transgene (Baum et al., 2007). This approach might provide an alternative for Bt protection in crops like cotton and maize, in which insects are continuing to develop resistance to Bt. Resistance to plant parasitic nematodes, such as the root-knot and cyst nematodes, achieved by targeting plant genes that are involved with the infection process (Sindhu et al., 2009), or essential genes within the nematode (Fairbairn et al., 2007), might be another successful outcome of this technology, if the resistance holds true in the field.

Another area where RNAi technology has been widely used is metabolic engineering and fine-tuning of metabolic pathways. Some examples are the improvement of human health attributes of cottonseed oil by increasing stearic and oleic acid contents and decreasing palmitic acid, which can increase cholesterol levels (Liu et al., 2002); the modification of starch composition of wheat, by altering its amylose-amylopectin ratio, to reduce the incidence of cardiovascular disease and colon cancers (Regina et al., 2006); the production of coffee bean plants with reduced levels of caffeine by silencing a key gene in the caffeine biosynthetic pathway (Ogita et al., 2004); the reshaping of the morphine pathway in opium poppy (*Papaver somniferum*) to increase the yield of pharmaceutically significant compounds by using RNAi to concomitantly silence several genes and interfere with multiple steps in a complex biochemical pathway (Allen et al., 2004); the production of transgenic tomatoes with increased carotenoid and flavonoid content, by suppressing an endogenous photomorphogenesis regulatory gene using a fruit-specific

promoter (Davuluri et al., 2005); the fine-tuning of the biochemical pathway of the pigment anthocyanin in an ornamental plant to generate the blue rose (Katsumoto et al., 2007).

RNAi technology for crop improvement - potential issues

One major concern related to the use of RNAi for crop improvements is the risk of off-target silencing of siRNAs that might silence non-target genes. While this is specially true in animal systems, where off-target activity is a real issue (Jackson and Linsley, 2010), in plants, fewer cases have been reported so far (Xu et al., 2006), which are counterbalanced by other examples showing no off-target effects from hpRNAs (Li et al., 2004; Kumar et al., 2006; http://www.pi.csiro.au/rnai/benefits.htm). This might be explained by the differences in the mode of action of siRNAs between plants and animals. In both systems, siRNAs share full sequence complementarity with their targets, and trigger enzymatic cleavage of the perfectly matched mRNA at the nucleotide opposite position 10 of the siRNA guide strand. But in animals, siRNAs, like miRNAs, can also bind to partially complementary sequences that reside primarily in 3'UTR regions of mRNAs, through its seed region, which involves residues 2-8, and this could lead to translation inhibition of non-target mRNAs (Birmingham et al., 2006). Because only short regions of sequence complementarity are required for this type of off-target silencing, several transcripts could be potentially affected. In plants, both siRNAs and miRNAs seem to mainly act through cleavage of coding sequences with high degree of complementarity in the target mRNAs, hence minimizing the possibility of off-target effects (Bartel, 2004; Schwab et al., 2006). Another advantage of the use of RNAi in plants compared to animals is that, while plants allow the use of longer hpRNA constructs that give rise to a population of different siRNAs targeting the same gene, animal cells, to avoid immune responses, require the use of short hpRNAs, which will give rise to high levels of a single type of siRNA against a specific target (reviewed by Jackson and Linsley, 2010). By using longer hpRNAs, the concentration of each specific siRNA in the cell is lower, which might help minimize off-target effects (Praveen et al., 2010), specially in crops whose genomes still remain to be sequenced . For crop plants whose genomes have already been sequenced or EST collections are available, a more effective alternative to avoid off-target silencing might be the use of artificial miRNAs.

Perhaps the most important aspect when assessing the risks of using RNA silencing in plants is the change in expression levels of one or more components in a biochemical pathway, due to feedback-loop responses, after a specific gene in the pathway has been silenced (Liu et al., 2002; Allen et al., 2004). These changes are not off-target silencing effects.

Conclusion

The profound impact of RNAi technology on animal and plant research is unquestionable. The use of hpRNAs in plants has proven to be an effective tool for gene function studies, genetic engineering of pathogen resistance and manipulation of metabolic pathways to improve agronomic traits and to produce products of pharmaceutical value in plants. Next-generation sequencing, transcriptome profiling and bioinformatic analysis are now so advanced that they will provide detailed insights into any changes brought about by RNAi and should enable the risks of using this technology in any crop to be comprehensively assessed.

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6.4 Synthetic Genomics: Science and Governance

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Along with the potential of significant benefit, all new technologies raise societal concerns. For biotechnology generally, these are generally recognized as concerns about bioterrorism, laboratory safety, potential harms to the environment, distribution of benefits, and a constellation of ethics and religious views that need to be considered by policymakers in considering how to govern new biotechnologies.

Synthetic genomics is a group of technologies that allow for the construction of very long pieces of DNA, making the acquisition of gene-length or even genome-length DNA relatively straightforward. This DNA can then be used to construct viruses, and, as shown recently, bacterial cells. Viruses such as poliovirus, influenza virus, and the bacteriophage phiX-174 have been constructed using DNA ordered from commercial firms or even from stretches of DNA made in laboratories using DNA synthesizers. Synthetic genomics is currently focused on this kind of construction, starting with the genes and genomes of known organisms; it is related to synthetic biology, a field more focused on programming systems.

Recently, scientists at the J. Craig Venter Institute constructed the genome of the small bacterium *Mycoplasma mycoides*, transplanted that chromosome into a related cell, and drove replication of bacteria from the constructed genome (Gibson et al., 2010). This proof of concept will now allow scientists to focus on constructing bacteria with minimal genomes, that is, chromosomes reduced to allow for replication in laboratory settings with a minimum number of genes (Hutchison et al., 1999). Such minimal genomes will permit these bacteria to serve as platforms for producing useful products, such as pharmaceuticals, alternative fuels, or new industrial materials.

All of the societal concerns expressed about biotechnology, including the potential benefits, have been discussed with respect to synthetic genomics (Cho et al., 1999). Synthetic genomics may raise few new societal concerns, but any new concerns may be important for the governance of the new technology (Garfinkel et al., 2007). For those concerned about bioterrorism, synthetic genomics provides a new way to acquire pathogens, and potentially a simpler way to construct pathogens with increased virulence. Although the application of synthetic genomics means that access can no longer be physically limited, for now, the concerns are about a select few viruses that cannot be obtained any other way (1918 influenza, smallpox, Ebola).

Biosafety or laboratory safety concerns are mostly focused on the speed and scale that synthetic genomics brings to research, and some concerns about workers in the field who were not trained as microbiologists. The concerns are related to the type of microorganism being constructed, not the DNA itself.

Concerns about harm to the environment from accidental or planned releases of engineered microbes date to discussions at the Asilomar meetings in the mid-1970s. Synthetic DNA itself is not harmful in the environment; again, the concern is the nature of the engineered microbe itself, and speed and scale are the sources of concerns. Government agencies in several countries are currently reviewing several sets of regulation and guidance to understand whether they are sufficient to deal with the use of many new microbes in open environments.

In addition to the set of biosafety concerns, there are a number of concerns about risks that are of a non-physical nature. Distribution of benefits is a potential problem much larger than synthetic genomics or biotechnology. Ownership as defined by intellectual property rights, concentration of knowledge and resources in a small number of firms or institutes, and how and whether these resources should be shared, is a discussion surrounding many technologies. Finally, hubris (sometimes called “playing God”) is a concern that might be the key non-physical concern in synthetic genomics, even if it is not unique for synthetic genomics. While these concerns are not directly related to biosafety, policymakers will be looking at regulation and oversight for synthetic genomics over the next several years. As questions of regulation of product only (as done in the United States) as opposed to looking at process as well (as is the case in many countries), these non-physical concerns may become more relevant in risk assessment.

Although the publication of the construction of a synthetic cell has raised few if any new risks with respect to these concerns, it is important for scientists to be aware of the concerns and evaluate them as they design their research (Cho and Relman, 2010). Further, in addition to scientists situated at universities or companies, synthetic genomics and synthetic biology generally have attracted new practitioners, specifically amateurs who may be working at home, or in community-run laboratories. As these technologies develop, it will be critical for that community as well to be aware of these concerns, especially as related to biosafety and potential harms to the environment.

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6.5 Effects of transgene insertions and their detection using genomics tools

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Keywords: Gene insertion, metabolome, transcriptome, transgenic plants, unintended effects, variation

Introduction

The recent emergence of genomics and transgenic research together offers unprecedented opportunities for the expansion of our basic knowledge of plant genetics and physiology and consequently the commercialization of crops with novel traits. The complexity of the impacts of transgene insertions on the underlying processes that govern plant performance and composition needs to be understood and research in this area is now being defined. The initial results have provided insights into the effects of transgene insertions on plants and in some cases have revealed the amazing ability of plant processes to adapt to changes introduced by transgenesis. This understanding can eventually lead to the development of processes to reduce un-intended effects and optimize intended effects. The scientific knowledge will be useful for developing processes for biosafety assessments.

Materials and Methods

The use of profiling technologies is beginning to reveal the fluctuations and variations in the transcriptome, proteome and metabolome that exist as plants develop or interact with environmental factors such as stress. Furthermore, the redundancy that exists among gene family members has revealed the complexity of signal transduction networks that integrate and coordinate the orderly changes in gene activity that control plant development and composition. Arabidopsis provides a model system for genetics and allows for the large-scale controlled-environment studies needed to perform controlled studies.

Results and Conclusions

The phenotypic effects of a cloned gene depend on a number of factors, including the relationship of the gene to resident genes in the host genome and the design of the transformation vector. The desired phenotype may be modulated by potential pleiotropic effects or position effects resulting from unintended genetic interactions. There is no evidence for undefined or novel effects on the transcriptome resulting from the insertion of cloned DNA alone. The transcriptome variability that results from the insertion of transforming DNA alone is far below the variability found when wild-type plants respond to environmental stresses. Indeed, changes in gene expression that can be directly attributed to the insertion of a transformation vector with common selectable marker genes could not be found even under conditions of stress. Although certain transcription factors could create dramatic changes in plant morphology and transcriptomes others that regulate stress through fundamental hormone signal transduction had very little effect. The data indicates that knowledge of the transgene, transformation vector, genome insertion site and transformation processes are critical for predicting the kinds of effects that may be generated by the transgene inserted into a given plant. Transgenesis alone has no effect on the Arabidopsis transcriptome.

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3.9 Poster Sessions and Abstracts

Session 1: Regulation, Detection and Capacity Building Issues

1.1 Assessment of capacities for regulatory assays in Argentina

Betiana Parody[1], Viviana Pedroarias[1], Moisés Burachik[2], Carmen Vicien[3], Clara Rubinstein[4], Inés Kasulín[5], Gabriela Levitus[6], Miriam Yoshida[7], Esteban Hopp[1], Raúl Ríos[8], Dalia Lewi[9]

1.2 Identification of key elements for understanding and improving of decisions concerning the introduction of genetically modified crops in Colombia

Javier A. González-Cortés

1.3 New Challenge to the Old Paradigm: Environmental Safety, Socioeconomics and Public Awareness of GMOs in Brazil – The LAC-Biosafety Project context

Débora P. Paula1, Deise M. F. Capalbo2, André N. Dusi3, Jose Maria F. J da Silveira4, Olivia M. N. Arantes2,5, Eliana M.G. Fontes1, Edison R. Sujii1, Carmen S.S. Pires1

1.4 Uruguayan Genetically Engineered Crops Regulatory Framework

Alejandra Ferenczi

1.5 Global cultivation of genetically modified plants and detection strategies for non-authorized GMO in the European Union

Patrick Guertler, Esther Meissner , and Ulrich Busch

1.6 Coexistence of traditional and biotech crops in the Czech Republic: measurements and control

Ovesna J., Pouchová V., Kucera L., Hodek J., Demnerova K.

1.7 Regulatory Framework for Genetically Modified Organisms in Taiwan

Wen-Sen Chu

1.8 EFSA's work on the safety assessment of genetically modified organisms

Yann Devos, Jaime Aguilera, Anna Christodoulidou, Zoltan Diveki, Christina Ehlert, Antonio Fernandez Dumont, Andrea Germini, Ana Gomes, Karine Lheurex, Yi Liu, Sylvie Mestdagh, Claudia Paoletti, Nancy Podevin, Reinhilde Schoonjans, Ellen Van Haver, Elisabeth Waigmann, Per Bergman

1.9 Assessing intangible risks: Including cultural and social effects in a risk assessment

Rachel Helson and Andrea McNeill,

1.10 Comunication and public awareness within the context of a research project on biosafety in Latin America.

Olivia M. N. Arantes1,4; Dilaine R. S. Schneider1,5; Deise M. F. Capalbo1; Débora P. Paula2; Jose Maria F. J. da Silveira3; Izaias de C. Borges3 ; Nilce C. Gattaz1; Eliana S. Lima1; Maria Fernanda D. Avidos2.

1.11 **Regulatory dossier of a public sector-developed GMO product in South Africa**
Lynelle van Emmenes1, Inge Gazendam1, Hector Quemada2, Muffy Koch3 and Johan Brink4

1.12 **Sustainable Governance of Biotechnology and Risk Communication Strategies in Asian Developing Countries: the Case Study of Biotech Papaya in the Philippines**
Chia-Hsin Chen1*, Suneetha M. Subramanian1, Kazuo N. Watanabe1, 2

1.13 **Public perception vs. quantitative risk assessment and impacts on policy decisions related to agricultural biotechnology**.
Ania M. Wieczorek, Neal K. Akatsuka (2), and Mark G. Wright (3)

1.14 **Environmental impacts of GM crops: Defining damage criteria for decision-making**
Olivier Sanvido, Jörg Romeis and Franz Bigler

1.15 **Food safety assessment of PAT gene product in herbicide resistant pepper**
Hyun-Suk Cho*, Si-Myung Lee, Hyo-Jin Kim, Jae-Kwang Kim and Tae-Hun Ryu and Seok-Cheol Suh

1.16 **Development of support systems for risk assessment of GM crops**
Kathy Messens[1]

1.17 **BioOK: Risk assessment on transgenic plants combining effective new and traditional methods in a computerized decision support system**
Kerstin Schmidt[2,] Jörg Schmidtke[2], Christine Höflich[1], Inge Broer[1]

1.18 **A Risk Analysis for the T25 GM corn event released in Brazil**
Sarah Zanon Agapito-Tenfen 1, Brigitta Kurenbach 2, Camilo Rodriguez-Beltran, Jack Heinemann 2 and Rubens Onofre Nodari 1

1.19 **Biosafety Status and Identified Gaps in Knowledge & Expertise in sub-Saharan Africa**
Dennis Ndolo1, Lilian Nfor1, Sylvia Uzochukwu1, Marianela Araya Quesada2, Francesca Farolfi2, Decio Ripandelli2 and Wendy Craig2

1.20 **Biosafety of Genetically Modified Organisms in the Latin America and Caribbean Region: An Opportunity for Strategic Capacity Building**
Marianela Araya Quesada, Decio Ripandelli and Wendy Craig

1.21 **ELSI Associated Capacity-Building Activities for Biosafety: A Case Study in Taiwan**
Chia-Hsin Chen

1.22 **E-learning Master in Biosafety in Plant Biotechnology: a unique biosafety training network**
George Tzotzos †, Magnus Bosse†, Sylvia Burssens*, Bruno Mezzetti**,

1.23 **Biosafety capacity building: experiences and challenges**
Ine Pertry,

1.24 **Validation of detection methods for genetically modified soybeans, maize and cotton newly approved in Korea**
Mi Gyeong Kim, Soon Keun Hong, Il Hyun Kang, Tae Sung Kim, Hye Seon Nam, Ae Rie Moon, Kyoung Sik Park, Ja Young Jung, Hae Jung Yoon

1.25 **GMO Detection as a biosafety tool: activities of the GMO Detection Lab of the National Institute for Agricultural Technology in Argentina (INTA).**
Pedroarias, V; Medina M; Fretes V; Pelissier G; Hopp HE; Fernández P; Martinez MC.

Poster session 2: Environmental Interactions

2.1 **Does Bt maize have an effect on arthropod biodiversity? : a case study from South Africa.**
Truter, J., Van Hamburg, H. & Van den Berg, J.

2.2 **Effect of cry 1ab protein from Bt maize (MON810) on the biology of *Chrysoperla pudica* (neuroptera: chrysopidae)**
R. Keulder & J. Van den Berg

2.3 **Low levels of refuge compliance contributed to the evolution and spread of *cry 1ab* resistance in the maize stem borer, *Busseola fusca* (lepidoptera: noctuidae) in South Africa.**
Kruger, M.1 and Van Rensburg, J.B.J.2 and Van den Berg, J.1

2.4 **Effects of genetically modified maize (MON810) and its residues on the diversity of microorganisms in the rhizosphere bulk soil: A glasshouse study**
Puta U1, Muchaonyerwa P2* and Bradley G1

2.5 **The effect of Bt maize on non-target soil pests of maize seedlings in South Africa**
Erasmus, A.1,2. and Van den Berg, J.2.

2.6 **Indirect exposure of the maize stem borer parasitoid, *Sturmiopsis parasitica* (Tachinidae), to Bt maize: an case study from South Africa.**
Erasmus, A.1,2. and Van den Berg, J.2.

2.7 **Using an ecological model to improve risk assessments for Bt maize: an example from South Africa.**
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2.8 **Identifying Patterns in Non-target Arthropod Communities using the Principle Response Curve (PRC) Method.**
Miles D. Lepping and Nicholas P. Storer

2.9 **Levels of Cry1Ab in Bt maize, in different tissues and between plants, at different growth stages**
Chris Viljoen & Grant Richardson

2.10 **Cry1Ac levels on Bt cotton leaves according to the storage condition and cotton phenological growth stages.**

Débora P. Paula,*, Renata V. Timbó, Carmen S. S. Pires, Eliana M. G. Fontes, Edison R. Sujii

2.11 **Field evaluation of Bt Cotton effects on non-target pests**
Edison Ryoiti Sujii*, Pedro Henrique Brum Togni, Paulina de Araújo Ribeiro, Thiara de Almeida. Bernardes, Kelly R. Cavalcante, Paloma Virgínia G. N. Milane, Carmen Silvia Soares Pires, Eliana Maria Gouveia Fontes, Débora Pires de Paula

2.12 **Diversity of arthropods in genetically modified BR and RR cotton crops in northern Santa Fe**
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Steffen Hagenbucher1, Dawn M Olson2, John Ruberson3, Felix L Wäckers4 and Jörg Romeis1

2.14 **Assessing the impact of insecticidal GM crops on non-target arthropods: characterizing exposure levels**
Michael Meissle & Jörg Romeis

2.15 **Laboratory toxicity studies demonstrate no adverse effects of Cry3Bb1 and Cry1Ab to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae)**
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Simone von Burg1, Fernando Alvarez2, Frank van Veen3 and Jörg Romeis2

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J.C. Tian, Y. Chen, G. Y. Ye

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2.21 **Transgenic *cry*1Ab rice "KMD2" can not result in the outbreak of its non-target herbivore the brown planthopper *Nilaparvata lugens***
Y. Chen, J. C. Tian, G. Y. Ye

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Stephan Härtel1, Harmen P. Hendriksma1, Ingolf Steffan-Dewenter1

2.23 **Honeybee maize pollen foraging in differently structured landscapes: Colony and single worker exposure to an important GM crop.**
Stephan Härtel1, Nadja Danner2, Andreas Schneider2, Ingolf Steffan-Dewenter1

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V.G.Lebedev(1), A.G.Mannapov(2), A.A.M.Abdulla(2)

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Hans Hoenicka, Denise Lehnhardt, Matthias Fladung

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Cristina Vettori*, Matthias Fladung**,

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Geoffrey Ecker1, Collin Ahrens1, Jinwon Chung2, Thomas Meyer2, and Carol Auer1*

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Armin Spök

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Heidrun Bückmann, Christian Kobbe, Alexandra Hüsken

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Chris Viljoen1 & Lukeshni Chetty2

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Hong-Gyu Kang1, Tae-Woong Bae1, Ok-Chul Chung2, Tae-Gun Cho1, Pyung Ok Lim1,3, Hyo-Yeon Lee1,2

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Yann Devos, Anna Christodoulidou, Christina Ehlert, Antonio Fernandez Dumont, Yi Liu, Sylvie Mestdagh, Nancy Podevin, Reinhilde Schoonjans, Elisabeth Waigmann, Per Bergman

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T. H. Ant1,2, M. Koukidou1, S. A. Morgan1, L. Alphey1

4.3 **Transgenic Mosquitoes: Risk and Benefits.**
Bianca Burini Kojin1, André Wilke1, Camilla Beech2, Mauro T. Marrelli3, Margareth Lara Capurro 1

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Margareth L Capurro1, Camilla Beech2, Megan Quinlan3, Luke Alphey2, Vicente Bayard4, Janine Ramsey Willoquet 5, Mauro Marrelli6, Kenneth Ombongi7, Rachel Reuben8, Pattamaporn Kittayapong9 & John Mumford3

4.6 **Transgenic Mosquitoes: Best Practice Guidance. Data Requirements for Field Release and Monitoring.**
Jon Knight3, Jack Rhodes1, Andrew McKemey2, M. Megan Quinlan1, Camilla Beech2, John Mumford1, Janine Ramsey Willoquet3, Margareth L Capurro4, Pattamaporn Kittayapong5, Luke Alphey2 Mauro Marrelli6, Vicente Bayard7, Kenneth Ombongi8 & Rachel Reuben9

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Alejandra Ferenczi1 and Rebecca Grumet2

5.2 **Introgression of crop (trans-)genes into wild relatives: Environment specific effects and stress sensitivity in Lactuca.**
Y. Hartman, B. Uwimana1, D.A.P. Hooftman, and P.H. van Tiende

5.3 **Differential physiological responses of transgenic a maize variety compared to its non-transgenic**

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Vinicius Vilperte1, Aline Mabel Rosa1, Vítor Gabriel Ambrosini1, Rafael Benevenuto1, Sarah Zanon Agapito-Tenfen1, Haroldo Tavares Elias2, Rubens Onofre Nodari1

5.4 **Seed longevity and dormancy of transgenic rice and chili pepper lines .**
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5.5 **Potential of crop-to-wild gene flow in soybean in Korea**
Sung-Dug Oh1, Soo-In Sohn1, Ki-Jong Lee1, Myeong-Rae Cho2, Hyeong-Jin Baek3, Jong Sug Park1, Seok-Cheol Suh1, Tae-Hun Ryu1.

5.6 **Reflections on a Gene Flow Study.**
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5.7 **Study of glyphosate impact on the genomic evolution of the target species Johnsongrass (*Sorghum halepense*) in Argentina**
Fernández, L1; Distéfano, A.J1,2; de Haro2, L., Hopp, H.E1,2,Tosto, D1,2.

5.8 **Applicability of Weed Risk Assessment system for host crops as first step of evaluating impacts of genetically modified crops on biodiversity in Japan**
Yoshimura Y.1, Mizuguti A.1, Nishida T.2, Ohigashi K.1, and Matsuo K.1

Poster Session 6 : New Technologies and Implications

6.1 **Risk mitigating genetic modification technologies: will they impact on risk assessment strategies and regulation?**
Anita Burger, James Rhodes, Jill Johns, Nwabisa Mehlomakulu and Hennie Groenewald

6.2 **Stabilizing transgene expression by using S/MAR elements.**
Antje Dietz-Pfeilstetter

6.3 **Regeneration and genetic transformation via organogenesis of different varieties of *Vitis vinifera* and *Prunus persica.***
Daniela Palma1, Luca Girolomini2, Silvia Sabbadini2 Tiziana Pandolfini1,Oriano Navacchi3, Bruno Mezzetti2

6.4 **The toxicology of interfering RNAs from transgenic plants: Perfectly duplexed dsRNAs over 30bps in length are specific but sequence-independent inducers of the mammalian interferon response.**
J.R. Latham and A.K. Wilson

6.5 **Organisms developed using oligonucleotide-mediated mutagenesis: challenges for regulation and enforcement in Europe.**
Katia Pauwels1, Sylvia Broeders2, Didier Breyer1, Nancy Roossens2, Philippe Herman1

6.6 **Molecular method development for the detection of genetically modified pollen in bio-aerosol.**

Silvia Folloni, Bojan Rajcevic, Maddalena Querci, Marc Van den Bulcke and Guy Van den Eede

6.7 Greenhouse and field cultivations of potato expressing different antigens

Heike Mikschofsky[1], Jörg Schmidtke[2], Kerstin Schmidt[2], Inge Broer[1]

POSTER SESSION 1

Regulation, Detection and Capacity Building Issues

1.1 Assessment of capacities for regulatory assays in Argentina

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Since 1996 Argentina is one of the leading GM crop producing countries and has also been a pioneer in Latin America on the Biosafety assessment of GMOs. The National Biosafety Framework was implemented as early as 1991 and therefore has accumulated solid experience, not only in the assessment a great number of events, but also in assisting other countries in the Region to implement their own biosafety systems.

In addition, several public academic institutions in Argentina, such as INTA, CONICET and some universities have been developing a number of GMOs since the late 1980's, but none of them are yet on the market. Moreover, only very few of them have just recently begun to transit the initial stages of biosafety trials and laboratory assays, particularly by means of commercial agreements with the private sector (biotechnology companies). The complete biosafety package to be submitted for the regulatory assessment of a GMO for commercial authorization requires not only enormous amounts of money for all the necessary assays and trials but also the coordinated work of a great number of laboratories, facilities and professionals highly specialized in regulatory matters.

Beyond the budgetary reasons, another main cause for the current delay in reaching the market of biotechnology products developed at public institutions might be the lack of awareness about the availability of the necessary capacities coordinated in order to perform all the assays required for the complete biosafety assessment in our country.

With regard to this issue, we observe that Argentina has a high level of intellectual resources and research scientists at many prestigious public universities and institutions, which are potentially capable of performing many of the laboratory assays, and also ideal conditions for field trials on many different agro-ecological regions and a tradition in human resources highly trained in agricultural practices.

The question is then whether the proven scientific capacities and agricultural expertise potentially available in Argentina could really be efficiently directed to perform regulatory assays and trials under high international standards (such as the GLP of OECD) and methodologies. That is, if a complete dossier containing all of the regulatory studies could be developed in Argentina so that a new GM variety wholly developed in our country can be commercially approved and accepted for export to the most demanding markets (such as the EU, Japan, etc.).

With the purpose of answering this question, INTA is carrying out a project intended to conduct a survey on the local availability of the needed capacities to perform biosafety assays and trials according to the international standards, to train highly specialized professionals on these matters, and consequently to build a

platform of biosafety resources (databases for professionals, facilities, protocols) to be made available to scientists in the public sector, thus starting the development and strengthening of local capacities for GMO risk/biosafety assessments. This project entails different areas of scientific knowledge and intends to involve scientists working on GMO research programmes and making them aware of the regulatory processes, allowing them to achieve the appropriate biosafety standards required by the national and international regulations.

Abbreviations

INTA = National Institute of Agricultural Technology
CONICET = National Council of Scientific and Technological Research
GLP = Good Laboratory Practices
OECD = Organisation for Economic Co-operation and Development

1.2 Identification of key elements for the understanding and improving of decisions concerning the introduction of genetically modified crops in Colombia

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Current decisions involving the authorization of the sowing of genetically modified (GM) cotton in Colombia have been taken improperly, especially if we take into account that one of the country's priorities, as stated by governments, is to guarantee that the objects of public interest, like biological diversity and human health, are not to be exposed to any risks.

Two flaws, that are key elements in the GM's introduction decision making process, were identified in our research. The first one relates to the variables included in the risk assessment studies, which are referred in the resolutions expedited by the competent authority, for every crop in different zones. Given that the variables or issues studied in one case are not always included in the remaining ones, we analyze some of the implications that this kind of unsystematic procedures can have over human health and biological diversity. The second flaw refers to the ambiguous, imprecise and irregular use of several terms present in the resolutions that approve the commercial sowing of GM cotton. We approach this problem by examining the use of several ill-defined categories of land classification that the competent authority has granted to one specific geographic zone.

Given that the decisions related to the use of technologies like GM cotton seeds would have to be made in ways that biological diversity and human health be preserved, we conclude, by means of the flaws found for Colombia, that epistemic and normative coherence should be reconsidered in order to guide procedures, such as risk assessment studies, that help to inform decisions in the country.

1.3 New Challenge to the Old Paradigm: Environmental Safety, Socioeconomics and Public Awareness of GMOs in Brazil – The LAC-Biosafety Project context

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Brazil has been continuously investing in GM products to improve the supply in farm sector for 20 years or more. The regulatory biosafety issues regarding the use of GM crops are covered by the National Biosafety Law since its implementation in 1995, and reviewed in 2005. A lot of initiatives involving GMO biosafety has emerged mainly

focused on the risk assessment - environment and food safety - but very little has been done on risk communication, public awareness and on socioeconomic impact. In 2008 the Latin American project "*Multi-country Capacity Building for Compliance with the Cartagena Protocol on Biosafety*" (LAC-Biosafety, www.lacbiosafety.org) was launched and Brazil is part of this initiative in cooperation with Colombia, Costa Rica and Peru. All those countries are mega-biodiverse and are centre of origin and/or diversity of important agricultural crops. Besides, these countries are Parties of the Cartagena Protocol on Biosafety (CPB). The LAC-Biosafety is financially supported by GEF through World Bank, and is under general coordination of the International Center for Tropical Agriculture - CIAT. This project aims to strengthen technical capacity in scientific knowledge generation and in communication in compliance with the CPB. It is strategically structured in two components: one technical-scientific (gene flow, geographic information system - GIS, non-target organisms and socio-economy); and another for communication and public perception.

In Brazil the project is organized as a net of specialists from universities and from the Brazilian Agricultural Research Corporation (Embrapa), and the focal points in Ministries (mainly Agriculture, Environment, and Science and Technology), under the national coordination of Embrapa Environment.

In Brazil, the selected case studies for Brazil were cassava, cotton, maize and potato. For gene flow aspects the study will concentrate in an *ex-ante* evaluation of cassava (*Manihot esculenta,* Euphorbiaceae*),* as a model, since Brazil has no GM cassava under development nowadays. The results expected will support any risk analysis for a GM cassava as it will generate knowledge on distribution of varieties over the country (Brazil is considered the center of origin for cassava species).

Aspects about cassava biological reproduction among the close phylogenetically wild relatives will be studied, and maps of the cassava species and wild relatives geographical distribution will be draw. The determination of: visiting insects and pollinating agents; pollen viability and conservation; development of inter-specific hybrids between cultivated varieties and wild cassava; and of voluntary plants in commercial plantations of cassava, will be verified.

The study about effects on non-target organisms will focus on current protocols and methodologies for evaluating effects of GM cotton, maize and potato on such organisms. The studies will compare and critically analyze such methods to explore opportunities for regional standardization. The activities will comprise: the collection of baseline literature about risk endpoints for relevant ecological functional groups or ecosystem services; the collection and comparison of dossiers of GM released events, in order to detect information gaps that need further research; the organization of panel of specialists to discuss the key topics and for choosing the most adequate methodology(ies) for testing hypothesis, and their harmonization, if needed.

