LMOS and the Environment
Proceedings of an International Conference
LMOs and the Environment
Proceedings of an International Conference

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and the Environmental Protection Agency

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The Proceedings

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Craig R. Roseland, Editor
LMOs and the Environment. Proceedings of an International Conference

The Conference

I thank APHIS management, and among them, Kevin Shea especially, for their encouragement to hold the Conference and to publish the Proceedings from it. I also acknowledge the major contributions of the EPA, especially those of Elizabeth Milewski, Ina You and Denise Roush, who helped to bring about this Conference. I would also like to thank the USDA-APHIS and EPA for funding the Conference and providing for many speakers. A large number of people assisted with the arrangements before and during the meetings at the conference site, including Pat McQuillan, Tony Paris, Kathy Balderson, Betsy Randall-Schadel, Lauren Jones and Kay Peterson of the USDA, the technical specialists Dennis Trainum and Michael Hargett of USDA and Eric Haugh of the Sheraton Imperial, Peter Kearns, Rebecca Weiner, and Sally DeMarcellus of the OECD. I especially appreciate Ved Malik, who made the initial arrangements for the facility, the refreshments and meals. I thank Sam Taylor for arranging and securing funding for the welcoming reception event. Finally, I would like to thank Crop Life International, Syngenta Biotechnology, Vector Tobacco, Inc and Hutchison & Mason PLLC for their financial contributions.

Sally L. McCammon, Chair
OECD Steering Committee
USDA APHIS Biotechnology Regulatory Services
Preface

The Conference in Raleigh-Durham, North Carolina, *LMOs and the Environment*, was convened to discuss the science needed to assess the effects of transgenic organisms in the environment. The OECD and its Steering Committee have organized this meeting as one of an ongoing series. The conveners appreciate their efforts as well as the financial backing of the U.S. Department of Agriculture and the U.S. Environmental Protection Agency. A diverse group of speakers considered a range of topics, aiming to present policy issues, research findings, and the needs and international considerations that are relevant to risk assessment.

These Proceedings reflect the breadth of topics presented in the Conference and include a few additional contributed papers that subsequently were developed by participants. A broad range of experience was represented in the Conference, including that of academics, researchers, government regulators or policy makers and staff from independent nonprofit agencies. The participants represent countries that currently engage in risk assessment, some for more than a decade, but also others that were invited whose representatives come from countries that have only recently begun this work. More than 200 people attended the Conference and were part of the discussions and deliberations. In the Rapporteur’s Report, the three authors have attempted to come to a consensus on the outcomes and conclusions to be drawn from the meeting, and I will not add my opinions to their worthy summary. Their work identified areas of agreement and disagreement about the practice and science of risk assessment. They presented opinions for how practices of assessment of risk could be improved, especially by promotion of increased research into gene flow issues and nontarget effects of transgenic plants.

The conveners were pleased that these Proceedings received manuscripts deriving from some of the larger-scale, and multi-crop monitoring research efforts that were initiated to study the possible impacts of LMOs on sexually compatible plants and other organisms in the environment. These investigations are taking place in the United Kingdom and France. Such efforts may serve as a foundation for future research on additional crops for which evidence of environmental impact or benefits will be sought. Reports were also contributed on risk assessment for single crops, such as sugar beets and rice. Another report described how impacts of transgenic microorganisms on the soil environment have been monitored.

These Proceedings contain descriptions of the risk assessment process made for specific crops, as well as presentations for how the process should be undertaken for any engineered crop. These papers, in some cases, supply useful direction for ongoing risk assessment, and in others, provide more theoretical considerations. Some of the papers offer suggestions on how to deal with controversial issues surrounding risk assessment, such as the role of uncertainty. Other papers provide a rationale for considering impacts on social and economic factors when risk assessments are conducted.

One of the highlights of the meeting was the Session on Maize at the Center of Origin and Diversity. This session focused on the challenges confronting Mexico following the discovery of unauthorized maize (corn) varieties in areas of the country cultivating large
numbers of maize land races. Stakeholders raised numerous concerns following the discovery. I am pleased to present some important contributions by those closest to the issues, including representatives from agencies of the Government of Mexico, CIMMYT (International Maize and Wheat Improvement Center) and a perspective from an environmentalist. Another paper outside this section showed how biological databases are used in Mexico to assess potential for gene flow. The diversity of opinions about farm impacts, necessary considerations for a crop at the center of origin, and the role and importance of social issues are worthy contributions to the discussions centering on release of transgenic maize in the center of maize’s origin.

It was clear from many presentations that our knowledge of the consequences of engineered plants may be imperfect, and improving the risk assessment process needs further support through funding of research programs. This research could result in proposals for new data requirements prefatory to regulatory approval, or could help define and describe appropriate means to monitor environmental consequences of transgenic crops after approval. One paper distinguished research that focused on higher order and landscape ecological effects from research at the population level and onsite effects. Among the higher order effects are impacts on land management, which subsequently affect biodiversity. These higher order effects are not studied as frequently as the more local effects. Priorities need to be determined for which level of biological organization new research efforts should focus.

Finally, the need for capacity building to enhance the research and decision-making expertise by individual states is discussed. The issues that surfaced at this conference will need to be addressed by each country that is developing its own capacity to produce, purchase, manage, and monitor LMOs. While most of the issues discussed in these papers are those of LMO crop production, some are related to trees, fish, insects and microorganisms. Clearly, there is need for additional research and for policy decisions about how to predict the impacts resulting from some of these other engineered organisms as well as the impacts of the more familiar engineered plants.

The papers included in these Proceedings will condense and clarify the important issues, and I hope, will provide material for further discussion about risk assessment and for setting new research directions. Additionally, these papers may help shape the structure of programs as well as the policies of agencies that will make decisions on products of biotechnology. I expect that you will find these papers as useful I have.

I am most appreciative of all those who made an effort to describe the needs, challenges and future directions for risk assessment and research to support it. There is no doubt that these papers will be most helpful for countries that are currently setting up risk assessment and evaluation processes, some of whom were represented here. Other papers will resonate with those whose national agendas continue to raise questions surrounding the acceptance and use of transgenic crop commodities and products. The shared experience of different countries and regions of the world that are recorded in these Proceedings should provide some substance for such future discussions. The papers that have been included also offer an introduction to some of the leading scientists and policy makers who have contributed to the analysis of risk of genetically modified organisms.

Craig R. Roseland, Editor
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Steering Committee for the International Conference

The Steering Committee chose the topics for the Conference and made selections of the speakers who made presentations. The Committee was responsible for oversight of the Conference, as well as the work of the Site Coordinator and Local Arrangements Director, Sally McCammon. The Committee also provided directions for the OECD Staff who were instrumental in arranging this International Conference.

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Delegates, distinguished speakers, panelists, and guests—it is an honor and a pleasure for me to be here today to chair this important OECD-sponsored conference on living modified organisms (LMOs) and the environment. In the opening comments this morning we will have remarks by Mr. Donald Johnston, Secretary-General of the OECD, and Mrs. Joke Waller-Hunter, the Director for Environment of the OECD.

It is also my pleasure to make some overarching comments on science and science policy to set the tone for our deliberations and conversations today. Let me begin with the wisdom of the late Congressman George Brown of California. Some of you may remember him as science’s best friend and most constructive critic in the U.S. Congress. We in the science community sorely miss his foresight and vision. I bring his words to you because you are an international community of scholars and scientific experts. As always, he left us with important ideas. In a 1993 speech titled “A New Paradigm for Development: Building Dignity Instead of Dependence”, he said that

This work must begin first by viewing developing nations as partners instead of as step-children. Of all the many ways in which we can cooperate for the global good, the case for science and technology cooperation with science-poorer nations is perhaps the most compelling.

To do so, we must abandon the instinct to judge others by their past accomplishments or to judge our own accomplishments as the proper path for others. We know that science and technology are an important force to help balance the world’s inequities. The job of the science community, and our nation’s leaders is to find a host of mechanisms to make use of the knowledge and benefits working as partners.”

I come to you today in that spirit and in the hope that our deliberations will be guided by George Brown’s thoughts.
In the long sweep of civilization, science and engineering have had an ever-increasing influence on the life of society. We’ve used most of that knowledge to remediate an existing problem or to address a current need. Currently, biotechnologies have been designed to address nutritional deficiencies and to combat disease. We all know the example of golden rice, engineered to reduce vitamin A deficiency. Rice is the staple food for most of the world’s population—in fact, 80 percent of the global population. Golden rice could prevent nearly half a million cases of childhood blindness and a startling one to two million deaths each year. UNICEF estimates that some 124 million children around the world are dangerously deficient in vitamin A. Bioengineered fruits and vegetables are being developed into edible vaccines for a host of debilitating and deadly diseases. Vaccines for hepatitis B and rabies are notable examples.

The same techniques are being applied in veterinary medicine to protect valuable livestock and fish crops. Plants that resist pests and herbicides promise to reduce contamination from harmful pesticides and boost crop production. This is just a small smattering of the potential that biotechnology holds.

However, we now recognize that we also need to draw on one of science’s most potent capacities—prediction. If we can predict, we frequently can prevent. The centuries of our accrued knowledge can and should increasingly be directed toward prevention.

In an old Icelandic saga there is a description of the character Snorri. It was said of him, “He was the wisest man in Iceland without the gift of foresight.” To me, this has always meant that Snorri had a great deal of knowledge but he didn’t quite take his knowledge to the next step. He didn’t use it to see implications, to anticipate the future. Without foresight, he could easily be caught by surprise, and obviously without a plan.

As a community of nations, we need to develop a broader, more anticipatory perspective in our research. We need to increase our emphasis on envisioning future possibilities, good or ill, as a mechanism to predict. Undoubtedly, this will open new vistas in our exploration and discovery. This must take place at the same time that the research community maintains a freedom and passion for new frontiers and the rigor of merit review. As all of you know so well, knowledge is our strongest insurance for preparedness.

Without new knowledge we cannot develop foresight. As we evolve increasingly into a knowledge-based society, our economic growth, our national security, and our social well-being will depend on the most advanced discoveries in every field. Knowledge is the bedrock. Our ability to use foresight gives us a kind of early warning system – a guard against unintended consequences. For example, we know that devastating floods are frequently caused by intense over logging of an area. Our science knowledge can accurately predict such consequent flooding and devastation.

Science can be an effective predictor. To prevent requires more. The research community needs to find more effective methods to use its capacity to predict in order to meet real-world needs through prevention. Everyone in this room knows that by solving a present problem we can easily sow the seeds of genuine dilemmas for the next generation.
History is replete with examples. When foresight directs our actions and the use of knowledge, we are a lot less likely to fix the present at the cost of the future. There’s good reason, then, to be thoughtful about the use of all new knowledge, techniques, and technologies, including biotechnologies.

Thoroughly evaluating potential risks and reducing uncertainty about unintended effects is just plain good science. Assessment is an important component of the process. And we know that we can never think of our current knowledge as a security blanket for the future. It will help us in the present, but as the renowned mathematician Alfred North Whitehead said, “Knowledge doesn’t keep any better than fish.” New, more complete knowledge replaces it—a process of constant renewal and at an ever-accelerating pace. This makes an unshakeable case for consistent research in all eras, at all times. We are just discovering the vast implications of what I call “biocomplexity in the environment.” This term refers to the dynamic web of often surprising interrelationships that arise when living things at all levels—from molecular structures to genes to organisms to ecosystems—interact with their environment. Links within and between different systems at different levels of organization often exhibit features of complexity characterized by abrupt changes, thresholds, and nonlinear dynamics. My own research on cholera has convinced me that a better understanding of these complex phenomena can help us to understand and eventually predict the web of relationships that connect an engineered molecule, the plant that contains it, the human who eats it, together with its effect on the ecosystem in which the plant grows. This frontier science that looks at the whole system of interrelationships is absolutely essential for the future of biotechnology.

Despite our vast knowledge base, we likely still know very little of what there is to know. This should prevent us from being arrogant about what we do know. That doesn’t always happen. In fact, we do ourselves a global disservice when we educate and train our scientists and engineers only in science and technology. The world in which our work bears fruit is a world of integration and overlapping consequences. Narrow knowledge can become incorrect knowledge.

In the 21st century, success will be determined increasingly by science and technology. Therefore, economic survival for all of us means being on the cutting edge of discovery and knowledge creation. Choosing otherwise is not frugal; it’s just shortsighted. The alternative to not utilizing the power of science and technology is the alternative of being left behind. It does not matter if the field is biotechnology, advanced computing, nanotechnology, or any number of other new or emerging fields. That is why George Brown’s concept of partnering between and among nations is so critical in this new era. No one culture or country has a monopoly of capable workers. Globalization has proven this repeatedly in the last decade. There is a reservoir of talent in other cultures whose languages we may not be able to speak but whose ideas and objectives are important to include.

As we seek the greatest advantage from our research enterprise we should never mistake science and technology for a linear process. Although science often leads to the development of new technology, new technology just as frequently enables science to explore new realms previously unreachable. Science does not enter a tunnel and come out the other end as technology. These two distinct forces historically have functioned in complement. Their
relationship is symbiotic. The word that is the very linchpin of this conference—biotechnology—is the deft fusion of the science of biology and the exquisite technology of genetic manipulation. Together they form a new whole.

And the advances continue like a braid of skeins winding back and forth across each other. This intertwining of knowledge—ideas, if you will—and tools has moved us to new understanding. We recognize that many disciplines converge to unlock the complex operation of systems—everything from climate patterns to terrorist movements. In my own research on cholera, technology played a crucial role. I could not have identified the cholera bacterium as water-borne and tied cholera outbreaks to the rise in sea surface temperatures without satellite remote-sensing technology to scan expanses of ocean. I have done most of that research over the last 25 years in the developing world, primarily in Bangladesh. There, deadly pandemics of cholera devastate villages and traumatize urban areas. For Americans, news of these dreaded scourges was sad statistics from far away. Since September 11, deadly disease scenarios are no longer implausible in our own backyard. No nation is immune from danger. In a world driven by science and reduced to a village by instant communication and lightening-swift transportation, safety is either for all of us or for none of us.

Less than 3 months ago, we saw a glaring example of why it is also important to have a public educated to the issues of science and technology. The surprise emergence of anthrax in the mail set in motion a race for information. It is vital that the global citizenry and all our leaders have a better working knowledge of the science and technology that defines our very existence. Although anthrax is not an everyday occurrence, there were many, including public officials, who thought it was contagious. Without correct information, we breed chaos and hysteria—neither of which fosters appropriate responses. In the United States, we have a new battle to fight and that is to prevent man’s deliberate turning back the clock of progress in public health.

A citizenry literate about science and technology serves several goals. It gives the nation a workforce educated and trained to compete in the increasingly competitive global marketplace. It promotes good judgment as voters on both issues and candidates. It serves as strong defense against delusions of safety as well as threats. I cannot stress enough the primary importance of a scientifically literate citizenry. I cannot stress enough the responsibility of the science community to help meet that goal.

In multiple aspects, September 11 was a knife-sharp awakening for our nation and its leaders. Not the least of those surprises was how little people outside of the science community and those on the periphery understand science and technology issues. At this time of uncertainty, the need for all of you is greater than ever before. Your experience, wisdom, research, and measured debate can bring both historical context and analytical order to precipitate public discussion and debate.

Alfred North Whitehead said of science that “the aims of scientific thought are to see the general in the particular and the eternal in the transitory.” And so we must ask how science can elucidate these times. We know that science brings fresh knowledge of our planet and ourselves and thus what is newly possible. That, however, is not enough. Science and technology are neutral. They are neither inherently good nor bad. What we choose to do with the potential that scientific knowledge offers is another matter. We have seen that so clearly in the last several weeks.
Modern biotechnology allows us to feed the world with improved nutrition but also allows terrorists to make more lethal bioweapons with greater ease. The same fertilizers that make our agriculture more productive were the mechanisms for destroying the Federal building in Oklahoma City just a few years ago. Scientists and nonscientists alike are all guardians over such choices. The world has always been a delicate balance of many complex forces, not the least of which is humanity—in all of its diversity of cultures, goals, and behaviors. Today, sophisticated knowledge, powerful tools, and high-speed transportation and communication amplify that complexity.

Two things will not change: humanity depends upon the complex and diverse systems of the planet to survive and prosper; the survival of the planet depends on the knowledge and know-how that humanity brings to the delicate environmental complexity that sustains us. I look forward to lively discussions that shed new light and knowledge on both.
Living Modified Organisms and the Environment—
An International Conference
Welcoming Address

James G. Butler,
Deputy Under Secretary
Marketing and Regulatory Programs
United States Department of Agriculture
United States of America

Conference Objective

Good morning and welcome. The stated objective of this conference is to bring together a diverse group of people to talk about the underlying science for assessing transgenic organisms in the environment. Looking at the list of attendees, I believe we have already met part of this goal. We have here today more than 230 registered participants from more than 40 countries representing a wide range of disciplines. Thank you all for coming.

Organization for Economic and Cooperative Development

Thank you also to the Organization for Economic and Cooperative Development (OECD) for once again bringing such a diverse group together. For almost 20 years, OECD has been addressing the issue of biosafety at the international level. Through its Working Group in the Harmonization of Regulatory Oversight and the Task Force for Novel Food and Feed, it has developed technical information useful for environmental safety assessments. By promoting international forums such as the one we are taking part in this week, OECD encourages us to learn from the experiences of other countries and, it is hoped, take home the best approaches to safety assessment available.

Thank you also to Dr. Rita Colwell, the Director of the National Science Foundation, who has graciously agreed to be the conference chair; Mr. Donald Johnston, the Secretary-General of the OECD; and Ms. Meg Scott Phipps, Commissioner of the North Carolina Department of Agriculture.
U.S. Government’s Role in Regulating Biotechnology

I think all of us here would agree that, as new agriculture technologies are developed, safety is a major concern. To ensure the safety of our people and our resources, there are questions we must ask ourselves before introducing transgenic plants and other living modified organisms (LMOs) into the environment. Achieving production goals and creating successful applications for biotechnology are important, but just as important is our ability to assess the impact LMOs may have on the environment. The foundation for all such assessments must be science.

For more than 15 years, The U.S. Department of Agriculture (USDA) has been aware of the driving need to ensure the safety of LMOs through sound science. As the Deputy Under Secretary of Marketing and Regulatory Programs for USDA, one of my responsibilities includes the oversight of a regulatory agency known as the Animal and Plant Health Inspection Service. We are tasked with reviewing LMOs before they are field-tested or commercialized. The United States has been proactive in establishing a regulatory system for the safe development and commercialization of plant biotechnology. Since 1987, when USDA established its regulations for the field testing of transgenic plants, we have authorized more than 6,000 field trials at 27,000 sites in the United States. All have been conducted safely. Our regulatory system is not static, however. As new products are developed and new scientific information becomes available, our system and assessments evolve.

This conference presents us with a unique opportunity to participate in a constructive dialogue on issues of biotechnology and the environment. It is an opportunity to look at the most up-to-date information and root our discussions firmly in a scientific framework. In the coming days, the meeting will highlight the products being developed, the practice of environmental assessment, the scientific framework for assessing transgenic organisms in the environment, and the unique challenges and opportunities for the future.

Conclusion

This conference is meant to foster a dialog among developed and developing countries, industry, and environmental groups about transgenic organisms and the environment. Working together in a forum such as this one – with participants from all over the world – allows us the chance to understand the environmental assessment processes we have in place. I look forward to learning from you and hearing your views on this important topic.
I am very pleased to be with you today at the opening of this important Organization for Economic Cooperation and Development (OECD) conference on living modified organisms (LMOs) and the environment. I thank the U.S. Government for its generous support in sponsoring this event.

We have here in Raleigh today a wide range of globally acclaimed scientists in relevant areas of biotechnology and the environment. We have responsible government officials from many countries. We are joined by representatives of important public interest groups concerned with preserving the environment. I am pleased to note that our participants come not only from OECD countries but also from around the world. This is right because developments in biotechnology and LMOs will have important implications for all people and all environments.

In opening this conference I would like to comment on the following:

- The potential importance of these technologies;
- What governments and international organizations must do to make it possible to realize this potential; and finally
- How this conference is an important part of that process.

Importance of Biotechnology or Living Modified Organisms

Perhaps the most important thing to say about the potential for LMOs is that we really do not have a very good idea of what that potential is.

In the early 1980s I was the minister responsible for Science and Technology in the Canadian Federal Government. In that capacity, I convened an international conference in Ottawa entitled, “Canada Tomorrow.” The purpose of the conference was to look to the scientific advances that we would welcome over the course of the 20 years till just beyond the end of the century. That period is almost behind us,
and I can report that the “Canada tomorrow” we envisaged is far different from the world we now see. That is because advances in science and technology are almost never linear and hence are unpredictable. So it will be with biotechnology and LMOs.

In 1983 we focused on the arrival of the information society and thought it had arrived! Some of us had begun to use computers or word processors in our offices, which are all antiques for today’s generation. But no one contemplated nor mentioned the possibility of the virtual explosion of the information and communication technology that we have seen and continue to witness. The Internet, the World Wide Web, did not exist, and no one as I recall had heard of Moore of Moore’s law!

At that time biotechnology was already showing promise. In 1983 we saw the potential in pharmaceuticals, the use of bacteria in waste control, and the cleanup of oil spills. Even in mining biotechnology, applications could leach valuable minerals from their environment. In fact, at the time, many governments expected that biotechnology would be the major area of economic growth in most of our countries by the 1990s. That has not been the case. Our timing may have been too ambitious. But compared with its likely ultimate role, biotechnology is probably in its infancy.

I think we will be able to compare its impact with the tremendous changes and economic, social, and scientific progress that have flowed from information and communications technologies. International Communications Technology (ICT) has permitted the dissemination of knowledge across the planet at an unprecedented rate. This has allowed us to take and apply successes in medicine rapidly and agriculture—in fact in all areas of human endeavor—Through ICT we have created a powerful global knowledge network that allows researchers and innovators to build upon the accomplishments of others at an unprecedented rate. What took decades, sometimes centuries to disseminate and apply now takes a day, a week, a month—seldom a year. And this process creates a highway for advances in biotechnology.

**Government and Stakeholders**

Will the scientific community be able to realize these advances on its own? Or does it need government—national and international—as well as other stakeholders?

This brings me to one of my concerns and the principal reason I see this conference as very important. Change is taking place so quickly, with effects on so many aspects of daily life, that many people are frightened and insecure. The average person cannot stay abreast of, and understand, these developments. I worry that the rapidity of change is the major obstacle to change itself in democratic societies. As always, ignorance is the enemy of progress.

Furthermore, the ICT revolution has inundated each citizen with information, including misinformation and exaggerated claims and alarms. It becomes difficult even to hear the scientific community. And we have the problem of making the truth sound convincing. Public fear can stampede governments into policies that will fetter new technologies and make them fall short of realizing human progress. How can the scientific community answer the
legitimate questions raised by many with respect to the environmental dimensions of biotechnology as used, for example, in agriculture? The public deserves answers. That is in large measure what this conference is all about.

In June 1999, the Heads of State of the G8 at the Cologne Summit asked the OECD to provide input for their discussions on biotechnology and food safety. At that time, I wrote to OECD Governments to make clear: in order to offer effective assistance to governments in developing approaches to biotechnology—in particular related to issues of food safety, GMOs and trade—the OECD and other international forums must address the interaction of three elements:

Science: findings on the implications of the technologies for human health and the health of the environment need to be presented clearly and underpin policy considerations.

Regulation: regulations need to be consistent with scientifically defined risks to health and the environment, and the similarities and differences in regulation across countries need to be analyzed in relation to rigorously defined standards.

Public information: governments and the scientific community must be transparent in presenting findings on risks and in putting in place measures to address them.

The first action we took in responding to the G8 request was to organize a meeting in November 1999 with representatives from all the stakeholders in the biotechnology issue. I was present throughout, and I must say that I was very impressed by how thoughtful the contributions were.

In March 2000 the United Kingdom hosted an OECD Conference in Edinburgh titled, GM Food Safety: Facts, Uncertainties and Assessment that again involved all the stakeholders and many non-OECD countries to discuss specifically the health aspects of genetically modified (GM) foods. The Chairman’s Report of this conference concluded that worldwide many people are eating GM food with no adverse effects on human health reported in the peer-reviewed scientific literature. The report goes on, however, to state, that in theory there could be long-term effects of GM foods on human health that have not been detected because these foods have been available for less than 10 years. Environmental impacts were also identified as an area of uncertainty. The report of this meeting was an input to the July 2000 Okinawa Heads of State G8 Summit.

In early July this year, again the United Kingdom generously hosted in Bangkok an OECD conference titled, on the New Biotechnology Foods and Crops: Science, Safety and Society. This conference specifically sought to involve scientists, officials, and stakeholders from non-OECD countries. The Chairman’s report of this conference recommended that all stakeholders commit to greater transparency on GMOs and that governments increase their support for independent and publicly funded scientific research into the risks and benefits of GM foods and crops. The output of this meeting was provided as input to the July 2001 G8 Heads of State summit in Genoa.
From the preceding it is evident that the OECD has been deeply involved in furthering the science-based debate on this new technology. This is not surprising because biotechnology is a crosscutting issue, and in the international arena, OECD, by its multidisciplinary nature, is well placed to organize such a debate. In fact, the OECD has taken up the scientific, agricultural, environmental, health, trade, and development aspects the various aspects of biotechnology.

An important task of OECD, as an intergovernmental organization, is to assist countries to ensure that their systems of regulatory oversight are not duplicative internationally and make sense together. The OECD has been working on this since 1982, and good science has always been the basis of our efforts. By bringing together experts from many countries to discuss how to bring the latest science into regulatory efforts, we also assist countries to improve the quality as well as the efficiency of their regulatory instruments. In doing this work we can count on close cooperation with the other intergovernmental organizations that are working in this field. For example, we were pleased to contribute to setting up the Inter-Agency Network on Biotechnology, which includes nine organizations.

After the three major conferences described above, it is now time to discuss what might be one of the most difficult issues in relation to biotechnology, namely, the question of the environmental impacts this technology may have. I thank the United States for providing the possibility here to discuss this issue in the same participatory way as at the earlier OECD conferences.

Finding answers to the environmental questions related to biotechnology is a key challenge and will indeed weigh heavily in the balance between opportunities and possible risks of biotechnology. We have a wealth of scientific expertise assembled here that can look at the issue from different perspectives. I hope that by Friday afternoon we will have a better idea whether there are gaps in our knowledge with respect to the environmental impacts of LMOs and, if so, what these knowledge gaps are and what needs to be done to address them. And in keeping with my earlier comments, it is important that the public be informed about what science knows, where there may be gaps, and how risk in this area is to be assessed and by whom.

The conference results will form a basis for discussing followup work in various fora. The OECD Working Group on Harmonisation of Regulatory Oversight in Biotechnology, in which relevant stakeholders and a number of non-OECD countries participate, is certainly one of the bodies that will carefully look at the outcomes of this conference. It will then decide on how it can contribute to addressing the unanswered questions that might come out of this conference.

We have an exceptional collection of experts here, an excellent chair, and great hospitality from our U.S. host. We have all the conditions united here that are necessary to have a good dialog. Therefore, we have good reason to look forward with great expectations to the contributions from all of you.

I will conclude with three quotes from the late American philosopher Lewis Mumford. He has said that
Western society has accepted as unquestionable a technological imperative that is quite as arbitrary as the most primitive taboo: not merely the duty to foster invention and constantly to create technological novelties, but equally the duty to surrender to these novelties unconditionally, just because they are offered, without respect to their human consequences.

I believe that Mumford’s view would not hold today where we all recognize the importance of examining the opportunities and challenges of new technologies and assessing their benefits and risks. And while doing this I think we can all be inspired by a second quote from the same philosopher. He also wrote that “however far modern science and technology have fallen short of their inherent possibilities, they have taught mankind at least one lesson: nothing is impossible.”

And I conclude with a final quote from Mumford, which I am often disposed to use, namely, “I am optimistic about the possibilities, pessimistic about the probabilities.” I hope that at the outset of this conference we can declare that we are optimistic about both insofar as the potential of biotechnology for improving the human condition on a global basis.

Keeping this in mind, I wish you all a very good conference.
Introduction

This report has been produced by the co-rapporteurs — Calestous Juma, Audia Barnett, and Iain Gillespie. It represents their personal interpretation of the key issues brought out in the conference. This final version has benefited from comments made by participants to the Conference over the following six weeks. The decision to incorporate specific comments in the text has been the sole responsibility of the three rapporteurs.

The objective of the Conference was to bring together a diverse group of participants for a constructive dialogue on the underlying science for assessing living modified organisms (LMOs) in the environment. The emphasis was on transgenic crops because these are the most common applications at the current time. However, other applications were also considered, such as the use of transgenic trees in forestry and fish in aquaculture. The conference promoted a dialogue between developed and developing countries in order to identify unique assessment needs and experiences of different countries and regions. The Conference was attended by around 250 participants from some 20 OECD countries and around 25 non-OECD countries drawn from government, industry, academia and civil society.

The conference was chaired initially by Rita Colwell and in the later stages by Calestous Juma and sought answers to four general questions:

- What are the current trends and future prospects for applications of LMOs and what are the potential benefits and risks?
- What are the current scientific data, information and hypotheses underlying the assessment of LMOs in the environment?
- What are the particular issues with respect to the environmental assessment of transgenic crops and what are the similarities or differences between environmental assessments conducted on transgenic crops and other types of LMOs?
- What future work on scientific environmental assessment is necessary?
In opening the conference, Dr. Colwell emphasized the value of a broad inclusive dialogue between countries. Science needs to refocus from enabling remediation and amelioration and do more to support prediction and prevention.

Accurate, accessible and high quality data and information could help create knowledge and development. Notwithstanding recent concerns about misuse of biological data, the clock must not be turned back on exchanging knowledge and information. On the contrary, science can elucidate our times and contribute to the further development of our world, but the scientific community clearly needs to do more to bring this message of promise to a wider public audience.

In his opening remarks, the OECD Secretary General, Donald Johnston, also emphasized the need for public dialogue, not least to help engender more confidence and less insecurity about biotechnology especially in view of its rapid pace of development. There is a need to communicate knowledge convincingly to the public and to continue the debate on issues raised by biotechnology with all stakeholders. The environmental impacts of the technology continue to need to be considered. The key challenges are to achieve balance between scientific opportunity and safety and to identify gaps in knowledge and what more needs to be done to address these gaps.

Structure of this Report

This report is divided into four parts. The first section provides a status review of trends in the development of LMOs, the practice of risk assessment, the scientific framework for assessment and challenges and opportunities for environmental assessment. It is, in effect, a précis of the proceedings of the conference. The second section summarizes the key areas of general agreement among participants while the third section covers areas where there is as yet no general agreement. The final section charts the way forward focusing on measures which might help to improve ways of assessing the impact of LMOs on the environment, areas requiring further investigation and opportunities for international harmonization as well as cooperation.

I. Status Review

Trends in the commercialization of transgenic crops

Agriculture has always been based on selecting and modifying plants to develop useful crops. Agricultural science is utilizing biotechnology.

Advances in genomics and informatics are helping push back the limits to agricultural production. A new generation of potential traits is being addressed – including factors affecting yield, quality, tolerance to environmental stress. While there has been a great deal of research work on transgenic varieties in many important plant species, large scale and commercial experience to date has been predominantly based on a relatively few crops modified in the main for herbicide tolerance or pest resistance. Changes in technology, including plastid transformation and better gene targeting may contribute to improved safety of LMOs.
Adequate food availability and food security continue to be considerable challenges for many developing countries. Biotechnology potentially offers “packaged technology in a seed” that could improve quality and quantity without compromising local traditional or established cultural practices.

In China, one LMO crop has been commercialized (Bt cotton) and work continues on development of several others. Data that were presented at the conference suggests that there are benefits to the health of agricultural workers (from reduced pesticide poisoning) and that economic benefits appear to accrue mainly to small farmers.

The agricultural priorities in developing countries – including food security and supply, nutritional and post-harvest quality, and appropriate pest resistance - are not always the same as those of developed countries. Most commercialization of LMOs has focused on the needs of the latter. Local farmers are important actors in uptake of technology and better ways need to be found to improve communication between scientists and society. While biotechnology is not likely to be the whole answer to human and environmental needs, international dialogue is required to ensure developing countries’ priorities are not ignored.

**Future trends and applications**

The exponential growth in genetic data has enormous potential to deliver improvements for crops. The key challenges are, in an era of agribusiness consolidation, to move to a more precise discovery model for new agrochemicals, develop better ways to process the flood of available genetic data, and deliver useful new traits and genes to meet the expectations of those investing in research.

A number of current trends were identified. Functional genomics and the ability to sequence whole plant genomes provide a powerful model system for discovery. “Industrialization” of phenotype analysis is increasing the rate of trait assessment. There is greater integration of information scientists with biologists in discovery teams, and combining genomics, proteomics, “transcriptionomics” and “metabolomics” heralds the age of “system biology”. The key drivers for these developments are the increasing trends towards narrower, more targeted markets and increasing “democratization” of discovery and information sharing.

**The practice of environmental assessment**

Many countries have systems of risk/safety assessment in place to evaluate release of LMOs into the environment. The Cartagena Protocol on Biosafety, as well as many national systems, lays down a methodology for risk and safety analysis including a number of systematic steps and a list of points to consider. Different regulatory systems base their assessments on very similar sets of data requirements concerning the organism, insert, trait and environment. Although there is variability between the detail of risk assessments, the issues that they address are common across OECD countries and beyond. Several initiatives are in place to offer capacity building on the practical application of risk assessment to LMOs. Even in relatively developed non-OECD economies human capacity remains a challenge.
There is over ten years of field release data on LMOs. The available information includes data on agronomic and environmental effects. The current regulatory systems have dealt with these releases. Some participants were of the view that the systems need to be looked at to ensure that they are able to cope with future introductions of increasingly complex genetic constructs in LMOs. For example, crops containing stacked genes for herbicide tolerance could be assessed for unexpected secondary functions of introduced sequences. A number of participants were of the view that the regulatory frameworks in their countries allow for these broader and more complex issues to be taken into account.

Much debate continues to focus on gene flow between LMOs and other plant species in the environment and on the extent to which increased weediness might occur. To assess gene flow, when plants with which genes might be exchanged in the environment are present, more knowledge is often required on the biology and spatial location both of the LMOs and such plants. To assess the potential impacts of gene flow, the characteristics of the introduced genes and related altered traits have to be taken into account. Uncertainty about the implications of gene flow is more of a concern when there are wild relatives in the environment and most particularly when such wild relatives are within centers of diversity.

The availability of robust data on the potential for gene flow – and particularly on the location of wild relatives – is sometimes patchy. However, it is feasible to construct databases of the biology and location of wild relatives, landraces and LMOs. Such databases can be used to identify areas where there is a high or a low probability of introgression following the release of LMOs, although the predictive ability of such systems for environments that have not been rigorously mapped needs to be further tested.

Many countries have regulatory systems in place addressing the issue of gene flow. Different countries place different relative emphasis on various factors affecting gene flow.

Many experts emphasized that a distinction needs to be drawn between information necessary to reach a conclusion on safety or risk assessment and information that would simply be scientifically interesting. Lessons can be learned from chemical risk assessment experience, but there are important differences.

Many if not most experts consider that gene flow *per se* is not harmful. However, relatively few empirical data are available on the long-term consequences of gene flow. Uncertainty about possible consequences of gene flow may be higher for these potential long term effects than for short term effects. Assessment of whether flow of particular genes affect fitness, for example, could be done stepwise, including prospective assessment of wild populations to determine likely selection pressures and head-to-head fitness comparisons of transgenic with non-transgenic populations. Assessment might also address whether mitigation measures could be appropriate and available.

Some evidence suggests that there are environmental benefits associated with the introduction of some LMOs. For example, in South Africa there are indications that insect, bird and frog biodiversity may benefit from the use of Bt cotton rather than traditional varieties subject to normal insecticide regimes. Speakers also referred to work in Columbia and China that suggests some environmental benefit. However, more research is needed to validate this.
Risk assessments of genetically modified crops have in the main focused on agronomic characteristics in temperate regions. Comparative risks and benefits of the introduction of LMOs with alternative cultivation methods need to be assessed on a case by case basis, taking into account regional agricultural practices and, where appropriate, socio-economic considerations. Baseline data required for environmental impact assessment, including information on endogenous species and existence of sexually compatible wild relatives of agricultural crop plants are scant.

While the likelihood of harm is a function of both hazard and exposure, the public debate is dominated by hazard identification, often neglecting issues such as exposure and the likelihood of harm, an evaluation of the final consequence and a comparison with the existing situation. The press coverage of potential harm to the Monarch butterfly is a prime example of this focus on hazard identification.

In ecological impact assessments, it is problematic to extrapolate from small scale field trials to the commercial scale cultivation. Countries have taken a number of approaches to dealing with this problem. In the UK, the approach has been to hold farm scale field trials that address scale, and integrate regional cultivation practices and farmer behavioral issues. The research process takes into account factors affecting credibility. It is entirely government funded, foresees peer review and public review of results. The driving issue of this study is the assessment of the impact of herbicide tolerant crops on farmland biodiversity. An ecological model is being developed using specific species as indicators for this purpose.

The cost of these issue-targeted farm scale field trials may be prohibitive for routine assessments of impacts of individual LMOs in every case. More generally, regulatory requirements may impose a cost barrier for development of minor crops (e.g. many vegetables and fruits).

Risk assessments are based on the best available sound science. However, there remains debate about the extent to which subsequent regulatory approvals currently or in the future ought to draw on other factors such as public attitudes and socio-economic factors. One view is that the assessment of risk from LMOs is currently performed on too narrow a basis and that a more interdisciplinary approach is required that draws on more ecological data, considers long term effects, and considers risks alongside benefits in a more transparent and participatory manner.

There is a clear need for better communication on scientific and risk issues between scientists and the public. Public participation is essential in risk assessment and management. However, the debate continues as to how this can best be achieved.

There also remains a difference of view in how to cope with uncertainty in risk assessments. Some participants thought that lessons might be drawn from the introduction of chemical entities where harmful effects took some time to manifest themselves. According to this view, risk management might be applied in advance of assessment, so that risks that, based on current scientific knowledge, could not be assessed rationally were simply avoided. A number of countries apply such a “precautionary” approach. However, others thought that it is not possible to manage risks that cannot be assessed rationally and that governments should focus on assessing and managing identifiable risk.
Further issues that remain under debate are whether assessment of risk and uncertainty should be applied primarily to new technologies or might also be applied to conventional practices and the extent to which the use of concepts like life cycle assessment might contribute to sustainable agricultural development.

**Scientific framework for assessment**

There was a discussion about the science underpinning effective risk assessment.

Assessing ecological impacts of LMOs is not without problems, particularly long term or “secondary” impacts. One approach could be to compare LMOs with organisms produced using more traditional breeding techniques. However problems remain such as lack of reliable base line data, the relevance of extrapolation from small to large scale, ability to detect rare events within a relatively short experimental time scale, lags between introduction and manifestation of impacts and general ignorance about the complexity of ecosystems. Furthermore, there remain problems, concerns and/or disagreements around how to place any observed change in the context of changes occurring through traditional agricultural practices. There is a need for international consensus on how these difficulties might best be addressed.

Measuring ecological impact within soil systems is perhaps most challenging of all. Relevant indicators need to be selected that reflect changes in the rhizosphere and that affect crop performance or food quality. Accurate measurement of rhizosphere populations is difficult. Changes in soils have to be measured by changes in gene products and marker genes or both rather than as change in microbe populations.

Assessment of non-target impacts of LMOs needs to reflect the complexity of real environments. For example, exposure experiments need to consider how an organism accesses its food chain within a given environment as well as considering potential impacts on non-target species that play a significant role in ecosystem functioning when such impacts might plausibly introduce risks.

A more systematic approach is possible and necessary to assess non-target effects.

Constancy of yield is important for developing countries. An inclusive approach to use of technology may be required that integrates pest control, farming and social practices. LMOs that could impact on pest or weed control are best introduced within this integrated framework, cognizant of regional conditions and practices, so that there are adequate levels of control of the target.

Introducing into an LMO several genes that assist the LMO to resist a pest (“stacking” or “pyramiding” of these genes) rather than a single gene is one strategy for reducing the probability that the target pest population will develop a means of countering these genes. Adequate information exchange between public and private sector researchers is necessary to develop a battery of resistance genes for stacking. Risk assessment systems need to evolve to deal with such stacking or pyramiding strategies and international discussion may facilitate this.
For the most part, transgenic plants expressing pharmaceuticals are being developed to reduce production costs and may improve product safety. The gene products expressed by such LMOs may pose different challenges for the assessment methodology. Close monitoring will be required of such LMOs which are likely to be grown in sites dedicated to maintaining product quality through a variety of enhanced isolation procedures.

LMO trees may increase quality and yields and promote sustainability by concentrating planting areas. The technology for the production of transgenic trees is developing faster than the technology for conducting sound environmental risk assessment. OECD first considered genetically modified trees in 1999. The case by case and step by step approach was considered important. Monitoring is difficult because of tree longevity.

Though transgenic insect technology is promising as a possible method of controlling parasite vectors, we know very little about hazards and risks. There is a need for scientific peer review as well as government funds to support research on risk analysis.

Some argue that transgenic fish may pose negligible ecological risks as they are unlikely to be selected for in the presence of wild populations. However, large numbers of LMO fish interbreeding with natural populations may present an issue. Recovery after release is unlikely. Studies based on one individual environment, for example in contained facilities, are inadequate to predict behavior and performance in natural environments.

Advances in genomics raise questions about how we approach risk assessment, as novel genes identified by genomics and available for biotechnological applications will be characterized by novel standards. The general principles underlying risk or safety assessment, however, remain similar.

Maize at the centre of origin and diversity

There was a presentation about maize production in Mexico. Local farmers routinely incorporate genetic material in land races to maintain vigor. Preliminary data presented suggest that Mexican land races might contain introgressed transgenic sequences. These data, however, need to be confirmed through other methods of detailed molecular analysis.

The question was posed whether there are any unique risks posed by the introgression of transgenic traits into land races. Discussions centered around whether an adverse effect would be associated with introgression of the Bt trait into land races. Questions raised in this context included:

- will such introgression affect future levels of diversity in maize?
- will such introgression affect other organisms?
- will such introgression require adaptation of crop management practices to control the pests?

This discussion included questions about whether there had been any adverse effect demonstrated with the use of the Bt gene in any instance. In Mexico socio-economic considerations were said to be important since cultural practices of farmers greatly influence their farm management.
A number of questions were posed about the implications of these events for regulation. Stakeholder and public consultation on the appropriate course of action was considered paramount, as fundamental national values are attached to maize in Mexico. The appropriateness of a moratorium was debated, and the ability to enforce quarantine measures was discussed.

Mention was made of the need to continue to promote cooperation regarding implementation of the objectives of the Cartagena Protocol on Biosafety.

**Challenges and opportunities for environmental assessment**

Public wariness of new and novel food products is not a new phenomenon, but rather has occurred many times over the centuries. The story of the adoption of coffee is an eloquent illustration of earlier debate on the consequences for society of introduction of new types of products.

Monitoring can be a key element of risk management. Insect resistance management in Bt crops has been the topic of much debate during the meeting. Program designs, and the reasons for them, need to be well communicated to and understood by farmers as well as scientists. Farmers are likely to be one of the best early warning mechanisms for any adverse events and cooperation between neighboring farmers is invaluable. Different approaches will be appropriate for different crops and environments, and for different countries and regions. In the US, no field resistance to Bt in LMOs has been reported to date and farmer compliance with insect resistance management strategies has been very high.

Common elements of all pre-commercial and commercial monitoring programs include:
- stakeholder consultation on parameters and implementation
- scientific consultation
- peer review of monitoring plan
- close collaboration of public sector, private sector and farmers in implementing programs to take account of the regional diversity in ecological and agronomic environments and cultivation practices
- public workshops
- communication of results

The processes described were all iterative, to provide for continuous review of what is monitored and how monitoring proceeds according to the latest scientific developments and feedback from scientific and public consultation.

Challenges include sampling methodologies and cost effectiveness of methods in particular if they are to be carried out on a case by case basis. Common international methodologies could be developed for monitoring and sharing of data. Particularly in the tropics there is a real need for more baseline data. Integration of information derived by molecular techniques, global information system technology and ecological studies may help to map areas where gene flow could occur.

An interdisciplinary approach to use of the technology might take into account costs of labor, time, management skills, as well as income to farmers and public acceptability.
There is a need for a common understanding of what constitutes an adverse effect, as well as a common understanding of indicators, risk assessment criteria, and end points.

A key set of questions remain around the extent to which biotechnology will successfully deliver benefits to developing countries and represent a true public good. Delivering research and products that address local needs, but with an eye on international markets, creates great challenges to the leadership and available capacity in developing countries. Public research in Africa, South America and Asia is addressing a number of crops and traits. Progress is being made but few are yet commercialized. In countries carrying out such LMO research, biosafety systems are in place though capacity, management and administration and the wider legal system needs development.

2. Areas of Broad Convergence

Provided that the health and environmental impacts of the technology are responsibly addressed, LMOs can offer the opportunity to address global food security and supply challenges.

Recent advances in molecular and evolutionary biology have opened a wide range of opportunities related to biotechnology. Advances in genomics, informatics and proteonomics are being integrated into systems biology. These advances open possibilities for the development of products more suited to specific human and environmental needs. At the same time, advances in science offer possibilities to improve safety assessment.

Future trends in the economic application of LMOs will depend largely on the extent to which concerns about their environmental implications are addressed. The promise of biotechnology in addressing economic and environmental problems (as reflected in Agenda 21) has been superseded by public skepticism and caution in many countries.

There is common agreement that regulatory practices should be built on scientific knowledge.

Environmental concerns regarding the commercialization of LMOs are renewing interest in ecological research. As the range of products expands so will the need to better understand the functioning of ecosystems. This should be matched by adequate funding.

There is general agreement that case-by-case, step-by-step approaches are the best available tools for managing risks associated with LMOs. A great deal of experience in field trials has generated much information, but risk assessment needs to continue to make use of best available science as new, less familiar trait and organism combinations are developed. There is agreement that gene flow to wild relatives or other LMOs needs to be considered carefully as part of risk assessment. The view of many participants is that gene flow per se is not a particular concern. However, the impact of individual traits in individual circumstances does need to be considered. Centers of origin or diversity offer more potential targets for gene flow that require study and evaluation and so may be particularly vulnerable to gene
flow from LMOs.

Risk assessment practices need to evolve continually to take account of new developments. There is agreement that better communication with the public is needed to improve the understanding of scientific developments. The European Commission, for example, has launched a public consultation on life sciences and biotechnology that aims to include social and policy considerations in research and make such research activities as inclusive as possible.

Continued international co-operation amongst OECD countries and between OECD and non-OECD countries remains essential to harness the potential benefits of this technology in a safe and sustainable way. While assessments need to take into account country or region-specific environments, common approaches and methodologies can nevertheless be developed.

As the scientific basis for decision-making becomes increasingly evident, so does the global nature of the policy aspects of the economic use of LMOs. This is partly because of increasing globalization of the world economy and the associated interdependence among countries through international trade. Resolving many of the considerations associated with LMOs will require international action and cooperation. But the global nature of these concerns are also accompanied by the need to take into account diversity among nations, ecological systems as well as local or regional needs and priorities.

The role of LMOs in solving specific problems of the developing world is emerging as a major policy challenge for the international community. While most of the major crops in commercial use were developed for temperate climates, future technological developments must involve identifying traits of relevance to the needs of developing countries. Indeed, several developing countries are already engaged in research that reflects their own priorities. Nevertheless there is a need to strengthen scientific and technological capabilities in developing countries and foster appropriate partnerships between these countries and industrialized nations.

3. Outstanding Issues

While there is general agreement that scientific knowledge is essential for risk assessment and management, discussions continue on how to identify appropriate baselines and what constitute appropriate data sets for identifying risks and estimating the likelihood of the identified risk occurring. There was discussion of the distinction between “what it is necessary to know” versus “what it is nice to know” in order to make a determination of risk, and what differentiates the two. Discussion also continues about whether a generic approach to risk assessment might be explored for certain applications.

One of the unresolved issues is whether concerns about uncertainty should be applied primarily to crops developed using new technologies and LMOs or should also be applied to crops developed using conventional methods such as wide crosses in breeding.

Some conference participants thought that uncertainty over issues such as labeling and traceability might be resolved by using international standards. They considered that such standards could help to avoid disrupting international trade or undercutting the capacity of developing countries to use emerging technologies, while allowing all countries and regions to meet their needs. Many believed strengthened efforts in international co-operation are essential to improve harmonization of regulatory oversight. Many participants urged that any such international cooperation keep in mind the international framework that will be set up as a
result of the Cartagena Protocol on Biosafety.

Many of the general safety principles that guide debate over LMOs have been developed in fields such as chemical and nuclear safety. While these areas have provided an initial approach to risk or safety assessment and are a major source of information and experience, it is not clear the extent to which the lessons are really applicable to living organisms like LMOs.

4. The Way Forward
Improving Assessments

After a decade or more of work, much progress has been made in understanding the underpinning science and a great deal of experience has been gained with the application and safety assessment of LMOs, including on the environmental aspects of commercial use, though most is drawn from a relatively small number of crop-trait combinations. The time has come to look back on this work and evaluate the data that have been generated.

There is a significant body of knowledge on the environmental impacts of the commercial use of LMOs. Much of this information remains unpublished though some has been quoted widely in public discussions over the environmental safety of LMOs. Synthesis and communication of this existing information might be helpful to inform more authoritative biosafety assessment in the future. Research showing no impacts tends to be regarded as unattractive for publication in the scientific literature. To the extent possible, companies and other institutions should publish or synthesize unpublished information on the safety of LMOs and make it readily available.

In addition to synthesizing the available information, there is a need to improve on existing environmental assessment methodologies in the light of experience gained from commercial and research use of LMOs.

Most existing risk assessment and management methodologies do not consider in detail the benefits that LMOs might deliver. As a result, much of the policy debate about the risks of LMOs creates the impression amongst the public that such products only carry risks and offer no benefits.

Undertaking Further Scientific Investigation

There are a number of areas that require further scientific investigation. These include issues such as gene flow, development of resistance and impact on non-target species. The success of such investigations will depend on the development of agreed baseline data, appropriate databases, assessment methodologies that capture the diversity of ecological systems while at the same time allowing for comparability. Also important is the role of modeling (which is used widely in climate impact studies) as a way of dealing with lack of information and other limiting factors in ecological knowledge.

Specific issues might include:
(i) more scientific knowledge to establish the way in which ecosystems will respond to
introduction of more complex LMOs.
(ii) better understanding of the mechanics and potential impacts of gene flow from living organisms, including LMOs, (the individual trait being a key determinant in considering potential impact) where there are potential hybridization targets available.
(iii) refinement of research on non-target organisms to ensure that it is relevant to real ecosystems.

International harmonization and co-operation

The flow of ideas for new LMOs is not limiting new advances, but transfer from the laboratory to the glasshouse and then to the field is slowly drying up. An important factor remains the unresolved societal debate.

Early phases of technological development are often characterized by regulatory uncertainty and rapid social learning. This requires flexibility and adaptability in existing regulatory frameworks and institutional arrangements based on harmonization and cooperation in research and assessment methods.

A number of opportunities for such international cooperation have emerged from discussion. These include:
• giving further thought to whether comparative risk assessments might usefully be applied to LMOs
• developing consensus on more specific definition of the environment into which LMOs are released in the context of risk or safety assessment (in particular how non-target effects might best be assessed).
• discussing the extent to which current risk assessment techniques are sufficient and appropriate to deal with non agricultural LMOs, LMOs with stacked genes and products of “systems” biology.
• addressing appropriate baselines for assessment and determination of long term effects.
• working together towards a more harmonized understanding of what constitutes an adverse effect, as well as towards developing risk assessment criteria, assessment endpoints and biotic and abiotic indicators in the context of risk assessment and monitoring of LMOs.
• developing more predictive tools for environmental impact of LMOs
• considering how and when future monitoring schemes for research or commercialization of LMOs might best be designed
• developing cooperation on research methodology including, for example, detection methods, monitoring and sharing of data
• seeking agreement on how best to determine risk associated with release of “second-generation” LMOs into environments where other LMOs are already present
• encouraging a discussion of whether, in light of knowledge derived through genome analysis, unexpected secondary functions of inserted genes should further be pursued.
International co-operation is also essential in area of *capacity building* for science-based and evidence-based biosafety management. Such capacity building should involve cost-effective methods of institutional development. This could be done through the expansion of the mandate and capabilities of existing institutions to address biosafety concerns. Where such institutions do not exist, there may be a case to establish new institutions. Regulatory capabilities for biosafety need to co-evolve with developments in biotechnology competence.

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Session 1: Genetic Modification:
Current and Future Applications
Overview of Current Commercial Applications

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I want to start by quoting a statement from a gentleman who has never been known to mince words when he talks about starvation and suffering. He is the agronomist whose discoveries sparked the Green Revolution that has saved literally millions of lives. This individual, Nobel Peace Prize and Medal of Freedom winner Dr. Norman Borlaug, states that we cannot turn back the clock on agriculture and only use methods that were developed to feed a much smaller population. We cannot feed our global population unless farmers across the world have access to current high yielding crop production methods as well as new biotechnology breakthroughs that can increase yields, dependability, and nutritional quality of our basic food crops.

We need to bring common sense into the debate on agriculture and science and technology, and the sooner the better. I believe that this conference today—and thank you for inviting me—is going to focus on the application of common sense to improving food productivity and food security worldwide.

I think the African scientist Florence Wambugu of Kenya best describes the real potential for application of biotechnology insofar as developing countries are concerned, and indeed for any country. She states that the great potential of biotechnology to increase agriculture lies in its “packaged technology in a seed.” This is scale neutral and it ensures that farmers can adopt the benefits of these technologies without changing local social and cultural practices. Dr. Wambugu has stated that, in the past, all attempts to improve productivity, specifically in her region in Kenya, have, in large part, failed because they demanded massive cultural change that farmers were not willing or able to undertake.

To illustrate the amount of land that would be needed to produce the same amount of crops today if we were to use the technology that was available to us in 1929, look at the map of the United States of America. Over two-thirds of the country would be under plow. We would lose our national parks, wetlands, and our marginal lands if we were to try and achieve equal productivity using “natural methods of production.”

If you look worldwide, a more devastating picture develops. Every single second, we lose 3,000 square meters of forest and 1,000 tons of topsoil. The arable land shrinks by 20,000 hectares every single year, and erosion has made 1 billion hectares
of soil unusable for agriculture. These are devastating numbers. We lose land to irrigation-induced salinity every year. We have lost about 25 million acres of land this way. This is approximately one-fifth the size of California. At the University of California, Davis, scientists have developed tomatoes using a transport protein that will allow these plants to grow in up to one-third the salt concentration of seawater. This will have enormous potential for developing countries. Over 25 percent of the grain needed in Africa is imported, whereas up to 40 percent of the harvest is lost owing to postharvest damage. One cannot imagine how we can achieve adequate productivity while minimizing the impact on our environment without using the tools of biotechnology.

Here are some examples of the technology available today that can be used to address these issues. In 1906, Luther Burbank wrote, “We have recently advanced our knowledge of genetics to the point where we can manipulate life in a way never intended by nature. We must proceed with the utmost caution in the application of this newfound knowledge.” Burbank (1849–1926), contributed significantly to modern agricultural technology by introducing some of the first scientific approaches to crossbreeding and selection. In fact, we have been modifying the world around us, primarily agricultural crops, for thousands of years. The notion of referring to modern genetically modified crops as living modified organisms is a slight distortion of the truth. Everything you are looking at around you today, from a crop point of view, is a living modified organism. Burbank was one of the first to apply scientific principles to modified organisms, but we have been modifying organisms for about 10,000 years. It has taken 10,000 years to reach the current levels of production of about 5 billion tons per year. And we are going to have to double that level of production by the year 2025.

If many consumers in the street, and even many of you, were to look at this century’s applications of technology, it would boggle the mind. The current technologies we use to increase productivity lend themselves to the map of the United States of America referred to earlier. One example of technology that we have been using is embryo rescue and wide crosses. We are using technological methods in which genes are integrated from very wide crosses that would not cross in nature because of reproductive isolation. These genes are sexually incompatible in nature, and although sex is wonderful, it is limited as far as the farmer is concerned. Modern technologies provide means to bypass this sexual incompatibility barrier. A green-looking cauliflower, called brocciflower, is an example of a wide cross of two species that would not cross in nature.

An example from work conducted at UC Davis helps to illustrate this point. High soluble solids are the Holy Grail for processing tomatoes—the higher the solids, the more paste there will be for the cannery. The common processing variety of tomato, *Lycopersicum esculentum*, has about 5 percent soluble solids because it is a hexose accumulator. There is a wild variety of tomato, *Lycopersicum chimielevskii*, that has 10 percent soluble solids, for it is a sucrose accumulator. But that is the only good characteristic that it has. The other characteristics are rather undesirable: small size, bitter taste, lower yield, toxicity, because, like potato, the tomato is a member of the deadly nightshade family that produces glycoalkaloid toxins. Toxic pizzas would probably not pass muster with the Food and Drug Administration (FDA). The researchers in a laboratory at UC Davis (Alan Bennett, personal communication) decided to try to transfer the higher soluble-solids-accumulation characteristic from the wild tomato to the domesticated tomato and yet retain all of the other desirable characteristics of the domesticated variety. Using a classical breeding approach to achieve this, first the researchers crossed the wild tomato with the domesticated tomato and over many years of
backcrossing to the domestic parent came up with a tomato with higher soluble solids content. One of the other sequences contained in the introgressed genes caused reduced fertility in the resulting tomato. Also, it is difficult to determine how much of the toxic gene information is still in those introgressed sequences. This illustrates that with classical breeding, breeders do not always get just the characteristics desired; sometimes they also get somewhat undesirable characteristics. And the breeder has little control over this outcome.

In the second approach the goal again was the same: to increase the soluble solid content of the tomato. This time the researchers looked at the metabolic pathway for simple carbohydrate synthesis and determined that if one gene is switched off (one responsible for coding for an enzyme that converts the sucrose accumulator [high solids] into a hexose accumulator [low solids], they could engineer a more valuable high-solids tomato. Through modern molecular breeding technologies they were able to turn off that gene by adding a complement of it using antisense technology that acts like a complementary piece of Velcro to turn off the machinery that makes the sugar-converting enzyme.

The application of technology to improve in-crop agriculture experienced a paradigm shift with the advent of recombinant DNA technology. Technology is shifting yet again with the application of the knowledge developed through the Human Genome Project and the discipline of bioinformatics. We are using the power of computational knowledge to understand the genes that are important in agricultural crops. For example, we can look at centuries of gene maps of cereals and take genes from distant relatives to see how they could be applied to improving productivity in cultivated species. The Native Americans did this for years. The ancestor of modern-day corn, teosinte, has a very thin ear, yet it has nearly all the genetic information necessary to produce modern-day corn. It has taken many years of mutation and selection to achieve today’s large, bountiful ear of corn.

Most people do not realize how much we actually use mutation—chemical or irradiation mutagenesis—to modify our crops. Japan is one of the countries that is quite skeptical of genetically modified organisms. But in fact Japan has been using radiation mutagenesis to produce mutations in crops for some time. The Institute of Radiation Breeding in Ibaraki-ken, Japan, uses cobalt-60 at the center of a field that produces 89 TBq of gamma radiation for radiation breeding. This field has a shield dike 8 meters high, and the source is so powerful, that workers must retract it into the earth before going in to the field to select the plants. Breeders look for the plants produced by the grown seeds that have the specific advantageous morphological changes desired.

This particular last-century technology has an interesting bearing on the complex issue of labeling. The Minister for Agriculture in Italy was horrified when he read in a German magazine in the summer of 2001 that his spaghetti had been modified using mutagenesis. He said, “Absolutely not, our spaghetti is pristine.” Well, actually, the durum wheat used to produce the spaghetti was produced using artificial mutagenesis. In addition, the Asian pear was improved by irradiation breeding. All Asian pears are susceptible to black spot disease. Through irradiation mutagenesis, we now have a pear perfectly resistant to black spot disease. If it were to be labeled, it would read, “Pear bred through radiation technology produced in California.” This statement is scientifically accurate but is not informative to the consumer. In fact, this statement would probably be construed as a major warning by the consumer.
Current Commercial Applications

The estimated global area of transgenic or modified crops for 2001 is 130 million acres grown by 5.5 million farmers in 13 countries. Globally, the principal modified crops were soybean, occupying 33.3 million hectares (ha) in 2001 (63 percent of global area) followed by modified corn at 9.8 million ha (19 percent), transgenic cotton at 6.8 million ha (13 percent), and modified canola at 2.7 million ha (5 percent). In 2001, herbicide tolerance, deployed in soybean, corn, and cotton, occupied 77 percent or 40.6 million ha of the global modified 52.6 million ha with 7.8 million ha (15 percent) planted to Bt crops, and stacked genes for herbicide tolerance and insect resistance deployed in both cotton and corn occupying 8 percent or 4.2 million ha of the global transgenic area in 2001. Developing countries account for 24 percent. Although 24 percent is a large percentage, almost all of that production is from one developing country, Argentina.

The timeline of biotech crop traits can be divided into three categories:

• agronomic traits, (both biotic and abiotic stress), and yield,
• qualitative traits,
• novel crop products and the environment.

I want to focus on the top few crops that have been commercialized primarily in the area of biotic stress and speak specifically on insect resistance, herbicide tolerance, and virus resistance. The primary crops are soybean, corn, and cotton.

The area of abiotic stress is equally important—especially for developing countries. Work has been done at UC Davis to develop transgenic tomatoes with a transport protein that takes the sodium ions in the vacuole and allows the plants to grow in 200 mmol salt, which is about one-third the salt concentration of seawater. Because 40 percent of soil is lost each year owing to irrigation, this could be of great benefit in developing countries. Likewise, aluminum is a major problem with respect to poor soil. Research is being done in Mexico on the production of citric acid in the roots that would allow plants to grow in soils contaminated with aluminum.

Crop yield is a critical issue for developing countries. Currently, we have maximized our capability of increasing crop yield through normal physiological processes. However, metabolic engineering holds incredible potential for increasing yield. The majority of genetically engineered plants now under development are the result of single-gene transfers. Such efforts, although important to raising actual yields, are unlikely to raise potential yields. To break yield barriers, the plants will have to be thoroughly reengineered. Nordine Chiek, director of Calgene research, defines several parameters for yield increase, including water use efficiency, thermostability, source capacity, starch synthesis, seed weight, and nitrogen metabolism. He has taken two main approaches to increasing yield in maize. One is through increased starch biosynthesis, and the second is through improved nitrogen assimilation. For the former he has modified starch metabolism to increase sink strength, and for this again he has taken two approaches, both of which depend on a thorough understanding of carbon metabolism and starch biosynthesis.

One approach is to improve the activity of an existing enzymatic step, and the second is to alter the metabolic pathway. There are many intermediary enzymatic steps in the metabolic pathway from sucrose to starch, and Chiek has targeted the two ends of the pathway to enhance the efficiency of going from source to product by increasing sucrose hydrolysis through altering the pathway and increasing sucrose biosynthesis by improving an existing
enzymatic step. He achieved the former through the introduction of a new gene coding for sucrose phosphorylase, which takes the pathway straight to glucose-1-phosphate, thereby bypassing UDP-glucose. The second boost also was achieved by introducing a new gene, but this time it has an endogenous counterpart. The idea of the new gene is that, because it is from another source and under the control of a different promoter, its activity is not subject to the same degree of inhibition by the plant’s native regulatory machinery. This new gene from *Escherichia coli* codes for the enzyme ADP glucose pyrophosphorylase and is under the control of a seed-specific promoter. Taking this approach, Chiek found on average a 23 percent increase in grain weight. Taking the same gene and this time placing it under the tuber-specific patatin promoter in potatoes, Chiek increased starch content by over 30 percent. This has an added bonus, as the higher starch content results in a lower moisture content that in essence gives not only more potato for one’s money but also far less fat absorption during frying because moisture lost during the process is replaced by oil uptake.

Increased yields by improved nitrogen assimilation may be the next breakthrough. Benefits of improved nitrogen assimilation in crops include optimization of crop response to fertilizer, increased yield potential at low and high levels of nitrogen, positive environmental impact, reduction of nitrate in ground water, improved crop quality and seed composition, and higher protein in leaf and seed.

Nitrogen is fixed, or combined, in nature as nitric oxide by lightning and ultraviolet rays, but more significant amounts of nitrogen are fixed as ammonia, nitrites, and nitrates by soil micro-organisms. More than 90 percent of all nitrogen fixation is effected by them. The major sources of nitrogen fixation for plants are soil and symbiotic bacteria such as *Rhizobium* associated with leguminous plants. Nitrates and ammonia resulting from nitrogen fixation are assimilated into the specific tissue compounds of algae and higher plants. Asparagine and glutamine are the main forms of transported nitrogen in cereals. Higher plants are more versatile than animals; they can make all of the amino acids required for protein synthesis with either ammonia (NH$_3$) or nitrate (NO$_3^-$) as the nitrogen source. Chiek has improved the assimilation process by taking advantage of the importation of ammonia into the intermediates of metabolic pathways mainly via the glutamate dehydrogenase (GDH) reaction. He has introduced GDH from an algal origin, bypassing two intermediate steps in the metabolic pathway for the production of glutamine and has thereby increased kernel protein by 6–12 percent.

Research is even being conducted on oxygen assimilation by modifying stomata or introducing hemoglobin genes that would carry oxygen more effectively through the plants. That task is daunting enough, but other researchers would like to go even further and tinker with the mechanisms of photosynthesis itself. Controlling such basic multigene traits is a complex, unpredictable task. Photosynthesis is a process that evolution has not changed fundamentally in a couple billion years. To improve crops’ ability to turn atmospheric carbon dioxide into food, genetic engineers have focused on RuBisCo, the principal catalyst for photosynthesis and a notoriously inefficient enzyme. Laboratories across the world are trying to improve the RuBisCo in food crops either by replacing the existing enzyme with a more efficient form identified in red algae or “bolting on” what could be thought of as molecular superchargers.
Some critics, however, question whether this approach will benefit agriculture. Since at least 1970, research has shown little correlation between crops’ photosynthesis rates and their yields, suggesting that improvements in RuBisCo will not automatically translate into better harvests. Thus, even if the work is a technical success, the payoff may be minor because traditional plant breeding has already pushed up crops’ harvest index and ability to capture sunlight about as high as they can go. Still, altering photosynthesis remains a hope for the future of agriculture. Once all the relatively obvious steps have been taken, photosynthesis is what is left.