The activities will comprise the collection of baseline literature of risk endpoints for relevant ecological functional groups or ecosystem services, as well as the collection and comparison of dossiers from released events, of the selected GM crops, the detection of information gaps, the organization of a panel of specialists, the choice of best methodologies for testing hypothesis, and their harmonization, if needed. The management of cotton and maize crop strategies and operational guidelines to minimize effects on non-target organisms will also be developed in this study. The socioeconomic study comprises the evaluation of *ex-ante* and *ex-post* impact of the adoption of GM varieties in three different important cotton production regions, applying CGE-regional methods and risk analysis. *Ex-ante* studies will be carried out in regions with small scale and traditional cotton growers. Additionally, *ex-post* studies will be conducted on seed industry for three different kind of cotton growers: organic/colored cotton, transgenic cotton and conventional varieties.

For the public perception and communication component of the LAC Biosafety Project, the needs of information for the general public and for the organized society will be detected. Such needs detected and analyzed from an online questionnaire and individual interviews, for general public and organized society respectively, will drive the building and delivery of adequate communication products. A specific abstract and poster is available in this same ISBGMO Symposium.

The project also includes the training on environmental risk assessment, management and communication for competent authorities and practitioners.

The LAC Biosafety project is aligned with regional and national expectations in proposing the reduction of the scientific bottlenecks on biosafety of GM crops of high socio and economic status. It also proposes to support the decision making process by improving science knowledge and, at the same time, the channels for GMO biosafety discussion with stakeholders. These actions meet the new priorities established at the FAO Conference (Guadalajara, March 2010) on biotechnology in agriculture in developing countries. For the future it is expected that the project can be consolidated by unifying researchers from Latin America and communicators involved with biosafety.

1.4 Uruguayan Genetically Engineered Crops Regulatory Framework

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The Decree N° 353/2008 establishes the regulatory system that is now being implemented in Uruguay after an 18 month moratorium (suspension) period imposed since January 2007. The Decree N° 037/2007 suspended the treatment of any *new* applications for authorization. During that time an inter-ministerial working group was convened to develop a proposal for a biosafety framework which was approved under the above mentioned decree. The logic underneath the framework established in the Decree N° 353/2008, is the risk analysis (RA) methodology for genetically modified organisms. The RA has three independent, but highly connected components: risk management, risk assessment and risk communication (RC). The Uruguayan National Biosafety Cabinet and the Risk Management Commission (CGR) are the risk "managers". The Risk Assessment on Biosafety (ERB) and the Institutional Coordination Committee (CAI) are the "evaluators". Multiple strategies can be employed for risk communication. This presentation will describe how the risk assessment phase is organized for efficient use of human and infrastructure resources to prevent duplication of efforts while working in a multidisciplinary and inter-institutional scientific network. A comparative analysis with other regulatory systems of South America is also presented to identify strengths and weakneses, and differences and similarities in assessing the environmental risk in the different countries. This analysis also examines which information could be shared among regulatory agencies, as well as analyses that could be homogenized to move toward a harmonization among regulatory systems while maintaining a confident risk analysis process for a safe introduction of genetically engineered crops.

1.5 Global cultivation of genetically modified plants and detection strategies for non-authorized GMO in the European Union

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Since commercialization in 1996, genetically modified plants (GM) have gained importance worldwide, which is underlined by an increase of the global area planted to biotech crops from 1.7 million hectares in 1996 to 134 million hectares in 2009. Soybean, maize, cotton and rapeseed remain the most prominent biotech crops. Herbicide tolerant GM plants occupied up to 62% of the global area of biotech crops. The amount of insect resistant GM plants increased by 14%, whereas stacked trait products increased by 6% (James 2009).

Import and planting of biotech crops in the European Union (EU) is strictly regulated. In order to ensure the highest level of protection of human health, GMO products must undergo a safety assessment before being

placed on the EU market. Furthermore, information on the genetic modification and appropriate detection methods has to be provided by the applicant. Validated methods of detection for approved GMOs and products are available via the European Community Reference Laboratory (CRL).
However, only scare information on non-authorized biotech crops is available. Experiences in recent years have shown that non-authorized biotech crops (e.g. biotech-papaya, Bt10-maize, LL601-rice and Bt63-rice) were detected on the EU market. The Bavarian Health and Food Safety Authority is responsible for the detection of illegal imports and for labelling control of Food and Feed in Bavaria (Germany). Genetic modification in plants are most commonly detected at the DNA level by means of the quantitative real-time PCR (Alexander et al. 2007; Querci et al. 2010). Therefore, data on cultivation areas, novel DNA constructs, event specific detection methods, the status of authorization in the EU and the availability of reference material were gathered and assembled in a GMO database.

Currently, 267 GM plants are registered in our database. Herbicide tolerance is incorporated in 139 GM plants. Insect resistance is found in 85 GM plants. The future trend in plant biotechnology, however, are stacked traits like the SmartStax™, which was released in 2010 in the USA with eight different genes coding for insect resistance and herbicide tolerance.

Screening of genetically modified plants or products thereof could be accomplished by detection of common genetical elements. 102 events could be detected by screening for the presence of pat/bar/CP4 epsps/m-epsps, whereas 168 events would be found positive for the presence of P-CaMV 35S/T-nos. Hence, the P-CaMV 35S/T-nos screening system is the most applicable system for the initial detection of genetic modifications.

As a future prospect, data provided by several patent databases shall be increasingly used to gain information on incorporated genetic elements in plants which are not authorized in the EU. On basis of this information, new detection methods and screening systems shall be developed.

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1.6 Coexistence of traditional and biotech crops in the Czech Republic: measurements and control

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Release of biotech crops into the environment and cultivation are subject to strict regulations in the EC. Based on the precautionary principle, all applications undergo evaluation by EFSA GMO panel and decision of relevant competent authorities. Although experimental field releases are ongoing, field cultivation is not frequent. Farmers from only some Europeans countries have decided to use GM crops.

Biotech crops like Bt corn (MON810) or amylose potatoes (Amflora) are cultivated in the Czech Republic under EU coexistence rules between traditional, biotech and organic agriculture. These are based on the results of scientific projects (e.g. investigation of pollen flow, persistence in the nature) and European Regulations together with Recommendation of European Commission 556/2003 and a national Act on Agriculture and an amending decree. All growers are bound by these specific rules. Unlike deliberated release into the environment GMO commercial cultivation is supervised by the Ministry of Agriculture.

Cultivation of Bt maize MON810 began in the Czech Republic in 2005. Together 150 ha (area without buffer zones) was recorded. Cultivation for experimental purposes (2005 – 2009) was excluded from the statistics. Maximum value was reached in 2008 with 8 380 ha followed by a decrease in 2009. MON810 is cultivated mostly in the regions where European corn borer abounds.

Consent holders have to provide monitoring plans and collect relevant data to capture any information on potential risks associated with cultivation of GM crop. Along with that the Ministry of Agriculture performed a questionaire survey 2005 – 2008 to explore maximum information from farmers. Farmers are subjected to controls of their fields. The accredited laboratory developed a sampling strategy for efficient control and analysis of green samples. The data may be subjected to the evaluation of the Czech commission for GMO handling and Scientific Committee for GM food and feed established in the Ministry of Agriculture. Up to now no unintended effect upon the environment has been recorded in the Czech Republic

With increasing number of GMOs developed for cultivation and their probable entry into European region, more comprehensive tools will be required for the competent authority. Nowadays data collection is based on observation, in the future more analytical tools will be needed, suited for metabolic profiling.

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1.7 Regulatory Framework for Genetically Modified Organisms in Taiwan

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Most major countries establish genetic modification (GM) technology management policy with national characteristics according to its domestic politics, economics, and social environment and public views. Taiwan has adopted an interagency approach for GM technology management through the existing regulatory systems as follows:

Basic research

The National Science Council (NSC), Executive Yuan, oversees laboratory work on GM technology according to "The Rules for Conduct in Respect to Genetic Recombination Experiment". The Rules, designed to ensure GM laboratory safety, is only an administrative rule that has no legal power. It applies to research funded by NSC. Some of the research consigned or subvented by other government bodies would also be requested to follow the Rules. However, there are no legal obligations for non-government funded laboratory works on GM technology.

To address the rather limited application scope and the legal status of the Rules, NSC is going to set appropriate legal authority in the "Fundamental Science and Technology Act". Then, a "GM Technology Research and Development Management Regulation" will be drafted based on the Act. In short, NSC wants to strengthen bio-safety management from the beginning to secure personnel safety and improve public confidence in GM technology. As for the detail and technical aspects of the operational specification related to GM technology, "The Rules for Conduct in Respect to Genetic Recombination Experiment" will be taken in principle as the blueprint and reserves the flexibility to adjust the content along with technology development.

Field trials

Taiwan has no general management regulation on field trials of genetically modified organism (GMO), but there are various regulations set that target for specific type of GMO.

For genetically modified plants, Agriculture and Food Agency (AFA) of the Council of Agriculture (COA), Executive Yuan, sets explicit regulations on the management of field trials according to "The Plant Variety and Plant Seed Act" and its derived laws, such as the "Transgenic Plant Field Testing Guidelines", the "Transgenic Plant Hereditary

Characteristics Study and Biosafety Assessment Criteria", and the "Transgenic Plant Hereditary Characteristics Study and Biosafety Assessment Criteria Implementation Guidelines".
For genetically modified animals, Department of Animal Industry (DAI) of COA sets regulations on the management of field trials according to the "Animal Industry Act" and its derived laws, such as the "Regulations for the Field Trial of Transgenic Breeding Livestock (Fowl) and the Bio-Safety Assessment".
For genetically modified aquatic animals and plants, Fisheries Agency (FA) of COA sets regulations on the management of field trials according to the "The Fishery Law" and its derived laws, such as the "Rules for the Field Trial of Transgenic Aquatic Animals and Plants".
For genetically modified microorganisms, the Environmental Protection Administration (EPA), Executive Yuan, currently sets regulation only on the use of microbial organisms or their metabolic products modified by means of genetic engineering as environmental agents under the "Environmental Agents Control Act" and the "Genetic Engineering Environmental Agent Microbial Preparations Development Management Regulation". However, COA is revising the "Agro-pesticide Act" and the "Veterinary Drugs Control Act" to adopt the applications of GM technology on pesticides and veterinary drugs to reinforce the existing genetically modified microorganisms management mechanism.

Marketing Approval

GM technology has wide applications. Its final product types may include food, feed, ornamental products, pesticides, environmental agents, and animal drugs. For marketing approval of genetically modified products, Taiwan has no general management system, but applies existing laws and regulations for specific types of products. The most rapidly developing products are genetically modified foods and genetically modified feeds. Their management also receives the most attention.
Regarding genetically modified foods, the Department of Health (DOA), Executive Yuan, is responsible for marketing approval according to "The Act Governing Food Sanitation" and its derived laws, regulations, and letters. Accordingly, DOH published "DOH Food No. 0900011745" that genetically modified soybean and corn shall be registered for marketing approval according to Article 14 of the "Act Governing Food Sanitation" and provides the "Guideline for Food Safety Assessment of Genetically Modified Foods Derived from Recombinant-DNA Organisms" as review standard. DOH also presented an outline of new mandatory GM labeling regulations for genetically modified soybean and corn products in "DOH Food No. 0900011746" according to Article 17 of "The Act Governing Food Sanitation". Additionally, a "Food Safety Assessment Manual for Genetically Modified Foods Derived from Recombinant-DNA Organisms" was published by Food Industry Research and Development Institute (FIRDI) under the financial support of DOH to facilitate the preparation of dossiers for registration. DOH continues deliberating the "Guideline for Food Safety Assessment of Genetically Modified Foods Derived from Recombinant-DNA Microorganisms" (draft) and the "Guideline for Food Safety Assessment of Genetically Modified Foods Derived from Recombinant-DNA Animals" (draft). In the future, DOH will take concerted action in accordance with national policies to expand gradually the management items of genetically modified products.
To fulfill genetically modified feed management mechanism, COA is revising the "Feed Control Act" as the legal basis for management. In the future, genetically modified feed management will in principle follow the precedent of genetically modified food to ensure the safety of the food chain.
As for other genetically modified products, the vast majority of GM technology-related activities in Taiwan are in experimental research phase. It is difficult to envisage the time and possibility for their commercialization. Therefore, there is currently no need to consider the management demand of genetically modified products other than genetically modified food and genetically modified feed. However, the government ministries will continue to monitor closely the development of GM technology at home and abroad to timely assess the necessity and urgency for management.

Import and Export of GMO

With regard to the import and export of GMO, it is roughly divided into two parts. Firstly, COA sets additional regulations for specific categories of GMO to be released in the environment. For example, “The Rules for Import/Export Permit of the Transgenic Plants” and the "Transgenic Plant Labeling and Packaging Guidelines” derived from “The Plant Variety and Plant Seed Act” were set to regulate the import and export of genetically modified plants. The “Guidelines for Screening Application for Letter of Approval for the Importation of Breeding Livestock and Poultry and Genetic Resources” derived from the “Animal Industry Act” was set to regulate the import of genetically modified animals. As for genetically modified aquatic animals and plants and genetically modified microorganisms, there is currently no specific regulation for their import and export.

Secondly, if the purpose of the import and export of GMO is not for environmental release, there is relatively little concern on the bio-safety issue. There are general provisions in "The Act Governing Food Sanitation” and the “Feed Control Act” that can be applied to manage the import and export of GMO not for environmental release. Its related control measures and product listing process are roughly equivalent to those of the marketing approval processes for genetically modified products.

On the whole, there are currently rather limited regulations for the import and export of GMO and have yet to be consolidated. The government ministries will review the relevant legal systems based on the necessity and urgency of management and adjust the systems accordingly.

Others

In addition to the above scope, the Government currently has conducted the clarification and/or reinforcement of blank or gray zones of regulations to manage specific categories of GMO with urgent need. For example, there is currently no regulation for commercial planting of GMO, but the ministries responsible for managing GM technology have come to a major opinion trend based on the consultant of the “Interagency Advisory Genetically Modified Products Special Task Force” that commercial planting of GMO should be strictly managed. To comply with the opinion, COA will conduct an amendment of current regulations to manage properly the commercial planting of genetically modified plants.

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1.8 EFSA's work on the safety assessment of genetically modified organisms

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Introduction

Within the European Union (EU), the application of genetic engineering is regulated for domestic and imported goods. To ensure a high level of protection of human and animal health, the environment and consumer interests, the EU has established a legal framework that regulates genetically modified (GM) food and feed, as well as the release of genetically modified organisms (GMOs) into the environment. In this respect, the role of the European Food Safety Authority (EFSA) is to independently assess and provide scientific advice to risk managers on any possible risks of GMOs to human and animal health and the environment. In the EU, it is the role of risk managers such as the European Commission (EC) and EU Member States to decide whether a GMO or a derived product can be placed on the EU internal market.

EFSA's remit regarding GMOs

The Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) consists of 21 scientific experts, which are selected for their scientific excellence after an open call for interest from the scientific community. Experts mostly come from EU research institutes, universities or risk assessment bodies, and provide independent scientific advice on the safety of (1) GMOs such as plants and micro-organisms, on the basis of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms; and of (2) GM food and feed, on the basis of Regulation (EC) No 1829/2003 on GM food and feed.
The scientific advice of the EFSA GMO Panel, which are published and made available to all in the EFSA Journal, is mostly given in the form of *Scientific Opinions* or *Guidance Documents*.

Scientific Opinions

Scientific Opinions have been issued on: GMO market registration applications; national safeguard clauses; and specific safety issues.
GMO market registration applications
Currently, several GM plants or derived products are on the EU market, whereas a growing number of applications are in the assessment pipeline. Approximately 100 applications for GM plants have been submitted under the GM food and feed Regulation. These applications cover a diversity of crops (mostly maize, followed by cotton and soybean) and traits (mostly herbicide tolerance, insect resistance, or both). New traits include drought tolerance in maize, altered oleic acid content in soybean, or reduced amylose content in potato. Most of the applications are for import and processing of GM plants for food and feed uses, meaning that most GM plants are cultivated outside the EU, and subsequently imported and processed, mainly for feed uses. Seventeen GM plant applications submitted under the GM food and feed Regulation cover cultivation in the EU.
Approximately 30 applications involving GM micro-organisms (GMMs) have been assessed or are under assessment at EFSA level. Five of these applications are for feed materials submitted under the GM food and feed Regulation, whereas the other applications cover feed additives or food ingredients produced from GMMs.
The EFSA GMO Panel evaluates the safety of GMOs (for their intended uses) on the basis of the information provided in applications, scientific comments received from EU Member States and relevant scientific literature. During the evaluation of GM plant applications for cultivation, EFSA works in close collaboration with a specific EU Member State who has volunteered to take responsible for the initial environmental risk assessment of the

application, which is then considered by the EFSA GMO Panel. The EFSA GMO Panel considers the collaboration with EU Member States on environmental risk assessment extremely useful and constructive.

National safeguard clauses

EU Member States can legally invoke safeguard clauses based on new scientific evidence related to safety to provisionally restrict or prohibit the commercial use of previously authorised GM plants on their territory. So far, safeguard clauses have been invoked by Austria, France, Greece, Germany, Hungary, Luxemburg and Poland for several maize and oilseed rape events with the majority of safeguard clauses being invoked for maize MON 810. For all cases where the EFSA GMO Panel has evaluated the scientific information submitted in support of a safeguard clause, the Panel has as of yet not found new evidence for adverse effects caused by the concerned GM plant. Therefore, EFSA's advice to the EC was that the invocation of the safeguard clause was not justifiable on the basis of the submitted information.

Specific safety issues

Scientific Opinions are also issued on specific GMO risk assessment issues, such as: the safety of antibiotic resistance marker genes; the role of animal feeding trials for the safety assessment and nutritional assessment of GM plants and derived food and feed; the statistical analysis of results of field trials; the evaluation of GM plants cultivated for non-food/feed purposes; post-market environmental monitoring; statistical considerations for the safety evaluation of GMOs; the risk assessment of GM plants used for non-food or non-feed purposes; and the assessment of allergenicity of GM plants and micro-organisms and derived food and feed. A scientific opinion on the environmental risk assessment of non-target organisms exposed to GM plants is to be completed by the end of 2010.

Guidance Documents for the risk assessment of GMOs

The EFSA GMO Panel Guidance Documents intend to guide applicants in the preparation and presentation of GMO applications by describing principles, concepts, data requirements and issues to be considered in the frame of risk assessment.

So far, the following Guidance Documents have been issued, or are in preparation:

(1) Guidance Document for the risk assessment of GM plants and derived food and feed (issued in 2004; revised in 2006; revision to be completed in 2011);
(2) Guidance Document for the risk assessment of GM micro-organisms and their derived products intended for food and feed use (issued in 2006; revision to be completed in 2011);
(3) Guidance Document for the renewal of authorisations of existing GMO products (issued in 2006);
(4) Guidance Document for the risk assessment of GM plants containing stacked transformation events (issued in 2007);
(5) Guidance Document for the environmental risk assessment of GM plants (issued in 2004, revised in 2006 and 2010).

Working Groups of the EFSA GMO Panel

Several Working Groups provide scientific support to the EFSA GMO Panel. Each Working Group is composed of members of the EFSA GMO Panel and of external experts that are invited on an *ad hoc* basis.

Standing Working Groups on GMO applications for marketing

To address the complexity of GMO evaluations and to be able to conclude on the safety of the GMO under consideration, the EFSA GMO Panel is supported by 4 standing Working Groups on applications, each focusing on specific areas of the risk assessment:

(1) Molecular characterisation: This Working Group considers all relevant scientific information regarding molecular characterisation of the GM product. Detailed information is evaluated on the source of the donor DNA; the transformation method; the organisation of the inserted DNA at the insertion site(s); and

on the expression and stability of the insert.

(2) Food and feed: The Food and Feed Working Group focuses on the evaluation of food and feed safety aspects of the GM plant and/or derived food and feed. The comparative approach, on which the safety assessment is based, encompasses the evaluation of the agronomic and phenotypic characteristics, composition, toxicity, allergenicity and nutritional value of the GM plant and/or derived food and feed.

(3) Environment: Key elements for the environmental risk assessment are potential changes in interactions of the GM plant with the biotic and abiotic environment resulting from the genetic modification. Thereby, changes in persistence and invasiveness of the GM plant; potential for gene transfer; interactions between the GM plant and target organisms; interactions between the GM plant and NTOs; effects on biogeochemical processes and abiotic environment; and impacts of specific cultivation, management and harvesting techniques associated with the cultivation of the GM plant are considered. The environmental risk assessment is based on the biological and ecological characteristics of the plant, the nature of the introduced trait(s), the receiving environment in which the plant will be introduced, the scale and frequency of the proposed introductions, and the interactions amongst them. Such an evaluation also considers potential direct and indirect, as well as immediate, delayed and cumulative long-term adverse effects.

(4) Genetically Modified Micro-organisms: This Working Group evaluates data regarding the molecular characterisation and environmental risk assessment of GMMs, when appropriate in collaboration with Working Groups from other EFSA Panels which evaluate the food and feed safety of the product produced from the GMM. Key aspects that are evaluated include: the characterisation of the parental micro-organism, inserted sequence(s) and genetic modifications; the stability of the GMM; and the presence of recombinant DNA in the GM product.

Single mandate Working Groups

In addition to the 4 standing Working Groups on GMO applications for marketing, temporary Working Groups address specific questions generally called mandates. Currently, the following single mandate Working Groups are established:

(1) Self-tasking Working Group on non-target organisms;

(2) Working Group updating the environment sections of the Guidance Document for the risk assessment of GM plants and derived food and feed products;

(3) Working Group on statistics;

(4) Working Group updating the Guidance Document for the risk assessment of GMMs and their derived food and feed products;

(5) Working Group on the selection of comparators for the risk assessment of GM plants.

GMO Unit

The GMO Unit of EFSA provides administrative and scientific support to the EFSA GMO Panel and its Working Groups. The GMO Unit also provides support to EFSA's risk communication task and responds to external questions related to the GMO risk assessment work of EFSA. These questions come from various stakeholders such as applicants, environmental non-governmental organisations, the EC, Members of the European Parliament and EU Member States. Currently, 17 scientific officers and 7 administrative assistants are part of the GMO Unit.

1.9 Assessing intangible risks: Including cultural and social effects in a risk assessment

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The Environmental Risk Management Authority (ERMA) regulates genetically modified organisms (GMOs) in New Zealand under the Hazardous Substances and New Organisms Act (HSNO) 1996. The Act is environmental legislation and its purpose is to protect the environment and health and safety of people. One of the guiding principles of the Act is to maintain and enhance the ability of people and communities to provide for their own social, economic and cultural wellbeing. The intention is to manage the risks of introducing new organisms while not impeding innovation and economic growth.

The HSNO Act requires the decision makers to assess both risks and benefits across five impact areas:

1 The environment
2 Human health and safety
3 Māori culture and traditions
4 Society and community
5 The market economy

Benefits and risks are assessed using qualitative descriptors. They range from 'negligible' through to 'extremely high' depending on the magnitude and likelihood of occurrence. An important feature of the HSNO Act is the ability to weigh risks against benefits. An application can be approved if the benefits outweigh the risks.

Even with the use of qualitative descriptors, this system can be challenging when assessing intangible effects. *Weighing* intangible effects against tangible ones is an even greater challenge. This is often the situation ERMA finds itself in when assessing applications for genetically modified organisms.

A treaty was signed between Māori tribes (indigenous people) and the Crown in 1840. This Treaty is New Zealand's founding document and has shaped many aspects of New Zealand society. The HSNO Act specifies that the decision-maker must take into account the effects of application proposals on Māori culture and their traditional relationships with their ancestral lands and other taonga. Taonga is anything that is highly valued or prized by Māori, whether tangible or intangible. Examples include artifacts, land, flora and fauna, water, language, cultural beliefs and traditions.

ERMA gathers information about cultural concerns through direct consultation with Māori. Applications are sent to a network of approximately 180 iwi (local groups or tribes) throughout the country. This network is familiar with the application process as a result of ongoing engagement with ERMA. Approximately 10% of those contacted usually respond to the consultation and/or make a submission once the application is publicly notified. For some applications, a focus group may be formed from 4-5 of the submitters. The focus group meets with ERMA and the applicant to talk through any concerns.

Social concerns are usually raised through public submissions. An application is advertised on the ERMA website and in the major newspapers. A public hearing is held if requested by a submitter. The assessment of an application begins once the submission period has closed so the cultural and social concerns raised can be included in the analysis.

Cultural concerns about GMOs usually relate to the ability of Māori to maintain kaitiakitanga (guardianship of taonga) and inconsistencies with mātauranga Māori (Māori knowledge). Instead of trying to weigh these intangible effects against a tangible benefit, ERMA attempts to mitigate them by placing controls on the approval. These controls usually relate to providing Māori with more information and ongoing communication regarding all the risks and benefits of the application. Below is an example of a response ERMA may give to these concerns:

> *"To date we have not seen evidence of adverse effects to kaitiakitanga and mātauranga in that they continue to exist and be referenced and used in Māori communities. We consider a **minor** effect on kaitiakitanga and mātauranga to be **unlikely**. We acknowledge there are gaps in knowledge and some uncertainty about both the magnitude and likelihood of this effect. However, on considering the proposed controls, particularly those relating to containment, monitoring and iwi/Maori engagement,*

the uncertainty was not deemed to be significant. Therefore, the overall assessment is that the level of adverse effect is ***negligible.****"*

Social concerns can be even more difficult to include in a risk assessment. They usually relate to personal values regarding perceived risks and naturalness of GMOs. The information ERMA receives is usually not in a form that can be assessed—the effects are not well identified or articulated, and are often more about the "yuck" factor. ERMA's approach is to be as fair, transparent and ethical as possible in their assessment, demonstrating that they have considered these concerns but explaining why they cannot be assessed or why they were considered to be negligible. For example:

"We note that some members of the community have strong feelings around these issues. However, these concerns are personal opinions rather than concerns based on a particular framework of reasoning, or a body of documented opinion. One opinion cannot be seen as more meritorious than another and it is not ERMA's role to judge whose view is the most correct. Given the contained nature of this research we do not consider this effect to be significant".

While not everyone agrees with ERMA's assessment, on the whole mitigating risks that cannot be weighed, and communicating our reasoning has been effective when assessing applications to undertake research in containment. But ERMA has yet to assess an application for a commercial release of a GMO. How will we weigh benefits such as economic gain against a general dislike of the idea of GMOs? Are the benefits to the economy greater than the belief there will be a reduction in cultural guardianship?

One idea ERMA has been considering is translating cultural and social concerns into tangible effects on the market economy, the environment and human health and safety. ERMA would provide Māori and other members of the community a range of possible effects and ask them to place a quantitative value on each one. The difficulty would be gaining consensus from the varying views amongst Māori and the wider community and would require significant discussions with a wide range of interest groups.

The success of such an approach will depend on being able to clearly communicate the real risks and back them up with information that the wider community can both understand and trust. Having extensive research on the safety of genetically modified organisms is very important. But if we do not transfer that information to the wider community, regulators who try to assess intangible effects will always be trying to compare apples with oranges.

1.10 Comunication and public awareness within the context of a research project on biosafety in Latin America.

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The project *Latin America: Multi-Country Capacity Building for Compliance with the Cartagena Protocol on Biosafety* – LAC-Biosafety, is a pioneering initiative for cooperation among mega-biodiverse countries, Brazil, Colombia, Costa Rica and Peru It is financially supported by the Global Environment Facility – GEF through the World Bank under general coordination of the International Center for Tropical Agriculture - CIAT. The objective of the project is to strengthen technical capacity in scientific knowledge generation and in communications, to support informed biosafety decision-making in compliance with the Cartagena Protocol on Biosafety – CPB. Therefore it was strategically structured in two components: technical; and communication and public perception. Through its Technical Component the project will disseminate approaches and innovative tools for risk assessment, develop a database for sharing knowledge on biosafety, and provide scientific training materials for the region. The

Communication Component (CC) aims to reinforce the capacity in communication and raising public awareness on biosafety in Latin America in general, and specifically in the four participating countries.
To achieve its objective, the CC is leading the development of strategies in communication and public awareness, and a plan for knowledge sharing. The project will be conclude with the organization of a regional conference on biosafety. The CC strategy is based on collaborative actions within national institutions of the four participating countries, regional international centers of agricultural research, and other committed entities that have a high level in complementary abilities. So, in Brazil the project is organized as a net of specialists from universities and from the Brazilian Agricultural Research Corporation (Embrapa) units, with focal points in Ministries (mainly Agriculture, Environment, and Science and Technology), under the national coordination and operation of Embrapa Environment. The Project was presented in the Meeting of the Parties - MOP3/2006 in Curitiba and at Embrapa Headquarters in Brasilia, 2009. In this case this project could become known by many people in Brazil.
The public perception approach attached to the communication initiative and to the technical component of a research project is a pioneer initiative in Brazil. The actions proposed for the CC are in agreement with the country priorities for improvement of biotech products and the creation and maintenance of channels of dialogue with different segments of the society.
The CC activities, initiated officially by the end of 2008, focused on the general public and the organized civil society. For the general public it was provided an online questionnaire to detect the public perception of biosafety of GM plants and its regulations. Its objective is to analyze the gaps and then provide the information needs detected, based on scientific results. With regard to the organized civil society the selected strategy was to interview some entities, directly and indirectly related to the decision making of public policies. The aim of such interviews was to identify: the perception about the proposed project activities; the reflections of such activities at country level; and any specific information needs in each area of interest for those organizations consulted.
The questionnaire showed (partial data based on responses till May 30th, 2010) that research and educational institutions, compared to NGOs and government entities, have very much credibility by the general public, mainly regarding the provision of information. The information provided is more trustful when obtained and presented by scientists and specialists. Also, there is a lack of adequate language to reach a large variety of interlocutors. Another important point detected is that there are critical gaps around CPB goals and improvement, even on National regulation and its implementation.
In regard to the organized civil society interviews (partial analysis based on interviews application till April 2010, meaning around 35% of the proposed ones), results indicate similar trustfulness around scientific research and data provided by public institutions. Those interviews also pointed out the importance of clear information and adequacy of the language to the specific audience, so that the consumers can understand the information and make the best choice.
Supported by such conclusions, the Communication Component, in Brazil, is preparing new documents utilizing appropriate language for each kind of audience, including the demands detected in the online questionnaire and in personal interviews. These information documents are being available on printed and electronic formats. Detailed information on the topics and the documents already developed will be presented at the ISBGMO Congress.
It is foreseen that the communication initiative could be the necessary bond agent and a good initiative to better organize the scientific information on ERA of GM plants. It is expected that the CC would be the facilitator in accessing scientific information and in building a better dialogue. It also opens the possibility to the project members, in Brazil, to subsidize policy decision makers with scientific information, to contribute to sustainable agriculture geared to the needs of the general society and a better management of biodiversity. These kinds of communication and scientific based information have been highlighted as priorities by the conference on biotechnology in agriculture in developing countries, organized by FAO in March 2010 in Guadalajara, Mexico. So, as a general conclusion of the CC activities of LAC Biosafety project, it is expected that will increase awareness and

sensitivity to biosafety by sharing knowledge throughout the region, and in this sense it will contribute to the conservation of biodiversity.