Let us now look at some environmental benefits of engineered crops. The biggest environmental impact of quality traits research is in the area of phytase. One might ask, What importance has this from an environmental point of view? Seeds store the phosphorous needed for germination in the form of phytate, a sugar alcohol molecule having six phosphate groups attached. In terms of food and feed, though, phytate is an antinutrient because it strongly chelates iron, calcium, zinc, and other divalent mineral ions, making them unavailable for uptake. Also, in this form phosphorous is not bioavailable to animals, and one must supplement it in feed in most instances—especially for monogastric animals—with phosphate. But the problem with that, from an environmental point of view, is the animals end up excreting most of that phosphate into the environment. This excreted phosphate can lead to environmental pollution and eutrophication. Introducing a phytase enzyme into the plant breaks down the phytate and renders it bio-available and also makes divalent ions available. The food does not have to be supplemented with phosphate, thus eliminating the excretion of phosphate and reducing eutrophication. This is an environmental benefit.

From a broader environmental perspective, I think the greater potential for biotechnology is to use the incredible amount of biomass in the environment as a source not just for biofuels but also as feedstocks for the production of raw materials such as synthetics and chemicals. But this is far into the future, for it is not economically feasible right now.

Currently, as far as the United States is concerned, the three main biotech crops are soybean, corn, and cotton. Despite predictions of doom and gloom from the European Union perspective, the only crop that decreased this year, in fact, was corn. It went down not because farmers had a fear about the technology (farmers are very wise) but because the farmers did not consider it necessary in 2001 to buy insurance in the form of the technology premium. The prime reason to purchase Bt corn is to protect against loss due to the European corn borer. Farmers felt the pressure from the European corn borer was going to decrease this year, and therefore they did not need to pay the premium cost to get this guarantee.

But if you look at the actual adoption for soybean and cotton, it has skyrocketed this year. It has increased to 64 percent for cotton and 63 percent for soybeans. In fact, as the numbers come in, it is now probably closer to 70 percent in real terms. Rapid adoption and planting of transgenic crops by millions of small and large farmers around the world; growing global, political, institutional, and country support for biotech crops; and data from independent sources confirm and support the benefits associated with biotech crops, which according to estimates exceeded $700 million for growers in the 1999 growing season. These results demonstrate that even with issues to acceptability—especially in Europe—the benefits are so great for farmers that they are still increasing their use of this technology. As to the actual overall numbers with respect to environmental impact, growers have found a reduction of
greater than 20 million applied-acre treatments of pesticides. This represents an enormous cost reduction, to the farmer and particularly in cost to the environment. Adopting this technology, as opposed to relying on traditional insecticides, has proven beneficial.

Another advantage of Bt corn, which was not unexpected by scientists and was, to an extent, not expected by farmers, is the collateral reduction in fumonisins. People often think of something natural as good, but believe me, some of the worst toxins in the world are produced by fungi. This particular toxin, fumonisin, has been known to cause liquefaction of horse’s brains and liver cancer. It is rather unpleasant stuff. But by protecting the plant and the ear of corn from being nibbled by insects, the spores of the fungus do not have access to the corn. Thus, you can gain a reduction of up to 90–95 percent of contamination of fumonisin. This is a significant improvement, and thus your corn flakes will be free of fumonisin. Right now, growers must use fungicides to control it.

Much attention was devoted to the recall of taco shells and other food products possibly containing StarLink corn. The Bt protein in StarLink, Cry9C, does not resemble any known allergens and was not derived from an allergic source, nevertheless the protein is more stable in some digestion tests (one attribute of allergenic proteins) and thus needs more research before receiving approval for human consumption. Allergenicity experts say that a high level of exposure to a protein is needed over a considerable period of time for an individual to be sensitized. The amount of Cry9C in corn kernels was less than 0.03 percent with considerably less than that in the shells themselves because the protein was only a small component of all the corn and other ingredients used. In addition, as this corn is processed at high temperatures, the protein is denatured and therefore no longer in a form that could cause an allergic response. Those who claimed to have suffered an allergic response having consumed products containing corn from the Cry9C cultivars had their blood tested by the Centers for Disease Control to determine if it contained Cry9C-specific antibodies. None were found in any example. Although this does not completely eliminate the possibility of a potential for allergenicity in a subpopulation, a 100 percent negative finding is relatively strong evidence of a nonevent. In effect there was little cause for concern, and the various companies’ quick action in recalling the product and Aventis’ decision to stop all sales of the seeds eliminated any possibility of harm.

Likewise, the other area of concern with respect to Bt corn was the potential impact on Monarch butterfly larvae. Six papers were published in October 2001 Proceedings of the National Academy of Sciences to indicate that Monarch butterflies are not in danger from this technology. In fact, the numbers of Monarchs have increased in the fields of farmers who have adopted Bt technology because, of course, now growers are no longer using broad-based pesticides that kill Monarch butterflies.

Another significant area that has proven advantageous, and in fact the one that has probably the greatest impact on field acreage, is the glyphosate-tolerant soybean. The advantage of this is that not only does it improve weed control but also actually helps farmers adopt no-till technology. Using no-till growers is improving the probability of diminishing soil erosion, for farmers do not need to plow into the surface anymore. Additionally, this means that the debris remains in the field from year to year, which allows beneficial insects to come back to these fields. Scientists have found small mammals and birds returning. Farmers are not using fossil fuels, and growers are not compacting the soil with heavy machinery.
But as far as the farmers are concerned, the real saving is to their pocketbook. In 1998 farmers saved $280 million by adopting this technology because they were no longer dependent on having to make multiple applications of complex herbicide cocktails. They only have to apply herbicides if weeds are present. In addition, this technology saves farmers’ time and does not affect crop rotation. There is less damage to the crops using this herbicide because it does not persist in the environment and is not carried on to the following year.

Herbicide concentrations have also decreased markedly since 1998 in groundwater, of farmers who adopted this technology as evidenced by a reduction in the number of groundwater label advisories for herbicide residues. Contamination in groundwater is decreasing substantially. In fact, in Illinois water monitoring samples, these herbicides are no longer measurable which is another success story.

Outside the three principal modified crops the most significant impact of a lesser crop has been demonstrated in Hawaii. In that State, a virus called papaya ring spot virus was devastating the Hawaiian papaya crop. There is no natural resistance to this virus. One could look in all the plants in all the countries of the entire world without finding a resistance gene. Dennis Gonsalves isolated the coat protein of the papaya ring spot virus, which he used to protect the plants. This is similar to a person being vaccinated against a virus. Plants, of course, do not have an immune system. But interestingly enough, this coat protein confers immunity against the virus. By inserting the gene coding for this coat protein, Gonsalves succeeded in protecting the crop, and the papaya economy is thriving in Hawaii.

Of course, the problem with using a coat-protein-mediated resistance is that it depends on a single gene. Increased selection pressure will be imposed on the virus to overcome protection; just one gene is involved. Scientists are now focusing on developing multiple strategies and taking a pyramid approach to reduce the probability of developing a resistance against this coat protein. They are looking at ways of using alternate techniques such as antisense technology and ribozymes. Ribozymes are catalytic ribonucleic acids (RNAs) that can be used to break down the virus. Scientists are looking at ways of modifying movement proteins to stop the virus from going from cell to cell. They are also looking at protease inhibitors. This is similar to the technology used in AIDS research to stop replication of the virus. These approaches all have different modes of action, thereby increasing the probability of reducing selection for resistance to the system.

You already heard about Pam Ronald’s work from Dr. Chen. Dr. Chen talked about the advantage of this particular gene, which confers resistance against bacterial blight caused by the bacteria *Xanthomonas oryzae*, which is responsible for a highly destructive disease of rice that often causes 50 percent yield losses in some areas. In fact, by taking the gene XA21 from the wild resistant variety and introducing it into the japonica variety (which is susceptible as manifested by about an 85 percent harvest loss each year), scientists have found the japonica variety developed 10 times the resistance of the donor plant. Pam has insisted that companies licensing this technology set up a scholarship fund for the country where the germplasm originated, India. (Some scientists believe in payback too.)

Another plant we are working on at UC Davis is called *Striga gesneioides*, which is a major biotic constraint on cowpea production. *Striga* is a parasitic weed that results in losses of $7 billion worth of crops in the savannah regions of Africa each year. Herbicide tolerance will not work in this plant because the seeds of the weed itself form a very close association
with the plant. It is a very intimate association. When the farmers harvest the seed each year and put it back into the ground, they compound the problem by putting the parasite back as well. These parasitic weeds depend on signaling systems from the host plant. The parasites are actually very cleverly using the signaling systems plants normally use called “xenonosins,” which is a term deriving from the Greek word meaning to recognize the stranger. *Striga* has turned the signalling system on its head, and instead of allowing the host to recognize the stranger, *Striga* uses its to hone in on the host plants. John Yoder has developed a mechanism that actually interferes with the signaling system. By inhibiting the signaling system, researchers are hoping to generate parasite resistant crops.

I would like to address some of the concerns about biotechnology. From a scientific perspective, antibiotic resistance, in reality, is not an issue. In 20 years of trying, no scientist has succeeded in transferring the antibiotic resistance genes from any crop plants into our commensal bacteria, potentially turning them into the multiple resistance strains encountered in hospitals. There is a far greater chance of acquiring resistance from an antibiotic-resistant bug by walking through a hospital than from eating a transgenic plant. However, researchers are looking at other mechanisms such as transposon tagging or positive selection in which there is exclusive energy source—for example, phosphomannose isomerase. Plant cells without this enzyme will not grow on media that have mono-6 phosphate as the sole carbon source. This is a much better selection method.

Likewise, with respect to gene flow, the real focus is on the whole area of chloroplast transformation because chloroplasts have their own DNA. They stay in the maternal line; the genes cannot get out. Numerous tests have been conducted in this area. Chloroplast transformation is also more efficient because there are far more copies of chloroplast DNA than of genomic DNA. This could address the issue of resistance in, for example, *Bt*, because a very high dose results owing to the number of copies inserted into the chloroplasts. Thus, researchers are also going to be using site-specific recombination to get chloroplast transformation. One concern that arises involves not knowing where the gene has been inserted, which, of course, is an issue in traditional agriculture as well. It is not necessary to know how the genes are going to come together. Using site-specific recombination, or what is called the Cre–lox site-specific recombination system, one can be very specific about where these genes are being targeted. Likewise, with respect to effects on nontarget species, one can have tissue-specific expression, and again, chloroplast transformation. I talked about the issue of loss of effectiveness by using gene-pyramiding or gene shuffling, which entails actually using gene rotation to modify the environment to which the pests are being exposed at all times.

I will leave you once again with the a thought from Norman Borlaug, who stated that the affluent nations can afford to adopt elitist positions and pay more for food produced by the so-called natural methods. The billion chronically poor and hungry people of this world cannot. New technology will be their salvation, freeing them from obsolete, low-yielding, and more costly production technology. As Jimmy Carter has observed, “responsible biotechnology is not the enemy. Starvation is.”

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Overview of Crops Important to the Developing World

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We know that some of the challenges to agriculture in developed countries are also very familiar to people in developing countries as well. One of the foremost challenges is a rapid increase in the population along with a decrease in our farmland. Population issues are of concern especially in developing countries such as in China but also for other countries. A dependent problem is a severe shortage of water. We also see dramatic increases in the cost of farming, which is especially expressed in a need for increased pasture size and in increased fertilizer costs to farmers. The last challenge to those developing countries, especially to China, is entry into the World Trade Organization (WTO) and its associated requirements for agriculture.

Premier Zhu just last month stated that, after entry into the WTO, agriculture is the issue of most concern to him. He recognized that we are using 7 percent of available land to feed about 22 percent of the world’s population. And you know that the population increased dramatically in China as well as in India and other countries between the 1950s and 2002. Meanwhile, the average amount of farmland per person decreased since the 1950s up through the present. Grain productivity increased owing to production of hybrid rice and other technologies. However, beginning in 1996 and 1997, the production in grains has become quite stable. It has not increased while the population was increasing so dramatically.

Thus, the governments of developing countries, for example China, decided to establish agriculture as a top priority. In China, biotechnology was placed among the top technologies, which included lasers, space, information, and automation. However, biotechnology was put in the very top priority in the Government’s agenda in the past 10 years and for the next 5 years. The budget for biotechnology from our government increased dramatically from 2 billion to over 13 billion yuan, just for biotechnology research.

In the next 5 years, Government strategic planning identifies biotechnology as a national priority. The first area of emphasis for biotechnology is that of so-called molecular breeding, which basically refers to production of transgenic or genetically modified organisms. The next area is genomic research. We also have research funding in the National Program for Biosafety Research.

1from an edited transcript of the presentation
Since 1986, the two principal areas to which we pay attention, crop improvement and genomics, have been quite similar to those of importance in other developing countries. The first priority for the biotechnology program is developing hybrid rice, which is very important for China, and the second is for the development of transgenic cotton with resistance against insect pests. Additional crops include transgenic plants like rice, wheat, corn, and others. I was in charge of the transgenic plants program in China from 1986 to 2000.

I would like to present a brief background on transgenic plants in China. We started to release transgenic plants, both tobacco and tomato, in 1989. In 1993 the first regulation on biosafety was issued by the Chinese Government. In 1997, the Ministry of Agriculture started to enforce the law on commercialization—the approval, release, and commercialization of transgenic plants—1997 is the year that transgenic plants were first officially commercialized in China. And then in 1999, 2000, and 2001, zero approval was given to any new crops in China, owing to very severe problems that originated within the European Economic Union (EEU) and some other countries, and the pressure they exerted. I will go on to describe some of the issues.

On 9 May 2001 Premier Zhu Rongji issued a new national regulation applying to management of agriculture and genetically modified organisms (GMOs) (see the website http://www.chinafeedonline.com/dbnews/news_show.show_news_detail_en?newsid=5833&tmppt=forprint). I understand that Canadian officials and scientists are very familiar with this because they recently sent a letter to our Government about their concerns with this new regulation.

The area planted to commercialized transgenic plants in China was the fourth largest in the world. From 1997 to 2000 applications rose from 55 to 443 applications per year, which is a dramatic increase. Before commercialization is approved, environmental release, field trials and also pending productive release must be approved for field trials of the major crops in China. These large numbers are only field releases and are not allowances for commercialization.

For field release, the largest crop approvals are for rice. Two major problems have been addressed. One is insect pests, and another is bacterial disease. Line XA–21, for example, expresses both insect and bacterial resistance. Several other genes are also being tested and released in the fields.

And then we have corn, soybean, and wheat trials. At present, only four crops are approved for commercialization, and only six major licenses have been approved by the Chinese Government. The four approved crops are transgenic cotton plants with Bt against insects; tomatoes with Bt virus and a third expressing control over ripening sweet peppers; and another with resistant to the petunia with altered color expression.

Transgenic cotton has very dramatically influenced Chinese cotton production. The Bt cotton lines commercialized in Hebei Province are produced on over 99.7 percent of personal land. Hebei Province is a very significant location for transgenic cotton. I will go over some transgenic cotton data evaluating effects on the environment and also on the farmers.
Now, the second major (transgenic) crop is rice, and those lines being released are in Fujian Province in the southern part of China. Compared with nontransgenic rice, control of insect pests in transgenic rice is significantly improved. This crop has been allowed to be planted in field releases and tests. We have also transferred a second gene together with the Bt gene into hybrid rice. Again, we have seen significant control of insect pests and also bacterial diseases.

At present, we have some transgenic rice and wheat tests in the fields. We have a Bt corn and soybean, a tomato, a sweet pepper, and petunia flowers as well as continuing research with international cooperation on transfer of genes into rice for producing vitamins A and E. Now, vitamin D expression has attained importance as a goal so that it will be made available in Chinese rural areas. We are in the process of transferring the genes to synthesize vitamin D in rice.

Transgenic plants have been released in most parts of China from the southern part to the northern part. As I just mentioned, China is ranked the fourth largest country in the world right now for transgenic crop production next to other developing countries such as, for example, South Africa and Argentina.

In Asia, there are only two countries in which transgenic crops are officially approved for commercialization: China and Indonesia. Among the challenges to transgenic crops in China and other developing countries are four issues that people mention again and again: environmental safety, food safety, public acceptance, and finally—and this is a very key issue—the trade issue.

A very complicated situation has developed in the past 3 years. It involves the scientific community, the public, politicians, farmers, companies, and consumers. I took a photo at the last OECD meeting in Edinburgh (Scientific and Health Aspects of Genetically Modified Foods) with its contentious asides, and the photo has been published in Chinese newspapers. I took the photo to show the very complicated situation in Europe. We in China should be careful because of A, because of B, and because of C and D. However, the applications to China—perhaps with the most acute consequences—are those arising because of impacts on global relations. We thought that we might do much in response to these issues, but that has actually not been the case. The situation is difficult.

The consequence of the sanctions placed against transgenic tobacco produced by China was that no more transgenic tobacco would be produced. The sanctions were also placed on soy sauce because Europeans understood that we use American imported transgenic soybeans to produce soy sauce, and therefore EEU countries quickly placed sanctions on our soy sauce. That made people very concerned. So the Government introduced a new law, a new regulation, to enhance accountability and management of food content, and that got more ministries involved.

Originally, just the Ministry of Agriculture was involved in regulating biotechnology. Now, we have Public Health and Environment, Import–Export, Inspections, and also the Ministry of Science and Technology involved too, and thus the regulatory climate has become more complicated. It is even becoming difficult to approve any new crops for commercialization.
The new regulations have resulted in concern by officials from different countries about the details we are proposing. We will issue new regulations announced in advance by the premier on 9 May 2001, this year. These will include four additional requirements in addition to those established by the law in 1996. The first requirement is that several more ministries must together approve the new license for commercialization and field release. The second requirement is that we have one more trial in addition to a field trial, field release, and medium trials. The additional one is called a production trial, and requires a lengthy effort for its accomplishment.

The third requirement is a labeling system. We presently have a labeling system in the country similar to that of the United States and Canada. However, because, Japan and Europe have requirements, and especially now because we are a member of WTO, that must change. Obviously, after entering WTO, we needed to have labeling, and this is a very complicated situation now. The last requirement is for import and export certificates in this new regulatory system.

Thus many puzzling arguments arise in China, a developing country, as they also do in other countries. I am sure that many arguments arise. The first is that this technology only benefits the multinational companies, not consumers or farmers. The controversy over who receives the benefits from this technology comes up again and again along with many other similar arguments. Recently conclusions were reached that organic agriculture will be the production system of the future and that GMOs have no future. Those ideas were published in newspapers in China and other developing countries.

I will give you data, very simple data, from a 3-year study. One group cooperated with U.S. scientists, and another two groups in our university studied crop modification benefits for 3 years. The Guokang is a variety [developed by CAAS] released in China, another is the transgenic cotton jointly issued by the Monsanto Company and local government, and a third is a domestic transgenic cotton Zhongmian released for field production by the Cotton Research Center.

There are 3 years of data which are basically very similar for the three varieties on the benefits going to farmers, the percentages, and also the profits to the multinational companies. Benefits go to both the multinational companies and to the farmers. From products of the Monsanto companies, about 83 percent of the benefits go to the farmers, and again, about 10 to 17 percent to the company because it has increased the price of seeds. The benefits still go to farmers—especially to poor farmers. No profit goes to the Chinese seed developers (from their lines released by the Chinese Academy of Agricultural Science) because we do not collect money from farmers.

A second argument says that there is almost no need for the transgenic crops, or that insect resistance will quickly develop, or that some other problems will arise. As some of you may know, over 40 to 50 thousand people are poisoned each year in China. These data have been published in a public database. Over 400 to 500 people die each year because of poisoning by pesticides in cotton and rice fields.
In the Hebei Province, I just mentioned that over 90 percent of fields were planted with transgenic cotton in the 3 years after plants were first released in the fields. This is a family-based economy, a family system. If the crop is not good, the farmer will not plant it. Planting decisions are not controlled by the Government but by each family.

The area in which transgenic cotton has been planted has increased rapidly since 1997. For the first release, we allowed the Hebei Province to plant only 2 hectares. By 2000, 220,000 hectares were planted. In Shantung Province, and in other provinces, cotton land has also increased dramatically. For pesticide use, we also have 3 years of data. The yield we received from Bt cottons substantially increased. However, for the pesticides used, you can see that on Bt cottons, pesticide use dramatically declined compared with non-Bt cotton. This cotton production is family based agriculture, I would like to emphasize. And thus the farmers appreciated the crop. The reason that over 97 or 99 percent of fields transgenic cotton has been planted in is that farmers know that less pesticides will be needed.

Now let me discuss environmental and health impacts, and this is, again, 3 years data from two groups. For Bt and non-Bt crop production, the number of people poisoned, as reported in hospitals and from villages, decreased quite dramatically in cotton fields. These data are for poisoning after applying pesticide in both transgenic and nontransgenic cotton fields.

Very similar findings are reported in rice fields. More people are poisoned annually in rice fields than in cotton fields. As you all know, insects get resistant to the pesticides and, as a result, farmers ask the pupils from elementary schools to pick the worms that were not killed by pesticides. Local citizens are no longer allowed to feed chickens with worms that were originally provided because the chickens will be killed by the contaminated worms. The worms contain large amounts of pesticides and are very poisonous.

Another concern is whether transgenic crops are safe, this is difficult to answer. But in China, we sometimes answer that over 200 million people have consumed transgenic crops for years, and zero cases of poisoning have been detected and no product has been revealed to contain a toxin. But again, significant research is being done in this area to analyze safety in feeding assays. We have tested safety of sweet peppers, tomatoes, and rice on a case-by-case basis. In the feeding tests, we detected no significant effects on animals just as has been reported in the United States. All the testing has been done in China.

Labeling, again, a difficult issue. I am sure labeling is also difficult for other developing countries, although it works in Europe and in Japan. Still it is a difficult issue. First, we all know the public has a right to be informed. Second, how many genetically modified (GM) products should be labeled, or should every GM crop or its products have to be labeled? Should content be labeled to 1 percent or 5 percent? And we all know that the cost will be increased dramatically for such labeled products.

We assessed the costs of labeling systems. We estimate an over 40 percent increase in production costs if we were to label transgenic foods. Because we have to separate the products, costs would be about 10 percent more for retail prices if we sold labeled compared with nonlabeled products in the supermarket. Even this might not be accurate because China
has a free market. Every morning, people, the farmers, bring the food to the city and sell in a free market. It would be very difficult for farmers to label food in a basket and to tell those professors that it’s transgenic or nontransgenic.

A common feeling is that when you are concerned about the technology, both Western people and Chinese say, “Well, the stuffed people never understand the feeling of hungry people.” That is really an ancient Chinese saying very similar to the one Western people repeat.

So we really feel that there needs to be international harmonization as a basis for national regulation. One country cannot establish regulatory policies alone. Regulation has to be international in scope, and to have a national policy on GMO trading the policy must be harmonized. The GMO labeling system somehow also has to have a harmonized component. For developing countries, identity-preserved output (IPO) will be a very important issue.

The EEU is a key market that will determine the future for biotechnology applications in the world. That is what the developing countries feel, and most of China feels, because of the problems associated with this region. Lastly, I would like to say that biotechnology is a promising technology. On the one hand, the farmers, and we, the developing countries, are waiting for this technology—rice, wheat, corn, and soybean. We need the technology. On the other hand, we have problems in exporting, and so we wait, we are waiting.

But the good news from Brazil and from this OECD organized meeting is that we recognize that biotechnology will be very important for poor countries, for developing countries. I attended a meeting in September of EEU ministers of agriculture in Brussels. For those 16 countries, the situation looks very promising, but there is still a long way to go. We all know that a discussion will be going on continually, but we hope that the GM technology can be assessed by the poor farmers in developing countries.

Thank you again for the invitation.
Session 2: The Practice of Environmental Assessment

Session 2A—Risk Assessment Issues: Mechanisms of Assessment; Baselines; Appropriate Date Sets
Risk Assessment for LMOs:
A European Perspective

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Introduction

My purpose here is to describe the principles and philosophy that guide the process of risk assessment for the release and marketing of Living Modified Organisms (LMOs) in the European Union (EU). To do so I must first outline the key features of the EU regulatory framework, which I will illustrate mainly by reference to its implementation in the United Kingdom. Secondly, I will characterise the actual process of risk assessment, and finally I will discuss one or two current issues and future challenges. Limitations on space dictate that this presentation is less than comprehensive, and the perspective is somewhat personalised.

The Regulatory Framework

Release and marketing of LMOs in the EU are controlled under an EU-wide European Council directive. In February 2001, a new directive (2001/18/EC) was adopted, replacing the one that had been in force for 10 years (90/220/EEC). European Union member states now have a deadline of October 2002 to introduce national regulations that will implement the new Directive. In Great Britain, Directive 90/220 has been implemented by Part VI of the Environmental Protection Act 1990 and Genetically Modified Organisms (Deliberate Release) Regulations 1992 amended in 1995 and 1997 (there is equivalent separate legislation in Northern Ireland).

The essential point about this legal framework is that releases and marketing of LMOs can only take place in the EU with the explicit consent of the regulatory authorities (the body of specific legislation for genetically modified (GM) crops contrasts with some other countries such as the United States that have dealt with GM crops, for example, under existing legislation). The EU Directive covers both small-scale trials for Research and Development (under Part B) and the “consent to place on the market” in Europe (the so-called Part C releases). In the latter case, member States evaluate the application for market release only after it has been assessed as low risk by the member State to which the application was originally submitted.
made. Since 1993 the United Kingdom has dealt with almost 200 consents for releases under Part B of the Directive, and 18 products have been approved for commercial release in the EU. These include four GM maize types, oilseed rape, carnations, and chicory. The oilseed rape, soya, and maize have approval for import into Europe for processing and animal feed use.

At the heart of all applications providing the basis on which risk assessment is made is a technical dossier describing the proposed release in detail (some features of the dossier are discussed later). Figure 1, simplified somewhat for clarity, tracks the movement of such a dossier in the United Kingdom and helps to highlight the main features of the regulatory mechanism. Central to this process is the reliance on an independent scientific advisory committee, in the United Kingdom this is the Advisory Committee on Releases to the Environment (ACRE). Other member States have a similar system. The U.K. application is made to the Joint Regulatory Authority (JRA), which consists of science-trained professionals in a Government department and also provides the secretariat for ACRE. The JRA then distributes the application to other departments, including statutory consultees such as the Health and Safety Executive and the wildlife conservation agencies (English Nature, acting on behalf of U.K. National agencies); others include the Department of Health and the Department of Trade and Industry. Once the JRA is broadly satisfied with the application, going back where necessary to the applicant for clarification, it is passed to ACRE which usually meets to discuss each application. If the application is a product for human food or for animal feed, other advisory committees under the aegis of the Food Standards Agency (the Advisory Committee on Novel Foods and Proteins and the Advisory Committee on Animal Feeding Stuffs) may be involved. Similarly, whenever an agrochemical may be part of the crop’s management, appropriate advice is sought from pesticide specialists. On the basis of all this advice, ACRE will then advise the relevant Government ministers on the risks associated with the release and whether consent should be issued.

The Advisory Committee on Releases to the Environment is an independent, statutory science and technical committee. It currently comprises 12 members with a range of expertise, including leading academics and researchers in the fields of molecular biology and genetics, plant physiology, biochemistry, ecology and virology; an expert in sustainable agriculture; and a practising farmer. Unlike some other committees that advise government, there is no lay member or expert on ethical issues. In addition to advising on particular LMO releases, ACRE reviews developments in biotechnology, often commenting on particular publications, advises on the deliberate release of non-native organisms (e.g., for biological control or conservation), and identifies research needed to inform risk assessment. From time to time the committee has established subgroups to provide strategic advice and specific guidance. Recent examples of this include a guidance note on the potential impacts of LMOs on biodiversity and farmland ecology and a document establishing some general principles of best practice in the design of GM crops (ACRE Guidance Note 13, 2001, - www.defra.gov.uk/environment/acre/bestprac/guidance).

The European regulatory system strives to be as open and transparent as possible (within the limits of commercial confidence) and is becoming increasingly inclusive (see later). For example, ACRE places its agenda and minutes on the World Wide Web and publishes an annual report and all members declare their interests.
The Risk Assessment Process

General Features

Although much has been written on the subject of risk and risk assessment, it is perhaps worth listing below the main features of risk assessment as it relates to releasing LMOs in Europe.

1. The assessment is science based, utilizing both quantitative and qualitative evidence and data.
2. The assessment is comparative, that is the risks of releasing an LMO are compared with those of releasing its nonmodified equivalent under similar conditions (thus recognizing of course that absolute risk is difficult to define).
3. The approach is precautionary. The starting point for each assessment, as in most scientific inquiry, is complete skepticism, and all applications are subject to detailed scrutiny with the onus being firmly on the applicant to provide the evidence demonstrating that a release poses a negligible or low risk.
4. Each application is assessed individually on a case-by-case basis. Coupled with this is a stepped approach that exploits the increasing familiarity with each particular crop or construct as it passes from contained use, to glasshouse cultivation, to trial and unto commercialization.
5. The process responds to new information. It is iterative and continuous, requiring the applicant to forward any new information relevant to the risk assessment and the regulatory authorities to revisit earlier assessments in the light of such information.

Finally risk assessment follows a logical sequence of steps (of which there are arguably five or six) arriving at an assessment of the overall risk of a particular release. These are as follows:

1. Identify potential adverse effects (hazards) however rare these may be.
2. Evaluate the consequences of the adverse effects being realized, i.e., assess the magnitude of harm.
3. Evaluate the likelihood of the adverse effects being realized (a measure of exposure).
4. Estimate the risk (a function of the two preceding steps).
5. Assess any proposed risk management strategies.
6. In the light of the preceding step determine the overall risk of the release.

Despite the universal acceptance of these steps it is not uncommon in the wider literature to see hazards (e.g., harm to Monarch caterpillars from Bt pollen) treated as risks with no estimate or measurement of the degree of exposure.

Baseline Information

As mentioned earlier, applicants wishing to release LMOs in Europe must provide detailed information to the appropriate regulatory authority, usually in the form of a scientific dossier. Whilst the detail varies between member states (in the United Kingdom an applicant is required to address a series of 41 questions), the essential information required for a small-scale trial
under the EU directive covers the LMO, the proposed release and receiving environments, plans for monitoring and control of the release, and a statement evaluating the risks to the environment and human health. With regard to the LMO itself, information must be provided on the characteristics of the recipient organisms, the details of the modification, and the way in which the modification has altered the organism (i.e., what effect it has had on performance). The information about the environment in which the release will take place and the proposals for monitoring the release, including postrelease monitoring to ensure material from the trial is disposed of, must address ways of reducing risk if these are necessary (e.g., removing flower heads, establishing borders of nonGM barrier plants, minimizing cross-pollination with other crops of the same species by maintaining agreed separation distances).

The new EU Directive (2001/18) has introduced several key, novel elements for assessing marketing releases as well as calling on member States to harmonize their approach to risk assessment such that decisions are more obviously consistent and transparent. Particularly important are the need to consider possible longer term, indirect, delayed, and cumulative effects on the environment and the requirement for applications to include a postmarket monitoring plan (a program of monitoring following market release to confirm the assumptions of the risk assessment and identify unanticipated events). Both elements present something of a challenge to applicants and regulators alike. The new Directive also covers issues such as the requirements for traceability and labeling of LMOs and products derived from them, the provision of accessible information to the public about LMO releases, the methods of public consultation on releases, and the use of antibiotic resistance markers (target dates of 31 December 2004 and 31 December 2008 for part C and part B releases, respectively, have been set for phasing out antibiotic resistance markers, which may have adverse effects on human health and the environment).

Returning to the current process of risk assessment, let me briefly consider the type of baseline information that experience has taught us is required for an effective risk assessment. Whilst it is difficult to be prescriptive without a specific example, the technical dossier—at least for GM crop plants—must provide adequate data to address, where appropriate, the following issues:

The Genetic Modification—

There is absolutely no doubt that a well-conducted molecular analysis forms the basis for good risk assessment and helps to speed the application through the risk assessment process. The details required include the transformation method, the nature and source of the vector, and the size and source of donor organism(s) and intended function of each constituent fragment of the region intended for insertion. One expects well-characterized data on the trait and sequences actually inserted or deleted, including any extraneous or vector DNA, copy number, and location in the cell as well as information on the stability and expression of the insert throughout the plant’s life cycle (and whether there is any unintended expression of flanking genes or junction sequences). The Advisory Committee on Releases to the Environment has recently offered some draft guidance on best practice for the presentation of molecular data in submissions for release of LMOs (www.defra.gov.uk/environment/acre/molecdata).
The Effect on the LMO’s Persistence and Invasiveness—

Data that consider the effect of the insert on the biology of the plant should include an assessment of comparative performance in agricultural and peri-agricultural environments— in particular, the extent to which the modification may enable the plant to be more persistent, possibly creating a “volunteer” or weed problem, or more invasive, leading to the establishment of persistent feral populations. Analyses of changes in plant fitness are not trivial undertakings as evidenced, for example, by the extensive studies on herbicide-tolerant oilseed rapes to compare modified and unmodified plants (see Gray and Raybould 1999). Where a problem is identified, it is important to give details of procedures to minimize or manage the risk (e.g., use of an alternative herbicide).

Gene Flow and Hybridization—

The risk assessment clearly needs to address the issue of gene flow—especially the potential for cross-pollination and hybridization with wild relatives—but also in Europe the issue of crop-to-crop gene flow is one of emerging concern. Most crops (e.g., 12 out of the 13 most important world crops by area [Ellstrand et al, 1999]) hybridize with wild relatives, often antecedents, somewhere in the world, but modern agriculture and plant breeding have led to growing many crops considerably outside their native areas or centers of genetic diversity. Thus, the problem of gene flow to wild relatives is largely regional. In Europe it is not an issue for crops such as maize and potatoes but is to a varying degree for sugar beets and oilseed rapes. The potential for gene flow from crops grown in the United Kingdom, the Netherlands, and Switzerland has been assessed by analysis of their wild flora (Raybould and Gray 1993, de Vries et al., 1992, Jacot and Ammann 1999).

The consensus among scientists advising the Government is that, where hybridization and introgression are possible, the risk assessment should assume that they will happen and therefore address the question of the consequences (the so-called “So what?” question). The effect on the fitness of genes conferring tolerance to a herbicide is likely to be very different from that of genes coding for say virus or insect resistance. Understanding the impact on wild and feral plant fitness of traits acquired by gene flow requires research that, in my view, lies along the critical path of risk assessment. The issue of crop-to-crop gene flow has gained importance in Europe as part of the debate on traceability and the separation of the GM and non-GM food chains.

Target and Non-target Organisms—

When the LMO has been engineered to express an antifeedant or insecticidal protein, a whole raft of issues arises in relation to the potential environmental impact. They range from the possible evolution of resistance in the target pest to the direct and indirect effects on nontarget species. Where such a risk can be identified e.g., the evolution of resistance to Bt in a target lepidopteran pest, the dossier should propose measures for managing or reducing the risk. As stated earlier in relation to the Monarch butterfly research, it is important to move from laboratory-based studies that identify possible hazards, particularly harm to nontarget species, to more field-based assessments of the organisms’ exposure to such hazards. Effects on nontarget species may be subtle and indirect and difficult to measure in the short term (e.g., reduction or removal of prey of beneficial insects), but the commonsense approach to date has been to assess risk within a comparative framework, that is, alongside current agricultural practice.
Biogeochemical Processes—

The possible impact of GM crops on soil processes and biodiversity has raised several concerns, and the research base to help address some of these is the subject of other contributions to this conference.

Crop Management—

When applying for consent to place an LMO on the market, the applicant’s risk assessment may need to address questions that arise from the way the crop is managed. Of current concern in Europe in this respect is the potential impact of crops that are tolerant to broad-spectrum herbicides. In addition to issues of safety, the possibility that such crops might exacerbate the decline in farmland biodiversity has led in the United Kingdom to the establishment of a series of farm-scale trials to compare the effects on biodiversity of three herbicide-tolerant crop species (maize and winter- and spring-sown varieties of oilseed rape tolerant to glufosinate ammonium and sugar and forage beets tolerant to glyphosate) with their conventional counterparts. These trials, the U.K. Farm Scale Evaluations, are described in detail in the proceedings of this conference (Firbank et al. 2002), and the first results will be reported in 2003.

Human Health, Animal Health and Food and Feed Issues—

Issues relating to food and feed safety are outside the scope of this meeting and presentation. However, data may be required to assess environmental risks to human health from exposure to novel proteins by perhaps harvesting or handling the LMO or breathing in pollen. Thus, the risk assessment must indicate what is known about the allergenicity of any such proteins.

The preceding examples illustrate the type of baseline information an acceptable risk assessment should contain. Not all issues will be relevant to all releases, and the technical dossier is likely to vary considerably from application to application, thus emphasizing the importance of the case-by-case approach.

Current Issues and Future Challenges

Assessment of the environmental risks posed by any new technology must be viewed as an evolving process. It is important to build on experience and to share information and data on an international scale. That process is happening, not least through meetings such as this one. Nevertheless, scientists charged with making evidence-based assessments of risk can expect to deal in the future with novel areas of uncertainty and to be asked questions that increasingly challenge their understanding of natural systems.

For the ecologists there is the challenge of assessing the potential environmental risks from the so-called second generation of LMOs engineered to tolerate a range of stresses such as drought, salt or frost, and altered Darwinian fitness. Additionally, the development of
nonfood crops producing oils, plastics, or pharmaceuticals will bring new challenges. We are already dealing with the first of what may be a rising tide of requests to release Living Modified Micro-organisms into the environment for a variety of purposes ranging from bioremediation through veterinary medicine to human clinical trials that may involve the shedding of live, disabled, genetically modified viruses.

Arguably, the major current issues surrounding LMOs in Europe are not concerned with risk assessment but with risk perception and with public acceptance and confidence. This reality is reflected in the revised EU Directive with its emphasis on transparency, accessibility, and inclusiveness. The need for scientists and regulators alike to communicate perhaps more clearly than they have in the past includes the responsibility to deal in an open way with the problem of uncertainty. At some point along the road that charts the scientist’s desire to know everything about every possible effect of a release, harmful or beneficial, a decision must be made about what is an acceptable risk or course of action. For some that point is sooner than for others. In my view that decision should also factor in any potential benefits of the release of the LMO and a range of other factors that lie outside the regulatory framework.

References


Figure 1

Simplified diagram of the links in the UK regulatory mechanism for assessing the risks from releasing LMOs (see text).
JRA = Joint Regulatory Authority
ACRE = Advisory Committee on Releases to the Environment
Gene Flow and Transgenic Crops—How Can Potential Impacts on Fitness Be Assessed?

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Introduction

Gene flow rates from crops to crops and from crops to wild plants have been measured over the past 10 years. This research has shown that gene flow at a relatively low level takes place by pollen transfer over long distances—sometimes up to several kilometers. The research has been prompted by concerns that transgenes might move from transgenic crops to conventional varieties, or to wild relatives of transgenic crops, or to both. The answer to the concern of “Does it occur?” now seems clear: gene flow is inevitable from those crops that naturally outcross both to conventional varieties of the same crop and to a small number of wild relatives, although this latter phenomenon is usually a rare event. However, for ecologists and agronomists the key question is “Does it matter?” More specifically, does outcrossing of transgenes affect fitness of recipient offspring in both natural and agricultural ecosystems?

This is a key issue because, if the transfer of specific transgenes to conventional crops and to wild relatives increases fitness, there is the possibility that the offspring of recipient plants will become either agricultural or ecological “weeds”, potentially disrupting natural ecosystems. This is because some of the desirable traits in transgenic agricultural crops confer the ability to be tolerant to insect, fungal, and viral attack and to be resistant to cold, drought, and salinity, which are all traits that could increase fitness (Cooper and Raybould 1997). Although some of these traits may exist naturally, transgenic technology can introduce them to plants with such genes absent from the gene pools of the crop–ancestor complex. Perhaps the most immediate issue is whether there could be increased fitness of the offspring of some agricultural crops that appear later in crop rotations as “volunteer” plants. Unless some volunteer crops (such as oilseed rapes and beets and some transgenic grasses) can effectively be controlled, they may become “weeds” in other crops. Until recently there has been little research into impacts of gene transfer on the population dynamics and habitat requirements of recipient crops and wild plants and still less on the possible implications for farmland and natural ecology. The lack of research on this topic has partly been the result of regulatory difficulties in obtaining consent for experimental releases of transgenic crops and especially for hybrids between transgenic crops and wild plants. There has also been considerable, but as yet unresolved, debate in scientific circles about methodologies to assess fitness in plants containing transgenes.
**Case-By-Case Approach**

Generalized statements about the impacts of transgenic plants and transgenes are confusing and unhelpful in the debate about biosafety. So far as the potential impact of gene flow is concerned, each genetic transformation carries different potential risks. These stem from the species and variety of plant transformed and the nature of the transgene (and associated genes such as promoters and markers) inserted into the transformed plant. Gene flow between transgenic crops and conventional crops and wild plants can only normally occur if they are sexually compatible in nature and grown in close enough proximity for pollen transfer to take place. Potential impacts of gene transfer will depend on the effects (if any) of the specific transgene on the plant phenotype as a whole. It is essential therefore, that any assessment of the potential impacts of gene flow be made on a case-by-case basis. As new methods of plant breeding based on genomic knowledge are developed, there may be a need to extend case-by-case risk assessment to this sector.

**Assessing the Likelihood of Gene Flow**

The inherent characteristics of a crop and its proximity to closely related plants are some of the factors that determine the likelihood of gene transfer to other plants. The key to understanding gene flow is knowledge of the sexual compatibility of the crop with other species growing in the same landscape. For example oilseed rape (canola) *Brassica napus* growing in Europe is sexually compatible with several other species, some of which are wild (such as *B. juncea* and *Raphanus raphanistrum*) and others of which are probably derived from crop plants such as wild turnip (*B. rapa*). Research (e.g., Scheffer and Dale 1994 and Thompson et al. 1999) has shown that, whilst several commercial varieties of this crop freely cross and may be artificially crossed with several related species in the laboratory, in nature this crop forms hybrids with very few wild relatives. These hybrids are only rarely found and may not be fully fertile (Raybould and Gray 1993). However some hybrids have been found in the wild (e.g., Wilkinson et al. 2000 and Chevre et al. 2000), and thus it may be assumed that transgenes from oilseed rape can be transferred into some wild relatives with the risk presumably increasing in proportion to the area of transgenic crop being cultivated. In contrast, crops such as maize have no wild relatives in Europe, and so the risk of gene transfer is zero.

**What is the Gene of Interest? Possible Fitness Effects**

If gene transfer does occur, the key questions for regulators are, “What will be the effect on the phenotype of the recipient plant?” and, “Will any effect change the fitness of the recipient?” This is not only an important issue for those charged with protecting wildlife resources in the landscape, but also a key issue for agriculture. Not surprisingly, many wild relatives of crops and feral populations of different varieties of the crop grow in close proximity to farmland, often sharing the field margins with the crop. Wild beet (*Beta vulgaris*) and sea beet (*Beta vulgaris* ssp maritimum) are sexually compatible with commercial beets and can be found in field margins and coastal fringes (Bartsch and Pohl-Orf 1996), whilst in North America wild sunflowers occupy the same habitat as the commercial hybrid crop (Snow 2002). If these species were to have increased fitness as the result of gene transfer from
transgenic plants, then they could become weeds of agriculture or might change their population dynamics in natural ecosystems. This risk also applies to crosses between transgenic plants and conventional crops. Increased fitness could also lead to the transgenes going to fixation in recipient populations.

Risk Assessment

Assessment of risk from gene transfer is a twofold process. Firstly, an assessment can be made of whether the transgene is likely to transfer to wild relatives. This is relatively straightforward and can be done in the field by estimating gene flow to wild plants using markers. Rates have been found to vary greatly, depending on the crop variety and factors such as distance from wild relatives, presence of pollinators, and weather conditions (Department of the Environment 1995, European Environment Agency 2001). The second part of risk assessment should be estimation of the impacts of the transgene on the fitness of recipient plants. This is much more difficult to predict and relies on the development of protocols for estimating fitness in the habitats where the recipient plants are likely to survive. This latter factor is notoriously difficult to predict—even for alien species that could potentially invade new habitats (Williamson 1996). Changes in the fitness of plants owing to the acquisition of new genes can enable them to colonize new habitats. One of the best examples can be found in the Rhododendron complex of species (Ellstrand and Schierenbeck 2000), where hybridization in Western Europe between species imported for ornamental purposes (R. catawbiense from North America and R. ponticum from Iberia) led to the acquisition of cold tolerance genes that allowed R. ponticum to invade, and in some cases overwhelm, native oak woodlands and wet heaths in oceanic regions of France and the United Kingdom. Because it is very difficult to predict the environment that might be favored by a plant acquiring new genes, regulators may need to draw upon experienced botanists to make a “best guess”.

Fitness Estimation

Theoretical assessments of the impacts of gene introgression on fitness are very risky—but not only because we cannot be certain of the ecological context into which a transgenic hybrid might spread. At present we do not have enough knowledge of the relationship between the plant and its habitat at the molecular level. This may improve as more research into environmental genomics emerges. For some transgenes such as cold, drought, and salt tolerance, it is also difficult to predict what the effect of the transgene might be on the recipient phenotype because the genetic background into which the transgene moves may be different from that of the original transformed plant.

Given the uncertainty surrounding the theoretical prediction of fitness impacts there is a need for experimental work to assess fitness of crop–wild plant hybrids containing transgenes. Perhaps the most obvious way to approach this research would be to construct transgenic crop–conventional crop and crop–wild plant hybrids artificially and then backcross them to conventional crops and wild plants to yield populations of plants with and without the transgene within the same genetic backgrounds. There would need to be properly designed experiments i.e. placing plants in relevant ecological situations, where the “best guess” would place the new plant in the ecological landscape. In many cases this would be the field margin and
disturbed ground habitat. Experiments will need to be closely controlled and inherently safe—possibly by recreating suitable habitat in contained conditions or by placing experimental plots in remote conditions where the plants in question could not survive naturally. There is an important role for regulatory systems to ensure that risks from such experiments are minimized.

**Measurement of Key Fitness Components**

Fitness experiments such as those described above would be assessing the relative fitness of two plant populations: those containing the transgene compared with those that do not. Fitness is defined as genetic fitness, that is the ability of a plant to reproduce relative to the nontransgenic population. There is often confusion about defining fitness. It is not simply variation in plant vigor, although this may be a component of fitness. In some habitats, especially those with harsh windswept conditions and poor nutrient status, high plant vigor is negatively correlated with fitness. Plants living successfully in these conditions put more resources into reproductive structures and less into vegetative vigor.

The key factors in any assessment of fitness will focus on those characters that impact on reproductive success. In plants these include the following:

- Identification of the habitat into which the plant is likely to spread. This is perhaps the most difficult and arguably most important part of any risk assessment.
- Consideration of plant survival to seed production stage from germination to adult plant. It is important that early stages of development be included because most mortality may take place at this time.
- Number of flower heads.
- Number of seeds per head.
- Seed viability and size.
- Predation of seeds on and off plants—mollusks, arthropods, fungi, mammals.
- Seed survival over winter which is important if cold tolerance and other genes are introduced.

**Importance of Relating Results to Population Dynamics of Plants in Typical Habitats**

If sufficient data are obtained by field experiments, it should be possible to model whether the gene in question would be likely to go to fixation. The population dynamics of plants can be predicted and documented by gathering sufficient ecological data to construct life tables that capture the survival of plants at the population level during all stages in the development of the plant. Changes in fitness detected experimentally can be used in conjunction with life tables to predict what effect any fitness change might have on the population dynamics of the plant in specific habitats. The choice of habitats is crucial and for crop plants may include not only field margins but also disturbed ground habitats that may be similar in character. These could include coastal and cliff areas, erosion areas, and fluvial margins. For hybrids between transgenic crops and conventional crops, field margins and disturbed track margins are the obvious choice. Where risks from transgenic hybrids with wild plants are concerned, this approach may reveal whether the hybrid is capable of surviving or increasing in the
defined habitat. It may then be possible to make estimations of potential effects on all populations of the plants in question within a specific biogeographic area.

Although the preceding protocol may seem excessive and burdensome for any regulatory system, it should be stressed that it would only be necessary to embark on such an approach if there were a reasonable risk of gene transfers leading to impacts on fitness from the introduction of transgenic plants into a biogeographic area. For many transgenic crops there may be no risk that gene transfer to wild plants can occur because sexually compatible wild relatives do not grow in the area. For many transgenes, there may be a vanishingly small risk that gene transfer would increase fitness in recipient plants. If those involved in the production of transgenic plants were to incorporate gene restriction mechanisms such as those suggested by the U.K. regulatory body Advisory Committee on Releases to the Environment (ACRE) (DEFRA 2001), then risks of gene transfer from transgenic crops would be greatly reduced and in some cases could be eliminated, making estimation of fitness impacts unnecessary.

References


The Use of Biological Databases to Assess the Risk of Gene Flow: The Case of Mexico

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Abstract

Gene flow from living modified organisms to their wild relatives is one of the risks associated with genetically modified crops—especially when the crop spontaneously hybridizes with its taxonomically related species. In countries like Mexico, which is a megadiverse country and a Vavilov center of origin where many wild relatives of the major crops can be found, proper assessment of this risk is very important. We outline the methodology to assess the risk of living modified organisms (LMOs) developed by the National Commission for the Knowledge and Use of Biodiversity (CONABIO) in Mexico. We highlight the importance that biodiversity databases have in the context of developing countries.

Introduction

It is known that living modified organisms (LMOs) may represent environmental risks. These risks depend on the new genes they contain in their genomes, the technology used to transform the organism, the features of the parent crop such as the reproductive biology, ecological parameter and genetic variation, and the specific ecological situation in which the LMO is released (Persley et al. 1993).

Commonly mentioned risks include the LMOs will become invasive (Wolfenbarger and Phifer 2000, Royal Society of Canada 2001) or cause geneflow to wild relatives (European Commission 2000, Ellstrand 2001), which may decrease their genetic diversity (Ervin et al. 2000), transfer weedlike traits to wild relatives (Rissler and Mellon 1996, Warwick et al. 1999) or simply alter their original genetic constitution; or have unintentional effects on nontarget species (Ellstrand 2001, Obrycki et al. 2001); or induce new viral diseases (Wolfenbarger and Phifer 2000).
To assess all the theoretical risks that releasing LMOs may pose for the environment systematically is a daunting task. In megadiverse developing countries comprehensive assessment of the risks is simply impossible because of the intrinsic complexity of the ecological factors and the incipient or nonexisting technical capacities. However, this difficulty has been acknowledged even in the developed world: “Given the complexity of biodiversity, the assessment of environmental impact of LMOs can only be completed indirectly” (CFIA DIR-94-08).

The countries in the North American Free Trade Agreement (NAFTA) recognize gene flow to wild relatives as one of the risks associated with field releases of LMOs. Although this risk is particularly significant and relevant in centers of origin of cultivated species in megadiverse countries, other countries have developed protocols to assess the risk of gene flow. An example of these is the NAFTA countries (Canada, Mexico, and the United States), which have such methodologies, although only one (Mexico) is megadiverse as well as a Vavilov center of origin (Vavilov 1951). Therefore, Mexico has special interest in the development of practical procedures to prevent such gene flow, including assessment methods, legislation, and policies.

Among the Mexican institutions that oversee different aspects of LMOs, there are specialized subcommittees for specific matters like science and technology, agriculture, health, environment, and industry. As a part of the group of institutions in charge of dealing with risks related to LMOs, the National Commission on Biodiversity, CONABIO, provides the risk assessment of gene flow from a biodiversity perspective.

Since CONABIO was created (1992), most of its budget has been directed to support studies and projects related to biological inventories and databases. By these means, CONABIO has computerized the main Mexican zoological collections and herbaria. Specifically, at this moment the database for vascular plants includes 836,600 specimens from herbaria and museums in Mexico (57 collections) and abroad (87 collections; see figure 1 for an example).

**Assessing the risk of gene flow**

There is evidence of gene flow between crops and their wild relatives, suggesting that even the most domesticated plants will hybridize naturally with their cross-compatible wild relatives when they come into contact (Ellstrand et al. 1999). The level of gene flow is highly variable and depends on a variety of spatiotemporal factors. To assess the risk of gene flow it is necessary to have biological information of the species concerned such as population and species variation parameters, the potential and current spatial distribution of populations of wild relatives, and the spatial location of the LMO site.

In addition, specific and detailed information about the reproductive biology of both the wild relatives and the LMO, in as many experimental or field situations as possible (NOM—056–FITO–1995) is needed to determine the possibility of gene flow.
Information about the proximity of LMOs and wild relatives is highly important. The three North American countries request different information about proximity in their respective regulations. Canada asks for the “geographic scope” of the effects of releasing LMOs (CFIA DIR 94–08); the United States requires “a detailed description of the intended destination” of the LMO (7CFR340), and Mexico requires information on the “wild relatives and their distribution” (NOM–056–FITO–1995), among other information important for the risk assessment. The requirements of information are established by the Mexican regulation NOM–056–FITO–1995.

In view of these requirements, every application for a permit to release LMOs in Mexico is evaluated using the following information to develop the risk assessment to wild relatives:

**Taxonomy of LMOs and Wild Relatives and Known Geographical Distributions.**

The Organization for Economic Cooperation and Development (OECD) reports 94 plant species that have been genetically modified (OECD, BioTrack database of field trials). Of these, Mexico has 889 species that are congeners to those LMOs. After checking for correct taxonomic identification, a search of databases is done to obtain georeferenced localities for the species taxonomically related to the LMO (www.conabio.gob.mx/remib).
Bioclimatic Modeling to Obtain Regions of High Ecological Similarity to the Known Localities.

The specimen points provide insufficient and biased estimates of biological distributions. To infer the potential distribution of species we first evaluate the geographical expression of the fundamental niche of the species (MacArthur 1972). This is done using an artificial intelligence algorithm called GARP (Genetic Algorithm for Rule Production; Stockwell and Peters 1999) and Geographic Information System (GIS) technology. Application of the algorithm creates GIS coverages of high similarity of physical attributes (climate, soil, slope, etc.) to the data points where the wild relative has been observed. This geographical coverage is interpreted as the area in the country where the fundamental niche of the species is present.

Predicting Distributions from Niches.

To predict species distributions we “cookiecut” the GIS coverages generated with the GARP algorithm by first using biogeographical coverages to include only the GARP regions belonging to the historical distributions of the species (thus discarding regions of high niche similarity but low “historical” or biogeographical affinity) and then using primary vegetation obtained from recent satellite images (Landsat ETM 1999–2000). In the final step, we consult experts to validate the resulting maps. These maps provide information about the likely presence of wild relatives. The current technology has a resolution of pixels of about 4 km. This technique has been evaluated for several taxonomic groups in Mexico, and it has been shown to have a good predictive capacity (A. T. Peterson and K. P. Cohoon 1999, Sanchez-Cordero and Martinez-Meyer 1999, Feria 2001).

Literature and Database Search to Obtain Reproductive Biology Criteria Regarding Likelihood of Gene Flow Given Proximity.

Of the 94 LMOs reported by OECD and their wild relatives in Mexico, we have databased the biological information from scientific sources for 28 species. This database is a growing body of information maintained by full-time staff. We include information about the descriptions of the novel trait of the LMO, comparative descriptions of the LMO and its counterpart, and information related to reproductive biology like pollinators, seed dispersal, pollen movement, genetic variation, and hybridization and gene flow. One of the important features of this database is that the biological attributes may be associated with the species or populations at a specific point in space and time.

On the basis of reproductive biology information in the database, we determine the risk of gene flow, assuming that the LMO and the relative are in close proximity. We also obtain estimates of how close the proximity should be to present a significant risk based on the presence of their wild relatives and biological features like movement of pollen.

Overlap of Data and Regions of Intended Introduction of the LMO

In the distribution maps for the wild relatives generated with GARP algorithms, we overlap the release location of the LMO. In these maps we observe if a given location is inside or close to a “risk area” that would allow gene flow with wild relatives. The determination of the risk is based on the biology and potential distributions of wild relatives of the LMO.
Since 2000, the preceding procedure has been applied to 105 introductions of different LMOs, including *Gossypium hirsutum*, *Glycine max*, *Cucurbita pepo*, *Carthamus tinctorius*, *Cucumis melo*, *Musa acuminata*, *Nicotiana tabacum*, and *Zea mays ssp. mays* (only to transport seeds between two research centers). The risk assessment of gene flow is performed on a case by-case basis (table 1).

Table 1. Number of requests evaluated following the procedure developed by CONABIO

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Number of requests</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gossypium hirsutum</em></td>
<td>70</td>
</tr>
<tr>
<td><em>Glycine max</em></td>
<td>21</td>
</tr>
<tr>
<td><em>Cucurbita pepo</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Carthamus tinctorius</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Cucumis melo</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Musa acuminata</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Zea mays ssp. mays</em></td>
<td>1</td>
</tr>
</tbody>
</table>

This methodology has been presented to scientists, non-governmental organizations (NGOs) and members of the private sector. It appears now to be accepted as a way of highlighting regions of high risk, where field research is indispensable, as well as zones of very little risk, where the LMO can be released with low or no risk to wild relatives.

**Conclusions**

The approach of CONABIO for the assessment of gene flow is feasible for developing countries and provides a quick assessment, which is nevertheless based on large quantities of scientific information. The accuracy of this methodology largely depends on the availability of data collections and on biological information of species. Sharing the “presence” information of wild relatives from museums and herbaria is something reasonably cheap and very useful.

We also require reproductive biology databasing and much more research on the population biology of LMOs and their relatives.

The extrapolation algorithms to generate the distributions of the species are predictive at mesoscales (about 10 km²) thus, we need to improve the technology to increase the resolution. High resolution also means that the proposed sites for release of LMOs must be known with precision.

In the midterm, CONABIO intends to make all the databases and algorithms available through its Web site (www.conabio.gob.mx).
References


Transgenic Rice and Gene Flow Assessment to Wild and Weedy Rice Species in Costa Rica

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Abstract

Costa Rica has made strategic decisions to develop agricultural biotechnology tools over the past 10 years. Development of transgenic rice lines was part of the effort to obtain local rice varieties resistant to the hoja blanca virus disease (RHBV) and to the herbicide phosphinothricin (PPT). However, deployment of transgenic crops in biodiverse tropical ecosystems raises several concerns. Conscious of this situation, we had identified and characterized potential *Oryza sativa* transgene recipients: native wild *Oryza* species populations and the weedy rice complex associated with rice cultivation.

This paper describes the advances obtained by developing an integrated strategy for the assessment and management of gene flow from transgenic rice lines to the closely related wild relative of rice, *Oryza glumaepatula* and to the weedy rice complex that contaminates rice fields. Future aims include promoting the conservation and utilization of wild rice populations and their ecosystems and providing science-based information on this strategy to local rice growers, policy makers, community leaders, and the international community.
Introduction

Costa Rica is one of the 20 countries with the greatest biodiversity, comprising 5-percent of the world biodiversity, with an estimate of more than 11,000 species of plants. As part of a national policy to preserve its biological resources, 25-percent of the territory has been protected through the Siculma Nacional de Areas de Conservación. In addition, a systematic national inventory has been taking place for the past 12 years as part of an effort to obtain information about the biological diversity of the country. More recently, public and private institutions have been engaged in finding uses for this biodiversity in the agricultural, pharmaceutical, and chemical industries. The country has also established laws for the appropriate use of its biological resources. Furthermore, the Government has invested in research development, supporting the establishment of research institutes in public universities and autonomous institutions for the past 25 years with the purpose of developing biotechnology to solve regional problems. In addition, the University of Costa Rica has developed the capacity for crop improvement by genetic engineering. Small biotech companies are also emerging, particularly for micropropagation of species of economic importance.

On the other hand, the National Biosafety Committee (NBC) has developed regulations, granting permits to agribiotech companies for transgenic seed increase for nearly a decade. Although the NBC has considerable experience in this area, no transgenic products have yet been released for commercial purposes in Costa Rica. Costa Rica is a small country of 51,100 km² of which one-fourth of its territory is devoted to national parks; therefore, the potential for expansion of its agricultural boundaries is very limited. In addition, the improper use of the land, the abuse of toxic agrochemicals, and low productivity are some of the factors that contribute to a nonsustainable agriculture. To reduce the impact of such human activities on the environment, it is necessary to introduce important changes in the agricultural practices. The development and utilization of transgenic crops, as part of an integrated pest management program, may contribute to a more sustainable agriculture.

It is important to stress that there is lack of information about the impact that transgenic crops may have in tropical environments, which are centers of origin for many plants. There is also lack of knowledge among consumers about living modified organisms (LMOs), their production, commercialization, consumption, and related food safety issues. Public perception is an important issue in Costa Rica for the acceptance of transgenic products in the country. Although general information has been broadcast in the mass media and debates organized by academic institutions, no data are available about public perception of LMOs in Costa Rica. Other constraints involve the negotiation of licenses and freedom to operate from patent holders of the proprietary technology used in the genetic transformation of the new varieties as well as the establishment of mechanisms for production and distribution of transgenic seeds among farmers. Unless these constraints are properly resolved, they may prevent the use of improved varieties by genetic engineering in Costa Rica and other developing countries.
The Case of Transgenic Rice in Costa Rica

Rice is a very important staple crop in Costa Rica, providing approximately 25-percent of the daily caloric intake to the population. Currently, rice production faces several phytosanitary constraints, which include the rice hoja blanca virus disease (RHBV) among others. The distribution of this viral disease is limited to tropical America, and there is no natural resistance to RHBV among indica rice varieties. Therefore, an alternative approach is to use nonconventional strategies such as the genetic transformation of commercial rice varieties with RHBV antiviral or insecticidal genes against the virus insect vector. The Centro de Investigación en Biología Celular y Molecular (CIBCM) of the University of Costa Rica has produced transgenic rice lines of Costa Rican cultivars CR–1821 and CR–5272 containing fragments derived from RHBV RNA–3 (Muñoz 2000).

Parallel to the development of transgenic rice lines, CIBCM has mapped and characterized wild Oryza species throughout the country. This study demonstrated that Costa Rica is home for three of the four wild Oryza species native to tropical America: O. latifolia, O. grandiglumis, and O. glumaepatula (Zamora 2001). O. glumaepatula is the most closely related to cultivated rice, O. sativa, sharing an AA-type genome, whereas the others are allotetraploid with CCDD genomes. The genetic proximity of O. glumaepatula to cultivated rice raises concerns about gene flow from cultivated rice to these natural populations, particularly northwest of Costa Rica in the Los Chiles wetlands, where there is proximity of this species to rice-growing areas. CIBCM has also conducted an exhaustive inventory of weedy rice biotypes associated with rice fields. Weedy rice is a complex of rice plants showing characters of O. sativa and other wild relatives of rice with different degrees of seed shattering, seed dormancy, phenology, outcrossing rates, and productivity. Weedy rice biotypes are the most likely potential recipients of transgenes if genetically modified rice lines are deployed in the field. Risk assessment and gene flow analyses should be focused on these potential target populations. In the case of transgenic rice lines produced by CIBCM, field tests were established under the supervision of the NBC. Public concerns and attitudes towards biotechnology and agriculture also need to be addressed properly before genetically modified crops are adopted by our society.

Our working hypothesis is that baseline studies about the distribution, population genetic structure, reproductive biology, and phenology of the wild and weedy Oryza species could help to design strategies directed to mitigate or delay gene flow from transgenic rice lines to closely related species. At the same time, such studies would increase the lifespan of new transgenic varieties thorough the implementation of science-based management strategies. This knowledge will also help to promote an inclusive assessment through the conservation and utilization of wild rice populations and their ecosystems, involving rural communities and farmers. The purpose of our research is to develop an integrated strategy for the assessment and management of gene flow from transgenic rice lines to the closely related wild relative of rice O. glumaepatula and to the weedy rice complex that contaminates rice fields. In addition, the studies promote the conservation and utilization of wild rice populations and their ecosystems by providing information to local rice growers, policymakers, community leaders and the international community.
**Material and Methods**

**Confined Field Trials of Transgenic Rice Lines:**

Two field trials of the transgenic rice lines containing RHBV-derived sequences and the *bar* gene conferring resistance to the herbicide PPT as a selection marker were performed in San Antonio, Alajuela (Central Valley, 900 meters above sea level), under the supervision of the NBC (figure 1). The lines were tested for RHBV and herbicide resistance and were also used for seed increase. The first trial involved 36 T1 lines that contained the RHBV coat protein gene in sense and antisense orientation or flanked by nuclear matrix attachment regions as well as 3’ and 5’ fragments of this gene. The progeny of those plants (T2) were evaluated in the greenhouse for selection of homozygous lines for the herbicide-resistance trait. The herbicide-resistant T2 lines were transplanted to a second confined field trial and are being evaluated for agronomical performance through an assessment of plant morphology and phenology.

**Figure 1.** Map of Costa Rica showing the country’s main rice-producing regions, weedy rice collecting sites and the location of *O. glumaepatula* populations, that provided the material used in this study.
Morphological Analyses of the Weedy Rice Complex:

Over 1,200 samples of weedy rice, commercial varieties, and landraces in the plant maturity stage were collected from the main rice-growing areas of the country (figure 1). Biotypes were classified and coded according to awn presence and color, color of the apiculus, and lemma and palea color. Twenty-one morphological traits were then evaluated based on previously established descriptors (IRRI 1980; UPOV 1985). The morphological variables were evaluated by a multiple discriminant analysis (Systat 8®). A classifying function was established based on the morphological characteristics of the commercial varieties of rice. The coefficients of the resulting function for the varieties were used to calculate the discriminating points for each weedy rice specimen and to separate it from the commercial rice varieties.