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1.11 Regulatory dossier of a public sector-developed GMO product in South Africa

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Introduction:

South Africa ranks third in potato production in Africa. From 1990 to 2006, production in South Africa has increased from 1,2 million tons to 1,8 million tons annually and current average yields are approximately 30 tons per hectare. The ARC supports a potato breeding program that ensures the identification of new characteristics to add to farmer-preferred varieties. In accordance with this goal, the ARC has evaluated the tuber moth resistance available in the genetically modified (GM) SpuntaG2, developed at Michigan State University, USA.

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), is a serious insect pest of potatoes in South Africa, and are responsible for losses of up to R40 million per annum to the South African potato industry. Commercial producers rely on insecticide applications, generally applied at weekly intervals for PTM control. Control is not always satisfactory and damage levels vary between seasons and years. The only control strategy that gives consistently good control against the PTM is the use of GM insect resistant potatoes containing the *cry1Ia1* gene. SpuntaG2 was one of several events transformed with the *cry1Ia1* gene and selected for its efficacy in field trials and storage tests in the United States and Egypt in 1999 and 2000. SpuntaG2 produces high levels of mortality in first instars of PTM in detached-leaf bioassays, laboratory tuber tests, and field trials in Egypt. Efficacy of the *cry1Ia1* gene in the SpuntaG2 genome was also demonstrated during field and storage trials over six growing seasons in South Africa. In all cases, control of PTM in the field and storage was 100%, and remained stable throughout several years of field testing in different agricultural conditions and in different agroecosystems. Evaluation of the resistance and agronomic traits of SpuntaG2 was performed by the ARC with the aim of enabling producers to test this trait and determine its value to specific growing areas. The ARC plans to use this trait in its breeding program to add value to the already established varieties in South Africa. All safety studies consistent with Codex Alimentarius guidelines and the Cartagena Protocol were also conducted, and the results of these studies were supplied as supporting documentation when the ARC applied for “General Release of Genetically Modified Organisms (GMOs) in South Africa”. The details of those studies and their conclusions will be presented and the impact of regulatory decision making delays on public sector research will be illustrated. Delays experience in regulatory decision making will be illustrated as well and the impact of this on public research and farmers' access to improved planting material will be discussed.

Discussion

One particular advantage of protection of tubers in storage is that it protects the harvested yield and enhances the ability of saved potatoes to be used as planting material in subsequent generations. General release will enable developers to work with transgenic plants in unconfined conditions, and commercial release is one activity that can be initiated when an applicant has a general release approval. The necessity of farmer participatory trials has been identified to enable farmers to evaluate the improved variety alongside preferred varieties in order to assess the

value of the new trait. General release approval will enable farmers to plant and grow the new variety without confinement restrictions and with confidence in the environmental and food and feed safety of this new planting material.

Conclusion

All the safety studies that were performed on SpuntaG2 indicated that it is safe for food and feed use, and safe to the environment. However, delays during the development and general release stages result in setbacks. Delays in confined field trial approvals set research back by a year when approvals are not received in time for planting. Delays in general release approvals keep a project on hold with no income for months to years and can completely derail farmers' access to the improved crop. Delays in appeals leave a project in limbo and, for public sector research with no profit incentive, this can mean a complete shut down of the project.

1.12 Sustainable Governance of Biotechnology and Risk Communication Strategies in Asian Developing Countries: the Case Study of Biotech Papaya in the Philippines

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Key words: biosafety governance, risk communication, living modified organisms (LMOs), Biotech papaya

The development of living modified organisms (LMOs) has gained significance in both developing and developed countries worldwide to complement skyrocketing needs for food, feed, and processing materials. Implementation policies play an important role in ensuring biosafety and sustained development of biotech crops, from research and development (R&D) to commercialisation. Additionally, regulations related to international trade in environmental and food safety also impact development of biotech crops. The objective of this research is to review oversight policies relevant to the market release of biotech crops in light of international instruments and guidelines, and to suggest policy options for biotechnology development, using biotech papaya in the Philippines as a case study. This research explains the regulatory process that new products of biotech papaya go through prior to commercialisation, describes consumers' attitudes, and identifies opportunities and challenges in the adoption of the new technology. Furthermore, it highlights key factors and risk communication strategies that drive sustainable commercialisation of biotech crops within sustainability frameworks during the introduction of new biotech crops, and facilitate harmonisation of international trade and environmental agreement in countries belonging to the Association of Southeast Asian Nations (ASEAN).

It is hoped that this paper will contribute to enhanced information sharing among countries that are in the process of developing implementation policies and a biosafety regulatory framework. It will also facilitate a reexamine action of the current regulations and situations in selected countries with the goal of enhancing the sustainable development of crop production systems and increasing global crop productivity and food security.

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1.13 Public perception vs. quantitative risk assessment and impacts on policy decisions related to agricultural biotechnology.

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The Hawaiian archipelago is the most isolated island group in the world, separated by almost 1,600 km to other islands and nearly 4,000 km to the next major land mass. Due to this isolation, and 1-5 million years of evolution (McDonald *et al*., 1986), Hawai'i is home to more than 1,000 native flowering plant species. Of the 88 families and 211 genera represented by these species, over 90% are endemic (Wager et al. 1990). Currently, 273 native plant species are threatened or endangered, over one-third of all federally listed plant species (USFWS 2006).

There are growing concerns that gene flow may occur from agricultural products that have been modified through recombinant-DNA or biotechnological techniques, to native Hawaiian plant species. Hawaii has the highest number of experimental permits for biotech crop development (*http://www.nbiap.vt.edu/cfdocs/fieldtests1.cfm*) in the USA, and had the first developed and commercially adopted genetic engineered (GE) fruit tree - papaya.

By April 2010 a total of 2,712 field-test release permits have been requested for Hawaii. Even though the majority of these requests (some requests have never been granted) are for corn and soybean, other crops are being or have been tested in Hawaii (anthurium, barley, coffee, cotton, sorghum, lettuce, lime, Mexican lime, papaya, pineapple, potato, rice, sugarcane, sunflower, tobacco, tomato, wheat). There are a number of major international seed companies operating in the State of Hawaii and most of them are continuously developing new GE varieties.

Environmental concerns are significant in Hawaii, yet at the same time, Hawaii has a vested interest in sustainable agriculture and agricultural biotechnology development. Eighty-90% of the food needs of Hawaii are met through imports, and ensuring improved sustainability in the islands requires improved agriculture and reduced dependence on importation. Agricultural biotechnology does offer some possibilities for improved food security in the islands, and already employs a large number of people in the agricultural sector.

Data available in 2010 show that about 60% of papaya grown in Hawaii is GE and that the seed industry is the largest agricultural sector in the state, currently growing at a rate of 11% annually, and contributing almost 30% of the total value of all Hawaii produced crops. The seed industry utilizes a total of about 15,000 acres with about 4,000 acres being grown at any specific moment. Over 80% of all the corn grown by the seed industry in Hawaii is GE. Thus although Hawaii's acreage in GE crops is actually limited, biotechnology plays an important role in local agriculture production. This role for GE crops in local agriculture will probably increase. Currently, the University of Hawaii College of Tropical Agriculture and Human Resources (CTAHR) is engaged in genetic engineering projects that hold promise for improved future crop varieties. All of these projects are in the

early, laboratory-based stage of development; to date, transgenic pineapple, banana, and taro have not been grown in field trials or released to farmers.

However, there may be a clash between proponents of native ecosystem conservation versus the needs of industrial agriculture. The fact that land is required for agriculture is an immediate and obvious conflict with preservation. GE crops add a new dimension to the argument. In the last ten years, numerous campaigns to discredit GE crop development in Hawaii have depended on the propagation of misinformation, fearmongering, and the notion that GE crops grown in Hawaii will have negative effects on Hawaii's native plant species.

During the 2003 through 2007 Hawaii state legislative sessions, 105 bills and resolutions were introduced, to ban GE crops from Hawai'i (which is the highest number of such bills in the nation). These legislative decisions may have far reaching national impact if precedents are set that other states might follow.

Public perception is often easily swayed by their perception of risk. Much has been made of potential health and environmental risks of GE technology in Hawaii, and this has been reported in the popular media extensively. While researchers may conduct quantitative risk assessments using the best procedures available, and provide assessments of risk that are realistic, the general populace may reject these. In many cases, the public perspective is that any risk is too much risk. A major concern in this regard is that people are easily impressed by warning of negative impacts in the popular press; they are less likely to encounter and appreciate risk assessment reports published in

less accessible media.

As these issues are discussed, it is important to remember that agriculture is an essential industry in Hawaii representing the state's third largest source of income after tourism and the military sector. Agriculture diversifies the economy and reduces dependency on tourism and imported produce. Risk assessments of GE technology products do not necessarily represent what the public perceives and feels and do not necessarily determine the outcome of political decisions. Ultimately, the impact of risk assessments on the legislative process may be marginal compared to public concerns and feelings. Examples of this can be seen in Hawaii with taro and coffee. Although no risk, measured scientifically, was associated with GE taro, two counties (Hawaii and Maui) with the highest acreage in farm land (2008 State of Hawaii Data Book), passed bills prohibiting testing, propagation, cultivation, planting growing and introducing of GE taro in that county. This bill also prohibits laboratory research on taro. This bill was passed in spite of the fact that there is essentially zero chance of negative impacts arising from the implementation of GE technology on these crops. It is therefore important to remember that scientific risk assessment forms only part of the discourse on GE; there are other public and private parties such as consumers, the seed industry, and activists that affect the political process.

The scope of the discourse on GE at any particular time can be gauged by examining the amount of the media (i.e. newspapers), internet, and legislative bills, surrounding GE crops. This presentation centers on how to evaluate what public perceives as a risk and how to evaluate public acceptance of GE. Nine years of data of public opinions associated with risk/acceptance of GE crops in the state are presented. Four different public opinion surveys (2001, 2006, 2007, 2010) were run in the state of Hawaii, and included an assessment of risk and risk acceptance of GE data. These data are compared to surveys conducted on the continental US by the International Food Information Council Foundation, in the same years. The thesis of this presentation is that risk perception and risk acceptance cannot be expressed simply as yes or no answers, but that it is contingent upon societal factors like ethnicity, socioeconomic class, and geographic location, as well as in relation to broader concerns to environment and food consumption.

This paper examines how public attitudes to GE technology change over time within Hawaii, and how Hawaii attitudes from mainland attitudes. We investigate the relationship between these attitudes and age, income, awareness of the prevalence of GE technology, and ethnicity in Hawaii. We present data on the above variables from four surveys, and relate the trends detected to media attention to GE technology at relevant times, addressing questions related to the impact of negative media on public opinions.

We also review public perceptions of risk and public perception of quantitative risk assessment. Possible reasons for the disconnect between trusting popular media reports versus quantitative risk assessment, and ways that this may be overcome. Finally, the impact that negative public perceptions of GE have on legislative actions is addressed. It is concluded that a very active and appropriately targeted educational program that explains the pros and cons of GE technology is required. This program should include components that provide the public with an enhanced appreciation of the philosophy and mechanisms of risk assessment. Formal risk assessment procedures are complex and are typically described in intricate technical documents, (e.g. EPA Guidelines for Ecological Risk assessment, EPA 1998). An increased appreciation of the true nature of GE technology and the way that risks are measured and managed should assist people in making better-informed decisions about new technology.

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1.14 Environmental impacts of GM crops: Defining damage criteria for decision-making

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The legal frameworks regulating the approval and use of genetically modified (GM) plants demand regulatory decisions to decide what kind of environmental changes are relevant and represent environmental damage. The current debate on the risks of GM crops for biodiversity illustrates that consensus on criteria that would allow a commonly accepted evaluation of environmental damage is presently lacking. Especially in Europe the risks of GM crops for biodiversity have been a constantly debated issue and the interpretation of scientific data is discussed controversially by the different stakeholders involved. Considering the vast amount of scientific data available, one can argue that the current debate is not primarily due to a lack of scientific data, but more to a lack of clear criteria that allow to put a value on impacts of GM crops on biodiversity.
Ultimately, any decision by regulatory authorities on what they judge being unacceptable is based on the relevant legal frameworks. Usually such decisions are taken in a political context that weighs scientific, ethical and economical criteria with cultural, religious, aesthetic and other relevant social beliefs and practices. Terms such as risk and safety are linked to a conception of damage. Damage, however, can not be defined on a purely scientific basis. The normative character of the term "damage" implies that both choice and definition of what constitutes a risk are impossible without a value judgement. Damage has thus to be defined together with an ethical evaluation as ecological analyses alone can not determine "correct" or "objective" criteria for damage.
The project VERDI (Valuating environmental impacts of GM crops - ecological and ethical criteria for regulatory decision-making) is an interdisciplinary collaboration between environmental biosafety and ethics that intends to offer European policy-makers and regulatory authorities guidance on how decision-making related to GM crops

could be improved. Concentrating on environmental impacts of GM crops on biodiversity, the project addresses both the ecological and the ethical questions involved in finding an operational approach to the evaluation of environmental damage recognizing that certain questions specific to the European context might not apply to other national regulations that have different legal bases.
Two case studies with the currently most prevalent GM traits (insectic-resistance based on Bt and herbicide tolerance) are used to discuss the open questions involved. Considerable scientific data on the environmental impacts of these two traits have been gathered in the past 15 years that allow to determine how such an evaluation could be performed to be generically applicable to different types of GM crop traits including new applications of biotechnology.

Stakeholders from different European countries including regulatory authorities, agricultural biotech companies and academia were invited to two expert workshops. In the first workshop, current approaches and challenges in the regulatory decision-making process related to GM crops were analysed. The second expert workshop aimed at determining what particular effects of GM crops on biodiversity are to be judged as unacceptable harm based on an ecological and an ethical valuation comparing effects of GM crops on biodiversity to environmental effects of current agricultural management practices. Results and feedback obtained during the workshops were used to elaborate a synthesis of the relevant ethical and ecological aspects when evaluating impacts of GM crops on biodiversity.
The results obtained reveal that both protection goals and baselines are two consistently emerging issues when discussing a definition of damage. Protection goals as specified by existing legislation are the exclusive starting point for a definition of damage for regulatory authorities. Yet, the legislative terms to describe the protection goal “biodiversity” are too vague to be scientifically assessed. To determine the relevant protection goals, a matrix allowing for an operational definition of biodiversity is proposed. The matrix allows specifying the area of protection, as well as assessment and measurement endpoints based on a number of defined criteria. While the initial proposal what to protect has to be framed by the regulatory authorities, the operational definition of protection goals should be defined in a transparent process involving a dialogue between all relevant stakeholders. The presented matrix can thereby be used as a tool to structure the dialogue, especially when defining both assessment and measurement endpoints. The process could include stakeholder meetings where stakeholders would compile and rank different conservation goals and ecosystem services.
Baselines are recognized to be the second crucial point in decision-making processes as they determine what makes a change to be a damage. Due to their vague definition, the use of baselines as a decision support tool remains nevertheless ambiguous necessitating a more precise characterisation. Common to all baseline definitions is the term “comparison”. Theoretically, decisions are always taken relative to a comparator that determines the current practice that is judged being acceptable. According to this baseline conception, the impacts of a specific technology can only be compared if the impacts of current practices are known. However, as GM and non-GM-based management practices are regulated according to different regulatory frameworks in Europe, a direct comparison of the effects of a GM cropping system to its conventional non-GM counterpart is impossible under the current EU regulatory framework. The baseline approach is thus principally not always applicable as, for example, the EU regulations demands to assess potential indirect or cumulative long-term effects of GM crops while this is not a requirement for conventional pest management practices. Nevertheless, an important point to consider in such a comparison refers to the fact that all regulatory frameworks differentiate between “intended” effects of a specific management practice and “unintended” effects that are to be minimised. This differentiation allows outlining a generic scheme that permits to evaluate whether the effects of different agricultural management practices are to be regarded as intended effects (which are judged acceptable) or as unintended effects that could represent environmental damage. This differentiation may help to overcome the principal difficulties of the initial baseline conception. In a first case study with Bt-maize, a flow chart is presented that can

help risk assessors to differentiate the effects of various pest management practices used for European Corn Borer management in maize on the arthropod fauna in agricultural landscapes.
In a second case study with herbicide-tolerant GM maize, criteria for regulatory decisions for non-insecticidal GM crops were determined. In contrast to Bt-maize, the assessment of the direct effects of the genetic modification is not the primary concern for non-insecticidal GM crops. Rather changes in agricultural management may be the primary cause of possible indirect effects on farmland biodiversity. The evaluation of indirect impacts prior to approval of the GM crop is challenging. It might be difficult to perform such an evaluation within the time frame normally available for pre-market risk assessment as long time periods are usually needed for indirect environmental changes to become apparent. These types of effects might moreover only become apparent during the large scale cultivation of GM crop events under real agricultural management. The establishment of risk mitigation measures appears thus to be a valid option to increase the level of safety. The goal of these risk mitigation measures should be to avoid the risk of reduced crop yields and the long-term build-up of problematic weed communities while supporting a sustainable degree of farmland biodiversity. Four risk management options are proposed that can help to achieve a balance between agricultural production and the support of desired non-crop plants in arable fields.
All technologies that could potentially harm the environment should be evaluated according to the same legal criteria (e.g., according to their novelty) and not to the process of development. What constitutes environmental damage should not be defined by the technology that causes it. The here elaborated ethical and ecological criteria may allow a generally acceptable evaluation of damage that can be applied to a wide range of different GM crops. The criteria could help regulatory authorities to improve decision-making and to take more accurate and coherent decisions. This may ultimately avoid decisions on environmental risks of GM crops being arbitrary in comparison to other technologies.

1.15 Food safety assessment of PAT gene product in herbicide resistant pepper

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Pepper is very important spice and condiment in Korea. It is the basic material of Kimchi. The aim of this study was to evaluate the effect on toxicity, allergenicity and stability of inserted gene product of Phosphinotricin acetyltransferase(PAT) introduced in transgenic pepper. We have studied the toxicity and allergenicity using Database. Deduced amino acid sequences of PAT are compared to the protein known or suspected to be allergen or toxin to determine if it has significant sequence identity. These proteins do not share overall sequence homology with any known allergenic and toxic proteins.
Large scale protein extraction was performed by using PAT protein expression vector. We could obtain large amount of PAT protein using pPSET-PAT vector. About 1g of PAT protein was purified by Q-sepharose and Ni-charged column chromatograph and provided for toxin test. The level of PAT protein expression was also evaluated by enzyme-linked immunosorbent assay (ELIZA). All generations show regular expression and the consistency of protein concentrations and the inherent stability of transgenic protein expression across multiple backcross generations. Stability of PAT protein was confirmed by SIF, SGF as same condition in digestive tract and heat treatment. All proteins were rapidly degraded within 5 second in SIF and SGF. Heat treatment also shows instability of this protein under 100℃ condition.
Finally, there was no significant difference between GM and non-GM pepper in nutritional properties. This study provides guidance on how to assess the safety of GM foods derived from GM crops.

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1.16 Development of support systems for risk assessment of GM crops

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Risk assessment is the pillar for adequate risk management and decision-making. Hazard identification is the most important component of any risk assessment and is initially inductive. As operating experience grows, the analysis can also adopt deductive approaches. The most common deductive approaches for hazard identification are checklists and unstructured brainstorming. Current checklists for GMOs are quite short and tend to mislead the analyst that all aspects of the systems have been questioned without confirming this to be true.
In this context a methodology was developed for problem formulation and risk assessment based on a computer-assisted decision tree and decision support systems. These methods can be used in streamlining and standardising approaches identifying potential hazards and focusing on their risk assessment. In addition specific case studies to test the efficacy of the DSS system in guiding risk assessment will be included.

1.17 BioOK: Risk assessment on transgenic plants combining effective new and traditional methods in a computerized decision support system

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Transgenic plants need specific approaches to analyze their potential impact on environment and consumer. The procedures used to date are often too extensive and time-consuming and associated with high costs.
The BioOK network was established to develop an effective and competitive risk assessment on transgenic plants based on interdisciplinary research. New and effective methods for the risk assessment were established which focus on transgene specific effects measured in *in vitro* systems in the laboratory or greenhouse whenever possible. Nevertheless event specific field experiments have to be carried out in addition to implement environmental effects e.g. on non targets or the variability of transgene expression.
The novel methods are combined with traditional procedures to form a multiplicative integrated decision support system (DSS) for the analysis of transgenic plants. The DSS is supported by a computerized tool using specific algorithms following a decision dendrogram. The decision rules are based on baseline, indicator and threshold data identified for the specific plant species. The prototype of the DSS is developed using potato as a model plant and to be validated on a cereal. The final decision is made by scientific expert judgment.

1.18 A Risk Analysis for the T25 GM corn event released in Brazil

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In 1998, Bayer Crop Science sent an application to the Brazilian Biosafety Technical Committee for the commercial release of T25 a herbicide-resistant GM corn event. In February 2008, after consideration of the committee advice, permission was granted by the Brazilian government for its environmental release.
We have performed a scientific analysis of the information accompanying the application provided by the company. We have analyzed the relevant information related to the molecular characterization of this event, the food safety studies and the environmental risk evaluation. The objective of this study was to perform an assessment of the safety of the T25 event, guided by the *Biosafety Assessment Tool* developed at the Centre for Integrated Research in Biosafety (INBI) (**Biosafety Assessment Tool**, GenØk - Centre for Biosafety, http://bat.genok.org/bat/, date accessed: 2010-08-25).
T25 was created by inserting the phosphinotricin acetyltransferase (*pat*) gene and other regulatory elements in the maize parent tissue culture line He/89 genome. We analyzed the various studies describing integration and stability of the insert. Our study provided details that indicate possible differences between how the DNA sequence of the insert is reported and how it is actually integrated into plant cells. There are differences between the patterns of the fragments generated after digestion with restriction enzymes, and the locations of the restriction sites used in the Southern blots, from what would be expected from the insert sequencing results. We hypothesize from these profiles that there is an unintended 250bp second partial copy of the novel gene (pat), inserted between the reported copy of the *pat* gene and the T35S terminator. Additionally, we comment on other aspects of the studies, such as the absence of positive or negative controls, or known molecular weight markers. We considered how these genomic-level differences might affect the amount or quality of expressed protein, and the implications for any subsequent analysis attempting to measure exposure, toxicity or allergenicity. For

example, the putative truncated copy of the pat gene, as well as the intended full length copy, could give rise to proteins with different post-translational modifications.
Studies of the agronomic performance in a few Brazilian environments were conducted but we considered there was insufficient information on the identity of the cultivated lines and the cultivation conditions in the field studies, including information about the use and management of the herbicide (glufosinate-ammonium) during the experiments.
We also concluded that the studies that were used to evaluate the environmental impact did not follow the recommendations of Annex III of the Cartagena Protocol of Biosafety. For example, no studies related to the impacts on biodiversity, non-target organisms, adaptation of new cultivars and coexistence were found.
Our assessment, using a new tool, appeared to detect potentially significant inconsistencies within the studies accompanying this application. T25 has been accepted by the regulators of some countries, such as US and Argentina, but has also been rejected by other regulators. This highlights how different regulatory authorities consider the available data presented in submissions and issues such as uncertainty. Our study indicates the need for scientific debate in Brazil related to the stringency of the biosafety risk assessment of T25; but also a public debate related to the regulatory process in general.

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1.19 Biosafety Status and Identified Gaps in Knowledge & Expertise in sub-Saharan Africa

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The objective of this study was to identify the status, needs and knowledge gaps regarding biosafety in sub-Saharan Africa (SSA), as the first part of a 3-year initiative to help support the development of effective safety and regulatory systems for biotechnology in the region.

More than 800 relevant stakeholders in SSA were consulted in an e-mail and telephone survey, which was supplemented with information from recently-published literature and various Internet resources. Overall, results suggested that there is a major need for capacity building in biotechnology and biosafety. For instance, at least 20 of the 48 SSA countries surveyed have the capacity to carry out agricultural biotechnology research and development (R & D), 12 of whom reported having a minimum of 6 licensed laboratories in which to carry out such work. However, technical capacity for R & D involving genetically modified organisms (GMOs) was found to be generally lacking across the sub-continent. Notably, South Africa and some Eastern African countries were found to have made significant advances in the deployment of modern agricultural biotechnology, as well as in the development of biosafety legislation and regulatory frameworks. Nonetheless, only South Africa and Burkina Faso have, to date, the regulatory experience of authorising the commercialisation of transgenic crops.

From a legislative perspective, most SSA countries were found to have a National Biosafety Framework, but only 13 have comprehensive biosafety regulations. In the absence of stand-alone biosafety laws, decision-making is based on existing cross-sectoral policies and laws of relevance, as well as on interim biosafety regulatory systems. There is a pressing need for countries with draft laws to now push them through parliament. On the whole, local technical expertise for the implementation of the regulatory systems appears low, especially with respect to having competent and academically-qualified personnel for the preparation and review of applications. There is also insufficient access to pertinent scientific and technical data and information upon which to base comprehensive and reliable risk assessments of new GM products, although recently improved IT infrastructure in the continent is expected to greatly enhance accessibility in the near future. It was also found that many SSA countries intend to include socio-economic considerations in the decision-making process regarding GM products.

The study results are currently being used to guide the implementation of the various training opportunities offered in the current ICGEB biosafety capacity building project, aimed at addressing locally-identified gaps and needs throughout SSA. These include organising training workshops for GMO developers, application reviewers, regulators, inspectors and members of both National and Institutional Biosafety Committees, to address the paucity of competent personnel for GMO regulation in the region. Further, fully-funded, full-time 1-year MSc fellowships in 'Managing the Environment', with special reference to risk assessment of GMOs, have been provided to 11 scientists and/or regulators from the region, to meet the need for academically-qualified personnel. A limited number of research fellowships are also available for the generation of much-needed biosafety data to accompany dossier evaluations. The project also sponsors delegations of scientists, dossier reviewers and regulators to international conferences, such as the ISBGMO, to familiarise them with internationally-accepted standards and practices in GMO regulation, and to facilitate their entry into the international biosafety debate. It is expected that such interventions will enhance the ability of countries in SSA to effectively regulate the products of modern biotechnology.

1.20 Biosafety of Genetically Modified Organisms in the Latin America and Caribbean Region: An Opportunity for Strategic Capacity Building

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Biotechnology has the potential to help improve food production in Latin America and the Caribbean (LAC), a continent where agriculture plays a dominant role. It is therefore critical that transgenic biotechnology is applied in such a manner as to capture its benefits and to minimise any potential risks from its application. To ensure this, regulatory oversight is therefore required in those countries intent upon using such technologies or their derived products.

An email-based stakeholder consultation to identify biosafety needs in the LAC region was carried out late 2009-early 2010, the results of which were then supplemented with data from recently-published literature. The study tracked developments in biotechnology and biosafety in the region and reviewed previous biotechnology/biosafety capacity building projects to culminate in a snapshot of the present situation. The study demonstrated that approximately half of responding countries are not carrying out R & D in GMOs, and further, a greater number have not developed commercial GM products beyond the proof-of-concept stage. Also, the majority of countries in the region have developed biosafety regulatory frameworks which describe risk assessment, risk management and decision-making processes. Nevertheless, the reality is that most have inadequate resources i.e laboratories, local experts and personnel, IT facilities (to access relevant scientific and technical information), established regulatory documents, insufficient funding, etc. to make these frameworks function. Only 36% of countries have operational biosafety regulatory systems in place, whilst, independent of the former, around two thirds of countries are currently developing biosafety legal instruments. During the interim period, pre-existing legislation is being relied upon to cover any activity in genetic modification. Overall, the study showed that the region is very heterogeneous in terms of biosafety proficiency, and as such, there has been no consensus at the regional level on how best to respond to global developments in genetic engineering and, particularly, whether to permit the importation and/or development of GM products.

Obviously, regulatory capacity must be enhanced in order to provide interested LAC countries with the necessary tools to assess and apply biotechnology. Acknowledging that methods of delivering capacity building should be tailored to specific demand-driven needs, the study therefore identified possible knowledge and expertise gaps in the region, to be used as a basis for possible training/support interventions. Efforts are continuing, via additional consultations with either knowledgeable individuals or via brain-storming sessions at relevant fora (e.g. the ISBGMOs), to elucidate the finer detail necessary to constructing a strategic approach for biosafety capacity building.

1.21 ELSI Associated Capacity-Building Activities for Biosafety: A Case Study in Taiwan

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Key words: biosafety education and capacity-building, ELSI (ethical, legal and social implications), Taiwan

Commercial biotech crops have been developed for fourteen years. As the biotech crop industry is still rapidly growing, society faces new challenges in policy-making processes. Because the commercialization of biotech crops involves considerations for consumer's attitudes, perceptions, and ethical dilemma, these often result into controversies over the risk and safety of biotech products along with the question of food security in the face of the growing population in the world. Researchers and educators need innovative education and training initiatives for their capacity building so that they can better incorporate biosafety strategies for developing ethically, legally and socially appropriate biotechnology. This paper reviews the current status of biosafety education and training programs in Taiwan, and addresses the challenges and requirements it faces in implementing ELSI associated education policies for biosafety-capacity building.

This paper proposes strategies to improve a biosafety education and training program in Taiwan partly to respond to the recommendations of the Parties to the Cartagena Protocol on Biosafety. The proposal includes the possibility of developing a model for biosafety education programmes that incorporate the needs of different regions. This proposal is not only related to biosafety education but also the issues of the rapidly developing biotech crop industry and its social roles.

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1.22 E-learning Master in Biosafety in Plant Biotechnology: a unique biosafety training network

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Biotechnology is transforming industry. It is an integral part of the knowledge-based economy and the main driver for the creation of new types of enterprises and the revitalization of old industries. Numerous new products are emerging from the use of modern biotechnology, such as:

1. Life-saving medicines and vaccines
2. Improved crops and new biofortified foods
3. Biofuels and high-added value chemicals
4. New products to replace fossil fuel derivatives as inputs for industry.

Biotechnology applications are also creating new opportunities in the process industries (e.g. food, paper and pulp, textiles and leather, mining) as well as in environmental improvement (bio- and phytoremediation). For the developing world, biotechnology promises better health, food security as well as wealth creation through increased industrial productivity. However, capturing the benefits of biotechnology ultimately depends not only on technological capacities, but also on institutions that ensure that public and environmental health are not compromised by its application.

The ability of developing countries to institute adequate safety systems and enforce regulation in biotechnology are vital elements in their efforts to exploit current and emerging biotechnology opportunities. It is widely recognised that the lack of expertise in regulation is a major bottleneck for these countries to reap the benefits of biotechnology at the research, industry and government level. The need to strengthen professional expertise in biosafety has thus become a priority of national and international efforts.

The UNIDO e-Biosafety training network is intended to meet this need. It addresses the demand of biosafety regulatory systems in developing countries for intensive training in biosafety. International experts with decades long experience in modern biotechnology have developed the curriculum in modular format drawing from best practices in distance education and instructional design.

The University of Ghent (BE) and the Marche Polytechnic University, Ancona (IT) are participating in this program by releasing yearly university certified diplomas in Biosafety in Plant Biotechnology.

1.23 Biosafety capacity building: experiences and challenges

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Biotechnology is revolutionizing industrial and agricultural practice as year by year the number of commercial biotechnology products is exponentially increasing. The anticipated wide-ranging socio-economic impacts require putting into place adequate safety standards, allowing technological advancement while preserving public health and the environment. This demands the presence of institutional capacities and professional competence in exercising regulatory oversight. While industrial countries have addressed biosafety with a number of regulatory approaches, developing and/or emerging countries frequently lack the standards for development, handling and commercialization of these biotechnology products. As a consequence they often face major barriers to access technologies and products. Therefore, many developing and/or emerging countries are currently setting up National Biosafety Frameworks in compliance with the Cartagena Protocol on Biosafety, which requires multi-disciplinary expertise and the ability to deal with a rapidly growing body of relevant information. This results in a need for professionals who can deal effectively with the assessment and management of biological risks. In order to address this need, intensive biosafety capacity building is required.
Different educational approaches can be used to train individuals in biosafety. Long-term education at Master level delivers an in-depth specialization in the subject and a profound development of the required competencies through very intensive interactive teaching and hands-on practical training. Although this type of education leads to academic accreditation, it does not make up a very appealing approach for biosafety education as it can be very expensive not only for students but also for course providers. Moreover, it is not attractive to professionals as it requires a full time study period. Short term courses overcome these disadvantages as they can rapidly address specific skills and needs. In addition, the costs arising from their organization are much lower compared to long-term education at Master level. The Institute of Plant Biotechnology for Developing Countries (IPBO, Ghent University) organized 3 summer courses of two-weeks on Biosafety in Plant Biotechnology (2004, 2005, 2006). While the first week provided an introduction on plant biotechnology and biosafety, international biosafety regulatory systems and GMO detection, the second week covered practical training on the preparation of notification for GMO releases.
However our experience showed that, due to the short training period, these courses cannot deliver an intensive training to deal effectively with the complexity of issues related to the assessment and management of biological risks. Therefore, IPBO decided in 2006 to switch to a different educational system based on e-distance learning, which combines both advantages of short- and long-term courses. This online accredited postgraduate course is organized annually at Ghent University in cooperation with the United Nations Industrial Development Organisation (UNIDO) (UGent) as part of their e-Biosafety network with additional nodes in Brazil and Italy (The Pontifical Catholic University of Sao Paulo and the Marche Polytechnic University Ancona). It delivers a solid basis to set up and implement regulatory biosafety frameworks related to plant biotechnology, and to assist in the legislation and interpretation of biosafety risk assessment, risk management and communication to policymakers or the public while minimizing geographical constraints and costs. Moreover, it makes up an attractive approach to reach professionals as it allows them to combine studying with their daily profession.
The course covers the entire range of disciplines related to biosafety by providing a program that includes an introductory section on plant biotechnology and its applications for agriculture and industry, while the main core covers the basics of risk assessment and regulatory structures, food and feed safety assessment, and

environmental safety assessment. In addition, an overview of national and international regulatory systems is included and the final section deals with risk perception and communication. Since the Academic year 2006-2007, 31 students participated in the course originating from 18 different countries. 16% of the participants originates from Europe, 13% from Asia and the Middle-East, and 71% from Africa. After three academic years 69% of the students have passed the course successfully.

Despite the advantages of e-distance learning and the fact that the students engage in real-time online tutorials and assignments, some educational challenges remain. We have experienced that there is a need for hands-on training. Therefore Ghent University hosts two on campus sessions of two weeks per year, which allows addressing tailored content responding to regional needs and priorities, hands-on practical training and evaluation of the students. In addition to these educational challenges, the course faces several logistic challenges, which may account for the modest rate of success. First of all students need to have access to a suitable internet connection. Secondly, although e-distance learning can minimize geographical restraints it cannot lift existing linguistic barriers. The UNIDO-UGent course is currently solely delivered in English. We do, however, experience a need for delivering the course in other languages like French, especially for the French speaking community in Africa, Spanish and Portuguese. Thirdly, the developed curriculum requires constant maintenance to ensure the continuous application of quality standards and streamlining of the material for specific needs. Finally, the sustainability of this project appears to be the biggest challenge. Despite the fact that biosafety in plant biotechnology is a major issue in society and decision making on the implementation of the technology, resources are decreasing year by year since the start of the project in 2006. This unfortunately resulted in a drop in scholarships, although they are essential to give students from developing countries the opportunity to participate in this course. Therefore it is important to keep bringing the value of biosafety education under the attention of governments and international organizations and to encourage them to mobilize resources for these projects.

1.24. Validation of detection methods for genetically modified soybeans, maize and cotton newly approved in Korea

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We have validated a qualitative detection method for nine different events of genetically modified (GM) soybeans (MON89788, A2704-12, DP356043-5, DP305423-1), four GM maizes (Mon89034, MIR162, Event3272, DP098140-6) and one GM cotton (GHB614), for which safety evaluations were approved in Korea. The information about the gene sequence, primer sequences, qualitative detection method and standard reference materials were acquired from their manufacturer.

The specificity and sensitivity were examined in the results of the validation using polymerase chain reaction. The detection limits were 0.005 % in MON89788, DP356043-5, DP305423-1, MON89034 and DP098140-6, 0.01 % in A2704-12 and E3272, and 0.05 % in MIR162 event. The 35s promoter and nos terminator sequence were detected in only MON89034. MON89788, DP356043-5, DP305423-1, DP098140-6 did not have both 35s promoter and nos terminator. All of the detection methods for 9 GM items have specificity to other GM events for which detection method had been validated or other crops, such as rice, wheat and barley. The detection limit of GBH614, which is GM cotton, was 0.016% by real-time PCR. Cross-contamination with other GM standards did not happen.

The validated method was shown to be suitable for screening of GMOs in processed food, and the approved 50 GM items in Korea can be detected by PCR.

1.25 GMO Detection as a biosafety tool: activities of the GMO Detection Lab of the National Institute for Agricultural Technology in Argentina (INTA).

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The GMO Detection Lab of the National Institute for Agricultural Technology in Argentina (INTA) was created as a service in 1998 based on the experience of a group of researchers in GMO development, in response to an increasing demand from the private and public sectors.
The Lab has standardized protocols for GMO detection and quantitation and has achieved accreditation for ISO/IEC 17025:2005 since 2007. As a consequence, the Lab validates all its methods, implements quality control protocols and participates in international proficiency tests.
The GMO Detection methods applied involve qPCR and PCR. The most frequently performed assays are screening for 35S Promoter and NOS Terminator. In addition, the Lab carries out other kinds of assays like detection of specific events (i.e. authorized GM events in Argentina) and specific genes present in unauthorized events.
The GMO Detection Lab receives samples from different sources such as farmers, food industries, brokers, breeders, seed companies, traceability certifying agencies, organic farmers, specialties farmers, etc. The INTA GMO Detection Lab also works as a referral laboratory for others GMO detection labs.
Samples analyzed are mainly seeds and grains. The main crop samples consist of soybean and maize but the Lab also analyzes canola, cotton, sunflower, tobacco, wheat, rice, among others. Moreover, derived and processed products such as flour, meal, starch, lecitine are also analyzed. Food industries also request detection of GMO in raw materials. Food products like corn-flakes, crackers, cookies, candies, oils, dehydrated soup and animal feed products are also analyzed for the presence of GMO. Samples come from different local regions and neighboring countries (Chile, Paraguay and Uruguay).
The most requested GMO detection assays are performed based on the requirements of the companies to export seeds or grains to different markets that have specific GMO regulatory policies and for seed quality control (no-GMO). In addition, the Lab services are required by companies that have traceability programs for their identity-preserved products, performing analysis in different steps of the productive chain. In this context, the Lab is able to respond quickly and efficiently in order to meet the challenging demands of today's market with the coexistence of GMO and non-GMO products in our country.
The Lab is involved in biosafety related activities together with national competent biosafety authorities. Therefore, the Lab assays samples provided by the Argentine Regulatory Agency (SENASA) in order to detect adventitious presence of GMO in imported materials mainly canola seeds.
The GMO Detection Lab provides training with special attention to the needs of local and Latin American professionals and technicians.
The Lab takes part in IRAM committee round table "Horizontal method for molecular biomarker analysis" (TC34/SC16) for international harmonization of methodologies in GMO detection.
The present work provides a statistical and qualitative analysis of the outcome data derived from the different Lab activities during the last years related to:
-Geographic distribution of the analyzed samples
-Sample types
-Events
-Client types
-The aim of GMO analysis

These results contribute to the GMO monitoring activities related to distribution and commercialization that might be useful for future decisions over biosafety issues in our country.

POSTER SESSION 2
Environmental Interactions

2.1. Does Bt maize have an effect on arthropod biodiversity? A case study from South Africa.

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Introduction

South-Africa is ranked number eight in the world with regard to total genetically modified (GM) crop area with 2.1 million hectares planted in 2009, equivalent to year-over-year growth rate of 17% (James, 2009). Bt maize (MON810) has been planted since 1998 in South Africa and has been favourably received by farmers (Kruger *et al*., 2009). This very high adoption rate by farmers reflects the fact that GM crops have consistently performed well and delivered significant economic, environmental, health and social benefits to both small and large scale farmers in developing and industrial countries (James, 2009).

The biodiversity of an agroecosystem is not only important for its intrinsic value but also because it influences ecological functions that are vital for crop production in sustainable agricultural systems and the surrounding environment (Hilbeck *et al*., 2006). Species assemblages in an agro-ecosystem fulfil a variety of ecosystems functions that may be harmed if changes occur in these niche assemblages (Dutton *et al*., 2002). Guild rearrangement due to the elimination of a target pest and subsequent changes in guild structure can lead to development of secondary pests. For this reason it is essential to assess the potential environmental risk the release of a GM crop may hold and to study its effect on species assemblages within that ecosystem (Van Wyk *et al*., 2007).

In order to identify possible future secondary pests and non-target effects, knowledge is needed on arthropod biodiversity in maize. This information can be used to evaluate the possible impact of Bt maize on non-target organisms at different trophic levels. Assessment of the impact of GM Bt maize on the environment is hampered by the lack of even the most basic checklist of species present in maize ecosystems.

The aims of this study were to determine arthropod diversity and abundance on maize and to compare diversity between Bt and non-Bt maize.

Materials and Methods

Collections of arthropods were done during the 2007/2008 and 2008/2009 growing seasons on Bt and non-Bt maize plants at two localities, i.e. Vaalharts in the Northern-Cape - and Venda in the Limpopo province of South Africa.

While arthropods were collected from Bt maize plants in commercial maize fields at the Vaalharts irrigations scheme, plants were collected from small fields of Bt maize planted with seed provided to resource-poor farmers in the Venda area. Pest pressure (target and non-target) is very high in the Vaalharts irrigation scheme and provides an unique opportunity for this study. In the Venda area resource-poor farmers have not been introduced to GM crops prior to this study.

Three maize fields were sampled per locality during each season (12 fields in total). Twenty plants, each of Bt and non-Bt maize (from the refuge area of the field), were randomly selected from the fields at each field. Each plant was bagged and all arthropods removed later. Arthropods were classified to morpho-species level as well as into functional groups that will assist in assessment of the potential exposure of species to Bt toxin in GM maize.

Data on species abundance and diversity was compared between Bt and non-Bt maize by means of the Shannon and Margalef diversity indices. Rank abundance graphs were also compiled.

Results and Discussion

Over the two year study a total of 6230 arthropods were collected that consists out of 19 orders and 281 morpho-species.

During the first growing season in Vaalharts a total of 2566 arthropods were collected which consisted of 126 morpho-species. The diversity indices indicated that there were no significant differences between species richness and diversity for Bt and non-Bt maize. Although there was a trend of higher numbers of individuals per plant on non-Bt maize than Bt maize, the numbers did not differ significantly. Although the incidence of Lepidoptera larvae was low, there was no significant difference between the mean numbers of Lepidoptera larvae that occurred on Bt and non-Bt maize plants.

During the second growing season a total of 1533 arthropods were collected from plants that consisted out of 281 morpho-species. The diversity indices indicated that there were no significant differences between species richness and diversity for Bt and non-Bt maize.

A total of 2131 arthropods were collected on maize in the Venda area that consisted out of 242 morpho-species of which 7.83% also occurred at Vaalharts. The diversity indices indicated that there were no significant differences between species richness and diversity of arthropods in Bt and non-Bt maize.

The arthropods on maize were divided into different functional guilds. The proportion made up by the different guilds was similar on Bt and non-Bt maize. Herbivores were the predominant group followed by natural enemies.

Conclusions

Arthropod biodiversity in maize was high and no difference between Bt and non-Bt maize was observed.

Acknowledgement

This work forms part of the Environmental Biosafety Cooperation Project between the Republic of South Africa and the Kingdom of Norway coordinated by the Department of Environmental Affairs, Directorate of Nature Management and the South African National Biodiversity Institute. We accordingly give due acknowledgement.

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2.2 Effect of cry 1ab protein from Bt maize (MON810) on the biology of *Chrysoperla pudica* (neuroptera: chrysopidae)

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Resistance development of target pests and possible non-target effects has been of concern since the first deployment of genetically modified crops with insecticidal properties. It is especially at the 3rd trophic level and with important predators such as lacewings where negative effects of Cry 1Ab protein could have adverse effects in agro-

ecosystems. Larvae of green lacewings, *Chrysoperla* spp. (Neuroptera: Chrysopidae), are voracious active predators with a high effective searching capacity. Among the pray consumed, are pests of economic importance such as aphids, whiteflies, mites and mealy bugs. *Chrysoperla* larvae also have a very high prey consumption rate, for example larvae of *C. zastrowi* may consume an average of 488 aphids or 906 potato tuber moth eggs during their larval life stage. These characteristics enable larvae of *Chrysoperla* species to be effective biological control agents. Previous studies on the effect of Bt proteins on the performance and biology of *Chrysoperla* spp. showed contradicting results. Some studies ascribed poor performance of *Chrysoperla* to poor quality prey, caused by the effect of Cry1Ab on the prey. Since Bt-resistant maize stem borer (*Busseola fusca*) larvae were available in a rearing colony, it provided a unique opportunity to study the effect of Cry 1Ab protein, consumed by healthy prey, on biology and fitness of *Chrysoperla pudica.*

An experiment was conducted to determine the effect of consumption of Bt protein-fed prey on *Chrysoperla* larval and pupal development. The experiment consisted of five treatments (diet groups) with 50 *Chrysoperla* larvae in each treatment. Larvae were fed on a daily basis after hatching until pupa formed. One treatment consisted of only *E. cautella* eggs. In two of the other treatments (Groups 1 & 2), *Chrysoperla* was fed 2^{nd}-3^{rd} instar Bt-resistant or Bt-susceptible *B. fusca* larvae reared on Bt-maize and conventional maize (control for Group 1) respectively. The other two treatments (Groups 3 & 4) were similar to the above but *E. cautella* eggs were provided on alternate days. Larval mass was determined at 3-day intervals. Larval and pupal development time as well as larval mortality and overall mortality (including mortality during the adult emergence phase) was also determined. Mean larval mass as well as percentage survival and number of days to pupa formation were calculated. Data were analyzed by means of ANOVAs and Cox's binomial test.

The optimum diet was *E. cautella* eggs and larval survival was highest and development time shortest on this diet. Results showed that there were no significant differences between mean larval mass, development time as well as overall mortality between treatments in the four groups. The pupal period in Group 1 that were fed only Bt-resistant borer larvae was however, significantly shorter than that of its control treatment (Group 2) which consumed only borer larvae exposed to non-Bt maize. Larval development time to pupation was however significantly shorter in Groups 3 and 4 compared to Groups 1 and 2, and is ascribed to the fact that these *Chrysoperla* larvae were also provided with *E. cautella* eggs.

The overall result of this study, in which the possible effect that food quality could have had was excluded, showed that Cry1Ab protein had a negligible effect on larval development and survival of *C. pudica*.

The likelihood of chrysopid larva feeding only on Lepidoptera that consumed Bt protein is low. Aphids are the main food source of these predators and they do not ingest the Bt proteins when feeding on Bt-plants. Thus under field conditions it is unlikely that the larvae will consume such a high number of Bt exposed Lepidoptera larvae as done in these laboratory experiments. It is more likely that they will feed on various pray species including aphids and other soft bodied arthropods. It is however important to assess the effects at field level where prey is more diverse.

2.3 Low levels of refuge compliance contributed to the evolution and spread of *cry 1ab* resistance in the maize stem borer, *Busseola fusca* (lepidoptera: noctuidae) in South Africa.

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Introduction

The first report of resistance of maize stem borer (*Busseola fusca*) to Bt maize (MON810) was made in South Africa during 2007 (Van Rensburg, 2007). Within one year of the first report of resistance of *B. fusca* another reportedly

resistant population was observed by farmers at the Vaalharts irrigation scheme, approximately 50 km from the initial site. No effective management strategy currently exists to limit the spread of resistance and prior to this study, no investigation to quantify the status of resistance has been done.

Bt maize has been grown at the Vaalharts irrigation scheme in South Africa since its first release during 1998. Research into aspects such as the level refuge compliance, pest incidence and production practices at Vaalharts followed on the first report of field resistance of *B. fusca* to Bt maize. Although the planting of refugia is compulsory to limit resistance development, the level of compliance at areas where resistance was reported was not known. The current refuge requirements are either a 20% refuge planted to conventional maize which may be sprayed with insecticides, or a 5% refuge area that may not be sprayed. The reasons for development of resistance by *B. fusca* to Bt maize are not known. It is however possible that non-compliance to refuge requirements played a role.

The objective of this study was to evaluate the status of resistance of different populations of *B. fusca* to Bt maize and to evaluate the level of resistance of borer populations occurring on the refuge-plantings of maize at the locations where resistance was reported. A survey was also conducted to determine farmers perceptions of the regulatory aspects guiding the planting of Bt maize and refugia, its benefits and disadvantages.

Material and Methods

Greenhouse study on levels of resistance

The experiment consisted of eight treatments. Four stem borer populations were used on each of a Bt- and conventional hybrid. The four stem borers populations were the Vaalharts Bt-resistant, Vaalharts refuge and two known susceptible populations. The following two hybrids were used: DKC 78-15B (transgenic, MON810), CRN 3505 (non-Bt iso-hybrid for DKC 78-15B).

The study was conducted in a greenhouse using potted maize plants (90 for each treatment), each artificially infested with 10 neonate larvae. Nine plants of each treatment were dissected 2, 4, 6, 8, 12, 16, 20, 25, 30 and 35 days after inoculation. The numbers and mass of live larvae per plant were determined at each sampling date. The experiment was terminated on day 35 when the first pupae started to form. Mean larval mass was calculated for each sampling date and survival was expressed as percentages.

Farmer survey

A survey was conducted amongst 80 farmers at the Vaalharts irrigation scheme where resistance to the target pest was reported. The questionnaire addressed signing of contracts upon purchasing genetically modified (GM) seed, refuge compliance, refuge design and general farming practices. Farmers were also questioned on the perceived benefits and disadvantages of Bt maize and their perceptions of the pest status of *B. fusca*.

Results and Discussion

Results from the greenhouse study confirmed observation of resistance of *B. fusca* at the Vaalharts irrigation scheme to Bt maize. Larval survival of the F1 populations collected from the field indicated that the population with expected resistance to Bt maize (Vaalharts Bt-resistant) as well as its refuge population (Vaalharts refuge) successfully completed their life cycles on Bt maize in the laboratory, with no significant difference observed in either the level of survival or mean larval mass (Kruger *et al*., accepted for publication).

Larvae collected from Bt maize at Vaalharts used in the greenhouse study were reared successfully for another three generations on Bt maize after completion of the experiment. The question arises whether the use of refugia could still serve its purpose in the Vaalharts irrigation scheme where resistance now seems to be wide-spread. If a large enough susceptible pest population is not maintained, alternative strategies will have to be investigated to manage the further spread of resistance (Kruger *et al*., accepted for publication).

The survey conducted on refuge compliance and farmer perceptions yielded interesting results. Farmers indicated that the two greatest advantages associated with Bt maize was convenient management (88 %) and increased productivity (61.3 %) while 42.5 % indicated that they perceived Bt technology to be environmentally friendly.

Initial levels of refuge compliance was low, and even though farmers were obligated to plant a refuge area for each Bt maize field, only 7.7 % of farmers planted refuges during 1998. This number increased to 100 % during 2008 (Kruger *et al*., 2009). Eight percent of farmers however indicated that they did not plant a refuge field for each Bt maize field which was justified on the basis of too small farm sizes (25 ha). Farmers preferred to plant the refuge option where 5% of the field area is planted to conventional maize which is not sprayed. In South Africa stewardship programs instituted during the 2008/2009 growing season, involve grower education programs as well as the compulsory signing of contracts between companies and farmers that contractually bind farmers to comply with refuge requirements accompanied by on-farm inspections (Kruger *et al*., 2009).

Conclusions

This study confirmed resistance by *B. fusca* to the *Cry*1Ab toxin, indicating the geographical distribution of resistant populations to include at least the Vaalharts area, in addition to the original report by Van Rensburg (2007) for the Christiana area. It appears that stem borer resistance to Bt maize in the Vaalharts area resulted from a combination of a late general planting date with consequent increased levels of infestation and variance in time of planting, providing a continues supply of moths (Kruger *et al*., 2009). Further research is needed on insect resistance management and the high/dose refuge strategy in order to limit the evolution and spread of resistant stem borer populations in South Africa.

Acknowledgements

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2.4 Effects of genetically modified maize (MON810) and its residues on the diversity of microorganisms in the rhizosphere bulk soil: A glasshouse study

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The cultivation of genetically modified mazie has raised a lot of questions regarding their effects on the microbial diversity. A glasshouse study was carried out at the University of Fort Hare (South Africa) to determine effects of Bt maize residues on soil microbial diversity. Residues of white Bt maize (PAN 6Q-121B), and its near-isogenic non-Bt maize (PAN 6Q-121) were incorporated into soil in pots and seeds of the same cultivars were planted in the corresponding pots. Two other treatments were pots containing soil without residues but planted with either the Bt maize or the near-isogenic non-Bt maize. Fertilizer was applied to all the treatments and water was added to replenish losses due to evapo-transpiration. Rhizosphere and bulk soil was destructively sampled from each treatment and analyzed for microbial community level physiological profiles using Biolog plates with 31 different

carbon substrates and water as a control. Absorbance in the Biolog plates was recorded after 120 h of incubation at 20°C.
Cluster analysis dendrograms showed that the presence of residues had an effect of rhizosphere microbial diversity throughout the 13 weeks, with a lower effect of the type. Microbial diversity in the bulk soil followed the same trend except that, at the last sampling, the non-Bt maize with or without residues were clustered together away from those with a growing Bt plant which were separated by presence or absence of residues. These findings suggest that residues and root exudates from Bt maize have similar effects on microbial diversity in the rhizosphere and only minor, non-recurring, differences in bulk soil.

Keywords: Bt maize, bulk soil, microbial diversity, rhizosphere, substrate utilization

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2.5 The effect of Bt maize on non-target soil pests of maize seedlings in South Africa

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Keywords: *Agrotis segetum*, Bt maize, *Heteronychus arator*, maize seedling pests, *Somaticus angulatus*, South Africa.

Introduction

The lepidopterous stem borers *Busseola fusca* (Fuller) (Noctuidae), *Sesamia calamistis* (Hampson) (Noctuidae) and *Chilo partellus* (Swinhoe) (Crambidae) are effectively controlled by Bt maize that express the Cry1Ab insecticidal protein. Many studies have been done on the controlling effect of Bt maize on the target pests of maize, but literature dealing with the effect of Bt maize on non-target pests of maize is scarce in South Africa. Another lepidopterous species, the cutworm *Agrotis segetum* (Denis and Schiffermüller) (Noctuidae), which is the most common and injurious pest of maize seedlings in South Africa, is exposed to Bt toxin for a part of its life cycle. The same with *Heteronychus arator* Fabricius (Coleoptera: Scarabaeidae) and *Somaticus angulatus* (Fahraeus) (Coleoptera: Tenebrionidae) which, are regarded as sporadic but serious pests of maize seedlings. However, the effect of this exposure to Bt maize has not been studied yet. The objective of this study was to determine the effect of Bt maize expressing Cry1Ab on these three non-target pests of maize seedlings.

Methods

Direct effects of Bt maize on non-target pests can easily be measured in laboratory experiments that stretch over the entire lifecycle of the pests. Laboratory studies were conducted with first- and fourth instar larvae, and moths of *A. segetum*. Feeding studies were conducted to determine the effect of Bt maize on mortality, growth and fertility of *H. arator*. Larval survival and mass gain as well as beetle fertility were also determined for *S. angulatus*.

Results

The effects of Cry1Ab toxin on the biology of *A. segetum* larvae and moths were largely insignificant. The effects of the two Bt maize events (MON810 and Bt 11) on the different parameters measured in the cutworm study was not

similar between the Bt events and their respective iso-hybrids. Compared with larvae that fed on conventional (non-Bt) maize, Bt maize did not affect survival of first instar larvae. However, mean mass of cutworm larvae that fed on Bt maize (Bt11) was significantly lower. Feeding on Bt maize did not have a significant effect on development period to *A*. *segetum* pupa formation. Fewer eggs were laid by *A*. *segetum* moths fed as larvae on maize event Bt11 compared with MON810. The results for the beetle study showed that the effect of Cry1Ab toxin on the biology of *H*. *arator* and *S*. *angulatus* was insignificant. No significant differences were observed in any of the parameters measured in this study.

Conclusions

This study indicates that Bt maize will most likely not have any significant effect on the control of *A*. *segetum* under field conditions and that Cry1Ab toxin targeting lepidopteran pests will not have an adverse effect on either *H*. *arator* or *S*. *angulatus*. Therefore, other control measures still need to be taken in South Africa to control these pests of maize seedlings.

2.6 Indirect exposure of the maize stem borer parasitoid, *Sturmiopsis parasitica* (Tachinidae), to Bt maize: an case study from South Africa.

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Keywords: Bt maize, *Busseola fusca*, South Africa, *Sturmiopsis parasitica*, tri-trophic study.

One of the most important components of integrated pest management is biological control and the preservation of natural enemies of pest arthropods. *Sturmiopsis parasitica* (Curran) (Diptera: Tachinidae), is an important larval parasitoid of gramineous stem borers in Africa. The large-scale cultivation of transgenic crops may carry potential ecological risks to natural enemies. To date no tri-trophic study was conducted in South Africa to determine if there is any effect of Bt maize on parasitoids. Such studies are essential and form part of the toxicity screening at higher-trophic-level species. This is because organisms at higher-trophic-levels are exposed to the toxin in an altered form due to processing by the herbivores. If no tri-trophic experiments are conducted, the effect of toxin processing in the herbivore gut is ignored entirely and, thereby, important ecological interactions among plants, herbivores, and natural enemies may be missed. The presence of Bt-resistant populations of the target pest, *B*. *fusca*, in South Africa provides the ideal opportunity to evaluate the possible indirect effects of the Cry 1Ab protein on the 3rd trophic level. The objective of this study was to determine if there is any effect on *S*. *parasitica* when parasitizing Bt-resistant *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) diapause or fourth instar larvae that have fed on Bt maize.

Bt-susceptible and Bt-resistant *Busseola fusca* larvae, originating from different rearing populations were parasitized (inoculated) with two to four *S*. *parasitica* maggots each. Host larvae were screened daily until parasitoids emerged. Parameters measured for parasitoids were duration of maggot stage in host larvae, duration of the parasitoid pupal stage, as well as pupal mass and pupal size.

Although not always significant, the percentage parasitism of Bt-consuming host larvae was always higher compared to host larvae that fed on non-Bt maize. It could be that Bt toxin affected the *B*. *fusca* larval fitness to such an extent that the immune systems were weakened, but that the larvae were still suitable for parasitization. The different parameters tested indicated only one case where maggots originating from diapause host larvae feeding on non-Bt maize had a greater mass compared to maggots from host larvae that fed on Bt maize. The same applied to *S*. *parasitica* pupal length. For the rest of the parameters tested there were no significant differences.

Although some adverse effects were observed on *S. parasitica* mass and pupal length it is most likely that this will not contribute to adverse effects in the field, but that there may rather be synergism between Bt maize and *S. parasitica*. Decreasing the target pest populations to minimal numbers, however, can drastically change existing multi-trophic interactions in the field. The impacts of this elimination of parasitoid host larvae that are totally controlled by the Bt plant are not yet clarified. One of the most obvious ways in which transgenic maize may affect the level of tachinid parasitism is by decreasing density of the host larvae. However, *S. parasitica* parasitize more than one lepidopteran species and all of these host larvae attack various crops and wild grasses. Therefore, even if the number of tachinid parasitoids decline due to stem borer depletion in Bt maize fields, their persistence in the environment is probably not threatened.

2.7 Using an ecological model to improve risk assessments for Bt maize: an example from South Africa.

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Introduction

South-Africa is ranked number eight in the world with regard to total production of genetically modified (GM) crops. Bt maize (MON810) has been planted since 1998 in South Africa and has been favourably received by farmers (Kruger *et al*., 2009). This very high adoption rate by farmers and the large area planted to Bt crops necessitates effective risk assessment for future releases as well as post-release monitoring of the possible effects of Bt crops on target pests (resistance development) and non-target arthropods.

Different models can be used to assess the risk of transgenic crops to non-target organisms. The ecotoxicological model aims to evaluate potential non-target effects of chemicals released into the environment and non-target species effects of GM crops (Andow & Hilbeck 2004b). In the ecotoxicological model universal indicator species are chosen because of their supposed sensitivity to chemical toxins, their wide availability, their ease of culture, and their genetic uniformity. Such species are supposed to provide information on the likely effects of the chemical on a wider range of species (Andow & Hilbeck, 2004b). The most serious problem with this approach is that it is not consistent with the need for case-by-case risk assessment that considers the relevant transgene, crop plant, and cropping environment. In the ecotox model, the primary end point is mortality or some other acute response to short-term chemical exposure. One of the shortcomings of the ecotox model is that indicator species are often not present in the environment where new technologies will be adopted.