Phenological Analyses of the Weedy Rice Complex:

The growth cycles of 27 weedy rice biotypes collected from Guanacaste (North Pacific) and Parrita (Central Pacific) (Sánchez 2001), 5 Costa Rican commercial varieties, 18 landraces and 2 wild Oryza species were compared in a field trial in San Antonio (figure 1). The following characters were evaluated: number of days from seeding to 50-percent of seed emergence, percentage of total emergence; number of days from seeding to 50-percent of tillering, booting, anthesis, heading, and maturity. The duration of anthesis was also recorded. Plant size, number of tillers, and number of leaves per plant were evaluated every 15 days during the vegetative cycle.

Optimization of Molecular Analyses of the Weedy Rice Complex:

DNA extraction from leaf tissue of 1-month-old plants was performed on samples of all weedy rice biotypes, landraces, commercial rice varieties, O. glumaepatula, O. glaberrima, and O. rufipogon. The extraction method by Lodhi et al. (1994) was modified to eliminate polyvinyl pyrrolidone (PVP) and mercaptoethanol. Polymerase chain reaction (PCR) was performed on a subsample of the preceding materials using 14 microsatellite primers: RM22, RM41, RM11, RM230, RM20, RM19, RM1, RM222, RM167, RM200, RM164, RM5, RM168 and RM123 (Mappairs®). PCR amplification was visualized in 1-percent agarose gels stained with ethidium bromide.

Flowering, seed set and sample collection of O. glumaepatula:

Flowering periods were monitored in three locations of the country: two natural populations in Los Chiles and Murciélago and a field trial used for seed increase in San Antonio, Alajuela (figure 1). Seeds from individual panicles along with their respective flag leaf were collected randomly throughout the area covered by O. glumaepatula in Los Chiles (figure 1). The seeds were labeled and kept for later germination and the flag leaf was stored separately at -30°C to conduct progeny studies.
Collection of Wild Oryza Species for Organoleptic Studies, Industrial Processing and Nutritional Analyses:

Seeds of *O. glumaepatula* were collected from three localities: two natural populations in Los Chiles, Alajuela and Murciélago, Guanacaste (figure 1), and a field trial used for seed increase in San Antonio, Alajuela. Seed samples of *O. latifolia* were collected from two localities on the province of Guanacaste: Bagaces and San Miguel, Cañas (figure 1). The seeds were stored at room temperature with low humidity. Seed availability of *O. grandiglumis* in the Caño Negro wildlife refuge was also monitored.

Results

In the first field trial for the evaluation of transgenic lines, T1 seeds were tested for PPT- and RHBV-resistance. Herbicide resistance was segregated in Mendelian fashion, and some lines were resistant to virus infection. Interestingly, neither RNA nor coat protein was detected. The T2 generation was planted in the greenhouse and the seedlings were evaluated for their PPT resistance 4 weeks after germination. It was observed that, as occurred in the T1 generation, the *bar* gene is active in the T2 transgenic lines and that these were resistant when PPT was applied in the recommended dose for weed control (1 l/ha). On the basis of these results, homozygous T2 lines were identified for herbicide resistance, and a replica of these lines was planted in a field trial for evaluating their agronomic performance and selection of true-to-type lines. This study is still in progress and the results are expected to be complete in the following months.

Twenty-seven weedy biotypes (WB) were identified according to the morphological characteristics of the mature grain. The discriminant analyses revealed that the most useful traits to separate the groups, were awn presence, color and distribution, total culm number, total number of culms with panicles, plant height and panicle exsertion. Using these variables, we grouped the commercial rice varieties into three clusters: V–I (varieties CR–1821, CR–4110, CR4338, CR 5272 and Camago 8); V–II (variety CR–1113, which shows awned grains and low number of culms); and V–III (landrace Caloro, which is taller than the others and has a lower average exsertion when compared with the commercial varieties). These same analyses discriminated the weedy rice population in five clusters: Group WB–I is constituted by one biotype (4110, identified as *O. rufipogon*), WB–II to WB–IV are conformed by the 7 awned biotypes separated according to awn distribution, length, and color. Finally, WB–V groups the 12 awnless weedy rice biotypes that have different plant heights: tall (> 150 cm), intermediate (100 to 150 cm), and small (65 to 99 cm) (figure 2).
The phenological analyses of the weedy rice complex indicated that 24 weedy rice biotypes germinated between 6 and 9 days after seeding (DAS). These germinated earlier than commercial varieties and landraces that required 9 to 20 DAS. In general, weedy rice biotypes reached 50-percent booting several days earlier than commercial varieties. It was observed that the weedy rice biotypes from Parrita significantly required fewer days to reach 50-percent booting than those from Guanacaste. The weedy rice biotypes were classified into four groups depending on the days to reach 50-percent anthesis: I. 90–100 (2 biotypes); II. 100–110 (5 biotypes), III. 110–120 (14 biotypes) and more than 120 (4 biotypes). Flowering time overlaps occurred between the commercial variety CR–5272 and fourteen weedy rice biotypes and three landraces. Nevertheless, for the same trait the rice varieties CR–1821 and CR–1113 overlapped with only one biotype (figure 3).
Optimization of molecular analyses of the weedy rice complex included the development of a DNA extraction protocol that preserves DNA quality for PCR amplification and that allows the processing of a large number of samples in a short time. PCR amplification of the following microsatellite primers were positive for all samples: RM22, RM230, RM20, RM19, RM167, RM164, RM5, and RM168. The amplification of primers RM222, RM200, RM123, RM11 and RM1 was negative for at least one of the wild Oryza species evaluated but positive for all the weedy rice biotypes and commercial varieties. No amplification was observed on any sample for primer RM41. Other primers remain yet to be tested.

Flowering and seed set monitoring of O. glumaepatula revealed that the flowering of O. glumaepatula occurred simultaneously from October to December 2001 in the populations growing at Los Chiles, Murcielago, and a field trial in the San Antonio. Approximately 192 progeny groups were obtained randomly at the “El Muro” population in Los Chiles along with the flag leaf of the respective mother plant. Seeds are currently undergoing humidity reduction for storage and future germination, and flag leaf samples were stored at -30ºC. Molecular progeny analyses using microsatellites will be performed in the following months, and the results are expected to provide information about the genetic structure of this population as well as on its reproductive biology.

Seeds of O. glumaepatula and O. latifolia have been collected and stored for organoleptic, industrial processing, and nutritional analyses. O. grandiglumis began the flowering period in the first weeks of December, and thus mature seeds were available in January. The following analyses will be performed proximately: carbohydrate content, starch, total protein content, dietetic fiber, cooking and milling properties, and flavor and aroma quality.
Discussion

Transgenic plants were resistant to both RHBV and to the herbicide PPT. This demonstrated that rice genetic transformation has been successful in Costa Rican varieties. In the field trial of the T1 lines, neither RNA nor coat protein was detected, indicating the effect of gene silencing for the viral protein. The herbicide resistance trait was observed in the T1 generation and was inherited in Mendelian fashion in the progeny (T2). The PPT resistance of the T1 and T2 transgenic lines indicated that the bar gene was expressed in the transgenic lines, and the enzyme phosphinothricin-N-acetyltransferase reached sufficiently high concentrations to detoxify PPT when used in the recommended dose for weed control (1 l/ha). This allowed the selection of homozygous PPT-resistant lines for the depuration of this trait. Morphological and phenological analyses of the T2 plants are yet to be performed and will provide valuable information for the selection of true-to-type herbicide-resistant lines. Some of these lines will also be useful for performing crosses with other elite commercial varieties. The location of the field trials in the Central Valley was chosen because it has adequate climatic conditions for rice development and is devoid of natural wild rice populations, and the nearest rice plantations are about 100 km away.

The morphological characterization of weedy rice populations allowed the identification of 27 biotypes based on mature grain characteristics. Previous publications have classified weedy rice according to lemma and palea color (Smith 1981, Montealegre and Vargas 1993). In this study, additional characters were included to obtain a broader morphological characterization mainly because weedy rice populations are highly polymorphic, and grain characters alone would not be sufficient (Noldin et al. 1999, Galli et al. 1982, Lago 1982). This method allowed the identification of clusters within a highly polymorphic weedy rice population and the separation of weedy rice from the highly related commercial varieties (Cuevas-Pérez et al. 1992).

The results obtained in the phenological analyses suggest that the fast and early emergence of weedy rice seeds indicate that these do not exhibit dormancy. However, early seed emergence may be helpful for weedy rice control because selective practices can be performed before the emergence of commercial varieties (Diarra et al. 1985). A wide heterogeneity on flowering periods was observed on landraces and weedy rice biotypes. The information on the overlapping of flowering periods between weedy biotypes and commercial varieties will be useful to select the weedy rice biotypes that may be used in field experiments for assessing gene flow from transgenic rice to weedy rice populations. Other characters such as the crossing compatibility between weedy rice and commercial rice varieties, plant height, similarity of grain size and color, panicle shattering and susceptibility to the herbicide PPT must also be considered. Because much of this information is still unknown, future experiments will be performed to answer these questions.

Modifications of the DNA extraction protocol allowed obtaining DNA with adequate quality for PCR reactions that did not require the use of organic solvents in the protocol, possibly owing to its plant age and the low presence of secondary compounds in rice. The low amplification efficiency of microsatellites observed in wild rice species may result from rice microsatellite primers obtained from cultivated rice varieties (Chen et al. 1997, McCouch et al. 1997), and the primer annealing may be affected in wild species due to its specificity to rice cultivars. Nevertheless, under these conditions at least eight primers can be used for analysis in all
available material. An additional five primers can be used for the analysis of weedy rice biotypes and commercial varieties, though modifications of PCR conditions may allow the amplification of the wild rice species. More microsatellite primers are yet to be evaluated.

*O. glumaepatula* flowering occurred simultaneously during October and November in all three sites evaluated. During these months, the daylight time is 30 minutes shorter in comparison with the longest days of the year. This change in photoperiod seems sufficient to trigger flowering in *O. glumaepatula* in the three locations because these sites have different climatic conditions. In addition, these regions have adequate environmental conditions for rice cultivation. Therefore, the annual flowering period of *O. glumaepatula* is important to define the rice-sowing cycles in areas close to these natural populations, and consequently minimize the possibility of gene flow between these species. Another possibility for minimizing gene flow is to provide additional options to rice cultivation in the area such as the use of wild rice species as alternative sources of income that may substitute for the cultivation of *O. sativa* in the area. The analyses of the nutritional, organoleptic, and milling properties of the wild species of rice could provide valuable information towards exploring the possibility of using these species as “gourmet” rice. This would also involve the rural communities in the conservation of the natural habitats of wild *Oryza* species.

**Conclusions**

The results and strategies presented represent a unique contribution in which the development of transgenic rice lines has paralleled a baseline study for obtaining useful information needed for the deployment of new genetically modified rice varieties. Weedy rice and *O. glumaepatula* are the most likely potential transgene recipients if genetically modified lines are deployed in the field. Therefore, the baseline studies aim towards the characterization of rice-related populations and are focused on their distribution, genetic structure, and reproductive biology. In addition, this study will help determine the magnitude of gene flow between transgenic rice and its wild and weedy relatives before the crop is commercialized.

The case of transgenic rice in Costa Rica is an example of progress in developing science-based information for the deployment of modified crops to local farmers. In this paper we presented results on environmental impacts, and ecological risk assessment, public perception, food safety, and intellectual property rights (negotiation of proprietary technology, protection of intellectual property, and freedom to operate) as well as food safety and organoleptic studies. We are also preparing information for the general public to generate objective facts about biotechnology practices that could provide a base for the future incorporation of transgenic organisms may be incorporated in the agriculture of the country.

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Gene Flow Assessment of Living Modified Organisms in the Neotropics

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Abstract

Transgenic plants, or living modified organisms as currently known (LMOs, according to the Cartagena Protocol), may offer improved traits and benefits for agriculture and consumers in the tropics; however, their introduction may also entail potential risks. Hybridization between crops and their wild relatives sometimes brings novel genes into wild populations, occasionally resulting in the evolution of aggressive weeds or endangerment of rare species. Widespread deployment of transgenic crops could also lead to similar outcomes, but only limited information is available regarding gene flow and distribution of wild populations in crop areas that might lead to the prediction of such outcomes. The likelihood of crop-to-wild hybridization depends on the outcrossing rate and on distance and direction of wild and crop populations. A careful assessment of potential impacts of gene flow from LMOs on population genetics of natural crop plant biodiversity is needed to design strategies for the safe use of LMOs in the Neotropics.

Introduction

Neotropical biodiversity has contributed by the mechanism of human domestication up to one-third of all plant crops grown worldwide. Mesoamerica, the Andean region, and the Amazon have been the centers of origin or diversification of well-known crops such as maize, beans, potato, sweet potato, tomato, cassava, groundnut, pineapple, cotton, cacao, and chili peppers (Harlan 1992). Many landraces still exist there, as well as most of the ancestral wild relatives. Gene flow usually occurs across the primary gene pool of wild relatives (i.e., genetically compatible), and its derived cultigens, to maintain genetic diversity and thus to ensure survival and adaptation to changes. More than 70 percent of plant species may be descended from hybrids of wild species ancestors. Nonetheless, even if hybridization is common, it is not ubiquitous (Ellstrand et al. 1999). Hybridization between crops and their wild relatives sometimes can also bring genes into wild populations, occasionally resulting in the evolution of aggressive weeds or
endangerment of rare species. The likelihood of crop-to-wild hybridization depends on the outcrossing rate, and on the distance and direction of wild and crop populations (Ellstrand and Hoffman 1990).

Transgenic crops offer an alternative for introducing traits that can reduce the need for chemical pesticides and fertilizers and can improve agronomic performance. This technology could contribute to a decline in the conversion of currently unused land (which bears areas of biological diversity) into new agricultural lands. Just as is true for the introduction of any foreign trait, so also may transgenic crops pose four potential types of adverse consequences of gene flow into the wild and weedy relatives (Ellstrand and Hoffman 1990). The foremost issue is the possibility that the traits inserted and expressed by the wild or weedy relatives might increase their aggressive weediness. A second issue is the possibility of diversity reduction in the wild or weedy gene pool, affecting their fitness and thus long-term survival. The third is the possibility of disturbing ecological relationships within natural communities. The fourth consequence is the possibility of unexpected pleiotropic effects associated with the introduced gene. There is a need to evaluate the potential effects of using LMOs under tropical conditions where there is continuous cropping and where pest, disease, and weed problems are higher.

Risks associated with gene flow presuppose physical proximity of the crop to its wild relative(s), or among varieties or landraces of the crop itself, so that pollinating agents can effect gene transfer. A second important assumption is the genetic compatibility of the crop and its immediate wild relative(s), which may or may not be fertile. In the former case, accurate mapping of the crop and above all the location of its different wild relative(s) having genetic compatibility is a prerequisite for the introduction and management of transgenic crops. With few exceptions (teosinte, wild potatoes) the range of distribution and ecology of wild relative(s) of several neotropical crops are still only partly documented. These deficiencies are true also for the limited numbers of American species of rice. If creation of transgenic varieties of principal tropical crops is expected, then the mapping of the genetically compatible wild relative(s) is a basic step towards their sound management.

The likely consequences of gene transfer to other crops or to wild and weedy relatives, including impacts on biodiversity, depend on both the specific genes and the environment. To address these questions, we are using beans and rice as models. Beans have a center of origin and biodiversity in the neotropics. Rice is an introduced species from Africa and Asia but with wild and weedy relatives that include wild native species in Central and South America. The aims of this work are as follows:

1. To analyze the gene flow from transgenic crops to wild and weedy relatives in crop centers of diversity using beans and rice as models
2. To monitor changes due to transgenes in population genetic structure and dynamics of wild and weedy relatives under confined field plots and local agricultural field conditions
3. To develop management practices for the use and handling of transgenic crops in the tropics
4. To advise biosafety entities of neotropical countries on the safe use and management of transgenic crops
Discussion

Bean Crop-Wild-Weedy Complex in the Neotropics

*Phaseolus* beans and wild relatives have their center of origin and diversity in Central America and the tropical Andean countries, they display a spectrum of reproductive biology, lifespans and agro-ecological niches (Debouck and Smart 1995). The common bean is usually reported as an autogamous species; however, rates of outcrossing up to 30 percent and above have been reported in subtropical environments (Wells et al. 1988). High temperatures might be directly or indirectly involved through higher insect activity (bees and bumblebees) acting as pollinators (Debouck et al. 1989). The wild relative of the common bean might have slightly higher rates of outcrossing in comparison with its derived cultigens (Triana et al. 1993). Traditional bean landraces are grown sympatrically with their direct wild ancestors in many places of Latin America—especially in Mexico (Delgado Salinas et al. 1988), Guatemala (Gentry 1969), Colombia (Debouck et al. 1993), Peru (Berglund–Brücher and Brücher 1976), and Bolivia (Freyre et al. 1996). Crosses have been shown to occur between the bean crop and populations of wild relatives in many places of their wide range of sympatric distribution (Beebe et al. 1997). In certain places in Latin America, the hybrid swarms resulting from such crosses are used by farmers—either as additions to their original seed stocks or as emergency food just in case of a crop failure (Debouck et al. 1989). The presence of a specific type of phaseolin (a seed storage protein) from the Peruvian wild *P. vulgaris* gene pool in the cultivated bean in Colombia suggests that gene flow from wild and weedy beans may have occurred before pre-Colombian times (Beebe et al. 1997). The extent of such wide crosses between bean landraces and wild forms, in terms of geographical range and timespan, as well as their significance for the maintenance of genetic diversity in bean landraces and for increased fitness of farmers’ mixtures need to be quantified (Beebe et al. 1997). Crosses between landraces have also been reported (Paredes and Gepts 1995).

Natural variation in seed storage proteins has been exploited in studies on bean evolution (Gepts and Bliss 1988). One group partly related to the lectins is arcelin, which confers high levels of resistance to pests of stored grains: the bruchids (Cardona et al. 1990). Bruchids exist on wild and cultivated *P. vulgaris* crops in the Americas from Mexico to Argentina. Arcelin proteins are not known to occur naturally outside Mexico (Acosta-Gallegos et al. 1998). There are several variants of arcelin protein known so far, and they do not confer even levels of resistance to bruchids (Cardona et al. 1990). Currently, we are conducting gene flow analysis of bean crop-wild-weedy complexes under local agricultural field conditions at the Central Valley of Costa Rica. Populations of the three target species—*P. vulgaris*, *P. polyanthus*, and *P. costaricensis* as well as cultivated beans had been mapped to specific locations. Simulation experiments under confined experimental field plots are also underway. To determine the gene flow rate, selected materials were planted in specific designs for the simulation experiments. A wild bean accession containing an arcelin type with almost no insecticide activity against bruchids is being used. It would thus behave neutrally if this arcelin type were to migrate through gene flow into the commercial varieties planted adjacent in the external concentric squares. Various bean varieties differing in flower color are also used. Hybrids are identified using the arcelin gene and segregation for flower color.
Cultivated rice, *Oryza sativa* L., is an autogamous plant, with a low outcrossing rate of 0–1 percent (Roberts et al. 1961). Such low outcrossing rates are not, however, typical of the wild relatives of rice. Outcrossing rates can be as high as 56 percent (Roberts et al. 1961). Several genome combinations are found among *O. sativa* and its wild relatives. Hybridization can be expected within the genomic group that includes *O. sativa*, namely, the AA group. Recent reports indicate the presence of rice wild relatives in Central and South America and the diversification of an American AA genome wild *Oryza* species, *O. glumaepatula* Steud, in Brazil, Colombia, Costa Rica, and Venezuela (Vaughan and Tomooka 1999). Some taxonomist have considered *O. glumaepatula* the American *O. rufipogon* Griff (*O. rufipogon* is of Asian origin), and its classification is still confusing in some Latin American countries, such as, Venezuela (Vaughan and Tomooka 1999). The wild relatives of the AA genome, which are found in Central and South America and may hybridize with the rice, include *O. rufipogon* (AA, hybrid seed set 19 percent without and 73 percent with embryo rescue), and *O. glumaepatula* (AA, hybrid seed set 39 percent without embryo rescue) (Chu and Oka 1970, Vaughan and Sitch 1991, Vaughan and Chang 1992). Gene transfer from *O. sativa* to *O. rufipogon* under field conditions has been documented in Asia. Spontaneous intermediates between cultivated rice species and their wild relatives occur frequently in and near rice fields when wild taxa are present. The intermediate plants usually appear as hybrid swarms. Natural rates of hybridization can sometimes be substantial, and the hybrids usually show hybrid vigor (Sitch 1990). Natural hybridization with cultivated rice has been implicated in the near extinction of the endemic Taiwanese taxon *O. rufipogon* ssp. *formosana* (Ellstrand et al. 1999). Throughout Asia, typical specimens of other subspecies of *O. rufipogon* and the wild *O. nivara* are now rarely found because destruction of natural habitats and extensive hybridization with the crop (Ellstrand et al. 1999). Recently, knowledge of the *Oryza* species in Latin America has increased greatly since the series of germplasm-collecting missions in Brazil, Paraguay, and Argentina and subsequent research on collected germplasm from Colombia, Costa Rica, and Venezuela (Vaughan and Tomooka 1999). *O. rufipogon* is abundant in the middle reaches of the Orinoco—particularly in Apure and Guarico States. Considerable variation is apparent from specimens, and *O. rufipogon* is found as a weed of cultivated rice. The biodiversity erosion of Asian *Oryza* species highlights the relevance of documenting the Latin American *Oryza* diversity, which may represent new sources of genes of interest for breeding that have not yet been used.

The weedy red rice is also readily found with the cultivated crop in Latin America. Red rice (*O. sativa* f. *spontanea*) is a weedy rice with a red pericarp and dark–colored grains. Its seeds shatter readily and possess dormancy characteristics; the plants typically are tall and late maturing and have pubescent leaves and hulls (Langevin et al. 1990). In contrast to Asia, where manual transplanting is still predominant, in tropical America direct seeding of red rice–contaminated seed is common for a high proportion of rice farmers in Latin America, ensuring field reinfestations and making it one of the most serious weed problems in this region (Fisher and Ramirez 1993). There are indications that genes placed in cultivated varieties of rice have transferred quickly into red rice in Asia (Clegg et al. 1993). In the United States, an allozyme progeny analysis of experimental mixed stands of the rice crop and the red rice weed indicated that the natural rates of hybridization could range from 1 percent (with early season variety, flowering at 72–76 days) to 52 percent (with late season variety, flowering at 82–86 days) (Langevin et al. 1990). Thus, cultivated varieties that flower and mature late, like those mainly grown in Latin America, may enable hybridization with red rice to occur throughout several generations (Langevin et al. 1990).
Studies to define the red rice and rice wild relatives complex in the crop contact zone would likely be important to design biosafety guidelines for the neotropical region. Current work conducted by our group on the characterization of the red rice biotypes found in the major rice-cropping regions of Colombia and Costa Rica suggests differences between red rice populations that are associated with the crop history of each field. Some of the red rice populations are clearly distinct from the rice variety crop in the plot and resemble the wild species *O. rufipogon*. Other red rice populations are more diverse. Although some biotypes are not significantly different from *O. rufipogon*, others look like the variety grown in the plot. Some biotypes phenotypically fall in between *O. rufipogon* and the cultivated variety. Complete characterization of these populations is underway to identify potential indicators of gene flow between rice and red rice.

**Tracing Gene Flow**

Measuring hybridization rates is critical for the assessment of the risk of weediness or extinction by hybridization. The appropriate way to assess hybridization rates under field conditions is to create experimental stands of the crop and wild or weedy taxon under conditions comparable to those under which the crop and the wild or weedy taxon will coexist when field release occurs. Progeny testing of the wild or weedy taxon for crop-specific genetic markers can then be used to measure gene flow (Ellstrand et al. 1999). A more precise estimation of gene flow is obtained by using specific molecular markers. Of the molecular markers available nowadays, microsatellites are valuable genetic markers because they are simple and codominant, allow detection of high levels of allelic diversity, and are easily and economically assayed by polymerase chain reaction (PCR) (McCouch et al. 1997). Spatial distribution of alleles can be used to study local gene flow, including pollen dispersal distances. Microsatellite markers have been used to detect polymorphism within crops, to detect population expansion, and to trace crop-to wild gene flow and wild-to-crop hybridization rate under confined experimental settings as well as under natural conditions (Guadagnuolo et al. 2001).

Our research group has used bean microsatellites to characterize the diversity of the different bean gene pools (unpublished). Clear differences were noted between the Mesoamerican and Andean gene pools. Gene-pool-specific microsatellites were identified. Results suggested that, although gene flow between cultivated and wild bean species occurred in natural environments, no indication of erosion of diversity of the wild pool was noted owing to gene flow. The diversity of the bean crop was rather narrow in contrast to the wild bean gene pool, which was widely diverse. At this point, intervention and destruction of the natural habitats may be a more important cause of diversity erosion. In the case of rice, a set of microsatellite markers is being used to detect polymorphisms between different rice varieties, wild species, and red rice. Genotype-specific markers have been identified and selected, allowing the identification of handmade crossed hybrids from individual genotypes. This set of microsatellites is being used to characterize the genetic structure of the experimental populations before gene flow and to detect outcrossing rates in the field. The spatial distribution of alleles is used to study local gene flow, including pollen dispersal distances. Microsatellites are used to trace crop-to-wild or to red rice gene flow and red rice or wild-to-crop hybridization rates under confined experimental settings as well as under natural conditions. Similar analyses were conducted to assess transgenic-to-non-transgenic variety gene flow.
Conclusions

The quantification of gene flow from the transgenic crop plants to the related weeds and wild species, its effect(s) on the population genetic structure of the recipient species, and maps describing spatial distribution of potential areas of gene(s) movement in the targeted countries are key elements to design strategies for the safe use of LMOs in the neotropics. Besides the mapping of crop and wild or weedy populations in the study sites, it is necessary to analyze the genetic structure of the wild or weedy relatives before and after gene flow. In this case, data analysis of gene flow under the controlled conditions of confined field plots and under local agricultural field conditions should be seen as complementary. A Geographic Information System facilitates fine mapping of wild or weedy diversity distribution in the region and helps target areas with risk potential for gene flow. The information should be translated into procedures and protocols for risk assessment and made available to regional developing countries. Research and monitoring of gene flow and introgression using non-LMOs will give us a suitable base line. Case studies making a comparison of LMOs and non-LMOs carrying the same trait (i.e., herbicide resistance) will serve as a base line to elucidate the effects due to the trait itself. For that purpose equivalent counterparts should be used which that be identified through microsatellite molecular markers and micro-array analysis.

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Practice of Risk Assessment

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Abstract

In this paper we describe the general methodology of risk assessment for living modified organisms (LMOs) as it is outlined in several internationally agreed documents such as Organization of Economic Cooperation and Development recommendations, the United Nations Environmental Program Technical Guidelines, and the Cartagena Protocol on Biosafety as well as the day-to-day practice of applying that methodology. Risk management is discussed along with the writing of a concluding evaluation of environmental impacts. This contribution is part of a training manual developed for training workshops on the development and implementation of biosafety frameworks. The training workshops are part of the project “Implementation of Biosafety Frameworks in Preaccession Countries of Central and Eastern Europe,” which is funded and implemented by the Dutch Government. The complete training manual and background information about the project can be found on the Central and Eastern Europe CEE Biosafety Web site (www.biosafety-CEE.org; sub page “Events, Links and Projects,” under “Matra project”).

Introduction

Risk assessment in the field of biosafety is a scientific process used to identify and evaluate the impacts that activities such as releases into the environment with genetically modified organisms (GMOs) may have on the environment, including humans. Risk assessment can be carried out by those who plan to accomplish those activities with GMOs as well as by authorities with responsibilities to regulate and assess such activities. To provide a meaningful tool for decisionmaking, risk assessment needs to be practiced in a scientifically sound and transparent manner and needs to make use of the best up-to-date scientific knowledge and experience.
Although the details of a risk assessment vary from case to case, the overall methodology followed in doing a risk assessment for GMOs usually involves several systematic steps. An outline of this overall methodology can be found in a variety of documents, including the following:

- UNEP International Technical Guidelines for Safety in Biotechnology,¹
- The Biosafety Protocol (Annex III),² and

As these documents show, the steps taken in a risk assessment are as follows:

1. Identification of potential adverse effects⁴ on the environment, including humans.
2. An estimation of the likelihood that these adverse effects will be realized.
3. An evaluation of risks based on the evaluation of the likelihood and of the consequences of the identified adverse effects of being realized.
4. Consideration of whether any identified risks are acceptable or manageable, including, where appropriate, an identification of risk management strategies.
5. Assessment of the overall potential environmental impact.

In addressing these steps, the relevant characteristics of the following are taken into account:

- The recipient organism,⁵
- The inserted genes and other relevant sequences,⁶
- The resulting GMO,
- The application (e.g., small-scale field trial or marketing),
- The receiving environment, and
- The existing situation, including consideration of the use of the nonmodified recipient organism

**Practice**

How is the methodology described above applied in practice in countries where biosafety frameworks have been in place for many years? This section focuses on risk assessment for the release of genetically modified plants into the environment. The reason for this focus on plants is that currently the bulk of requests for permits deal with releases of genetically modified plants. However, the methodology presented can, to a large extent, also be applied to releases of other genetically modified organisms.

The practical approach described below follows two steps:

1. Preparation of a cover note for the request and the dossier,
2. The actual risk assessment
Preparation of a Cover Note

As a first step in the risk assessment it is useful to ‘set the scene’ of the assessment by listing the following on a cover note:

- the name of the applicant,
- the type of application (e.g., field trials under controlled conditions or a commercial release),
- the name of the recipient organism, including whether the recipient plant can cross fertilize with wild flora with cultivated, or both, crops in the receiving environment;
- the inserted genes or sequences

The last part of the cover note is a list of the inserted or modified genes and sequences, and—where known—the corresponding traits for which these genes code or may code. What is important in this stage is to get a complete list of any inserted genes or sequences regardless of whether the genes are actually expressed in the plant.

It is recommended that the assessor note the pages of the request on which relevant information is found. Assessors should be aware that in some requests the relevant information about inserted genes may be given in different places within the request. Applicants should be urged to concentrate similar types of information when possible in one part of the request. Experience shows that the use of such a cover note facilitates the risk assessment and in fact the entire handling of the request. An example of such a cover note is attached as “worksheet A” to this article.

The Actual Risk Assessment

Once the main elements of the request are included in a cover note, the actual risk assessment can start. The risk assessment starts in a systematic way whereby for each of the inserted genes and sequences the questions described above are addressed:

1. Identification of potential adverse effects.
2. An estimation of the likelihood that these adverse effects will be realized.
3. An evaluation of the identified risks.
4. Consideration of risk management.

After this systematic gene-by-gene assessment, a broader and more holistic approach follows whereby the potential impacts of the genes together are evaluated with a view to an assessment of possible synergistic effects. Finally, the overall environmental impacts are evaluated. These steps are discussed below.

Identification of Potential Adverse Effects—

The risk assessment process starts with the identification of potential adverse effects that will be considered in the risk assessment. This is done in a systematic way by identifying each of the inserted genes or sequences of genetic material that have been introduced. Any
resulting changes in the plant metabolism are examined along with any resulting new or changed traits (phenotype), taking into account that gene products through their interaction with the physiology of the host may cause multiple traits that may differ from the traits expressed in the host organism.

Unlike risk assessments for chemicals, there is not yet a fixed “cookbook recipe” for the identification of potential adverse effects related to a gene or sequence. Whether or not a particular gene or sequence may have the potential to cause adverse effects depends on the characteristics of the gene, of the gene product, of any resulting changes in the phenotype, of the receiving environment, and of the type of application.

In identifying potential adverse effects, the following types of questions are addressed on the basis of the case:

- Can the inserted gene or sequence cause the recipient plants to become more persistent in agricultural habitats or more invasive in natural habitats (weediness) with the related potential adverse effects of changes in management of weeds, changes on population level, or both in natural populations? An inserted gene may confer a selective advantage or a change in survivability.
- Can the inserted gene or sequence cause the recipient plant to be toxic, allergenic, or both to humans or animals?
- Can the inserted gene or sequence cause changes in susceptibility of the recipient plant or—after outcrossing—of other plants to pathogens, which in turn can cause the dissemination of infectious diseases and creating new reservoirs or vectors?
- Can the inserted gene or sequence cause negative effects on populations of nontarget organisms, including indirect effects on population level of, where applicable, predators, competitors, herbivores, symbionts, parasites, and pathogens?
- Can the inserted gene or sequence cause unintended effects on the target organisms (e.g., resistance development)?
- Can the inserted gene or sequence result in a change in management of the genetically modified crop plant that has a negative impact on the environment?
- Can the inserted gene or sequence cause changes in biogeochemical processes?
- Can the inserted gene or sequence cause other unintended side effects such as
  - the potential reduced effectiveness of an antibiotic used in medicine as a result of horizontal transfer of antibiotic-resistance genes?
  - the development of new virus strains owing to the introduction of viral sequences in a plant genome and possible recombination of genetic material?
  - potential insertion effects?

In this stage of the risk assessment it is important to consider the potential adverse effects that are scientifically conceivable on the basis of the gene characteristics involved regardless of whether it is likely that this effect actually would occur during the proposed activities. The question of likelihood will be addressed in the next stage of the risk assessment. In the process of identifying potential adverse effects it should also be remembered that such effects can be direct, indirect, immediate, or delayed. Adverse effects may occur directly or indirectly through mechanisms such as
• the spread of the GMO(s) in the environment
• the transfer of the inserted genetic material to other organisms
• phenotypic or genotypic instability
• interactions with other organisms
• changes in management, such as agricultural practices

The identification of potential adverse effects to be considered in the risk assessment can be addressed in a way that is scientifically sound as well as transparent to the public. The scenario in which this potential adverse effect might occur should be described sequentially, that is, beginning with the causal steps that could culminate in the adverse effect. The scenario should begin with the trigger: What is the scientific reason to assume that a certain adverse effect may occur? The scenario should show the chain of causal events that may lead to its occurrence. As with all scenario writing, this is a creative process, which in this case also requires a rigorously scientific imagination.

It is also important to formulate clearly which potential adverse effect is being considered. For example, the mere mention of the “horizontal gene transfer” of an antibiotic resistance gene does not clarify the potential adverse effect. Transparency is established when the assessor identifies potential adverse effects of reduced effectiveness of an antibiotic used in medicine that might arise as a result of horizontal transfer of antibiotic-resistance genes to pathogenic micro-organisms. Sometimes it may be useful to indicate whether the adverse effect, should it occur, is deemed to be either severe or insignificant.

Although assessments are done on a case-by-case basis, information and analyses from previous assessments can be very useful. There are many such sources of existing knowledge and experience such as

• decision documents of earlier cases, which can be found on the Web sites described under topic 4c;
• search engines such as the SWISS-PROT/TrEMBL Full text search (http://www.expasy.ch/cgi-bin/sprot-search-ful) and the ICGEB database on risk assessment (http://www.icgeb.trieste.it/biosafety/rasm.html);

As was noted earlier, it is strongly recommended that the risk assessment be started systematically, focusing on each of the genes or sequences separately and listing for each gene or sequence any potential adverse effect or effects that the assessor may wish to consider in the risk assessment. Here too, a cover note has proven to be a useful tool. An example of such a cover note (worksheet B) is attached to this article.

Estimation of Likelihood—

Once a potential adverse effect has been identified for inclusion in the risk assessment, the next step is estimating the likelihood that the identified potential adverse effects will actually occur in the proposed application. This stage follows the same systematic approach. For each of the identified potential adverse effects of each of the inserted genes or sequences, an
estimate is made within the proposed application of the likelihood that particular potential adverse effect will actually occur. Here the term “estimation” is chosen, because, given the “nature of nature,” exact numbers of the frequency with which something will happen in nature can rarely be given. Therefore, terms are used such as likely, unlikely, and negligible or effectively zero (or ‘zero’ for that matter, but many scientists are uncomfortable using the “zero” in the context of risk assessment).

The likelihood that a certain inserted gene or sequence will actually result in a potential adverse effect is influenced by many different factors such as the following:

- The characteristics of the inserted gene. For example, a gene that is not involved in toxicity of the donor organism is very unlikely to cause the recipient organism to be toxic. On the other hand, it is likely that a gene product known to be toxic for one insect, such as the endotoxins produced by Bacillus thuringiensis, will also be toxic for other closely related insects. Assumptions related to toxicity or allergenicity can usually be verified with the information presented in the request or dossier as in feeding studies.
- The characteristics of the recipient organism. For example, the potential for outcrossing with wild relatives is negligible for sterile plants or in regions where no relatives exist but is likely for fertile plants in an environment where wild relatives are present in the environment.
- The characteristics of the size or the scale of the application. For example, the likelihood of a genetically modified plant with a certain built-in pesticide resulting in the development of resistance by the target organism is negligible in a small-scale field trial but can be quite likely in a commercial application if no resistance management is applied.

Several tools are available that can provide useful information on the characteristics of recipient organisms such as

- the OECD consensus documents on the biology of plants (www.oecd.org/ehs/service.htm),
- the so called botanical files (De Vries et al. 1992, Van den Meijdan 1994)
- files on the biology of several crop species (see www.aphis.usda.gov/biotech)

In cases in which the estimation of the likelihood does not result in the conclusion “negligible” or “effectively zero”, the risk assessment continues with the next step described in the next section. In cases in which the estimation of the likelihood does not result in a clear conclusion, it is sometimes recommended to proceed with the next step of the assessment by assuming a certain effect will occur. For example, rather than spending much time and effort to determine the frequency of outcrossing, it is assumed that if the plant can outcross, then it will outcross. The attention is then focused on the next step in the risk assessment, that is, the potential consequences of such a transfer.

Another example is the assessment of the possible transfer of antibiotic resistance genes from plant material to microbial organisms. In case there is no scientific consensus about the likelihood of the transfer from plant material to microorganisms, then it may help to continue the risk assessment by asking what the consequence would be if such a transfer would occur. (See next section).
Evaluation of the Identified Risks—

In the cases where a potential adverse effect has been identified and the estimation of the likelihood did not lead to the conclusion “negligible” or “effectively zero”, the risk assessment proceeds to the next step, namely, the evaluation of that particular risk. Note that at this point the term used is “risk” instead of “potential adverse effect”. Risk is the combination of a potential adverse effect and the likelihood of it occurring. Here too, it is recommended that the assessor follow the same systematic approach as before. For each of the identified risks (i.e., the cases in which the likelihood of an identified potential adverse effect is not negligible or effectively zero) of each of the inserted genes or sequences, an evaluation is made of the actual consequence on a component of the environment.

It is important to differentiate between risks related to human health and risks related to the environment. Key issues in risks to human health are toxicity and allergenicity. For required toxicity data, experimental tests are often available. For allergenicity, a specific risk assessment, including specialized assays, is required because allergenicity can usually only be definitively assessed by patients who have the allergic reaction.

For an evaluation of the potential consequence of possible toxicity or allergenicity, the type of application is taken into account. For applications such as small-scale field trials in which the material resulting from the field trial is not consumed by humans or animals, toxicity and allergenicity would generally be of no consequence. For large-scale and market releases, toxicity and allergenicity would be of consequence and would therefore need to be addressed. It is for this reason that, in requests for market approvals, the results of toxicity and allergenicity tests are usually included. Assessors should bear in mind that there is a difference in looking at toxicity in terms of food safety for which it is assumed that large quantities may be consumed frequently (i.e., scenarios in which even low levels of toxicity may have a consequence) and toxicity in the context of environmental safety for which the focus is on the effects of incidental consumption.

Evaluating the impacts that the introduction of a genetically modified plant may have on the environment is less straightforward for several reasons:

1. The different types of effects that can be considered such as weediness, susceptibility to diseases, effects on nontarget organisms, effects on target organisms, and changes in agricultural management differ markedly.
2. Agricultural and natural ecosystems are very dynamic systems in which many changes occur constantly.
3. Every agricultural activity has an impact on the environment in which it takes place. For example, a straightforward agricultural practice such as ploughing and tillage in general has a severe impact on the soil organisms. However, natural processes such as immigration of soil organisms restore these impacts usually quite quickly.

In order to evaluate the possible consequences of the introduction of a GMO in the context of these dynamic processes, the concept of a “baseline” plays an important role. The assessment of the transfer of antibiotic resistance genes from plant material to microbial organisms can serve to illustrate this. Apart from the discussion of whether or not it is likely that such genes present in decaying plant material can be taken up by bacteria so that the gene will still function in the bacterium, one could assess the consequences for a bacterial population that received the transgene.
For the previous case it is important to identify the baseline. What is the existing presence of antibiotic resistance genes in the soil population? It is known that certain antibiotic resistance genes, such as kanamycin resistance, are so abundantly present in the environment that any addition would theoretically make no measurable difference (i.e., would be of no consequence). Some other antibiotic resistance genes, on the other hand, are not present in the environmental isolates of relevant species at such high numbers, and in those cases a (hypothetical) transfer of antibiotic genes could have a measurable consequence such as on medically important micro-organisms. This example illustrates that the assessment of antibiotic resistance gene presence cannot be done in a generic way but depends on the type of antibiotic resistance involved.

**Consideration of Appropriate Risk Management Strategies—**

In the previous step of the risk assessment, whether the introduction of a GMO would have a measurable adverse impact in the background of the baseline of the existing situation was evaluated. In cases in which this is true, the risk assessment continues with the next phase, which is a consideration of whether the identified risk is acceptable or manageable, that is, a consideration of appropriate risk assessment strategies.

It should be emphasized that the term “acceptable” plays a role twice in the evaluation of a proposed activity with GMOs, but in different ways. First, it plays a role in this phase of the risk assessment when the risk management strategies that would be appropriate are considered. Second, it plays a role in the final decisionmaking when an identified risk for the environment or human health is compared and weighed against any potential benefits that the proposed activity may have for the environment or human health.

In this phase of the risk assessment, the question of whether risks are identified that require additional risk management measures is addressed and, if so, a risk management strategy is defined. This step should also be conducted in the systematic gene-by-gene and potential adverse effect by potential adverse effect way, as described previously. For cases in which a risk management strategy has been identified, the risk assessment “loops back” to the earlier steps in the risk assessment to check whether the proposed risk management strategies sufficiently reduced the likelihood or the consequences. This is why risk assessment is often called an iterative process. There are many different strategies for risk management of genetically modified plants, including reproductive isolation by removing of flowers, use of isolation distances or border rows, and reduction of the size or duration of an application. Annex 5 of the UNEP guidelines gives examples of risk management strategies.

**Assessment of the Overall Potential Impact: Conclusion—**

After the systematic gene-by-gene assessment described in the previous steps, a broader and more holistic approach follows whereby the potential adverse effects of the genes together are evaluated with a view to possible synergistic effects. Finally, the overall environmental impacts are evaluated by placing any identified risks in the context of risks posed by the nonmodified recipients and by taking into account any beneficial effects the proposed activities with GMOs may have on the environment.
Synergistic Effects

New traits may enhance or suppress each other. This may have effects on the overall behavior of the genetically modified plant. This is why after the systematic gene-by-gene approach two more questions are considered in the risk assessment focusing on the GMO as a whole. The first question is, Do the introduced genes or traits have characteristics that may enhance the effect of the GMO in the environment? For example, a plant with one newly introduced abiotic stress resistance trait, such as drought resistance, may behave differently than a plant with several different abiotic stress resistance traits. Whether this is the case depends on the type of traits introduced and on the biochemical pathways involved. The second question is, How does the GMO behave in practice? For this part of the assessment, data obtained from greenhouses, field trails, and attempts to market the GMO in other countries are often included in the request. Assessors should be aware that, although the GMOs that have been developed to date are usually relatively simple constructs with one or sometimes two new traits, more complex cases will likely be offered for assessment in the near future.

Overall Environmental Impact—

In the last step of the risk assessment, the overall environmental impact is evaluated. Note that at this point there is a change in terminology. Although in the previous steps the focus was on potential adverse effects, in this last step the focus is on the overall environmental impact, that is, consideration and comparison of potential adverse effects as potential beneficial effects on the environment. This is done by placing any risks identified in the context of risks posed by the nonmodified recipients and taking into account any beneficial effects the proposed activities with GMOs may have on human health or the environment.

The risk assessment usually ends with a summary or a conclusion. It should be emphasized that this is not the same as the final decision. The summary or conclusion will “spell out” the type of risks that the proposed activity with GMOs may have, including, where appropriate, proposed risk management strategies. The summary also describes any potential beneficial effects the proposed activity with GMOs may have on the environment or human health. It is usually up to the decisionmakers to weigh these potential risks and benefits.

Endnotes

1 http://www.unep.org/unep/program/natres/biodiv/irb/unepgds.htm
2 http://www.biodiv.org/biosafe/protocol/protocol.html
4 In some countries and documents the terms “potential harm” or “hazard” are used.
5 In some documents the term “host organism” is used. Both these terms refer to the organism in which genetic material from a donor organism is introduced.
6 With the “other relevant inserted sequences”, reference is made to inter alia (a) open reading frames (ORFs) that code for proteins (i.e., that encode a protein in the host from which the sequence has been derived); (b) promoter, terminator, and enhancer sequences; and (c) sequences that code for RNA transcripts that are not functional in translation (e.g. anti-sense RNA).
Practice of Risk Assessment

For example, Directive 2001/18/EC describes these terms as follows:

- “Direct effects” refer to primary effects on human health or the environment that are a result of the GMO itself and do not occur through a causal chain of events.
- “Indirect effects” refer to effects on human health or the environment occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management. Observations of indirect effects are likely to be delayed.
- “Immediate effects” refer to effects on human health or the environment observed during release of the GMO. Immediate effects may be direct or indirect.
- “Delayed effects” refer to effects on human health or the environment that may not be observed during the release of the GMO but become apparent as a direct or indirect effect either at a later stage or after termination of the release.


See for example, annex III of the Biosafety Protocol.

See for example, annex II of Directive 2001/18.

References


WORKSHEET A (Cover note)

**Applicant (dossier number)**

………………………………………………………………………………

**Type of use:** field trial / commercialisation.

**Host plant:** ………………………………………

- Potential for outcrossing
  - With wild relatives ……………
  - With cultivated relatives …………

- Potential for persistence in the environment …………

**Inserted genes and sequences:**

- ……………………………..
- ……………………………
- ……………………………..
- ………………………………

WORKSHEET B (ASSESSMENT PER GENE)

**Dossier/Applicant:** ……

**Type of use:** ……..

**Plant:** ……

**Gene:** ……

<table>
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<th>Estimation of likelihood</th>
<th>Evaluation of identified risk</th>
<th>Consideration of risk management</th>
<th>Assessment of overall impact</th>
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**OVERALL CONCLUSION FOR THIS GENE:**
Session 2: The Practice of Environmental Assessment

Session 2B—Risk Management Issues
Resistance-Breaking Pathogen Strains and Identification of Mitigation Measures

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Abstract

Traditional pathogen resistance genes usually are effective for only a short time. The breakdown of resistance is an expected consequence of the release of resistant varieties. Durable resistance remains a goal that is yet to be achieved for most plant–pathogen systems. Traditional pathogen resistance genes are also usually strain specific. Varieties are released with the recognition that they are not resistant to all strains and that new strains existing in areas where a variety has not been tested might have the capability to overcome the resistance gene(s) possessed by the variety. Transgenic pathogen resistance is expected to exhibit the same characteristics as traditional genes in terms of limited time of use and strain specificity.

The most extensive experience to date has come from transgenic virus-resistant crops because these have already been commercialized. Evidence from the past experimental literature has revealed the strain specificity of engineered virus resistance, which parallels that of traditional virus resistance. Strain specificity has been noted for currently commercialized transgenic squash as well. Consequently, the detection of a field isolate that appears to overcome coat protein-mediated resistance in commercial transgenic squash is consistent with expectations. It is not known whether this strain has appeared as a consequence of selection caused by the presence of the resistant variety or whether it is an established, preexisting resistance-breaking strain.

The plant breeder’s response to strains that overcome resistance genes has typically been to seek new, more effective genes that can be bred into new resistant varieties. The availability of transgenic strategies to incorporate resistance to new strains could make this approach more effective. Continued commercial use of transgenic strategies to mitigate the effect of resistance breaking pathogen strains will require the rapid and cost effective introduction of transgenes. Regulatory policies that enable these conditions to be met will ensure the continued development of pathogen resistance traits in transgenic plants.
Introduction

The development of crops resistant to pathogens is one of the most important applications of crop genetic engineering—particularly for developing countries. Pathogens destroy a significant portion of crop output and are a major cause of the variability of crop yields from year to year. Resistance genes have been introduced in many crops, and the development of pathogen resistant varieties is an ongoing goal of traditional plant breeding.

The extensive history of breeding for pathogen-resistant varieties has shown at least two characteristics of traditional resistance genes:

1. Resistance genes are usually not durable; their effectiveness against target pathogens is often short-lived. One reason for this phenomenon is that, for many pathogens, the number of generations per unit time is significantly greater than that of the host. This fact provides the pathogen with a greater opportunity to generate mutations for overcoming resistance. In other words, the pathogen has the potential for evolving more rapidly than the host.

2. Resistance genes are usually strain specific. A resistance gene rarely confers resistance to all pathogen genotypes.

Results

Examples of Traditional Fungal Resistance Genes

Traditionally bred fungal resistance has often proven to lack long-term effectiveness. For example, the wheat cultivar gene, was resistant to the fungus, Mycosphaerella graminicola when it was released. However, after only 3 years of use, the resistance in this cultivar deteriorated (Cowger et al. 2000). In beans, the pinto bean cultivar pinto olathe, containing the rust resistance gene Ur-6 was released in 1981. The resistance in this cultivar was overcome by the target pathogen, Uromyces appendiculatus, within 10 years (Steadman et al. 2001). Finally, data from a recently completed 2-year study in Ohio indicate that the soybean resistance genes Rps1a, Rps1b, Rps1c, Rps1k, Rps3a, and Rps6 conferring resistance to specific strains of Phytophthora sojae, are losing effectiveness against this economically important pathogen (Pollock 2001).

The multiplicity of resistance genes in soybean highlights the second characteristic of traditional genes mentioned above, namely, their strain specificity. Although the Ohio survey indicates that the resistance genes studied are losing effectiveness against strains to which they conferred resistance in the past, these genes were not effective against all strains in the first place. For example, Rps1, Rps3, Rps4, Rps5, and Rps6 of the soybean can be defeated by race 7 of Phytophthora sojae (Young et al., 1994).
Another example of strain specificity is the apple Vf gene, which confers resistance to *Venturia inaequalis* races 1–5. This resistance gene has been found to be overcome by *V. inaequalis* race 6 in some apple varieties (Parisi et al. 1996). Another study indicates that other resistance genes residing in apples confer varying degrees of resistance to different *V. inaequalis* field isolates (MacHardy et al. 2001).

It is important to note that the existence of a multiplicity of strains of this and other pathogens makes it difficult to distinguish between selection for a mutant that is able to overcome the resistance and the selection of an already established ("preadapted") strain that might predominate in a particular geographic location and then spread to a new location because resistance genes eliminate competing strains. Indeed, these two possibilities are simply two sides of the same coin.

**Examples of Traditional Bacterial Resistance Genes**

Traditional bacterial resistance genes follow the same pattern as that described for fungal resistance genes. For example, the resistance gene Rb in cabbage, conferring resistance to race 1 of *Xanthomonas campestris* pv. *campestris*, can be overcome by race 0 of the same pathogen, thus demonstrating the strain specificity of this particular resistance gene (Dzhalilov et al. 2000). In a field experiment illustrating the evolution of resistance breaking as well as strain-specificity, Leach et al. (2000) showed that in the case of rice possessing the resistance R genes Xa4, Xa10, or Xa7, which confer resistance to *Xanthomonas oryzae* pv. *oryzae* several new strains could be selected that overcame these resistance genes. All selected strains were capable of overcoming Xa4 resistance, whereas all strains derived from one specific clonal lineage overcame resistance R gene Xa10, and only a few strains of that same lineage overcame resistance R gene Xa7.

**Examples of Traditional Virus Resistance Genes**

As with fungal and bacterial resistance, virus resistance genes are also eventually overcome. Furthermore, as with fungi and bacteria, multiple strains of virus exist. Virus resistance genes are not effective against all of these strains.

Aside from the potential that several rounds of viral replication (several “generations”) will occur for each generation of host plant, thus providing viruses with a better opportunity for generating diversity that can include resistance-breaking mutants, the replicating mechanism of ribonucleic acid (RNA) viruses in particular affords additional potential for generating high diversity. First, RNA-dependent RNA polymerase (RDRP) possesses no editing function. This lack of editing leads to a high mutation rate among RNA viruses. For example, the mutation rate for tobacco mosaic virus (TMV) has been calculated to be 0.15 per genome (García Arenal et al. 2001). Thus, if the number of viral particles per cell is $10^6$ (a conservative estimate), a potential exists for at least $10^4$ mutants per cell in an infected plant. On the basis of the number of infected cells within a single plant, there is a high potential for the generation of mutants, some of which might be able to defeat a resistance gene.

In addition to the high error rate it causes during RNA replication, RDRP also exhibits a high frequency of strand switching during this process, thus producing a high recombination rate. Estimates range from $10^4$ to $10^8$ per nucleotide (García-Arenal et al. 2001). For a
member of the Potyviridae, with an average of $10^4$ nucleotides per genome, and again on the basis of the conservative estimate of $10^6$ molecules per cell, between $10^3$ and $10^6$ recombination events per cell can occur. This potential is borne out well by the observation that RNA molecules arising from infectious transcripts of cloned viral cDNA are highly variable (Schneider and Roossinck 2000, Ambrós et al. 1999, Kearney et al. 1999, Palukaitis and Roossinck 1996, Kurath and Dodds 1995, Kurath and Palukaitis 1990, Kurath and Palukaitis 1989).

Examples of the consequence of this variability in viral genomes include the following:

1. The use of tomato mosaic virus resistance in tomatoes selected for mutants that overcome the resistance gene (Pelham et al. 1970),
2. The lettuce gene mo12 conferring resistance to lettuce mosaic virus, is overcome by one strain, LMV–E (German-Retana et al. 2000),
3. The tomato spotted wilt virus (TSWV) resistance gene Sw-5 in tomatoes is resistant to isolate D but susceptible to isolate A (Moyer et al. 2001),
4. The Cry locus in cowpeas confers resistance to CMV–Y but is overcome by CMV–L (Karasawa et al. 1999).

Because there has been greater success in engineering effective resistance against viruses than against either fungi or bacteria, there is a better opportunity to compare the performance of these engineered resistances to viruses with traditional resistance genes. With respect to the two characteristics mentioned thus far, the performance of transgenic resistance is identical to that of traditional resistance genes. First, as with traditional virus resistance, transgenic resistance is strain specific. For example, the coat protein gene of papaya ringspot virus (PRSV) confers resistance to the Hawaiian strain (PRSV–HA) in transgenic papaya but not to strains from Mexico, the Bahamas, Florida, Australia, Brazil, China, Ecuador, Guam, Jamaica, and Thailand (Gonsalves and Slightom 1993). This strain specificity is also observed in transgenic plants engineered with genes other than the coat protein gene. Transgenic plants expressing the nucleocapsid protein gene (N gene) of the BL strain of TSWV are resistant to that strain but not to the Arkansas, 10W Pakchoy, or Begonia strains (Pang et al. 1992). Finally, resistance to cucumber mosaic virus (CMV) conferred by the expression of a replicase gene was shown to be overcome by a genotype belonging to CMV subgroup I (Hellwald et al. 1999). Selection specifically for a genotype that can overcome resistance has been shown experimentally by Moyer et al. (1999), who were able to select a resistance-breaking genotype that could overcome transgenic N-gene resistance against TSWV.

Strain specificity is true of virus resistance genes now in commercialization. Initial work with the CMV–C coat protein presently in commercial virus-resistant squash (Cucurbita pepo) hybrids containing the constructs ZW20 or CZW3 (marketed by Seminis Vegetable Seeds) was shown in a tobacco model system to be resistant to another strain in the same subgroup (CMV–Chi) but not to a strain in the other subgroup (CMV–WL) (Nambo et al. 1991, Quemada et al. 1991). More extensive studies of the coat protein genes in squash itself corroborated the results in tobacco. For example, transgenic line ZW20 was challenged with various isolates of zucchini yellow mosaic virus (ZYMV). The results of these experiments, summarized in table 1, show that at least one isolate is capable of overcoming the transgenic resistance. A study of the transgenic line CZW3 summarized in table 2 similarly shows the strain specificity of resistance against CMV, ZYMV, and watermelon mosaic virus 2 (WMV2).
Table 1. The resistance or susceptibility of transgenic squash line ZW20 to different geographic isolates of ZYMV.

<table>
<thead>
<tr>
<th>ZYMV isolate</th>
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<tr>
<td>California</td>
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<td>Florida</td>
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Table 2. The resistance or susceptibility of transgenic squash line CZW3 to different geographic isolates of CMV, WMV2, and ZYMV.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Isolate</th>
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<td></td>
<td>Egypt</td>
<td>Resistant</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

These studies of field isolates in greenhouse inoculations therefore lead to the prediction that other virus genotypes capable of defeating the transgenic resistance will be encountered upon deployment of transgenic squash over an increasingly wide area. This prediction has apparently been fulfilled after only a few years of commercialization. Figure 1 shows the results of inoculation of a transgenic hybrid, Destiny III, with a virus strain isolated from an infected field of transgenic squash growing in southern Illinois. The virus, which appears to be an isolate of WMV2, is capable of overcoming the resistance conferred by the WMV2 coat protein gene.
Discussion

The evolution (or detection) of strains that can defeat specific viral resistance genes was not unexpected by the developers of transgenic squash and should be expected by developers of future crops engineered to be resistant not only to viruses but other pathogens as well. To mitigate the breakdown of resistance, a strategy of pyramiding genes was foreseen and should be incorporated into the plans for maintaining any long-term benefits of transgenes against pathogens. Breeders of traditional resistance genes have followed this strategy, but the use of transformation technology has the potential for making it an even more effective means of developing crops with enhanced disease resistance.

As applied to coat protein-mediated resistance, the pyramiding strategy is simple: one has merely to isolate the coat protein gene(s) of new strains that are encountered and add them via transformation to the array of resistance genes already present in a plant. This strategy allows the incorporation of genes that may be modified based on new information, thus adding more effective and broader resistances. More important, this strategy allows the incorporation of resistance gene constructs that better address safety concerns such as recombination and transencapsidation.

The pyramiding strategy also permits incorporation of genes that confer resistance not only against new viral strains but also against new virus problems that might arise either because of expansion of cultivation into an area where new viruses are encountered or because new viruses take the place of those that have effectively been eliminated by the resistance genes. A similar approach can be envisioned for genes that might be deployed in the future against fungal or bacterial disease.
The effective execution of a gene-pyramiding strategy requires that at least two conditions be met: (1) the introduction of new transgenes must be sufficiently rapid to respond effectively to new strains or new viruses, and (2) the incremental cost of introducing new genes should decrease, to allow their introduction to be economically feasible. Neither of these conditions exist today—especially for the “minor crops”—that is, the crops other than soybeans, corn, and cotton. Consequently, disease resistance is not being pursued vigorously by industry,—particularly that segment concentrating on “minor crops”. Data for the United States from the Information Systems for Biotechnology (ISB) field test database (http://www.isb.vt.edu/cfdocs/fieldtests1.cfm) for the period 26 November 2000 through 25 November 2001 provide evidence for the low level of industry activity. Figure 2 shows the proportion of notifications and field test applications for all pathogen-resistant transgenic crops filed by the public sector and by industry. The majority of activity appears to be in the public sector. This disparity of activity is even greater when corn and soybeans are eliminated from the data (no notifications or applications for disease-resistant cotton were filed during the period studied). Figure 3 shows that for crops other than soybeans and corn, roughly 75-percent of the development activity is being carried on in the public sector. This same proportion is seen for virus-resistant minor crops (figure 4). These statistics signal that the industrial sector, which is more sensitive than the public sector to the costs versus economic gain of developing a crop, realizes that the cost and time required to develop and maintain the effectiveness of transgenic pathogen resistance are often not economically feasible.

Figure 2. The proportion of notifications or field test applications for all pathogen resistant plants filed by the public sector versus industry for the period 11/26/00-11/25/01.
The cause of the cost and time problems does not appear to be technical. The ability to transform various crop species increases each year, and the efficiency of this technology likewise improves. The involvement of public institutions in the United States and of publicly funded research institutes in developing countries is testimony to the relatively low cost involved in accomplishing the basic technical tasks required to develop and deploy pathogen-resistant crops. Rather, the primary constraint appears to be the regulatory hurdles that prolong the development time and increase cost.
**Conclusions**

Because publicly funded institutions in the United States and other parts of the world appear to be the principal means by which pathogen-resistant crops will be developed, the cost of fulfilling regulatory requirements must be considered in any plans to apply this technology. Public institutions must recognize that they will have to bear those costs in addition to those they have already borne for the technical development of the crop. In many cases, given the current regulatory framework, the regulatory costs could account for the more significant portion of the total project funds.

Funds are rarely set aside in public projects for fulfilling regulatory requirements. If the regulatory framework requires excessive expense and development time, so that transgenic pathogen resistance cannot be deployed by even publicly funded institutions, then the benefits of this technology will be lost to those who are in the most critical need of this technology—the developing countries.

Developed countries can better afford to implement traditional solutions to crop disease. If transgenic pathogen resistance were not developed, farmers would be able to return to their reliance on traditional resistance genes; they could shift areas of production to new disease-free areas; and they could grow different crops not susceptible to prevailing diseases. Furthermore, farmers could continue their reliance on traditional chemical means of control: fungicides, antibiotics, and insecticides or other chemicals to kill or inhibit disease vectors. The harm of some of these alternatives, especially chemicals, has been clearly established—particularly by data presented at this conference. The real potential for transgenic technology to reduce this demonstrated harm needs to be weighed against its postulated risks.

**Acknowledgments**

Dr. Rosario Provvidenti of Cornell University conducted most of the strain inoculations shown in Tables 1 and 2. The author also wishes to acknowledge the help of Dr. Alan S. Walters of Southern Illinois University, who located virus-infected transgenic squash fields in Illinois.

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Young, B. A., St Martin, S. K., Schmitthenner, A. F., Buzzell, R. I. and McBlain, B. A.  1994.  Absence of residual effects of defeated resistance genes on phytophthora rot of soybean.  Crop Science 34, 409-414. Table 1.  The resistance or susceptibility of transgenic squash line ZW20 to different geographic isolates of ZYMV.
Risk Management from a Developing Country’s Perspective

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Abstract

Risk assessment and management in South Africa is the responsibility of the National Department of Agriculture under the Genetically Modified Organisms (GMO) Act. The Minister is advised by an executive council consisting of officers from the six departments involved. A Registrar administers the Act, and an advisory committee, consisting largely of academics experienced in working with living modified organisms, considers all applications for releases and advises the registrar. An example of risk assessment and management in the case of cotton resistant to cotton bollworms will be given. In addition, an example of trade barriers to export from an African country to Europe will be discussed. Risk assessment and management legislation in other sub Saharan African countries will be mentioned. Finally, research needs to improve risk assessment and management will be presented. These include the development of insect resistance, effects on nontarget insects and on other animals and birds, socioeconomic impacts, and environmental benefits.

Introduction

This paper will deal mainly with risk assessment and management in South Africa. Before the introduction of the Genetically Modified Organisms (GMO) Act in 1997 and its implementation in 2000, living modified organisms (LMOs) were handled by the South African Committee on Genetic Experimentation (SAGENE). This was a governmental statutory body that handled requests for contained use, field trials, or general releases of LMOs. Although compliance with SAGENE regulations was voluntary, no known violations were perpetrated. The author was a member and former chair of this committee. After the 1994 elections in South Africa, the Government decided that legislation was required to enforce compliance with regulations. This paper will deal with risk assessment and management after the implementation of the GMO Act.
Discussion
South Africa

The GMO Act was passed by the South African parliament on 23 May 1997. However, it took until November 1999 for the regulations to be approved. Hence, the Act was only implemented at the beginning of 2000. The Act stipulates that there will be an Executive Council, a Registrar, and an Advisory Committee. Their composition and functions are as follows:

Executive Council

The executive council consists of one officer from each of the National Departments of Agriculture (which administers the Act), Arts, Culture, Science and Technology, Environment, Health, Labour, and Trade and Industry. These officers should be knowledgeable of the implications of the GMO Act with respect to their individual departments. The Executive Council decides on the issue of permits (on the basis of advice from the Advisory Committee via the Registrar), oversees the office of the Registrar, is involved in intercountry liaison, advises the Minister of Agriculture, and ensures law enforcement under the Act.

Registrar

The Registrar, who is appointed by the Executive Council, is responsible for administering the Act. He or she issues permits, acts on contraventions of the Act, appoints inspectors to do site inspections, and ensures that the conditions of permits are complied with.

Advisory Committee

The advisory committee consists of up to eight members knowledgeable in the field of GMOs. It includes two persons from the public sector with knowledge of ecology and GMOs. Among the areas of expertise represented are biochemistry, biotechnology, cell biology, ecology, entomology, microbiology, molecular biology, and plant pathology. The functions of the advisory committee are to advise the Minister of Agriculture and the Executive Council (via the Registrar) of the environmental impacts of introducing GMOs; the contained use, import, and export of GMOs, and regulations and guidelines concerning all of these.