An ecological model on the other hand is highly appropriate to improve environmental risk assessment and to tailor it to specific environments. In such a model local species can be classified functionally and prioritized using ecological criteria to identify potential test species, assessments and end points in the evaluation process (Andow & Hilbeck 2004b). The environmental risk assessment process described for Bt maize in Kenya by Andow & Hilbeck (2004a) assessed the possible risks of transgenic crops on biodiversity. In that model it was recommended that species be selected from assemblages, that the potential for risk be identified and that research protocols be developed to assess these risks before release of GMOs.

The aim of this paper is to illustrate the applicability of the ecological model approach to prioritize species for evaluation in risk assessment of Bt maize. Data that will be presented in this case study deals with identifying priority Lepidoptera species that is most likely to be affected (adverse or beneficial) by Bt maize, evaluation of priority species for potential exposure and possible effects that may be caused by exposure. Knowledge gaps regarding the biology and ecology in maize is also identified and the possibility of secondary pest development highlighted. The methods described in this paper provides a framework for selecting priority species for risk assessment studies as well as post release monitoring of GM maize.

Discussion

The diversity and incidence of target and non-target Lepidoptera on maize is continuously being studied. Through surveys a list of species of Lepidoptera that are directly consume maize as food source was compiled. Seventeen species of Lepidoptera have so far been recorded on maize. These have been prioritized for their close association with maize, general occurrence in the maize growing regions and their potential for economic damage if they become secondary pests. Through use of a selection matrix knowledge gaps were identified for future research and guidance of the design of ecologically realistic experiments. Non-target species populations with the highest maximum potential exposure to Bt toxin are *Sesamia calamistis* (Lepidoptera: Noctuidae), *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Acantholeucania loreyi* (Lepidoptera: Noctuidae). Because of their sporadic occurrence, *Agrotis segetum* (Lepidoptera: Noctuidae) and *Spodoptera exigua* (Lepidoptera: Noctuidae) are considered to be of lesser importance than *H. armigera* and *A. loreyi*, but should also be considered during pre- and post-release monitoring.

Through use of the ecological model and after considering life strategies and insect dependence on maize in certain agro-ecological zones, it was hypothesised that the *Busseola fusca* (Lepidoptera: Noctuidae) was a high-risk species for resistance development. The continuous use of transgenic crops may result in an increased risk that insect species directly exposed to Bt maize may evolve resistance to Bt proteins and that secondary pests may develop. Of the three stem borer species on maize in South Africa *B. fusca* and *C. partellus* are the most dominant while *S. calamistis* occur at very low levels. The risk of resistance development differs between species based on geographical area and cultivation practices. The species most likely to develop resistance in the Higveld region was predicted (by the ecological model) to be *B. fusca* while *C. partellus* will be the species most likely to develop resistance in low-altitude subtropical and arid-areas. *Busseola fusca* is the dominant and often the only stem borer species in the South African Highveld region where Bt maize was released in 1998 while *C. partellus* is the dominant species in low-altitude regions where *B. fusca* often does not occur. The first report of resistance of *B. fusca* to Bt maize was subsequently made by Van Rensburg (2007) in of South Africa. Studies on gene-flow between different geographic populations of the resistant target pest is under way.

Field surveys indicated that a non-target pest, *H. armigera*, which only attacks maize ears, is suppressed by Bt maize. This may result in resistance development and complications with regard to H. armigera resistance management in areas where Bt cotton is planted in proximity to maize.

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2.8 Identifying Patterns in Non-target Arthropod Communities using the Principle Response Curve (PRC) Method

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A limitation in nontarget arthropod monitoring programs has been analysis and interpretation of species abundance at the community-level rather than on a species-by-species basis. The Principal Response Curve (PRC) method enables analysis of all populations simultaneously, and provides a clear presentation of species patterns as they relate to a pre-assigned control. Results from a PRC analysis are presented for two field studies conducted in the Argentina maize belt for transgenic maize lines producing insecticidal proteins from *Bacillus thuringiensis* (Bt). Field studies examined abundance of key nontarget arthropod groups in maize plots containing a stacked event line, the individual events that are part of the stacked event, and the near-isogenic line as the pre-assigned control. Arthropod abundance was monitored throughout the growing season using pitfall trapping, sticky card sampling, and visual plant inspections. Additionally, crop phenological events were correlated with arthropod activity. Species data was analyzed using Redundancy Analysis (RDA), from which species patterns were plotted as PRCs. Monte Carlo permutation tests were used to examine the significance of patterns as they related to explanatory variables. Consistent across sampling methods, arthropod communities associated with the Bt maize products were comparable to those of untransformed maize. PRC trends illustrated the natural variability of environments in which maize cropping systems are established. Additionally, a critical crop phenological event (pollen shed) was identified as a key determinant of arthropod distribution for certain taxa. The PRC method improves upon traditional analyses by readily identifying species patterns and distinguishing those patterns from the variability inherent in ecological systems. The utility of this method is discussed in the context of a tiered approach to nontarget risk assessment.

2.9 Levels of Cry1Ab in Bt maize, in different tissues and between plants, at different growth stages

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Background and aim Insect resistance development is a concern to farmers, since it negates the benefits of Bt crop. In 2007, Van Rensburg (2007) published the first report of resistance to *Buseola fusca* in South Africa. It has been suggested that insect resistance to Bt maize may have developed due to the survival of larvae in the presence of sub-lethal levels of toxin. This may be due to low levels of production at late stages of plant growth or the migration of larvae from Bt maize to non-Bt maize refugia (Kruger et al. 2009; Van Rensburg 2007). It appears that there was increased selection pressure for resistance to develop in South Africa, especially in maize grown in irrigation schemes, due to non-compliance with refugia. Although it has been suggested that gene stacking may

increase the life expectancy of Bt varieties, not knowing the underlying reasons for resistance development may also negate the durability of second generation insect resistant transgenic plants. Nguyen and Jehle (2007) published an extensive study on the levels of Cry1Ab toxin produced in maize roots, stalks, leaves, anthers and kernels over time up to ripening. However, there is still important outstanding data in terms of Bt toxin production in silk, cob sheath and soft cob tissue as well as later stages of plant growth and how this may impact insect resistance development. For example, Van Rensburg (2001) found that *B. Fusca* larvae had an increased survival rate on Bt after using silk as primary food source. In addition to this, it appears that migration may play an important role in larval survival (Kruger et al. 2009; Van Rensburg 2007). Bates et al. (2005) also suggested that non-expressing Bt 'off-types' may compromise the high dose strategy. Compared to this, we hypothesize that a major factor in insect resistance development is the fluctuation in levels of Cry1Ab, in different tissues between plants as well as at different growth stages. Thus the aim of this study was to determine the levels of Cry1Ab in roots, stems, leaves, tassels, silks, cob sheaths and cobs within and between plants at different growth stages pre-flowering (V20), flowering (R1), green cob stage (R4) and seed maturity (R6).

Materials and methods A MON810 converted maize variety was grown according to commercial farming practice in South Africa, but without the use of herbicide or insecticide spraying, at Bainsvlei during 2008/2009 and 2009/2010. Plant tissue was collected at four growth stages: pre-flowering (V20), flowering (R1), green cob stage (R4) and seed maturity (R6). A total of 55 plants were sampled per growth stage and tissue collected on ice and kept at -20^{o}C before freeze drying where after they were stored at -20^{o}C. Lyophilized plant material was homogenized in a Waring blender at 4^{o}C and protein extracted from 100 mg tissue in a PBS buffer (pH 7.2) containing 0.55% Tween 20. The Cry1Ab concentrations were determined using the QuantiPlate ELISA Kit for Cry1Ab/Cry1Ac (Envirologix) according to a standard curve of 1 ng/ml, 2 ng/ml, 6 ng/ml and 10 ng/ml of Cry1Ab toxin (Biosence). Statistical analysis of the data included ANOVA and Bonferroni-Holml test using Daniel's XL Toolbox (version 2.60) for Excel 2007.

Results and discussion There was a significant difference in the level of Cry1Ab between the different growth stages as well as different types of tissue. The lowest level of Bt toxin was found in roots at seed maturity (6.7 ug/g), stems at preflowering (7.0 ug/g), leaves at seed maturity (9.4 ug/g), silk and tassel at floweing (31.5 ug/g and 31.4 ug/g, respectively), cob and cob sheath at seed maturity (1.0 and 1.6 ug/g, respectively). There was an overall increase in the level of Bt toxin from pre-flowering up to flowering followed by a decrease up to seed maturity in roots, stems and leaves. For example, levels of Cry1Ab increased in stems from 7.0 ug/g at pre-flowering to 18.6 ug/g at flowering and decreased to 9.8 ug/g at seed maturity. Similarly, the levels of Bt toxin in leaves increased from 57.0 ug/g at pre-flowering to 65.4 ug/g at flowering followed by a decrease to 9.4 ug/g at seed maturity. There was a considerable range of Cry1Ab production in the same tissue between plants. For example, at flowering the level of toxin ranged from 5.9 ug/g to 30.0 ug/g in stems and from 36.4 ug/g to 99.9 ug/g in leaves compared to 1.4 ug/g to 23.1 ug/g in stems and 27.2 to 102.1 ug/g in leaves at pre-flowering.

Conclusions The results of this study have shown that there is a significant change in the production of Cry1Ab in different tissues at different growth stages with an overall increase up to flowering followed by a decrease until seed maturity. This may have a severe impact of the survival ability of the target insect. The levels of toxin production in different tissues is congruent with the findings of Van Rensburg (2001) that the larvae feeding on silk tissue (31.5 ug/g toxin) which migrate to the cob sheath (8.5 ug/g toxin) or cob tissue (2.7 ug/g tissue) have a higher survival rate. A further point of consideration is that the difference in toxin production between plants may benefit insect survival in a population where resistance is developing. We hypothesize that while other factors my contribute to resistance development, once resistance has developed, a major factor is the ability of larvae to survive as a result of migration from tissue containing higher to lower levels of Cry1Ab. Finally, in terms of gene

stacking, as a solution to ensure sustainable insect resistant management, it is important to determine whether gene stacking may affect the toxin production from different stacked *Cry* genes.

2.10 Cry1Ac levels on Bt cotton leaves according to the storage condition and cotton phenological growth stages

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Key-words: Cry monitoring, Bt cotton, storage condition.

This study aimed to test foliar preparation for shipment to remote analysis of the Cry toxin level in transgenic plants and the monitoring of the toxin foliar level along the transgenic crop phenology in order to support research in areas of commercial cropping of Bt cotton in post-release monitoring.

Materials and Methods

The varieties used were transgenic Bt NuOpal and non-transgenic DeltaOpal croped in December 2007 in Brasília – DF Brazil, with five plots per variety sowed in randomized blocks with 90 cm between rows and 8-10 plants/m. The leaves used in the experiments were collected from the most apical pair.

The storage test of the cotton leaf samples intended to simulate the farmers' shipment conditions. The treatments consisted of Bt and non-Bt cotton leaves collected 120 days after plants emergency, seven replicates in triplicate each: a) leaves collected immediately before analysis (control group), b) leaves stored in ice for 72 h styrofoam box; c) leaves stored without refrigeration for 72 h in a styrofoam box; and d) leaves dried in an oven at 60 °C for 24 h. The samples weights were standardized to 1 g, macerated in liquid nitrogen and homogenized in 3 mL of cold 1X PBS pH 7.4, following subsequent removal of the particulate material by centrifugation at 10,000 x*g* for 15 min at 4 ° C. The estimation of Cry1Ac foliar average was performed by sandwich ELISA using the kit Bt1Ac SDI (Gehaka ®), according to the manufacturer's protocol. To construct the calibration curve, purified Cry1Ac was solubilized in 0.05 M sodium carbonate buffer pH 9 in quantities 25, 12.5, 6.25, 3, 1.5, 0.75, 0.375 and 0 ng (reading at 450 nm). It was estimated the average levels of toxin in cotton leaves in µg of Cry1Ac per gram of fresh leaves by linear regression equation between the patterns of Cry1Ac and their absorbance values.

The monitoring of Cry1Ac amount along the Bt cotton phenology was made by regular collection (20-30 days) of the Bt and non-Bt varieties, in five replicates in triplicate, in the following stages: a) vegetative; b) beginning of the reproductive phase; c) flowering; d) fruiting; and e) senescence. The leaves were collected freshly and frozen in liquid nitrogen prior to storage at -20 °C. The samples preparation and the molecular analysis were performed according to the methodology described above, except for the new calibration curve of 20, 10, 5, 2.50, 1.25, 0.675, 0.3125 and 0 Cry1Ac ng.

Results

The storage condition of the Bt cotton leaves did not affect the mean level of Cry1Ac [CI (95%) = 1.114 ± 0.012 µg/g of fresh leaf] (ANOVA, F = 4.04, *P-value* = 0.010). The preservation of the equivalent Cry1Ac level was due to short-term storage prior to molecular analysis, demonstrating that there is no need of special preservation methods of the plant material extracted from the field and sent for analysis in remote laboratory, facilitating therefore the analysis of toxin expression in commercial farms. However, the study about the time and conditions of storage must be made on a case-by-case according to the host plant species, tissue and kind of transgene to be monitored.

The monitoring of the toxin concentration throughout the NuOpal Bt cotton phenological stages showed that there was a reduction in the Cry1Ac concentration throughout the plant development. The Cry1Ac amount was higher in the early vegetative stage (4.36 µg/g of fresh leaf), decreasing sharply in the early reproductive phase (0.50 µg/g of fresh leaf), recovering in the middle of the reproductive phase (2, 28 µg/g fresh of leaf), and decreasing again in the fruiting (0.64 µg/g fresh of leaf) and finally, was lower than all other phases at the end of the cycle (0.15 µg/g

fresh of leaf) (ANOVA, F = 34.66, *P-value* = 0.025). The Cry1Ac amount in the NuOpal variety declined approximately 29 times (96% decrease) between the early vegetative stage to the senescence. Similar pattern of decay of the Cry1Ac level through the physiological cotton maturing has been reported elsewhere. Some authors warn that this decreasing is due to the reducing of the total protein content following the tissues aging; however the proportion of the toxin in relation to the total amount of protein becomes progressively larger.

Discussion

Fluctuations and, especially, the temporal decrease in the Bt toxin level are of extreme relevance to the pest and toxin resistance managements, might being sufficient to favor the increasing of resistance of target organisms in Bt cotton varieties. Additionally, the reduction in crop protection in the most susceptible stage to attack the target organisms, like *Heliothis virescens* (tobacco budworm) and *Pectinophora gossypiella* (pink bollworm), pests of reproductive structures in the advanced stages of crop phenology, goes against the technology of genetic modification for resistance to insect pests.

Data regarding the Cry1Ac content may be correlated to the pests and their natural enemies monitoring. For comparative analysis proposed above, this study demonstrated the importance of collecting the samples from plants at the same phenological development.

2.11 Field evaluation of Bt Cotton effects on non-target pests

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Key-Words: biosafety, Cry toxin, pest, non-target species

Bt cotton expresses a Cry 1Ac protein highly specific for control of Lepidopteran caterpillars, although it may present unexpected effects on non-target species such as the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) and the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). The present study aimed to compare the colonization rates and population dynamics of these non-target pests in field plots of Bt and non-Bt cotton. The experiment was conducted on an experimental field of the Embrapa Recursos Genéticos e Biotecnologia - Cenargen in Brasilia, DF, Brazil for two consecutive years (2007/8 and 2008/9), under a randomized block experimental design with five replications per treatment (Bt and non-Bt cotton). Each plot had 12 rows of 10 m, with 10 plants/m in an area of 100 m^2. The number of winged and wingless aphids/plant and the presence of ants and ladybeetles were monitored every 2-3 days. The sampling was conducted in sets of a sequence of 10 plants in the planting row and 5 sets of plants were randomly selected per plot. The boll weevil population was monitored by collecting cotton squares and bolls in the top of the plant, and the structures dropped on the ground under the plant. On each plot, five points were selected in a Z form track and the closest and more developed plant was chosen in each sampling point. A sub-sampling of two reproductive structures (square or boll) with boll weevil attack (oviposition marks) was taken from each sample. Each structure was kept in a plastic cage (250 mL) in an heated room (25 ± 2ºC, 60% RH and 13 h of photofase) for evaluation of adults hatching. The colonization rate of plants by winged aphids did not present differences between the treatments. There was also no difference in the abundance of wingless aphids and in the production of winged aphids between the treatments. The results suggests that the parameters that control population establishment such as initial plant choice, fecundity, survivorship, predation and dispersion was not affected by the Bt gene insertion in the cotton plant. The abundance of coccinelids, specific predators of aphids and ants that spread aphids among the plants did not differ significantly between the treatments, supporting the above statement. Reproductive structures such as cotton

squares were produced in the same amount in both Bt and non- Bt plants. The boll weevil attack measured through the number and percentage of reproductive structures and the average number of adult boll weevils emerged from the squares, did not differ significantly between the treatments. We conclude that the Bt cotton did not affect the population parameters evaluated of these two key-pests of cotton.

2.12 Diversity of arthropods in genetically modified BR and RR cotton crops in northern Santa Fe

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Genetically modified crops have been widely expanded within the agricultural systems in Argentina in the last few years. The production and marketing of cotton with two biotechnological events (MON 531 x MON 1445) resistant to Lepidoptera and glyphosate was authorized in Argentina in February 2009 (ArgenBio, 2007). The adoption of cotton BG / RR marks a turning point in the production of cotton and this is the first year of commercial production in our country, it is necessary to obtain information on abundance, richness and diversity of arthropods with stacked events for the environmental conditions the northern region of Santa Fe. The aim of this study was to determine the abundance, richness and diversity of arthropod present between genetically modified cotton crops BR and RR. This research work was carried out during the cotton season (2009-2010) in the field of Agricultural Experimental Station of INTA Reconquista, Santa Fe (Argentina), located at 29 ° 11 'S and 59 ° 52'W. The experimental design was randomized complete block with five replications. The plot included 12 rows spaced 0.52 m by 15 m long. Treatments used were two GM varieties: 1) BR NuOpal Lepidoptera and herbicide-resistant and 2) G2000 RR herbicide resistant. Delinted seed to acid was used, treated with systemic insecticide and fungicide. Sowing was done in late December to test drill for conventional tillage. No pesticide application was made after planting; only was applied growth regulator and defoliant. Sampling of arthropods was conducted from February to April 2010, at 30, 60 and 90 days after emergence. For the survey of soil stratum, 2 pitfall traps with saline solution were placed per plot, separated by 6 m, during 7 days. For the aerial plant samples was used a G-Vac (garden-Vacuum). Two samples of G-Vac for each plot using a STIHL blower. Each sample by suction for one minute represents an area of one square meter. The collected material was preserved in individual containers with 70% ethyl alcohol, properly labeled and taken to the laboratory for further identification. Placed a total of 20 traps and 20 sites were aspired by sampling date. With individual data for each treatment per replication from pitfall trap and G-Vac were calculated H diversity indexes (Shannon) and r (richness) by using the PAST program. InfoStat/P (Di Rienzo ***et al***, 2010) as statistical software was used to perform variance analysis, and averages were compared with Tukey test ($\alpha <= 0.05$). We captured a total of 14,469 arthropods. Using pitfall traps were captured 7705 individuals while 6864 with the G-Vac, In the pitfall tramps, the most abundant were the ants (66%), collembolans (12%) and Diptera (10%), while the G-Vac were aphids (67%) and Diptera (17%). No differences were found between treatments for both indexes (H r), however the mean values were always higher in G2000 wealth. Diversity indexes were found: H = 2.23 (NuOpal BR) and H = 2.22 (G2000) G-Vac and H = 1.48 (NuOpal BR) and H = 1.41 (G2000) with traps fall. The richness index values that were found r = 25.60 (G2000) and r = 22.20 (NuOpal BR) with G-Vac while in pitfall traps were r = 34.80 (G2000) and r = 30,20 (NuOpal BR). According to the results obtained in this growing season, the diversity and richness of arthropods present in the crop would not be affected by using varieties with two events (resistant to lepidopteran and herbicides) compared with varieties with only one event (herbicide resistant).

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2.13 Reduced foliage herbivory in *Bt* cotton benefits phloem-feeding insects

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Genetically engineered cotton plants that express Lepidoptera-active Cry toxins from *Bacillus thuringiensis* (*Bt*) are grown on 15 million hectares worldwide. Numerous studies have established that these plants pose a negligible risk to non-target arthropods due to the narrow spectrum of activity of the expressed Cry toxins. A potential indirect effect of *Bt* cotton that has received little attention is the interactions between the introduced insecticidal trait and the natural insect defence system of the cotton plant.

An integral part of the cotton defence system is a group of closely related terpenoids: gossypol, hemigossypolone and four heliocides. Those terpenoids are efficient toxins against a broad range of pest organisms and cotton varieties with low terpenoid levels are highly susceptible to insect attack. Terpenoid production in cotton is induced by insect damage. The defence induction, however, is not equally distributed over the entire plant. Cotton follows the optimal defence hypotheses by protecting valuable plants parts, like young leaves, better then less valuable parts such as old mature leaves.

Interestingly, not all insects can cause the terpenoid induction in cotton. While most caterpillars with chewing mouthparts induce the cotton defence system, phloem-feeding insects like aphids and whiteflies do not. In the field, cotton plants are usually under the attack by a broad range of insect pests and there is evidence that phloem-feeding herbivores are to some extent controlled by the terpenoids produced in response to caterpillar attack. We have thus hypothesized that the reduced damage caused by caterpillars to *Bt* cotton would lead to a lower concentrations of cotton terpenoids and subsequently would benefit other herbivores.

We tested this hypothesis by monitoring the population dynamics of cotton aphids (*Aphis gossypii* Glover) on Lepidoptera-damaged, mechanically damaged and undamaged *Bt*- and non-*Bt* cotton plants under greenhouse conditions and in a field experiment conducted at the USDA/ARS research station in Tifton (Georgia, USA). As study plants, we used a Bollgard II© variety expressing Cry1Ac, Cry2Ab and an herbicide-resistance trait. As control we used the nearest non-*Bt* expressing isoline containing only the herbicide-resistance trait.

One week after the respective treatment of the study plants in the greenhouse, mixed stages of *A. gossypii* were released on the youngest fully-developed leaf of the plants. Aphid abundance was then recorded after two weeks. As hypothesized, aphids performed better on *Bt* cotton plants that were less damaged by caterpillars compared to the non-*Bt* control plants. Similar differences in the aphid populations were observed in the field experiment. Samples of plant material will be analysed for their terpenoid content using HPLC to confirm that the observed differences in aphid populations were caused by differences in the defence induction.

2.14 Assessing the impact of insecticidal GM crops on non-target arthropods: characterizing exposure levels

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When assessing the risks of insect-resistant genetically modified (GM) plants on non-target arthropods, both exposure to the expressed insecticidal protein and the toxicity are considered (Romeis et al. 2008). This poster illustrates major exposure pathways of non-target arthropods to plant-expressed insecticidal proteins using examples from our own research with Bt maize.

Herbivores, and many predatory species, ingest plant-expressed insecticidal proteins by directly feeding on plant tissue, including pollen (Li et al. 2010, Romeis et al. 2009). Species with chewing mouthparts (e.g., caterpillars or beetles) or with piercing-sucking mouthparts that feed on epidermal or mesophyll cells (e.g., thrips, mirid bugs, or spider mites) generally ingest the insecticidal protein (Meissle and Romeis 2009). In contrast, in the case of Bt plants, phloem-feeders, such as aphids, take up at most traces of the expressed Cry proteins, since they are not transported in the phloem (Romeis & Meissle 2010). Whether or not pollen feeders are exposed depends on the promoter that controls the transgene and the transformation event. For example, Cry3Bb1 concentrations in pollen of corn rootworm-resistant Bt maize MON88017 are close to the concentrations measured in leaves (Meissle and Romeis 2009), while pollen of corn-borer resistant Bt maize MON810 contains less than 1% of the concentration in leaves (Romeis et al. 2009).

The major route of exposure for many entomophagous arthropods is via their prey or hosts, which can be either herbivores that have fed on the GM plant, or other entomophagous species (Romeis et al. 2009). The toxin concentration contained in herbivores depends on the species´ mode of feeding, on the site and time of toxin expression, the amount of plant material ingested, and the rate of proteolytic degradation and excretion (Romeis et al. 2009). Consequently, exposure of entomophagous species is highly variable and depending on the species and life stages of consumed prey and hosts. For example, Bt protein concentrations in field collected *Theridion impressum*, a generalist European spider, were shown to range between 0 and 2.5% of the concentration in plant tissue (Meissle and Romeis 2009).

The apparent complexity in calculating the exact level at which non-target arthropods are exposed to insecticidal protein produced by GM plants has lead to simplifications for current non-target risk assessment. Many studies have shown that transgenic proteins are diluted along the food chain. Consequently, the highest expression level in the plant is considered adequately conservative as a basis for the calculation of the concentration to be tested in laboratory toxicity studies to inform the regulatory risk assessment (Romeis et al. 2009).

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2.15 Laboratory toxicity studies demonstrate no adverse effects of Cry3Bb1 and Cry1Ab to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae)

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For the two most common insecticidal Cry proteins that are expressed in *Bacillus thuringiensis* (*Bt*)-transgenic maize varieties, i.e., Cry1Ab and Cry3Bb1, most peer-reviewed laboratory and field studies have revealed no detrimental effects on several non-target ladybird beetles (Coleoptera: Coccinellidae). However, a recent study raised concern in the scientific and regulatory communities because it claimed toxicity of purified Cry1Ab and Cry3Bb1 to larvae of the two-spotted ladybird beetle *Adalia bipunctata* in direct feeding studies. The study was subsequently cited by the German authorities as new scientific evidence for potential environmental harm that supposedly justified the suspension of *Bt* maize (event MON810) cultivation in 2009. However, the study has been criticized because of methodological shortcomings that make it questionable whether the observed effects were due to direct toxicity of the two Cry proteins. We therefore conducted tritrophic experiments assessing whether an effect of the two proteins on *A. bipunctata* could be detected under more realistic routes of exposure. Spider mites that had fed on *Bt* maize (events MON810 and MON88017) were used as carriers to expose young *A. bipunctata* larvae to high doses of biologically active Cry1Ab and Cry3Bb1. Ingestion of the two Cry proteins by *A. bipunctata* did not affect larval mortality, weight, or development time. These results were confirmed in a subsequent bioassay in which *A. bipunctata* were directly fed with a sucrose solution containing dissolved purified proteins at concentrations approximately 10 times higher than measured in *Bt* maize-fed spider mites. Hence, our study does not provide any evidence that larvae of *A. bipunctata* are sensitive to Cry1Ab and Cry3Bb1 or that *Bt* maize expressing these proteins would adversely affect this predator. Our results show that the harmful effects of the two Cry proteins reported in the previous study were likely false positives, i.e., artifacts of poor study design and procedures. This shows the importance of following minimum quality standards in conducting laboratory non-target studies for assessing the environmental risk of GE crops. It is thus important that decision makers evaluate the quality of individual scientific studies and do not view all as equally trustworthy. Consequently, not all peer-reviewed scientific studies are equally relevant for environmental risk assessment.

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2.16 Structure of an aphid-parasitoid food web on transgenic disease-resistant wheat

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Ever since the introduction of genetically modified (GM) crops their use has brought up concerns about their potential effects on non-target organisms. Many studies have looked at the influence of GM plants on single insect

species. However, agricultural ecosystems are characterized by numerous species which are all involved in complex interactions forming so-called food webs. In ecology such interactions have always gained attention since the diversity and complexity of such insect food webs are considered to be important factors that determine ecosystem function and stability.

In this study we looked at transgenic disease-resistant wheat (*Triticum aestivum*) and its effect on aphids and their associated parasitoid food webs. It is known that insect host-parasitoid systems are influenced by plant traits and that plant nutritional quality directly affects trophic interactions by influencing morphology, behaviour and life-histories of insects. Furthermore, genetic modification can cause compositional differences between GM varieties and their conventional counterparts which can affect organisms feeding on the plant. It may also alter phloem-sap composition which in turn is known to affect aphid performance. We thus hypothesized that the genetic modification of the wheat lines directly or indirectly affect aphids and that these effects cascade up to change the structure of the associated food webs.

We planted different experimental wheat lines in the field as well as under semi-field conditions and allowed natural colonization by insects. The aphid population was regularly monitored and parasitoid mummies were collected. The mummies were kept under room temperature until adult parasitoids emerged which were then identified. We constructed quantitative aphid-parasitoid food webs for the different experimental wheat lines and compared their properties to the corresponding non-transgenic sister lines.

2.17 Compatibility of transgenic legumes and natural enemies to control bruchids (Coleoptera: Bruchidae)

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Starchy leguminous seeds are an important staple food and source of nutrition in many countries. Bruchid beetles (Coleoptera: Bruchidae) are responsible for the greatest post-harvest losses to stored legumes. Surface and fumigant chemical applications are thought to be the most effective methods for managing bruchid infestations. However, prohibitive costs and the risk of adverse secondary effects from such treatments have led to the exploitation of alternative control measures and the adoption of more integrated management approaches. In this context, the combination of plant resistance factors and biological control provided by hymenopteran parasitoids has been suggested to control bruchid infestations (Schmale et al., 2003). Potent plant resistance factors include α-amylase inhibitors (αAI) which disrupt the carbohydrate metabolism of several insect pests, including bruchids. Genetic engineering has been used to transfer an α-amylase inhibitor (αAI-1) from the common bean, *Phaseolus vulgaris,* to other leguminous plants. Several studies have confirmed the resistance of genetically modified (GM) legumes expressing αAI-1 against some major bruchid pests, e.g. *Callosobruchus maculatus*. However, other bruchid species (e.g. *Acanthoscelides obtectus*) are resistant to αAI-1 (Ishimoto and Kitamura, 1992; Shade et al., 1994). Parasitoid larvae or host-feeding females might therefore get in contact with the inhibitor when attacking resistant bruchid larvae that have ingested αAI-1. However, whether bruchid parasitoids rely on α-amylases for carbohydrate digestion and whether they are significantly exposed and harmed by the inhibitor has so far not been investigated. Therefore, we have developed a conceptual model describing a pathway on how the presence of αAI-1 in GM legume seeds might negatively interfere with the biological control services provided by bruchid parasitoids (Lüthi et al., 2010). The steps of our model include the characterization of α-amylase activity in parasitoid extracts, the *in vitro* sensitivity of the digestive enzyme to αAI-1, as well as the assessment of the hazard and exposure to the plant-expressed αAI-1 in tritrophic experiments.

At the moment we have characterized the α-amylase activity in five different parasitoid species commonly used to control bruchids: *Anisopteromalus calandrae* (Pteromalidae), *Dinarmus basalis* (Pteromalidae), *Lariophagus distinguendus* (Pteromalidae), *Eupelmus vuilleti* (Eupelmidae) and *Heterospilus prosopidis* (Braconidae). α-Amylase activity was found in females and larvae of all parasitoid species, suggesting that both

larvae and females rely on α-amylases for carbohydrate digestion. *In vitro* inhibition studies have confirmed that α-amylase activity of both larvae and females of all parasitic wasps are strongly susceptible to αAI-1. Future research will include tritrophic experiments to evaluate the impact of GM chickpeas and cowpeas expressing αAI-1 on the parasitoids *in vivo*.