Figure 1 shows the process followed when the Registrar for Genetic Resources receives an application for a trial or commercial release. He or she chooses a member of the Advisory Committee to chair an ad hoc subcommittee to review the application. This Chair chooses two or three members of the South African scientific community skilled in the particular application under consideration from a list of names supplied by the Registrar for this purpose. All relevant documentation is supplied to the subcommittee, which is required to submit its recommendations within 2 months. These recommendations are summarized by the subcommittee Chair, who then submits a report to the Registrar. This report is sent to all members of the Advisory Committee for comment. The Registrar then compiles a final report for submission to the Executive Committee.
Table 1. Risk assessment of Bt cotton in South Africa

<table>
<thead>
<tr>
<th>Environmental Impact</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Effect on sustainable agriculture</td>
<td>• Positive; less input and ‘peace of mind’ management</td>
</tr>
<tr>
<td>• Effect on soil, water, and air</td>
<td>• Positive; less pesticide load</td>
</tr>
<tr>
<td>• Socio-economic effects</td>
<td>• Benefits rural, small scale farmers</td>
</tr>
<tr>
<td>• Stability</td>
<td>• Stable for 10 years worldwide</td>
</tr>
<tr>
<td>• Other specific concerns (e.g., development of insect resistance)</td>
<td>• Compulsory integrated pest management to minimise development of resistance</td>
</tr>
<tr>
<td>• Spread of gene (pollen, seed, or vegetative propagation)</td>
<td>• No negative impact</td>
</tr>
<tr>
<td>• Out crossing to weeds or natural flora</td>
<td>• No compatible local relatives; not invasive</td>
</tr>
<tr>
<td>• Effect on insects, birds, and other consumers</td>
<td>• Only lepidopterans affected; renews biodiversity (insects and birds) in and around crops due to reduced use of insecticides</td>
</tr>
</tbody>
</table>

Figure 1. The process followed when the Registrar for Genetic Resources receives an application for the trial or commercial release of a LMO
Before an applicant can submit a request to the Registrar for a field trial or commercial release of a GMO, public notification has to be given in three different newspapers in the geographical area affected. Copies of such notifications have to accompany the application. Comments or objections are submitted by the registrar to the Executive Council, which will take these into account when deciding whether or not to award a permit. It is at this stage that socioeconomic impacts of such permits are considered.

Should the Executive Council approve a permit, the Registrar appoints one or more inspectors to ensure that the trials are carried out in accordance with the GMO Act. Inspectors maintain records and issue warrants for violations of the Act. The inspectors conduct routine and surprise inspections.

Organizations or companies conducting the trials are required to ensure that measures are taken to avoid adverse impacts on the environment, and they are responsible for any damages. Conviction of offenses carries penalties, including fines or imprisonment.

A summary of the applications for trial releases, commercial releases, and commodity imports in South Africa is shown in figure 2.

![Figure 2. Applications for trials and releases in South Africa from 1990 to 2000.](image)
Risk Assessment in South Africa

Risk assessments and risk management are currently both the responsibility of the Department of Agriculture. It is hoped that in time the Department of the Environment will become more closely involved. An example of risk assessment leading to risk management is shown in table 1 for cotton resistant to cotton bollworm species (*Helicoverpa armigera*, *Diperopsis castanea*, *Erias biplagar* and *E. insulana*). This crop expresses the gene encoding the *cry*1Ac gene of *Bacillus thuringiensis* and is known as Bt cotton.

As a result of the preceding risk assessment, the following risk management procedures are being implemented:

**The Development of Bollworm Resistance to the Bt toxin—**
Commercial farmers will plant the required percentage of non-Bt cotton to prevent the development of insect resistance. Originally it was considered feasible to sell small-scale farmers a mixture of non-Bt and Bt cotton to provide suitable refuges in situ. However, data presented by Janet Anderson of the U.S. Department of Agriculture at this conference showed that this could, on the contrary, speed up the development of insect resistance. Instead, a procedure is recommended whereby a group of farmers join to form a consortium and together set aside a given area of land in which to plant non-Bt cotton in order to provide the required refuges.

**Effects on Nontarget Insects and Other Animals—**
An international research grant has been applied for to determine the effects that Bt cotton might have on nontarget insects. This research will be carried out among both small-scale and commercial farmers.

**Socio-economic Impacts—**
Ismael et al. (2001) recently published a study of the socioeconomic impacts of growing Bt cotton by small scale farmers in South Africa. They showed that farmers who adopted the Bt cotton variety in the 1998 and 1999 seasons benefited from the new technology according to all the measures used. Average yield per hectare and per kilogram of seed was higher for adopters than for nonadopters. The increase in yields and reduction in chemical application costs outweighed the higher seed costs, and thus gross margins were also considerably higher for adopters in the second season. This was a bad year owing to unusually heavy rainfall, and the Bt adopters suffered far less fall in yields than those who did not adopt.

Because yields and gross margins are only partial measures of efficiency in that they fail to take account of inputs such as labor, the preceding data were supplemented with other studies. These found that Bt cotton adopters were considerably more efficient than those who used non-Bt varieties. For 1998 the adopters averaged 88 percent efficiency compared with 66 percent for nonadopters. The relative numbers for 1999 were 74 percent and 48 percent.

A similar risk assessment was carried out for Bt maize grown in South Africa. At present this is only yellow maize used predominantly for animal feed. Risk management scenarios are much the same as for Bt cotton except for the following economic implications. Namibia exports most of its beef to Europe and imports almost all its maize feed from South
Africa. Europe will not accept any beef from Namibia fed with GM maize. One of the reasons for this is that GM maize carries a gene coding for antibiotic resistance. This, however, is a spurious argument, for the \textit{Bt} maize grown in South Africa does not carry an antibiotic resistance gene. Therefore, Europe is excluding Namibian beef solely on the basis of trade barriers.

**Sub-Saharan Africa**

South Africa is currently the only country in Africa with GMO legislation in place. However, several countries have drafted legislation, and these are in various phases of implementation. These countries include Namibia, Zimbabwe, Zambia, Kenya, Uganda, Cameroon, Nigeria, and Egypt. Genetically modified crops trials are, however, underway in Zimbabwe, Zambia, Kenya, Uganda and Egypt.

**Conclusions**

The situation in South Africa is reasonably acceptable. However, we need research into the following:

- Insect resistance
- Effects on nontarget insects
- Effects on other animals and birds
- Socioeconomic impacts
- Environmental benefits.

The situation in other African countries is quite variable, and they need support to develop biosafety guidelines and regulations for the import, trial, and commercial releases of LMOs.

**Acknowledgments**

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**References**

Current Status of Biosafety Framework in Brazil

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This paper represents the views of the authors and not the views of the Government of Brazil

Biodiversity, a resource that has proven essential for human survival, comprises the wealth of organisms that are a source of food, medicine, and shelter among other essentials. The economic importance of biodiversity has significantly increased with the development of modern biotechnology because sexual barriers have been bypassed, and thus genes discovered in diverse biota can be expressed in genetically modified organisms (GMOs). Brazil is a country rich in genetic resources with its diverse ecosystems, harboring close to 20 percent of the planet’s species and the highest number of plants and amphibians besides abundant species of birds, reptiles, and mammals (Groombridge 1992).

In 1992, Brazil hosted the Rio Conference (UNCED) when Agenda 21 and the Convention on Biological Diversity (CBD) were signed, international instruments which were later ratified by 170 countries. The CBD emphasizes the preservation and sustainable use of biodiversity and also recognizes the state’s sovereign right to its own genetic resources and the share of benefits generated by the use of biodiversity in a fair and equitable way. The concept of safe use of biotechnology is stressed in the text of the CBD in article 8 (g) regarding the need to regulate, manage, or control the use and release of living modified organisms (LMOs) resulting from modern biotechnology. The focus is on those LMOs that are likely to have adverse effects on the conservation and sustainable use of biodiversity, but risks to human health are also taken into account. The intentional transboundary movement of LMOs is addressed in article 19 (3) of the CBD in which the need to develop a protocol setting appropriate procedures for the safe transfer, handling, and use of LMOs that may have adverse effects on biodiversity is stated. Such a protocol was finalized and adopted in Montreal in January 2000, and it is known as the Cartagena Protocol.
Biosafety Framework in Brazil

Brazil is a CBD member State, and it has adopted national policies toward the conservation of biodiversity and the sustainable use of genetic resources. Biosafety policies have been addressed by the establishment of a legal regulatory framework. Significant investment is being made in capacity building, biotechnology research, and development programs. The country participates in the discussions for the implementation of the Cartagena Protocol, although it has not yet ratified the instrument. In this paper we report the main features of the Brazilian Legal Biosafety Framework and make a brief evaluation of the operability of the system and the current status of genetically modified (GM) crops.

National Legal Biosafety Framework

In Brazil, a Biosafety Legal Framework has been in place since 1995 (Law 8,974/95, Decree 1,752/95), complemented by other legal instruments (MP 2,237–9/01 and Norms) that set the standards for controlling the use of genetic engineering techniques in the construction, cultivation, manipulation, transportation, marketing, use, release, and disposal of GMOs with the objective of protecting the life and health of humans, animals and plants, as well as the environment. All activities and projects, including scientific research and industrial production, are subject to regulation under such a framework. Activities with GMOs can only be performed by legally established institutions, not at the individual level. The Biosafety Law forsees, in case of non-compliance, the application of fines and penalties. Besides the specific Biosafety legislation, the Brazilian model also includes a harmonized approach with legal instruments, such as those originating in the inspection agencies of the Ministries of Agriculture, Health, and Environment. The competent authorities from other branches of government have to comply with the technical report originating in the National Technical Biosafety Committee (CTNBio), elaborated on a case-by-case basis.

Features of the National Technical Biosafety Committee (CTNBio)

The National Technical Biosafety Committee (CTNBio) is a Federal body consisting of 18 full members and their alternates with the following representation: 8 scientists presently working with biotechnology (2 in human sciences, 2 in animal sciences, 2 in plant sciences, and 2 in environmental sciences); representatives from the following ministries: Science and Technology (1), Health (1), Environment (1), Education (1), Foreign Affairs (1), and Agriculture, Livestock, and Supply (2); representative of an agency for the consumer’s defense (1); a representative from the biotechnology business sector (1); and a representative from an agency for worker’s health protection (1).

National Technical Biosafety Committee (CTNBio) members are designated by the Minister of Science and Technology from a short list recommended by the members of the Committee and based on recommendations received from scientific, public or private institutions and associations, or, in case of representatives from other ministries, by the respective minister of that organ. The Chair of the Committee is designated by the Minister of Science and Technology. The members have a 3-year mandate and can be nominated for another term. CTNBio deliberates with a minimum quorum of two-thirds of its members. Board members are not paid to work for the Committee, for it is considered an honorific duty to do so. Petitions and other biosafety related demands are initially analyzed by the members according to the issues and to their area of expertise and discussed within the specific
subcommittee (CSE—Human, Animal, Plant and Environmental), before a consensus or majority position is reached on the subject by the CTNBio board.

The CTNBio has the following legal responsibilities:

- To propose a national biosafety policy and a code of ethics on genetic manipulation
- To follow the developments in biosafety and related areas,
- To issue Biosafety Certificates (CQB) and to establish procedures for operating the Internal Biosafety Committees (CIBio),
- To classify the biosafety risk level of the genetically modified organisms (GMO) and determine the need for environmental impact studies,
- To issue expert technical reports on deliberate release of genetically modified organisms (GMOs) and projects involving pathogenic GMOs,
- To provide technical support to inspection agencies,
- To publish the petitions and expert reports in the official journal (D.O.U.),
- To request adhoc consultants,
- To propose changes to the biosafety law and norms, among other demands involving modern biotechnology.

Box 1 - Norms (IN) issued by CTNBio:

- IN 1—The Certificate of Quality in Biosafety (CQB) and functioning of the Internal Biosafety Committee (CIBio).
- IN 2—Importation of GM Plants for Research. The CIBio issues expert reports on the importation of risk Group I GMOs and the CTNBio of risk Group II GMOs. Authorizations are issued by the Ministry of Agriculture.
- IN 3—Risk Assessment for Field Release of GMO (microorganisms, plants, and animals). Information required: taxonomy, objective of the release, location of the experimental area, habitat and ecology, GMO genetics, data on previous experiments, experimental design, monitoring, safety procedures, information to the public.
- IN 4—Transport of GMOs. Information on the GMO and a valid CQB are required.
- IN 5—Links importation of GMOs to the approval for field trial.
- IN 6—Classification of GM plants according to their risk groups and norms for the contained use of GM plants.
- IN 7—Classification and norms for the contained use of GM microorganisms. GMOs are classified under Group I (Risk Group 1) or Group II (Risk Groups 2, 3, or 4); work in Large Scale is not allowed with Risk Group 4 GMOs;
- IN 8—Genetic manipulation and human cloning. Genetic manipulation of germinal cells and radical cloning by any technique are not allowed.
- IN 9—Gene therapy. Genetic manipulation or gene therapy in humans is only allowed on somatic cells, respecting the Ministry of Health’s Resolution 196/96 (ethics of research with human beings).
- IN 10—Fast Track rules for field release of GM plants that have been previously approved by the CTNBio according to IN 3,
- IN 11—Importation of GM microorganisms for contained use. the CIBio issues expert reports for the importation of Group I GMOs and the CTNBio for the importation of Group II GMOs,
- IN 12—Procedures for the contained use of GM animals. Establishes the requirements for work under Biosafety Levels 1 to 4,
- IN 13—Importation of GM animals for contained use. The CIBio issues expert reports for the importation of Group I, and CTNBio for the importation of Group II animals,
CTNBio’s Activities

CTNBio had its first meeting in June 1996. Since then, the Committee had 57 ordinary meetings, which now take place once a month and 5 extraordinary meetings. The institutions working with GMOs in Brazil have been certified and visited by CTNBio members with the support of the Committee’s Executive Secretary staff. Usually, an inspection agent of the competent area is part of the team. The CTNBio has issued 163 Biosafety Certificates (CQB) to institutions involved with GMO activities and has analyzed their annual reports.

As a rule, all petitions for importation or any other activity with risk Group II GMOs need to be evaluated by the CTNBio. The Committee has, so far, elaborated 20 norms (Box 1) to regulate different activities with GMOs. Additionally, CTNBio members have actively participated in meetings sponsored by other branches of the Government and acted as consultants in an attempt to harmonize the legislation concerning registration of products, labeling, importation, and compliance with international agreements pertaining to GMO activities.

Risk assessment for field releases and commercialization of GM crops

Over 1,000 field release proposals have been analyzed by the Committee using a case-by-case and step-by-step approach. The field releases of GM crops approved to date are listed in table 1. The proposals are evaluated for approval by CTNBio, and after a conclusion is reached a report is also submitted to the Committee. The main information required for analysis of petitions regarding deliberate field release of GM plants is listed in Box 2. The information required for analysis of a petition is based on norms established by the United Nations Environment Program (UNEP) guidelines (1995), on other relevant government documents, and on scientific literature. For the release of GM plants intended for use as pesticides or as biological control agents, in addition to CTNBio evaluation, the petitioner also needs to comply with further legal requirements from other Government agencies (Ministries of Health, Agriculture, and Environment).

To date, five petitions for the commercialization of GM crops have been submitted to CTNBio: one for glyphosate-resistant soybean, one for herbicide-tolerant corn, and three for insect-resistant corn. In 1998, the Roundup-Ready Soybean was approved by CTNBio for commercialization with a requirement to implement an environmental monitoring program...
However, the transgenic seed product is not yet on the market owing to legal requirements that include registration and further environmental impact studies. The petitions for the commercialization of transgenic corn are under evaluation by CTNBio.

**Food safety assessment**

CTNBio is required to perform a safety evaluation of the production, importation and marketing of GM plants and their products, intended to be used as food or feed or in processing. Basically, the substantial equivalence concept is applied to the risk assessment analysis (Tomlison 2000). The main information required when a company submits a petition for analysis by CTNBio is indicated in Box 2. After evaluation by CTNBio, and according to Decree 3,871/01, all packed GM food or food products containing GMOs in the concentrations of 4 percent or higher should be labeled as “genetically modified (product)” or “contains genetically modified (ingredient).” The regulation applies to the unintended presence of GMO in food products. Labeling is perceived in this context as a consumer’s right to have access to information, and it is not related to risk factors.

CTNBio analyzed food safety aspects of a commercial transgenic corn shipment intended for use in Brazil during an emergency shortage of feed. On that occasion, the Committee issued an expert report approving the importation based on food safety data provided by other countries that commercialized such products. The GM corn cargo was transported under the control and jurisdiction of the Ministry of Agriculture from the port of entry directly to the milling factory, avoiding accidental environmental release of the grain.

**Capacity building**

Board members are continuously updated on biosafety issues by attending courses, workshops, and conferences on both the national and international levels. The members are expected to deliver oral presentations on the Brazilian Legal Framework and risk assessment procedures when performing technical inspections on institutions requesting Biosafety Certificates. Board members also participate in scientific meetings on biotechnology, biosafety, and related areas to discuss the operational aspects of the Committee. CTNBio has organized a workshop on bioethics, sponsored several events organized by universities and scientific associations, and cosponsored several initiatives of the National Biosafety Association (ANBio). CTNBio members have been participating, as part of the Brazilian Delegation, in meetings for negotiation of the Cartagena Protocol among other international activities.

**Operational aspects**

The Executive Secretary of CTNBio is located at the Ministry of Science and Technology within a suitable environment for operating a multidisciplinary advisory committee. Administrative matters are efficiently handled by the Executive Secretary. Pitfalls in CTNBio’s modus operandi could be stressed, including the difficulty in harmonizing legislation of other Government branches regarding regulation of GMOs. Another relevant drawback is the high turnover of members of the Committee, which is in part due to their work serving on a voluntary basis and not receiving compensation in any form for their activities; on the other hand, such professionals still have to fulfill their main job obligations. Furthermore, members may feel discouraged by the extremely polarized public perception and emotional debate on transgenic crops and food products, bringing constant visibility in the press and frequent legal obstacles.
BOX 2- Risk assessment information required for analysis of petitions on GM crops

a—Deliberate field releases
• Taxonomic characterization (subspecies level) of donor and receptor organisms
• Classification of donor, receptor organism, and GMO into biosafety risk level
• Objective of the proposed release
• Detailed location of the experiment with information on vicinal habitats
• Habitat and ecology of the receptor organism and its interactions
• Genetics of the GMO, including sequences of inserted genes, origin, and methodology
• Vector, its restriction map, method of insertion, and host range
• Inserted gene products and their effects on humans and the environment
• Survival, dispersion, and gene transfer data on the GMO
• Measures for containment, safe disposal of the GMO, and monitoring of voluntary plants
• Field trials done in other countries and history of safe use
• Plant propagation mechanisms
• Indirect phenotypic effects
• Mitigation measures
• Information to the public excluding confidential data

b—Food safety information
• History of safe use as food of the receptor organism
• Toxicity, allergenic reactions, and metabolites affecting humans and animals
• Nutritional aspects and digestibility
• Possibility of gene transfer to microbiota associated with the intestines

c—Postcommercialization environmental monitoring (Roundup-Ready Soybean)
• Monitoring for 5 years, including annual reports
• Areas representative of soybean crop regions in Brazil
• Population dynamics of weeds and seeds in the soil
• Population dynamics of insects, plant pathogens, and microorganisms
• Gene transfer to compatible plants
• Gene transfer to soil microorganisms
• Environmental impacts of glyphosate
Table 1. Field releases of genetically modified plants in Brazil

<table>
<thead>
<tr>
<th>GMO</th>
<th>Number</th>
<th>Area (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize (Zea mays)</td>
<td>842</td>
<td>427</td>
</tr>
<tr>
<td>Soybean (Glycine max)</td>
<td>64</td>
<td>208</td>
</tr>
<tr>
<td>Cotton (Gossypium hirsutum)</td>
<td>52</td>
<td>97</td>
</tr>
<tr>
<td>Sugarcane (Saccharum sp.)</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Beans (Phaseolus vulgaris)</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Eucalyptus (Eucalyptus sp.)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Potato (Solanum tuberosum)</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Papaya (Carica papaya)</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Rice (Oriza sativa)</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Tobacco (Nicotiana tabacum)</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>989</strong></td>
<td><strong>751.2</strong></td>
</tr>
</tbody>
</table>

Main Traits Inserted in GM Plants, CTNBio, 2002:
Herbicide tolerance (HT), Insect resistance (IR), (HT+IR), Virus resistance (VR)

**Concluding Remarks**

Brazil has an operational Biosafety Legal Framework compatible with both the development and use of modern biotechnology and the sustainable use of biodiversity, ecosystem preservation, and human health. The country has adopted a multidisciplinary biosafety committee model nominated to deliberate on all activities involving GMOs. The federal biosafety regulatory body (CTNBio) is part of the structure of the Ministry of Science and Technology, where regulatory and administrative matters are handled with efficiency and transparency. To date, all institutions involved in activities with GMOs in the country have been mapped and are certified, for new applications are analyzed promptly. This has been accomplished by CTNBio’s applying an educational rather than a punitive approach because Biosafety is perceived as a new concept necessary for the safe use of modern biotechnology.

A relevant challenge faced by CTNBio and by other entities dedicated to the development of biotechnology in Brazil has been the negative public perception regarding transgenic plants. Intense publicity on the risks posed by biotechnology has not been matched by a comprehensive analysis of the benefits that can result for human health and the environment in comparison with conventional agriculture practices. Furthermore, agreement among different branches of the Brazilian Government on the legislation for commercialization of GM crops has not yet been achieved; nevertheless, efforts are in progress with the aim of obtaining a legal harmonization. Throughout the years of operation, a deficiency has been identified in CTNBio’s predominantly consultative status. The Committee, not being an executive organ, has been challenged on the limits of its legal competence concerning GMO authorizations in Brazil. This has had the consequence of delaying the commercialization of LMO products owing to additional requirements for their approval.
References


Risk Management Strategies for Living Modified Organisms that Take Uncertainty into Account

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Abstract

There are different types of uncertainty in risk assessment and of risk management. It is helpful to distinguish between a quantitative and a qualitative type of uncertainty. Quantitative uncertainty analysis addresses descriptive errors such as the variability in baselines or laboratory experiments. Qualitative uncertainty concerns ignorance and indeterminacy, which cannot be addressed through risk assessment but must be covered by appropriate risk management practice. Many of the previously unpredicted adverse effects of chemicals (e.g., DDT, methyl bromide) refer to the qualitative type of uncertainty. This paper outlines risk management strategies dealing with ignorance: the implementation of both an early warning system after the approval of the living modified organism and also an approach to finding precautionary criteria before the approval to avoid effects of substantial and unmanageable stressors due to the lack of mitigation measures.

Introduction—Why Uncertainty?

“While the duty of preventing damage to the environment is based on a known risk, the notion of precaution is based on “lack of certainty” (OECD 2000).

All human activities have an impact on the environment. Some of the impacts can be addressed proactively by ecological risk or environmental impact assessment; others become visible after a substantial delay. Some impacts of human activities are impossible to identify owing to the complexity of ecosystems. The history of risk identification and regulation of different pesticides shows the following: from 1939 up to now the risk identification of new (previously unknown) harmful effects to humans and the environment continues to evolve. The depletion of the ozone layer by methyl bromide and the hormonal effects of many chemicals (for example Vinclozolin, see figure 1) are well known examples of these new risk identifications within the last two decades. Furthermore, the history of pesticide regulation shows the relevance of the following sequences of events:
Risk Management Strategies for LMOs

- The time lag between introduction of a chemical and exposures in the environment;
- The occurrence of a harmful effect not immediately associated with the pesticide;
- The identification of that deleterious effect;
- The scientific proof of the cause of that effect;
- The political reaction to that new scientific evidence;

Figure 1 gives a short review of these aspects for three examples.

Owing to the complexity of ecosystems the overall ecotoxicity can never be fully assessed. Risk assessment and risk management follows the precautionary principle only when it acknowledges the limits of knowledge (e.g., food web) and hence takes the possibility of error into account.

In the view of the U.S. Environmental Protection Agency (EPA), “Risk assessments explicitly evaluate uncertainty. Uncertainty analysis describes the degree of confidence in the assessment and can help the risk manager focus research on those areas that will lead to the greatest reductions in uncertainty” (U.S. EPA 1998, 7f). The U.S. Department of Energy (DOE) noted that the current Environmental Protection Agency (EPA) policy for risk characterization (issued by Carol Browner, EPA Administrator, in February 1995) requires all risk assessment to have the core values of transparency, clarity, consistency, and reasonableness. To attain these core values, Agency risk assessors and risk managers are instructed to have a full and open discussion of uncertainties in the body of each risk assessment, including a prominent display of critical uncertainties (U.S. DOE 1996, p.1).”
Apparently there is a need for more explicitly addressing uncertainty in risk assessment of living modified organisms (LMOs).

There are many papers regarding the uncertainty in risk assessment. Some of these documents (EU-Commission 2000, OECD 2000, UNEP 2000) deal with the question of how to apply the precautionary principle in the light of uncertainty in risk assessment. Other, more technical documents give examples of reasons for (or sources of) uncertainty and how to address uncertainty in the risk assessment (e.g., U.S. DOE 1996, U.S. EPA 1998, Warren-Hicks and Moore 1998a).

**What is Uncertain?**

The term “uncertainty” in the context of risk assessment has been used since the mid 1980s (Morgan and Henrion 1992). However, there is no common understanding of the use of this term. Covello et al. (1992) distinguished four primary sources of uncertainty in risk assessment and management:

- Uncertainties about definitions;
- Uncertainties about scientific facts;
- Uncertainties about risk perceptions and attitudes;
- Uncertainties about values.

Uncertainties about definitions derive primarily from disagreements about meaning and interpretation of key concepts such as probability. Uncertainties about scientific facts derive primarily from disagreements about failure modes, the probability and magnitude of adverse health or environmental consequences, cause and effect relationships, dose–response relationships, and exposure patterns. Uncertainties about risk perception and attitudes derive primarily from disagreements about what constitutes a significant or acceptable level of risk. Uncertainties about values derive primarily from disagreements about the desirability or worth of alternative risk management actions or consequences.

Very different from that are U.S. EPA definitions on sources of uncertainty (U.S. EPA 1998) as follows:

- Unclear communication
- Descriptive errors
- Variability
- Data gaps
- Uncertainty about a quantity’s true value
- Model structure uncertainty (process models)
- Uncertainty about a model’s form (empirical models).

The Scientific Committee of EEA (European Environmental Agency) (EEA 1998) distinguishes different basic types of uncertainty:
Risk Management Strategies for LMOs

- Risk: Odds known
- Uncertainty: Odds not known, may know the main parameters.

May reduce uncertainty but increase ignorance

- Ignorance: What is not known is not known. Ignorance increases with increased commitments based on given knowledge.
- Indeterminacy: Causal chains or networks open.

(For further definitions on uncertainty see, e.g., Hrudely 1998, Warren-Hicks and Moore 1998a, EU-Commission 2000).

From the perspective of reducing uncertainty it is possible to distinguish between two different groups of sources of uncertainty:

- A quantitative uncertainty (see U.S. EPA 1998 above) in comparison with the tools from QUA (quantitative uncertainty analysis), which are used to reduce uncertainty by Monte Carlo analyses, and Bayesian statistics, or both (e.g., Warren-Hicks and Moore 1998b; Warren-Hicks and Moore 1998a).
- A qualitative uncertainty (e.g., ignorance and indeterminacy), which cannot be reduced by QUA (quantitative uncertainty analysis) and other technical tools. Figure 1 reveals that most errors in risk assessment are caused by ignorance.

**How to Address Ignorance**

Quantitative uncertainty has to be addressed mainly during the risk assessment process. Ignorance, on the other hand, cannot be addressed during risk assessment but can be covered by risk management (which includes postapproval activities and activities before risk assessment, i.e., definition of hazard and thresholds). Risk assessment analyzes “how it is” and estimates “how it will be” and risk management aims at “how it should be” and “how to act” to achieve that goal (definition of harm and thresholds). There are two ways to address ignorance during risk management. One way is that of the “quick response” after the approval of LMOs supported by the implementation of an early warning system. The other way is to define precautionary criteria in advance for the risk assessment such as thresholds for persistence (see the section, ‘Before Approval—Precautionary Criteria’).

**Post Approval—Early Warning System**

Two different monitoring schemes exist within the European Union (EU): “case specific monitoring,” which mainly focuses on the validation of case-specific hypotheses of the risk assessment, and “general surveillance” for “unanticipated adverse effects” of LMOs (EU-Parliament and Council 2000). Case-specific monitoring would assess, for example, the risk derived from a hypothesis that predicted a negligible impact for the release of Bt toxins in the soil. General surveillance has to be seen as an early warning system. Key problems for such a system are
• defining key parameters that would show major ecosystem disruptions with greatest sensitivity,
• describing sensitive “trigger points” or thresholds and appropriate methods to reduce the timelag between release of the transgenic organism and first identification of an exposure, and
• detecting adverse effects and implementing mitigation measures, (See, for example, the discussion about the F2 screen, which is the most sensitive method for monitoring changes in the frequency of resistance alleles in insect populations, and also other less sensitive monitoring schemes like the dose–response test (Roush and Miller 1986, Andow and Alstad 1998, Marcon et al. 2000).

To facilitate a quick response there is a need for clear responsibilities to ensure the implementation of mitigation measures without major delays. Furthermore, the newly detected adverse effects must lead to adjustments in the models of risk assessment. In addition, improved error reporting needs to be accomplished, a so-called soft factor in the improvement of the risk assessment process. A recent survey lists the following key soft factors used in highly reliable operations to manage unexpected events (Weick and Sutcliffe 2001):

1. Pay close attention to errors. Encourage reporting errors
2. View errors as a window to the system as a whole
3. Have clear instructions for how to reevaluate your risk assessment

Before Approval—Precautionary Criteria

In some cases the early warning system reveals new, unanticipated adverse effects without opportunities for effective mitigation of these effects. For example, if the seed bank in various soil ecosystems were contaminated with feral plants expressing transgenes (synthetic genes), long-term impacts would occur without easy remedies. The implementation of precautionary criteria is the only way to avoid unmanageable and long-lasting adverse effects.

The historical review (see figure 1) shows clearly that persistent chemicals—especially if they are mobile and can be accumulated—are major risk factors in a continuously changing environment. Owing to the complexity of ecosystems, the overall ecotoxicity can never be fully assessed. The persistence of chemicals is a central criterion for assessing ecotoxicity because exposure to persistent chemicals cannot be terminated or removed if new harmful effects are identified in the future (Klöpffer 1994). Because transgenes may be transferred to feral populations by outcrossing, their persistence may cause a more serious or more long lasting effect given the lack of knowledge about the half-life of a synthetic gene. This has to be taken into account in the risk assessment.

Analyzing and Identifying Uncertainty During Risk Assessment—a Brief Example

The traditional approach in assessing risks from outcrossing of transgenes asks, Is there any potential for an adverse effect (i.e., weediness) from the spread of, for example, a synthetic herbicide-tolerant (HT) transgene to wild relatives of canola? The answer of contemporary regulatory systems at present is no!. There is no selective
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advantage of that gene that might allow plants expressing the HT gene to become more weedy
as asserted in the following observation by the U.S. Department of Agriculture (USDA):

In nature, the gene that results in accumulation of CP4 EPSPS and GOXv247 proteins will
not provide glyphosate tolerant canola or its progeny with any measurable selective
advantage over "nontransformed" canola plants in their ability to disseminate or to
become established in the environment. There is no reason to believe that glyphosate-
tolerant canola exhibits any increased weediness relative to that of traditional varieties.
The use of glyphosate-tolerant canola or its progeny in agriculture will not lead to an
increase in weediness in any plant with which it can successfully interbreed (USDA
1999).

While analyzing and identifying uncertainty during risk assessment, firstly the potential
hazard (effect profile) and secondly the potential exposure (exposure profile) must be
addressed. In analyzing the exposure profile, two areas must be investigated:

1. The potential exposure of the genetically modified organism (GMO) itself and
2. The potential exposure of the transgene (synthetic gene) or, in other words, What is
   the fate of the gene? Will it spread and persist in feral populations?

With respect to the fate of synthetic transgenes in feral populations, considerable evidence
from computer modeling indicates that synthetic transgenes will persist in feral populations.
At migration rates between 10 and 20 percent after positive selection, selectively neutral or
even up to 20 percent selectively disadvantageous synthetic transgenes will be fixed in a
feral receiving population at rates between 70 and 90 percent (Adam and Köhler 1996).
Experimental data show that transgenes do not necessarily contribute to selective
disadvantage. In some cases a slight fitness advantage for hybrids (carrying the transgene)
has been detected. This leads to the conclusion that transgenes will likely persist for many
generations in feral populations (Klinger and Ellstrand 1994, Arriola and Ellstrand 1997, Snow
et al. 1999).

On the basis of polymorphism in allozymes, the theoretical foundation for assuming that
"synthetic genes" act as selectively neutral traits and therefore will spread and persist in
feral populations was laid down in 1968 by Kimura (1991a). In contrast to the Darwinian
theory of evolution by natural selection, the neutral theory claims that the overwhelming
majority of genes evolved by continued inputs of mutations are selectively neutral and therefore
randomly fixed owing to random sampling drift in finite populations (Kimura 1991b). After
changes in the environment, some of the genes turn out to be useful.

Intergroup competition and individual selection lead to extensive adaptive evolution. Nevo
(2001) rejected Kimura’s hypotheses. He declared that polymorphism can also be preserved
in small, long-isolated populations by “stabilizing selection” or “cyclical selection.” Both
theories contribute to the same conclusions. Neutral transgenes are maintained in feral
populations. The way genes are maintained is seen differently as occurring randomly or by
“stabilizing selection.”

After environmental changes, some “synthetic” transgenes may turn out to be useful.
From the standpoint of uncertainty, the half-life of that gene is a key factor in assessing the
degree of uncertainty. But there are limits in estimating the half-life or even the fitness of a
gene. Because fitness is a function of an organism and its corresponding environment, lab
tests give quite limited security for extrapolations to a wide range of possible affected ecosystems. Figure 1 shows clearly that ecotoxicity of a chemical, and also for LMOs, can never be fully assessed in advance of a release. Therefore, more risk may be associated with outcrossing than weediness (e.g., extinction by hybridization; Rhymer and Simberloff 1996). Once a release occurs, there are limits in the ability to estimate the potential for weediness of a “synthetic gene” from lab experiments alone just as is true for an estimate of fitness.

Some scientists argue that crop genes derived by mutations or derived by genetic engineering are equal with respect to uncertainty and persistence. From an evolutionary perspective the creation of genes by enhanced induced mutation is “just” speeding up evolution 100–10,000 times faster than it would occur naturally. The creation of synthetic transgenes in plants with combinations of virus promoters, bacterial expression sequences, and so forth is not known to occur as a consequence of evolutionary forces. Persistence of synthetic transgenes must be considered a more serious hazard to biodiversity than persistence of crop genes derived by induced mutations simply because possible impacts of exotic genes may be highly uncertain.

The U.S. EPA (1998) recommends

1. To articulate major differing viewpoints of scientific judgments clearly;
2. To acknowledge uncertainties and assumptions in a forthright manner; and
3. To identify reasonable alternatives and conclusions that can be derived from the data.

These points should be incorporated into the risk assessment, especially when outcrossing may be at issue. The following short example should indicate specifically how risk assessment reports that consider outcrossing for decisionmakers could be improved:

**Clearly Articulate Major Differing Viewpoints of Scientific Judgments:**

There are different scientific judgments about the extent to which outcrossing must be seen as a risk in itself or not.

**Acknowledge assumptions and uncertainty:**

There are major restrictions in estimating the fitness and weediness of a “synthetic gene” because of the difficulties of performing fitness tests in a varied and continuously evolving environment. The ecotoxicity cannot be fully assessed. Persistence of transgenes in feral populations is likely to occur. Unpredicted long-term effects arising from persistent transgenes may be associated and cannot be excluded by current risk assessment methods. If adverse effects from persistent transgenes occur, no effective ways to mitigate adverse effects are known.

**Reasonable alternatives and conclusions:**

Some scientists conclude that outcrossing is a risk in itself. Genetically modified crops with the potential for outcrossing should not be approved because there would be no (or at least very limited) options for mitigation measures.
Conclusions

Acknowledging uncertainty in the risk assessment of LMOs should lead to new risk assessment frameworks as suggested by the U.S. EPA (1998) among other agencies. This should also lead to a redefinition of risk that might be represented as follows:

\[ \text{Risk} = \text{hazard} \times \text{likelihood} + \text{uncertainty} \]

Risk assessment reports should include the adverse effect, exposure, and uncertainty profile. The implementation of an early warning system and the definition of precautionary criteria like “persistence” should reduce the probability of major unanticipated adverse impacts that cannot be fully excluded by risk assessment methods.

Acknowledgements

I thank USDA for paying travel expenses allowing me to give a presentation at the OECD Conference at Raleigh as well as U. Niggli and W. Klöpfer for providing useful hints for the figure on pesticide regulation. I would also like to thank C. Roseland, A. Hartl, and M. Lehner for reviewing the manuscript.

References


A Perspective of Civil Society

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Abstract

In Europe there is an intense debate about the use of transgenic plants in agriculture. An impression is conveyed that ecological aspects are neglected in the overall evaluation process and possible long-term effects are not taken into account sufficiently. There are strong hints that double standards are used when evaluating the evidence submitted in the context of risk assessment. Improvements in the risk assessment procedure are needed.

Introduction

I am here as a scientist and also as a representative of civil society. I would like to provide you with a different evaluation process for the biosafety of engineered plants and with viewpoints that have emerged specifically in Europe. I will contrast the views of those scientists working in the life science sector and industry on the one hand with those scientists representing quite often the majority of civil society on the other. The differences in viewpoints have much to do with an alternative weight given the associated risks of transgenic plants in the environment, but, in addition, with a conflict or debate on the future directions for the development of agriculture. To frame it in popular terms, we deal with a conflict between different sets of shareholders and stakeholders.

Aspects of the Debate

Transgenic plants fit into the paradigm of an industrial agriculture based on a high-input, resource-intensive means of production. They are meant to optimize this form of agriculture. On the other hand, important international institutions, including FAO, the Food and Agriculture Organization of the United Nations, demand major changes and improvements as stated, for example, in their report Towards 2010 — The Future Development of Agriculture (1995). In the long run the agriculture that the industrial world has developed is destroying the resources on which it is built and on which it is depending (FAO 1995). This assertion refers to soil depletion, soil erosion, and loss of biodiversity as an overall loss and specifically as a loss in plant genetic and animal genetic resources as well as contamination of water and soils with pesticides and fertilizers.
Therefore, we deal with two sets of questions in the context of risk assessment:

- First: Do transgenic plants pose unprecedented short- or long-term risks, and may such plants contribute to or even aggravate the addressed problems?
- Second: Do transgenic plants have the potential to optimize certain aspects of an industrial agriculture or even transform it into a sustainable form of agriculture without the negative side effects that are often cited?

There are serious doubts about the context surrounding both sets of questions.

For 20 years genetic engineering has been extensively discussed. Since the very beginning there have been serious concerns by those scientists involved in the first GMO experiments. Since then, billions of dollars have been invested in the development of different applications, and millions of dollars have been spent in public education and acceptance.

An international analysis of associated risk research, that is, research on possible ecological and health impacts (Sukopp and Sukopp 1997), came to the conclusion that less than 1 percent of the worldwide development budget has been used for research regarding safety effects. In other words, before the first commercial plantings in 1996, 10 years of field testing had been conducted without looking in depth into possible ecological consequences. That was also the outcome of an evaluation done by Mellon and Rissler (1995) published in *Nature Biotechnology* (table 1).

Table 1

- **Since 1987:**
  - 850 applications and notifications approved
  - 269 reports only 139 available to the public
  - 85 most recent reports analyzed

- **Problem of weeding**
  - 86% general observations
  - 14% aspect not mentioned

- **Gene flow**
  - 24 reports concerned crops with wild relatives in the U.S.
  - 23 reports did not address possible impact of gene flow

- **Problem of virus recombination**
  - 19 reports dealt with virus resistant plants; in no case were special experiments or monitoring done during the release—17 reports did not even mention the risks

- **Nontarget effects**
  - 15 reports dealt with insect-resistant plants
  - Failure to mention the possible adverse impact of nontarget effects

Conclusion by Mellon and Rissler (1995) Risk Assessment in Official Documents: **“the field tests do not provide a track record of safety but a case of ‘don’t look, don’t find’.”**
Five years later a review published in *Science* came to nearly the same conclusion: “A review of existing scientific literature reveals that key experiments on both the environmental risks and benefits are lacking” (Wolfenbarger and Phifer 2000). In short, there has not been much progress. The consumer or the public as a whole feels more and more uncomfortable given these facts.

In dealing with risk assessment over the last 15 years there appears to be considerable disconnection between the emerging data and the handling of these data in the context of evaluation and decisionmaking. There are strong hints that double standards are used when evaluating the evidence submitted for market approvals. To arrive at the following summary I refer both to a study performed by Les Levidow and Susan Carr commissioned by the European Commission (Levidow and Carr 2000) and to our own study done for the German Technology Assessment Bureau (Vogel and Tappeser 2000). The main outcome of both studies is the following: studies or statements that underline the benefits are readily accepted by regulators in the U.S. and the European Union even if those studies are not peer-reviewed and rely only on laboratory experiments. Studies indicating risks and possible negative ecological or health impacts are heavily criticized no matter that they are peer-reviewed and published in scientific journals. These studies are criticized when they rely only on laboratory experiments.

In addition there has been a shift in judging certain impacts. For example, a central issue that has figured in the discussion on the cultivation of transgenic plants since its very beginning is that of outcrossing of such plants and the introgression of the recombinant genes into related weed and wild plants. It was more or less agreed at least in the beginning of the debate that pervasive spread of transgenes should be avoided if at all possible, for this may have problematic effects on species networks and on biodiversity in general. A point now attracting increasing attention is the implication of resistance development through outcrossing and the consequences of that development for agricultural land use systems. In Europe canola is at the center of interest because several related species are prevalent there. All experience and data gained in the course of the past years point to a high probability of transgenic rape populations becoming established outside cultivated areas and the subsequent possibility of gene flow into nontransgenic populations and related wild herbs. Nowadays, gene flow as such is no longer judged as being of special concern. It is said that gene flow only constitutes a risk when the outcome, the possible impact in the complex networks, can be described and the impacts are judged as having specific negative consequences. Otherwise such gene flow is qualified as a “so what” type of conclusion.

But the demand to describe the impacts of gene flow can only be met with a broad long-term research program because of the multiple knowledge gaps and uncertainties that exist. Given the current level of investments in the field of biosafety and assessment of ecological impacts, such a research program would extend into the next 20 years at least. But the decision to pursue such research has to be taken now.

I would like to develop a scenario describing the possible risks of deploying herbicide tolerant crops:
Nearly all of the world’s major crop plants have been equipped with the same herbicide resistance genes. Their large-scale use will therefore produce an enormous selective pressure towards resistant weeds (Begon et al. 1991). Although those transgenic plants with wild and weedy relatives in a region will be the plants that initiate rapid resistance development via outcrossing, other plants expressing the same resistance genes but lacking crossable wild relatives in the region will promote, on a continuing basis, the one-sided selection of these weeds. I would dare to say that the use of herbicide-resistant plants may even accelerate resistance development compared with conventional agricultural practices. Furthermore, the cloning of different resistance genes into one and the same crop species also gives related wild herbs the opportunity to acquire multiple resistance traits. In Canada, double- and triple-resistant canola have already developed and are qualified by some as superweeds (Orson 2002, Hall et al. 2000).

This development may also be accompanied by a further impoverishment in farmland-associated floral species and insects because of the constantly increasing usage of broad spectrum herbicides instead of selective herbicides. The additional weed shift to less sensitive species will further contribute to a diversity-poor agricultural ecosystem with less stability and much more need of additional management inputs.

If that is a scenario you are willing to accept along with a short-term reduction in pesticide use—although this claim of benefits is challenged, too (Benbrook 2001 EU; Directorate-General for Agriculture 2000)—the long-term outcome of herbicide-resistance may be further biodiversity loss and the next round in the chemical treadmill.

Conclusions and Recommendations

I elaborated on this example of canola to provide some additional background for the mixture of conclusions, demands, and open questions with which I would like to finish:

- Risk assessment has been done on too narrow a basis.
- Risk assessment needs other scientific competencies than those necessary for the development of transgenic organisms; interdisciplinarity has to be strengthened in the risk-assessment procedure.
- The datasets are inappropriate. The main focus of evaluated data has been on the agronomic aspects of the transgenic plant. There are only sparse data on ecological impacts.
- The significance of, and the relationship between, laboratory experiments, greenhouse experiments, and field experiments are not clear, especially when there are data that question the use of transgenic plants as an unproblematic issue.
- Possible long-term systemic effects and effects due to changes in management practice and parallel use of different transgenic crops have not been elaborated.
- There is no consensus about the baseline, and there is no consensus about the environmental goals in agriculture. Maybe we have to accept that there are different options and goals and should shift part of our discussion to the goal of making it possible to follow different roads—that means differential requirements for segregation, traceability, and labeling.
The framework of a risk–benefit analysis has to be defined thoroughly and the evaluation has to follow scientific principles.

Transparency and public participation has to be improved.

And last but not least;

- How much uncertainty is acceptable?
- What is enough certainty to decide not to accept or use a certain type of transgenic plant?

Climate, soils, and ecosystems but also the tradition of agriculture are very different in various regions of the world. This diversity is part of our heritage and the riches of the Earth. Risk assessment and the decisions based on the risk assessment have to take that into account and may therefore have different answers in the different regions.

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Modern Biotechnology in Agricultural Development: A Latin American Perspective

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Abstract

The countries of Latin America and the Caribbean have many needs for the development and adoption of biotechnological strategies to improve agricultural production. The introduction of national regulatory authorities and the training of interdisciplinary assessors will be needed before the appropriate risk assessment can be done.

Introduction

The Latin America and the Caribbean Countries (LACC) are strategically placed centers for global food security. Three of the 12 global centers of origin for crops of major socioeconomic importance are found here, and their enormous biodiversity is highly significant (Leon 1987). Although representing only 7-percent of the earth’s surface, the LACC contain a large amount of the planet’s biodiversity. These resources are concentrated primarily in 18 countries, 9 of which are in the American hemisphere (Alarcon et al. 1998).

Recent studies by the World Bank estimate that more than 70-percent of the nearly 500 million inhabitants of the region live in urban zones and daily dispose of over 250,000 tons of waste. Less than 55-percent of this garbage is treated, which in turn contaminates surrounding water bodies. Almost one-third of the population lives in levels of absolute poverty, and more than 40 million indigenous people are excluded from the development process. These populations do not have access to basic public services such as education, health, and social assistance. From this perspective, the region is significantly challenged to find a suitable economic development plan that will also foster social equilibrium as well as the sustainable use of this region’s biodiversity.

The agroindustrial sector contributes slightly more than 25-percent of the region’s gross domestic product (GDP). Therefore, the consequences of the agricultural advancements derived from research and technological innovation are of the utmost importance for the region. The general consensus is that conventional technologies themselves will not provide a sufficient increase in the quantity and quality of food production to satisfy a population that is estimated will double in the next 50 years.
The Food and Agriculture Organization (FAO) has projected that over the next 25 years the population of the LACC will increase from 490 to nearly 680 million. It is possible that more than 30-percent of the cereal consumption of the LACC will be imported by 2020. The same FAO studies predict that the arable land in the region could be expanded by only 12-percent at acceptable economic and environmental costs (although such expansion would inflict damage to the remaining biodiversity). The increase in food demand expected to occur in the region during the same period is 61-percent. In the LACC the only potential cultivable lands are the Brazilian Cerrados and the Llanos of Colombia and Venezuela (Kendall et al. 1997), which may be marginal areas without the need for substantial improvements needed for agricultural production.

The Developing Issues

The challenges and opportunities for the LACC are large, given the high participation of the agricultural sector in the region’s GDP. In addition, the LACC possess a rich base of flora, fauna, and micro-organisms essential to obtaining new products for the pharmaceutical and food industries.

The scenarios for the agricultural production of the region are not homogeneous. Those in the temperate zones of the north and south differ from the scenarios for those of the high mountain plains. The wet and dry tropical lowlands and medium-elevation hillsides, such as those in Central America, the Andean countries, and some Caribbean nations present yet a different scenario.

Technological engagement in temperate agriculture by industry and Government is greater than that occurring in the tropical areas. The biotechnological expertise of other countries has been consequently deployed in the LACC. In the LACC, for example, in the case of soybeans and wheat, a transgenic “RR soybean” has recently been imported. In tropical areas, there is no available technological counterpart for the region, although transgenic rice could be imported. Consequently, the technological gap with the world’s leading countries is widening with respect to many crops (see tables 1, 2).

Table 1. Basic Grains. Current yields in LACC and leading countries of the world

<table>
<thead>
<tr>
<th>Products</th>
<th>Average current yield in the LACC (ton/ha)</th>
<th>Current yields of world leaders (ton/ha)</th>
<th>Annual growth rate in the LACC 1985–97(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>3.2</td>
<td>6.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Bean (Dry)</td>
<td>0.6</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Corn</td>
<td>2.7</td>
<td>7.7</td>
<td>2.9</td>
</tr>
<tr>
<td>Wheat</td>
<td>2.4</td>
<td>6.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Source: IICA, Technical Management, Area II. Supported data from FAO. STAT.
Table 2. Main Crops. Average current yields in LACC, South American, Colombia and USA countries. Year 2000.

<table>
<thead>
<tr>
<th>Crops</th>
<th>LACC (ton/ha)</th>
<th>South America (ton/ha)</th>
<th>Colombia (ton/ha)</th>
<th>United States (ton/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (cereals)</td>
<td>2.79</td>
<td>2.99</td>
<td>3.05</td>
<td>5.86</td>
</tr>
<tr>
<td>Wheat</td>
<td>2.67</td>
<td>2.52</td>
<td>2.17</td>
<td>3.82</td>
</tr>
<tr>
<td>Rice</td>
<td>3.60</td>
<td>3.58</td>
<td>4.77</td>
<td>7.04</td>
</tr>
<tr>
<td>Barley</td>
<td>1.87</td>
<td>1.94</td>
<td>2.17</td>
<td>4.29</td>
</tr>
<tr>
<td>Corn</td>
<td>2.72</td>
<td>3.12</td>
<td>1.75</td>
<td>8.60</td>
</tr>
<tr>
<td>Rye 1.28</td>
<td>1.28</td>
<td>1.17</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>Oat 1.67</td>
<td>1.68</td>
<td>ND</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td>Millo</td>
<td>1.52</td>
<td>1.52</td>
<td>ND</td>
<td>2.11</td>
</tr>
<tr>
<td>Sorghum</td>
<td>2.86</td>
<td>3.16</td>
<td>3.17</td>
<td>3.82</td>
</tr>
<tr>
<td>Quinua</td>
<td>0.69</td>
<td>0.69</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Triticale</td>
<td>1.38</td>
<td>1.39</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Roots and Tubers</td>
<td>12.63</td>
<td>13.23</td>
<td>12.20</td>
<td>42.79</td>
</tr>
</tbody>
</table>

Source, FAOSTAT, 2000

As we can conclude from analyzing the data in the tables, the Latin American region urgently needs improved agricultural biotechnology to increase food production. In the LACC, progress in biotechnology research has been particularly rapid for some of the most valuable crops of the region. Scientists hope that the development of transgenic plants will help to alleviate both the heavy use of pesticides and the susceptibility of traditional cultivars to many biotic and abiotic stresses.

Conclusions

The characteristics of modern biotechnology provide both opportunities and challenges. If the LACC are able to build capacity for their national research systems, biotechnology holds the promise of supporting national efforts towards food security and sustainable development in the region as well as increasing the export potential.

The issues of agricultural biotechnology are being actively debated—but mostly in the rich industrial world. However, it is in the developing world where the greatest value of this new technology may lie. Consumers in the industrial world can afford, if they wish, to take a highly skeptical view toward this new technology. A majority of farmers and consumers in the LACC, on the other hand, are not yet wealthy or well fed. This suggests they would have much more to gain from agricultural biotechnology for the following reasons:

- Poor farmers in the LACC currently lose a large share of their crop production (probably more than 30 percent) to diseases and pests. Biotechnology makes possible the development of plants resistant to pathogens and pests.
- Low average crop yields are in part caused by biotic stresses (such as salt or drought) on plants. This constraint may be overcome by engineering plants better adapted to such stresses.
The use of crops protected against insects and disease offers potential agricultural, economic, and environmental benefits to the LACC farmers.

The countries of the region require appropriate infrastructures that will permit them to acquire, absorb, develop, and efficiently manage biotechnologies. The creation of enabling conditions must be addressed to obtain the potential benefits of these new technologies and to minimize any possible adverse effects on the environment, on human health or on the agricultural production systems.

The adoption and expansion of biotechnology in the LACC have increased in recent years. One indicator used to measure the progress in the agricultural biotechnology sector is the number of field tests of transgenic crops, which has been estimated to be near 870 in the region since 1997. Nevertheless, with very few exceptions, transgenic crops tested in agricultural ecosystems of the LACC have been those developed in the northern industrialized countries.

If we take into account that the cultivated area for the majority of conventional crops is greater in the developing countries than the cultivated area in the industrialized countries (14.5 times greater in rice, 3 times greater in cotton, 2 times greater in corn, and almost the totality of cassava and sweet potato), we can assume that the demand for transgenic cultivars will increase in developing countries.

The LACC must take advantage of these technologies if they want to move forward in agricultural development. However, the region must also make an objective, technical evaluation of possible risks for human health, the environment, and agricultural and cattle production that could result from the introduction of these technologies—especially when introduced into the tropical ecosystems. Every country in the region should analyze the necessity for having systems in place to identify and monitor potential adverse effects from crops protected against herbicides, diseases, and insects, through modified modern biotechnology or conventional breeding practices.

Although some countries in the LACC have biosafety regulations, the majority do not. What is even more critical is that many do not have the sort of multiply trained and interdisciplinary personnel needed to carry out risk analyses and risk management within a methodological framework, as stipulated by contemporary international regulations. Because of this limitation, the potential advantages of engineered crops may not be obtained to guarantee necessary biosafety requirements to protect the environment, human health, agricultural production, and the equitable distribution of the benefits for the welfare of the region’s inhabitants.

It is clear that the LACC must continue to develop and perfect existing regulatory instruments on a par with related international agreements to prevent or minimize possible risks derived from the use and handling of transgenic products. For this to occur, competent national institutions must also develop institutional capacities to manage and evaluate field trials. Only then will countries in the region be able to take full advantage of transgenic crops capable of enhancing agricultural production and improving food security.
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Field Application of LMOs: Developing Country Perspective

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Abstract

About 87 million people are added annually to this world (Kendall et al. 1997). A majority of these people reside in developing countries. Biotechnological interventions like living modified organism (LMO) technology offer tools for increased food production and can be of great benefit to the developing world facing the challenge of feeding the growing population.

Although the potential of LMO technology is accurately perceived, the benefits of this technology remain limited largely to the developed nations. Most developing countries remain deprived of the benefits of LMO technology and have witnessed limited field applications on a commercial basis. Efforts are needed to take the technology closer to the developing parts of the world. This can be successfully done by a holistic approach that keeps in view the social, economic, and ecological considerations of individual countries.

This paper points out some unique considerations and perspectives of the developing world and focuses on the need for “tailoring” the technology implementation strategy according to those unique considerations.

Introduction

It is estimated that the global food demand will double by the year 2050. This increasing demand with decreasing natural resources like arable land and water conveys the challenge of producing more food in a sustainable manner. Although the global population increases annually at a rate of 1.5 percent, the amount of cultivable land is expected to decrease to 0.15 hectare per capita by the year 2050 (Engelman and LeRoy 1995). In view of globalization and the concept of our “common future,” the challenge is global and is a concern for each one of us.
The need to produce more food in a sustainable agricultural system makes way for innovation in traditional agricultural practices. Incorporation of biotechnological methods like genetic engineering and its products like the LMOs can serve as one of the important tools, contributing to global food security. According to a report (Kendall et al. 1997) by a panel of experts commissioned by the World Bank, “it is likely that efforts to improve the rice yield in Asia through biotechnology will result in a production increase of 10 to 25 percent over the next 10 years.” This estimation has been proven realistic in many field trials of LMOs in which the yield increase has been significant.

Incorporation of LMOs in the existing agricultural system promises encouraging beneficial consequences for the future of agriculture. The technology is of greater importance in developing nations where the population growth is higher and the challenge of food security is more intense. Biotechnological tools like LMOs, combined with some conventional breeding practices, offer effective means to meet the future demand of food production. Integration of biotechnology into the agriculture system has the potential to offer an increase in food yield in view of the economic and environmental considerations.

Although a very small portion of the globe has received the benefit of LMO technology, in most parts of the world, especially the developing nations, it remains a concept far from commercial application. Introduction and incorporation of LMOs call for innovative strategies. The strategies will have to be based on the unique considerations pertinent to a particular country. These considerations include social, ecological, and economic ones.

This paper will focus on the perspectives of developing countries on the following:

- Current status of application
- Traits of priority
- Future research trends.

I will try to give the developing countries point of view on these topics cited while comparing them with the industrialized nations’ perspectives. The comparisons are made to differentiate the unique considerations of developing and developed parts of the world.

**Current Status of Application**

During the 5-year period between 1996 and 2000, the global area of transgenic crops increased by more than 25-fold from 1.7 million hectares in 1996 to 44.2 million hectares in 2000 (James 2000). Although the growth of technology adoption has been very rapid, it has been concentrated mainly in the industrialized nations. Figure 1 indicates the rapid yet uneven growth of transgenic technology adoption.
In view of the potential contribution of LMO technology towards food security and its economic benefits, it is of greater importance to the developing nations. According to a United Nations estimate, by the year 2050, 90 percent of the world’s population will reside in Asia, Africa, and Latin America (http://www.unfpa.org). This clearly emphasizes the need for technologies like LMOs that may contribute to an increase in food production in the less developed nations. Although presently 85 percent of the transgenic crops are grown in industrialized nations, most developing nations remain deprived of this technology.

The uneven growth of LMO adoption needs serious consideration. In a global context, the benefits of this technology need to be extended to most developing nations that await the field application of their first transgenic crop.

**Traits of Priority**

Table 1 shows the relative frequency of traits in transgenic field trials conducted in 1999 and 2000. Most of these activities have been concentrated on the area of crop protection, including traits like herbicide tolerance, fungal resistance, viral and insect resistance, and so forth. The traits incorporated in the crops and with the priorities chosen are by and large the industrialized countries’ priorities and considerations. Seventy-four percent of transgenic crops grown today are for herbicide tolerance trait. This trait is of greater importance for industrialized nations in which minimum human involvement is desired in order to cut the cost of labor in agriculture is one of the important considerations.
Table 1. Global area of transgenic crops in 1999 and 2000: by trait

<table>
<thead>
<tr>
<th>Trait</th>
<th>1999</th>
<th>%</th>
<th>2000</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicide tolerance</td>
<td>28.1</td>
<td>71</td>
<td>32.7</td>
<td>74</td>
</tr>
<tr>
<td>Insect resistance (Bt)</td>
<td>8.9</td>
<td>22</td>
<td>8.3</td>
<td>19</td>
</tr>
<tr>
<td>Bt–Herbicide tolerance</td>
<td>2.9</td>
<td>7</td>
<td>3.2</td>
<td>7</td>
</tr>
<tr>
<td>Virus resistance/Other</td>
<td>&lt;0.1</td>
<td>&lt;1</td>
<td>&lt;0.1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Global Totals</td>
<td>39.9</td>
<td>100</td>
<td>44.2</td>
<td>100</td>
</tr>
</tbody>
</table>


Although crop protection traits are also important in the developing nations, the ranking of priorities for agriculture in developing countries may be different from that of developed nations. For developing nations that host more than 80 percent of the world’s population, an increase in yield still remains a number one priority. This is especially true when 815 million citizens of the developing world are suffering from malnutrition.

The traits of high priority for a developing country are those that contribute towards increased food production. Although heading towards economic growth by way of ensuring food security, the developing nations also face the challenge of generating employment for their population that is growing at an alarming rate. Providing employment for the people, and thus making them capable of buying food, is also an important aspect of food security achievement. Therefore, unlike industrialized nations, where a trait like herbicide tolerance leads to a jobless economic growth (by minimum human involvement), the developing nations require job-led economic growth.

The traits of priority for the developing world should be considered with respect to their impacts on socioeconomic structure. The traits that will give maximum outcome in terms of quantity and quality of food and that use natural resources in a sustainable manner should attain highest priority in developing countries. The social considerations are also important when the introduction of technology will have to be focused on the needs of the people. Some examples of traits of high priority for the developing world are discussed in the sections that follow.

Traits with Improved Nutritional Value

About 30 percent of the people in the world suffer from iron deficiency and more than 40 million from vitamin A deficiency. A majority of these deficient people reside in less developed nations. Incorporation of a gene for ferritin, an iron-rich soybean storage protein, into the rice plant is a very good example of “need driven” biotechnology produce. This kind of iron-enriched rice can be one of the most socially and economically viable answers to minimizing iron deficiency in developing parts of the world.

Research work carried out by Ingo Potrykus and his team on development of vitamin A enriched rice can be used as an important tool to alleviate the serious health hazards caused by vitamin A deficiency in the developing world (Potrykus 1999). The development of vitamin
A enriched rice has the potential to save 250,000 children annually from becoming blind in the rice-eating Southeast Asian countries.

Improved nutritional qualities in the locally grown crops, like rice, can be a very effective tool for combating widespread nutritional deficiency in developing nations at a cost much lower than dietary supplement drugs.

**Preventing Post Harvest Loss**

Countries like India lose about 28 percent of their total food produce in postharvest losses. This is primarily due to a lack of infrastructure like transport systems, storage facilities, and so forth. Lack of proper storage mechanisms not only results in quantitative losses but also accounts for considerable deterioration in the nutritive quality of food.

Traits for increasing shelf life can contribute greatly to preventing food waste and making it accessible to food deficient people. These traits can help the harvest withstand the effects of poor storage and transport facilities in countries in which modern tools of quick transportation and environmentally controlled storage facilities are a luxury.

**Traits for Resistance Against Abiotic Stress**

Extreme climatic conditions are one of the major factors limiting world food production capacity, especially in the developing nations. It is difficult to find “stress free” areas in which crops may approach their potential yield. Abiotic stresses are considered to be the main source (71 percent) of yield reduction (Boyer 1982). Incorporating traits for stress resistance and tolerance can be an economically and environmentally viable approach to bridging the gap between actual and potential crop yield in marginal areas. Development of crops suitable to grow in conditions of abiotic stress can make a significant contribution toward utilizing the arable land to its maximum potential and thereby increasing crop productivity.

The foregoing traits combined with traits for pest resistance, increased fertilizer efficiency, and edible vaccine production are of prime importance to the developing nations and offer economically and environmentally suitable, sustainable solutions.

**Future Research Trends**

Future research that aims to incorporate newer traits in crops should consider the needs of both the developed and the less developed parts of the world. The current debate about and limited acceptance of LMOs in many parts of the world are largely a consequence of the lack of communication between scientists and the consumers of technology, including the farmer. Understanding and effective communication between the two would help in clarifying many misconceptions about the technology. The study of public perception of biotechnology and the development of a stronger to-land link needs to be considered as one important research direction for the future.
Ironically there is a global communications network that makes the latest findings of science available almost immediately to research workers around the globe. What is urgently required is a similar communications network at the service of farmers and consumers of technology. Serious efforts are needed towards setting up a “global” mechanism in which the involvement of international agencies can play a significant role. International agencies like Consultative Group on International Agricultural Research (CGIAR) and Food and Agriculture Organization (FAO) involved with agricultural development work can take a lead in educating farmers about revolutionary technology like LMOs. Consortia of agencies should be formed under the umbrella of international agencies with local government, industries, scientific organizations and educational media as its members in developing countries. The consortia can take up the challenge of educating the farmers. The consortia can also become a tool for communication between various research efforts, media, farmers, and policymakers. This role is similar to the one played by international agencies like the World Health Organization (WHO), in health education during the past two decades. Agricultural education needs similar attention and can optimize the advantage of technology in leading the less-developed world towards food security.

At the present time in countries like India there is a lack of educational mechanisms for farmers. This is one of the greatest reasons for relatively slow acceptance of concept technologies like LMOs. Traditionally, in the absence of efficient government-initiated education mechanisms, agro-industries have played an important role in the farmer’s education. The industry’s efforts (quite understandably) have been biased towards prototing a particular product rather than then a technology. This identifies the need for unbiased agencies to get involved with educational efforts and to facilitate public understanding of the variety of applications and issues associated with agricultural biotechnology. Such education must be based on an in-depth assessment of public awareness and attitudes about biotechnology. Existing research is not addressing the need to design and implement effective educational programs (Hoban 1992).

**Conclusion**

There is an increasing amount of compelling evidence that LMOs can deliver important economic and social benefits in addressing the global need for sustainable food security. The promise of the technology can only be fulfilled when the benefits of the technology will be extended evenly to all parts of the world. Because LMO adoption is now primarily concentrated in the industrialized nations, serious efforts towards taking the technology to the developing world are needed. That this important technology does not remain limited to the more advanced nations but gets incorporated into the agricultural systems of the less developed world should be seen as a global necessity.

Today, 85 percent of the land area covered under transgenic crops is in industrialized nations. Consequently, the crop and traits being incorporated are based on the priorities of the developed world. The developing nation’s priorities may differ from those of industrialized nations—especially because most developing nations host a very large number of food deficient people. Producing a greater quantity of food with limited natural resources remains a prime priority of food-deficient nations, but this may not be the case in industrialized nations. The traits and crops of importance should be considered from the standpoint of these unique considerations of developing nations and expected priority differences.
The technology transfer from laboratories to land should be done holistically on the basis of the individual country’s socioeconomic and ecological needs. This kind of approach will contribute towards extending the benefits of LMO technology more evenly to all parts of the world. Educating the farmer and the consumer about the benefits of the technology will help in promoting it in such a way that we do not have to “push” the technology but instead the people will “pull” the technology because it is seen as advantageous and profitable.

Acknowledgments

I wish to thank all my colleagues, especially the useful discussions with Dr. Ved Malik, Dr. Chandraprakash, and Dr. C.S. Prakash while writing this paper. My sincere gratitude to my gurus M.S. Swaminathan, Dr. Mehta, Vice Chancellor of Gujarat Agriculture University, and Dr. Manju Sharma, Secretary, DBT, for their guidance and support. I sincerely acknowledge the help of all the farmers with whom I work and learn the “real life” lessons of biotechnology. I acknowledge the OECD and USDA for giving me the opportunity to represent the farmers in developing countries at the Raleigh conference.