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2.18 Impact of six transgenic rice lines on four non-target thrips species attacking at panicle development in the paddy rice field

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Transgenic crops, since their origin, have been considered for their impacts on non-target arthropods. A two year field experiment was conducted in order to observe the non-target effects of six transgenic rice lines (KMD1 and KMD2, Huachi B1 and Huachi B6, expressing the Cry1Ab protein; TT9-3 and TT9-4 expressing the Cry1Ab/Cry1Ac protein) on four non-target thrips species *Frankliniella intonsa* (Trybom), *Frankliniella tenuicornis* (Uzel), *Haplothrips aculeatus* (Fabricius) and *Haplothrips tritici* (Kurd) as compared to their parental control lines. Sampling techniques to observe the non-target thrips were beat plate and plastic bag methods.

Results

There were some significant and non-significant differences in the total thrips population density of four thrips species over the entire period of sampling. More significant results were found in both transgenic KMD rice lines as compared to their control Xiushui 11 during two years sampling seasons by plastic bag sampling method as compared to other two groups of Bt rice lines. While there were no significant differences in the tested three groups of Bt rice lines by beat plate method over the two years of sampling seasons.

Conclusions

If transgenic rice is affecting the non-target piercing-sucking insects, it can be an integrated pest management approach to control these insects in rice. So further investigation is needed in order to clearly understand the mechanisms if the effects of *Bt* toxin are found upto third trophic level i.e. the predators. A tier based study should be conducted for biological agents in rice.

2.19 Prey-mediated Effects of Transgenic *cry*1Ab Rice on a Beneficial Spider, *Pardosa pseudoannulata* (Araneida: Lycosidae)

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One major concern regarding the release of *Bt* rice is its potential impact through tritrophic interactions on non-target arthropods, especially natural enemies. *Pardosa pseudoannulata* (Bösenberg et Strand) (Araneae: Lycosidae) is one of the dominant spider species in rice field in most of Asia. It moves around the rice paddy and usually hunts prey at the bottom of rice plants. It can effectively regulate the pest population of leafhoppers and planthoppers, and is the most important predator of brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) which is one of the most serious pests in paddy field in south Asia.

In this study, we investigated the effects of transgenic *cry*1Ab rice varieties, KMD1 and KMD2, on this predatory ground spider, *P. pseudoannulata*, supplied with *Bt* rice-fed *N. lugens* nymphs. Although immunoassays confirmed that Cry1Ab protein can be transferred from lower trophic levels (*N. lugens*) to higher trophic levels (*P. pseudoannulata*) through tritrophic interactions, no bioaccumulation of Cry1Ab protein occurred and no negative effects were found on spider survival and development. Furthermore, the fecundity of *P. pseudoannulata* fed prey reared on *Bt* rice was not significantly different from that of those fed prey reared on non-*Bt* rice. Additionally percentage of *P. pseudoannulata* testing positive for *N. lugens* remains under field condition was tested using ELISA and PCR. No significant difference was found between *Bt* rice and non-*Bt* rice field. Functional response results showed that their functional response all belong to Holling Ⅱ type reaction, and the attack constant and handling time were not significantly different.

In conclusion, the transgenic *cry*1Ab rice tested in this study had no negative effects on the survival, developmental time or fecundity of *P. pseudoannulata* in laboratory or on biological control in the field.

2.20 Bt-rice Effects on Insect Biodiversity in Paddy Fields

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In order to test the effect of a genetically modified Bt-rice variety, a field survey of the insect diversity in paddy fields was conducted by a motorized backpack suction trap (MBS trap) and sweeping net sampling. The total insect samples collected were separated by their function as pests, natural enemies (insects and spiders independently), and others. Samples were made every two weeks after plantation and compared by their function groups.
As a result, two Bt-rices did not show any effects on insect diversity in paddy fields in comparison to their mother varieties. It was also confirmed that the MBS trap is handy and useful to test the effect of GM rice on insect diversity in small sized experimental fields.

2.21 Transgenic *cry*1Ab rice "KMD2" can not result in the outbreak of its non-target herbivore the brown planthopper *Nilaparvata lugens*

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With the introduction of the insect-resistant cultivars, it is necessary to assess any impact on other members of the ecosystem. The brown planthopper (BPH), *Nilaparvata lugens* (Stål) is the non-target insects of Bt rice, and become a major pest in recent years. Thus, whether the Bt rice could develop outbreaks of BPH needs to be evaluated. In this study the non-target effects of Bt rice "KMD2" expressing a Cry1Ab protein on the performance of BPH over multiple generations were evaluated under laboratory and field conditions. In the laboratory, BPH was reared to observe the impact of the Bt rice as compared to its parental non-Bt cultivar Xiushui 11, while the population dynamics and oviposition performance of BPH were investigated in the field.

Results

At the rice seedling stage, the nymph duration of BPH feeding on KMD2 and Xiushui 11 was significantly affected by rice type and generation; adult longevity was significantly affected by generation and rice type with generation interaction; egg production was affected by rice type and generation. At the adult stage of rice, the nymph duration of BPH was affected by rice type and rice type with generation interaction; egg production was affected by rice type and generation. The results indicated that the nymph duration was significantly prolonged and the egg production was significantly reduced when the BPH fed on the KMD2 at both seedling and adult stage. In the field investigations, the average density of BPH in the KMD2 field was lower than that in Xiushui 11 field all the sample times in 2008 and 2009. The average density of nymphs, and nymphs plus adults showed a significant difference between KMD2 and Xiushui 11 in the field both in three sample times in 2008 and four sample times in 2009. By comparison with the non-Bt rice, both percentage of tillers with eggs and number of eggs per tiller in the Bt rice were significantly lower from jointing to mature stage for the former and from heading to mature stage for the later. The relative height of oviposition on the Bt rice was significantly higher than that on the non-Bt rice at the tillering, heading and filling stage, while it was significantly lower than that on the non-Bt rice at the rice mature stage.

In general, our results suggest that the transgenic *cry*1Ab rice KMD2 will not result in outbreaks of BPH in the field.

2.22 A new laboratory method to test direct GM crop effects on in-vitro reared honeybee larvae

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The honeybee (*Apis mellifera*) is a key non-target arthropod in environmental risk assessment of genetically modified insect resistant crops. Transgenic insecticidal proteins primarily affect the larval phases of target organisms and older honeybee larvae are directly exposed to GM pollen during their development. Therefore robust and highly standardized testing methods for in-vitro rearing honeybee larvae are required to minimize potential risks for this important pollinator. In the laboratory, we applied a new GM plant risk assessment method for honeybee larvae by adding transgenic pollen directly to the larval diet. We tested pollen of two transgenic and three conventional maize varieties. Assessment endpoints were survival and pre-pupae weights of the tested individuals. As positive control we fed toxic pollen to the larvae. In the pollen treatment groups, there was no increase in larvae mortality observed. The multiple insect resistant Bt-maize pollen treatments did not show any adverse effects on survival rates or pre-pupae weights of honeybees. In our positive control, a clear toxic effect was present. We recommend that laboratory risk assessments on non-target organisms should follow existent GM plant exposure patterns. The direct exposure to the plant produced insecticidal proteins via pollen feeding should

improve the strength of GM plant risk assessments on honeybees. We believe that our novel method has the potential to become a standard method in honeybee environmental risk assessments.

2.23 Honeybee maize pollen foraging in differently structured landscapes: Colony and single worker exposure to an important GM crop.

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Honeybees use pollen of a broad range of GM crops as protein source. This makes honeybees a key non target organism (NTO) in risk assessment of genetically modified crops. Despite its outstanding role as NTO, comprehensive information about the exposure of honeybee colonies or single worker bees to important GM crops in a landscape context is lacking so far. Here we studied the pollen content of the bee rectum and the amount of maize pollen collected in pollen traps in a range of maize field covers. We compared twelve complex agricultural landscapes with an increasing proportion of maize fields and varying availability of alternative food resources. A honeybee colony was placed in the centre of each landscape at the beginning of the maize pollen shedding period. Pollen traps were used to record the pollen spectrum collected by each colony. Pollen analysis of the recta of nine days old worker bees (N=10 /colony) was used to analyse the individual ingestion of maize pollen. Our results show a strong correlation between the pollen diversity in the pollen traps and the pollen composition within individual bees, indicating a fast turnover of collected pollen within honeybee colonies. Thus, pollen analysis of the rectum of individual worker bees could be used as a representative monitoring method to measure transgenic maize pollen exposure. Our data further indicate that under the condition of a complex landscape the contribution of maize pollen to the protein diet of honeybee colonies and individual honeybees is of minor importance. We suggest that studies to assess colony and single worker exposure on a landscape scale should be taken into account when establishing GM crop environmental risk assessment schemes for honeybees.

2.24 Environmental risk assessment of genetically modified pear trees using honeybees

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Honeybees are the most important pollinators of fruit trees and are a key test species used in the biosafety assessment of genetically modified crops. To assess potential impacts of transgenic pear trees for honeybee colonies, adult honeybees were fed with a sucrose solution containing non-transgenic pear pollen or pollen, expressing antibiotic resistance genes nptII and hpt. Transgenic pollen also contained marker genes gus and gus-intron, respectively. We evaluated weight, longevity and flight activity of worker bees, queen productivity, sealed brood, and productivity of bee colonies. Studies showed no negative effects of transgenic pollen on these parameters. Moreover hpt pollen-fed bees did differ significantly from control bees in the timing of their longevity,

flight activity, sealed brood, and honey production, whereas nptII pollen-fed bees - flight activity and honey production only. Our results suggest that the transgenic pear has no adverse impacts on honeybees. To our knowledge, this is the first report of testing transgenic pollen of fruit trees on honeybees.

2.25 Earthworm numbers, biomass and activity in soil with growing Bt maize (MON810) cultivars in the Central Eastern Cape, South Africa

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Keywords: activity, biomass, Bt maize, Cry1Ab, earthworm

The introduction of genetically modified crops in South Africa, for better pest and weed control, has raised concerns on the effects of such crops and their residues on soil ecosystems. A study was carried out at the University of Fort Hare Research Farm (South Africa) to investigate effects of growing genetically modified maize (MON810) on population, biomass and activity of local earthworms. The study was laid out in a randomized complete block design with two Bt maize cultivars (DKC61-25B and PAN6Q 321B) and their corresponding near-isogenic lines (DKC61-24 and PAN6777), which were replicated three times. Both Bt maize cultivars expressed the Bt protein, Cry1Ab, in the roots, stems and leaves.

Earthworm numbers did not vary significantly with growing season in all four treatments. At six weeks after planting, the DKC61-24 treatment had higher earthworm numbers than the DKC61-25B and the other two lines. However, at nine weeks after planting, the earthworm number and biomass in the DKC61-24 treatment was lower than in the DKC61-25B but compared well with the other two treatments. At 18 weeks after planting, earthworm numbers, casts were not affected by the maize treatments. While the DKC treatments had higher earthworm biomass that the PAN treatments, there were no effects of genetic modification on earthworm biomass. The findings suggest that growing maize with the MON810 transformation event would not have negative effects on earthworm population, biomass and activity in the Central Eastern Cape, South Africa.

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2.26 Genetic characterization of *Diatraea saccharalis* populations in Argentina and insect resistance management for Bt corn.

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Different events of transgenic insect resistant maize expressing the *Bacillus thuringiensis* Cry1Ab toxin have been grown extensively across Argentina during the last decade. The main lepidopteran target pest in Argentina is the sugarcane borer, *Diatraea saccharalis* (F.) (Crambidae). The efficacy of these transgenic events might be limited by the capacity of the target insect to develop resistance.

The evolution of insect resistance to Bt crops depends on many different genetic, biological and environmental factors. Some of the genetic variables are the initial frequency of resistance alleles, the mode of inheritance of resistance (degree of dominance, number of loci), fitness costs associated with resistance alleles, insect population structure, and gene flow among populations.

In Argentina, an insect resistance management plan for Bt corn is encouraged by the Regulatory Agency (CONABIA) and complied with by the seed companies by means of a monitoring program. So far, no resistance has been documented for field populations of *D. saccharalis* in this country. Nevertheless, there has been no research into the genetics of the local sugarcane borer populations intended to understand the population history and genetic processes of this species.

The problem to address is a product of the dynamics of an artificial system in nature where evolutionary processes are operating. In this context, a research project has been implemented at INTA with the aim of understanding the population genetic structure and processes of this target insect of transgenic Bt corn.

Samples of *Diatraea saccharalis* populations were collected from several locations all along the country from 2005 to 2009, including regions of high and low levels of Bt corn adoption, different host crops within the same locations and different generations within a cropping season. From each location and sampling date, a minimum of 30 individuals were processed for molecular marker analysis. Due to the lack of previous genetic studies of this species, the molecular markers used were random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), which are anonymous, neutral and dominant markers, and mitochondrial sequences (COI). Recent developments in population genetic statistics enabled the use of robust software for the analysis of many population genetic parameters using these sorts of molecular markers. Populations were grouped either by location of sampling, geographical area, by host crop, or by generation within a season, according to the hypothesis to be tested. Thus, the following estimates could be determined: genetic diversity within populations, allelic frequencies, genetic differentiation among populations, hierarchical genetic diversity partition, effective population size, migration rates, etc. A phylogeographic analyses was also performed. The results are being further analyzed, but the preliminary results so far suggest that there is no significant genetic differentiation among host races (e.g. Φ_{pt} 0,0075; p=0,24 for the mitochondrial marker), that, at the national scale, most of the genetic variability lies within populations (e.g. >83% with RAPD marker), that there exists a moderate degree of genetic differentiation among populations (>10%), and that there is also important gene flow among populations. In the context of resistance evolution, the lack of host race differentiation suggests no mating restriction for insects developing on different crops in neighboring fields, which are considered as functional refuges. Also, gene flow

through migration is one of the main forces to avoid genetic isolation, one of the key genetic processes which could have led to the evolution of Bt resistance in other species.

2.27 How to consider long-term effects in Problem Formulation

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The Environmental Risk Assessment (ERA) of GM plants should follow a step-by-step assessment approach. In their latest guidance document version, the EFSA GMO Panel describes the various ERA steps, as described in Directive 2001/18/EC (EC, 2001), starting with a Problem Formulation step including hazard identification (EFSA 2010). Problem Formulation (PF) includes the identification of characteristics of the GM plant capable of causing potential adverse effects to the environment (hazards), of the nature of these effects, and of pathways of exposure through which the GM plant may adversely affect the environment (hazard identification). It also includes defining assessment endpoints and setting of specific hypotheses to guide the generation and evaluation of data in the next risk assessment steps (hazard and exposure characterisation). In this process, both existing scientific knowledge and knowledge gaps (such as scientific uncertainties) are considered. More detailed guidance for applicants on how to apply problem formulation on specific areas of risk to be addressed in the ERA is provided in the EFSA guidance document (EFSA 2010)

A general requirement of an ERA is that an analysis of the "cumulative long-term effects relevant to the release and the placing on the market is to be carried out" (EC, 2001). Predicting and assessing (adverse) long-term effects requires information about the GM plant and the receiving environment(s), both in terms of the baseline conditions in the receiving environment(s) and temporal changes in these conditions following GM plant introduction. The rate and degree to which the baseline is likely to change independently of the GM plant (e.g. as a result of new crops and agronomy) will vary among production systems. The consideration of long-term effects in the ERA should address effects that might arise up to at least 10 years after the start of cultivation, i.e. corresponding to the time frame of the consent authorisation (EC, 2001, EFSA, 2008), but should also cover the time period over which progeny of the GM plant might persist after harvest in the seedbank and appear as volunteers or ferals.

According to EFSA (2010) long-term effects might result from a diversity of primary causes and secondary interactions, which make it difficult to generalise on methods of investigation. Such effects can be considered in two broad categories:

1. In the first category, long-term or chronic exposure to a particular GM plant or practice results in a delayed response by organisms or their progeny (Category I). It may be in some instances that a response occurs immediately, but is not detected by the measuring tools or the particular indicators employed. For example, exposure over time may affect a species or community by suppressing certain functional forms in relation to others, or acting on natural mutations that exist at very low frequency such as occurs when pests develop resistance to a pesticide.
2. In the second category, effects occur as the result of an inevitable increase in spatial and temporal complexity, determined by the number of possible interactions that a GM plant would have with the biota and the physical and chemical environment as it is grown more widely throughout the landscape and in more extended sequences of cropping (Category II). There may not necessarily be a chronic or delayed effect as in the first category; rather, the effect occurs in certain contexts that are outside those experienced in the initial testing, or that have arisen as entirely new contexts due to global environmental change or the adoption of new forms of management. The latter may indeed arise as a downstream effect of the introduction of the GM plant cultivation itself if this causes a change in the sequence or range of

plants grown in the production system.

Some effects of Category I might be investigated, at least in principle, in constrained experimental systems maintained over several generations of the organisms under study. Questions will still remain; however, as to how much the constrained system restricts the range of possible reactions or encourages untypical reactions, such as caused by a reduced choice in the foraging range and food available to invertebrates that are kept for months or years in controlled environment chambers or restricted to intensely managed field plots.

Category II, by definition, cannot be investigated through an initial experimental phase of testing, even at the scale of the field plot, half-field or paired field, none of which can provide the range of complexity experienced after full commercial release.

Appropriate desk-based studies within PF might frame the assessment of long-term environmental effects of the GM plant in relation to both categories. Examples of the type of information that could be used in PF are:

1 Experience of cultivating the GM plant or long-term environmental exposure in other regions;
2 Experience from cultivation of similar plants (GM and non-GM);
3 Long-term ecological or environmental datasets applicable to the receiving environment(s); e.g, government statistics on cropped areas, pesticide usage, nutrient inputs, agrochemicals in water; ecological surveys showing change in organisms range or abundance;
4 The results of major field experiments on GM plants or other factors that have examined effects or GM plant events similar to those of the GM plant under assessment;
5 Quantitative examples of the degree to which previous agricultural change, even if not involving GM plants, has affected the appropriate ecological and environmental indicators;
6 The results of meta-analyses drawing together data from different sources
7 The use of models of ecological processes to explore or test scenarios: mathematical models of ecological processes are unlikely to be considered sufficient justification on their own, but may be used to demonstrate that possibilities have been explored.
8 Foreknowledge of relevant change in the production system and wider environment that can be expected in the years following release; an example is the withdrawal of pesticides from commercial usage.

PF should take into account that long-term effects of GM plant cultivation (or any other innovation) are not likely to be revealed in highly constrained experimental systems, while the assessment of long-term impacts in the field are hampered by incomplete knowledge of the dynamical states of arable ecosystems. The long-term effects of any one GM plant may be difficult to measure given the comparatively short time-scale over which other large changes habitually occur in crop type, management and weather. The PF may conclude that research studies, modelling and monitoring are appropriate tools to investigate long-term environmental effects during GMO cultivation close to practice. In more than 20 years of experimental field releases and more than 10 years of commercial cultivation, adverse long-term effects reported in the scientific literature concern (i) the development of resistance in Bt crop target organisms and (ii) tolerance in weeds to complementary herbicides used in HT crops. No other adverse long-term effects have yet been established. However, other potential long-term effects are discussed in the relevant scientific literature and in scientific fora in general (BEETLE reports, 2009).

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2.28 Benefits and risks of genetically modified wheat with improved powdery mildew resistance – a joint research project

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The Swiss National Research Programme 59 “Benefits and risks of the deliberate release of genetically modified plants (GMPs)” includes research concerning the ecological, social, economic, legal and political conditions of GMPs in Switzerland (www.nrp59.ch). Part of the programme is a joint research project (the “wheat consortium”), which consists of 11 research groups that work in large field trials at two sites.
In 2008-2010, the field sites in Zurich-Reckenholz and Pully (near Lausanne) were planted with selected genetically modified (GM) spring wheat lines with enhanced resistance to powdery mildew (Pm3- and glucanase/chitinase-transgenic wheat). Up to 14 GM wheat lines were compared in the field with their near-isogenic sister lines, their genetic background, four conventional Swiss wheat varieties, spring barley and triticale. Included in the complex experimental design were treatments with fungicide as well as natural infection and artificial inoculation with a specific powdery mildew isolate.
The wheat consortium includes nine projects: Two projects analysed the effects of the resistance genes in the field. The other seven projects dealt with biosafety aspects.
“Analysis of Pm3 resistance gene function in transgenic wheat” (Beat Keller, University of Zurich, bkeller@botinst.uzh.ch). Six alleles of the Pm3 gene are investigated in the field.
“Analysis of powdery mildew resistance induced by chitinase/glucanase” (Christoph Sautter, ETH Zurich, csautter@ethz.ch, Fabio Mascher, Agroscope ACW, abio.mascher@acw.admin.ch). The efficacy of these resistance genes against different fungal diseases and the agronomic traits of the wheat lines and varieties were investigated.
“Interplay of arbuscular mycorrhizal fungi with transgenic and non-transgenic wheat” (Thomas Boller, University of Basel, thomas.boller@unibas.ch). First results: Song Wilson et al. (2010): Different nitrogen fertilization levels affected mycorrhiza whereas the differences of root colonization between transgenic and non-transgenic plants were not significant.
“Impact of genetically modified wheat on soil fertility sustained by plant-beneficial bacteria” (Monika Maurhofer, ETH Zurich, monika.maurhofer@agrl.ethz.ch, Christoph Keel, University of Lausanne, christoph.keel@unil.ch). Abundance, frequency and diversity of disease suppressive and phosphate-solubilizing pseudomonads are investigated.
“Effects of GM wheat cultivation on the decomposition of GM biomass by soil arthropods and annelids” (Wolfgang Nentwig, University of Berne, wolfgang.nentwig@iee.unibe.ch). First results: Peter et al. (2010): Leaves of six GM wheat lines and their control lines were fed to larvae of two different fly species (*Drosophila melanogaster*,

Megaselia scalaris). No differences were detected in development and fertility during the following four generations.
"Transgenic wheat and non-target impacts on insect herbivores and food webs" (Jörg Romeis, Agroscope ART, joerg.romeis@art.admin.ch). First results: von Burg et al. (2010): In 30 aphids clones fed with four GM wheat and control lines no major impact of GM wheat on aphid performance was detected.
"Influence of abiotic and biotic environment on the ecological performance of GM and non-GM wheat" (Bernhard Schmid, University of Zurich, Bernhard.Schmid@uwinst.uzh.ch) First results: Zeller et al. (2010): Transgenes can have an effect on the phenotype of a plant. These effects can be different under glasshouse or field conditions.
"Genetic and ecological consequences of introgression of transgenic wheat in a wild relative, *Aegilops cylindrica*: an open field experiment" (François Felber, University of Neuchâtel, francois.felber@unine.ch). Fitness parameters of hybrids of *Ae. cylindrica* x GM wheat lines and their back-crosses with GM wheat are investigated.
An additional project investigates the possible out-crossing of the chitinase/glucanase wheat lines into surrounding fields (Andrea Foetzki, Agroscope ART, andrea.foetzki@art.admin.ch).

First results:
- The *Pm3* wheat lines showed a higher resistance against powdery mildew.
- Depending on the insertion event some of the *Pm3* wheat lines showed phenotypic effects (chlorosis, reduced fertility).
- In many projects, the differences between GM wheat line and its near-isogenic sister line were smaller than between different conventional varieties.

30 No out-crossing event was found outside the field so far.
The results of the wheat consortium projects will allow better understanding of the interactions between transgenic wheat plants with an enhanced fungal resistance and their environment. They will make a contribution to the scientifically based discussion on the deliberate release of GM plants.

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2.29 Assessment of local biodiversity in the Migliarino - San Rossore – Massaciuccoli regional park (north Tuscany, Italy): development of a quick monitoring index as a tool to assess environmental impacts of transgenic crops

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Key words: Biodiversity, target species, inbreeding level, trophic links, GMP

In relation to the LIFE08 NAT/IT/342 DEMETRA project, 3 test areas were defined to evaluate plant (weeds and trees), animal, and soil microorganism biodiversity. As the final aim of the DEMETRA project is to develop a quick monitoring index (QMI) to rapidly assess the potential risk generated by a selected range of transgenic crops in well determined ecosystems or biotopes, test areas were chosen also taking into account their proximity to cropped surfaces in which Genetically Modified Plants (GMPs) could be used in the near future. In particular, direct or indirect target species of the GMPs will be considered, and trophic links analysed. Monitoring of the plant biodiversity (especially herbaceous plants and weeds) in the three sites will be investigated starting from the census of the flora and proceeding to the landscape analysis. Plants with pollinators present on the cropped areas or possibly breeding with local crops will be the object of a detailed investigation. Concerning tree biodiversity, the major effect of GMPs on local tree populations can be direct as in the case of poplar or indirect in the case some pollinator target species which become reduced in numbers and abundance. A drastic reduction of insect pollinators of some trees species could cause a drastic reduction in their seeds production and therefore on future natural regeneration of the species determining a decrease of biodiversity or species loss. Therefore, some tree species with entomophily pollination will be studied. These investigations assess the outcrossing and inbreeding level of the population, and predict if the reduction of outcrossing in consequence of a decrease of pollinator species can strongly reduce tree regeneration ability. The most important species will be chosen strictly in relation with the possible pollinators present at the study site and in accordance with the plant biodiversity task. Target species, will be identified through information on their abundance and functional role in the trophic chain. Furthermore, trophic levels of the most abundant species will be identified at each site. Species with different functional roles such as scavengers (Isopoda, Coleoptera), detritivores (Isopoda, Coleoptera), granivores (Coleoptera), herbivores (Mollusca, Hemiptera, Lepidoptera), pollinators (Hymenoptera, Lepidoptera) and predators (Chilopoda, Arachnida, Coleoptera) will be analysed. In addition, plants are known to have an effect on the abundance, diversity and activity of soil microorganisms living in close proximity with their roots (rhizosphere), resulting in a distinct microbial population that is larger and more active than that found in the surrounding zones. The rhizobacteria/michorriza biodiversity of the tree and weeds species identified as possible target for breeding or correlated to pollinator target species will be studied. The data will individuate sensitive or relevant species providing the necessary information to define a QMI in order to assess the potential risk generated by the use of GMPs in the ecosystem.

POSTER SESSION 3
Non Food Crops, Gene Flow and Confinement

3.1 Biosafety research on genetic containment of forest trees

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Gene flow from non-native plants and taxa resulting from traditional breeding has been cause for concern in the past (Hoenicka and Fladung, 2006). The introduction of a small number of *Populus* × *euramericana* clones and *P. nigra* varieties, which can intercross with wild *P. nigra* trees, has generated preoccupation regarding the integrity of the *P. nigra* 'gene pool' in Europe (Lefevre et al. 1998). These concerns have increased recently as private companies and research institutes worldwide show interest in incorporating genetic engineering for breeding of forest tree species. Release of transgenic trees into forest ecosystems would represent an additional risk factor to forest ecosystems.
Until now, gene flow avoidance strategies for non-native trees and taxa derived from traditional breeding have been limited to geographic separation of sexually compatible species. The development of "gene containment" strategies using genetic engineering is a promising solution to more efficiently avoid undesired gene flow.
Biosafety research carried out in our institute is currently focused on the evaluation of genetic containment in early flowering poplar. Early flowering lines allow studies under controlled conditions (safety level 1, S1), which are necessary in countries having very restrictive biosafety legislation, e.g. Germany, in order to substitute experimental field release. We generated transgenic lines with gene containment constructs using transgenic and non-transgenic early flowering poplars for our research. This approach has allowed us to initiate evaluation of gene flow avoiding methods.
Probably one of the most promising prospects offered by genetic engineering of forest trees is on overcoming of the long reproductive phase (juvenility), which has been a severe impediment for tree breeding. Tree species produce flowers (Reproductive Phase) only after a prolonged juvenile phase (Vegetative Phase). The duration of the vegetative phase is quite variable lasting in some tree species until 40 years (e.g. *Fagus sylvatica* L.).
Early flowering strategies have been developed to accelerate reproductive phase in tree species using different gene constructs. We have tested the early flowering gene constructs 35S::*LFY* (Weigel and Nilsson, 1995), HSP::*FT* (Zhang et al., 2010), 35S::*FT*, 35S::*BpMADS4* (Hoenicka et al., 2008), 35S::*MdFT* and SucII::*MdFT* in poplar with different levels of success. HSP::*FT* allowed shortening the vegetative phase in poplar from 7-10 years to 1-2 year maintaining at the same time a good plant performance. The remaining gene constructs induced a less efficient or no early flowering at all. Hybrid aspen *Populus tremula x tremuloides* clone T89 showed better performance than HSP::*FT* poplar but also a longer vegetative phase of 3-4 years.
Several containment constructs are currently been evaluated in our institute. Gene constructs tested aimed at sterility induction or the complete elimination of transgenic sequences in pollen grains. Our results indicate that sterility constructs CGPDHC::*STS* and TA29::*Barnase* disturbed pollen production in poplar, eliminating or reducing significantly the amount of pollen contained in anthers (Hoenicka et al., 2006). Gene construct MALE1::*STS* (Höfig et al., 2006) avoided pollen development completely in about 70% of catkins. A significant pollen reduction can be achieved using genetic engineering. However, there is still a need for more research in order to develop more efficient containment strategies specific for forest trees. The number of publications on sterility induction in forest trees is still very low (Fladung and Hoenicka 2004; Hoenicka et al., 2006; Lemmetyinen et al., 2004; Meilan et al.

2001; Skinner et al. 2003; Wei et al., 2007). Most sterility approaches reported until now were based on gene constructs used successfully in crop plants. Heterologous promoters can direct activity of cytotoxic gene expression in non-target, vegetative tissues generating in some cases a lower performance of transgenic poplar (Meilan et al. 2001). Use of floral promoters from forest trees (Skinner et al. 2003) or use of other sterility genes may overcome handicaps detected.

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3.2 COST Action FP0905: Biosafety of forest transgenic trees: improving the scientific basis for safe tree development and implementation of EU policy directives

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The potential for unintended consequences of spread of foreign genes (via vertical or horizontal transfer) and of pleiotropic effects following transgene expression may be enhanced in long-lived forest trees. This Action will focus on four key aspects related to the biosafety of genetically modified trees (GMTs): (a) analyses of the efficiency of existing gene containment strategies to avoid or if not possible to minimize gene flow; (b) facilitate efforts to develop site-specific integration of transgenes in tree genomes to minimize variability of transgene expression and pleiotropic effects, (c) evaluate possible methods to monitor GMTs in the whole production chain, and (d) conduct socio-economic and cost/benefit analyses in relation to the use of GMTs in plantations. This Action combines multidisciplinary knowledge generated with transgenic lines of forest trees (such as, *Populus* spp., *Pinus* spp., *Eucalyptus* spp., *Betula* spp., *Castanea* spp., *Picea* spp., etc.) as well as extensive expertise in correlated topics. The information gained should contribute to strengthen the scientific basis for the execution of the EU policy directives related to transgenic trees intended for cultivation in Europe. The main objective of the COST Action is to evaluate and substantiate the scientific knowledge relevant for GMT biosafety protocols by putting together already existing information generated in various European and Non-EU Countries as basis for future EU policy and regulation for the environmental impact assessment and the safe development and practical use of GMTs.
To reach its aim, the work plan of the Action is organised in 4 Working Groups (WGs) to implement collaboration of scientists.

WG 1 - Biological characterization of GMTs: to characterize the GMTs in respect to their genetic and phenotypic features relevant for gene flow, gene containment and gene targeting.
WG 2 - Environmental impact assessment and monitoring of GMTs in the whole production chain from plantation to final products: to study environmental risk assessment strategies and monitoring the GMTs along the whole production chain.
WG 3 - Socio-economic implications of and recommendations for the use of GMTs: to make socio-economics analyses of the use of GMTs considering the concerns and acceptance by the public, the economic potential for GMTs and R&D efforts to be invested, as well as cost/benefit analyses, and propose recommendations for the use of GMTs.
WG 4 - Management of intranet - internet websites and dissemination: through a website (www.cost-action-fp0905.eu), provide science-based information and increase public awareness in the utilization of GMTs in forest plantation and at the same time safeguarding the environment

The knowledge gained will be summarised in a book as a final output of this Action which will report the state of art of knowledge and research on GMTs with suggestion on how to effectively implement present EU directives on GMO considering the problematic of forest trees and their environmental impacts.

The Action started the 12th of April 2010 and it will end the 13th of April 2010. Actually, 22 COST countries (Austria, Belgium, Bulgaria, Croatia, Denmark, Estonia, Finland, France, Germany, Greece, Israel, Italy, Latvia, Netherlands, Norway, Romania, Serbia, Slovak Republic, Slovenia, Spain, Sweden, United Kingdom) and 1 COST neighbouring country (Bosnia and Herzegovina) have signed the Memorandum of Understanding (MoU). Three countries (Australia, New Zealand, South Africa) having an agreement signed with COST and other NON-EU countries (Canada, USA, China) are participating to the Action.

With integration of all this countries the EU COST Action FP0905 is expected to generate important benefits as it also foresees a strong collaboration among R&D bodies and legislative directives. This kind of collaboration will be fundamental, on the one hand, to address policy-making efforts and, on the other hand, to allow the scientific community to discuss to public concerns in a responsible way, particularly concerning socio-economic implications and biosafety issues of transgenic tree plantations.

3.3 Switchgrass (*Panicum virgatum*) biogeography and gene flow in the Northeastern United States.

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A native of North America, switchgrass (*Panicum virgatum)* is a hardy perennial grass with significant potential as a low-input lignocellulosic biofuel crop. Breeding programs have produced cultivars that are widely grown for livestock forage and wildlife habitat, and recent genetic engineering (GE) projects have developed new traits such as bioprocessing enzymes. With regard to GE switchgrass, regulators and stakeholders need an ecological risk assessment framework (ERA) that includes problem formulation, exposure characterization, hazards characterization, and expression of uncertainty. However, there is insufficient information to develop such a framework because basic questions about gene flow and species distribution remain unanswered. The goal of our work is to support credible ERAs for GE switchgrass. At present, we are focusing on questions relevant to *Panicum* distribution and pollen-mediated gene flow in cultivated plants, wild populations, and sexually-compatible relatives in the Northeastern U.S. The results of two projects are reported here: 1) biogeographic surveys to determine the distribution of *Panicum* in natural and cultural landscapes, and 2) studies on pollen release from the 'Blackwell' cultivar growing in semi-natural plant community in a conservation area.

Our biogeographic survey (2009-2010) was conducted using replicated transects in seven habitat types in the Southern New England Coastal Hills and Plains Ecoregion (CHP) and three habitat types in the Coastal Lowlands Ecoregion (CL). Plant species were identified and their stems counted along 50 m transects providing information about distribution, abundance, and plant community associations. Results from the CHP ecoregion showed that the seven habitats represented distinct plant communities and included four *Panicum* species (*P. virgatum*, *P. dichotomiflorum*, *P. capillare*, and *P. rigidulum*). *P. virgatum* was most often identified in roadside habitats, and it frequently co-occurred (92%) with closely-related *Panicum* and *Dichanthelium* species. *P. dichotomiflorum* was the most abundant species and often found in maize fields. *P. capillare* and *P. rigidulum* were associated with maize fields and herbaceous meadows respectively. In the coastal (CL) ecoregion, switchgrass was more abundant and was found in both natural habitats and roadsides. The closely-related species *P. amarum*, a threatened species in this region, was found in several habitats types. The major conclusions from this study were: 1) switchgrass is more common in the coastal ecoregion than inland, 2) switchgrass often grows along roadsides and this distribution pattern could provide a corridor for gene flow, and 3) switchgrass often co-occurs with closely-related species, including species of special concern, suggesting that interspecific hybridization (gene flow) could contribute to risk.

A study of switchgrass flower development and pollen release (2010) was conducted in an established planting containing switchgrass 'Blackwell', big bluestem (*Andropogon gerardii*), and other forbs. Fifty randomly-chosen switchgrass stems were observed every five days for panicle development. Only 4% of the stems had open flowers on July 28, and 6% had some open flowers on August 30. Thus, switchgrass plants in a semi-natural wildlife conservation grassland had asynchronous pollen release for more than four weeks with a peak in early August. Because switchgrass is relatively common as native or cultivated plants in the Northeastern U.S., pollen-mediated gene flow will be an important aspect of exposure pathways and ERA.

Our research goals include understanding the origin of switchgrass populations, modeling pollen emission, and tracking interspecific hybridization. Taken together, these studies will support predictive ecological risk assessments for GE switchgrass. The authors gratefully acknowledge research funding from the USDA Biotechnology Risk Assessment Grant and the University of Connecticut.

3.4 Pharming the field - Challenges for risk assessment and risk regulation

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The idea of cheap and almost limitless production of biopharmaceuticals and other valuable industrial substances from genetically modified plants grown on the field, also known as plant molecular farming, has motivated some 400 field trials in a broad range of plant species with maize, tobacco, rice, and safflower being the most frequently used production hosts. Besides open field cultivation there are also initiatives to explore more contained systems using hairy roots, Lemna, moss, algae and plant cell culture. About a dozen biopharmaceutical products, vaccines, nutraceuticals, and additives for human or animal use have already arrived in advanced stages of product development with a vaccine against New Castle Disease in chicken as the only authorised product, so far. Minute amounts of some substances are, however, already marketed as fine chemicals.

A number of important differences in risks and risk assessment compared to first generation GM crops have been proposed. In plant molecular farming protein concentration in the plant tissue can be extremely high and thus exposure of farmers, workers and wildlife can be increased. Low-level leakage of a vaccine can lead to immune tolerance and jeopardise the efficacy of vaccination. Multiple genetic modifications aiming at improving biomass production, bioprocessing, glycosylation, and introducing molecular confinement mechanisms could increase the likelihood of pleiotropic effects. On the other hand, area requirements are much lower and product value is much higher compared to normal agriculture allowing the use of more extensive confinement measures. A key concern though is adventitious presence of such plant material in the food/feed chain. Risk mitigation measures including molecular, physical and organisational confinement measures, stewardship concepts, monitoring, inspections etc. are therefore moving into the focus of risk assessors. Given the US experience these measures will not only consider safety but also include economic risk.
This paper is focussing on open field cultivation as this type has triggered the most concerns. It will analyse the challenges for risk assessment and risk regulation. Most recent developments in the EU, the USA, and at the international level will be considered. The research work draws on and extents the work conducted in two research projects on behalf of the German Parliament and the European Commission.

3.5 Applicability of cytoplasmic male sterility (CMS) in maize as a reliable biological confinement-method

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Introduction
In consideration of biosafety of transgenic plants with improved traits such as active pharmaceutical ingredients (PMPs – plant made pharmaceuticals), non-food crops (PMIs – plant made industrials), functional ingredients (functional food) or bio-energy production, the reduction of an unwanted spread of genes (biological confinement) is vital.
An effective biological confinement method requires a high level of gene stability and the knowledge about its reliability especially in crop species which release huge amounts of pollen (e. g. maize).
Cytoplasmic male sterility (CMS) is a maternally inherited trait that prevents the development of functional pollen and occurs naturally in many plant species. CMS was originally introduced for the production of hybrid seeds. CMS

alters in the presence of one or more nuclear restorer genes (*Rf* genes) which restore the fertility of the plant and results in fluctuated or fertile tassels with more or less vital pollen. The presence of *Rf*-gens depends on the type of cytoplasm. In maize three main CMS-types are known, CMS-T, CMS-S and CMS-C (Sofi et al., 2007). They can express different *Rf*-gens. Furthermore fertility can be restored by environmental impacts like heavy rain, extreme heat et cetera.

The objective of this study is to verify whether cytoplasmic male sterile maize hybrids are a reliable confinement method for the prospect cultivation of PMPs, PMIs or further GMOs. Therefore, in 2009 field experiments were conducted in three different environments in Germany and will be continued in 2010. The objects of the investigation were

1. the characterisation of the tassel (fertile, fluctuant or sterile) depending on regional environmental impacts,
2. the vitality of pollen, if it is released by fluctuated or fertile tassels,
3. the occurrence of cross-pollination and its distance into the recipient field,
4. possible growing recommendations for PMPs and PMIs.

Materials and Methods

Field experiments were carried out in Groß Lüsewitz, Mecklenburg-Vorpommern (North-Eastern Germany), Braunschweig, Lower Saxony (Northern/Middle Germany) and Freising, Bavaria (Southern Germany). According to results of a pre-experiment with 10 CMS-maize hybrids at each location in 2008, three CMS-maize hybrids (DSP2: CMS-T, Torres and Zidane: CMS-S) were selected and tested in comparison to a conventional fully fertile maize hybrid (Delitop). All hybrids develop yellow kernels. White maize was grown as pollen recipient. If it is pollinated by yellow maize pollen it produces yellow kernels due to the fact that the yellow kernel colour is hereditary dominant over white kernel colour.

The trials were designed downwind which required the position of CMS-maize hybrid plots westwards of the white maize plots. The distance between these plots was 3.5 m. Each plot of the field experiments measured 48 m width to 69 m length. To prevent cross-pollination between the test units (CMS-maize hybrid and white maize) cannabis was grown as a natural pollen barrier. The experiments were conducted under local agricultural conditions.

Tassel characteristics were tested during male flowering time. If anthers were developed, self pollinations were carried out to investigate the vitality of the pollen. At defined distances within the recipient plots 20 cobs were harvested and yellow and white kernels, respectively, were counted out to determine cross-pollination. Weather data were collected at all locations.

Results

In 2009 no tested CMS-hybrid was 100 % sterile. The highest level of CMS-stability was estimated for Torres, CMS-S-cytoplasm, within all environments. Torres developed fluctuant tassels with a small amount of pollen. Self-pollination resulted in less than 1 % kernel per cob. No environmental impact on tassels was found for Zidane either (CMS-S-cytoplasm). Fluctuated and fertile tassels were estimated and 20 – 40 % of kernels were developed after self-pollination. Contrary to the expectation and results of the pre-experiment, the CMS-maize hybrid DSP2 (CMS-T-cytoplasm) developed different tassels depending on the location. In Braunschweig the majority of plants were sterile, except single plants with fully fertile tassels and a lot of pollen, equally spread within the plot. Fluctuant tassels with a few amount of pollen were measured in Groß Lüsewitz. In Freising the majority of plants developed fertile tassels containing a lot of pollen.

Depending on the mild climate in Freising, the vegetation coursed efficient and fully flowering was reached early (averaged 90 days after sowing), followed by Braunschweig (approx. 100 days after sowing) and Groß Lüsewitz (approx. 105 days after sowing). Flowering synchronization between male flowering of the tested hybrids and female flowering of the white maize was estimated in either case.

According to the exposed site of the trial, the highest cross-pollination rates were measured at harvest in Braunschweig compared to the other locations. Nevertheless, at all locations the highest cross-pollination rate was found for DSP2 (on average 1.0 to 1.8 % of the whole plot), and the lowest for Torres (< 0.2 %). As expected, higher cross-pollination rates of all tested CMS-maize hybrids were found in the first row of the white maize plots and at 3.5 m distance, respectively, and declined more or less rapidly with further distances to the donor plot. After 30 m all determined cross-pollination rates showed < 1%.
In relation to the conventional fully fertile maize hybrid Delitop cross-pollination of the CMS-maize hybrids was strongly reduced. Within the first 30 m of the white maize plots Torres reduced cross-pollination by 96.5 % on average of all three locations. Zidane realized a reduction of 83.7 % on average and DSP2 of roundabout 77 %. Hence, no CMS-maize hybrid could realize a cross-pollination reduction of 100 %.

Conclusions
The 2009 results suggest that the cultivation of CMS-maize hybrids can be proposed (with reservations) as a useful tool for cross-pollination reduction. The applicability of CMS as a reliable biological confinement method in transgenic maize cultivated for PMPs or PMIs should be realizable in combination with other confinement methods such as small isolation distances or buffer zones of non-GM maize.

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3.6 An African perspective on GM Maize gene flow

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Background and aim South Africa is one of a few countries in Africa that has introduced genetically modified (GM) crops. First generation GM maize has been commercially grown in SA since 1997. In 2008, South Africa was ranked eighth in terms of global commercial GM production that included cotton, soybean, yellow and white maize. Gene flow from GM crop to non-GM crop may have several consequences including: the development of resistance in target insects for Bt crops; the contamination of landraces; loss of trade in processed and bulk grain commodities; the contamination of the food chain by experimental, industrial or pharmaceutical GM crop. Thus, similar to other GM producing countries, SA has to deal with considerations to minimize or prevent comingling through the use of isolation distances, where necessary, for GM field trials and coexistence. A further consideration is that specialist GM crops including pharmaceutical production, nutritional enhancements, and bio-fuels are expected to become a reality within the near future. Minimizing gene flow for different applications from contained use through to environmental release is an important consideration. In the past, several studies have recorded different distances of cross pollination for maize, using a variety of field trial designs under different environmental conditions. However, these trials have usually been small plots and not on the scale of commercial farming. Furthermore, very few of these studies have made specific recommendations with regard to the ideal isolation distance required in terms of different stringencies for minimizing cross pollination. For example, different tolerances for comingling

may apply to field trials under contained use compared to the production of maize engineered for bio-fuels. There is also no published data regarding the extent of cross pollination for maize in South Africa and regulators have to base decisions on available data not necessarily applicable to South Africa. Thus the aim of this study, conducted from 2005 to 2007, was to determine the extent of maize cross pollination under South African conditions in the context of commercial farming practice, that could inform the regulatory decision making process with regard to GM field trials.

Materials and methods Field trials were planted with a central plot of yellow GM maize (0.0576 Ha) surrounded by white non-GM maize (13.76 Ha), in two different geographic regions over two seasons with temporal and time isolation to surrounding commercial maize planting. Cross pollination from GM to non-GM maize was determined phenotypically, across 16 directional transects, every 2 m up to 100 m and thereafter every 10 m up to 300 m. Pollen was captured during flowering in four wind directions and genotyped using PCR. Pollen counts during flowering were compared to weather data as well as percentage cross pollination. The data was transformed logarithmically and mean percentage cross pollination compared to high cross pollination.

Results and discussion Although there was general congruency between wind data, pollen load and cross pollination, it is evident that wind data and pollen load do not solely explain the directional extent of cross pollination. We suggest that swirling winds and other biotic factors may have contributed to this incongruence. The highest cross pollination ranging from 54% to 82% occurred at two meters from the pollen donor and declined sharply up to between 20 to 25 m. Interestingly, a low percentage plateau of cross pollination was observed up to the furthest distance sampled. There was a high correlation of logarithmically transformed mean percentage cross pollination of distance (R^2=0.97). Based on the logarithmic transformation of cross pollination over distance, 50 m is sufficient to minimize cross pollination to between <1.0% to 0.1%, 159 m for <0.1% to 0.01% and 501 m for <0.01% to 0.001%. However, an important consideration when using mean cross pollination values is that the potential of cross pollination to occur, may be under estimated. To test this hypothesis, we performed a logarithmic transformation of high values of cross pollination over distance. It is interesting to note that there was a high correlation for high values of cross pollination over distance (R^2=0.95). Based on these values, a theoretical isolation distance of 135 m is required to ensure a minimum level of cross pollination between <1.0% to 0.1%, 503 m for <0.1% to 0.01% and 1.8 km for <0.01% to 0.001%. However, it is not practical to apply such stringent isolation distances, especially when different minimum levels of comingling may be required. We therefore suggest that a combination of temporal and distance isolation be combined, taking into account the GM maize pollen sources within the radius of the most stringent isolation distance required. We also investigated graphical shifts in percentage cross pollination over distance, over the different locations at which trials were planted. We noted that a shift in percentage cross pollination over distance was similar to the comparison of mean compared to high values for cross pollination.

Conclusions Based on the incongruence between pollen load, environment and cross pollination, as well as taking into consideration the comparison of mean compared to high values of cross pollination, we suggest that pollen load, environment and reproductive physiological characteristics are factors in determining cross pollination. Furthermore, considering the challenges facing agriculture as a result of climate change, it may be useful to revisit gene flow studies for a particular region from time to time to ensure that the potential for cross pollination has not changed significantly. Finally, this study highlights the importance of geographic specific data.

3.7 Evaluation of pollen-mediated gene flow from GM herbicide-tolerant zoysiagrass to non-GM population

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Environmental risk of a GM herbicide-tolerant zoysiagrass has been assessed (*J Environ Qual* 37: 207-218, 2008). In this study, we have evaluated the longevity and dispersal of pollen from genetically modified (GM) herbicide-tolerant and wild-type zoysiagrass. The pollen was most predominantly shed at approximately 10:00 h, with viability declining to nearly 0% at 12:00. All ability to germinate was lost within 150 min when stored at 25°C. No significant difference was found between GM and non-GM plants in their pollen viability or longevity. The pollens were distributed mainly within around 50 cm height from the ground of the zoysiagrass field. In addition, they did not fly easily off more than 5 m distance from the field. By testing with Basta spray, a non-selective herbicide, the gene flow rate has been assessed in 2007 and 2008, resulting in each 2.1% and 11% at 2 m, 1.7% and 5.8% at 4 m, 0.8% and 3.8%, 1% and 2% at 8 m, 1% and 1% at 12 m, 0.5% and 0.5% at 16 m, 0% and 0% at 20 m, 0% and 0.05% at 22 m from the GM field (12 x 24 m^2), and 0% at distances over 22 m on average. The large difference of gene flow rate between 2007 and 2008 seemed to be due to the difference of the direction and speed of wind during the flowering period (May) of each year. However, there was no difference of gene flow between the two years at the distances over 10 m. In addition, no basta-tolerant zoysiagrass has been discovered in the places within 20 km radius of the test field of 15,000 m^2 yet. On the basis of these on-going studies, we conclude that the GM zoysiagrass does not appear to pose a significant risk when released or cultivated outside of test field. This work was supported in part by grants from the Bio Green 21 Program (code 20080401034014) and from National Research Foundation of Korea (NRF 2009-0094062).

POSTER SESSION 4
GM Insects and GM Animals

4.1 EFSA's work on the safety assessment of genetically modified animals

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Introduction
Within the European Union (EU), the application of genetic engineering is regulated for domestic and imported goods. To ensure a high level of protection of human and animal health, the environment and consumer interests, the EU has established a legal framework that regulates genetically modified (GM) food and feed, as well as the release of genetically modified organisms (GMOs) into the environment. In this respect, the role of the European Food Safety Authority (EFSA) is to independently assess and provide scientific advice to risk managers on any possible risks of GMOs to human and animal health and the environment. In the EU, it is the role of risk managers such as the European Commission (EC) and EU Member States to decide whether a GMO or a derived product can be placed on the EU internal market.

EFSA's remit regarding GM animals
Currently, no GM animals or derived products from GM animals are legally on the EU market, but in a proactive measure, EFSA has been requested by the EC to develop guidance for the risk assessment of GM animals covering the following areas: (1) safety of food and feed derived from GM animals; (2) safety of releasing GM animals into the environment; and (3) possible health and welfare implications on animals related to their genetic modification. As specified in the EC mandate, EFSA will build upon the work carried out thus far at international level, including that of the *Codex Alimentarius*. The guidance will outline the risk assessment approach to be used should EFSA receive a GM animal market registration application. It will describe how the risk assessment of GM animals shall be carried out and will define the data to be provided by applicants when preparing an application for risk assessment to EFSA. To date, no GM animal applications have been submitted to EFSA.

EFSA's work on GM animals
As a first step, draft Guidance Documents will be prepared by specific Working Groups of EFSA's Panels on Genetically Modified Organisms (GMO) and on Animal Health and Welfare (AHAW) who will develop these in close cooperation. As for all draft Guidance Documents, EFSA will consult EU Member States and relevant stakeholders on these draft Guidance Documents during public consultations. Comments received during public consultations will be taken into account when finalising the Guidance Documents.
Working groups
To address the broad scope of the EC request, dedicated Working Groups have been created on:

(1) Human and animal safety: EFSA's GMO Panel has established a Working Group on guidance for the human and animal health safety assessment of products derived from GM animals. Further information on the Working Group members and meeting minutes can be found on EFSA's website.
(2) Environmental safety: To effectively assess environmental safety, taking into account the diversity and specificity of animals, the EFSA GMO Panel is setting up 3 dedicated Working Groups responsible for the development of Guidance Documents for the environmental risk assessment of GM animals. These groups will draft specific guidance for GM fish, GM insects, and GM mammals and birds, respectively.

In order to gather the necessary background information in the area of the environmental risk assessment of GM animals, EFSA has launched and awarded 3 separate calls for tenders, with the aim of: identifying GM animals or derived products that may be the subject of a EU market registration application within the next decade, and relevant scientific disciplines and fields of expertise that might feed an environmental risk assessment of GM animals; and of defining risk assessment criteria for GM fish, GM insects and GM birds and mammals. In the course of development of its Guidance Documents, the EFSA GMO Panel will take into consideration the reports submitted by the selected contractors. So far, the external reports on GM fish and GM insects have been finalised and published on EFSA's website.

EFSA has organised a workshop with the aim to discuss and review the draft external report on GM fish. The Meeting Report and attendance list of this workshop have been made available on EFSA's website. A similar workshop will take place for the evaluation of the draft external report on GM mammals and birds in October 2010.

(3) Health and welfare aspects of GM animals: EFSA's AHAW Panel will prepare a Guidance Document for the assessment of possible health and welfare implications on animals related to their genetic modification.

Public consultation and finalisation of the Guidance Documents

All draft Guidance Documents will be subject to public consultation in 2011 before the final adoption by the respective EFSA Panels concerned. These will be announced on the EFSA website and in EFSA's weekly eMail newsletter (EFSA highlights). The final Guidance Documents are foreseen to be adopted by the end of 2011.

4.2 Genetic engineering of the olive fruit fly, *Bactrocera oleae*, for use in the sterile insect technique (SIT).

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The olive fruit fly, *Bactrocera oleae*, is an invasive pest of olive fruit, causing considerable crop damage world-wide. At present control is largely based on the intense use of insecticides. The sterile insect technique (SIT) is a highly effective, species-specific and environmentally non-polluting method of pest control that involves the mass release of sterilized insects. Sterile insects reduce the reproductive potential of the wild population through infertile matings, causing a rapid decline in population density. However, past SIT attempts targeting *B. oleae* have achieved limited success. These relied on the release of irradiated mixed-sex insects, which resulted in the released sterile males mating with released sterile females, instead of dispersing and seeking the wild-type females. A genetic sexing system to allow male only release is therefore seen as essential for olive fly SIT.

Oxitec has successfully developed a genetic engineering approach to improve the utility of SIT called Release of Insects with Dominant Lethality (RIDL®). RIDL® provides a highly effective genetic sexing system, and additionally provides easy monitoring of the released insects in the field by a heritable fluorescent transformation marker. This technology has already been effectively applied to several important pest species, including the Mediterranean fruit fly, *Ceratitis capitata*, and the Mexican fruit fly, *Anastrepha ludens*. We are currently in the process of transferring, and optimising this technology for use in the olive fruit fly.

4.3 Transgenic Mosquitoes: Risk and Benefits.

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Insect born diseases have been causing endemic countries enormous problems for public health, such as high mortality and economics issues. The failure of existing control methods has lead to overwhelming burdens for scientists that have been asked to establish a new method for vector control.

The concept of implementation of the use of genetically modified mosquitoes as an alternative measure or as a part of integrated vector management (IVM) has gained support in the past years since controlling malaria and dengue vectors remains a challenge in disease endemic countries.

Culicinae and Anophelinae has been transformed to generate transgenic lines that can be used in two different approaches in an attempt to reduce population size or replace an existing population for one that are incapable to transmit a disease causing agent. Both of them represent a new interesting way to decrease the transmission of pathogens and if used concomitantly with biological, physical and chemical methods can shed a light for eradication.

Every control method has specific limitations and virtues for each particular event and this has to be taken into account so the best strategy can be chosen. According to this an accurate assessment of benefits and risks involved in its use need to be done in order to foresee real advantages and potential hazards.

We intend to profoundly evaluate benefits and risks for population replacement and population suppression considering their potential impact on environment and human health.

4.4 Preparations for open field trials of genetically sterile insects for control of mosquito species.

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Dengue fever, an arbovirus, is an increasing public health problem worldwide, without either a vaccine or remedial treatment. WHO estimates there are 50 -100million cases every year, with a range of symptoms from severe flu-like aches and pains to fatality. The only way to combat dengue fever is the control of the mosquito (*Aedes aegypti*) that transmits the disease. *Aedes aegypti* is extremely anthropophilic, residing in houses, shops and other human habitations. Traditionally control is carried out using a combination of methods, such as space-spraying pesticides, treatment of water storage vessels with larvicides or biological controls such as copepods or fish, and behavioural activities such as emptying water containing vessels, such as flowerpots. New controls are urgently needed.

Recent advances in insect genetic engineering have opened new possibilities for the control of mosquitoes. Oxitec has developed strains of *Aedes aegypti* and *Aedes albopictus* which are homozygous for one or more dominant lethal genes, which are “sterile” unless provided with the repressor molecule, tetracycline, in the diet. This method, known as RIDL®, is based on the Sterile Insect Technique (SIT) which has been used successfully for the suppression or local elimination of several insect species in agriculture. Sterile male mosquitoes are released continually over a wide area to mate with the target pest population; no adults result from these matings, and the target population declines. Mathematical modelling and preliminary trials indicate SIT based approaches can be effective against *Aedes* mosquitoes.

The first RIDL strains have been successfully tested in confined conditions for mating competitiveness with wild-type mosquitoes, suppression and a range of life history and behavioural traits in a range of locations and

conditions. Preparations are underway for field trials to demonstrate suppression of wild populations and preliminary data may be available at this meeting. This poster will summarise the results of experiments to date and discuss preparations required for open field trials.

4.5 Transgenic Mosquitoes: Best Practice Guidance. Technology research and production phase

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MosqGuide is a WHO/TDR funded project to develop and validate best practice guidance relating to the requirements for deployment of genetically modified mosquitoes to control mosquito-vectored disease, specifically malaria and dengue. Drawing on risk/benefit methodologies from related fields, expert consultation and experience, the project is preparing a modular approach on best practices for the testing, import, deployment and monitoring of genetically modified mosquito vectors. A total of 7 Modules are in various stages of preparation and validation:

Module 1: Overview of technology options, social and regulatory issues
Module 2: Technology Research and production phase decisions
Module 3: National decision making
Module 4: Data handling and environmental monitoring
Module 5: Role of stakeholder and community engagement
Module 6: Capacity Building (in conjunction with WHO/TDR training courses on GM Vectors.)
Module 7: Prototype decision tools.

The objective of the Module described here (Module 2) is to highlight best practice in procedures for the research and production phase of GM Vectors. It is targeted at the researcher, operator, developer, technician etc as they move through the phases of basic laboratory research, through open field trials through to large scale rearing and production of genetically modified mosquitoes

Rationale

The key issues addressed within this Module are the control of the modified vectors within the laboratory, during shipping and large scale production, as well as in the field. This Module will not provide a manual, but rather give points to take into account and highlight where further information may be obtained. It presents issues from laboratory to field from the researcher's perspective, but does not address programmatic use of GM vectors for routine control (discussed in Module 3).

The processes that are covered in this Module are:

Laboratory:

1. Obtaining materials, import, export and shipping
2. Contained use
3. Setting up insectaries
4. Quality control procedures

5 Institutional Biosafety Committees; Risk assessment and risk management

Confined Testing

1 Confined field research
2 Mass rearing of insects
3 Legal authorities for obtaining confined field tests of mosquitoes

Open field testing

1 Scientific considerations for site selection

Further information on the project can be found at www.mosqguide.org.uk

4.6 Transgenic Mosquitoes: Best Practice Guidance. Data Requirements for Field Release and Monitoring

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MosqGuide is a WHO/TDR funded project to develop and validate best practice guidance relating to the requirements for deployment of genetically modified mosquitoes to control mosquito-vectored disease, specifically malaria and dengue. This poster relates to the project's **Module 4: Data handling and environmental monitoring**.

The objective of the Module is to catalogue and justify the types of data necessary for decisions regarding contained and open field release of GM mosquitoes for the purpose of disease-vector control. Data needs are presented in terms of commonly required documents such as import application, biosafety commission dossier, environmental impact assessment, etc. The poster is aimed at government regulators and other interested parties, including those working in environmental and biodiversity protection.

Rationale

Guidance on data needed to understand, predict and monitor potential environmental impacts of the field release of a novel organism is proposed, covering various forms of GM mosquitoes. A harmonized approach to data requirements can support government decision making and ensure that all key issues are taken into account in a transparent and evidence-based manner.

In addition to supporting safety considerations, measurements of efficacy and cost effectiveness will facilitate further development and testing of these novel vector control interventions without the undue burden of compliance with varying national or regional requests.

Further information on the project can be found at www.mosqguide.org.uk

POSTER SESSION 5
Introgression, Persistance and Invasion

5.1 Biosafety parameters when assessing environmental risks of complex traits.

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The continuous scientific progress has enabled the development of the second wave of biotech crops with new genes and new traits, new crops and new environments, raising new opportunities for farmers and challenges in risk assessment as well. These new traits being tested and developed, such as dehydration stress tolerance, may involve the use of genes for which the protein product is indirectly responsible for the desired phenotype, in contrast to traditional transgenic crops such as insect- or herbicide tolerant crops. Such genes may encode transcription factors that regulate expression of other genes; they may code for signaling factors that initiate response to perceived changes in the cellular environment; they may produce metabolic pathway enzymes that result in the production of new cellular compounds, among others. This kind of genes can initiate a cascade of cellular changes and thus present the potential to produce unanticipated effects on plant metabolism, physiology, and/or development with biosafety implications. Adding to this complexity, the interaction between genotype and the highly variable environmental conditions might affect the expression of the phenotypes they contribute to. Observations during the pre-release performance testing period from a range of locations and years will help to determine whether these possible pleiotropic effects in the plant could cause any ecological harm. In addition, post-release monitoring provides further opportunities for safety observations in the relevant production environments. This presentation discusses the use of biosafety parameters for determining the likelihood of unforeseen effects occurring with biosafety implications. It is discussed from a regulatory point of view the inclusion of these parameters in a post-planting monitoring plan or general surveillance program in order to contribute to the decision-making process of these soon-to-be-commercialized second generation complex traits.