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Life Cycle Assessment (LCA) of the Cultivation of Transgenic Crops as a Tool for a Comprehensive Assessment of Potential Environmental Effects

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Abstract

In a study commissioned by the Austrian Federal Environment Agency comparative life-cycle assessment was tested for the first time as a method to evaluate the short-term and long-term environmental effects of conventionally bred and transgenic crops in a given, specific agricultural system.

The methodology developed was based on life-cycle assessment in accordance with ISO 14040 ff. A scenario-based approach was chosen. To increase the usefulness of the life-cycle assessment, a risk assessment was added to the impact assessment. Insect-resistant maize (BT-176, Novartis) and herbicide-tolerant rapeseed (BASTA-tolerant, AgrEvo) were chosen as model plants.

Inventory analysis was performed using a set of data specific to Austrian agriculture and so-called generic data on energy, transport, and commodities. The quantitative impact assessment for a selection of broadly accepted impact categories was enlarged by adding a qualitative risk analysis on the human and ecotoxicological impacts of genetically modified organisms and those pesticides that can be replaced by the use of such organisms.
Introduction

The application of genetic engineering in plant breeding has triggered a discussion on the potential benefits and risks of using transgenic plants in agriculture. Comparative life-cycle assessment (LCA) may be a method to assess the short-term and long-term environmental effects of conventionally bred and transgenic crops in a given agricultural system.

Therefore, the Austrian Federal Environment Agency in Vienna commissioned a team of scientists from C.A.U. GmbH, Öko-Institut e.V. and ÖVAF (Österreichische Vereinigung für Agrarwissenschaftliche Forschung) to perform a study (Klöpffer et al. 1999) titled “Life-Cycle Assessment of Genetically Modified Products as a basis for an Assessment of Potential Environmental Effects.”

A comparative assessment of genetically modified plants and “naturally” bred plants by LCA cannot replace public discussion. But it can identify the most important measurable factors, quantify mass and energy flows, and compare the environmental impacts of different systems. Therefore, it can be a meaningful instrument for an extensive evaluation.

An LCA takes into account all the relevant energy and mass flows, including emissions and waste during the whole life cycle “from cradle to grave” of a product or service. It relates the numerical results obtained to “functional units”—a measure of product utility. The “functional unit” is laid down in the “goal definition” together with the system boundaries of the product systems under study and other information. The “cradle” in the case of replenishable raw materials and agricultural produce is usually taken to mean the seed. This “holistic” approach is what distinguishes LCA from other assessment methods that only consider partial aspects of environmental protection and are thus liable to misinterpret results. Furthermore, LCA is the only internationally standardized method of analyzing product-related environmental effects.

An LCA, as defined in ISO 14040 (ISO, 1997)(see figure 1), consists of four steps:

- Goal and scope definition
- Inventory analysis
- Impact assessment
- Interpretation.

Applications of the method are, among others,

- Product development and improvement
- Strategic planning
- Public policymaking
- Marketing.
Goal and Scope Definition

The main goal of the study was to adapt the LCA methodology to the special questions connected with the cultivation of genetically modified crop plants. Such an approach posed a methodological challenge. First, on a European scale there are few data available on the performance of transgenic plants under normal agricultural conditions. Second, the problem of how to integrate the currently undertaken risk assessment according to the European Union (EU) directives into the framework of the LCA has to be solved. In selecting the model plants for this study several aspects were considered as follows:

- Relevance among applications for release of genetically modified crops in the European Community
- Environmental relevance of the receiving plant
- Environmental relevance of the genetic modification
- Expected agricultural practice
- Economic potential.

Grain maize and winter rape were chosen. They both represent crops with large areas under cultivation in Austria. The genetic modifications are the introduction of an insecticidal protein into grain maize by using the delta-endotoxin gene of *Bacillus thuringiensis* (Bt-176, Novartis, now Syngenta) and, in the case of winter rape a resistance gene against the herbicide Basta or Liberty (AgrEvo, now Aventis).

The two model plants were studied on the basis of three different cultivation methods appropriately adapted to reflect conditions in Austria:
• Conventional cropping
• Conventional cropping with genetically modified organisms (GMOs)
• Organic cropping.

Organic farming was included in the analysis to do justice to the importance that this method has attained in Austria. All quantitative results are referred to a functional unit, defined here as 1,000-kg grain maize (e.g., used as animal feed) and 1,000-L rapeseed oil (for food purposes), respectively. To illustrate the systems under study, Figure 2 gives an overview of the life cycle of grain maize.

Figure 2: Overview of the processes in the life cycle of grain maize; the lower part was not quantified in inventory analysis, but considered as target compartments in risk analysis.

The geographic system boundary in the context of agricultural production is the national border of the Austrian Republic. As with almost all life-cycle assessments with national system boundaries, the supply of fossil fuels and raw materials constitutes a transgression of
the present system boundary. The temporal system boundary primarily applies to the agricultural scenarios, which were developed with reference to the period from 1994 to 1996. Most available data were selected for their up-to-dateness; older data (before 1990) were only used in exceptional cases where newer ones were unavailable.

**Inventory Analysis**

Key features of inventory analysis in LCA are data collection about

- raw materials, energy, and land use;
- emissions to air, water, and soil;
- waste;

and include the quantification of inputs and outputs for all modules of the system under study.

Inventory analysis here was performed in accordance with ISO 14041 (ISO 1998) using a set of data specific to Austrian agriculture (supplemented with information from other EU member states and Switzerland (EC–DG VI 1997) and so-called generic data on energy, transport, and commonly used materials. Great effort was made to get representative data on Austrian cultivation of grain maize and winter rape. The specific agricultural data collected by ÖVAF derive from official statistics, official model calculations, advisory papers, responses to questionnaires, and expert talks with chambers of agriculture, the Federal Office and Research Centre for Agriculture, University for Agriculture Vienna, and Raiffeisen Ware Austria.

In the case of grain maize, four scenarios for conventional cultivation, three for the use of GMOs and one for an organic scenario were analyzed. On the basis of data reflecting typical Austrian growing conditions, these different scenarios gave attention to variable infestation by the European corn borer and resulting yield loss or to plant protection strategies (application of the insecticide Decis or straw flailing). The scenarios investigated are listed in table 1. The scenarios GM 6 and 7 represent a typical Austrian average with and without use of a genetically modified organism (GMO), and scenario 1 is a worst-case assumption. For winter rape, one conventional, two GMO, and one organic scenario were defined. The two GMO scenarios differ in the extent of Basta application. Weed control in the organic scenarios was mechanical.
Table 1: Overview of the scenarios for the two crops in the Austrian study

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain maize</td>
<td></td>
</tr>
<tr>
<td>GM-Sc. 1</td>
<td>conventional, 100% infestation of corn borer</td>
</tr>
<tr>
<td>GM-Sc. 2</td>
<td>as 1, but application of insecticide</td>
</tr>
<tr>
<td>GM-Sc. 3</td>
<td>conventional, no infestation</td>
</tr>
<tr>
<td>GM-Sc. 4</td>
<td>GMO, no infestation</td>
</tr>
<tr>
<td>GM-Sc. 5</td>
<td>GMO, 100% infestation</td>
</tr>
<tr>
<td>GM-Sc. 6</td>
<td>conventional, 25% infestation, insecticide use on 10% of area</td>
</tr>
<tr>
<td>GM-Sc. 7</td>
<td>conventional, 25% infestation, GMO on 10% of area</td>
</tr>
<tr>
<td>GM-Sc. 8</td>
<td>organic</td>
</tr>
<tr>
<td>Winterrape</td>
<td></td>
</tr>
<tr>
<td>WR-Sc. 1</td>
<td>conventional</td>
</tr>
<tr>
<td>WR-Sc. 2</td>
<td>GMO, one Basta application</td>
</tr>
<tr>
<td>WR-Sc. 3</td>
<td>GMO, two Basta applications</td>
</tr>
<tr>
<td>WR-Sc. 4</td>
<td>organic</td>
</tr>
</tbody>
</table>

GMO-specific data provided by the company Novartis showed that it is reasonable to assume that the GMO scenarios only differ in plant protection from the conventional scenarios. Inventory analyses were performed for all the preceding scenarios.

The most widely used generic dataset employed originates from the “Eco-inventories of Energy Systems” developed at ETH Zürich (ESU–ETH 1996). Infrastructure was generally taken into account, for example, by balancing machinery production, maintenance, and farm buildings for accommodating the machinery. The generic datasets of ESU–ETH, which are largely used, also take infrastructure into account, and thus data compatibility was ensured.

**Impact Assessment**

Life-cycle impact assessment (LCIA) entails the following:

- Classification of interventions from the inventory like CO₂ emissions to an appropriate impact category like climate change.
- Characterization—modelling the potential environmental impact of interventions from inventory within an impact category (e.g., Global Warming Potential (GWP) of CO₂ = 1).
- Optional elements: normalization, sorting, grouping, weighting.

A quantitative impact assessment was conducted for a selection of impact categories, as described in table 2. The assessment was largely based on the method published by Klöpffer and Renner (1995) with modifications in the categories resource depletion and nutrification.
In the category of resource depletion, each resource is weighted with the reciprocal value of its static lifetime (Lindfors et al. 1995). The static lifetime of a resource is expressed in relation to that of crude oil, which serves as a standard.

Table 2: Selected Impact Categories for Impact Assessment in an LCA of genetically modified plants

<table>
<thead>
<tr>
<th>Impact Category</th>
<th>Relevant Exchanges in Inventory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative Energy Demand (CED)</td>
<td>Fuels</td>
</tr>
<tr>
<td>Resource Depletion</td>
<td>Raw Materials</td>
</tr>
<tr>
<td>Climate Changes (GWP)</td>
<td>CO₂, CH₄, N₂O</td>
</tr>
<tr>
<td>Acidification (AP)</td>
<td>SO₂, NH₃, NOₓ</td>
</tr>
<tr>
<td>Eutrophication NPA, (NPT)</td>
<td>N, P</td>
</tr>
<tr>
<td>Human Toxicity (HTP)</td>
<td>Heavy Metals, Pesticides, GMO</td>
</tr>
<tr>
<td>Ecotoxicity (AEP), (TEP)</td>
<td>Heavy Metals, Pesticides, GMO</td>
</tr>
<tr>
<td>Appropriation of Environmental Space</td>
<td>Land Use</td>
</tr>
</tbody>
</table>

Cumulative energy demand (CED) is defined in Verein Deutsche Ingenieure (VDI) Guideline 4600 (VDI 1997). It does not include manpower, metabolic energy (e.g., the energy content of food), and passively used solar energy. The CED is normally calculated on the basis of the net calorific value (NCV).

For characterization in the categories human toxicity and ecotoxicity (aquatic and terrestrial), the critical surface time method conceived by Jolliet and Crettaz (Jolliet and Crettaz 1997, Jolliet et al. 1998) was applied.

The impact category of “appropriation of environmental space” is intended to embrace impact potentials caused by land use pertaining to the spheres of nature conservation, species diversity, soil protection, erosion, and direct landscape consumption. It is meant in particular for possible damages that go beyond the toxic effects of emissions.

**Risk Analysis**

The risk analysis was done on the basis of the risk categories defined by the Organization for Economic Cooperation and Development (OECD) in 1993, EU Directive 94/15 EC (Commission of the EU 1994), and the “Framework Approach to Environmental Risk Assessment for the Release of Genetically Modified Organisms” jointly elaborated by the member States (Doc XI/0877/96—Rev. 4). According to these sources, the release of transgenic plants merits an examination of the following types of risks:
Life Cycle Assessment of Transgenic Crops

- Pathogenicity for other organisms
- Altered host ranges
- Potential for adverse health effects on human beings
- Questions of allergenicity and toxicity
- Population dynamic effects and effects on biogeochemical cycles
- Effects on target and nontarget organisms
- Pathogen–host interrelationships
- Predator–prey relationship
- Competition and displacement effects
- Interactions with the abiotic environment
- Possibilities of survival, establishment, and dispersal
- Introgressive tendencies and competitive advantages of transgenic plants
- Gene transfer to natural cross-breeding partners
- Horizontal transfer of recombinant genes to microorganisms
- Phenotypic and genetic stability
- Pleiotropic and position effects

Not all of these categories are applicable to the two selected transgenic plant groups (maize, rape).

Following Torgersen’s suggestion (1996), this study analyzed alterations in agricultural practice with a view to identifying possible additional effects of transgenic maize and rape cultivation on the environment.

For the Bt-176 maize scenario the possibility of resistance development in the corn borer as the target pest and to resistance management strategies was also considered. Risk evaluation was undertaken to estimate the effects of Bt-176 maize cultivation and Basta-resistant rape on the environment, giving due consideration to possible damage to natural ecosystems as required under EU Directive 90/220/EEC. To have considered natural ecosystems alone, however, would have meant neglecting many other effects with environmental consequences. Following Torgersen’s (1996, p. 41ff) demand, the present study therefore took a broader view in evaluating the possible effects of transgenic maize and rape cropping as practiced in their GMO scenarios. This was done by considering the potential effects on agricultural ecosystems, ruderal habitats, and small island populations. Furthermore, it discusses the environmental effects of the altered methods used in GMO cropping. Nevertheless, any prospective risk assessment is inevitably subject to multiple uncertainty factors. These problems of prospective risk assessments are attributable to

- the complexity of the matter;
- lack of knowledge on essential factors governing a system and their interactions;
- lack of unequivocal cause-and-effect relationships (which cannot be established in complex systems that are governed by very many parameters; if at all, they can only be identified retrospectively at a statistical level);
- the difficulty of transferring contained systems (e.g. a greenhouse) to open systems;
- the difficulty of transferring results from small areas to large ones.
Prospective risk assessment becomes more convincing the more it can be backed up by demonstrable effects or data. A matrix was used to make a differentiated presentation of what are proven, or experimentally demonstrable phenomena, or both, on the one hand and, on the other, associated with the former, effects that are presumed or plausibly explainable or have been demonstrated in individual cases.

Results

Working from the inventory analyses, impact category results were calculated for the categories of cumulative energy demand (CED), resource depletion (RES), appropriation of environmental space, global warming potential (GWP), acidification potential (AP), eutrophication potential (aquatic NPA and terrestrial NPT), human toxicity potential (HTP), and ecotoxicity potential (aquatic AEP and terrestrial TEP). Save for the semiquantitative part aspects, risk analyses were performed on a qualitative basis, covering the issues of outcrossing, resistance development, uptake of transgenes by micro-organisms, resistance management, diminished effectiveness of biological plant protectants, and human and ecotoxicological impacts of those pesticides that can be replaced by the use of a GMO.

As an example for the results of quantitative impact assessment, the resource depletion (RES) for the eight-grain maize scenarios is shown in figure 3.

Figure 3: Resource depletion (RES) for the cultivation of grain maize including upstream chains (referred to the functional unit of 1,000 kg grain maize)
Interpretation

For interpretation of the quantitative impact assessment, the investigated systems have been compared within each impact category to identify significant differences that indicate advantages or disadvantages of one system with regard to environmental burdens. Significant here means differences of at least 20 percent.

Evaluation of the impact assessment shows that in most of the impact categories the result is determined, apart from the level of the yield, by the nitrogen fertilization. Whereas the data basis for the mineral fertilizers is satisfactory (apart from some data gaps in the process emissions), the uncertainty about heavy metal content in the case of the organic fertilizer has to be pointed out.

The upstream chain of the pesticides has only a minor influence on the results. Because herbicides dominate in the cultivation of maize and the scenarios 1 to 7 differ in the use of insecticides, there are only small differences in the categories on human toxicity and ecotoxicity.

In table 1 a list is presented for the grain maize scenarios. The conventional scenarios 1, 2, 3, and 6 are distinguished by corn borer infestation, yield, and measures of insecticide use or straw flailing. The GMO scenarios 4 and 5 correspond to the conventional scenarios 2 and 3, and the GMO scenario 7 corresponds to the conventional scenario 6. The scenario 6, which mirrors the average situation for the conventional cultivation of grain maize in Austria, is selected as the standard. In figures 4 and 5, the percentage deviation of the other scenarios from the standard scenario shown. Bars pointing to the left indicate smaller ecological burdens relative to the reference scenario; bars pointing towards the right indicate greater burdens.

The deviations of the scenarios 1 to 7 are mainly due to different yields. However, they have to be judged as not significant because the deviations are smaller than 10 percent for all impact categories. No ecological advantages for Bt-maize in Austria can be deduced from the results.

Figures 4 and 5 show examples for grain maize scenario 1, which represents worst-case assumptions and the Bt-grain maize scenario 4/5.
Figure 4: Comparison of impact category indicator results of grain maize scenario 4/5 (GM-Sc. 4/5) relative to the Austrian standard scenario (GM-Sc. 6)

Figure 5: Comparison of impact category indicator results of grain maize scenario 1 (GM-Sc. 1) relative to the Austrian standard scenario (GM-Sc. 6)
Discussion and Conclusions

The essential results of the present LCAs on the systems of grain maize and rape oil, considered for the cultivation methods conventional farming, conventional farming using GMO, and organic farming under Austrian conditions, respectively, were as follows:

- The chosen methodology (LCA according to ISO–EN 14040 [1997] supplemented by a risk analysis on the nonquantifiable impact categories) is suitable for arriving at a meaningful comparison of systems.
- The inventory analyses and impact assessments show that in terms of the quantifiable parameters the differences between GMO and conventional farming are small, whereas organic farming performs significantly better in some categories such as cumulative energy demand, acidification and eutrophication.
- In all systems studied, fertilizer use was found to contribute the greatest burden. Data on this point were particularly deficient in the case of organic farming.
- Verbal risk analysis on the basis of risk categories revealed a considerable degree of uncertainty regarding the ecological behaviour of GMOs. These must be taken seriously as risks associated with GMO release and commercialization as required by the precautionary principle.

As political action usually proceeds after a weighing of the potential benefit of a proposed measure with its associated risks, the question emerges whether the risks of GMO farming identified in the study are compensated by any ecological advantages. Under the agricultural and environmental conditions prevailing in Austria the examples studied show no significant advantages. On the basis of the study’s recommendations a further approach to integrating the risk analysis of the use of transgenic crops in agriculture into the methodological framework of life-cycle impact assessment was made (Klöpffer et al. 2000, Renner et al. 2001). This was done by creating a new impact category called “effects of genetically modified crop plants.” This impact category enables taking into account the risks of the deliberate release of genetically modified crop plants in the course of agricultural production and the comparison of different genetically modified crop plants. To calculate a factor for characterizing a specific genetically modified crop plant, a risk number is determined on the basis of the likelihood of each risk category’s being realized. This depends on the likelihood of dissemination in a specific climate zone as well as on the number of transferred or modified genes. This risk number is combined with the number of the potentially affected safeguard subjects (natural environment, human health, manmade environment). The data are gained from the respective notification dossiers for the specific genetically modified plant.

Life-cycle assessment is now applied to the cultivation of transgenic maize in several European countries in a project subsidized by the European Commission (CAMPLES 2000-2002).
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Advisory Group
The project team was complemented by an advisory group that contributed substantially to the selection of systems, discussion of interim results, and data gathering. The advisory group included the following institutions

• University for Agriculture, Vienna (Institute for Organic Farming)
• Austrian Academy of Sciences (Institute for Technology Assessment)
• NOVARTIS International AG

Reviewer
According to ISO 14040 § 7.3 (ISO 1997) the publication of an LCA study report containing comparative assertions necessitates a critical review by external experts. The review was conducted by Dr. Gérard Gaillard, FAT, Tänikon, Switzerland, now Forschungsanstalt für Agraröleologie und Landbau (FAL), Reckenholz, Switzerland).

References


Living Modified Organisms and the Environment: Social and Economic Issues to Consider

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Abstract

The typical approach to risk analysis does not explicitly incorporate socioeconomic factors as a required step. A common argument against their incorporation is that socioeconomic considerations are more elusive and difficult to assess using sound science. Nevertheless, some of the possible effects of socioeconomic factors may be identified through adequate methodologies. Society’s traditional practices and cultural responses to perceived risks may alter the overall risk as well as the net benefits of a new technology and increase the associated cost of risk mitigation strategies. Therefore, risk analysis will be inaccurate unless assessors adequately consider relevant socioeconomic factors. Differences in socioeconomic conditions across regions in which new technological products are to be applied must be known, understood, and properly incorporated into existing methodologies for risk analysis. A wider multidisciplinary approach to evaluate these issues and facilitate communication among experts in different fields or agencies must be ensured.

Introduction

Traditionally, approaches and methodologies in risk analysis, which comprises risk assessment, management and communication, have largely ignored the socioeconomic factors, also known as the “fourth criterion” or the “peoples’ factor” National Research Council 1994, Lalcy 2000 in Batie and Ervin 2001). The incorporation of socioeconomic aspects into risk analysis is necessary to understand how humans create, react to and redistribute risks and hazards and decide on the best courses of action. People react to risk, and the collective probability they associate with health or environmental consequences affect their choices.

In the face of uncertainty, consumers may decide not to purchase some products, or peasants may opt for using different plant varieties, adopt different technologies, change the plots of land that they cultivate, and so on, in an attempt to protect themselves against risks. As a result, the overall risk and the net benefits and costs will change. Even more important, not all people and societies will react the same
way, nor will they balance the costs and benefits of a new technology in the same manner. The only way to make these factors apparent is through their incorporation as part of risk analysis. Therefore, risk analysts must explicitly incorporate socioeconomic factors into their methodologies to take into account the consequences and desirability of adopting a new technology in a specific context.

In the case of new biotechnologies resulting in the production of genetically modified organisms (GMOs), adequate risk analysis is particularly important given the uncertainties and potential risks surrounding their adoption. Incorporating socioeconomic considerations into the analysis is also necessary given the large range of socioeconomic as well as environmental conditions under which these technologies are intended to be introduced. This is imperative also because globalization among other factors is accelerating the diffusion of new technologies and the range of environments in which they operate.

This paper will attempt to show the relevance of socioeconomic factors in risk analysis for new biotechnologies, particularly for genetically modified crops, and to highlight some of the methodological implications. The first part of the paper deals with the nature of technological developments and risk. The second part focuses the analysis on biotechnologies and GMOs. The third section provides some specific examples of socioeconomic factors that affect the outcome of risk analysis and consider its implications.

**Technology Adoption and Risk Analysis in Perspective**

Technological developments arise from the conjunction of human creativity and human needs in a race to outcompete natural forces. Human creativity is constantly producing new solution concepts that, if successful, will improve social welfare. These new solutions, however, often have unexpected outcomes or do not always fulfill their promises. Why? Two main factors behind failing technologies are either that (a) they were applied in contexts (both natural and socioeconomic) for which they were not designed, leading to poor performance of the technology, or (b) unexpected negative outcomes were detected once they were adopted even when applied in appropriate contexts, leading to costs not previously accounted for.

With regard to the importance of the context for technological applications, it has been recognized that variations in skills and capacities and in cultural differences related to labor practices as well as differences among property rights systems do imply variations in costs and levels of productivity across various alternative production technologies (Hodgson 1988). Therefore, the same technology cannot be expected to perform in the same way in different contexts, and this will affect its benefits and relative costs. These differences may also lead to variations in associated risks.

The second factor, unexpected outcomes, is related to the state of knowledge at the time of adoption, and the negative impact of this factor on society depends on its willingness to adopt the new technology despite risk and uncertainty. Although it would be better to have full certainty about outcomes before making decisions, there is a limit to our capacity to
understand the natural world therefore, society’s attitude to uncertainty must be adequately considered. Limited knowledge of the implications of new technologies has led in the past to unexpected outcomes not considered at the time these technologies were introduced.

One example of unexpected outcomes was the introduction of pesticides like DDT during the so-called green revolution that were considered risk-free by scientists and manufacturers. As time passed, however, evidence of negative impacts arose that led to different decisions regarding adoption of these pesticides. New information on the safety and performance of new technologies allows for better decisions to be made. It should not be forgotten, however, that some of the negative impacts of technology may be irreversible or very significant. Hence, it is desirable to find means to reduce the knowledge gap before the adoption of new technologies. This not only involves knowing more about the technology–environment and technology–society interactions but also about the “type” of information needed by society to make a decision about the adoption of the technology. It is important to bear in mind that this information may not be the same for different socioeconomic contexts.

As a result, we can conclude that there are three elements of risk analysis for which socioeconomic factors play a significant role:

1. The extent and nature of perceived risks;
2. The extent of costs and benefits; and
3. The information needed for an adequate balance between overall risks and net benefits.

When transferring technology to other national contexts, we have to analyze the social, institutional, and economic impacts of doing so to ensure that risks are bearable and manageable and are offset by the benefits of the technology in terms of the society in the new context, not from the point of view of the society that produced the innovation.

If socioeconomic factors are included in risk assessment, differences in socioeconomic conditions across the regions in which the new technological products are to be applied must be identified and understood so that these issues may be properly incorporated into risk analysis.

However, consideration of these factors has not been required in the typical risk assessments of new technologies. A common argument for not including socioeconomic considerations into risk assessment and management is that socioeconomic factors are more elusive and cannot be assessed in the same way as “hard” sciences. Nevertheless, (1) the impossibility of assessing socioeconomic factors in the same controlled way as some biological aspects does not in itself diminish their importance, and (2) developments in social and behavioral sciences in the past decades allow for a broader set of analytical tools and methodologies to address these issues.

Future discussions of risk analysis must take into account socioeconomic aspects and enable a fluent and effective dialog across disciplines to construct a common understanding of the implications of socioeconomic factors and to expand current risk analysis procedures and develop integrated assessment methodologies.
From the government perspective, an integrated risk assessment methodology is essential to ensure that regulation based on risk analysis achieves the socially desirable outcome. The regulatory framework is an instrument that potentially could alter the perceived costs, benefits, and the possibility of different outcomes of new technologies, and it is therefore important that the policymaking process be guided by adequate risk assessments. To the extent that policy decisions take into account results from risk assessments, including socioeconomic considerations, decisionmakers will be able to consider strategies to distribute costs and benefits of a risk or hazard equitably among social and economic groups in the implementation of the risk management strategies. Consequently, Crocker and Shoffgren (1999) concluded that “risk management in this area should take both biological and social-economic aspects into account, in order not to be (at best) inaccurate or (at worst) ineffective.” It must be recognised that public opinion and social perceptions are also important elements to be taken into consideration. In the face of choice under uncertainty, the decision about what level of overall risk is considered appropriate, given potential benefits, is inherently a social, not a scientific one (Lalcy 2000 cited in Batie and Ervin 2001).

New Technologies, New Risks: Biotechnologies

In the case of modern biotechnologies, like the new range of GMOs, adequate risk analysis incorporating socioeconomic considerations is particularly important given the profound potential changes, both socioeconomic and ecological, that may arise from the use of these technologies. Biodiversity loss is a general category of impacts, which covers damages to crops, wild relatives, nontarget organisms, and to wider ecosystem processes. For instance, herbicide-resistant organisms stimulate the use of herbicides, antibiotic-resistant species create health risks, and genetic engineering in general stimulates homogeneous monocultures that in turn promote erosion and quickly spread diseases (van den Bergh and Holley 2001). These ecologically related potential consequences are among the most serious risks since insurance against them is often impossible. Effects such as genetic erosion, extinction, or loss of ecosystem functions are irreversible and cannot be compensated in financial terms (Crocker and Shogren 1999). It is therefore important to review the risk analysis procedures for these organisms.

Risk Assessment for GMOs and Conventional Improved Varieties

A controversy continues over whether genetic engineering is different from ordinary or traditional techniques used during domestication of organisms through controlled breeding and selection. This is an important starting point because disagreement with this statement could lead to different conclusions with other implications. If there is no intrinsic difference, then there is no need to respond differently to transgenic plant introductions than to the new variety introductions derived from conventional techniques. There would still be a need to improve the way in which we analyze the risk associated with new varieties, but probably to a lesser extent.
We believe, however, that there are intrinsic differences between traditional breeding for domestication and genetic engineering (table 1). Therefore, we have to analyze whether this new technology presents any new hazards to human health or the environment and how socioeconomic aspects might influence the levels of risks associated with the use of GMOs that are different from those generated through traditional breeding. For each case we have to consider a different three-fold interaction: (1) the genetically modified organisms (GMO), (2) the modification or inserted genes, and (3) the environment in which the GMO will be released. Here we propose an extended perception of the environment that includes, besides the typical biological factors, particular cultural, social, and economic factors, including characteristic farming practices.

Table 1. Comparison among traditional breeding and genetic engineering

<table>
<thead>
<tr>
<th>Traditional Breeding</th>
<th>Genetic Engineering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limited by compatibility and phylogenetic relationships</td>
<td>Not limited by reproductive barriers</td>
</tr>
<tr>
<td>Leads to the interaction of groups of genes in the same evolutionary lineage</td>
<td>Often includes the interaction of genes and genetic sequences from very distant lineages</td>
</tr>
<tr>
<td>Produces new gene combinations that are possible</td>
<td>Produces new gene combinations that are not possible</td>
</tr>
</tbody>
</table>

Socioeconomic Considerations in Risk Analysis for GMO Technologies: Methodological and Conceptual Challenges

Socioeconomic considerations are pertinent to risk assessment and risk management activities in relation to biotechnological products, as has been already recognized for risk communication. The socioeconomic considerations of most importance to risk analysis include the evaluation of economic costs and benefits of different decisions, development and communication of decision criteria for the release of a GMO (e.g., cultural considerations) and the cost-effectiveness of risk management strategies.

Socioeconomic considerations also include the analysis of economic risks that, although preexisting in the production systems under consideration, may be exacerbated or reduced by the introduction of a new biotechnology. For instance, consider that the market for biotechnological inputs in the crop sector is highly concentrated, that is, with only a few major companies. The result of the wide diffusion of biotechnologies is linked to an increased dependence of farmers on a limited number of suppliers of inputs for crop production (European Commission 2001). This dependency adds economic uncertainty for producers and processors who no longer have the same degree of technical and economic influence over their suppliers. Although the effect is more of a market nature rather than an ecological one, it is nonetheless
important to consider as part of a more holistic approach in risk assessment changes in markets may have effects on ecological processes and vice versa.

Some biotechnological applications tend to be nonreversible. Their irreversibility has both economic and ecological–evolutionary implications. Economically, irreversibility associated with the practical impossibility of eliminating released GMOs that may have transferred their transgene constructions may lead to the foreclosure of options to use alternative technologies or the access to specific markets demanding GMO-free products. Ecological irreversibility

Table 2. Examples of the relevance of socio economic factors in risk assessment and management

<table>
<thead>
<tr>
<th>Risk Analysis</th>
<th>SocioEconomic Factor</th>
<th>Potential Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Assessment</td>
<td>Consumption patterns.</td>
<td>Risk effects related to allergenic reactions are related to consumption patterns, which in turn are culturally determined. Different human populations may have different thresholds for allergenic reactions.</td>
</tr>
<tr>
<td></td>
<td>Technology choice is a function of socio economic conditions.</td>
<td>Different technologies may affect levels of exposure or levels of risks, e.g. may increase gene flow (see next section).</td>
</tr>
<tr>
<td></td>
<td>Traditional practices vs. biological means to protect intellectual property.</td>
<td>If some grains from “terminator” crops were to be imported and planted by farmers, they will inadvertently have a reduced output, since the seeds won’t germinate.</td>
</tr>
<tr>
<td></td>
<td>Economic strategies to diversify risk.</td>
<td>Exposure factors may be different in multicropping systems.</td>
</tr>
<tr>
<td></td>
<td>Liability rules (legislation) and economic capacity.</td>
<td>Capacity resist or recover from impacts of a hazard in the long as well as in the short term may vary across regions.</td>
</tr>
<tr>
<td>Risk management</td>
<td>Participation mechanism in the decision making process.</td>
<td>Imbalance of voices in decision making process may lead to inefficient/undesirable outcomes.</td>
</tr>
<tr>
<td></td>
<td>Social perception and values.</td>
<td>Attitudes towards risk may vary.</td>
</tr>
<tr>
<td></td>
<td>Technological patterns and economic conditions.</td>
<td>Differentiated cost of risk mitigation strategies.</td>
</tr>
<tr>
<td>Risk communication</td>
<td>Literacy and educational levels.</td>
<td>Efficacy of labels may vary.</td>
</tr>
<tr>
<td></td>
<td>Social perception and values.</td>
<td>Public acceptace of the methods.</td>
</tr>
</tbody>
</table>
involves changes in the genetic composition of species, domesticated crop varieties, wild relatives, and interacting species (van den Bergh and Holley 2001). Both sets of impacts need to be taken into consideration by assessing the foregone alternatives when choosing to release a GMO into the environment.

Table 2 includes some possible effects of new biotechnologies involving GMOs associated with socioeconomic factors. The list, far from being exhaustive, is an indicative one showing how socioeconomic factors are relevant for all three stages of risk analysis: assessment, management, and communication.

A Subtle but Profound Impact of Culture on Risk: Seed or Grain?

To provide one example of the influence that social traditions may have on risk assessment and risk management, consider the difference between seed and grain. Peasants throughout Mexico, but especially in the southern part, usually save some of their harvest to be used as seed for the next cycle. They can just as well use some of the saved seed for human consumption if needed in extreme situations. They may also exchange seeds on a seasonal basis in order to avoid inbreeding depression. These three simple elements of traditional cropping systems, however, would need to be changed if we are to accommodate the requirements imposed by modern agricultural practices and biotechnological developments: seeds coated with a seed-protecting pesticide, or carrying an herbicide resistance gene are no longer suitable for human consumption. Seeds with pesticide would be toxic and should not be consumed, while herbicide resistant seeds have to be planted given the “extra” value for the included biotechnological quality that would be lost if consumed. Also, intellectual property protection and biosafety measures imply that some of the grain produced can neither be freely replanted nor traded as seed. Hence, seed or grain, conceptually the same in traditional cropping systems, would have to be differentiated. Full transfer of the GMO technological package would therefore imply significant changes in the way that traditional cropping systems have been operating over hundreds or maybe thousands of years. Limiting seed exchange and interfering with the mechanisms for diversification of traditional varieties could have a strong impact on world food security and also impact rural communities. In addition to these considerations we must also consider that in many cultures the use of some crops has deep social and religious meanings that may be affected by changes in practices required by modern biotechnology. Partial transfer of the GMO technological package, namely the seed only, does not affect the practices themselves, but it does have more subtle impacts, such as the increase in the gene flow from GMOs to the landraces with uncertain consequences.

Should We Ask: are GMOs Desirable?

Biotechnological developments are therefore technology-pushed processes rather than processes induced by social demand. In other words, biotechnology is a ‘technology in search of applications’ (Batie and Ervin, 2001). Private companies engaged in technological developments do not necessarily direct their efforts to flow to the areas where they are needed or direct them in ways that maximize welfare in all contexts where technologies are to be introduced. Therefore, the evaluations of GMOs prior to release have a double task of not only considering the risks involved, but also the social desirability of the organisms given the social objectives that were not considered in its development. Evaluations must
Social and Economic Issues to Consider

consider the social context surrounding the release. This task is not trivial and the speed of biotechnological development may catch public regulatory bodies unprepared for unintended social consequences.

Hence, environmental regulators are currently not focusing on the process, but simply introducing end-of-pipe type of measures, i.e. once the technology is here, they have to see which measures are needed to mitigate the risks involved. A more efficient approach for regulators would be to induce changes in the processes themselves and direct research efforts to those fields that are more desirable, or to induce research efforts to generate relevant information for risk analysis from the design stage, including socio economic data. This typically calls for public intervention in correcting potential market failures associated with biotechnological developments and ensuring that the developments are directed towards the most socially beneficial goals and take social risks into consideration. Once again, socio economic assessments are meant to shed light into the extent and nature of the tradeoffs between benefits, costs and risks that societies are willing to consider, and how these are in turn influenced by society’s reaction to new technologies.

Conclusions

Clear and reliable methodologies need to be designed and implemented to incorporate socioeconomic considerations into risk assessment and risk management activities regarding GMOs.

It is important that the field tests carried out to determine the potential risks of GMOs do provide relevant information that can be extrapolated to the kind of socioeconomic and environmental contexts in which each GMO is likely to be released. As of today, field experiments with genetically modified crops in confined conditions do not provide sufficient information about ultimate ecosystem and socioeconomic impacts of using such crops in normal agricultural circumstances. This is an area that needs further consideration. Another policy implication for risk assessment and management which is related to socioeconomic factors is the need for more public involvement in the decision process. This is linked to the issue of risk communication where of course, it needs to be recognised and respected that different societies and individuals may perceive risk differently. An adequate risk communication strategy must enable society to take decisions and transmit them to policy makers, and not pre-empt outcomes by assuming that society will necessarily assess risk the same way as the evaluators. Lalcy cautions that “[However,] any public participation requirements would have to be designed carefully to control excessive transaction costs and to balance powerful lobbying groups that espouse narrow views (Lalcy 2000, in Batie and Ervin 2001).”

The incorporation of socioeconomic factors into risk analysis also implies that risk analysis must have a wider multidisciplinary approach. The objectives of environmental risk analysis and human health risk analysis are different, and in most countries, different agencies or ministries undertake them. Effective communication is required among them, and also among experts on socio economic issues included in the analysis.

Several aspects of harmonization are desirable: the information generated in one country
must be relevant for the countries in which the technology is to be released. Additional considerations that must be incorporated in order to consider context specific factors, both environmental and socioeconomic, must be explicit in the methodology. Similarly, the definitions related to these methodologies must ensure that socioeconomic factors are taken into account and that social rather than private costs and benefits are used when assessing the technologies. Failure to incorporate these elements will most likely create an obstacle for the development of common criteria and principles in national legislation. If regulatory harmonization is to be feasible, the definitions and methods for risk analysis must be broad enough to accommodate the diversity of socioeconomic and environmental contexts in which new biotechnologies are adopted. A critical review of these issues in the context of national regulatory frameworks is a step in the right direction.

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References


Session 3: A Scientific Framework for Assessing Transgenic Organisms in the Environment

Session 3A—Transgenic Crops in the Environment
A Scientific Framework for Assessing Transgenic Organisms in the Environment

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Abstract

The ecological impacts of living modified organisms (LMOs), both good and bad, will ramify through ecosystems and away from their site of release. To study these impacts, we must be prepared to think on a large scale and over long timeframes. Risk-based research must be expanded in scope and amount. Predictability of the effects of introducing organisms that are not LMOs is generally low, but so is the probability of their causing harm (and benefit). It is, however, not clear whether LMOs are more or less predictable than organisms that are not LMOs. Lastly, because the community does not calculate risk on the basis of probability but on the basis of “outrage,” the results from risk-based environmental research will never be more than part of the picture.

Background—Implications of Living Modified Organisms for Biodiversity

The debate about the impacts of living modified organisms (LMOs) on biodiversity has taken place in a largely data-free arena. When studies are published, they are seized upon by proponents or opponents of the technology and overextrapolated in a way that makes many ecologists uneasy. No single study can have all the answers (science usually progresses by a series of incremental steps), and nowhere is this more true than in ecology. Ecological systems are complex and have feedback loops, thresholds, and damping mechanisms that mean they respond to perturbation rather unpredictably. This is not only an argument for caution in implementing the technology and in extrapolating the risks but also for more risk-based research.

One hears the scientific debate about ecological hazards posed by LMOs polarized around “sound and unsound science,” and those urging caution are usually accused of being “unsound.” This is not a useful dichotomy (Levidow and Carr 2000). It may take decades, or even centuries, for the full ramifications of a new biological introduction to be played out. The history of biological invasions shows that changing circumstances over time can cause hitherto benign organisms to cause ecological disasters, and thus to describe hazard identification as “unsound science” is to ignore the lessons of history. Ecological hazards vary in probability over time, such that a hazard that seemed unlikely once can become very real decades later (see Non-LMO introductions as model systems). It is much more productive to consider a posited hazard as real and focus on its likelihood and how that might change.
Typically, the ecological impacts from LMOs that have so far been most studied have tended to be those felt at the population level, or onsite (table 1), such as the following:

- Spread of introduced genes (e.g., for herbicide resistance) to wild relatives, which then become “superweeds.”
- Loss of insecticide resistance in nontarget species through ubiquity of insecticide in the plant–soil system.

However, few studies have focused on what may be termed the higher order and landscape scale risks (table 1) such as:

- Environmental benefits from promised reductions in inputs (herbicides, insecticides, fertilizer, etc.) not achieved because of ecological feedback loops.
- Changes to land-management practice through living modified organism (LMO) cropping that reduces or affects biodiversity.
- Land degradation through LMOs’ allowing use of marginal land.
- The impact of the use of LMOs in the natural environment (e.g., as agents for feral pest control).

Table 1: Schema for considering the ecological impact of an LMO (Lonsdale, W.M. and Andersen, A.N., unpublished). Most research has focused on population level studies and on-site impact.

<table>
<thead>
<tr>
<th>Ecological level</th>
<th>On-site</th>
<th>Off-site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals/populations</td>
<td>Decreasing number of studies</td>
<td></td>
</tr>
<tr>
<td>Communities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecosystems</td>
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An objective of the Australian National Biotechnology Strategy (Commonwealth of Australia 2000) is to ensure that the potential risks from the introduction of genetically modified organisms (GMOs) are accurately assessed and are managed effectively. Some of the strategies under this objective are the following:

- To establish a framework and a methodology for risk assessment.
- To identify priorities for an environmental risk assessment program.
- In collaboration with Commonwealth Scientific and Research Organization (CSIRO) and other agencies, to improve basic knowledge and assess environmental risks associated with GMOs.

Commonwealth Scientific and Research Organization (CSIRO) Australia has established an LMO ecology group that networks ecological modelers, risk analysts, and ecologists working in systems ecology and on the ecology of pests and weeds. Their brief is to study the environmental risks of LMOs if adopted at the large scale into agricultural and natural ecosystems.
The program consists of two key research areas (KRAs).

**KRA 1—Robust Risk Assessment Tools for LMOs**

Aim: To develop robust risk assessment tools for LMOs that consider effects at the wider, landscape scale and at the longer timeframes at which environmental interactions occur.

- New risk assessment tools will be developed based on a critical analysis of existing tools used for genetically modified organism (GMO) risk assessment from around the world (see, e.g., table 2)
- Technical and policy workshops involving scientists in KRA2 and regulators will be used to test these alternative risk assessment tools against results from pathfinder GMO studies.
- Deductive datamining and metaanalysis will be used to analyze risks and benefits of past introductions of organisms and agricultural technologies into Australia in order to deduce new generalizations that will lead to the development of improved, more quantitative approaches for assessment of risks of GMOs.

**KRA2 - Pathfinder Studies for the Risks of New LMOs**

Aim: To initiate several theoretical and field-based case studies of LMOs to predict or measure their consequences for biodiversity. This will provide data and insights to test and refine the risk assessment tools as well as to help develop guidelines for a national monitoring system for ecological impacts.

The case studies will involve the following:

(a) Field and laboratory studies of LMOs that are released or likely to be released shortly (field crops and pasture species), which will be studied in the field. The following topics are being covered: Impact of Bt cotton on beneficial arthropods (pollinators, predators, parasitoids or other nontarget pest species), impact of genetically modified (GM) clover pasture legumes on their rhizobial symbionts and herbivorous pasture insect pests, and impacts of GM cotton and canola on key soil processes.
(b) Theoretical studies on four very diverse LMOs that are much further from field release (5–10 years):
   - Sterile feral work on mice and carp
   - Modified cattle rumen biota
   - Insect-resistant eucalypts.

All the chosen technologies are part of the current research projects of CSIRO. This selection will give the required balance between immediate relevance and expanding the long-term strategic capacity in risk analysis.

(See [http://www.biodiversity.csiro.au/2nd_level/3rd_level/plan_gmos.htm](http://www.biodiversity.csiro.au/2nd_level/3rd_level/plan_gmos.htm) for more information.)
Table 2: List of some GMO risk assessment models. See Hayes (1997) for a brief comparison of each.

<table>
<thead>
<tr>
<th>Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRC Risk assessment model for genetically modified plants and micro-organisms</td>
<td>NRC 1989</td>
</tr>
<tr>
<td>Cornell/ICET Risk assessment schema for release of biotechnology products</td>
<td>Strauss 1991</td>
</tr>
<tr>
<td>Population dynamics model for assessing the risks of invasion for genetically engineered plants</td>
<td>Parker and Kareiva 1996</td>
</tr>
<tr>
<td>GENHAZ – a system for critically evaluating genetically modified organism hazards</td>
<td>RCEP 1991</td>
</tr>
</tbody>
</table>

**Risk Analysis Context**

**Four Pillars of Risk Analysis**

Not every environmental problem can be addressed, and priorities need to be set by agencies and land managers. In recognition of this, risk analysis has forced itself onto the agenda for governments around the world over the last 20 years. Risk is the likelihood that damage can be caused by some behavior or action (including no action). Hazard is the agent that causes damage. When describing risk-based disciplines, risk analysis is the most general term and consists of the following four components, which are referred to as the “four pillars of risk analysis” by Davies (1996):

1. **Comparative risk analysis** entails comparing two or more types of risk and is principally a tool for policymakers to decide on resource allocation.
2. **Risk assessment** is a set of analytical techniques for estimating the frequency of undesired events and their consequences (damage or injury) and is properly accompanied by a description of uncertainty in the assessment process.
3. **Risk management**, in contrast to risk assessment, risk management considers social, economic, and political factors to determine the acceptability of damage and what action can be taken to mitigate it.
4. **Risk communication** entails conveying information about risk.

To these four terms I would add the following:

5. **Monitoring**, to detect the impact of hazards at an early stage (although purists might include such monitoring under the heading of risk management) or to provide data to refine future risk assessments.

For Session 3, the most important aspect is risk assessment, and so I will now consider this in more detail.
Ecological Risk Assessment

At first, risk assessment of LMOs was envisaged to be moving as a linear progression from testing to predictability to commercialization. As data accumulated, it was thought that commercialization would become easier and easier. However, as time has gone on, LMO risk assessments have been framed increasingly broadly—particularly in Europe (Carr 2000).

A useful starting point for understanding risk assessment is the National Academy of Science’s human-health risk assessment process. It was developed for chemical pollutants affecting human health and consists of a four-step procedure (NAS 1983):

1. **Hazard identification**—What type of damage can a substance cause?
2. **Exposure assessment**—How long will a target population be exposed to how much substance?
3. **Dose–response assessment**—How does the target population respond to this exposure?
4. **Risk characterisation**—This is a process that combines information from the steps above to estimate the likelihood and magnitude of damage.

During the recent past, GMO risk studies have appeared amid great controversy. A good example was the studies of the risks posed by Bt corn for migratory Monarch butterflies (*Danaus plexippus*), in the United States. One laboratory study applied pollen from Bt corn to leaves of the butterfly’s host plant, milkweed (*Asclepias* spp.), and found larval mortality was increased (Losey et al. 1999). Another study collected leaf disks from potted *Asclepias* exposed to Bt corn in the field and again found elevated mortality (Jesse and Obrycki 2000). Both studies were useful within limits, but a consideration of the preceding basic risk assessment methodology highlights those limits.

What is most clearly absent from these two Monarch studies is the exposure assessment. The studies were both preliminary in that they did not attempt to collect data on questions of field exposure such as

- Where do milkweeds grow in relation to corn crops?
- How do Monarchs select milkweeds in nature?
- What is the distribution of Monarchs in relation to contaminated milkweeds?
- Which leaves do they eat (upper, middle, lower?)
- What concentration of Bt toxin would exist in pollen?

In essence, these were risk assessments only in a narrowly defined sense (see Sears et al. 2001). By the same token, risk assessments carried out by proponents tend also to be limited in the range of hazards addressed and in the broader ecological framing of the assessment.

As ecologists, we must take into account the distribution and abundance of organisms in nature, as well as scale effects, and the possibility that cascade effects and feedback loops will occur. Indeed, the simple model above, useful though it was in highlighting the deficiencies of the Monarch studies, is itself inadequate for ecological risk assessments when we are interested in ecosystem effects from living organisms. The “pollutant” can self-
replicate, and it might affect many possible species, not just humans. One model that attempts to consider these factors is that of the U.S. Environmental Protection Agency (EPA) (US EPA 1992). Others specifically developed for GMO risk assessment are cited in table 2.

**Community perceptions of risk**

Scientists measure risk as a hazard’s magnitude multiplied by its probability. The community, on the other hand, calculates risk as a hazard plus outrage. Outrage is increased if

- Exposure to the hazard is coerced;
- The hazard is industrial;
- The hazard is exotic;
- The consequences are dreaded;
- The consequences are catastrophic;
- The regulating process is unresponsive,

and so on (Sandman, P., Rutger’s University; see http://www.psandman.com/getpubs.htm).

Consequently, there comes a point at which no amount of science or advocacy can counteract the community’s perception of the undesirability of a particular technology.

**Non LMO Introductions as Model Systems**

**Impacts**

Williamson (1996) has argued that we can use non-LMO introductions as model systems for understanding the risk profiles of LMOs. Broadly, of the thousands of organisms introduced to a new region, a tiny minority will become harmful, but this probability of harm varies with the organism, the region, and the mode of introduction. Impacts from introductions result from an interaction between the organism and its environment; for example, the same organism may be harmful in one region but not in another. This is also true of its beneficial effects. Obviously, an insect-resistant plant introduced where insect pests are rare will have no advantage over nonresistant plants. Small genetic changes can be critical in determining outcomes of introductions. It took several tries to get a rabbit population to breed in the wild in Australia, but, the right population being found, it became one of Australia’s worst vertebrate pests (Williamson 1996).

Predictability of harm for biological introductions is low for various reasons, including the following:

- Cascades in ecosystems—food webs, and so forth amplify or damp effects;
- Scale effects are paramount in ecology—what is true in field plots at 1 ha is unlikely to be true at $10^4$ km$^2$;
- Lag phases—It may take 150 years for trees, for example, to become invasive (Kowarik 1995);
- Base-rate effect (see section on Decision theory).
Because technologies are not always taken up and used successfully, the rate at which introduced organisms become useful is also probably quite small. Therefore, predictability of both harm and benefit is generally low.

**Are LMOs Less Predictable than Non-LMOs?**

For a non-LMO being introduced to a new region, say Australia we use our experience of the organism in a similar environment outside Australia to predict potential harm in Australia:

\[ \text{Organism} \times \text{"similar" environment elsewhere} \rightarrow \text{organism} \times \text{Australian environment.} \]

Typically, for an LMO we know how its parent organism behaves in Australia and use this to predict how the novel organism will behave:

\[ \text{Parent organism} \times \text{Australian environment} \rightarrow \text{novel organism} \times \text{Australian environment} \]

The answer to the question posed in the heading for this section will depend on how much of the variance in invasion impact is explained by genetic differences and how much by differences between ecosystems (Lonsdale, W.M. and Richards, A., unpublished). We do not know the answer to this, and so the question we have posed, though an interesting one, remains unanswered. What is clear, though, is that the predictability of an LMO of which the parent organism is not known in Australia will be lowest of all because we are extrapolating both for the environment and for the organism:

\[ \text{Parent organism} \times \text{"similar" environment elsewhere} \rightarrow \text{novel organism} \times \text{Australian environment.} \]

It could be argued, therefore, that introductions of LMOs of which the parent organism is not known in a region should be prohibited as being too unpredictable in their consequences.

**Decision Theory**

Weighing up the risks and benefits of an action is the domain of decision theory. Smith et al. (1999) adapted a decision-theoretic analysis of the value of earthquake prediction to explore the basis for heeding predictions about damage resulting from introduced organisms. They showed that the decision on whether to heed a recommendation to exclude a new crop plant depended on the following:

1. The damage that would be caused if a useful plant were excluded;
2. The damage that would be caused if a weed were allowed in;
3. The background probability (also called base rate or prevalence) that a plant will become a weed;
4. The accuracy of the system that predicts whether the plant will cause problems.

This simple but powerful analysis deserves further exploration. It may turn out to be too simplistic, but it certainly suggests aspects of the risk–benefit research agenda. **We should**
be carrying out economic evaluations of the costs of different types of pests and weeds and
the benefits of differing types of agricultural animals and plants, conducting
metaanalyses of the rate at which different kinds of organisms become pests or weeds, and
aiming to increase the accuracy of our predictions of harm. Note that the estimate
of harm could also embody social perceptions (outrage, etc,) and policy initiatives such as the
Precautionary Principle.

Concluding Remarks

Those wishing to release living organisms into the wild must be prepared to deal with
uncertainty and to acknowledge that what we do not know vastly outweighs what we
know. As Lao Tzu said, “Knowing our ignorance is the greater part of knowledge.”

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Appendix—Points for Discussion

The points below emerged during Session 3 discussions.

• A useful context to think about LMOs is in terms of triple-bottom-line sustainability—What will be their impact on the environmental, economic, and social well-being of a region or country? This broadens the discussion from one centered on one aspect or another of biotechnology uptake to a more holistic one.

• Prediction systems early warning and monitoring systems—is predictability so low that we must move from a risk assessment to a risk management system? This would be one in which we move forward with a potentially hazardous technology but monitor it carefully, being prepared to rapidly reverse our decision to proceed if we detect harm.

• Gene-by-environment interactions are important for determining both harm and benefit.

• Can we move from a case-by-case to a generic approach in risk assessment?

• How do we measure secondary effects, and which ones? Scientists carrying out the British farm scale evaluation of biodiversity impacts of LMOs spent a considerable amount of time planning the methodology for their study to ensure that effects would be detectable and that the right organisms had been selected for study.

• How the farmers use the technology (e.g., herbicide-tolerant and Bt crops) is an important variable for determining environmental outcomes.

• Impacts of LMOs on stability of agricultural system (e.g., overreliance on a few varieties)—The non-GM introduction of Texas cytoplasm corn is a cautionary tale here.

• Global food politics—Are GMOs the way to feed the world? On the one hand, losses to pests, weeds, and diseases are huge across the developing world. On the other hand, hunger in the developing world is rarely simply a consequence of food shortage but more of inequalities of wealth and food distribution.

• What constitutes an adverse effect? Even biodiversity scientists might consider the loss of individuals of one species acceptable because of concomitant gains for other
species elsewhere, but such a rationalization might not be acceptable in the wider community.

- What is the baseline for comparison? The predominant view among regulators globally has been that the impacts of GM crops should be compared with the impacts of conventional (high-impact) agriculture as the baseline. Thus, a Bt crop would be judged unlikely to cause more harm than a conventional crop sprayed with chemical pesticide. However, some countries such as Denmark and Austria believe that GM crops should offer an improvement over conventional agriculture, and Austria, in wishing to move towards organic agriculture, believes that this should be the baseline for comparison (see Carr 2000).

- Should intrasectoral impacts be within scope for regulators? Should regulators consider the impact of GM agriculture on other agricultural subsectors such as organic farmers?

- For some countries, such as the United Kingdom, so much of the landscape is agricultural that the agricultural landscape is the natural environment. In Australia, by contrast, large swathes of the landmass are under conservation. For example, World Heritage-listed Kakadu National Park in northern Australia is roughly the size of Israel.
Session 3: A Scientific Framework for Assessing Transgenic Organisms in the Environment

Session 3A: Transgenic Crops in the Environment
The Farm-Scale Evaluations of Herbicide-Tolerant Genetically-Modified Crops in Great Britain

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Abstract

The Farm-Scale Evaluations were established in response to concerns about possible impacts of genetically modified herbicide-tolerant (GMHT) crops on biodiversity in British farmland. This project involves growing genetically modified (GM) and comparable non-GM beet, maize, and winter and spring oilseed rape in around 70 representative fields per crop over 3 years. At each site, the fields are split and the GM and non-GM crops allocated to each half field at random. The crops are managed by volunteer farmers as under commercial conditions subject to the regulations concerning GM crops. Crop management is monitored, along with biodiversity indicators—notably plants and invertebrates in and around fields. The first results are due in 2003 and will be peer-reviewed before publication. The division of responsibilities within the project is summarized. This study should make it easier to design environmental risk assessment studies that are better targeted to local agronomic and ecological situations.

Introduction

By October 1998, the first genetically modified herbicide-tolerant (GMHT) crops had cleared most of the regulatory hurdles needed before commercial growing could be permitted in the United Kingdom. These crops (maize, beet, and spring and winter oil-seed rape, or canola) have been modified to make them tolerant to broad-spectrum herbicides—either glyphosate or glufosinate ammonium. Such crops have the potential to allow greater flexibility in the timing of herbicide use, to facilitate the control of herbicide-resistant weeds, and to reduce reliance on persistent and relatively hazardous chemicals—notably atrazine in maize. However, concerns were voiced that this change in weed management might exacerbate the recent declines in biodiversity of arable fields—especially by reducing weed numbers—and thus reduce plant and invertebrate food resources for farmland birds (Krebs et al. 1999). This indirect risk to the environment of growing such crops had not been considered specifically under the existing regulatory system. However, other research suggests that GMHT crops might benefit biodiversity during the growing season because they facilitate later applications of herbicide compared with conventional weed treatments. Thus, the weeds may be allowed to persist longer than in conventional crops, providing food resources and habitat structure for animals during an important part of the year for invertebrates and nesting birds (Dewar et al. 2000). The overall balance of these potentially positive and negative effects of GMHT crops on biodiversity remains uncertain (Firbank and Forcella 2000).

To test these possible effects on biodiversity, the Farm-Scale Evaluations were established (Firbank et al. 1999). This study began in April 1999 and was immediately one of the most controversial agroecological studies ever undertaken because of the background of public concern about genetic modification (Krebs 2000). The study has become the focus of intense media attention and public debate as well as the target for direct action by groups opposed to growing GM crops. The last field season begins in 2002, and the first results due to be reported in 2003.
The study is designed to assess the effects of the agricultural management of field-scale releases of GMHT maize, beets, and winter and spring oilseed rapes (canola) on farmland wildlife abundance and diversity in Great Britain. The project is therefore concerned primarily with comparing the indirect effects of managing GMHT and non-GMHT crops on species diversity, abundance, and trophic relationships (Walker and Lonsdale 2000); it does not constitute a complete environmental risk assessment. The project does not focus on any effects of these specific crops on biodiversity arising from gene flow in Great Britain, as has already been addressed by a growing body of research (e.g. ACRE 1999), although gene-flow monitoring is taking place at the field sites.

The purpose is formalised through the null hypothesis that: there are no significant differences between the biodiversity associated with the management of GM winter oilseed rape, spring oilseed rape, maize, and beet crops that are tolerant to particular broad-spectrum herbicides and comparable non-GM crops at the farm scale.

The research methodology for each of the crops is the same, as far as possible, allowing the results to be presented both separately for individual crops and together for different combinations of crops.

**Project Design**

**Selecting Biodiversity Indicators**

It is impossible to assess the range of biological variation in all living creatures in and around GMHT crops. Therefore, indicators are required to represent larger groups of organisms and to elucidate processes that may lead to significant ecological shifts not detectable directly given the time and spatial scales available for the study. The experiment focuses on the effects of weed management on weed populations and hence on higher trophic levels. We assume that the major ecological effects of GMHT crops result from the direct and indirect effects of the different herbicide regimes on the arable weeds (Firbank et al. 1999; see also Watkinson et al. 2000). The regimes differ in timing and specificity; the herbicides glyphosate and glufosinate ammonium are broad spectrum and can be applied later in the development of tolerant crops than can those herbicides applied to nontolerant crops. The potential advantages to the farmer are the simplification of weed management (because the timing is less critical, and the number of applications required may be reduced) and the option of an additional method of bringing more severe weed infestations under control (Firbank and Forcella 2000). The weeds are important for farmland biodiversity, partly in their own right (Firbank 1999), and partly for their contributions to food resources, cover, and microclimate for other organisms (Potts 1997). The indicators of these weed populations must be sensitive to the differences in weed management and be capable of providing data that can be related to resources for higher trophic levels. These include data on the weed seedbank, seedlings (before and after postemergence herbicide application), adult plants, seed set, and dissemination. The biomass of mature arable plants is also recorded, for this is considered a potential measure of food resources available to animals within the crop towards the end of the season.
To quantify effects on different trophic levels, a broad range of invertebrate groups must be sampled at a variety of habitats in the field, including the soil surface, on the weeds, and on crop plants. The sampled taxa include carabids, collembola, and other soil-surface arthropods; arthropods on vegetation; gastropods; and crop pests. Birds, small mammals, and some insects involved in the food webs have territories and foraging areas that are too large for changes in populations to be detected readily at the scale of the experiment. Bees and butterflies are being monitored, but this work quantifies foraging behavior rather more than effects on populations. In general, potential effects on wideranging species will have to be inferred from changes further down the food webs, using data on biomass as well as abundance of species (see Watkinson et al. 2000).

To monitor treatment effects on field boundary fauna, such as herbicide spray drift, and interactions between field boundary and crop species (e.g., Marshall 1988, Thomas and Marshall 1999), assessments are made of vegetation in the field boundaries. Plant species composition and availability of flower and seed heads are recorded along with gastropods and arthropods.

Any ecological effects due to differences in palatability to herbivores or differences in growth form and phenology of the varieties selected in the experiment will be subsumed within the overall results. Soil organisms were largely excluded from the farm-scale evaluations. This is partly because differences due to cultivation regimes need several years to become apparent (Mele and Carter 1999) but also because very large sample sizes are required to test the null hypothesis adequately, and the phenology of the crop makes surveying very difficult in practice.

**Experimental Design**

The heart of the project is the test of the null hypothesis for biodiversity indicators between pairs of treatment units. The experimental design is, therefore, a randomized block with two treatments (GM and conventional crops) per block. The blocks are represented by individual fields on farms that typify the range of soil and environmental conditions and crop management strategies employed for each crop within Great Britain. The experiment also includes variation between years to take into account variation due to effects of weather on species abundance and crop management. Thus, the total number of sites needs to be spread over the 3 years available to the project, but not necessarily equally. This value was determined through a power analysis using a range of scenarios that encompassed combinations of treatment differences, numbers of sites, and random variability. Perry et al. (in prep) concluded that the use of 60 sites over the course of a 3-year experiment would enable a 1.5-fold multiplicative treatment difference to be detected with greater than 80-percent probability for characteristic levels of variability represented by coefficients of variation around 50-percent. The research program is currently aiming to sow around 75 sites per crop to account for site wastage and also to allow for both upward and downward adjustment of the power estimates as data accumulate during the experiment.

The pilot trials included halved and paired field sites, and from these it was concluded that halved fields were preferable as the experimental unit largely because of the reduced variability between treatments. The fields are split to try to keep biodiversity resources as similar as possible between the two halves (e.g., both halves should have roughly the same
amount of hedgerow or woodland adjacent to them). The allocation of GM crop to field halves is strictly at random and cannot be influenced by the farmer or field surveyor. The number of blocks corresponds to the number of sites at which the crop is grown.

**Choice of Study Sites**

The study sites themselves are designed to represent the conditions under which the crops are likely to be grown commercially should this be approved. Therefore, we are using volunteer farmers growing the crops within appropriate rotations and with a wide geographic spread across Great Britain. Organic farms are excluded because GM crops are not allowed within their current standards. The target populations of farms for each crop have been characterized using existing data in terms of regional distribution and agronomy. We assume that low-intensity, high-biodiversity farms are of particular importance because of their potentially high contribution to regional biodiversity (nota bene Watkinson et al. 2000) and because these may be of particular value in establishing the effects of GMHT crop management on scarce species and more diverse communities.

**Crop Management**

It is important that crop management be representative of how the crops would be managed commercially. Our approach is to allow farmers maximum flexibility to manage both GMHT and non-GMHT crops as they consider appropriate under commercial conditions. The control crop variety is selected by the farmer according to local conditions and can vary between farms.

Although the main differences in crop management between the treatments are most likely to be restricted to different herbicide regimes, differences in rotations, field-margin management, or cultivation are allowed between the two half fields if there are good agronomic reasons. Any insecticides, molluscicides, or fungicides required should be applied on both treatments at the same time unless there is an agronomic reason for any difference (e.g., if there are more pests on one treatment than the other). Any pesticide seed treatments are the same on both treatment and control crops. All crop management is audited to check that it has conformed to good agronomic practice—in particular that the herbicide regime has been appropriate to deliver cost-effective weed control.

**Program of Field Sampling**

Because the half fields are far too large to allow complete biodiversity censuses, data are collected from sample locations and are pooled to provide total values for each half field for each set of observations. The field sampling uses a range of recording procedures to collect data according to the program summarized in Table 1, starting before the crop is sown and continuing into the following crops.
Table 1. Summary of the Field-Assessment Program

<table>
<thead>
<tr>
<th>Survey</th>
<th>Timing and frequency</th>
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<tbody>
<tr>
<td>Crop assessment</td>
<td>At every biodiversity assessment</td>
</tr>
<tr>
<td>Margin attributes</td>
<td>Once</td>
</tr>
<tr>
<td>Soil seedbank</td>
<td>Before sowing and a year later</td>
</tr>
<tr>
<td>Weed seedling counts</td>
<td>Preherbicide, late winter (winter oilseed rape only), mezzanine (a survey undertaken in any lengthy gap between the application of herbicide on one treatment and the application on the other), postherbicide on both treatments</td>
</tr>
<tr>
<td>Weed biomass</td>
<td>Once, before crop harvest</td>
</tr>
<tr>
<td>Seed rain</td>
<td>Continuously from late May until harvest</td>
</tr>
<tr>
<td>Subsequent vegetation</td>
<td>Once, summer after harvest. If significant effects are found, these will be repeated in the year afterwards</td>
</tr>
<tr>
<td>Edge vegetation</td>
<td>Three for spring crops, four for winter oilseed rape</td>
</tr>
<tr>
<td>Gastropods</td>
<td>Three for spring crops, four for winter oilseed rape (within crop and within verge)</td>
</tr>
<tr>
<td>Bee and butterfly</td>
<td>Three for spring crops, four for winter oilseed rape, coincidental with edge vegetation</td>
</tr>
<tr>
<td>Crop pests</td>
<td>Two per year</td>
</tr>
<tr>
<td>Invertebrates on vegetation</td>
<td>Two during spring / summer</td>
</tr>
<tr>
<td>Soil surface arthropods</td>
<td>Three per year</td>
</tr>
</tbody>
</table>

Statistical Analysis of the Data

At its simplest, the analysis consists of a statistical test for each biodiversity indicator assessment for each crop with a wide range of potential covariates, including location, crop growth stage, year, and management variables. The tests are paired, because we are looking at the differences between the two halves of the field, and are two-tailed in as much as we are looking for both increases and decreases for biodiversity indicators on the GMHT crops. These tests will need further interpretation, however, because both positive and negative results may occur by chance. We therefore need to distinguish between results that represent signals of ecological processes and random patterns of significant and nonsignificant results.
Such work will require an understanding of the ecological system as a whole, which may be expressed at different levels of complexity from a decision tree to a formal mathematical model of the dynamics of the ecological components of the system.

The ideal endpoint is one in which ecological models can be generated for each crop that suggest the long-term and large-scale implications of growing the four GMHT crops at a commercial scale across Great Britain. This is an ambitious target and is not required for the project as a whole to be successful. Nevertheless, it is possible in principle given data from other experiments and surveys (see Watkinson et al. 2000, Firbank and Forcella 2000).

**The Division of Responsibilities within the Project**

One of the concerns that has been expressed concerning the farm-scale evaluation (FSE) study is that the biotechnological industry has had an undue influence on its conduct (Anon 2001). In fact this is not the case, and the project has been designed carefully to have clear divisions of responsibility (Firbank 2001).

**The Role of Government**

The research is fully funded by the British Government through the Department for the Environment, Food, and Rural Affairs and the Scottish Executive. The Government established the project specification, which was opened to competitive tender. The Government also has a regulatory role by providing the risk assessments and regulations within which the experiment is conducted and by providing a monitoring service to ensure that these are complied with. The Government is also responsible for disseminating information about the project, including the locations of the field sites. This has involved the establishment of a Web site and also a substantial program of public meetings across the country.

**The Scientific Steering Committee**

The project is supervised by a scientific steering committee (SSC) made up of independent scientists. Their role is to monitor the progress of the work, including the selection of sites and development of the methodologies. These scientists will accept the results of the project only once they have also been accepted for publication by a peer-reviewed scientific journal.

**The Project Consortium**

The research is undertaken by a consortium comprising the Centre for Ecology and Hydrology, the Institute for Arable Crops Research, and the Scottish Crop Research Institute. The consortium is responsible for the conduct of the research, including its publication.

**SCIMAC**

The GM seeds are supplied by companies under the umbrella organization SCIMAC (Supply Chain Initiative for Modified Agricultural Crops), which is legally responsible for ensuring that the crops are grown within the regulations. Farmers apply to SCIMAC to take part in the project, but their acceptance is up to the research team, which has to provide an
adequate sample for approval by the SSC. The farmer is then contracted by SCIMAC to grow the crop. Although SCIMAC provides the seeds and can give some advice concerning crop management, this can only be done for the herbicide regime of the GMHT crop (this is appropriate because so few farmers or advisers have experience of growing these crops). This advice is audited. The SCIMAC is also responsible for appropriate disposal of the crop and has also undertaken a 3-year voluntary agreement with Government not to plant GM crops commercially to allow time for the FSE study.

**The Farmer**

The farmer is responsible for managing the GMHT crop within the guidelines provided by SCIMAC and, more generally, ensuring that both the GM and the conventional crops are managed according to sound agricultural practice. The farmer is also required to provide crop management information to the research teams.

**Discussion**

The Farm-Scale Evaluations are one of the largest ecological experiments ever attempted (J.N. Perry, pers. comm.). Because of the range of biological indicators being studied and the number and variety of field sites, the farm-scale evaluations will provide a detailed study of the relationships between the management of arable crops and the species associated with them; they will also provide the kind of data that are required to model the effects of different crop management regimes on species and ecological communities.

The FSE study will not provide a comprehensive risk assessment of GM crops. The results apply only to herbicide-tolerant crops grown in Britain and only address one particular environmental impact, namely, indirect effects on biodiversity within and around the fields. The results cannot be extrapolated to other crop traits, to other locations, or to other environmental risks.

Fortunately, this does not imply that studies of this scale are going to be required for every kind of GM crop in every country. We are confident that this study will provide a conceptual framework for designing studies appropriate to the ecosystems under study and the likely perturbations that will result from GM cropping. Moreover, it should be possible to use the FSE work to identify those elements of the system that seem to be particularly useful indicators, are easy to measure, and are sensitive to the most likely effects of the GM crops. In other words, we can learn from the FSE study how to design experimental and monitoring programs targeted for local situations.

The FSE study has other important lessons for studies of living modified organisms (LMOs) in general. The first is the importance of partnership between Government, scientists, the biotechnological industry, and the farmers, but this partnership must be created in ways that are transparent to the general public so that they can have confidence in the quality and integrity of the results. The second is that the study of the impacts of LMOs need to take into account the behavior of people; indeed, the role of people deciding how LMOs are taken up and managed may prove the most important variable in many risk assessments. Moreover, no single study can, or should, be expected to provide all the answers concerning risks and benefits of LMOs.
Finally, important limits to our knowledge remain that restrict our ability to forecast the ecological effects of LMOs, or indeed of many other forms of ecosystem disturbance. Thus, work on LMO risk assessment will help inform fundamental research just as fundamental research will help inform risk assessments. However, this can only be done if the results of the many experiments and surveys around the world can feed into metadatabases and ideally have some common structure to allow effective data mining.

Acknowledgements

This work is funded by the Department for the Environment, Food, and Rural Affairs. We thank our many colleagues in this project; they cannot all be listed as authors! We also thank the conference organizers and participants for their comments on this work. Further information is available on the FSE website, http://www.defra.gov.uk/environment/fse/index.htm

References


Monitoring Case Report: Impact of Transgenic Plants Within Cropping Systems

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Abstract

With the development of living modified organisms in the field of agriculture, new concerns about the environmental impact of novel plants and their crop management have been raised and are further addressed here. It is determined that besides the typical case-by-case evaluation already implemented within the regulation process before marketing, a more systemic approach taking into account global, cumulative, and long-term effects is required. Therefore, a French case report from a multiyear, multicrop experimental study is being carried out and suggests that a challenge is open for science to design new approaches, methods, and tools for assessing an overall cost–benefit balance, providing adequate crop management guidelines, and building new monitoring systems. These issues are presented and discussed through the herbicide-tolerant rapeseed case.

Introduction

After about 15 years of biotechnology research carried out by public research teams as well as by private companies, the first marketing releases of living modified organisms (LMOs) occurred in North America in 1995, and they are now planted on a significant part of the arable land. Meanwhile, Europe has strictly limited the commercial releases of LMOs and, apart from Spain, where Bt corn has been cultivated to some extent in 1998, only cultivation for experimental purposes is, in practice, carried out. A moratorium has been decided in different European countries and, owing to environmental and food safety concerns, new regulation rules are reinforcing the premmarket evaluation and traceability of novel products.
More generally, with the development of LMOs, new concerns have been raised and, even if most of them are not specific to recombinant DNA techniques, LMOs are now at the heart of major debates and processes as manifested by the following:

- The drastic increase of knowledge in biology;
- The industrialization and evolution of agricultural systems;
- Food and feed safety of novel products or processes;
- The power of analytical tools and their challenge for traceability and labeling;
- The relationship between science and society and decisionmaking rules; and
- New requirements for environmental sustainability.

**Towards a More Systemic Approach**

With respect to these concerns, the evaluation process still has to be performed on a case-by-case basis to take into account the specific characteristics of each living modified organism (LMO) in terms of traits or plant biology. However, if a considerable amount of knowledge is now available on the impact of each LMO, it is necessary to address the interactions between LMOs within agricultural systems by taking into account the diversity of soil and climatic conditions, of cropping systems, and of farmers’ practices. As a matter of fact, any new technology used in agriculture, even if limited to a single and simple action, may lead to significant changes in ecosystems through various ecological processes and interactions. New methodological tools for assessing systemic effects within the diversity of environmental systems in which LMOs may be cultivated are thus needed. Furthermore, we claim that sustainability of such an innovation requires the ability to anticipate future changes, as far as it is possible, such as changes of environmental conditions due to modification of agricultural practices or future traits to be introduced in plants (e.g., introducing a herbicide resistance gene in wheat would lead to significant changes in the overall risk assessment balance for other herbicide-tolerant crops).

Addressing these objectives is a real challenge for science because new approaches, methods, and tools are required for such a systemic evaluation of the cost–benefit balance of LMOs. Of course, field experiments are a key component of that global evaluation, whereas in the meantime it is necessary to ensure that possible negative effects on the environment remain reversible in case the outcome of the overall balance evaluation indicates that a commercial release is not desirable.

Analyses of the impact of LMOs within cropping have been carried out in France since 1995, when the first files were submitted for clearance in the European Union (Bt176 corn from Novartis, Herbicide-tolerant rapeseed from Monsanto and Rhône–Poulenc). Although the evaluation process was mainly focused on the behavior and impact of each specific LMO, the following objectives were addressed:

- Evaluating the impact of different genetically modified (GM) crops and different traits within cropping systems,
- Assessing cumulative and long-term effects of GM cropping systems,
- Detecting potential and unexpected adverse effects,
• Constructing crop management guidelines for farmers, and
• Providing regulatory bodies with a framework and tools for postmarketing monitoring—the so-called biovigilance or biosurveillance.

**Different methods for such a systemic analysis**

Assessing long-term effects of LMOs on farmers’ crop management of transgenic plants and designing adequate agricultural practices have been addressed by carrying out three main kinds of studies as follows:

1. Specific experiments have been conducted on a particular phenomenon to obtain basic scientific results. This has been done for establishing pollen dispersion curves (Scheffler et al. 1993, Lavigne et al. 1996, Klein 2001) or for assessing the ability of rapeseed to hybridize with wild relatives (Jorgensen and Andersen 1996, Chèvre et al. 2000).

2. Modeling is an essential tool for forecasting the systemic and long-term impact of transgenes within cropping systems. By gathering all available results into dynamic models, it is possible to identify those domains for which basic knowledge is still needed, to predict long-term effects of LMOs and to test, through simulations, the efficiency of mitigation measures. All the results from specific experiments have thus been gathered in order to build the Genesys model, which aims at forecasting the fate of rapeseed volunteers within agricultural systems by taking into account a wide range of landscape patterns and technical practices (Colbach 1998; 2001a,b).

3. Because systemic effects are necessarily taken into account in real situations, monitoring the fields in which LMOs have been introduced or performing retrospective analyses of previously introduced traits in agriculture is thus a powerful tool. Even if results are sometimes difficult to analyze, it is useful to validate our basic conclusions and to detect unexpected or unintended events. Besides the postmarketing monitoring studies performed for commercial releases in the United States or Canada, a survey is being performed in a small region of France to followup on the fate of the high-erucic trait in landscape feral plants since it was removed from rapeseed varieties (Pessel et al. 2000).

**A multicrop and multiyear study for a systemic analysis**

In addition to such approaches and to assess the ecological and agronomic effects of LMOs cultivated under agricultural conditions, a monitoring study has been designed and implemented for various transgenic crops on three platforms located in different regions of France: Champagne, Burgundy and Midi-Pyrénées (southwest). Each platform has a 5- to 6-ha acreage, and transgenic corn, rapeseed, and sugar beets are cultivated under the current regional cropping system and practices (Messéan 1995, Champolivier et al.1999). The transgenic traits considered are as follows:

1. Glufosinate and glyphosate resistance for corn, rapeseed, and sugar beets;

2. European corn borer tolerance (using the Bt system) for corn.
A 500-m area around the field where LMOs are cultivated is monitored to assess the spatial and temporal impact of transgenic crops. This multiyear experiment aims at

1. Assessing the impact of these transgenic crops when they are cultivated together in the same field area;

2. Designing the weed control strategy to be applied to volunteers remaining in subsequent crops that are resistant to the same herbicide (e.g., glyphosate-resistant rapeseed volunteers in the subsequent sugar beet resistant to glyphosate);

3. Estimating the multiple resistance rate observed in plants when we cultivate two adjacent rapeseed fields with two different herbicide resistances;

4. Estimating the crop-to-wild-relative gene flow under natural and local conditions; and

5. Estimating the cost–benefit balance of herbicide resistance technology with respect to conventional techniques.

This study has been carried out since 1995 with wide cooperation, including public research teams (INRA Dijon & Rennes, University of Orsay), agricultural technical institutes (CETIOM, AGPM, ITB, ITCF), and competent authorities. This long-term study is funded by both government and by farmers. A scientific steering committee designs the protocols, discusses results, and validates the final synthesis. A close relationship has been established for several years with various similar European projects—particularly the British network for farm-level risk assessment. A first report was issued at the end of 2000 within the framework of the French moratorium of herbicide-tolerant traits for rapeseed and sugar beets (Astoin et al. 2000, CETIOM 2000, CGB 2000).

A Tentative Cost–benefit Analysis for Herbicide-Tolerant Rapeseed

Available results have been used to estimate an overall cost–benefit balance for each major crop, and the rapeseed case is briefly presented and discussed here.

Weed control

In addition to the increased efficiency observed with the use of broad-spectrum herbicides (better control of weeds not under control today), the herbicide tolerance technology allows farmers to switch from current systematic, preemergence weed control to postemergence weed control when the kind and extent of weeds actually present in the field are known.

From current weed control practices observed in France, it has been estimated that the direct costs for weed control could be reduced by an average 30 percent (from 75 euros/ha to 52 euros/ha). However, this estimate does not take into account either the cost of the technology (seed costs and fees) or additional costs for controlling tolerant volunteers in the
rotation and for other specific management measures. Again with these additional measures excluded from consideration, the amount of active measures used for weed control would decrease from 20 up to 85 percent according to the situation.

It has also been stressed that indirect effects such as minimum soil tillage practices (which are much easier to carry out with the new technology) or manpower requirements could be higher than the direct effects. These effects cannot easily be estimated through experiments or simulations because they depend highly on interactions within the production system and on typical farmer strategies. Even experience from countries already using this technology, although very valuable, is not sufficient to forecast these effects.

**Volunteers**

Seed loss in the field (50 to 300 kg of seeds/ha, or 1,100 to 6,700 seeds/m² on average remaining on the plot at the time of harvest) is a well-known phenomenon that farmers already have to manage in conventional crops by controlling volunteers through mechanical or chemical means during the intercrop periods and through weed control (chemically) in the subsequent rotation crops. However, herbicide-tolerant volunteers have two major specific effects:

1. They can no longer be controlled by the herbicides to which they are tolerant even if conventional herbicides currently used would remain effective on these volunteers. Selection pressure should be avoided by not using a broad spectrum herbicide alone.
2. Owing to higher requirements coming from the marketplace in terms of thresholds for an unintended presence of LMOs in conventional seeds, the volunteers level of control within the subsequent crops must be higher than for conventional farming systems in which farmers only take into account their agronomic competitiveness.

**Seed Dispersal Outside Fields**

Seed dispersal outside fields through wind (short distances) and through machinery (over longer distances) results in a gene flow—particularly to noncultivated areas located near the plots (edges of paths and roads). Genetic profiling of feral plants from these nonagricultural areas was carried out in a regional area (center of France), and it was demonstrated that phenotypes corresponding to conventional varieties that had not been sold for at least 8 years could subsist there (Pessel et al. 2000).

Even if these feral plants produce less pollen than cultivated fields, they contribute to long-distance dispersal of LMOs—particularly if selection pressure is favored by treating such areas with a broad-spectrum herbicide using only glyphosate or ammonium glufosinate.

**Crop to Crop Gene Flow Between Fields**

Dispersion of pollen by wind and insects was mainly studied in continuous field conditions, both in specific experiments and in the platforms previously described. Results show that most of the pollen released remains within several meters of the emitting plant. At 30 m, less than 1 seed out of 100 bears this trait. At 120 m, less than 5 seeds out of 1,000 bear this trait.
Observations of dispersal over long distances (between 400 and 1,000 m) indicate that the dispersion of pollen becomes highly irregular but that tolerant seeds can be detected in most of the commercial fields that have been surveyed (from 0 to 1 seed out of 1,000 bearing this trait). Rates depend on the size of the emitting and receiving fields as well as the pattern of the landscape. No distance could be determined beyond which there would be no dispersion of pollen at all.

The major concern about gene flow between commercial fields is the quality alteration of harvested seeds—particularly for those farmers from the neighborhood who are supposed to market products free of genetic modification. Furthermore, unintended genetically modified (GM) volunteers would appear in conventional fields (owing to pollen flow from GM fields), and selection pressure could occur if agricultural practices were not adapted.

**Crop to Wild Relatives Gene Flow**

Under French conditions, it was established that rapeseed could hybridize with various species—in particular the wild radish (*Raphanus raphanistrum*) and hoary mustard (*Hirschfeldia incana*) (Chèvre et al. 1996). These hybrids cannot be controlled by those herbicides to which they are tolerant, although one of the purposes of this strategy is to control this type of crucifer, which is uncontrolled or poorly controlled today. As for rapeseed volunteers, these hybrids could even be selected if these herbicides are widely used, thereby modifying the flora of nearby cultivated or semicultivated areas.

At our platforms, we are surveying wild relatives and looking gene flow from crop to wild relatives. More than 75,000 seeds of wild mustard (*Sinapis arvensis*) have been collected over a 4 year period from plants sampled inside the field where transgenic rapeseed is cultivated as well as in the monitoring area. None of them has become tolerant to the nonselective herbicides (Astoin et al. 2000). In the case of the wild radish, a very common weed in French rapeseed fields, even if the probability of the appearance at a given location of interspecific hybrids with a tolerance to an herbicide is low (Chèvre et al. 2000), we must consider that it will occur. Thus, the fitness of such hybrids and their fate within cropping systems have to be assessed, and selection pressure should be avoided.

**Constructing Crop Management Guidelines**

Herbicide tolerance technology has potential benefits in the chemical weed control of rapeseed under the current French agricultural models: better weed control efficacy, a postemergence weed control strategy, higher flexibility in timing and cultivating practices, less chemical pressure, and lower costs. However, the indirect effects of herbicide tolerance on crop management and farmer’s practices could change the overall balance. Furthermore, its efficiency with respect to alternative practices or agricultural systems is a major issue that must be addressed—particularly in the European context.

Undesirable effects related to gene flow result mainly in **agronomic** considerations (persistence of resistant volunteers, creation of new weeds, multiple resistance) and **commercial** considerations (unintended presence of LMOs in conventional rapeseed production affecting the plant’s competitiveness in the marketplace).
Many results are now available for these concerns, but more research is required on the impact of larger field size for pollen dispersal and on gene flow from the crop to wild relatives under natural conditions.

From the available results, it can be stressed that herbicide-tolerant rapeseed cannot be cultivated without applying specific guidelines for crop management. However, and even if research is still required, specific crop management guidelines have been suggested to limit the undesirable effects by achieving two main objectives:

1. The development or extension of practices aiming at reducing, in time and space, the persistence of undesirable plants (volunteers and hybrids with wild relatives; and
2. The avoidance of selection pressure on these undesirable plants.

Five mitigation measures have been defined by Centre Technique Interprofessionnel des Oleagineux Métropolitains (CETIOM) (Messéan et al. 2001) for building global guidelines for management of herbicide-tolerant rapeseed:

1. Favor the immediate emergence of seeds remaining on the soil after harvesting in order to withdraw them from the seed bank: no tillage until the first rain and then repeated minimum soil tillage to avoid seed dormancy (Lutman and Sweet 2000).
2. Increase control of rapeseed volunteers within the subsequent crops. The requirement is not only made to avoid competitiveness of weeds but also to reduce the seed bank.
3. Avoid other crops resistant to the same herbicide within the rotation to make the control of tolerant volunteers easier in the subsequent crops.
4. Organize the spatial location of crops through adequate isolation distances, through regional specialization, or both.
5. Dedicate the use of broad-spectrum herbicides with their active material used alone (glyphosate or glufosinate) to tolerant crops and associate another active material with these for their nonselective uses (preharvest applications, fallow land management).

These mitigation measures are being gathered to build scenarios for herbicide-tolerant rapeseed management. Their effectiveness is currently estimated through the Genesys model (Colbach et al. 2001a,b) by simulating the ability of each scenario to keep the impact of transgenes within agro-ecosystems under control and to manage the coexistence between LMOs and non-LMOs (Angevin et al. 2001). This effectiveness is highly dependent on the threshold level that will be finally adopted for LMO presence in conventional products. Furthermore, the capability of the economic agents (farmers, cooperatives, agrochemical and seed companies) to carry out such measures and to cooperate has to be taken into account.

**Tools for Postmarketing Monitoring**

Even if the premarketing evaluation process must be improved by taking into account systemic effects on the environment, it will never ensure that no unintended event
will occur. A postmarketing monitoring system (or “biovigilance”) must be implemented in order to

1. Monitor the undesirable agro-environmental impacts (development of volunteers of rapeseed or tolerant hybrids) and detect discrepancies with respect to the premarketing evaluation and

2. Detect as soon as possible the unintended or unexpected effects that have not been identified during the premarketing process

The general framework for such a monitoring system is widely agreed upon, but the precise threshold between the premarketing process and the postrelease monitoring system is one major issue on which countries certainly diverge. But, in fact, the premarketing evaluation, mitigation measures, and postmarketing monitoring are parts of a continuous process aiming at ensuring an efficient and sustainable development of new technologies.

Conclusions

Results from various risk assessment studies suggest that there is no direct major ecological risk in the case of herbicide-resistant oilseed rape, because the presence of a gene for tolerance to an herbicide in oilseed rape (or a related species) does not appear to increase its fitness in natural ecosystems (CGB 2000). However, various agronomic and commercial concerns have been raised, and specific crop management guidelines are required. Mitigation measures have been defined and are being evaluated in terms of efficacy to keep under control the unexpected or undesirable events and in terms of feasibility and acceptability.

Furthermore, even if North American experience provides us with data that, after evaluation, may be of equal value for European agriculture and these data are implemented to some extent, large-scale experiments are required under European agro-ecosystems to assess the effect of scaling-up as well as to establish an overall environmental balance, to design guidelines for crop management, and to build methods and tools for monitoring. Furthermore, a global consideration has to be given to the impacts of large-scale use of those two nonselective herbicides whose resistance is being introduced in various crops.

References


Abstract

Biosafety studies typically show no difference in hybridization between genetically modified plants (GMPs) or non-GMPs with related wild species. Because risk is a product of both exposure and hazard, biosafety research should clearly not only target gene-flow exposure but specifically concentrate on expected hazards emerging from successful transgene flow to wild relatives of GMPs. The conventional sugar beet (Beta vulgaris ssp. vulgaris) has now been cultivated for 200 years. This cultivar has not shown unwanted ecological effects despite the introduction and spread of this European species to the New World. The only realistic way of assessing the environmental effect of transgenic beets is a comparison with classically bred cultivars. In particular, we compared the ecological performance of rhizomania-resistant genotypes under various environmental conditions with regard to parameters such as winter hardiness, seed production, and ecological relevance of virus resistance.

Introduction

The number of biosafety-related publications concerning transgenic organisms has increased within a decade (1990 to 2002) more than 3,700 citations according to one of the most comprehensive databases (http://www.icgeb.trieste.it/~bsafesrv/). Because risk is a product of both exposure and hazard (Sharples 1991), it is clear that biosafety research on environmental effects should not only target the probability of gene flow but must also focus on the consequences (and potential hazards) of successful transgene flow to relatives of transgenic crops. This means that biosafety research should address the phenotype (especially the fitness phenotype) of the transgenic hybrid versus that of nontransgenic controls.

In their outstanding review Ellstrand et al. (1999) demonstrated that 12 of the 13 most important crops worldwide hybridize with wild relatives somewhere within their cultivation area. These events could be defined as baselines in the sense of evolutionary references for crops with transgenes. However, with the exception of worst-case laboratory studies, no adverse effect has been reported for transgenic plants so far; but hazard is based on anthropocentric assessment and value judgement.
that is very often a sociological–political compromise made by different stakeholders. Scientific biosafety research can therefore not answer a question such as Can we observe an unwanted spread of transgenes? Instead, biosafety research has a better chance of providing answers to questions such as Is there a difference in the spread and environmental effect of transgenic in comparison conventional plants?

The definition of “unwanted” is a political one, and nature conservation has more to do with public perception than with scientific observation. Today, unwanted ecological effects are commonly manifested by the decline of rare species or loss of genetic diversity. These phenomena are not new for they are a well-known aspect of human activity that has increased within agricultural practice over the last 10,000 years. Today, the situation requires a case-by-case and step-by-step assessment of GMPs. Here, we present the example of transgenic sugar beets resistant to rhizomania virus.

Conventional sugar beet (*Beta vulgaris ssp. vulgaris* L.) has now been cultivated for 200 years. This cultivar has not shown unwanted ecological effects despite the introduction and spread of this European species to the New World (Bartsch and Ellstrand 1999). The only realistic way of assessing the environmental effect of transgenic beets is a comparison with classically bred cultivars. In particular, we compared the ecological performance of rhizomania-resistant genotypes under various environmental conditions with regard to parameters such as competitiveness, winter hardiness, and seed production. Our results are summarized below.

**Results**

**Winter Hardiness**

The biennial sugar beet needs to survive cold winter temperatures in order to produce offspring. Winter hardiness is an important ecological factor for the geographical distribution of cultivated and wild beets in Europe. The natural distribution range is limited to mild areas at the seacoast in the Northern Hemisphere. Some of our experiments focused on overwintering of transgenic and nontransgenic sugar beets at different locations in Europe experiencing mild to cold winters in the years 1994–99. We found no survival differences even under virus infestation conditions (Pohl-Orf et al. 1999; fig. 1A).

**Sexual Reproduction**

Transgenic attributes are transmitted by natural reproduction and by gene flow to all sexually compatible relatives. One prerequisite is sympatric growth of cultivars and their hybridization partners. Only a few plants can cross with the sugar beet: the Swiss chard, fodder beet, table beet, and wild *Beta* species belonging to the Section Vulgaris. No difference was found in the hybridization ability of transgenic in comparison with non-transgenic controls (Bartsch and Pohl-Orf 1996, Dietz-Pfeilstetter and Kirchner 1998). No significant differences among the three plant genotypes were found at a given virus infestation level in terms of seed production (fig. 1B).
Fig. 1: Ecological parameters (A) winter hardness and (B) seed production of two wild beet hybrids and sugar beet cultivar grown with or without virus infection. Mean levels of characters with the same indicator letter are not significantly different by 2-way Anova / Tukey Test. Mean and Standard Error are given, n.d. = not detected)
Development of Weediness Due to Early Bolting

Weeds are simply plants in the wrong place in either agricultural or nature conservation areas. Interestingly, the same species can be protected as a plant genetic resource in one country and eradicated as a weed in another.

Beet seed bolters pose problems for mechanical harvest machinery and reduce yields; therefore they are regarded as weeds in sugar beet fields. In contrast early bolting and seed production in the first vegetation period is an important attribute for the ecological distribution of beets, because freezing temperatures can be better tolerated by seed in the Northern Hemisphere. In addition, the development of an annual habit is also important for the weediness of beets in disturbed habitats such as agricultural fields.

The unwanted annual habit can evolve in two ways: random introgression of genetically dominant genes from wild beets or selective reevolution towards wild characters (genetic drawback). The latter phenomenon was targeted by one of our field experiments. We found that the transgenic genotype had a much “safer” performance, owing to its higher resistance to early prebolting, than the isogenic control (Bartsch et al. 2001). Because the physiological background is still unknown, this pleiotropic effect should be carefully considered and cannot be related to transformation events *per se*.

Ecological Relevance of Virus Resistance

If virus resistance is ecologically significant, we should detect rhizomania virus in sea beets. We checked 40 populations of sea beet and could not detect virus infested plants. Therefore, there is no ecological advantage for transgenic hybrids in sea beet habitats. The reason for this is that the habitat of sea beets is found in coastal areas. There the plants are regularly exposed to showers of salty water and temporary flooding. Indeed, the level of virus infection is influenced by salt concentration. In our experiment we irrigated a highly susceptible sugar beet variety and sea beets from six different populations from Italy with water (both 0.5 percent and 1 percent salt solutions). Plants were grown in virus-contaminated soil. Our results showed that in every population the level of virus infection depended upon salt concentration. With increasing salt concentration virus infection decreased. But this picture is not consistent, population C was more susceptible when irrigated with 0.5 percent solution than with the 1 percent solution, and population A showed no susceptibility to rhizomania virus infection (fig.2) most likely due to an inherent natural resistance.

Discussion

Generally, the ecological behavior of transgenic sugar beet plants is similar to that of non-GMPs if the modified trait confers a neutral advantage under environmental or experimental conditions. However, GMPs perform better than non-GMPs if the new phenotype is challenged by conditions ecologically advantageous for the modified trait. So far, we have no evidence that the use of GMPs has an adverse impact on sustainable agriculture and nature conservation *per se*.
There is still a gap in basic long-term knowledge about how conventional pest management strategies influence nontarget species in agricultural systems. We also have little knowledge of how past gene flow from cultivars may have influenced the genetic diversity of related wild species (Bartsch et al. 1999). Biosafety research and monitoring may become the driving force for comprehensive studies encompassing traditional and modern agricultural systems. Overall, new extensive field studies support the view that harmful effects on nontarget organisms in the laboratory are rarely detected in the environment—at least not so far (Bartsch and Schuphan 2002). Biosafety studies have typically demonstrated that there is no difference in the hybridization ability of GMPs compared with non-GMPs with crossbreeding wild populations. Indeed, numerous reports describe cultivars that have escaped into natural ecosystems (Bartsch and Ellstrand 1999). In this respect transgenic plants will be no exception, and we have no evidence that the use of GMPs is contrary to sustainable agriculture and nature conservation per se. No field study has reported a severe effect caused directly by transgenic plants, but this could be based on the poor ecological relevance of traits such as herbicide, virus, and insect resistance (Saeglitz and Bartsch 2002). In the future, more significant implications may arise with the introduction of ecologically more important traits such as drought and salt tolerance. Because biosafety research is a time-consuming and resource-intensive process, we will have to concentrate on well-thought-out and thorough experiments as well as on targeting the ecologically “riskier” organisms.

Biosafety research cannot solve every open and basic question of general ecology (Kareiva et al. 1997). After the best pragmatic use of the case–by–case and step–by–step approach, a well-designed monitoring program is necessary following commercialization. This monitoring must prove, on a larger scale, the prognostic assumptions made by former biosafety research and assessment (Marvier et al. 1999). We know for certain that containment strategies do not work properly and provide no justification to avoid monitoring (Sukopp and Sukopp 1993;
Saeglitz et al. 2000). Monitoring must be flexible enough to recognize unpredictable phenomena such as pleiotropic effects. Currently, we have no evidence that transgenic plants systematically express more pleiotropic effects than plants from classical breeding programs (Bartsch and Schuphan 2002).

References


Nontarget Food Chain Effects and the “GMO-Guidelines Project”

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Abstract

Current biosafety procedures are inadequate to test the impacts of transgenic crops on their environment. For instance, current U.S. models rely on pesticide methodologies and consider novel transgenic products, such as the Bt toxin, as equivalent to the toxin when used as a pesticide. However, toxin exposure for prey and their natural enemies from transgenic plants is very different from pesticide exposure as shown by the effects of Bt toxin on Chrysoperla carnea (the green lacewing) when it ingests the toxin via Bt maize fed to prey species rather than an artificial Bt-containing diet. The example illustrates the need for new biosafety methodologies that go further than pesticide-based approaches. This paper launches an international initiative of public sector scientists that aims to develop comprehensive scientific guidelines for prerelease biosafety testing of transgenic plants. Public sector scientists from developing and developed countries are invited to join the group and participate in this unique effort.

Introduction

Currently, commercially produced transgenic plants are mainly cultivars of corn, cotton, soybeans, and canola that are tolerant to herbicides, insect resistant, or both (James 2002). Insect resistance is mostly due to a novel, highly bioactive compound, the Bacillus thuringiensis (Bt) toxin. This toxin is produced in high concentrations throughout most parts of the transgenic plant and throughout most of the growing season. Consequently, most if not all organisms feeding and living on these transgenic plants are likely to be exposed to the expressed novel toxin for a prolonged period, potentially leading to so-called nontarget effects. Nontarget effects are any unintended side effect of the transgenic plant on organisms other than the target pest species. These include detritivorous organisms, pollinators, other herbivores, and higher trophic level organisms such as insect natural enemies. Nontarget effects are well known from the use of pesticides. These have led to serious agroecological problems (e.g., the development of pest resistance and disruption of naturally occurring regulation mechanisms, leading to secondary pests and pest resurgences) and have resulted in an ever increasing dependency on synthetic chemicals in industrial agriculture. On the basis of this experience, nontarget effects of novel insecticidal transgenic plants need to be carefully investigated.
The constitutive expression of an insecticidal gene, such as the Bt toxin product, can be expected to have highly complex impacts on the insect community associated with the introgressed transgenic plant in the region where it is grown such as changes in community composition and structure (fig. 1) (Obrycki et al. 2001, Hilbeck 2001, 2002). Although Bt toxins are typically most effective against the larval stages of particular herbivore species, they may have subtle sublethal effects on other herbivores. Ecologically, these can be as disruptive for population dynamic processes and trophic interactions as lethal effects.

Natural enemies will be exposed to novel plant compounds in transgenic plants predominantly via their prey or hosts, although some natural enemies also feed to a limited degree directly on the host plants as a source of water and certain additional nutrients. Thus, our major concern is so-called tritrophic effects. These are effects which the first trophic level (the host plant, here the transgenic plant) exerts on the third trophic level (the natural enemies of the plant-feeding herbivores) mediated via the second trophic level (herbivores feeding on the transgenic plant). Tritrophic interactions are more than the sum of two bitrophic interactions, that is, host plant–herbivore and herbivore–natural enemy interactions (Kareiva and Sahakian 1990, Malcolm 1992). Although the primary concern in agricultural ecosystems is that such tritrophic effects may offset the benefits from biological control if natural enemies are adversely affected by the Bt toxin (or any other insecticidal compound for that matter), an additional concern in unmanaged natural ecosystems is a decline in biodiversity due to adversely affected insect species at whatever trophic level.

Exposure of, and impact on, natural enemies could be highly complicated and composed of several overlaying factors, as outlined in figure 1. When herbivores ingest the novel insecticidal compound, natural enemies can be affected in various ways as follows:

1. The insecticidal compound, or any metabolite of it, may affect the natural enemy directly;
2. The insecticidal compound, or any metabolite of it, exerts an interaction effect in concert with other secondary or primary compound(s) of the plant (e.g. 2,4-dihydroxy-7-methoxy-(2H)-1,4-benoxazin-3(4H)-one (DIMBOA) in corn);
3. The insecticidal compound affects the nutritional quality of the sublethally affected prey or host herbivore and thus affects the natural enemy indirectly;
4. The natural enemy may be affected by any combination or all of the above. It will be very difficult to distinguish between these different levels of impact, and the limited resources for research in this field may simply render it unfeasible.
**Current Biosafety Testing Procedures**

Current prerelease biosafety testing procedures draw heavily on the testing protocols for pesticides, that is, short-term testing of a few indicator species for acute, direct (bitrophic) effects. In the U.S., an additive two-part model for regulating transgenic Bt plants is applied consisting of the conventional crop plant and the Bt-toxin. The conventional crop plant is considered safe, and consequently no additional testing is required; thus the “added” expressed Bt toxin is considered simply as a pesticide outside its plant context and tested as such. This means that prerelease, nontarget testing is conducted with either microbially produced purified toxins or with highly processed, lyophilized, ground-plant protein typically in a bitrophic experimental setup (i.e., the toxins are administered directly to the natural enemy and not via a prey or host species).

This pesticide paradigm is deficient in several aspects. First, pesticide release is controlled by the applicator, who determines timing, point location, concentration, frequency, and much more. Spray coverage of the crop plant is rarely ever complete, and thus unsprayed refuges remain where nontarget and target organisms can survive. Second, pesticide degradation begins immediately after application. And third, the mode of action for most synthetic pesticides is typically acute and immediate also for nontarget organisms. In contrast, transgenic Bt-plants release the Bt toxin continuously and in almost all plant parts. The tissue-specific toxin production varies over time and in different environments, and the mode of action is not immediate (it takes 2 days or longer before even the target pest dies) and not necessarily acute. Sublethal, chronic effects become more important for nontarget organisms. The resulting dynamics and types of nontarget effects therefore differ from those caused by pesticides.
These deficiencies of the additive concept for prerelease testing and environmental impact assessment can lead to under- or overestimation of the real impact. Any position, pleiotropic, or epistatic effects as well as any interaction effects are also ignored. An illustrative case example is the impact of Bt proteins and transgenic Bt-corn on *Chrysoperla carnea* (1998 a,b; 1999; for an overview see Hilbeck 2002).

**The *Chrysoperla carnea* Example**

The effects of transgenic Bt-expressing maize and microbially produced Bt-proteins on an important, very polyphagous natural enemy species, *Chrysoperla carnea* (the green lacewing), were studied in a tri- and bitrophic model system approach. Three series of no-choice experiments were carried out using different Bt-delivery systems, transgenic Bt-maize, and Bt-incorporated diets. Prey-mediated effects of Bt-containing diets for herbivorous prey and direct effects of a Bt toxin on *C. carnea* larvae were investigated. The results of all three series of experiments consistently demonstrated the susceptibility of immature *C. carnea* to Bt proteins (Cry1Ab toxin and protoxin, Cry2A protoxin) either provided via prey or directly (Hilbeck et al. 1998a, b; Hilbeck et al. 1999). The degree of mortality varied depending on the Bt-delivery system, and an increase in toxicity of the Bt protein through the food chain was observed. Prey-mediated mortality of immature *C. carnea* was highest when the prey food source was transgenic Bt-maize (59-66 percent) relative to the concentration of the Bt toxin Cry1Ab, which was the lowest in plants (<5 ug/g fresh weight [Fearing et al. 1997]) compared with all other concentrations in the other diets. When feeding the Bt toxin (100 mg Cry1Ab/mL artificial diet) directly to chrysopid larvae at a concentration approximately 10–20–fold higher than in the transgenic plants, the induced mean total immature mortality of *C. carnea* was significantly higher than in the respective control but lower than expected, and similar to the prey-mediated mortality induced by transgenic Bt maize expressing lower Bt concentrations. But when the comparable Bt toxin concentration was incorporated into a meridic diet (100 mg Cry1Ab toxin/g meridic diet) and provided via lepidopteran prey to *C. carnea*, total immature mortality of *C. carnea* was 21 percent higher (78 percent) than when feeding this concentration directly to *C. carnea* larvae (57 percent). At this high concentration, *C. carnea* mortality may also have been confounded by increased intoxication of *Spodoptera littoralis* (42 percent) that was observed at that concentration only. But similar effects were observed when incorporating Cry1Ab toxin at lower concentrations (50 and 25 g into meridic diet), where *Spodoptera littoralis* was not lethally affected by these Bt concentrations. However, they did exhibit a sublethal effect: stunting of growth (for more details see Hilbeck et al. 1999).

Also Bt protoxin-incorporated diets (Cry1Ab and Cry2A) caused significantly higher prey-mediated mortality in immature *C. carnea* than in the untreated control, although to a lower degree than the Cry1Ab-toxin-incorporated diet. *S. littoralis* was not lethally affected by the protoxins, regardless of the concentrations applied, but exhibited similar sub lethal effects as in the toxin-treatment. Further, when comparing control mortalities of all studies, prey-mediated *C. carnea* mortality in the trials using transgenic Bt maize plants was approximately 11 percent higher (37 percent for both *S. littoralis* and *Ostrinia nubilalis* fed predator larvae) than when using the meridic diet (26 percent), suggesting that plant-fed prey larvae were less suitable for optimal nutrition of immature *C. carnea* than meridic diet-fed prey.
The need for new biosafety methodologies to accommodate the differences between transgenic plants and pesticides and that consider the novelty of the technology is evident. In the following section, a new initiative of an international group of public sector scientists aiming to develop such new guidelines will be presented.

The GMO Guidelines Project of the IOBC Global Working Group

The guidelines project “Development of International Scientific Biosafety Testing Guidelines for Transgenic Plants” is an international initiative of public sector scientists organized within a global working group on “Transgenic Organisms in Integrated Pest Management and Biological Control” under the umbrella of the International Organization of Biological Control (IOBC). This project is funded by the Swiss Agency for Development and Cooperation.

The project aims to accomplish the following:

1. Develop comprehensive, transparent scientific guidelines for prerelease biosafety testing of transgenic plants such as could serve as an international standard.
2. Facilitate the development of scientific capacity in the contributing countries that can guide the implementation of the guidelines.
3. Test the application of the guidelines in real policy contexts to assist in the evaluation of particular transgenic crops.
4. Publish the guidelines and periodically revise them in response to new developments, thereby keeping them up-to-date and providing for their long-time use.
5. Extend the guidelines for possible use in postrelease monitoring.

The guidelines will give a series of questions and corresponding methodologies by which any particular genetically modified organisms (GMO) issue can be evaluated scientifically. The questions will start by addressing broader issues and will become progressively more specific and be structured as a series of interlinking modules. The guidelines will have no regulatory legitimacy themselves, but regulatory authorities can choose to implement parts or all of the guidelines as they desire or need with confidence in the scientific soundness behind the evaluations. The guidelines will also be tested in real policy contexts using case studies from countries in Africa, Asia, and South America. This will take place in 3 workshops over the next 2 years.

The development of the guidelines will be an open process that incorporates scientific and technical capacity building and communication between scientists and policy-makers in developed and developing countries. The project is coordinated by a steering committee of public scientists and invites contributions from public sector scientists from all countries, who will form the core group of the project. Figure 2 illustrates the organization of the first year of the project. An advisory committee comprising of representatives from various international and national organizations will accompany the process to critique the drafts constructively and advise on their improvement.
Scientific Approach to the Guidelines

The scientific work is divided into five sections: needs analysis or good agricultural practices, transgenic plant characterization, nontarget and biodiversity effects, pest-resistance management, and gene flow and its effects (figure 1).

Needs Analysis/Good Agricultural Practices

This section sets the context for the rest of the analysis. It will provide a framework for evaluating the need for the transgenic plant in specific crop production contexts. This includes providing an approach to evaluating projected changes in crop production practices such as tillage systems or insecticide use.

Transgenic Plant Characterization

This section will assess (1) how a transgene should be described to enable evaluation of its stability and inheritance; and (2) how the phenotypic effects of the transgene in the plant should be specified to facilitate assessment and management of environmental effects (what, how, what plant parts, and when product concentrations should be measured in transgenic plants).

Typically, transgenes are comprised of integrating elements, a marker gene and its promoter, a target gene and its promoter, and possibly other genetic elements. Questions arising here are, How many copies of the transgene elements are incorporated into the plant? Where is the transgene integrated in the genome? Where might the transgene break into parts (by recombination or other genetic mechanisms)? How is stability evaluated or proved?

Non-target Effects and Biodiversity

In this section, there are two main tasks to accomplish for each of the identified categories of organisms: (1) Specify a procedure to determine the nontarget species or function or processes that should be tested (= selection procedures). (2) Specify scientific procedures for testing these species, functions, or processes (= testing procedures). Seven categories of organisms that need to be addressed have been identified: (a) natural enemies, (b) pollinators, (c) soil organisms, (d) species of conservation concern, (e) species of cultural significance, (f) nontarget pests, (g) other non-target species. Routes of exposure need to be identified. Exposed organisms are determined through suspected causal chains of impact. On the basis of this information, protocols and methodologies for appropriate testing can be developed.

Pest Resistance Management

To determine the resistance risk and management responses needed to reduce this risk, it will be important to address the feasibility of implementation. In addition, approaches for developing a practical monitoring and response system to detect resistance and to adapt management appropriately should be considered. Although this primarily addresses resistance development in pests, resistance development of weeds as a result of commercial production of transgenic herbicide-tolerant crops will also be considered.
Figure 2: Organisation and Product Development in the First Year of the GM0 Guidelines Project
Gene Flow and Its Effects

Gene flow is the route along which transgenes can spread genetically into populations of related species and geographically into other regions, including protected areas of sensitive ecological value. Gene flow is considered a risk because of the great uncertainties associated with the possible consequences in the recipient ecosystems. Successful transgene flow will simultaneously affect both recipient plants and their associated organisms. Protocols need to be developed for establishing (a) the likelihood of intra- and interspecific gene flow, (b) the possibility of subsequent geographic and genetic spread of transgenes, (c) the potential ecological effects resulting from gene flow, and (d) the effectiveness of sterility mechanisms, their breakdown, and management.

Launch of the GMO Guidelines Project and Invitation to Participate

Current biosafety procedures are inadequate to test the impacts of transgenic crops on their environment. Current U.S. models rely on pesticide methodologies and consider novel transgenic products, such as the Bt toxin, as equivalent to the toxin when used as a pesticide. However exposure is very different when the product is continually expressed in the plant tissues as against when it is periodically applied as a pesticide. This is illustrated by the effects of Bt toxin on *C. carnea* (the green lacewing) when it ingests the toxin via Bt maize fed prey species as compared with an artificial Bt-containing diet. When feeding the Bt toxin directly to chrysopid larvae, the induced mean total immature mortality of *C. carnea* was similar to the mortality from prey food fed Bt maize that contained Bt concentrations approximately 10- to 20-fold lower than in the artificial diet. In addition, the chrysopid larvae exhibited a sublethal effect of the Bt toxin: stunted growth. Such tritrophic effects on natural enemies could be composed several of interacting factors that are difficult to distinguish, and they can be as disruptive for population dynamic processes and trophic interactions (leading to serious agroecological problems) as the direct lethal effects of the Bt toxin. This example illustrates the need for new biosafety methodologies that go further than pesticide-based approaches and that consider the novelty of the technology.

This paper presents the launch of an international initiative of public sector scientists that aims to develop comprehensive scientific guidelines for prerelease biosafety testing of transgenic plants such as could serve as an international standard. The guidelines will be tested in real policy contexts using transgenic-crop case studies from countries in Africa, Asia and South America over the next 2 1/2 years. The guidelines will present a series of questions and corresponding methodologies by which any particular GMO issue can be evaluated scientifically. The guidelines will be published in the open literature and on the project Web site and will be revised regularly to ensure their continual relevance.

For further information on the GMO Guidelines Project, please contact Angelika Hilbeck (hilbeck@geobot.umnw.ethz.ch) or Evelyn Underwood at the project secretariat (underwood@geobot.umnw.ethz.ch), or go to our Web site (http://www.gmo-guidelines.info). Public sector scientists from developing and developed countries are invited to join the group and participate in this unique effort.
References


Session 3: A Scientific Framework for Assessing Transgenic Organisms in the Environment

Session 3B—Transgenic LMOs in the Environment:
Additional Considerations
Effect of Living Modified Organisms on the Soil

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Abstract

Methods to determine baseline perturbations of soil microbial communities and functions are illustrated briefly. The impact of living modified organisms (LMOs), whether genetic modification is in the plant or rhizosphere microorganisms, is usually small compared with changes induced by conventional agricultural practices. Notwithstanding, plant nutrition can be improved by LMOs and the ACC deaminase gene can decrease plant stress and facilitate bioremediation. Modification of organisms by insertion of marker genes can decrease biological fitness of the organism, and therefore the environmental impact of microbial LMOs is likely to be less than those produced by wild types.

Introduction

Micro-organisms have been used extensively in crop protection as well as bioremediation and are seen as primary targets for genetic modification to improve performance. However, it has also been quickly realized that the useful genes could also be incorporated into plants and that this might provide a more predictable and economically viable route for delivery. Thus, the vast majority of release requests received by most national regulatory committees have been for living modified organisms (LMO) plants rather than micro-organisms. However, the principles of determining any perturbations on the baseline soil ecology are essentially the same irrespective of the type of LMO being introduced. The rhizosphere is a site of particular interest because 40 percent of the plant’s captured photosynthate is released as rhizodeposition products and is therefore available to the soil biota (Lynch and Whipps 1990). This is therefore the energy powerhouse of the soil ecosystem and is also the site at which introduced genes, plant or microbial, may exert greatest influence.

Horizontal Gene Movement

In the early investigations, the primary concern was whether there would be horizontal gene transfer from the introduced living modified organisms (LMOs). To investigate LMO effects, marker genes were used as the initial targets. In our own studies we focused on two common soil–rhizosphere organisms,
Enterobacter cloacae and Pseudomonas cepacia. The donor strains had tetracycline and trimethoprim resistance on plasmids, and the recipients had nalidixic acid resistance. Continuous-flow columns packed with coarse sand or glass beads, with or without a hollow fiber down the center pumped with glucose to stimulate rhizodeposition from a root were used (Sun et al. 1993, 1999; Pearce et al. 1997, 2000, 2001).

For E. cloacae one transconjugant was produced per 10⁴ and 10⁶ donors or recipients, but the level was heavily dependent on the flow rate down the column and the distance from the hollow fiber (Pearce et al. 2001). This rate is comparable with the plasmid exchange frequencies that have been observed for other soil bacteria. Gene exchange has generally not been observed from chromosomally borne genes. Plasmid fluidity between wild types is a natural occurrence, but antibiotic marker or functional gene marking would have uncertain consequences; therefore, it seems reasonable that chromosomal genetic modification is the safest option.

Need for New Technologies for Baseline Population Studies

Traditionally, monitoring of microbial populations in soil has centered on the enumeration of specific populations. However, for a significant perturbation to be measured, changes of between 100 and 300 percent (0.3 and 0.5 on a log scale) are necessary. Moreover, there is the problem of nonculturability. In one study (Troxler et al. 1997), only 0.08 percent of Pseudomonas fluorescens CHAO–Rif cells were culturable after 200 days, 4.77 percent were viable but nonculturable, and 95.2 percent were dormant or nonviable of the methods relying on culturability. We have found the most effective way to assess culturables quantitatively is to determine the r and K strategists from different habitats and to formulate an ecophysiological index (EPI) (De Leij et al. 1993), which is a technique finding increasingly wide acceptance (e.g., Van Elsas et al. 2002). A range of nonculture techniques that do not rely on culturability of micro-organisms such as fatty acid, methyl ester organic phyrophosphate content with ms pyrolysis, immunofluorescence, and cellular protein profiles have been used, but these seldom give a perspective on the perturbations that affect the ecosystem. There is a similar problem with the nucleic-acid-based methods such as restriction fragment length polymorphism (RFLP), DNA fingerprinting, amplified ribosomal DNA-restriction analysis (ARDRA), analysis of randomly amplified polymorphic DNA markers (RAPD), polymerase chain reaction (PCR), DNA–RNA sequence analysis, or hybridization probes and community hybridization using the reannealing of DNA samples.

Methods that target ecosystem functions or gene products can be very useful. Commonly these can involve enzymes as the gene products or nutrient cycle analysis. Ultimately the impact on the plants themselves can be most readily determined by a plant bioassay. Similarly, soil faunal assays can be carried out. It is important in this respect to use several methods to determine the ecosystem baseline and the way in which it is perturbed. What follows are some examples of a few studies in which this has been done.

Marker Genes

The first release of a free-living LMO bacterium in the United Kingdom (UK) was carried out during 1993 and 1994 on spring wheat in a silt loam at Littlehampton, West Sussex, and on sugar beet in a heavy clay soil in Oxford. The bacterium Pseudomonas
fluorescens SBW 25 was isolated from the phylloplane of sugar beet, but it was also shown to colonize the rhizosphere of the sugar beets readily as well as the phylloplane and rhizosphere of wheat. The marker genes (lacZY and kan’xylE) were chosen to facilitate identification and detection of the LMO by simple culture methods and positioned 1 Mb apart on the 6.5Mb chromosome to ensure genotypic and phenotypic stability as well as to facilitate any gene exchange between microbial populations associated with the two crops. At the Ee site, lacZY (4.0kb) was inserted, and at the 6 site, kan’–xylE (7.2kb) was inserted (Rainey and Bailey 1996).

Prerelease studies were carried out to determine the natural ecology of the phylloplane of the sugar beet (Thompson et al. 1993) and wheat (Legard et al. 1994). Before the release, studies were conducted to determine any perturbation effects under contained glasshouse conditions (De Leij et al. 1994 a,b). Subsequently, the release trials were carried out with the consent of the UK Advisory Committee for Releases into the Environment and reported (De Leij et al. 1995 a, b; Thompson et al. 1995). The full account of the studies has been reviewed (De Leij et al. 1998). In terms of impact, the conclusions drawn are summarized in table 1. The principal results were that the organism became established and disseminated, but gene transfer to other organisms was not detected. Subsequent studies (De Leij et al. 1998) investigated the potential metabolic burden of the inserted genes on the ecological competence of a variety of constructs modified with the marker genes used in the release-study strain of the bacterium (table 2). Whereas the kanamycin resistance did not seem to affect the fitness of the organism, both of the other marker inserts did reduce ecological competence. The table shows that the X-gene (in combination with Kr) caused a decline in SBW 25 population. The conclusion, therefore, is that even though the modified bacterium was competent in the field, the wildtype is even more competent. The marking was essential for monitoring purposes, but because the marker inserts added no beneficial function, it would not be sensible to use them in any exploitation of the bacterium.

### Table 1. Ecological Effects of Pseudomonas fluorescens SBW 25 EeZY-KX

<table>
<thead>
<tr>
<th>Effect</th>
<th>Wheat</th>
<th>Sugar Beet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival and establishment</td>
<td>( &gt;10^6 \text{cfu}^{-1} \text{g root during season and 7 months after harvest} )</td>
<td>Up to ( 5 \times 10^6 \text{cfu}^{-1} \text{g senscnet leaves} )</td>
</tr>
<tr>
<td>Dissemination</td>
<td>Vertical &gt; 45cm</td>
<td>Vertical &lt; 10 cm</td>
</tr>
<tr>
<td></td>
<td>Lateral &gt; 2m</td>
<td>Lateral &lt; 10cm</td>
</tr>
<tr>
<td></td>
<td>Colonised volunteer and resown plants and weeds</td>
<td>Colonised volunteer and resown plants and weeds</td>
</tr>
<tr>
<td>Gene transfer</td>
<td>None of markers</td>
<td>None of markers but on mercury resistance plasmids exchange</td>
</tr>
<tr>
<td>Community analysis</td>
<td>Small and transient, no effects on plant health</td>
<td>Small and transient, no effects on plant health</td>
</tr>
</tbody>
</table>
Table 2. Ecological competence of Pseudomonas fluorescens SBW 25 variants. (De Leij et al. 1998)

<table>
<thead>
<tr>
<th>SBW 25 variant</th>
<th>Percentage of total introduced</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>P. fluorescens SBW25 population</td>
</tr>
<tr>
<td></td>
<td>Time (days)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>SBW 25 – 6K</td>
<td>30.0a</td>
</tr>
<tr>
<td>SBW 25 – 6KX</td>
<td>44.8b</td>
</tr>
<tr>
<td>SBW 25 – EeZY-6KX</td>
<td>25.3a</td>
</tr>
</tbody>
</table>

Antifungal Genes

The DAPG gene

In 1993 the European Commission funded a major program on Biotechnology and Ecology of Microbial Inoculants (IMPACT) followed 3 years later with another titled Harnessing the Potential of Genetically Modified Microorganisms and Plants (IMPACT 2). The partnership has involved CSIC Spain, Irish Sugar, Agronomica (Italy), S & G Seeds (The Netherlands), TUV (Germany), and the Universities of Cork, Turin, ETH Zurich, Lausanne, Leiden, Surrey, Padua, Pisa, Bielefeld, Madrid Polytechnic, Leuven, and York. One target was to determine the impact of Pseudomonas fluorescens F113, which had been isolated from sugar beets and found to produce the antibiotic 2, 4 diacetylphloroglucinol (DAPG) (Shanahan et al. 1992). Besides being active against Pythium damping-off, DAPG was also active against the potato soft-rot pathogen Erwinia carotovora ssp. atroseptica (Cronin et al. 1997) and the potato cyst nematode Globodera rostochiensis (Cronin et al. 1997b). For comparative purposes, strain F113 G22 was constructed, which is a Tn5::lacZY DAPG-negative derivative of F113 that does not have the ability to inhibit the growth of plant pathogenic fungi (Shanahan et al. 1992).

The impact of Pseudomonas strains on the rhizosphere was carried out primarily at Surrey. One of the main approaches was to determine the effect on the rhizosphere–soil enzymes N-acetyl glucosaminidase, chitobiosidase, acid and alkaline phosphatase, phosphodiesterase, aryl sulfatase, and urease, which are representative enzymes in the carbon, nitrogen, phosphorus, and sulfur cycles in soil (Naseby and Lynch 1997). The results were published in a series of papers and are summarized in table 3 (Naseby and Lynch 1997, 1999, 2001; Naseby et al. 2000, 2001 a, b).
A further series of studies addressed the mineralization and uptake of $^{15}$N-enriched wheat residues (Brimecombe et al. 1998, 1999, 2000). Inoculation of pea seeds with *P. fluorescens* F113 or F113G22 increased mineralization and uptake of organic nitrogen in the rhizosphere. In contrast, the inoculation of the same strains onto wheat seeds reduced mineralization and uptake (table 4). The explanation seems to be that inoculation of pea resulted in an increase in the number of nematodes and protozoa in the rhizosphere, but for wheat there was a decrease in the microfauna, which stimulated the mineralization of organic nitrogen. The inoculants, when provided to peas could catabolize nematicidal compounds, produce a nematocide, or both. This is therefore a clear benefit of the inoculants but took place irrespective of whether they had been modified.

As a further aspect of inoculation effects on nitrogen cycling in the rhizosphere, the impact on nodulation of peas was studied (table 5). Nodulation was increased, but only with the DAPG-producing strain of the bacterium (Aldrade et al. 1998). Thus, this beneficial effect was canceled by the genetic modification of the wild type.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot dry weight (g)</th>
<th>Number of <em>Rhizobium</em> nodules/g root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>1.24&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Pseudomonas</em> F113</td>
<td>1.02&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>9.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Rhizobium</em> + <em>Pseudomonas</em> F113</td>
<td>0.89&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>20.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Pseudomonas</em> G22</td>
<td>0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Rhizobium</em> + <em>Pseudomonas</em> G22</td>
<td>1.22&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*P. fluorescens* F113 is the wild type which produces the antibiotic DAPG. *P. fluorescens* F113G22 has been modified to delete DAPG production. Values not followed by the same letter are significantly different at $P = 0.05$.

Table 6. Effect of *Brassica napus* (oil seed rape or canola) cultivar variation and transgenesis with antifungal proteins on rhizosphere nitrate and alkaline phosphatase before and after rainfall.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ppm nitrate/g dry soil</th>
<th>alkaline phosphatase (mg released/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>Border variety</td>
<td>63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wild type (Westor)</td>
<td>43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>150&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Null</td>
<td>43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>173&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acc AMPl</td>
<td>57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Null</td>
<td>80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dm AMPl</td>
<td>77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All, except the border are Westor wild types modified with antifungal genes Acc AMPl from *Allium cepa* or Dm AMPl from *Dahlia merckii*. The divergent null lines do not have chromosome conjugation. Values not followed by the same letter are significantly different in column, but all values significantly different across column ($P = 0.05$).

Table 7. The ethylene effect on plants and alleviation by ACC deaminase.

**Effects of ethylene in the rhizosphere**
- Root initiation
- Root length inhibition
- Promotes seed germination
- Inhibits modulation and mycorrhiza

**Ethylene producing stresses**
- Phytopathogens
· Low/high temperature
· High salt
· Flooding/drought
· Heavy metals/organic contaminants
· Insect predation

**ACC deaminase**
- Converts ACC to ammonia and \(\pm\)-Ketobutyrate
- Requires pyridoxal phosphate
- Native form is a 105 Kda trimer
- \(K_m\) 1 – 15mM
- Temperature optimum ~ 30°C
- pH optimum ~ 8.5
- Bacteria growing on ACC with ACC deaminase promotes root elongation

**Transgenic oil seed rape**

The soil enzyme methodology has been used to assess the impact of genetically modified plants on soil biochemistry (Naseby, D.C., Greenland A., and Lynch, J.M., unpublished data). Two modified oilseed rape lines were used (*Brassica napus*, var Westar), which produced small cysteine-rich proteins with antifungal activity specifically expressing either the DmAMPl gene from *Dahlia merckii* or the AceAMPl gene from *Allium cepa*. The null lines only have the genetic modification on one chromosome, whereas breeding following modification yields the genes on both chromosomes (chromosome conjugation). The field trial consisted of these transgenic lines compared with several controls, their divergent null lines, a wild type control (Westar), and a different variety of oilseed rape. Sampling of this trial for enzymatic analysis consisted of taking the rhizosphere soil of 10 replicated plants from each treatment. Sampling occurred over 2 days (i.e., five replicates from each treatment were taken on the first day and five on the second day of sampling). A range of soil enzyme activities were measured, and the available soil nutrients were analyzed.

The results (table 6) showed large differences between the two sampling days in soil enzyme activities (e.g., alkaline phosphatase) and available soil nutrients (e.g., nitrate). Differences were found in most soil enzymes measured and the available soil nutrients such as nitrate. Differences were also detected between the various oilseed rape varieties assessed. However, there was little difference between the enzyme activities in the rhizosphere of the genetically modified (GM) and non-GM plants. However, AMP-1 before the rainfall did differ from its null analog result. The major factor influencing the enzyme activities and soil nutrients between the two sampling days was the soil moisture content, which was increased by overnight rain. Therefore, in this field trial, the differences between soil enzyme activities were not attributable to plant genetic modification but to environmental variation and to differences in plant variety.

**ACC deaminase**

Ethylene has a range of effects on plants. It is produced endogenously in the plant and exogenously by soil micro-organisms, and both sources affect plant growth regulation (table 7). Most notably ethylene is the classical inhibitor of root growth in flooded soils, either from endogenous root production, causing arechyma (air spaces) to form in roots, or from exogenous
LMOs and the Soil

microbial sources. The substance 1-aminocyclopropane-1-carboxylate (ACC) is synthesized in roots and transported to plant shoots where it is converted to ethylene by ACC oxidase. The synthesis of ethylene can be inhibited by the enzyme ACC deaminase. The ACC deaminase has been found in a range of strains of rhizosphere bacteria (Enterobacter cloacae, Pseudomonas spp., Kluyvera ascorbata) that appear to promote plant growth by inhibiting ethylene stress (Burd et al. 1998, 2000; Grichko and Glick 2000, 2001; Li and Glick 2000; Ma et al. 2001; Shah et al. 1998; Wang et al. 2000). The plants not only become flood tolerant, but the destressing effect enables them to accumulate heavy metals and therefore become potential agents of bioremediation. Transgenic tomato plants have been produced with the bacterial gene under the transcriptional control of either two tandem 35S cauliflower mosaic virus promoters (constitutive expression), the rolD promoter from Agrobacterium rhizogenes (root-specific expression) or the pathogenesis-related PRB-lb promoter from tobacco to generate the flooding tolerance and ability of the plants to accumulate cadmium, cobalt, copper, nickel, lead, and zinc (Grichko et al. 2000; Grichko and Glick 2001). Thus, microbial inoculants or transgenic plants expressing novel products and with abilities to enhance bioremediation might become a very exciting new initiative to improve the soil environment.

Conclusions

Clearly, baseline ecology needs to be established to determine perturbation effects. Some conclusions that can be drawn from our studies and those of others thus far can be summarized as follows:

- Gene products are better indicators of population change than monitoring populations directly.
- Gene exchange is mainly mediated by plasmids.
- Field impacts of LMOs are generally smaller than impacts of meteorological conditions and agricultural practices such as ploughing, which is not discussed here.
- Living modified organisms can carry metabolic loads that reduce ecological fitness.
- Living modified organisms may influence microbe–faunal interactions that indirectly regulate plant nutrition.
- The enzyme ACC deaminase may decrease plant stress and facilitate bioremediation.

The focus of this very brief summary of research has been on Pseudomonas strains. One of the major issues not covered here is the significance of the Bt toxin gene to soils, but this product of a soil microbe has received attention in very recent publications from Guenther Stozky’s laboratory in New York (Saxena and Stozky 2000; Saxena et al. 2002 a,b).

Acknowledgments

I very much appreciate the inputs from my colleagues at Surrey (Frans De Leij, David Naseby, Melissa Brimecombe, Jose Pascual, John Way, and Nigel Bainton) in these studies. In addition, the late Michael Bazin (King’s College, London) John Whipps (HRI), Mark Bailey (CEH), Fergal O’Gara (University College, Cork) and the IMPACT Consortium, Andy Greenland (Syngenta), and Bernie Glick (University of Waterloo) have been excellent collaborators to work with.
References


LMOs and the Soil


Abstract

The use of genetics as an insect control tool emerged from the broader tradition of biological control more than 50 years ago. The limited use of genetic control strategies is due, in part, to the limited abilities of entomologists to create the necessary genotypes required for successful program implementation. Transgenic insect technology provides entomologists with new opportunities to execute genetic control programs. Transgenic insect technology is being considered in three types of applications: beneficial insect augmentation, population suppression and eradication, and pest-status modification. At least four broad host–range transformation systems are currently available for insects. Most of the safety issues associated with transgenic insects are analogous to those identified for transgenic plants. The limited ability to manage transgenic insects following their release presents unique challenges. Transgenic insects will permit the expanded use of genetics in biological control strategies, which is consistent with the growing demand to reduce chemical inputs into the environment and the development of sustainable agricultural systems.

Introduction

The intense interest in transgenic technology in agriculture has focused largely on crop improvement. In particular, efforts to confer on crops properties that result in their being more insect resistant and herbicide tolerant have dominated early development efforts. Pest management remains an important emphasis of transgenic crop development but future efforts will also likely focus on yield improvements. It is not surprising, therefore, because insect pest management remains a priority in agriculture, that strategies to exploit the power of transgenic technologies are being explored by entomologists. It should also be noted that the interest by entomologists in transgenic technologies is not focused solely on insects of agricultural importance. The emergence of insecticide-resistant insect vectors of disease, among other things, is leading to a resurgence in insect-borne diseases such as malaria and dengue fever. Currently there are over 1 million deaths from malaria and over 300 million acute cases of the disease annually. No less than 40 percent of the world’s population is now at risk of contracting malaria. Ninety
percent of the mortality associated with malaria presently is confined to sub-Saharan Africa (WHO/OMS 1998). Although the severity of this problem is reflected by its impact on human health, note that the economic impact of malaria is similarly severe. It has been estimated that the economic growth of Africa is reduced up to 1.3 percent annually as a result of this disease. The short-term economic benefits of controlling malaria are estimated to be between $3 and $12 billion per year (WHO/OMS 1998). Consequently, transgenic insect technologies are also being explored as tools for solving public health problems.