5.2 Introgression of crop (trans-)genes into wild relatives: Environment specific effects and stress sensitivity in *Lactuca*

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Since the introduction of GMO's there has been much controversy about the possible negative effects of transgene escape. Transgenes providing an increased tolerance to abiotic stress are of specific interest in this respect, as they could allow the carriers to invade new types of habitats. The persistence of a transgene under natural circumstances will depend on the degree to which the transgene confers any advantage to its carrier in a natural environment. However, after insertion those genes are not stand-alone units: they interact with those in linkage around them. Therefore, the selective advantage or disadvantage of whole genomic regions could likely be of more importance than the effect of the transgene alone. For that reason introgression risk needs to be studied in the context of the hybridization as a whole. Our goal is not only to look at a single species but highlight the possibilities of using selective disadvantages of whole genomic regions from crops in hybrid populations in order to prevent introgression of transgenes.

We use Recombinant Inbred Lines from a cross between (non-GM) *Lactuca sativa* cv. Salinas (Lettuce) and its wild relative *L. serriola*. Our work is based on a 1107 marker genetic map, consisting mainly of AFLPs and ESTs and evenly distributed over the nine linkage groups. This work is conducted in combination with other work shown at this conference (Uwimana et al.) and modeling work done at the University of Amsterdam.

Through a combination of greenhouse and field studies we identified a variety of QTLs for abiotic stress factors (salt, drought and nutrient limitation). Using this approach we can already identify a number of areas in the crop genome which are not suitable for transgene insertion. In addition we make comparisons between QTL-patterns found in greenhouse and field experiments assessing the predictive power of greenhouse experiments. Specifically, in this poster we show highlights of our work surrounding two main questions:

(i) What are the fitness effects of genes inherited from the crop? Is this dependent on (abiotic) stress?

(ii) Can small-scale contained experiments with transgenes be used to assess potential ecological consequences?

5.3 Differential physiological responses of transgenic a maize variety compared to its non-transgenic counterpart under abiotic stress during germination.

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There are no precise or official statistics about transgenic crops production in Brazil. However, according to the industry ISAAA agency, in terms of 2009 world cultivated area, Brazil retained its second largest position in the adoption of biotech crops. ISAAA estimated a total of 21.4 million ha, of which 16.2 million were cultivated with *Roundup Ready* soybean (40-3-2 event) and 500,000 ha with *Bollgard* cotton varieties carrying a single *Bt* gene (531 event), grown for the second time in 2009. In 2008, the National Technical Biosafety Committee (CTNBio) approved *Liberty Link* corn (T25 event, recently forbidden by Justice decision), *Bt11* corn (Bt11 event) and YieldGard corn (MON810 event). The National Health Surveillance Agency (ANVISA) and The Brazilian Institute of the Environment and Natural Renewable Resources (IBAMA) appealed against that decision, but The National Biosafety Committee (CNBS) ratified the CTNBio decision. Regarding health risk analysis, ANVISA appeal indicates inadequacy and insufficiency in all health studies, inconsistently with the conclusion of human and animal health safety. Regarding the environmental risk analysis, IBAMA pointed the lack of specific regulation for maize coexistence within different production systems, the lack of environmental risk studies in all Brazilian biomes, and inconclusive studies for the environmental safety of MON810. MON810 event has been progressively and widely assessed by many independent scientific studies that indicate adverse effects of its use and consumption. However, MON810 has also been widely assessed as safe by many other independent scientific committees (eg. see EFSA review on the renewal of MON810). CTNBio did not consider those issues raised by IBAMA and ANVISA in their appeal document. In addition, Brazil ratified and is Party of the Cartagena Protocol on Biosafety, and has in the first article of its own Biosafety Law the necessity of the Precautionary Principle compliance. Therefore, even when uncertainty or lack of knowledge are posed by scientific studies or either non-consensus documents exist, CTNBio should consider all evidences or arguments in the decision making process.

In order to broader investigate future impacts of the large scale adoption of GMOs in Brazil, we have accessed possible physiological alterations in response to salinity due to the presence of Cry1Ab transgene in MON810 maize. This study has been conducted in May 2010 in the Laboratory of Plant Developmental Physiology and Genetics at the Federal University of Santa Catarina, Brazil. Seeds of transgenic and the non-transgenic counterpart of the variety P30F53 (Pionner) commercialized in Brazilian markets was used. Germination percentage, germination rate, fresh and dry biomass of *in vitro* introduced seeds were analyzed in three different

salinity levels (0, 50 e 100 mM of NaCl). The seeds were rinsed with water and commercial detergent, placed in 1.0% NaOCl (v/v) solution for 15 min, and then rinsed three times with sterile water. The washed seeds were further introduced in a MS medium without any hormone or phytoregulator, and incubated at the following conditions: temperature of 25 ± 2ºC, air moisture adequacy index of 60 ± 5%, photoperiod of 16 h and light intensity of 100 µmol m^{-2} s^{-1}. Each experimental unit consisted of ten test tubes containing one seed per tube, in a total of six treatments arranged in four completely randomized blocks. Germination percentage was analyzed when the seed had its green hypocotyls. Mean germination time was calculated as the weighted mean of the germination time, using the number of seeds germinated daily, through the formula $\bar{t} = \sum_{i=1}^{k} n_i t_i / \sum_{i=1}^{k} n_i$, where t_i: time from the start of the experiment to the i^{th} day of observation; n_i: number of seeds germinated in the day *i* (not the accumulated number) and *k*: last time of germination. Mean germination rate was determined as the reciprocal of the germination time, $v = 1 / \bar{t}$, where $\bar{t}$ is the mean germination time. For the number of daily events, it was considered an event each day with germination $\geq$ 1 seed. Biomass was analyzed weighting plantlets material without any medium and endosperm that was left. No significant statistical differences were found for the percentage of germination and dry biomass between the two types of the maize variety. However, there were significant statistical differences when analyzing mean germination rate and fresh biomass. The transgenic variety had germination rate of 0,156 seeds.day^{-1} and the non-transgenic counterpart of 0,137 seeds.day^{-1}. The transgenic hybrid had also higher levels of fresh biomass (0,757g) when compared to its non-transgenic counterpart (0,625g). The presence of transgene(s) in commercial varieties is obtained by traditional breeding techniques such as controlled crosses between lines of interest, usually, backcross. Thus, single cross hybrid seeds, like P30F53, are genetically identical individuals obtained by the controlled crosses between two inbred lines.

According to CTNBio technical report for the approval of MON810. the information in the dossier indicates that transgenic plants do not fundamentally differ from genotypes of untransformed corn, except for the resistance to insects of Lepidopteron order. Our results show that these two hybrid types behave differently when germinated in *in vitro* conditions of salinity, probably because the transgenic hybrid show a different response under salinity stress. These possible pleiotropic effects of genetic engineering might be related to the alterations on lignin levels, since evidence for that has been obtained in commercial varieties of MON810 in Europe (Poerschmann et al., 2005). Our working hypothesis can be tested in future, although in the literature one can find support for pleitropic effects. Pleiotropic effects happen when genes are inserted into an organism, and unintended changes along with the modifications to the targeted trait(s) may occur, which means the effect of one gene on multiple traits. In spite of the difficulty to investigate and assess pleiotropic effects, additional precautionary research and approaches must be done in order to decrease the uncertainty related to the biosafety of GMOs.

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5.4 Seed longevity and dormancy of transgenic rice and chili pepper lines

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New traits of transgenic plants may alter seed longevity and consequently increase the possibility of weediness of the transgenic plants. Studies to estimate seed longevity and dormancy of transgenic rice (*Oryza stiva* L.) lines tolerant to abiotic stress and herbicide and transgenic chili pepper lines resistant to anthracnose and cucumber mosaic virus (CMV) were conducted from 2007 to 2009 at the isolated field in KRIBB.
Transgenic and non-transgenic parent plant seeds were buried at 5, 15 and 30cm depths in the field. Three different environmental field conditions were treated in the fields; black plastic mulching, weed management and no weed management. Rice seeds were buried in the paddy and upland fields.
Based on the ANOVA, differences in germination were not detected among burial depths, between field types, and also between transgenic and non-transgenic rice lines. Both transgenic and non-transgenic rice lines survived over one winter season and less than 5% of seeds were germinated five month after seed burial. Based on the ANOVA, differences in germination were not detected among environmental conditions. However, seed germination rate of transgenic and non-transgenic chili pepper lines depended on the seed burial depths. Though the germination rates were very low, seeds of chili pepper lines germinated after two winter seasons.

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5.5 Potential of crop-to-wild gene flow in soybean in Korea

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The aim of this study is to evaluate the gene flow from genetically modified soybean to wild soybean (*Glycin soja*) plants in a confined field located at the National Institute of Animal Science, Chungcheongnam-do, Korea. In the field, we planted the glyphosate tolerant soybean and around it, sowed wild soybean (*G. soja*) plants as a pollen receptor. From the various distances from pollen source, we harvested 219,015 seeds by each row and used *cp4 epsps* as a target gene. The cross-pollination frequency was evaluated by the survival rates the progenies of wild soybean (*G. soja*) plants after applying the glyphosate solution and then confirmed the presence of *cp4 epsps* gene by immunochemical chromatography and PCR analysis.

Under the conditions of this experiment, pollen flow from transgenic soybean plants to wild soybean (*G. soja*) plants was extremely low. 12 soybeans from 219,015 were determined to be hybrids between GM soybean and wild soybean and the frequency of gene flow was 0.0054% on average considering germination rate of wild soybean as 78%. The gene flow rates within 1.0m from the pollen source were 0.04% on average. From the studies, we can assume that the potential for gene flow to occur during soybean cultivation is assumed to be very low.

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5.6 Reflections on a Gene Flow Study

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Gene flow, one of the main concerns when evaluating the deregulation and introduction of transgenic events (Goodman and Newell, 1985), is defined as the change in frequency in a population due to movement of gametes, individuals or groups of individuals from one place to another (Slatkin, 1987). Gene flow is a naturally occurring phenomenon and isolation recommendations, both in space and time, are in place for seed production of many species. However, although it is difficult to detect gene flow between conventional crops, it is easier to detect the presence of a transgene. The transgene could move to feral plants of the same species or to a relative species with which the transformed one is compatible, as could occur between *Agrostis stolonifera* and *A. gigantea*, or between *Brassica napus* and *B. rapa* (Ellstrand, 2003). Once the transgene introgresses into the feral population, it might

provide a competitive advantage to the recipient population. If it confers a competitive advantage, the species that received the transgene could expand its ecological niche and its range. On the other hand, the transgene may not provide a competitive advantage to the species that receives it unless a selection pressure is applied (Cerdeira and Duke, 2006). Also, the transgene could impart a disadvantage to the recipient population that could result in its reduction or extinction. However, this last case is less probable because the transgene would likely be a disadvantage for the original transgenic plant as well, unless the transgene is one that alters reproduction in the new population.

There have been several controlled gene flow experiments, but it is hard to extrapolate the data obtained in small size research plots to what could occur at the landscape level once the transgenic crop is released. On the other hand, measuring the impact of the release of a transgenic organism at a landscape scale is not easy, and could result in the escape of the gene. The results could indicate that a particular transgenic event should not be deregulated but the gene can not be recalled.

We performed a four-year gene flow study at the landscape level taking advantage of a planting of regulated transgenic glyphosate-resistant *Agrostis stolonifera* (Zapiola et al., 2008). After four years of mitigation within and around a 4,500 ha control area, we found 62% of the 585 *A. stolonifera* plants tested *in situ* to be transgenic, implying that once the transgene is released in the environment it can not be recalled. There are several ways that a transgene can move in the environment and will vary depending on the species' characteristics. The size of the transgene source, and the potential recipient populations and the climatic conditions of the area where the species is situated will also impact the potential for gene flow. There are models for estimating gene flow, but they generally have a tail that tends towards zero but never reaches it thus preventing the estimation of the conditions that could result in no gene flow. There are clearly some crops where the risks of gene flow are reduced, like soybeans, wheat, cotton, but there are some others like corn, alfalfa, sugarbeet, canola, creeping bentgrass, and cross-pollinated crops in general that have greater chances to be involved in gene flow (Mallory-Smith and Zapiola, 2008). Once the gene moves to a feral population it can be introgressed or it can disappear from the population, which will depend on the characteristic it codes for and the fertility of the resulting transgenic feral plant. Therefore, gene flow will occur but we have to decide the level at which can be tolerated and design prevention measures to reduce it. We also have to accept that once a transgene is released in the environment it can not be recalled.

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5.7 Study of glyphosate impact on the genomic evolution of the target species Johnsongrass (*Sorghum halepense*) in Argentina

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Commercial cultivation of herbicide resistant GM plants have a complex impact in agroecosystems involving selective pressure over target species.

Glyphosate resistance in soybean, canola, cotton and corn is the most widespread character in GM crops and the selection pressure exerted on the weeds that are targets of herbicide action are unique in history. This harsh selective pressure has allowed the selection of resistant mutants. Johnsongrass (*Sorghum halepense* L.) is a widely distributed weed in many countries and represents a major problem to agriculture.

In Argentina, the first glyphosate tolerant Johnsongrasses were detected as a few foci in Tartagal (Salta) and in Estación Aráoz (Tucumán), in 2005. More recently, about 10 more locations were notified to SENASA (quarantine authority in Argentina).

The monitoring of the impact of commercial GM plants by molecular analysis of the population genomic, population dynamics, and phylogeographic characterization of target species, are very usefull tools to assess one aspect of the enviromental risk and to cary out a correct weed control management

The problem to address is a product of the dynamics of an artificial system in nature where evolutionary processes are operating.

One of the important points is to determine if the resistance has monophyletic or polyphyletic origins. In the first case, by founder effect (genetic drift) it is expected that invading resistant plants are much more alike (in their genetic background) than their neighboring native susceptible plants. Assuming polyphyletic hypothesis resistant plants have genetic backgrounds similar to their neighbor in ecotypes or populations.

Fifty geographically diverse samples were analyzed for 10 heterologous microsatellites representative of 20 loci of the tetraploid genome in an ABI automatic sequencer denoting an elevated polymorphism and suggesting a strong allogamic behavior. Most of the samples clustered according to their geographic origins, regardless of their resistance to glyphosate, thus supporting a polyphyletic origin hypothesis (i.e. independent mutant events were selected). A phylogeographic study by analising different polymorphic chloroplast regions is also being carried out.

Best studied resistance mechanisms are those derived from directed mutagenesis of EPSPS gene indicating that aminoacid positions 101 and 106 are critical to tolerate glyphosate while maintaining a good PEP substrate affinity. EPSPS genes from different sources of Johnsongrass (resistant and susceptible plants) and cultivated sorghum control were sequenced except for the leader peptide (90% of the total coding region). Different deduced aminoacid polymorphisms were detected, but none of them were located in positions 101 or 106, strongly indicating that resistance mechanism is not based on known mutations of the herbicide target enzyme gene.

5.8 Applicability of Weed Risk Assessment system for host crops as first step of evaluating impacts of genetically modified crops on biodiversity in Japan

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In Japan, the impacts of genetically modified (GM) crops on biodiversity are being examined according to the Cartagena Protocol domestic law in Japan,* which seeks to secure biological diversity by regulating the use of GM organisms. In the regulation, competitive superiority is evaluated based on a comparison with biological characteristics of the host crop and the literature. Abiotic-stress-tolerant GM crops such as drought-tolerant and cold-tolerant crops have recently been developed domestically and abroad. If these GM crops should escape from the fields, they could have advantages over host crops in survival and persistence under abiotic stress conditions and thus may affect the biodiversity around them. Therefore, novel methods are needed to evaluate the environmental risk of abiotic-stress-tolerant GM crops before their commercialization.

Nishida et al. (2009）developed a method to evaluate pre-introduced weeds using a modified version of the Australian Weed Risk Assessment (WRA) system designed to fit Japanese conditions. This method would be useful to demonstrate the potential weediness of GM crops from the literature or from web information before their introduction. In this study, we evaluated the applicability of the WRA to evaluating 26 host crops that have already been approved and are being developed in Japan and have been approved in US, the largest exporter of agricultural products to Japan. The WRA includes 49 questions about the history/biogeography and biology/ecology of plant species. We modified some questions to fit the cultivated crops, answered them and then presented the scores. We subsequently tabulated all valid information available to explain our answers. Although we obtained hardly any yield data outside fields, we could obtain most basic characteristics of host crops and could answer more than 35 of the total 49 questions. Scores of *Brassica. napus*, *B. juncea*, *Medicago sativa*, and *Agrostis stolonifera*, which are regarded as weeds by the WRA system, exceeded 10 (Nishida et al., 2009). They have already become naturalized and widely distributed in Japan, therefore the WRA system for host crops was considered to be a useful tool for evaluating the weediness of pre-introduced GM crops in Japan.

*Formally, “Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms.”

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POSTER SESSION 6
New Technologies and Implications

6.1 Risk mitigating genetic modification technologies: will they impact on risk assessment strategies and regulation?

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A multitude of technologies, associated with genetic modification (GM), is currently being developed to mitigate the potential health and environmental risks associated with genetically modified organisms (GMOs). These include targeted transgene integration, cisgenics, tissue-specific transgene expression, chloroplast transformation, grafting on genetically modified rootstock and microRNA gene silencing. With GMOs not containing any exogenous DNA sequences, no additional protein being expressed, tissue-specific and temporal transgene expression and reduced pollen mediated gene flow these technologies could decrease the potential biosafety risks associated with these organisms and their products. The question arises as to what extent these GM associated technologies will decrease the quantifiable risks and the regulatory requirements in South Africa.
In this regard, the impact of each of the mitigation strategies will be assessed according to their potential effect on decreasing the likelihood and consequence of specific risks. This evaluation will facilitate the assessment of the potential risks posed by GM technology.

6.2 Stabilizing transgene expression by using S/MAR elements

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Ensuring the stability of transgene expression is a major demand for the generation of genetically modified plants, as transgene expression may be lost in subsequent generations or under certain environmental conditions. The selection of single-copy transgenic events without rearrangements, preferably integrated into hypomethylated genome regions is the most common approach to prevent transgene silencing and to ensure expression stability in primary transgenic plant lines.

The generation of plants with stacked traits means an additional challenge regarding expression stability. Different genes/traits can be combined in one transgenic plant in a single transformation or in successive steps involving either re-transformation or conventional genetic crosses of different transgenic lines. Stacking transgenes by conventional breeding implies that the different primary events used in the crosses are mutual compatible with respect to a stable and reliable expression of the transgenes. The same or very similar promoter elements are often present in the transgenic lines that have so far been commercialized. Use of the same promoter in different lines brought together by sexual crossing, however, can in certain instances lead to transcriptional trans-silencing (Matzke et al. 1993, Daxinger et al. 2008). Avoidance of transcriptional gene silencing (TGS) therefore seems to be an important challenge for plant breeding.

Scaffold/matrix attachment regions (S/MAR) have been reported to reduce the loss of transgene expression from one generation to the next (Ülker et al. 1999, Levin et al. 2005). S/MARs are eukaryotic regulatory DNA elements located at the borders of chromatin domains, mediating attachment to the nuclear scaffold. Evidence suggests that S/MARs protect against transcriptional gene silencing, probably by preventing read-through transcription of complex gene loci, and against weak trans-silencing loci. One way of reducing the likelihood of TGS

after combining different genes with homologous promoter sequences may therefore be the use of S/MARs (Ascenzi et al. 2003).

An S/MAR isolated from petunia has been shown to be active as transcriptional enhancer in stably transfected mammalian cells (Dietz et al. 1994) and as creator of minidomains in mammalian cells and in plants (Dietz-Pfeilstetter et al. 2003). This Petun-SAR element was used to flank a CaMV35S promoter regulated *gus* gene on both sides in a binary vector construct. Transgenic tobacco plants obtained after *Agrobacterium* mediated transformation with the Petun-SAR construct as well as plants transformed with a S/MAR-less CaMV35S-*gus* construct were analysed with respect to gene copy number, number of integration sites and reporter gene expression. Compared to plants transformed with the S/MAR-less vector, slightly enhanced, limited copy number-dependent gene expression was found if the transgene was flanked by the Petun-SAR. Primary transformants with single gene copies were selected for the production of subsequent generations and for combining different events by genetic crosses. While the progeny of transgenic plants without the Petun-SAR showed progressive inactivation of the *gus* gene, stable expression including a gene dosage effect (i.e. additive effect of transgene expression in homozygous progeny) was found for Petun-SAR flanked transgenes up to the F_2 generation. Various types of interactions were observed for different combinations of transgenic events.

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6.3 Regeneration and genetic transformation via organogenesis of different varieties of Vitis vinifera and Prunus persica.

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Fruit production represents one of the most important sectors for the Mediterranean area, including fruits for fresh market and industrial processing. Often phytopathological problems penalize these crops with significant impact on production, quality and related costs. The genetic transformation of fruit trees provides huge potential for the improvement of fruit production and quality and for obtaining cultivars with increased resistance to pathogens. Furthermore, efficient methods of fruit tree transformation would facilitate functional genomic studies.

The aim of this research is to develop efficient regeneration and genetic transformation protocols to be applied to different varieties of table and wine grapes and to varieties and rootstocks of peach. The genetic transformation has been carried out by optimizing the method already developed for two table grape cultivars (Thompson seedless and Silcora) by Mezzetti et *al*., 2002. This method relies on *in vitro* production of meristematic bulk tissues with a strong capacity to differentiate adventitious shoots via organogenesis. Slices prepared from these bulks were used for *Agrobacterium*-mediated transformation of table and wine grape varieties (Vitroblack and Pinot Noir) and a commonly used peach variety (Big Top) and rootstock (GF677). The selection of the putative regenerants was performed on appropriate substrates, enriched with the selective agent (Kanamycin) at increasing concentrations (25mg l^{-1}, 50 mg l^{-1} and 100 mg l^{-1}). The tissues under selection were transferred monthly to fresh substrates to allow more efficient selection before starting the rooting phase. Several lines were selected which were able to proliferate and root on selective media were characterized for their transgenic state and considered for further studies on risk and benefit evaluation.

6.4 The toxicology of interfering RNAs from transgenic plants: Perfectly duplexed dsRNAs over 30bps in length are specific but sequence-independent inducers of the mammalian interferon response.

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Recent developments in the application of RNA interference (RNAi) to plants mean that the introduction of transgenes with defined double-stranded sequences can now reliably result in the inhibition of target RNAs. Consequently, there are now greatly enhanced opportunities for the use of this technology in agriculture and speciality crops. Applications demonstrated so far include not only the manipulation of plant metabolism and behaviour but also resistance to pathogens and pests, including bacteria, viruses, insects and other invertebrates. Realisation of the commercial potential of RNAi, however, depends in large part on the specificity with which transgene-derived double-stranded RNAs act. Specificity is important for biosafety both within plants but also towards exposed non-target organisms such as beneficial insects, livestock and, potentially, humans. To-date, although there have been reviews of human therapeutic RNAi off-target effects (OTEs), there has been little discussion of OTEs arising from plant transgenic RNAs (Karpala et al. 2005; Auer and Frederick 2009). Three classes of plant RNAi OTEs are potentially of concern:

(1) OTEs leading to non-specific downregulation of plant RNAs;
(2) OTEs affecting non-target invertebrates feeding on transgenic plant material and;
(3) toxicological effects on mammals.

This paper considers effects of transgenic plant-derived dsRNAs, focussing especially on mammals. In mammals, long (>30bp) perfectly duplexed RNAs (such as are typically produced by plant RNAi transgenes) are Pathogen Associated Molecular Patterns (PAMPS) and therefore potent triggers of innate anti-viral immune defences (e.g. Adamson and Fabro 1969; Alexopoulou et al. 2001). Mammals respond to the presence of such dsRNAs through specific and distinct intracellular and extracellular pathways. The effects of long dsRNAs on mammalian intracellular functions are well known and typically profound. Toxic responses extend to complete inhibition of protein translation and cell death, even at low doses, through receptors such as PKR and RIG-1 (Hunter et al. 1975). Less is known about the toxicological effects, presumably mediated by extracellular pathways, of dsRNAs on intact whole mammals. Especially poorly understood are the effects of different routes of administration of dsRNAs, effects on different organs and species-specific differences in responses.

A related toxicological question is the prevalence within the natural environment of perfectly duplexed RNAs. Presumably, PAMPS such as dsRNAs are evolutionarily selected because their rarity makes them useful markers for pathogens. This would suggest that transgenic dsRNAs may be rare or unique in their setting. We

therefore will also include the results of our attempts to identify from genome sequences and other sources naturally-occurring perfectly-duplexed dsRNAs of more than 30bps.

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6.5 Organisms developed using oligonucleotide-mediated mutagenesis: challenges for regulation and enforcement in Europe.

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In the European Union, genetically modified organisms (GMOs) and genetically modified microorganisms (GMMs) are defined respectively according to Directives 2001/18/EC on deliberate release of GMOs (1) and 2009/41/EC on the contained use of GMMs (2).

The definition of a GMO is both technology and process-based: an organism will fall under the scope of the GMO legislation only if it has been developed with the use of certain techniques. The EU Directives therefore include annexes that give additional information regarding the techniques that result in genetic modification, that are not considered to result in genetic modification, or that result in genetic modification but yield organisms that are excluded from the scope of the Directives.

The scope of these Directives is now challenged with the emergence of some techniques for which it is not always clear whether the resulting organisms are subject to the prevailing European GMO legislation or not. One of these techniques is the oligonucleotide-mediated mutagenesis (OMM), a technique that can be considered as a form of mutagenesis mediated by a chemically synthetized oligonucleotide and that is used to correct or to introduce specific mutations at defined sites of an episomal or chromosomal target gene. While recognizing that any political decision as to whether organisms developed through the use of OMM should be covered by the EU legislation of GMOs remains a matter of a broad and univocal reflection at EU level, we provided scientific arguments for not having those organisms falling within the scope of the EU Directives (3).

OMM not only poses challenges with regards to the EU definition of a GMO, it also addresses important questions as regards to the traceability and labelling of GMOs. Notwithstanding several methods provide for the detection of mutations (substitution, deletion, insertion of one or few basepairs), it is important to note that organisms developed through OMM cannot be distinguished at the molecular level from those developed through "traditional" mutation techniques (using chemicals, ionizing radiations) or from organisms in which spontaneous mutations or single nucleotide polymorphism occur. Therefore, the lack of an unambiguous detection and testing method that could make this distinction also hampers the enforcement of the GMO traceability and labelling provisions according to Regulation (EC) No 1830/2003 (4).

Resolving the regulatory status of new techniques such as OMM is also of utmost importance in terms of potential societal and economical consequences, especially for developers of novel organisms, given the higher

costs and increased level of procedural complexity of applying the GMO legislation in the EU. Absence of legal clarity might have ramifications for the development of commercial applications using new techniques.
All these aspects underscore the need to consider the regulatory, economical and societal impacts of these new techniques at international level in order to avoid discrepancies between regulatory jurisdictions. Moreover, such a coherent approach would benefit the international regulation of transboundary movement of GMOs as well (5).

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6.6 Molecular method development for the detection of genetically modified pollen in bio-aerosol

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Pollen represents an isolated ubiquitous reproduction means of higher plants. Pollen grains moreover contain a haploid blueprint of the plant genome and have an active metabolism comparable in complexity to mature plants. As such, pollen could represent a valuable tool in addressing a number of issues related to risk assessment in a lesser complex manner than in intact plant tissues or seeds but nevertheless in a more representative manner then in artificial systems such as protoplasts or tissue cultures. Mature pollen represent isolated physical entities that can be readily recovered and as such used as a study object for various purposes (transcriptomics, proteomics, physiology…). Also, being isolated segregating reproductive structures, pollen grains are a unique source of information on the nature of the genome of the parental plants (zygosity, SNP, copy number…). Due to their means of dispersion (by wind, insects, birds …), pollen allows to monitor the influx of genetic material by air into a defined area such as farming fields, natural reserves or any well-defined ecosystem.

In order to establish a common approach for applying molecular tools in monitoring airborne genetic influxes, the use of maize pollen grains (including genetically modified (GM) pollen) as a model system is being evaluated. Maize pollen represents a well-characterized biological entity with well defined genetics. Moreover maize represents a world-wide cultivated arable crop of which a substantial part is to date genetically modified, mainly towards insect resistance.

Two distinct means of capturing maize pollen from the air are being tested: a passive sampling system developed by Frieder Hofmann (Team Integrated Environmental Monitoring, Germany) and an active sampler commonly used in collecting airborne pollen for allergenicity forecasting (Burkard-type samplers). A suitable CTAB-based extraction method for maize pollen yielding PCR-grade DNA has been established. Compatibility of the DNA extraction method with the pollen capture matrices as present in the pollen traps is being assessed. For these

purposes, pollen from both field maize and from greenhouse-grown plants (including some GMO) has been collected during the summer of 2010.

Finally, to allow high-throughput analysis of GM pollen in a sample, DNA extracts from entrapped pollen samples are being analyzed using the so-called *Real-Time PCR-based Ready-to-Use Multi-Target Analytical System* developed at the European Commission's Joint Research Centre. This system represents an easy-to-use GMO screening tool allowing the simultaneous detection of 39 GM plants, including the currently EU-approved and unapproved genetically modified organisms (GMOs) known to the European Union Reference Laboratory for GM Food and Feed (EURL-GMFF).

QBased on the outcome of these analyses, a strategy and decision support system (DSS) for application of pollen entrapment combined with molecular analysis of isolated DNA will be developed.

6.7 Greenhouse and field cultivations of potato expressing different antigens

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Plants have great potentials to produce recombinant pharmaceuticals. Here, we report a comprehensive cultivation of antigen-expressing potato under greenhouse conditions and field trials. Potato is an appropriate model plant system because they are safe to use and to send under vegetative propagation, and no natural crossing partners exist in Europe. Using potatoes expressing VP60, the only structural capsid protein of the rabbit haemorrhagic disease virus (RHDV), CTB, the non-toxic B subunit (CTB) of the cholera toxin (CTA-CTB_5) and the model protein NPTII (neomycinphosphotransferase), we tried to identify optimal conditions for the production of plant-derived antigens. Although an environmental impact on transgene expression could be demonstrated for one event in the field, in general, transgene expression levels were higher than or similar to the greenhouse plants. Our data indicate that isogenic potato plants can be used to produce consistent levels of VP60 and CTB in the field.