Current interest in the application of transgenic technologies to insects represents a new phase in an area of insect pest management that began 50 years ago and has focused on genetics as a tool for biological control (Whitten 1985). Despite the proven success of certain insect genetic control strategies such as the control of the new world screwworm (*Cochliomyia hominivorax*) following the large-scale release of radiation-sterilized insects (the sterile insect technique), the widespread application of genetic control strategies has been limited largely by our inabilities to genetically manipulate pest species in ways that are compatible with certain programatic requirements. There was great enthusiasm for insect genetic control in the 1960s and 1970s which resulted in theoretical and conceptual advances but few programatic successes (Whitten 1985). Again, the limited abilities of entomologists to create the necessary genotypes and the fitness costs associated with these genotypes and their production were largely responsible for preventing the successful implementation of these programs. There have been significant advances in insect molecular genetics during the last decade, including the development of robust transgenic technologies for insects of agricultural and public health importance (Handler and James 2000). These advances have renewed an interest in insect genetic control strategies, and, although such strategies have many advantages over conventional chemical-based control methods, they can also pose some unique risks. Whether these risks can be minimized and managed to the extent required to make these insect control strategies acceptable and attractive remains to be seen.

**Applications of Transgenic Insect Technologies**

Transgenic insect technologies are being considered for a variety of applications, including beneficial insect augmentation, population suppression and eradication, and pest-status modification.

**Beneficial Insect Augmentation**

Silkworms and honeybees have been the targets of genetic improvement for thousands of years. Current advances in our abilities to create transgenic insects are likely to provide new opportunities for silkworm and honeybee breeders to create strains with useful characteristics such as resistance to certain diseases and pests. The use of transgenic technologies in these cases is analogous to the application of transgenic technologies to crop and livestock improvement. In addition to the genetic manipulation of domesticated insects, there has been an interest in genetically manipulating the natural enemies of insects such as parasitoids and predators (Beckendorf and Hoy 1985). Improvement efforts have focused on insecticide resistance with the hope that these insects could be deployed as part of an Insect Pest Management (IPM) program. The feasibility of beneficial arthropod augmentation has been well documented for the predatory mite *Metaseiulus occidentalis* (Hoy 1985). A strain of *M. occidentalis* was developed using conventional breeding and selection strategies
that was resistant to organophosphorus insecticides under field conditions. This strain was successfully deployed in California almond orchards and resulted in decreasing production costs by reducing the number of pesticide applications for spider mite control (Headley and Hoy 1987). Transgenic technologies are expected to provide more opportunities to employ beneficial insect augmentation strategies because the number of genotypes that can be created is almost without limit and the time required to produce these genotypes can be quite short.

Population Suppression and Eradication—

Population suppression and eradication remains the primary objective of most insect pest control strategies. Clearly, insecticides have played the major role in these efforts, but insecticide use is becoming increasingly difficult because of the emergence of resistant pests, the difficulty in developing new chemical control agents, and the growing levels of societal intolerance to their use in the environment. Consequently, alternative strategies for pest control need to be developed, and among those “alternative” strategies that hold great promise are genetic control methods. These methods can be divided into two categories: the sterile insect technique (SIT) and genetic load control (GLC) (Waterhouse et al. 1976). In both cases the pest species with an appropriate genotype (sterility in the case of SIT, deleterious genes or conditional lethal genes in the case of GLC) is reared en masse and released into the pest population in the field. Mating between wild and released insects either results in no progeny (as in the case of the SIT), fewer progeny, or progeny that will die prematurely (as in the case of GLC), depending on the genotype of the parents. As with strategies for augmenting beneficial insects, producing insects with the necessary genotypes fit enough to compete successfully when released into the environment is a major problem; however, the use of transgenic technologies affords entomologists the opportunity to better solve and manage these problems.

Pest Status Modification—

Perhaps the most ambitious proposal for the application of transgenic insect technology to pest management is to use it to modify the pest status of individuals within a population (Curtis and Graves 1988). This approach does not prescribe the eradication of the pest insect population but instead involves its conversion via the incorporation of a pest phenotype-altering transgene. For example, genetically altering the mosquito Anopheles gambiae such that it can no longer serve as a host for the human malaria parasite Plasmodium falciparum might serve to reduce malaria transmission and the incidence of disease. The successful implementation of this strategy not only requires the creation of a genotype that will ultimately prevent parasite development in the mosquito but also the spread of this transgene through natural populations of the insect, resulting in a stable genetic transformation of an insect population in nature. This application of transgenic technology, which might be called “environmental gene therapy,” is perhaps one of the most ambitious applications of transgenic insect technology and will present us with some rather unique risk issues.

The Technology

Interest in transgenic insect technology has a long history extending back to the 1960s (Handler 2000). In the 1970s the first report of the stable genetic transformation of the laboratory fruitfly Drosophila melanogaster appeared, but unfortunately the methods employed
did not constitute a “system.” In the early 1980s a method for systematically and repeatedly creating transgenic *D. melanogaster* was developed based on a gene vector constructed from a transposable element (the P-element). Unfortunately, the P-element has a very restricted host range and is unable to function as a gene vector in insect species outside the family Drosophilidae. Consequently, none of the major insect pests could be transformed with the *Drosophila* system, although it did provide a useful paradigm for subsequent insect gene vector development efforts. During the 1990s there were widespread efforts to discover, analyze, and test other insect transposable elements for their abilities to serve as gene vectors analogous to the *Drosophila* P-element. Several promising candidate elements were found, and the first reports of genetic transformation of insects of economic and public health significance appeared (Handler and James 2000). As of today there are four major insect gene vector systems other than the P-element system. All are constructed from different transposable elements and have broad host ranges. More than 15 different species of insects have now been genetically transformed using at least one of these vectors (Atkinson et al. 2001). It is fair to say that the technology for creating genetically transformed insects is widely available.

**Molecular Biology—**

Transposable elements comprise a large and diverse collection of genetic elements that share (either now or in the past) the ability to move within the genome. Certain types of elements such as Class II elements move through a process of element excision followed by element insertion (Berg and Howe 1989). This excision and insertion process is exactly what the genetic engineer attempts to do when creating a transgenic organism. Therefore, the inherent mobility properties of transposable elements make them attractive genetic platforms upon which to construct integrative gene vectors. All of the gene vector systems currently available for insects are constructed from Class II transposable elements (P, hobo, Hermes, mariner, Minos, piggyBac) isolated originally from insects (P and hobo—*D. melanogaster*; Hermes—*Musca domestica*; mariner—*D. simulans*; Minos—*D. hydei*; piggyBac—*Trichoplusia ni*). Each system consists of an integration vector comprised of a pair of element-specific, terminal-inverted repeat sequences that are essential for integration. These repeat sequences flank the transgenes to be integrated, conferring on them the same mobility properties as the native transposable element. The system also consists of an element-specific transposase-coding region under an appropriate promoter control system. The transposase-coding region encodes for system-specific proteins that perform the integration reaction.

**Biology—**

The two components of the system, the inverted repeat-containing vector and transposase-producing “helper” plasmid, are coinjected into the posterior pole of preblastoderm insect embryos, where integration will occur in primordial germ cells. The resulting adult insect is a chimera of transgenic and nontransgenic tissue. If the germ-line is transgenic, the insect will produce fully transgenic progeny from which stable lines can be established (Spradling 1986). Dominant visible markers such as the Green Fluorescent Protein are commonly employed to aid in the identification of transgenic individuals. The microinjection methods and subsequent animal husbandry and genetic manipulations can be extremely challenging and severely limit the applicability of existing gene vector systems.
Benefits

Genetic control methods, whether or not they employ transgenic insects, have a number of remarkable benefits when compared with traditional chemical-based control methods. By their very nature genetic control methods are species specific and minimize chemical use. In addition, genetic control methods tend to be most efficient when the target insect population is at low density owing largely to the ability of the released insects to locate and find conspecific individuals. Genetic control strategies that involve population replacement and pest status modification have the potential to be highly sustainable, requiring little or no input after the initial implementation of the program. The benefits of using transgenic technologies within the context of genetic control programs are the ability to construct insects with the desired genotypes rapidly, the possibility of constructing a much greater repertoire of genotypes, the ability to minimize secondary genetic alterations associated with conventional breeding practices, and the ability to apply genetic control strategies to a wide variety of insect species. In short, transgenic technologies now provide entomologists with the opportunity to customize the insects being used in a genetic control strategy to meet their programmatic requirements more precisely. Not only can insects be constructed with appropriate “effector” genes, but they can also be modified in ways that facilitate mass rearing and competitiveness.

Hazards and Risks

Hazards are associated with genetic control methods, some of which are rather general and common to many insect control programs. Insect eradication programs of any type result in the elimination of a species from an ecosystem and consequently can disrupt the ecology in ways that are undesirable. For example, the emergence of secondary pests may result in a pest problem as severe or worse than the original problem. Genetic control programs involving the massive release of a pest species (SIT and GLC) pose the potential hazard of increasing the size or range of the pest population. The probability of such an event in the case of SIT is directly proportional to the efficacy of the sterilization methods employed in the program. Consequently, the risk of increasing the size or range of the pest population can be reliably assessed. Programs designed to modify pest-status, whether or not they use transgenic technologies, also pose hazards that are similar to those encountered in conventional biological control programs such as unanticipated and unwanted invasion of new habitats. Because the released insects are fertile and, by design, competitive—as in the case of pest-status modification programs—the exposure component of any risk calculation becomes rather large. Consequently, programs involving the release of fertile and fit individuals must minimize hazards to keep the overall risk to an acceptable level. Consequently, pest status modification programs will be extremely challenging. Such programs will need to consider carefully the possibility of changing the pest status of an insect in an unintentional way, leading to an enhancement in pest status rather than a reduction. In the case of programs designed to modify the transmission capabilities of disease vectors such as mosquitoes, changes in vectoral capacity, host range, life history characteristics, and parasite–pathogen biology will need to be carefully considered (Hoy 2000).

The use of transgenic insect technologies in insect genetic control programs adds some additional hazards, many of which are similar to those identified for transgenic crops. The six safety issues (gene transfer, expression of genetic material from pathogens, weediness, trait effects, genetic and phenotypic variability, and worker safety) identified by the Organization
for Economic Cooperation and Development (OECD) as being of major significance to the release of transgenic plants are generally applicable to transgenic insect releases (OECD 1993). However, transgenic insects also can present us with some rather unique challenges.

Gene transfer and the expression of genetic material from pathogens are hazards arising as a result of a loss or movement of the transgene and a breakdown in biological containment. These hazards exist when deploying transgenic insects as they do when deploying transgenic plants and can be managed generally by using gene–vector systems that permit postintegration stability of the transgene to be maximized. For some applications of transgenic insect technology this will not be desirable or possible and represents a unique feature of some transgenic insects. For example, pest modification strategies rely on the introduction and spread of a phenotype-altering transgene in a wild population. One method of spread being considered is to link the transgene to a self-mobilizing transposable element. Transposable elements by virtue of their mobility characteristics under some conditions can sweep through natural populations. Linking a transgene to a “sweeping” transposable element is envisioned as a way of spreading the transgene through a natural population. Hence, stability is minimized and intraspecific spread is desirable. This represents a rather unique feature of transgenic insect technology.

The tendency of plants to spread beyond the fields where they were planted has been referred to as “weediness.” Containing or limiting the distribution of transgenic insects is an analogous issue, but the dispersal characteristics of insects make this particular hazard more complicated than that for plants. For all insect genetic control programs dispersal is a critical requirement for achieving success. The objective of all insect genetic control programs is to release insects and have them disperse and mate with conspecifics in the environment. In programs where released insects are sterile (e.g., SIT) biological containment will be maximized and weediness tends to be minimized. Genetic load control programs, although releasing fertile insects, are designed ultimately to kill them or negatively impact insect reproduction again, tending to maximize containment and minimize weediness. Pest status modification programs are likely to maximize the weediness threat because of the use of fertile and fit transgenic insects.

Trait effects derive from transgenic traits that are harmful to nontarget organisms. Effects on nontarget organisms are an issue for all insect control strategies and that will also be an issue to be considered in the release of transgenic insects. Transgenic insects present a unique situation for insect control specialists and risk assessors because the stability of the transgene will influence the likelihood of its being transferred to another species. For most applications of transgenic insect technology, stability will be a highly desirable trait, and efforts to engineer the transgenic insect and the vectors used to create it will tend to maximize stability. Only in programs in which the transgene is being spread through a natural population using an autonomous transposable element will stability be minimal—at least initially. The presence of a highly unstable transgene (as a result of linkage to an actively transposing transposable element) will increase concerns for nontarget organisms as well as transfer of transgenes to pathogens and symbionts. Perhaps the most prudent approach to the use of actively transposing transposable elements as genetic engineering tools is to maximize their species-specificity so that, should they be transferred to other organisms, they would be incapable of integrating. Thus the risks of trait effects and expression of transgenic material from pathogens due to horizontal transfer would be minimized.
Genetic and phenotypic variability can lead to unpredictable outcomes in the field and, as with transgenic plants, transgenic insects will need to be characterized carefully with respect to phenotype. Our abilities to describe and understand the phenotypes of transgenic organisms until recently had been fairly limited. Consequently, critics of releases of transgenic organisms into the environment point to this limitation and the ability of genes to affect more than one trait (pleiotropy) as reasons for justifying bans on such releases. Testing claims that transgenes are pleiotropic can be difficult. The advent of transcriptome profiling using microarrays now provides geneticists with more powerful tools to assess the degree of pleiotropy associated with transgenic organisms. Current genomics efforts will increase our potential to determine the effects of transgene integration and expression effects in transgenic organisms and to make decisions concerning deployment based on a sophisticated and more thorough understanding of phenotypes.

Conclusions

The environmental safety issues associated with the release of transgenic insects parallel those identified for transgenic plants. Most of these same issues arise during conventional (nontransgenic) approaches to genetic and biological control. What makes insect genetic and biological control programs challenging and somewhat different from the release of transgenic crop plants is the degree of management that is possible. Insect genetic and biological control programs attempt to release fit and sometimes fertile insects into the environment. Dispersal of released organisms is essential for these programs to be successful and as a result cannot be managed to the degree that plants in monoculture can be. Hazard identification and risk assessment of transgenic insects are likely to place a high demand on understanding the phenotype of the transgenic insect being released. In addition, a thorough understanding of the mobility characteristics of the gene vector being employed, the use of “suicide” vectors to maximize stability, and the construction of gene vectors with a very high degree of species-specificity will help mitigate many of the risks associated with transgenic insects, most of which stem directly or indirectly from potential transgene instability. Although the challenges associated with the development of safe and effective transgenic insects are significant, this technology may lead to the expanded use of genetic and biological control strategies in pest management programs. This would satisfy the growing demands to reduce chemical inputs into the environment and the development of sustainable agricultural systems.

References


Ecological Risk from Aquatic Living Modified Organisms

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Abstract

For more than one billion people, fish is the primary source of animal protein. Demand for seafood is now much greater than the yields from already threatened wild fisheries. Aquaculture and aquatic living modified organisms may greatly reduce pressure on ocean ecosystems. Some propose that aquatic living modified organisms (LMOs) may increase in the wild and cause irreversible damage—even extinction—and that case-by-case contained testing is necessary to screen these organisms. This paper does not recommend case-by-case contained testing because genotype * environment interactions limit prediction of fitness in the wild. Moreover, this case-by-case approach fails to generate generic predictions for a range of LMOs. This paper does suggest that a generic and falsifiable prediction of (negligible) risk can be drawn from genetic theory. What is unknown is how long it will take for the negligible risk hypothesis, without falsification, to be accepted as dogma by regulators and the public.

Introduction

Drivers of Aquatic LMOs

Most of the world’s capture fisheries are fully or over-exploited (Wijkstrom et al. 2000). Wild fisheries plateaued in the late 1980s at around 90 million tons per annum, but world seafood consumption is 140 million tons per annum. The failure of wild fisheries to meet world demand for seafood is a major reason for the recent expansion of aquaculture. Today, aquaculture is one of the fastest growing agriculture sectors worldwide. Per annum growth and value of aquaculture is 10 percent and US$ 50 billion, respectively (Rana and Immink 1984–1996). Against this backdrop of finite wild fisheries and increasing demand for seafood, aquaculture production will need to expand rapidly and continually for the foreseeable future. With only 1 percent of present aquaculture using genetically improved stocks (Gjedrem 1997), there is some prospect that genetics can accelerate aquaculture production to meet demand and do so in a sustainable way (i.e., with increased efficiency and reduced pollution and diseases). In particular, present marine aquaculture requires substantial fishmeal and thus adds to the pressure on
wild fisheries. There is some urgency to reduce this dependency. Options include the modification of terrestrial plants for fish feed or modification of fish (Knibb et al. 1998) to consume transgenic meal. Together, these factors translate into some interest to apply genetic engineering technologies in aquaculture.

There is also interest to use fish, particularly zebra fish (Danio rerio) and medaka (Oryzias latipes) as models for vertebrate gene discovery and function (Hackett and Alvarez 2000).

Previously I set out hypotheses (Knibb 1994, 1997) that predict negligible ecological risk from transgenic fish, and these predictions apply for living modified organisms (LMOs) in general:

"Despite concerns to the contrary, the following hypothesis remains to be falsified: "laboratory induced allele frequency/genotype changes and novel alleles or genes have a negligible probability of being selectively favoured in wild populations under natural selection, and accordingly, without sustained large scale releases, have little potential for ecological impact."

Now, some years later, I will review how the hypothesis stands—has it been falsified? In this way, I will address some of the stated objectives for this meeting—in particular, to consider hypotheses underlying the assessment of LMOs in the environment.

Discussion

What Aquatic LMOs Are There?

To date, most research on LMOs in aquaculture concerns the acceleration of growth in salmonids (Atlantic salmon [Salmo salar], Du et al. 1992; coho salmon [Oncorhynchus kisutch], Devlin et al. 1994; channel catfish [Ictalurus punctatus], Dunham et al. 1992; tilapia [Oreochromis niloticus], Martinez et al. 1996; and carp [Cyprinus carpio], Chen, et al. 1990) using heterologous growth hormone constructs. Resulting growth acceleration in salmonids is often dramatic (order of magnitude increase) but is less for other species. More preliminary work concerns cold (Wang et al. 1995) and salinity tolerance, disease resistance (Hew et al. 1995), metabolic modification, and fishmeal replacement (Pitkanen et al. 1999, Knibb et al. 1998). In model fish (medaka, zebra fish), most LMOs are generated to elaborate vertebrate gene function. Hence, model fish LMOs are more numerous and varied than those from aquaculture (Hackett and Alvarez 2000). Methodologies for gene transfer vary, although microinjection of transgenes into eggs and random incorporation of DNA is the most common technique.

Special Concerns for Aquatic LMOs

Should aquatic LMOs survive in the wild, then their recovery from oceans, lakes, and rivers no doubt would be challenging. Indeed, a special concern for fish and aquatic LMOs is that release may be irreversible. This concern is tempered by the likelihood that LMOs will increase in proportion in wild populations, and the consequences of such an increase. There are various selective and stochastic processes whereby transgenes could increase in frequency in the wild (Knibb 1997) such as the following:
• Continual or large-scale releases of LMOs with reduced “Darwinian” fitness;
• Drift (even for deleterious alleles) (Crow and Kimura 1970);
• Genetic drive (even for deleterious alleles) (Morita et al. 1992);
• Selective advantage either as (intraspecific) polymorphism in a species, or due to speciation.

It is selective advantage that attracts most interest and debate (Tiedje et al. 1989, Kapuscinski and Hallerman 1990, Kapuscinski and Hallerman 1991, Regal 1994), for two reasons. First, selective advantage is suspected of being more probable for transgenics than spontaneous changes, although all of the preceding processes can apply for engineered and classical changes. Second, the release of just a few LMOs with transgenes of increased Darwinian fitness may lead to a wide-scale spread.

Mesocosms and Risk Assessment

Following on from concerns that release may be irreversible, there is some interest to describe the relative wild adaptive values of transgenes before release. Some advocate contained mesocosms as the preferred vehicle for fitness assessment (Levin et al. 1987, Hallerman and Kapuscinski 1995, Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish). Intuitively, assessment by mesocosm is logical, for it presents opportunities for containment and empirical assessment. Indeed, research funds are now being channeled into contained testing, and the first data for fish are emerging (see section titled Experience and Data). But is intuitive reasoning sufficient? Does fitness in a mesocosm predict relative fitness in the wild? Are mesocosms adequate from a genetics perspective in view of unpredictable Genotype * Environment (G*E) interactions (Knibb 1999)?

As contained facilities, mesocosms are less complex than wild environments. Mesocosm testing should (but does not) encompass the following:

• A range of environments because a transgene with a high adaptive value in one environment may have a low value in another. Also, testing should consider environmental variation in space and time, including seasonal and long-term changes.
• A range of populations, strains, and genotypes, for relative fitness of the transgene in a population may depend on background genotypes (Dobzhansky 1970, Griffing 1967).
• This range should consider the different genotypes present in the wild (allele type and frequency) and, perhaps, the evolution of modifiers via multigeneration testing.

Fitness varies with further parameters, and thus mesocosm testing should also include the following:

• A range of frequencies of the transgene (fitness may change if the transgene is rare or common should there be frequency-dependent selection).
• Different transgenic lines because each line may represent unique insertion events (and unique chromosome locations), unique copy numbers, and unique genetic backgrounds of transgenic lines.
• Different life stages in as much as relative fitness for survival, reproduction, and so forth may vary ontogenetically (ideally, cross generation zygote to zygote data should be obtained).

For fitness assessment we may also need knowledge of the following:

• The role of drift (Crow and Kimura 1970).
• Closely linked elements near insertion sites to distinguish between selection on transgene and linked elements.

Hence, fitness is context specific. Any one mesocosm will represent just a small subset of environments and genotypes of the wild but not necessarily in the proportion they exist in the wild. Mesocosms are unique situations confounded with unique factors relevant to fitness. Thus, finding lack of evidence of risk in one situation, no matter how many times the observation is repeated, will not prove the absence of risk in the wild. Nor will finding evidence for risk necessarily predict risk in the wild because mesocosms may have selective forces not present in the wild (e.g., selection for stress and disease tolerance and other novel factors in containment; Knibb et al. 1987).

An inability to predict fitness or performance is not a new concept and is well described in the animal genetics literature. For example, unpredictable G*E interactions exist for carp, whereas European varieties outperform their Asian counterparts when fed rich but not poor diets (Moav et al. 1975). In lay terms, we can refer to this phenomenon as “horses for courses.” Also, when measured in two different environments, the same phenotypic character (e.g., growth) in a given population can be determined by different sets of alleles and genes. So when dealing with different environments, it may be prudent to consider a measured character not as one but as many different characters (Falconer and Mackay 1996).

**Risk Assessment—Better Options?**

The preceding discussion on empirical testing leads to the proposition that only release into the wild may be sufficient to assess ecological risk of transgenics. Perhaps this is so, but even for research purposes, wild release would not easily meet with public or regulatory acceptance—at least not without some knowledge or prediction that the chance for an irreversible event of significant consequence is not credible. Moreover, it is doubtful that empirical testing, even releases into the wild, will yield generic predictions for a range of LMOs.

**Genetic Theory**

Previously, Knibb (1994, 1997) suggested that genetic theory should be explored to predict the (generic) probability fitness distributions for transgenic LMOs. To recapitulate and summarize, we note that major mutations are usually pleiotropic (Dobzhansky and Holz 1943) (i.e., to some degree they influence many different characters and cellular and biochemical process). Hence, spontaneous mutations or transgenes that change one character will tend to alter different aspects of the phenotype. Because many aspects of the phenotype are adaptations for survival and reproduction in the wild, changes to them typically reduce overall fitness. Hence, we expect almost all genetic changes (that affect function) will be selected
against in the wild, although there are various ways beyond major or continuous release that deleterious alleles can increase in frequency (drifting, hitchhiking, driving). Presently, we have insufficient scientific knowledge of the genetic architecture of fitness to know a priori what constitutes an adaptive change or how to make one deliberately. Without this blueprint, genetic changes, classical or engineered are accidental with respect to fitness. In lay terms, this may be equivalent to tuning a piano without hearing. Hence, genetically engineered changes (that change a character) are expected almost inevitably to reduce fitness (or approximate genetic changes already produced in nature). From a lay perspective, this reasoning may seem counter-intuitive. Certainly, it is contradictory to present public perception that genetic engineering is planned, deliberate, and likely to increase fitness.

Concerning the continuing debate whether genetic engineering is qualitatively different from natural processes, it is often suggested that genetic engineering results in large and novel phenotypic changes, novel gene arrangements and combinations, lateral DNA transfer, and so forth that translate into a reasonable likelihood for fitness increase (Tiedje et al. 1989, Kapuscinski and Hallerman 1990, Kapuscinski and Hallerman 1991, Regal 1994). This is contentious (Knibb 1997), but argument whether or not nature can produce functionally complementary phenotypes may be quite secondary to the question of fitness equivalence. For genetic changes producing significant phenotypic, physiological, and biochemical change, theory and experience predict the probability for detriment to fitness will increase, not decrease, with the magnitude of the change (Fisher 1930, Endler 1986). Also secondary to the debate are the position effects and insertion mutagenesis which can accompany gene insertion and reduce fitness, for this will not apply for all LMOs. Similar processes apply for classical mutations.

Overall, there is no suggestion here that adaptive mutations did not occur in nature, nor that some laboratory genetic changes, classical or engineered, will not have increased fitness in the changed and novel selective environments of the laboratory, farm, or factory. It may well be a question of probability why genetic changes generated in the laboratory are not adaptive in the wild. The low intrinsic probability of generating an adaptive mutation is compounded by the low probability of making the mutation in the laboratory before a selectively equivalent change is produced in nature. And perhaps we should not underestimate the capacity of natural populations to “experiment” with new mutations. For example, a single brood of a single female scallop producing $10^6$ to $10^7$ eggs may have mutations in a large number of loci in the genome if mutation rates on the order of $10^{-5}$ to $10^{-6}$ per loci per generation are assumed (Voelker et al. 1980). There will be multiple mutations per quantitative trait on the assumption of a mutation rate of $10^{-2}$ per character (Barton and Turelli 1989). Nature seems to have much more time, opportunity, and numbers to produce mutations before we can make them in the laboratory. Moreover, consideration of the various natural mechanisms of gene shuffling has led to the question, With all the mechanisms that exist for moving the genes around, why is the genome so stable? (Crow 1984). Similar argument applies to the probability of speciation from “laboratory” genetic changes ahead of natural process.

In summary, considering fitness rather than the specific type of change and the unplanned and accidental nature of genetic changes with respect to fitness, pleiotropism, and the dynamic nature of genomes in natural populations, we reach a general prediction for new genetic changes, that is, a testable hypothesis of negligible probability that transgenes will be selected in the wild.
Experience and Data

In contained facilities, Atlantic salmon, transgenic for Chinook salmon (*Oncorhynchus tshawytscha*) GH, tended to feed in the presence of predators at a rate much greater than nontransgenic controls, which suggests less predator avoidance of the transgensics (Abrahams and Sutterlin 1999). Contained testing of channel catfish, transgenic for salmonid GH constructs, indicated less predator avoidance (less survival) of the LMOs compared with the nontransgenic controls ((Dunham et al. 1999). Others think their data indicate fitness reduction in transgenic or GH-treated fish (Guillen et al. 1999, Jonsson et al. 1996, Farrell et al. 1997)). Cranial deformities, opercula overgrowth, and reduced viability are evident for coho salmon engineered with GH constructs (Devlin et al. 1995, Ostenfeld 1998). The caveat with these data is that they derive from contained facility testing and do not include cross-generation fitness assessment. For future empirical risk assessment, it would be interesting to assess the feasibility of releasing model fish (zebra fish or medaka) transgenic for a great range of different constructs into natural (but isolated) environments and then monitor transgene frequency changes over generations (see Barker and East 1980).

In direct contrast to the preceding claims, Muir and Howard (1999 and 2001) argued that their experimental data from medaka transgenic for GH, and subsequent modeling, indicate credible risk. Specifically, a transgene causing growth acceleration could lead to population, even species, extinction on the assumption that transgenic males have a substantial mating advantage due to large size but their offspring have low viability (“Trojan gene hypothesis; Muir and Howard 1999). Some (Maclean and Laight 2000) have criticised this work (Muir and Howard 1999) on the grounds that the experimental data did not show adult size differences. Nor were any mating preferences for the transgenic medaka reported. One can add to this list a failure to assess fitness empirically across generations (zygote-to-zygote viabilities) let alone assess fitness outside the laboratory. With such short generation times, it is surprising and noteworthy that cross-generation competition data were not presented. More important, all models were based on many assumptions, including the absence of selection for modifiers and hence of genetic variance and mutations for modifiers, as well as the absence of G*E interactions. Indeed, some question whether modeling can predict fitness and evolution (Barton and Turelli 1989). But these criticisms are of a somewhat minor technical matter, for, in principle, a deleterious gene can increase in frequency, at least initially, under a range of conditions. This situation is well documented elsewhere for a category of rare classical mutations (Sandler et al. 1959, Braden 1958). So it is not entirely clear that the “Trojan gene hypothesis” scops a principle peculiar to transgenics or something already discussed elsewhere (Lande 1980, Barton 1990). If not conceptually novel, this work (Muir and Howard 1999) could make a new contribution should GH transgenes display selective characteristics not evident from natural processes, that is, a probability of risk different from that of natural processes. However, Knibb (1997) pointed out that natural processes readily mimic the growth acceleration of GH transgenes in vertebrates (i.e., gigantism from mutations in major genes or selection for existing additive polygenic genetic variance). Indeed, Knibb (Knibb et al. 1998, Knibb 2000) reported a spontaneous major locus variant in sea bream (*Sparus aurata*) that accelerates growth and that classical selection increases growth. Devlin et al. (2001) showed that trout from stocks with a prior history of classical selection grow about as fast as unselected fish transgenic for GH, indicating the presence of additive genetic variance in wild populations sufficient for dramatic growth increases (should selection occur).
Altogether, this work (Muir and Howard 1999, 2000) would generate less argument if the use of the word transgene had been generalized to transgene and other genetic changes. But then, the novelty would be lost, and these “Trojan gene” arguments would devolve to one of the preceding sections (i.e., the probability of generating selectively equivalent mutations before nature).

For the lay reader to assess the credible likelihood of risks implied by Muir and Howard (1999 and 2000) we can consider the implications of their suggestions (1), that existing species are at a credible short-term extinction risk from natural mutations causing meiotic drive, mating advantage, and so forth, and (2), that genetic engineering offers a credible prospect for pest eradication through the release of just a few animals. Certainly there would be great interest, public and financial, in eradicating noxious exotic carp and tilapia in Australia. Unhappily for these purposes, this type of genetic control, albeit using classical chromosome mutations, has been attempted before and has failed (Cantelo and Childress 1974, MacKenzie 1976)). Do we propose that by accident we will achieve something not possible by design?

**Likelihood of Risk and Consequences from an Adaptive LMO**

Knibb (1997) suggested a potential risk might exist whenever a transgene has a nonnegligible probability of increasing in the wild. Negligible probability events can include issues we do not regulate or insure against such as genetic damage from products of classical selection or damage from a meteorite strike. One lens through which we can glimpse the probably of adaptive mutations in nature is the rate of amino acid sequence divergence, which for coding regions is on the order of 1–2 percent per million years. This change represents an astonishingly small subset of possible mutations when considered in the context of population sizes, mutation rates, number of generations, and the probability that some divergence arises without selection. A requirement that transgenic changes happen before selectively equivalent “natural” changes would suggest that sequence divergence rates overestimate the likelihood of this type of risk.

Consequences of new adaptive intraspecific genetic change will vary and follow a probability distribution. Prima facie evidence (from the natural world today) is that new adaptive polymorphism almost invariably is not associated with significant change (e.g., extinction). Quite the contrary, new polymorphism may contribute to genetic variation important for long-term selection response. The probability for significant ecosystem change is the negligible probability of generating an adaptive genetic change before nature multiplied by the remote probability that a genetic change can alter the community. The likelihood that the change will be perceived as a benefit or cost is not considered here, for this requires value judgments. There is some probability that specific changes will be perceived as beneficial by all.

From experience, the probability of a given genetic change’s leading to speciation (reproductive isolation) is even more remote than that leading to adaptive polymorphism. The model used to predict environmental consequences of speciation is the one of introducing exotic species (Knibb 1997) into new environments (e.g., carp, tilapia, rabbits, cactuses or cane toads into Australia). Again the likelihood for change will follow a probability distribution. Only a small minority of introduced fish species cause significant community change (Welcomme 1988). Why do even the minority of species spread? Communities are not coevolved with the exotic, and some exotics can find absence of effective controls (predators, parasites, and competitors). Indeed, the cactus (*Opuntia* spp.) in Australia was controlled
once the moth *Cactoblastis cactorum* was imported and released. Accordingly, it is questionable whether the exotic model is entirely appropriate for cases of speciation within ecosystems or sympatric speciation. If so, then the probability of change from sympatric speciation will be less than that predicted using the exotic model and will be conditional on the probability of producing speciation before nature.

Inasmuch as we explore the hypothetical environmental risks from LMOs, so should we explore potential environmental gains and the consequences and opportunity costs from a philosophical rejection of transgenesis. There is an acute need to reduce pressure on wild fisheries (14 of our 16 major fisheries are overexploited). Aquaculture may reduce pressure, especially when we can find substitutes for the marine fish meal used in aquaculture. Engineering terrestrial plants as fish food, or engineering fish (Knibb et al. 1998) to consume terrestrial plants, may lead to major environmental dividends, as would the engineering of disease resistance, reduced feed conversion rates (FCRs) and so forth.

**Conclusion**

In part, the background leading to the hypothesis of Knibb (1997) was a perceived need, a gap, to describe a generic testable or falsifiable hypothesis and to do so in the traditional Popperian (Popper 1935) scientific fashion. That is, a falsifiable hypothesis should draw on existing theory and describe available data rather than start with a particular position. To illustrate, we have little evidence for Martians on Earth (albeit arguably more than for adaptive LMOs in the wild; Friedmann et al. 2001), and we do not set the null hypothesis as “there are Martians on Earth.” To date, empirical data for a range of species fail to disprove the hypothesis of negligible ecological risk (without large or sustained releases). What is unknown is how long it will take for this hypothesis, without falsification, to be accepted as dogma by regulators and the public.

This paper does not recommend the continuation of expensive case-by-case empirical testing in contained facilities because of potential GxE interactions. If the generic predictions based on theory and cumulative experience are considered inadequate for regulators, then testing should encompass multi-generation fitness assessment of large numbers of different transgenes in wild environments, possibly using model species.

A countervailing philosophy advocates the use of the so-called precautionary principle, which at its most extreme requires proof of universal safety. In this form, and following the canons of Popperian logic, the precautionary principle with its requirement of proof rather than disproof is inherently untestable and hence unscientific. Certainly, the issue of G*E effects makes the problem of comprehensive empirical testing intractable without wild releases. Of more consequence is the understanding that, far from being safe, the precautionary principle may be inherently risky through opportunity costs and failure to replace agricultural practices harmful to human health and the environment (Morris 2000).

Finally, a failure to separate or distinguish between the adaptive profiles of spontaneous and transgenic changes, as suggested here, may mean that science is not the appropriate forum for the transgenic debate, and we should turn to religious (Genesis I:26,28), cultural, or other values for guidance. This sentiment will be of little joy for regulators, but this recognition should advance our understanding of the very roots and nature of the debate. Here, perhaps
we may find consensus, though more likely, contradictions—and I conclude with two. First, do we preserve genomes and environments as static or look to an uncertain future and equate an increase in genetic variance with an increase in fitness (Fisher 1958), an increased ability to respond to selection and survive even without apparent phenotypic change? For the latter, will some view genetic engineering as a potential environmental management tool, redeeming small inbred demes or rescuing others from global climate change? Second, do we view “natural” genetic process as all benign to the environment? If so, how then do we describe spontaneous mutations leading to fitness reduction and possibly extinction? Is evolution dangerous?

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Endnotes

1 One billion people worldwide rely on fish as their primary source of animal protein
2 Fish represent 16 percent of animal protein consumed worldwide
3 From population growth. Also from increasing per capita consumption due to increasing affluence and recognition of sea food as a healthful food.
4 The Food and Agriculture Organization (FAO) defines aquaculture as “the culture of aquatic organisms, including fish, mollusks, crustaceans, and aquatic plants.”
5 “Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish” (1995) prepared by the Agricultural Biotechnology Research Advisory Committee of the U.S. Department of Agriculture (Documents 95–04 and 95–05).

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Transgenic Living Modified Organisms in Forestry—
A Canadian Perspective

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Abstract

Intensive forestry and allocation of forest land for different levels of management are receiving increased attention in Canada. Advances in tree genetic engineering could thus provide unprecedented opportunities. At the same time, we are very conscious that the environmental benefits of transgenic trees may be difficult to maximize because of the complexity of social, public opinion, and regulatory issues. Although the knowledge generated for crop species can to some extent be applied to forest trees on a case-by-case basis, some issues remain unique to the forestry context. The technology for the production of transgenic trees is developing faster than the knowledge required for thorough environmental risk assessments, and there are social acceptance issues. Several discussions have taken place in Canada and internationally on the issues surrounding the environmental impacts of transgenic trees and on research required to address them. These are discussed in this paper.

Introduction

Increasing movement has occurred worldwide towards tree farming or plantation forestry in recognition that this strategy may be necessary to meet future global demands for wood and wood products while addressing the global commitment to sustainable development of our forests (Sedjo 2001). Genetically modified (GM) trees will primarily be used in the tree farm or plantation setting to create high-yield, high-quality products in a sustainable manner. At the same time, if the proper policies are in place, protected areas would be expanded and conservation of natural ecosystems increased.

There are several categories of living modified organisms (LMOs) for use in forestry. These include genetically modified trees, pest control products, and the micro-organisms used to produce enzymes in pulp and paper processing and effluent treatment. This discussion will focus specifically on the potential ecosystem impacts of the release of genetically modified trees into the environment and a comparison with those of GM agricultural crops.
Discussion

Some of the traits of interest for genetic modification of forest trees are similar to those that have successfully been introduced into agricultural crops (e.g., insect and disease resistance, herbicide tolerance and stress tolerance). Other traits are of unique interest to forestry such as lignin modification, improved wood quality, and modification of tree size, form, and performance. It is important to consider not only the theoretical potential of trait modification but also the unintended effects. For example, modification of lignin is of importance to the pulping process for improving the ease of pulping and reducing the use of toxic chemicals, thereby reducing pollution. However, modification of this trait could change critical fitness properties of the forest tree, including tolerance to cold, rendering trees with modified lignin composition impractical for planting in countries like Canada.

Many species of trees have been genetically modified, the most common being poplar species and hybrids. The first report on poplar transformation was published over 15 years ago. Other forest tree species that have undergone genetic modification include pine, spruce, elm, walnut, chestnut, maple, eucalyptus, and birch. Several fruit tree species have been genetically modified, including apple, pear, citrus species, persimmon, plum, apricot, and papaya (Pena and Séguin 2001). The success story of papaya genetically modified for virus resistance, which saved the papaya industry in Hawaii, is well known. Papaya is the only case of a commercialized genetically engineered tree to date.

It is important to realize that the technology for the production of transgenic trees is developing faster than social acceptance and the generation of knowledge required for thorough environmental risk assessments. An integrated approach is provided in Canada through the Canadian Biotechnology Strategy, an active horizontal framework involving several Federal government departments and regulatory agencies that incorporates social, ethical, health, environmental, and regulatory considerations. The Canadian Forest Service is advancing the policy agenda towards the responsible deployment of forest biotechnology products by carrying out research and facilitating Federal, provincial, and ad-hoc expert committee discussions towards the development of sound, science-based regulatory frameworks. The Canadian Food Inspection Agency is responsible for the regulation of importation and environmental release of plants with novel traits, including trees. Several international discussions have taken place on the issues surrounding the environmental impacts of transgenic trees. The Organization for Economic Cooperation and Development (OECD) hosted an international workshop on the environmental impacts of transgenic trees in Trondheim, Norway, in 1999. The objectives and outcomes from this meeting are further described in the Proceedings of the OECD Workshop, 13-15 September, 1999, Environmental Considerations—Genetically Modified Trees. Both environmental and socioeconomic issues were recently discussed at an IUFRO (International Union of Forestry Research Organizations) meeting in Stevenson, Washington, July 22–27, 2001 (proceedings available on line at http://www.fsl.orst.edu/tgerc/iufro2001/eprocd.htm). The issues were further investigated at the workshop of the Pew Initiative on Food and Biotechnology entitled “Biotech Branches Out: A Look at the Opportunities and Impacts of Forest Biotechnology” which was held December 4–5, 2001 in Atlanta, Georgia (proceedings available on line at http://pewagbiotech.org/events/1204/).
Forest trees differ in many ways from agricultural crop plants. There are unique features of the forest trees, forest ecosystems, and forest tree breeding. Trees are large, long-lived perennials that are essentially undomesticated. Forest tree populations have tremendous genetic diversity, can adapt to seasonal environmental stresses, and are highly ecologically competent within complex ecosystems. Forest ecosystems have high species level biodiversity and complex interactions among the forest species. Forest tree breeding differs significantly from breeding of agricultural crops because of the long timeframes involved (i.e. decades) in genetic improvement programs. A rich pool of genes is available in the natural populations of forest trees that have not been tapped, and large gains can still be made by transferring genes within species and among close relatives (Mullin and Bertrand 1998).

Although forest trees differ significantly from agricultural crop plants, the major biosafety issues are quite similar, but with larger and more complex effects. These issues include the horizontal and vertical spread of transgenes, ecosystem interactions, species integrity, and biodiversity. Each of these issues will be considered in turn.

Vertical gene flow, that is gene flow from the genetically modified tree to non-modified relatives, is complicated by wind-borne pollen that travels hundreds of kilometers. Trees are perennial and long-lived, shedding pollen and seed repeatedly. Gene flow from genetically modified trees will occur unless they are completely unable to reproduce in any manner, i.e. through sexual or vegetative propagation. Horizontal gene flow, that is gene flow from the genetically modified tree to unrelated organisms, is also possible. Although there is scientific evidence for gene flow among soil microorganisms and from soil microorganisms to trees—as is the case for *Agrobacterium* infection—there is no scientific evidence for genes to flow in the opposite direction from trees to other organisms.

Control of flowering in transgenic forest tree species is being considered to prevent gene flow from pollen and seed. However, there is concern about the stability of the gene expression that controls flowering. Current regulations and the long life cycle of trees prohibit full scientific evaluation of stability of transgenes over a tree’s lifetime. There is some potential for increased yield as a result of energy diversion from flowering into wood production; on the other hand, lack of flowers, pollen, and seeds will have an impact on forest species that depend on these structures for food and other uses.

Forest ecosystems are complex, and the introduction of traits that have no coevolutionary history within the ecosystem, for example insect resistance, may have some unpredicted consequences. Increased fitness could cause changes in adaptive range, and species displacement, and changing competitiveness. However, trees have been evolving for millions of years, optimizing their genetic makeup for fitness, therefore genetic modification is more likely to result in reduced rather than increased fitness. Pest-resistant trees may encourage the development of new pests by opening new niches for previously innocuous organisms. Moreover, as insect pests become resistant to the transgenic tree, any biological control products based on the same product will lose efficacy or become ineffective.

Environmental safety assessment requires a multidisciplinary approach. It requires a baseline understanding of species and ecosystem interactions, a thorough characterization of the novel practices or products, a rigorous analysis of potential environmental consequences, and the development of appropriate tools, protocols, and criteria for risk assessments.
Environmental safety assessment research carried out at Natural Resources Canada’s Canadian Forest Service (CFS) in relation to genetically modified forest trees addresses issues such as gene flow from transgenic trees to natural populations, long-term stability of introduced genes, and the potential long-term effects of genetically enhanced trees in the ecosystem. Tools are being sought to analyze the impact of transgenic trees in intensively managed plantations in order to develop sound deployment strategies. Examples of the techniques used include laboratory bioassays, DNA monitoring techniques (DNA markers and microarrays), toxicity assays, modeling, and so forth. Small-scale field trials involving transgenic poplar, white spruce, and black spruce are carried out by the CFS under strict confinement conditions and monitoring protocols under the regulatory authority of the Canadian Food Inspection Agency. These trials are used to develop protocols for tracking the fate of genetically modified DNA in forest soil and litter, to monitor changes in soil microbial populations, and to enhance scientific understanding of the performance of the experimental trees (Bonfils 2001).

Conclusion

Social acceptance of transgenic trees will be highly dependent on our capacity to show that environmental and sustainable management issues have been properly addressed. The issues are more acute for products of genetic engineering. Over the past several years there have been numerous incidents of serious vandalism by radical environmental activists. The World Wildlife Federation has called for a global moratorium on research and development of transgenic trees. There are also international trade issues with the growing demand for certified wood products that are affecting acceptance of forestry LMOs. Several strategic questions arise. Firstly, do we have the tools and methods to predict and assess the potential impacts? Secondly, what is the magnitude and scope of potential environmental impacts of forest management practices and products of biotechnology? Finally, what research areas should be the focus of a sound, coordinated, strategic approach to the use of LMOs in forestry? These will have to be addressed in a well-integrated strategic research and policy framework in order to benefit fully from forest biotechnology opportunities while minimizing the risks. Further information on issues surrounding forest biotechnology, visit the Natural Resources Canada website at http://www.nrcan.gc.ca/biotechnology/english/discuss.htm.

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Summary of the Workshop on Genetically Modified Trees; Aim of the Norwegian Gene Technology Act

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Abstract

The presentation included two parts. First I gave a short summary of some of the main elements from the OECD workshop on genetically modified trees that was held in Trondheim 13th to 15th September 1999. Then there was a short presentation of the aim of the Norwegian Gene Technology Act.

OECD Workshop on Trees

At the OECD workshop on genetically modified trees that was arranged in Trondheim in 1999 presentations were given by 21 experts from 11 countries. The presentations were divided into three different sessions: 1) Present status and future possibilities in gene technology concerning GM-trees, 2) Contribution from forestry practice and silviculture in risk assessment and 3) Systems and challenges in assessing environmental considerations. Some of the main topics discussed and raised by the working groups were:

- Trees include both forest trees and fruit trees. Both can be used in different types of plantations. Their use is often dependent on level of domestication. Many tree species are keystone species in ecosystems, and some forest trees develop their own ecosystems. Basic knowledge of the species biology, interaction and function in the environment was considered very important for the risk assessments.
- Their longevity and size is different from other plants. Some trees (e.g. spruce and pine) can have generation times from 10-20 years and become 100-300 years old. Other tree species can live more than 3000 years (e.g. giant sequoias). Gene flow and spread of pollen and seeds usually occur over longer distances and longer time frames than for most other plants. Transfer into natural populations and introgression with possible effects on fitness was considered important. Possible long-term effects on non-target species can be among the consequences. The ecological significance of gene flow has to be evaluated in the context of the specific trait, rotation and management practices. Monitoring of effects will be difficult in connection with many tree species due to the long life span.
- The long term stability of the genetic modification was considered as a difficult and an important issue to take into account in connection with risk assessments.
• It was considered possible to have different approaches regarding risk assessments for trees in plantations (fruit trees) in comparison with trees under natural conditions or used in traditional forestry.

One important conclusion was to use the “old” OECD principles “case by case” and “step by step” when considering deliberate releases of genetically modified trees.

Norwegian Regulations

Due to many questions regarding socio-economic considerations raised in the discussion the previous day, I decided to present the aim of the Norwegian Gene Technology Act. The Norwegian act differs from most other regulations with respect to considering ethical, social and sustainable questions in connection with applications for commercial and deliberate releases of genetically modified organisms. In section one of the act the purpose is stated as:

“The purpose of this Act is to ensure that the production and use of genetically modified organisms takes place in an ethically and socially justifiable way, in accordance with the principles of sustainable development and without detrimental effects on health and the environment”.

Section 10 the Approval, States That:

“In deciding whether or not to grant the application, significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development”.

One attempt to transfer the aim of the act to practical management when treating application for releases of GMOs, has been done by the Norwegian Biotechnology Advisory Board (NBAB) in guidelines released last year. The NBAB has the opinion that the Norwegian Gene Technology Act should be understood in a way that the demand for sustainable development, socially utilitarian value and other ethical and socially considerations, are requirements for a decision that alone can give conclusive weight against approval of an application. However this shall also be considered in proportion to the risk for harmful effects, when this is low.
One way to interpret the broad aim of the act, and which is the goal of the consequence assessment, is as the following figure shows:

The left side represents input from the assessments considering risks in connection with a release, while the right side considers the social justification and the ethical part of the assessment. There can be linkages both between the right and left side and the top and bottom of the diagram. Together the different assessments will merge into the overall assessment of the consequences for sustainable development.
Plants in Uses Other than Food Production:
A Summary of Session 3B

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The speakers in Session 3B on plants in uses other than food production presented papers on the environmental use of living modified organisms (LMOs) about which we are not yet very familiar. The examples the participants presented were very interesting, not only for their own merit but also because they lead to more general considerations that are of interest for the discussion of LMOs and the environment. In my response to the session I pointed out these considerations, and several other issues that came to mind while listening to the presentations.

David Heron gave an overview of the regulatory situation around “plant pharming”. The regulations aim mainly at preserving the safety and identity of the LMOs during the entire process—from planting to pharmaceutical processing—and at preventing these living modified organism (LMO) plants from entering the food supply in some way, such as by outcrossing. Interestingly, it is anticipated that these plants will not be deregulated.

In the early 1990s the Organization for Economic Cooperation and Development (OECD) Group of National Experts on Safety in Biotechnology discussed safety issues for the scaleup of LMOs in the environment—from field trials to full-scale production. The results of the discussion were published, and one of the points stipulated in this document was that scaleup of “pharmaceutical plants” would require more discussion of the specific risk assessment issues for this application. Over the past decade we have seen that many of the earlier claims about the technique of genetic modification turned out to be untrue, such as the once-asserted precision of insertion using the Agrobacterium tumefaciens T-borders or the predictability of expression of cloned sequences. One consequence of risk assessment might be that requirements are imposed that these pharmaceutical GMOs be constructed with special techniques that restrict the possibility of imprecise genetic transformations.

For certain types of LMOs, we might want to require that more reliable strategies be taken for their construction. One emerging strategy is the targeted integration of the modifying sequences at previously selected sites in the genome. Some of the advantages of this method are the avoidance of unexpected gene disruption at the site of integration and “location effects” at the level of expression. The location of the modifying sequence in the genome may influence the rate of outcrossing and the stability of the trait in the population. This restriction of gene location will allow
more stability when different traits are integrated at the same genomic location. These techniques may offer many advantages for production of LMOs having predictable traits, but, as always, we should carefully consider whether new techniques are “nice to apply” or are a “need to apply”.

In his presentation on transgenic fish, Wayne Knibb raised the point that transgenic fish may not be that different from fish generated by more traditional genetic techniques. This is a very important observation that is true for LMOs in general. It is often forgotten that some traditional genetic techniques used in plant breeding had an enormous impact on the genome, causing massive rearrangements. Against this background, the so-called pleiotropic effects of genetic modification, (e.g., integrative disruption of genes and mutations caused by random insertion of fragments of the modifying DNA) cannot have many effects that we have not already seen in traditional plant breeding. On the other hand it is clear that genetic modification offers much wider and better focused possibilities for gene exchange. This should be the emphasis of LMO risk assessment and not the pleiotropic effects.

One item on the original program for the meeting but that unfortunately had to be eliminated was bioremediation. This application of LM micro-organisms is important, for it teaches us several important lessons. It turns out to be very hard to get micro-organisms, whether modified or not, to work the way we want them to after their introduction in the environment. And, once we know how to do this at one site, it is difficult to transport that knowledge to another site. If anything, all the research efforts on bioremediation have shown us the versatility of the microbial environment and the genetic plasticity of microbial communities that appear to “share” their gene pool. It is illustrative that bioaugmentation—that is direct injection of a DNA sequence into the soil—works; the sequence apparently is taken up by micro-organisms in the environment that can put them to use. The main problem, however, appears to be how to enhance the activity of the micro-organisms in the environment when it is limited by the supply of energy-rich substrates. An interesting possibility is to use plants for delivery of these substrates into the soil.

I pointed out the impact that genomics is likely to have on the risk assessment of LMOs. Genomics is already causing a revolution in our way of thinking about genomes. That one-third of the genes identified in each new prokaryotic genome sequence are totally new, one-third are only similar to known genes, and only one-third of the genes can be readily recognized as “previously known” shows how limited our genetic knowledge really is. For eukaryotic genomes the situation is similar, but an added difficulty is the recognition of what sequences make up a gene in the first place.

How will the functions of these novel genes be identified? When these genes will be used as donor genes for genetic modification, the paradigms for risk assessment as we have used them until now will no longer be valid. Risk assessment requires extensive knowledge about the role of the donor gene in the physiology of the donor; from that knowledge the role of the gene in the physiology of the host organism can then be predicted, which leads to prediction of the interaction of the resulting LMO with its environment.

We will probably found our knowledge about the properties of these new genes on different types of data derived from the new science of bioinformatics. This is the time for risk assessors to start thinking about what type of data is an acceptable basis for risk assessment and what type of validation of data is needed.
This advance recognition is important just because of the tremendous impact that genomics will have on the possibilities of applied genetics. Our new knowledge may cause revolutions in genetic modification but may also do so in traditional breeding. Linda De Verno was certainly right in pointing out the very extended time scale that tree breeders face in developing a new cultivar. But I have no doubt that genomics information will be applied to shorten that time span substantially so that within the breeder’s lifetime he or she may see the product of his or her efforts and maybe even in several rounds of breeding.

The LMOs treated in this session require rigid analysis of their environmental impact. That certainly goes for the transgenic insects that David O’Brochta talked about. In general, however, the call for prediction of environmental impacts of LMOs only points out a very basic lack of knowledge about ecological processes in general. The ecological behavior of LMOs is sometimes easier to study just because of their special characteristics that make them, or their genes, easier to trace. However, we cannot interpret the results of such studies, if we do not know the baseline: what is happening in the environment in the present context before LMOs are extensively planted. We have the feeling that there is great need for fundamental ecological research to supply the tools needed to measure the ecological impact of LMOs as well as to produce the background information needed to interpret the results of the experimentally measured impacts.
Special Session:

Maize at the Center of Origin and Diversity
Transgenic Maize in the Center of Origin and Diversity of the Crop

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Abstract

The need for research on the consequences of releasing transgenic maize in Mexico, located within the region considered as the crop’s center of origin, had been identified in the years before the commercial availability of that biotechnological product in the United States. For many years, there was no funding to initiate the scientific investigation required to answer critical questions on the risks and benefits of transgenic maize in its center of origin and diversity. Up to this date, and after the first report on the possible presence of transgenic maize in Mexico, the question of whether or not unique risks are posed by novel crops in a center of origin and diversity remains unanswered.

Introduction

As defined by Vavilov (Harlan 1992), a center of origin and diversity of crops is a biogeographic region where the crop has its largest diversity and a close relationship exists with its wild relatives. Mexico is located within the Mesoamerican region, which has been identified not only as a center of origin but also of domestication of crops (figure 1). Thus, there is also a close relationship with ancient civilizations flourishing in this area of the Americas and the domestication of crops. Likewise, the main centers of primary agricultural development in Asia and the Middle East are associated with the presence of cultures and consequently with domestication of crops.

Through millenia, human populations have intensively and extensively selected and managed plants that have formed the basis of agriculture. Together with the biological forces that shape the evolution of crop plants, human intervention has been a factor of considerable importance that drives diversity of these crop plants.
To this date, surviving practices in traditional agriculture in some regions of the world still generate and produce diversity in many crops. In Mexico traditional agriculture can be found in many regions—mainly in the south and southeastern parts of the country.

At least 300 landraces have been identified in Latin America (Goodman et al., 1988). In Mexico more than 40 landraces (figure 2) have been collected from the 1940s to the 1970s, and most of these collections are preserved ex situ in gene banks of international public institutions such as International Maize and Wheat Center (CIMMYT) in collaboration with

Figure 1. Centers of origin and diversity of crops in the world.

Figure 2.  
a) Races of maize in Mexico and their relationships according to Wellhausen et al. (1952): 1) Ancient Indigenous Group; 2) Pre-Columbian Exotic Group; 3) Pre-Historic Mestizos; 4) Modern Incipient Group.
Mexican national agricultural research programs. At the same time, projects related to *in situ* conservation of maize have been established, and the dynamics of maize diversity is actually being observed, described, and analyzed.

With the advancement of biotechnology and the production of novel genetically engineered crops, the Mexican Secretariat of Agriculture in 1988 initiated an *ad hoc* committee to cope with the first requests of permits for testing of these biotechnology developments. Soon after these first requests, it was evident that a more formal committee was needed.

With the submission of the request for testing of transgenic maize material in the field, the discussion and assessment of risks for the maize crop in Mexico began. The highly social, cultural, economic, and agricultural importance of maize prompted the interest of the National Agricultural Biosafety Committee (NABC), which started a close consultation with experts on maize crop from many different scientific disciplines. In 1994, the NABC had been consolidated and an official national standard for the release of genetically modified organisms to the environment had been discussed. In 1995, a forum was organized to analyze the implications for maize diversity in Mexico in view of the imminent release of transgenic maize in the United States (Serratos et al. 1997).

Many unresolved matters relating to biosafety and regulation of transgenic maize triggered a new workshop in 1997 organized with the support of the North American Plant Protection Organization (Serratos et al. 2000). After this workshop, several discussions within the NABC and the Secretariat of Agriculture led to the decision in 1998 of a *de facto* moratorium of transgenic maize testing in Mexico.

In 1999, an *ad hoc* committee was organized with the aim of elaborating a report on the status and update of biosafety and regulation of genetically modified organisms, with a particular emphasis on the assessment of transgenic maize. Deriving from that report, the Intersecretarial Commission of Biosafety and Genetically Modified Organisms (CIBIOGEM, in Spanish) was created by Presidential Decree. This Commission overtook the duties of the NABC and expanded its regulatory and policymaking mandates to the environmental and health sectors. With regard to maize, the CIBIOGEM initiated a new consultation with experts from different scientific disciplines in 2001 to elaborate the terms of reference for the introduction, release, and management of transgenic maize in Mexico considered as the center of origin of the crop.
The biosafety and implications of the release of transgenic maize in Mexico, as the center of origin and diversity of maize, had been discussed for many years but only at the academic level. In the next section, some of the ideas that were discussed during these years are presented.

Discussion

The main conclusions and recommendations from the workshop organized at CIMMYT in 1995 (Serratos et al. 1997) were grouped in three sections as described in the following paragraphs.

Gene Flow from Transgenic Maize to Teosinte and Maize Landraces

1. Bidirectional introgression between maize and teosinte should be considered as present in the field even though this may occur at low frequency.
2. Always consider that the probability of gene flow between transgenic maize and landraces is much higher than that between transgenic maize and teosinte.
3. Design and conduct studies to obtain precise quantitative information on gene flow between Zea species and varieties to elucidate any possible effects from interactions between transgenes and “native” genes before releasing transgenic maize for commercial use in Mexico.
4. Assign different risk levels in the Mexican territory for field testing with transgenic maize.
5. Place the existing *ex situ* collection of the National Agricultural System (INIFAP) in proper long-term storage and build a national plant germplasm bank to preserve native species.
6. Before commercial release of transgenic maize, collection of the approximately 20 percent of teosinte diversity that was presumably not being collected in Mexico was recommended.
7. Establish a collaborative program to monitor teosinte populations and to salvage the knowledge of communities associated with the management of this germplasm.
8. First target landraces and then teosinte in deciding research and conservation priorities given that the transgenic flow will presumably occur in this order.
9. Begin conservation and characterization of maize and teosinte in zones close to settlements with high demographic growth and in areas with significant ecological changes.

Research in the Area of Risk, Impact and Biosafety

1. The effect of transgenes on teosinte cannot be anticipated or inferred until these transgenes are incorporated into its genome. Therefore, research on maize–teosinte introgression focusing on currently available transgenes should be established in two lines of investigation: (a) insecticidal protein in Bt maize to determine if introgression of these genes contributes to the development of insect populations resistant to the toxin; (b) resistance to herbicides, which could imply two different situations for teosinte populations. In one scenario teosinte could be at danger of extinction because of the application of herbicides that would accompany the
herbicide-resistant maize, and in the second scenario teosinte would develop a
greater fitness or increase its potential as a weed because of the introgression of
the transgenes.

2. Set up experiments to determine the frequency of migration (m) of maize pollen to
fertilize teosinte, the fitness or selective coefficient (s) of maize–teosinte hybrids
independent of the selective coefficient of the transgene, and the selective coefficient
of the transgene in the hybrid. These parameters could be used in population
genetic models and risk analysis. Some of these experiments can be done in situ.

Regulation and Safety Measures in Transgenic Maize Tests

1. Field tests with transgenic maize could be carried out in Mexico as long as proper
measures were adopted to prevent gene flow to other Zea species.
2. Critical questions for transgenic maize in Mexico are not at the laboratory or
experimental level, where conditions can be controlled, but at the stage of deciding
whether to permit commercial release when there cannot be containment.
Therefore, careful analysis of the consequences of deregulation is recommended.
3. The workshop recommended establishing an education and communication program
to inform the public about the introduction of transgenic maize and to clarify the
decisionmaking process regarding deregulation.
4. The workshop recommended that research on gene flow and the analysis of
biological risks derived from the use and release of transgenic plants be a coordinated
multi institutional task. This would involve the participation of biotechnologists,
ecologists, plant breeders, and other scientists from diverse disciplines.

After this workshop and for some time thereafter some proposals for projects aimed to
study the lines of research that were discussed in this forum were advanced. Unfortunately,
there was a lack of interest from possible donors, and the necessary funds to finance this
research were not available to follow up the recommendations given at the workshop. The
only project that was developed after the meeting was financed by the Mexican Seed
Association, and the main objective was to determine the frequencies of pollination from
maize to teosinte; however, no publications resulted from that investigation nor a report to the
Ministry of Agriculture.

Between 1995 and 1997, most of the field testing of transgenic maize was carried out in
very small plots within public research institutions under the supervision of the General
Directorate of Plant Health. Questions about the impact of transgenic maize on maize diversity
were not specifically discussed. Predominantly, the issues related to the analysis of scenarios
on the impact of Bt maize and herbicide-tolerant maize in the agroecosystem were discussed
at length in different fora.

The workshop organized in 1997, which had a broader scope involving the regulatory
systems of the three countries within NAFTA, had the main objective of reviewing the status
of transgenic maize in Mexico. The recommendations from this meeting were as follows:

1. That NABC together with a panel of experts establish a working plan for specific
research in the area of biosafety.
2. That the framework of a Mexican risk assessment model be elaborated.
3. That maize genetic resources be preserved in situ, not only with the view of transgenic
maize impact, but to identify the factors involved in the genetic erosion of the crop.

During the meeting, the opinion of the participants was that not much had been advanced in terms of research after the workshop in 1995 and that, consequently, the main concern was how to get the necessary funding from different sources. Actually, the situation regarding the research on biosafety, genetically modified organisms and genetic diversity, and risk assessment and management did not change that much in all these years from 1994 to 1997.

For several years, however, it had been noted within the NABC that the deregulation of transgenic maize in the United States could be a significant source of grain-containing transgenic material. Also, in the two workshops devoted to transgenic maize in Mexico, the possibility for this mechanism as a port of entrance of transgenic maize into the country was foreseen, but banning the importation of transgenic maize from the United States was not formally proposed. In any case, through NAFTA quotas for grain importation from the United States had been imposed on Mexico.

Again, within NABC several discussions took place among the members to define a position on risk assessment, management, and research related to transgenic maize in Mexico. The NABC sent a report containing different scenarios of transgenic maize regulation to the General Directorate of Plant Health (GDPH). The GDPH, after analyzing the report, stopped receiving submissions for field testing of transgenic maize. With the support of the Under Secretary of Agriculture, a moratorium on the release to the environment of transgenic maize in Mexico was established in 1998.

Reports in the newspapers about the presence of transgenic maize seed in commodities from the United States prompted the creation in 1999 of an ad hoc committee that produced a document on the status of the Mexican biosafety system for the president (Sarukhan-Kermez and Larson-Guerra 1999). By the end of that year, the Intersecretarial Committee on Biosafety and Genetically Modified Organisms (CIBIOGEM) was created by presidential decree.

Once again, countless fora were organized to analyze the issues of transgenic organisms, transgenic food, risk assessment, risk management, and policies involving LMOs and products of biotechnology in Mexico. At the same time, the controversy was filled with just opinions from different angles of the problem (Martinez-Soriano and Leal-Klevezas 2000). However, the hard data from research on fundamental questions of gene flow, pest resistance to transgenic maize, gene–transgene interactions, diversity, and human intervention in maize agricultural systems in Mexico were almost completely absent. Only a handful of reports related to basic questions posed since the workshop in 1995 were published (Garcia et al. 1998, Kato and Sanchez 2002, Louette et al. 1997, Luna et al. 2001, Ruiz et al. 2001, Sanchez et al. 1998, Serratos et al. 2001).

In 2001 there were confidential reports stating the possibility that transgenic maize crops were present in Mexico. The National Institute of Ecology and the National Commission for the Conservation and Use of Biodiversity started a survey to analyze these reports (Ezcurra and Soberon 2002, this proceedings). Almost at the same time, the CIBIOGEM organized a seminar to establish the terms of reference for transgenic maize in Mexico. To date, this document has not been published.

Despite the controversy around the work of Quist and Chapela (2001) on the validity of their results, undoubtedly this work pointed out a fundamental issue for the Mexican biosafety
system, which is the assessment, management, and monitoring of transgenic maize in Mexico. All these activities need strong support from scientists and the research system in Mexico. Despite the time that has elapsed it is still possible to address this situation.

**Conclusion**

The important issues such as uncertainty, risk assessment, risk management, gene flow, prediction, diversity and evolution, environmental complexity, and biotechnology in the maize center of origin have not been thoroughly investigated. Therefore, one question will remain for some time: do novel plants in a center of origin and diversity of crops pose unique risks?

**References**


Evidence of Gene Flow from Transgenic Maize to Local Varieties in Mexico

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Abstract

Maize, originated and domesticated in Mexico, is the basis of many food and feed products. Its development is correlated with the development of Mesoamerican civilizations. To date, traditional agricultural practices in Mexico promote and maintain maize diversity. Following a communication related to the presence of transgenic material in Mexican maize landraces, the National Institute of Ecology and the National Commission on Biodiversity started an investigation in collaboration with two national institutions. Here we present preliminary results of the first of a series of tests in progress. We obtained polymerase chain reaction amplifications of the CaMV 35S promoter and the NOS terminator from DNA extracted from maize seedlings grown from seeds collected in different localities at Oaxaca and Puebla in Mexico. Our preliminary data suggest that the frequency of transgenic constructs in the field might be low, although the geographic dispersion seems to be widespread. Further analyses will help to corroborate this pattern.

Introduction

Even though there is still some controversy on the origin and early history of maize, in general agreement exists that the domestication of Zea mays occurred in Central Mexico (Kato 1976, Mangelsdorf 1974, Dobley and Goodman 1984, Doebley et al. 1987, Doebley 1990). The development and improvement of maize are correlated with the development of cultural complexity
and the rise of highly organized civilizations in pre-hispanic Mesoamerica. A recent analysis (Piperno and Flannery 2001) using accelerator mass spectrometry to date maize cobs from the Guila Naquitz cave in the mountainous eastern part of the Valley of Oaxaca established that this sample from about 6,250 calendar years ago represents the oldest maize cobs known to date. Because of the absence of Zea macrofossils in earlier sediments of the cave, these phytoliths, unlike pollen grains, provide evidence of an early domestication process somewhere else before settlement in this region.

A high diversity of maize populations is still present in many regions of the Mexican territory, where more than 40 landraces of maize have been described (Ortega 1980, Benz 1986, Sánchez 1989, Wellhausen 1987, Hernández Xolocotzi 1998). Traditional agricultural practices in many parts of the country promote and maintain maize diversity. In places with high biological and landrace diversity, most of the land planted with maize occurs in relatively small units and often in combination with beans and squash. The small farmer and peasant communities are highly open to seed exchange, and it can be observed that the traditional management of varieties leads to a constant flow of genetic material among communities over large areas (Louette 1997). Farmers continually maintain cultivars through seed selection. Through the years Mexican races of maize have been used by Mexican farmers to generate new varietal mixtures as well as creolized materials, which are crosses between modern improved varieties and hybrids with traditional landraces.

The wild relatives of maize, the teosintes, are present in many areas of maize production. Because maize is primarily a wind cross-pollinating species, the possibility of a low-frequency introgression cannot be completely discounted. Maize and teosinte coexist sympatrically and form fertile hybrids (originated from maize plants fertilized by teosinte pollen) in many regions (Kato 1997). Although there are genetic barriers that hinder the fertilization of teosintes by maize pollen (Evans and Kermicle 2001), the risk of gene flow from the cultivated species into its wild relatives cannot be totally ruled out.

In late 2000, researchers from the Zapotec–Chinantec Union (UZACHI) and the University of California at Berkeley initiated a program to document the absence of transgenic markers in traditional maize in the Sierra de Juárez, Oaxaca, with the aim of opening a market for “transgenic-free gourmet corn.” However, during the process of setting up their experimental protocols they found that some ears from criollo samples gave positive results for the transgenic 35S promoter. Ignacio Chapela from the University of California at Berkeley, the coordinator of this research program, communicated his findings to the environmental authorities in Mexico. On the basis of this communication, the National Institute of Ecology (INE) from the Ministry of Environment and Natural Resources (SEMARNAT) and the National Commission on Biodiversity (CONABIO) started an investigation to corroborate the results and to evaluate and quantify the levels of the gene flow from transgenic corn to landraces from Oaxaca. The research performed by Chapela was published last year (Quist and Chapela 2001).

**Methods**

We sampled 21 locations as well as two grain distribution centers. Sampling of maize consisted of both complete ears and harvested seeds. Most locations were small rural communities in the Sierra de Juárez in the State of Oaxaca. Two localities were sampled in the State of Puebla in Mexico (table 1).
Two random subsamples were taken and sent to two independent laboratories: the Center of Research and Advanced Studies (CINVESTAV) from the National Polytechnic Institute at Irapuato and the Institute of Ecology at the National University of México (UNAM). The samples were blind-coded, and the whole procedure was notarized by a Mexican public notary. When the sample consisted of complete ears (i.e., seeds left attached to the cobs), the seeds arising from each different ear were tagged to preserve the maternal identity.

The work at each laboratory followed similar research protocols for better comparison of results. Seeds were treated with a fungicide and planted in controlled conditions. After germination the first leaf was used for DNA extraction. Subsequent polymerase chain reaction (PCR) analyses followed standard protocols.

The DNA was extracted and purified from a total of 1,876 seedlings, which included between 30 and 275 for each location. PCR analyses were performed with primers for the 3S promoter from the cauliflower mosaic virus (CMV) and the nopaline synthase terminator (T–NOS) sequence from Agrobacterium tumefaciens. Two series of primers for each DNA sequence were tested. T–NOS 118bp—GCA TGA CGT TAT TTA TGA GAT GGG; T–NOS 118bp—GAC ACC GCG CGC GAT AAT TTA TCC; 35S 195pb—GCT CCT ACA AAT GCC ATC A; and 35S 195pb—GAT AGT GGG ATT GTG CGT CA. We also amplified the 16S nuclear ribosomal gene to test DNA quality. Positive and negative controls were included in each PCR run.

Once both laboratories finished their initial analyses we compared their results for potential discrepancies and pooled them if they did not differ significantly. In the few cases in which significant differences were found in two subsamples, the analyses were repeated to discard contamination and other technical artifacts.

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<td>17°16'</td>
<td>96°28'</td>
</tr>
<tr>
<td>20</td>
<td>El Punto</td>
<td>Oaxaca</td>
<td>Ixtepeji</td>
<td>2422</td>
<td>17°13'</td>
<td>96°35'</td>
</tr>
<tr>
<td>21</td>
<td>Las Presas</td>
<td>Oaxaca</td>
<td>Tlalilac</td>
<td>1653</td>
<td>17°05'</td>
<td>96°39'</td>
</tr>
<tr>
<td>22</td>
<td>Nochixtlán</td>
<td>Oaxaca</td>
<td>Nochixtlán</td>
<td>1660</td>
<td>17°27'</td>
<td>97°13'</td>
</tr>
<tr>
<td>23</td>
<td>Santo Tomás Teipan</td>
<td>Oaxaca</td>
<td>Santa María Ecatepec</td>
<td>2380</td>
<td>16°15'</td>
<td>95°59'</td>
</tr>
</tbody>
</table>
Evidence of Transgenic Gene Flow

Results

We found PCR evidence for the presence of the 35S promoter in 95-percent of the localities sampled. For all different localities a total of 142 (7.6-percent) seedlings gave positive results for this sequence. All (100-percent) seedlings gave positive results for ribosomal gene 16S. Amplifications indicating the presence of the T–NOS sequences showed consistently lower frequencies (see table 2). A small sample of the PCR amplifications obtained with the 35S primers was cloned and sequenced and compared with the sequence of the 35S CMV promoter. Most of them showed sequence identity whereas one showed a single base pair difference.

In 15 localities we found that less than 10-percent of the seeds showed evidence of transgenic markers. However there was considerable variation in the frequencies found (from 1 to 35-percent). In the sample taken in a grain store at Ixtlán de Juárez, where maize grains for tortillas imported from outside the region are sold, 17-percent of the grains showed amplifications of the 35S promoter, whereas the sample from the local market, where we sampled locally grown *pozole* (stewed maize) grains, showed no evidence of the presence of either marker used.

In five localities (mostly outside the core of the Sierra de Juárez, Oaxaca) we found higher frequencies of transgenic introgression ranging between 10 and 35-percent. These localities are found in the Central Valleys of Oaxaca: in the Mixtec Region, in the southern portion of the Sierra de Juárez, and in the Tehuacán Valley in Puebla. However, the high frequencies observed in these last sites could also be caused by a sample artifact: our sampling involved only a few, randomly selected maize ears on which we arbitrarily sampled individual grains. Thus, there is a fixed experimental maternal effect (what statisticians call “plants-nested-within-sites”) that could be driving these results. A logistic analysis (Crawley 1993) of the data did detect significant maternal effects, but these effects were always detected at low-frequency sites. Thus, we can conclude (with some caution) that the sites showing high frequencies are probably places where the frequency of transgenic constructs is significantly higher.
Table 2. Observed frequency of PCR amplification products

<table>
<thead>
<tr>
<th>LOCALITY</th>
<th>SEEDLINGS</th>
<th>FREQUENCY 35S nos</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>0.044 -</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>0.039 -</td>
</tr>
<tr>
<td>3</td>
<td>94</td>
<td>0.149 -</td>
</tr>
<tr>
<td>4</td>
<td>97</td>
<td>0.072 0.04</td>
</tr>
<tr>
<td>5</td>
<td>89</td>
<td>0.022 -</td>
</tr>
<tr>
<td>6</td>
<td>91</td>
<td>0.011 -</td>
</tr>
<tr>
<td>7</td>
<td>128</td>
<td>0.062 0.03</td>
</tr>
<tr>
<td>8</td>
<td>105</td>
<td>0.038 -</td>
</tr>
<tr>
<td>9</td>
<td>84</td>
<td>0.107 0.05</td>
</tr>
<tr>
<td>10</td>
<td>275</td>
<td>0.036 -</td>
</tr>
<tr>
<td>11</td>
<td>37</td>
<td>0.351 0.05</td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>0.062 -</td>
</tr>
<tr>
<td>13</td>
<td>163</td>
<td>0.098 -</td>
</tr>
<tr>
<td>14</td>
<td>65</td>
<td>0.020 0.02</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>0.167 -</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>- -</td>
</tr>
<tr>
<td>17</td>
<td>60</td>
<td>- -</td>
</tr>
<tr>
<td>18</td>
<td>60</td>
<td>0.067 -</td>
</tr>
<tr>
<td>19</td>
<td>75</td>
<td>0.040 -</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
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</tr>
<tr>
<td>21</td>
<td>60</td>
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<tr>
<td>22</td>
<td>45</td>
<td>0.111 -</td>
</tr>
<tr>
<td>23</td>
<td>75</td>
<td>0.173 0.01</td>
</tr>
</tbody>
</table>

Discussion

These preliminary results present provocative evidence suggesting that the amplification of the 35S sequence and the T–NOS are due to the introgression of transgenic sequences into Mexican traditional maize populations. However, because our analysis was done through PCR amplification, the possibility of false positive results cannot be totally ruled out. If these results are corroborated by a series of other analyses currently in progress, the presence of transgenic elements planted in Mexico will be definitely confirmed in spite of a national policy that has put into place a standby moratorium on the planting and cultivation of transgenic maize in the country.

The ecological consequences of the possible flow of transgenic constructs into traditional varieties are not well known, and more research is clearly needed on the subject. Among other consequences, the possible introduction of transgenic constructs into populations of the different species and subspecies of teosintes (corresponding to all the wild species of the genus Zea, including all the wild subspecies of Zea mays; see Buckler and Holtsford 1996) needs to be studied in detail. Two of the possible consequences that need to be addressed are (a) the potential genetic erosion of the traditional landraces (e.g., Ortega Paczka 1999) and (b) the possible increased weeding of teosinte plants if insect-resistant or herbicide-tolerant transgenes were allowed to drift into the wild populations. Effects on biodiversity in general should also be evaluated.
Our preliminary data suggest that the frequency of transgenic constructs in the field might be low, although the geographic dispersion of the presence of the transgenes seems to be widespread. Further analyses in other parts of the country as well as monitoring and the sampling of additional localities will provide a clearer picture of the situation. However, we still need to know if enzyme-linked immunosorbent assay (ELISA) tests, as well as BASTA resistance experiments and Southern blot hybridization will further confirm this distribution pattern and rule out the possibility of false positives in the PCR analysis.

More extensive sampling—including milpas (traditional maize fields) in many parts of Mexico as well as wild populations of teosintes in successive planting seasons—will allow us to define in a more precise manner the trends and the risks involved for biodiversity.

Acknowledgments

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In Situ Conservation of Maize Diversity, Gene Flow, and Transgenies in Mexico

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Abstract

Mexico is within the primary center of domestication and diversity of maize (Zea mays L.). The knowledge, preferences, and farm management practices of small-scale Mexican farmers have played a key role in the evolution of maize and its diversity in the country—a role that is still present and widespread. This paper argues that these same conditions—farmers’ knowledge, preferences, and farm management practices—that promoted and maintained maize diversity in Mexico would be conducive to the diffusion of transgenes into maize landraces if they were introduced in Mexico. To assess the potential diffusion and impact of transgenes into maize landraces in Mexico, it is therefore fundamental to take farmers’ conditions and management into consideration. The paper describes the way Mexican small-scale farmers manage their maize populations, particularly landraces. It relates this management to the maintenance and evolution of maize diversity and in turn to its conservation in situ and explores the implications of farmers’ management for the potential diffusion of transgenes into farmers’ maize populations. A key message is that the parameters used in developed countries to assess the environmental impacts of transgenic maize varieties may not be appropriate for the situation in Mexico and Central America where these parameters may be different.

Introduction

Mexico is within the primary center of domestication and diversity of maize (Zea mays L.). This diversity is confirmed by the presence in Mexico of most maize races reported for Mesoamerica (Bretting and Goodman 1989). A maize race is the basic taxonomic unit used to describe the diversity of maize landraces. A maize “race” has been defined as “a group of related maize plants with enough to be recognized as a group” (Anderson and Cutler 1942:71). In Mexico 49 maize “races” have been identified (Sanchez and Goodman 1992). Both isozyme analysis (Doebley et al. 1985) and analysis of morphological characteristics (Sanchez and Goodman 1992) indicate that the variability between races is significant. A long history of coevolution connects maize and human populations in Mesoamerica.
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(Hernandez 1985, Wellhausen et al. 1952). Small-scale Mexican farmers’ knowledge, preferences, and management practices have played a key role in the evolution of maize and maize diversity—a role that is still present and widespread. The cultural significance of the crop, its multiple uses by rural communities, and specialized tastes and preferences for foods prepared from the crop are expressed in farmers’ selection criteria and the diversity present among the maize populations they grow.

Maize is the staple food of Mexicans—particularly the rural poor. During the rainy season of 2000, about 7.5 million hectares were planted to maize out of a total of 12.5 million hectares planted to annual crops (SAGARPA 2001). About three million small-scale farmers plant maize. Despite the availability of improved maize varieties over the last 40 years and repeated Government programs to encourage their use, today improved varieties are planted in only about one-fifth of the total maize area of the country. Most of this area is located in the commercial production zones of central and northwestern Mexico (Morris and Lopez-Pereira 1999). Hence, about four fifths of the area under maize is planted to landraces or recycled improved varieties (creolized varieties). Mexican small-scale farmers are not only heirs to the diversity of maize landraces but continue to maintain it.

This paper argues that small-scale maize farmers in Mexico play a key role in the maintenance and evolution of maize diversity and that the same farmers’ conditions and practices that have helped maintain and promote maize diversity in their fields will also be conducive to the diffusion of maize transgenes if they are introduced into Mexico. To assess the potential diffusion and impact of transgenes into maize landraces, it is therefore fundamental to take these farmers’ conditions and management into consideration.

The goals of this paper are as follows:

1. To describe the way Mexican small-scale farmers manage their maize populations, particularly landraces
2. To relate this management to the maintenance and evolution of maize diversity and therefore to its conservation in situ
3. To explore the implications of farmers’ management for the potential diffusion of transgenes into farmers’ maize populations

The rest of paper is divided into four sections. The first describes the way small-scale Mexican farmers manage their maize populations. This is followed by a discussion of on farm (in situ) conservation of maize diversity as a component of global strategy to conserve genetic resources. The third section discusses the implications of farmers’ conditions and management for the potential diffusion of transgenes into farmers’ landraces. Finally, the conclusions are presented.

Small-Scale Farmers and Maize Diversity in Mexico

Small-scale Mexican farmers’ knowledge, preferences, and management practices have played a key role in the evolution of maize and its diversity in Mexico, which is a role that is still present and widespread. Key maize management practices of small-scale Mexican farmers include planting numerous maize “varieties” within a small area or under management by a single farmer, seed recycling, seed flows, mixing seed of different origins, and creolization. Furthermore, farmers’ activities have a direct impact on teosinte—the wild relative of maize—”cultivation” and on regulating gene flow between maize and teosinte. A
discussion of the relationship between farmers’ practices and teosinte, however, is beyond the scope of this paper.

**Landscapes with Multiple Maize Populations.**

Many small-scale maize farmers simultaneously plant more than one “variety” to meet different needs and preferences (Bellon 1996). This is particularly important because most farmers consume what they produce, which means that their decisions of what to plant are not only influenced by the agronomic performance of a variety but also by the quality of the end-products such as tortillas, tamales, or atole.

Because even within a community farmers are not homogenous they may plant different varieties, which leads to a landscape in which numerous different maize populations coexist side by side (Bellon and Brush 1994, Louette et al. 1997, Perales 1999). Furthermore, because these farmers usually own several small plots scattered throughout the landscape, they are unable to prevent the exchange of pollen between varieties (Bellon and Brush 1994). This condition creates a landscape in which numerous different maize populations are planted side-by-side and an environment conducive to pollen flow among different maize populations.

**Seed Recycling**

Saving seed from one season to the next (also known as seed recycling) is an almost universal practice among Mexican small-scale farmers. Farmers usually follow strict procedures to choose what they keep as seed for the next season. Saving seed is not only a practice associated with landraces, and saving seed from hybrids is much more prevalent than generally believed (Morris et al. 1999). Seed selection has important genetic implications. First of all, it defines which individuals, and therefore which traits and alleles, go to the next generation and which do not, therefore affecting the genetic structure of the population. Farmers exert direct selection pressures on ear characteristics but only indirect pressures on related plant characteristics such as plant height given that seed is selected in the household and not in the field; hence plant characteristics are rarely taken into account (Louette and Smale 2000, Smale, et al. 1999). It may also be fundamental to maintain the integrity of a variety (at least from the point of view of the farmers), which can easily be lost owing to hybridization (Bellon and Brush 1994, Louette et al. 1997).

**Seed Flows**

Besides maintaining seed from their own stocks, Mexican farmers commonly acquire it from other farmers or sources in their own community or far away from it. For example, in our work we discovered the introduction of Zapalote chico (a tropical race found at sea level) of the Istmo de Tehuantepec into communities of the Central Valleys of Oaxaca 200 km away and at 1,800 meters above sea level. There are several reasons for seed flows. The risk of losing seed of an appreciated variety is a constant threat owing to pests, disease, drought, or frost. Farmers may plant small areas due to socioeconomic constraints, or in the case of particular varieties, such as black or red maize types, therefore easily finding themselves without enough seed to plant the next season (Aguirre Gómez 1999; Louette et al. 1997). There is a common belief among farmers that they must change seed regularly to maintain the productivity of the variety, as Louette et al (1997:31-2) recount “sow the same maize type but
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from new seed. As they report, the frequency of seed renewal varies from several cycles to several years.

Seed flows are important to understand the diversity in a given location because they are the basis of incorporating new varieties and obtaining materials that have been lost but are desirable. These flows may have important genetic implications because they may be an important mechanism for the migration of genes and may counter genetic drift and mutation accumulation in varieties planted over very small areas (Louette et al. 1997).

Mixing Seed of Different Origins

It is not uncommon for farmers to get seed from other farmers to plant alongside their own either because they do not have enough seed or with the expressed idea of modifying their maize population. Aguirre Gómez (1999) has described this practice as “partial seed exchange.” The modification may involve combining desirable characteristics of a foreign variety with one’s own, or it may be done to counter the loss of vigor in one’s variety. Many farmers said that after planting a variety for many consecutive seasons, it “gets tired” (se cansa), and therefore one needs to add seed from a foreign variety to it. For example, in our work with landraces collected in the central valleys of Oaxaca, when they are selfed they exhibit a high proportion of deleterious mutations probably owing to endogamy. An influx of foreign genes may enhance heterozygosity and hence avoid expression of these mutations.

Creolization

Although the adoption of improved maize varieties has been limited in Mexico, there is increasing evidence that small scale subsistence farmers have incorporated improved varieties into their farming systems, planting them alongside their landraces and, once adopted, managing them the same way as their landraces. These farmers, willingly or accidentally, have promoted the hybridization of improved varieties and their landraces. This process, through which materials produced by the formal plant breeding programs change when placed in the hands of farmers, has been termed “creolization” or “rustication” (Bellon and Risopoulos 2001, Wood and Lenné 1997). Farmers recognize the products of this process as “creolized” varieties (variedades acriolladas). They are appreciated because they are perceived to combine the advantages of improved varieties and landraces.

Farmers’ conditions and management practices described above can be summarized in the following factors:

- Multiple maize populations coexisting in the same landscape
- Fragmented landholdings (small plots and large border effects)
- Seed recycling
- Short- and long-distance seed flows among farmers
- Creolization
- Partial seed exchanges

These practices and conditions generate important gene flows among distinct, and sometimes distant, maize populations. These flows are fundamental to maintain the viability of these maize populations. Table 1 presents examples of farmers’ practices and management conditions that are conducive to gene flow from case studies carried out in Mexico.
Table 1. Examples of Farmers’ Practices and Management Conditions

<table>
<thead>
<tr>
<th></th>
<th>Chiapas a</th>
<th>Oaxaca a</th>
<th>Guanajuato b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1997</td>
<td>1997</td>
<td>1996</td>
</tr>
<tr>
<td>Number of households</td>
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<td>240</td>
<td>160</td>
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<tr>
<td>Varieties/household</td>
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<td></td>
</tr>
<tr>
<td>average</td>
<td>2.4</td>
<td>1.5</td>
<td>1.95</td>
</tr>
<tr>
<td>min-max</td>
<td>1-5</td>
<td>1-5</td>
<td>1-4</td>
</tr>
<tr>
<td>Fields/household</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>average</td>
<td>2.6</td>
<td>3.4</td>
<td>2.2</td>
</tr>
<tr>
<td>min-max</td>
<td>1-7</td>
<td>1-9</td>
<td>1-6</td>
</tr>
<tr>
<td>Field size (ha)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>average</td>
<td>3.4</td>
<td>0.92</td>
<td>4.22</td>
</tr>
<tr>
<td>min-max</td>
<td>.05-13</td>
<td>.062-6</td>
<td>.5-26</td>
</tr>
<tr>
<td>Partial exchange households (%) c</td>
<td>7.1</td>
<td>30.4</td>
<td>49.3</td>
</tr>
<tr>
<td>Total exchange households (%) d</td>
<td>43.9</td>
<td>19.6</td>
<td>39.8</td>
</tr>
<tr>
<td>Seed flows</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flows (% households)</td>
<td>36.7</td>
<td>37.5</td>
<td>nd</td>
</tr>
<tr>
<td>local (% households with flows)</td>
<td>50.0</td>
<td>97.8</td>
<td>nd</td>
</tr>
<tr>
<td>non local (% households with flows)</td>
<td>61.1</td>
<td>13.3</td>
<td>nd</td>
</tr>
<tr>
<td>Seed recycling (% households)</td>
<td>92.9</td>
<td>96.3</td>
<td>nd</td>
</tr>
</tbody>
</table>

Sources:

a Unpublished data CIMMYT;

b Aguirre Gómez 1999

c Planting a mix of seed from different origins including seed from one’s previous harvest

d Planting exclusively seed obtained outside the household.

Maize diversity in farmers’ fields is not a static condition but rather a dynamic process. Gene flow and farmer selection are the basis of this diversity. Furthermore, gene flow counters endogamy in maize populations planted in small areas. The introduction of “foreign” germplasm can be a source of morphological and agronomic diversity rather than genetic erosion (Louette et al. 1997). Gene flow can occur over long distances with very diverse materials, and even though some may not be appropriate for the environments where they are introduced, they may constitute a source of new alleles for local populations.

Maize landraces are not static and are continuously evolving owing to the gene flow that farmers favor and their selection of maize characteristics for changing conditions and preferences. Maize landraces are open genetic systems that continuously incorporate traits from exotic germplasm, including improved varieties. For example, morphological and genetic analyses of maize landraces collected in the central valleys of Oaxaca, Mexico, have shown that there is a strong selection for morphological traits of importance to farmers, mainly ear and kernel traits, which can be (or are perceived to be) related to culinary qualities. However, when the analysis was done with neutral molecular markers, no clear structure was detected among the landraces from different farmers—that is all the landraces shared the same genetic
neutral diversity, which can be explained by a very strong migration effect (either through seed or pollen, G. Pressoir, pers. comm.)

**In Situ Conservation of Maize Diversity**

There is a worldwide recognition of the importance of conserving crop genetic diversity. This has led to public investment in the creation and maintenance of gene banks around the world for many different crops (i.e., ex situ conservation; Plucknett et al. 1987). More recently, on farm (in situ) conservation has emerged as an important complement to ex situ conservation (Altieri and Merrick 1987, Maxted et al. 1997) and as part of a global strategy to conserve genetic resources (Brush 1999, IPGRI 1993, Maxted et al. 1997, Wood and Lenné 1999).

On-farm conservation involves farmers’ continued cultivation and management of a diverse set of landraces in the agroecosystem where they were developed (Bellon et al. 1997). This approach depends on farmers’ active participation because it only succeeds to the extent that farmers find it in their interest to maintain diversity (Brush 1991). On-farm conservation seeks to maintain the evolution of crop populations in response to natural and human selection. In the case of maize, conservation aims at maintaining farmer management practices and conditions associated with maize diversity and not necessarily any specific maize population. Hence, the practices and conditions described above are at the core of the conservation of maize genetic resources on-farm in Mexico.

**Maize Diversity, Farmers, and Transgenic Varieties**

The recent discovery of transgenic products in maize landraces planted by small-scale Mexican farmers has caused great concern (Quist and Chapela 2001). Although these results have been questioned (Christou 2002), they point out the need to look into the potential spread of transgenes into maize landraces in Mexico—the center of origin and domestication of maize—and their potential impact on the environment, biodiversity, and the livelihoods of small-scale maize farmers.

The management of maize germplasm by Mexican small-scale farmers is very different from that of their counterparts in the USA, Canada, and Western Europe where hybrids dominate maize farming, farmers purchase seed from commercial sources and have large landholdings (particularly compared with small-scale Mexican farmers). Clearly these conditions contrast with the conditions of Mexican farmers presented above. Therefore, the parameters used in developed countries to assess the environmental impacts of transgenic maize varieties may not be appropriate for Mexico. In this country, it is fundamental not only to take into account biological factors, but also the practices and conditions of small-scale farmers that inadvertently or even willingly may or could introduce transgenes into their agroecosystems. For example, a simple approach to assess the potential diffusion of transgenes may be to measure the distance at which pollen can flow and remain viable (e.g. Luna et al. 2001). However as illustrated above, the conditions and practices of Mexican farmers foster pollen and therefore gene flow, which is basic for the maintenance of the diversity and viability of their landraces.

One can hypothesize that if small-scale Mexican farmers have access to transgenic varieties,
and if these varieties are perceived as valuable by them, they will foster their diffusion wittingly or unwittingly into their local maize populations. The same farmers’ conditions and practices that maintain and promote diversity in their fields and farms may lead to the diffusion of transgenes into their landraces if transgenes are introduced. Clearly, this is a complex process that merits much research since there are many unknowns. For example the diffusion of transgenes may depend on the scale of introduction of transgenic varieties, on the genetics of the associated transgenes, and the fitness that those transgenes may confer to the populations they enter. However, this fitness cannot be assessed purely on biophysical factors, but also needs to be evaluated in terms of farmers’ management practices and their cultural preferences.

There are many questions that have to be addressed if the spread of transgenes to maize landraces happens. Some of those questions are: Is this diffusion positive or negative? For whom is it positive and for whom is it negative and why? Does the spread of transgenes jeopardize genetic diversity, and if so, how? What may be the impact of the diffusion of transgenes on the livelihood of small-farmers who depend on maize for their sustenance? How would the owners of these transgenes react to their diffusion into non-target maize populations? How would maize consumers react to this?

The answers to some of these questions should be addressed through scientific research, while others have to do with values and preferences of different members of society. This in turn requires a broad debate on the potential benefits and costs of introducing transgenes in areas such as Mexico. For all these reasons, it is important to recognize the complexity and uncertainty faced by scientists, policy makers and society in general in trying to assess the potential diffusion and impacts of transgenes in a center of maize diversity and domestication.

**Conclusions**

Small-scale Mexican farmers’ knowledge, preferences, and management practices continue to play a key role in the evolution of maize and its diversity. This role is fundamental for the conservation of this diversity on-farm. However, the same farmers’ conditions and practices that maintain and promote diversity may lead to the diffusion of transgenes into their landraces if transgenes are introduced. To assess the potential diffusion and impact of transgenes into maize landraces in Mexico, it is fundamental to take farmers’ conditions and management into consideration. These conditions are different from those of farmers in developed countries and the parameters used in developed countries to assess environmental impacts of transgenic maize varieties may not be appropriate for Mexico. Furthermore, recognizing the importance of farmers and their management for these issues, points out the complexity and uncertainty faced by scientists, policy makers and society in general in trying to assess the potential diffusion and impacts of transgenes in a center of maize diversity and domestication.
Acknowledgements

We thank Gael Pressoir for sharing his insights and results on the genetic structure of maize landraces in the Central Valleys of Oaxaca and Satwant Kaur for her editorial assistance.

Endnotes

1. The concept of a landrace is complex (Zeven 1998), and here we used this term for a locally grown maize population that a farmer cultivates and manages as a seed lot. A seed lot is defined as “...all kernels of a specific type of maize selected by a farmer and sown during a cropping season to reproduce that particular maize type” (Louette et al. 1997:24).

2. Here we use the term “variety” to refer to farmer varieties: crop populations that a group of farmers recognize as distinct units, regardless of whether they are landraces, improved, or creolized varieties. This definition contrast with the one used in the context of developed country agriculture, where a variety is defined as a plant grouping within a single botanical taxon of the lowest rank, which grouping can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes. Additionally for a commercial variety it should be new, distinct, uniform, and stable (UPOV, 1991).

3. These are traditional maize preparations common in Mexico.

4. Many of the issues described in this paper for maize farmers in Mexico are pertinent for many parts of Central America, which are also within the center of domestication and diversity of maize.

5. To be fair to Luna et al. (2001) they just focus their research for research scale plantings in the context of maize research activities.

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Potential Consequences from Contamination of Maize Landraces and Teosintes by a Bt Transgene

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Abstract

Scientists have recently found evidence of transgene contamination of Mexican maize landraces. Most likely the contamination is from a gene encoding a Bt-endotoxin. In this article we discuss the potential for further spread of the gene to other landrace and teosinte populations. We review the scientific literature on theoretical consequences of gene flow for rare populations. We also consider broader ecological impacts of the introgression of this specific transgene, which codes for an insecticidal protein. To prevent harm, we argue that steps must be taken similar to those of the U.S. Environmental Protection Agency in its efforts to prevent Bt-transgene contamination of a wild relative of cotton, Gossypium tomentosum. The genetic resources found in the Mexican maize and teosinte center of diversity are at least as valuable as those of G. tomentosum; similar provisions to prevent gene flow should be taken to safeguard this global heritage.

Introduction

In September 2001, the Mexican Government announced the discovery of transgenic sequences in maize landraces in Mexico; research published 3 months later in the journal Nature confirmed the findings (Quist and Chapela 2001). Environmental groups had long argued that uncontrolled release into the environment of engineered crops would lead to contamination of centers of diversity (see, for example, Rissler and Mellon 1996). Industry and Government officials alike made pronouncements about the undesirability of such an event. In 1998, the Mexican Government took the unprecedented step of placing a moratorium on all planting of transgenic maize within the country to prevent contamination of an important center of diversity for one of the world’s most essential staple crops.

Now that contamination has taken place, much scientific revisionism is occurring. Industry representatives who once swore never to sell transgenic crops in their centers of origin are now claiming that no harm will result from the introgression of the Bt transgene into maize landraces and teosintes.
Early on in the debate over the ecological consequences of engineered crops, scientists argued that most transgenes would have associated fitness costs and would quickly disappear from recipient populations (Tiedje et al. 1989; but see also Bergelson and Purrington 1996). Results from empirical research on this topic call into question this original assumption. Several studies conducted during the 1990’s show persistence and spread of monitored genes—even those that might have a fitness cost associated with them. It has also been argued that F1 hybrid sterility, in many cases, would serve as an effective barrier to gene introgression. However, many crop plants produce fertile hybrids when mating with wild relatives.

In this paper, we review the published results of empirical research that challenges these assumptions. We also review recent contributions to evolutionary theory regarding the consequences of gene flow and introgression for small and rare populations such as those of maize landraces. Finally, we consider the impact of the introgression of a particular transgene, the \textit{Bt} gene, with regards to broader environmental consequences it may pose.

\section*{Hybridization}

Introduced maize varieties can hybridize with local cultivated varieties, landraces, or teosintes. The only outstanding question regards the degree of hybridization that might occur. How much hybridization will take place depends on the proximity of the transgenic crop to landrace or teosinte populations. In the case of teosinte, it will also depend upon the frequency with which outcrossing occurs between the species. Outcrossing rates vary depending on the teosinte species (Wilkes 1972; Doebley 1990; Castillo, González and Goodman 1997).

\section*{Gene Flow and Introgression}

\textit{“Gene flow can be a potent evolutionary force.”} (Ellstrand et al. 1999)

There is little dispute over whether gene flow and introgression will result from hybridization between transgenic maize, landraces, and teosintes, though some scientists challenge whether introgression will be anything more than a transient phenomenon. Three main theoretical concerns over gene flow between transgenic varieties and landraces or teosintes—each with implications for maize diversity—have been identified: outbreeding depression, swamping, and permanent introgression of the transgene.

\section*{Swamping and outbreeding depression}

Outbreeding depression is a reduction in fitness due to hybridization (Ellstrand 1997); swamping is also known as genetic assimilation.

Outbreeding depression from detrimental gene flow will reduce the fitness of a locally rare species that is mating with a locally common one. An alternate route to extinction is by swamping, which occurs when a locally rare species loses its genetic integrity and becomes assimilated into a locally common species as a result of repeated bouts of hybridization and introgression. We would expect swamping to result from gene flow that is largely neutral or beneficial.
Both outbreeding depression and swamping are frequency-dependent phenomena and show positive feedback. With each succeeding generation of hybridization and backcrossing, genetically pure individuals of the locally rare species become increasingly rare until extinction occurs. **Both phenomena can lead to extinction rapidly** (Ellstrand et al. 1999, emphasis added).

Outbreeding depression in maize may be manifested as a general decrease in yield or as other agronomic effects on landrace populations. If a farmer is unhappy with the agronomic characteristics of seed from landrace–transgenic crosses, he or she is unlikely to maintain that lineage. When that happens, the genetic information contained in those seeds will no longer be reproduced year after year and will be lost (Rhymer and Simberloff 1996). Diversity may also be lost without selection by the farmer. When reproductive effort is spent on hybridization that results in less fit offspring, there can be consequent loss of diversity (Rhymer and Simberloff 1996).

Swamping can be expected if farmers save seed from the transgenic–landrace hybrid for replanting or if there is a continual influx of transgenic seed into the farming community.

Both landraces and teosintes are found in small populations and may be at risk from these processes. As noted by Arriola (1997), “this potential loss of genetic variation can be argued to be the most pressing biological threat to the populations of teosinte and maize land races at present.”

**Introgression**

Introgression of genes between crops and wild relatives is a well-documented phenomenon, including introgression between maize and teosinte (Doebley 1990, Kato 1997). Numerous researchers have considered the questions of hybridization and introgression as related to transgenes over the past decade (see, for example, Langevin 1990; Klinger and Ellstrand 1994; Mikkelsen et al. 1996; Arriola and Ellstrand 1997; Whitton et al. 1997; Linder et al. 1998; Ellstrand et al. 1999; Snow et al. 1999, 2001; Klinger 2002; and Bergelson and Purrington 2002). In particular, these laboratories have investigated the fitness of hybrids between crops and their wild relatives and questions regarding the persistence and spread of an introgressed transgene.

A significant factor determining whether introgression will occur is the fitness of the first-generation hybrids. For a gene to make its way into a wild population, it first must pass through the F1 hybrid generation, which is often of much lower fitness than either the crop or the wild relative. Research examining fitness of crop–weed hybrids in *Raphanus sativus* and between *Sorghum bicolor* and *Sorghum halepense* found hybrids with fitness equivalent to, or exceeding, that of wild siblings (Klinger and Ellstrand 1994; Arriola and Ellstrand 1997). In reporting results of their research on sunflowers (*Helianthus annuus*), Snow and colleagues described the F1 barrier to introgression as “quite permeable” (Snow et al. 1998). Linder et al. (1998) came to similar conclusions from their work on sunflowers: that there was a “lack of a strong correlation between hybrid fitness and potential for gene dispersal.”

Scientific advisors to the U.S. Environmental Protection Agency (EPA) (United States Environmental Protection Agency 2001a) have commented on the F1 hybrid barrier and the ecological significance of gene flow:
First generation hybrids may pose minimal threat if they have low vigor or are infertile. However, even infertile hybrids could pose a threat if they are able to reproduce asexually. The production of fertile F1 hybrids would create a genetic bridge between lineages that would promote introgression. Introgressive hybridization can occur when transfer of transgenes from one lineage to another requires the establishment of fertile F1s and backcross hybrids. Finally, there is a chance for polyploid speciation. The production of fertile F1 hybrids between normally incompatible lineages is possible via chromosome duplication after fertilization. Such polyploid species are fully fertile.

In most cases where wild relatives co-occur with transgenic crops, some gene flow would be expected even at substantial distances… Even if gene flow is low (<1%), it may result in evolutionary changes in recipient species if selection favors the new trait Rare hybridization events can be ecologically important—even a single event. (emphasis added)

In general, genes can be detrimental to the recipient population, they may be neutral, or they may be beneficial. As noted in the introduction, many scientists had originally assumed that transgenes were inherently problematic for a plant and would likely eventually be lost, that is, they considered that permanent introgression of a transgene was unlikely to occur. Linder et al. (1998) concluded otherwise:

A transgene will be prevented from introgressing into a sympatric wild population only if it lowers fitness or is tightly linked to a gene that lowers fitness. Advantageous or neutral transgenes will quickly spread into wild populations.

Whitton et al. (1997), also working in sunflower, agree:

We conclude that neutral or favorable transgenes have the potential to escape and persist in wild sunflower populations…cultivar genes are capable of persistence in weedy populations, and thus even low levels of hybridization may result in transgene establishment in weedy sunflower populations.

Even crop genes that reduce the fitness of a crop–weed hybrid have been shown to be maintained in weed populations over time (Snow et al. 1999; Snow et al. 2001).

What empirical evidence shows, and what researchers have predicted based on population genetics theory, is that genes associated with increased fitness, such as resistance to herbivores (including insects), herbicides, or environmental stress, may easily spread in recipient populations. An insect resistance gene such as the \( \text{Bt} \) gene is expected to confer a benefit on the recipient plant.

Some authors (Martinez-Soriano et al. 2002) have asserted that in nature there are no pests that limit teosinte in the wild. They argue that because the \( \text{Bt} \) gene will not confer any benefit on teosinte it will not persist in wild populations. The research cited above challenges this conclusion. Moreover, as Power (2002) has shown, initial assumptions about the pest resistance of crop wild relatives can be incorrect. Such assertions by Martinez-Soriano et al. (2002) regarding teosinte–pest dynamics are inappropriate without corresponding empirical evidence. Indeed, if teosintes are limited by insects that would be killed by the \( \text{Bt} \) toxin, the introgressed gene could prove advantageous to recipient populations. Those populations may pose increasing problems for farmers.
If the gene introgresses and persists in landrace or teosinte populations, the gene product may eventually be widely distributed in the environment across space and time. This geographic and temporal spread is cause for concern because of the numerous other ecological problems that could result from introgression of a transgene, including impacts of the transgene product on nontarget organisms (Obrycki et al. 2001, Letourneau et al. 2002). These potential impacts are detailed in the following section.

Environmental Effects of the Bt Gene

According to a recent U.S. EPA scientific advisory panel (U.S. Environmental Protection Agency 2001a), potential consequences of Bt transgenes include “increased fitness, increased invasiveness and weediness.” Such an insect resistance gene is considered by scientists to be a fitness-enhancing gene and thus be likely to increase in frequency and spread throughout local populations. Following introgression into landraces and teosinte, the Bt gene could have broader ecological impacts, through

- persistence of the Bt protein in the soil with toxicity to soil organisms
- toxicity to nontarget herbivores, predators, and parasites (natural enemies of affected pests)
- the development of resistance to Bt in affected pests

Impact on Soil Organisms

Because of the crucial role that soil organisms play in soil health, it is necessary to understand how different agricultural practices affect them. Bt crops may be problematic for long-term soil health, because they express proteins known to be toxic to certain insects such as lepidopterans (moths and butterflies) and coleopterans (beetles) and are suspected of being toxic to a range of nontarget organisms as well, including earthworms (Marvier 2001). An unknown number of species make up the soil food web and could be affected by Bt, yet, tests have been conducted on very few, in very few soil types and ecosystems.

If the Bt deposited in the soil by these crops has an impact on soil organisms—bacteria, fungi, insects, worms—there will necessarily be downstream effects. If you kill or otherwise reduce the activity of any of these soil organisms, you disturb the web of relationships necessary for carrying out essential ecosystem functions such as decomposition and nutrient cycling.

According to the U.S. EPA’s scientific advisory panel, Cry proteins “are likely to be present in the rhizosphere soil not only throughout the growth of the crop, but perhaps long after the crop is harvested” (U.S. Environmental Protection Agency 2001a). Therefore researchers and regulators must assume “that continuous exposure to Cry proteins is likely within the soil system.” The panel concluded that “it would be prudent to determine under operational field conditions in different geographical regions and soil types, the extent to which Cry proteins accumulate in soil” (U.S. Environmental Protection Agency 2001a). They drew attention to studies that showed Bt could persist in certain soil types for up to 234 days (Koskella and Stotzky 1997, Tapp and Stotzky 1998) and recognized that further studies needed to be done to determine whether the persistence of Bt would cause problems for nontarget organisms and the health of the soil ecosystem.
As noted by Benbrook (1999), in addition to long-term research on impacts of Bt in soils, research is needed on the short-term soil microbial community impacts of a big dose of Bt as corn trash and other crop residues break down in the spring and early summer. One might hypothesize that under some circumstances, Bt entering the soil will impact soil microbial communities in ways that lead to complex, multi-tier impacts on microbial and soil insect biocontrol, pathogen pressure, immune response and nutrient cycling. Even if the impacts last only 4 to 8 weeks, that is ample time to leave a lasting mark on the performance of the cropping system, both in one season and over many years as microbial communities evolve to a new steady state.

**Impacts on Non-target Organisms**

As noted in the previous section, genetically engineered crops can have impacts on organisms other than those they are intended to kill. The impact of Bt corn pollen on Monarch butterflies is the most well-known example of this phenomenon (Losey et al. 1999, Hansen Jesse and Obrycki 2000, Sears et al. 2001, Losey et al. 2002). Other organisms that have been shown to be affected by Bt crops are lacewings, which are beneficial insects that play an important role in the natural control of crop pests (Hilbeck et al. 1998a, 1999). Both earthworms and collembola (other small soil-dwelling invertebrates) have been shown to be affected by Bt crops (EcoStrat 2000, Marvier 2001).

Changes in populations of both other pests and of natural enemies have been documented in Bt cotton. Data from China show that use of Bt crops can exacerbate populations of other secondary pests, including aphids, lygus bugs, whiteflies, Carmine spider mites and thrips (Cui and Xia 1998). Cui and Xia (1999) have shown significant reductions in populations of the parasites Microplitis sp. (88.9-percent reduction) and Campoletis chloridae (79.2-percent reduction) in Bt cotton fields. Data being collected in India indicate higher levels of aphids and jassids in Bt cotton fields (Ghosh 2001). Wold et al. (2001) recently demonstrated impacts of Bt corn on field populations of Coleomegilla maculata, a predatory coccinellid commonly found in corn fields.

**Insect Resistance**

A large literature exists on the ability of pests to develop resistance to pesticides, including pesticides such as Bt that are engineered into the plant. Local lepidopteran pests of maize susceptible to Bt would initially be controlled by Bt expressed in transgenic maize or landraces or teosintes that were recipients of the transgene. However, owing to the continuous selection pressure exerted by the transgenic plants, populations of pests are likely to develop resistance to the Bt protein. Resistant pest populations would cause problems for those farmers deliberately using Bt as a topical insecticide.

**Defining Harm**

Muir and Howard (2002) define the potential harm of a transgenic organism as a composite measurement between the risk of transgene introgression and hazards posed by the transgene if permanent introgression occurs. They observe that a
single outcrossing event could pave the way for transgene introgression. They also point out that

“long-term hazards to the ecosystem are difficult to predict because not all non-target organisms may be identified, species can evolve in response to the hazard, and a nearly infinite number of direct and indirect biotic interactions can occur in nature” (Muir and Howard 2002).

This understanding leads the authors to conclude that the only way to ensure the environment will not be harmed is to release only those transgenic organisms whose fitness is such that the transgene will not spread. In the case we are considering here, Bt maize, it is clear that the only way to prevent the spread of the gene through landrace and teosinte populations, and to prevent harm, is to prevent the introduction of Bt maize into Mexico. Indeed, this has been the policy of the Mexican government to date.

Preventing Harm

The EPA recently published revised restrictions on the cultivation of Bt crops. Contained in the EPA decision document (U.S. EPA 2001b) are provisions to prevent gene flow from Bt cotton to wild and feral relatives of cultivated cotton. These provisions, and EPA’s justification for the restrictions, are detailed below:

Gene flow containment provisions

The most obvious concern is the development of weediness, but also concerns of biodiversity and loss of genes that might provide value in plant breeding have been considered.

Adequate data do not exist to complete a full risk assessment on the effects of the Bt Cry1Ac protein in wild cotton. Until thorough research on the impacts of gene flow can be completed, restriction [sic] on where Bt cotton can be planted are being implemented.

In light of the lack of basic biological data (e.g., pollinator ecology, compatibility/sterility factors, potential impact of Bt on herbivores, distribution of native populations) on G. tomentosum, the wild Hawaiian cotton, conservative measures are needed to mitigate hybridization with cultivated cotton on these islands. Similarly, the paucity of data on the distribution of feral cotton in the U.S. Virgin Islands and Puerto Rico indicates the following terms and conditions must be instituted to mitigate gene flow concerns:

a. No planting of Bt-cotton south of Route 60 (near Tampa) in Florida,
b. Commercial culture of Bt-cotton is prohibited in the state of Hawaii,
c. Test plots or breeding nurseries established in Hawaii must be surrounded by 24 border rows of a suitable pollinator trap crop regardless of the plot size and must not be planted within 3 miles of Gossypium tomentosum,
d. Commercial culture, experimental plots and breeding nurseries of Bt-cotton are prohibited in the U.S. Virgin Islands, and
e. Commercial culture of Bollgard cotton is prohibited in Puerto Rico. Test plots or breeding nurseries established on the island of Puerto Rico must be surrounded by 24 border rows of a suitable pollinator trap crop regardless of the plot size and must not be planted within 3 miles of feral cotton plants.
Certainly the genetic resources found in the maize and teosinte diversity of the Mexican center of origin are at least as valuable as *G. tomentosum*; nothing short of similar provisions to prevent gene flow should be in place in centers of origin of all of our most valuable crop plants.

**Conclusions**

1. “**Plant genetic resources for food and agriculture are a common concern of all countries**” (International Treaty on Plant Genetic Resources 2001).

2. “**Recognizing that states have sovereign rights over their plant genetic resources for food and agriculture, we also confirm our common and individual responsibilities in respect of these resources**” (Leipzig Declaration on Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture 1996).

Clearly questions remain regarding the potential impacts of *Bt* introgression into maize landraces. Population genetic theory predicts that the transgene is likely to spread throughout the landrace population. There may be consequences for those small populations from swamping or outbreeding depression. Additionally, the evidence for environmental effects of the *Bt* gene in the environment is wide-ranging, including impacts on nontarget organisms and alterations in populations of secondary pests, natural enemies, and parasites.

The international community, in numerous international agreements and declarations, has repeatedly emphasized the crucial importance of centers of diversity to future food security. The maize landraces and teosintes of Mexico are an essential component of this valuable diversity. To allow an open-air experiment on the impacts of transgene introgression to continue in a center of diversity is to abdicate the responsibility of the world community to protect this precious heritage for future generations.

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Potential Consequences from Contamination of Maize


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Session 4: Future Needs: Unique Challenges and Opportunities for Environmental Assessment
Research and Monitoring in the Industrialized World: 
European Commission Policy and Experience

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Abstract

The European Commission has been active in supporting genetically modified organism safety research for some 15 years. This paper examines how the Commission has gone about research on genetically modified organisms in the environment, considers the governance of this research, and identifies suggestions for future research priorities. Although no particular safety or environmental problems have been revealed, the multinational consortium approach is considered particularly valuable for this type of work. Communication of safety research results appears to be a particular bottleneck—especially from the perspective of public perception.

Introduction

It has been European Commission (EC) policy from the beginning to accompany its research programs in biotechnology with research on safety aspects. In the last 15 years, over 80 projects involving over 400 research teams and a European Community financial contribution of over €70 million have been supported in the area of genetically modified organism (GMO) safety. The research has covered investigations of plants, plant microbes, biocontrol, food, bioremediation, fish, and vaccines. A review of this work is given by Kessler and Economidou (2001).

Most of this work has dealt with the environment—even the food safety research. This is because many of the food issues actually derive from traits that had been introduced for environmental objectives such as pest control. Many consumers are now demanding choice, not only in the characteristics of the products they buy but in the characteristics of the production chain that produced them and, in particular, the environmental impact of that production chain.

In discussing GMO use, a message frequently repeated is that more research is needed or, worse, that no research has been done at all. Although further research may be justified, there is obviously a corresponding need for communicating research results that are already available, and this conference is particularly significant in this respect. This paper will examine how the EC has gone about research on GMOs in the environment, consider the governance of this research, and conclude by looking at some suggestions of future priorities for research.
The first question asked in the early 1980s was, Does genetic engineering make an organism riskier? Tackling this question revealed gaps in underlying knowledge in disciplines such as ecology, population genetics, soil science, and biodiversity. Research rapidly built up to a peak in the mid-1990s. However, by the later 1990s a public backlash to the technology set in, and new areas for concern were raised (e.g., virus resistance and nontarget effects); this gave further impetus for going into more detail in the research program.

In supporting this research the EC took no preconceived position and considered that genetically modified organisms (GMOs) are neither inherently risky nor inherently safe. Research followed the classical pattern of examining the organism, the insert, and the environment; taking a precautionary approach; and identifying the two phases in risk assessment: hazard identification and frequency of occurrence.

Several features make the EC approach to research funding particularly appropriate to GMO safety research. First, it is undertaken by multinational consortia of teams from any entity with an appropriate research capacity, it can include other organizations like nongovernmental organizations (NGOs), consumer groups, or farmers’ organizations that can contribute to exploitation or communication of results; and it is open to participation from third countries. Because this is publicly funded research, there is an obligation to exploit results. Second, in addition to standard peer-review criteria, considerable emphasis is placed on societal and policy criteria, which for modern life sciences are becoming increasingly important. This research is termed prenormative, that is, it precedes the establishment of regulations and embodies the idea that science underpins regulation. This idea is sketched in figure 1, which illustrates research as a dynamic entity driving regulation and management and leading to outputs in the form of accumulating experience and best practice as well as determination of long-term effects.

The European Union (EU) research activities complement national activities and the research undertaken in preparation for submitting dossiers to fulfill regulatory requirements. These research activities are an example of regional scientific cooperation that is implementable in other parts of the world, and through which groups of countries with common problems or common ecological conditions can benefit enormously through complementation, sharing of skills, and achieving a critical mass of activity, which are attainments smaller countries might not be able to realize on their own.
GMO safety research, regulation and practice: synergy and feedback

Examples of recent research include ecological effect of gene flow in virus-resistant plants, effects of *Bt* transgenes on nontarget biodiversity, the impact of biotechnological approaches to potato pathogen control on soil microbiota, evaluation of gene flow from transgenic (chloroplast transformation) plants, and biosafety assessment of novel plant growth promoting micro-organisms.

Three general comments can be made about the results of this research. First, no particular safety or environmental problem of the technology has been revealed. Second, analysis of the role of GMOs in agriculture has raised many questions about the environmental impact of conventional agriculture in Europe, and in this way attention has been drawn to certain environmental issues. Third, as a positive spinoff new knowledge has been generated in many different disciplines.

**Governance of Research**

The perception that science has run ahead of public opinion raises the question of governance. This is an issue that is given some prominence in the Commission’s recent Communication on Life Sciences and Biotechnology—A Strategy for Europe (Commission of the European Communities 2002) produced after a public consultation process. The use of GMOs in the environment is governed in the EU by Directive 90/220/EEC, which is now updated as Directive 2001/18/EC. The numbers of field trials authorized under the directive are shown in figure 2. Following the introduction of the directive, numbers of trials built up steadily, but there has been a sharp decline in recent years to the level of the early 1990s.
In an attempt to raise the voice of science in the debate on the use of GMOs, the EC has initiated a round table on GMO safety research. This aims to achieve a balanced discussion among all stakeholders of the results of safety research and the areas of uncertainty or concern. To avoid mixing issues and confusing the argument, each session focuses on a single topic. The first meeting examined the benefits and risks associated with \textit{Bt} maize and was structured around environmental, animal feed, and human food issues. Results are published on a Web site (http://biosociety.cordis.lu/).

\section*{Recently Identified Research Priorities}

As a result of various consultation processes and discussion forums the following priorities, concerning both the content of the research and the way it is carried out, have emerged. Many of the general issues are also mentioned in the Commission’s Communication on Life Sciences and Biotechnology—A Strategy for Europe (Commission of the European Communities, 2002), especially Actions 13, 17, and 23.

First, because there is concern over the speed of change in agriculture in Europe, baseline studies to define agro-ecosystems are needed to provide a known starting point from which to measure changes. Such studies would evaluate new systems of any sort—conventional, low-input, organic, and so forth as well as systems using GMOs. In particular, field experiments are needed to determine the benefits or risks of new components. For different systems to co-exist and be validated, separation distances and other buffering techniques need further
investigation. In the case of GM crops, soil impacts, particularly on nontarget organisms and in the long-term, and effects of gene stacking are a cause of concern. In this work, consensus on research methodology, including monitoring, is clearly desirable from scientific and public perception viewpoints.

A second approach relates to the inherent safety of products. Fewer concerns will be raised if safety issues and public perception issues can be tackled at the design stage by building in specific features. In particular, strategies for prediction will become essential if risk assessment measures are to cope with many new products in the future.

Finally, in order that research not be too far removed from the public and for communication purposes, stakeholders should be involved as much as possible.

Conclusions

The EC has been active in supporting GMO safety research for many years, and the multinational consortium approach is considered particularly valuable for this type of work. Although no particular safety or environmental problems have been revealed, further items for research and areas of concern have been identified. Communication of research results appears to be a particular bottleneck, especially because of a continuing public perception that little or no GMO safety research has been carried out.

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Abstract

The U.S. Environmental Protection Agency (EPA) has imposed an unprecedented insect resistance management (IRM) program for Bt crop products to delay or prevent the target insects from becoming resistant to the Bt proteins. Maintaining this IRM program requires the effective actions of farmers, pesticide companies, researchers, and Government regulators. The science of insect resistance management and insect resistance monitoring is complex and is continuing to develop. Insect resistance monitoring is expensive, and the extremely high costs can be offset by more reliance on farmer actions to carry out robust IRM plans, on compliance monitoring, and through remedial action plans. EPA will continue to monitor all of these activities closely for the Bt crop products.

Introduction

The development of pesticide resistance in insects, fungi, and weeds is well documented in agriculture. As resistance begins to develop, more pesticide is needed to achieve control until total failure of that pesticide occurs. Integrated pest management or IPM grew out of insect resistance to insecticides, and pesticide resistance management remains a common component of IPM programs today. Monitoring for the increased pesticide tolerance of the pest is a valuable asset in an IPM program, but it is rarely done proactively.

Insect resistance management (IRM) is the term used to describe practices aimed at reducing the potential for insect pests to become resistant to a pesticide. Bacillus thuringiensis Bt IRM is important because insect resistance poses a threat to future use of microbial Bt pesticides and Bt technology as a whole. Academic scientists, public interest groups, and organic and other farmers have expressed concern that the widespread planting of these genetically transformed plants will hasten the development of resistance to pesticidal Bt endotoxins. Effective insect resistance management can reduce the risk of resistance development.
An IRM plan is not specifically required under the U.S. pesticide laws or regulations. Rather, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the U.S. Environmental Protection Agency (EPA) is mandated to ensure that there will be no unreasonable adverse effects from the use of a pesticide when economic factors are taken into account. In this specific case, EPA has stated that we are working to prevent potential adverse effects if \( Bt \) could not be used and more toxic compounds were used to control the insect pests.

The goal of IRM is to have the target pest continue to be susceptible to the pesticide. Each IRM program consists of strategies to reduce the likelihood that insect resistance will develop and strategies to manage insect resistance once it occurs. At the EPA, IRM is an important tool in protecting against the loss of safer pesticide products. In 1992 we began to consider what would be an appropriate resistance management approach but one not limited to \( Bt \) crops. EPA has implemented an unprecedented IRM program for the \( Bt \) crops; however, we have not forgotten the conventional and microbial pesticides. We have recently published a final policy notice regarding labeling statements for most pesticides as part of a project under the North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (USEPA 2001a). This notice provides a numerical system to identify the pesticide’s mode of action and label statements to encourage users to rotate between pesticides with different modes of action.

**History of IRM for \( Bt \) Crops in the United States**

In contrast to all other pesticides, the IRM plan(s) for the \( Bt \) crops are truly unprecedented in detail, scope, and implementation. The program has been enhanced early on when EPA held public meetings on biotechnology products where public interest groups voiced concern that \( Bt \) crops under development could lead to the insect pests’ developing cross-resistance to microbial \( Bt \) products. EPA shared this concern and has always made IRM a key element in its regulation of \( Bt \) crop products. EPA has repeatedly consulted with our outside Scientific Advisory Panel and our public policy advisory group, the Pesticide Program Dialog Committee, regarding our IRM program for \( Bt \) crop products. In addition to excellent advice, EPA has received strong support for its work at each of these meetings.

At a Scientific Advisory Panel meeting in March 1995, the EPA laid out a multifaceted program we considered appropriate for \( Bt \) crop products. The elements are as follows:

- knowledge of pest biology and ecology, dose (level of toxin expressed in the \( Bt \) crop),
- refuge design and deployment (non-\( Bt \) plants producing \( Bt \)-susceptible insects),
- cross-resistance between different \( Bt \) proteins
- effective field monitoring for insect resistance
- remedial action if resistance occurs
- integrated pest management
- development of alternate modes of action
- grower education
Repeatedly, the Science Advisory Panel has agreed with EPA that an appropriate resistance management strategy is necessary to mitigate the development of insect resistance to *Bt* proteins expressed in transgenic crop plants. Resistance management programs should be based on the use of both a high dose of *Bt* endotoxin and structured refuges designed to provide sufficient numbers of susceptible adult insects. This so-called high-dose/structured refuge strategy assumes that resistance to *Bt* is recessive and is conferred by a single locus with two alleles resulting in three genotypes: susceptible homozygotes (SS), heterozygotes (RS), and resistant homozygotes (RR). It is also assumed in this strategy that there will be a low initial resistance allele frequency and that there will be extensive random mating between resistant and susceptible adults. Ideally, only rare RR individuals will survive a high dose produced by the *Bt* crop. Both SS and RS individuals will be susceptible to the *Bt* toxin. A structured refuge sets aside some percentage of the crop land for non-*Bt* varieties of that crop. The refuge provides for the production of susceptible (SS) insects that may randomly mate with rare resistant (RR) insects surviving the *Bt* crop to produce susceptible RS heterozygotes that will be killed by the *Bt* crop. This will remove resistant (R) alleles from the insect populations and delay the evolution of resistance. The scientific advisory panels held in 1998 and 2000 noted that insect resistance management strategies should also be sustainable, and to the extent possible, strongly consider grower acceptance and logistical feasibility.

**Scientific Basis of IRM**

To be effective, an IRM plan must be specific to the target pest, the crop, and to the class of pesticide being used. The use of a high-dose strategy is often not the best approach for chemical pesticides sprayed on a crop, and pesticide rotation is one of the preferred approaches. Annually rotating between *Bt* cotton and non-*Bt* cotton treated with conventional insecticides is likely to wipe away many of the environmental benefits of increased nontarget organisms we are seeing in areas where *Bt* cotton is frequently grown. As an IRM program is developed and implemented, each pest’s unique biology must be factored into the plan. For example, how far the larvae move within the field and how far the adults move affects the distance between the refuge and the *Bt* crop. The susceptible insects from the non-*Bt* refuge need to be in close enough proximity to randomly mate with the resistant insects that emerge from the *Bt* fields to produce heterozygous offspring that are fully susceptible to the *Bt* protein. Additional important issues that need to be addressed are the number of insect generations produced each year, the mating behavior and the egg-laying or oviposition behavior, the host range of the insect, population dynamics, pest ecology, and, if possible, the genetics and mechanism of resistance and the frequency of resistance alleles in the insect population. In addition, how the crop is grown, including other pest management practices, when it matures, the extent of the acreage, and the overlap in distribution with other *Bt* crops are all important in the development of an appropriate program.

Mathematical models to predict the potential for resistance development have helped EPA make decisions regarding the requirements for *Bt*-crop IRM. In general, the predictive models allow the EPA to compare the relative efficacy of different IRM strategies to mitigate the development of insect resistance. For example, these models allow for a qualitative comparison of different refuge sizes, the impact on efficacy of the refuge if chemical insecticides are used, and differences in having the refuge be within the field or external to the field.

EPA has just completed an extensive reevaluation of the *Bt* crop products (*Bt* plant-
incorporated protectants) registered in the U.S., including IRM (USEPA 2001b). The EPA’s IRM requirements are discussed in the next section.

The New IRM Requirements for Bt Crops in the United States

The EPA has determined that the 20-percent non-Bt field corn refuge requirements for Bt field corn grown in the Corn Belt and the 50-percent non-Bt corn field refuge requirements for Bt field corn grown in cotton-producing areas are scientifically sound, protective, feasible, sustainable, and practical to growers. EPA believes that the use of predictive models provides confidence that resistance will not evolve to any of the target pests (i.e., European corn borer, corn earworm, southwestern corn borer, fall armyworm, and other stalk-boring pests) under the time frame of the registrations.

For Bt sweet corn, no specific refuge requirements are necessary because sweet corn is typically harvested much earlier than field corn (18–21 days after silking) and before most lepidopteran larvae complete development (USEPA, 2001). However, to mitigate the development of resistance, EPA has determined that crop residue destruction is necessary within 30 days. This practice will likely destroy any live larvae left in Bt sweet corn stalks and prevent overwintering of any resistant insects.

At this time, EPA believes that available empirical data substantiate the success of the 5-percent external unsprayed, 20-percent external sprayed, and 5-percent embedded structured refuge options to delay insect resistance to Bt cotton. However, EPA believes that it is imprudent to allow the 5-percent external, unsprayed refuge option for more than a limited time because current data indicate that it has a significantly greater likelihood of insect resistance than either of the other refuge options. The 2000 Scientific Advisory Panel stated that the external, unsprayed option poses the highest risk to resistance evolution—especially for cotton bollworms. Because of the greater risk of resistance development, the external, unsprayed option will expire after three growing seasons (30 September 2004). During the next years, the registrant is required to develop considerable new data on alternative host plants as possible effective refuges.

In addition, the Agency is mandating additional improvements to the current Bt corn (field and sweet) and Bt cotton IRM programs that will require the following (USEPA, 2001).

1. Anyone purchasing Bt corn and Bt cotton must sign a grower agreement contractually binding the grower to comply with the IRM program and ensuring that there will be a mechanism by the year 2003 by which every grower will affirm his or her contractual obligations to comply with the IRM program,
2. An ongoing IRM education program will be implemented,
3. An ongoing IRM compliance monitoring program, including a third-party compliance survey and mechanisms to address noncompliance will be implemented,
4. An ongoing insect resistance monitoring program for each target insect pest will be designed,
5. Remedial action plans will be implemented if resistance does develop,
6. The IRM (and other) activities are to be reported annually. No other pesticide products besides the Bt crop products have such extensive IRM requirements.
The U.S. IRM Strategies for Bt Plant-Incorporated Protectants

In planning the new IRM regulatory program for these Bt plant-incorporated protectants (PIPs), EPA considered not only the science but also factors such as grower costs and compliance, resistance monitoring, and remedial action if resistance should occur. EPA considered four important areas: farmer actions, compliance monitoring, insect resistance monitoring, and remedial action plans. Each of these areas was considered in making the decision to continue the registrations of the Bt PIPs.

Farmer Actions

Farmer adoption of IRM requirements is critical to the long-term, sustainability of IRM strategies for Bt crops. Probably the first essential farmer action is to become familiar with the IRM requirements for a Bt crop. Without farmer implementation of appropriate IRM strategies, pest resistance cannot be mitigated. Each farmer must sign a contract or grower agreement indicating that he or she will abide by the IRM requirements, and the farmer receives a technical bulletin describing the latest requirements. Education also includes making sure that the farmer is aware of any changes that have occurred since he or she last grew the Bt crop—whether it was last year or 2 or more years ago. Educational materials are provided by each company, and education sessions are held by the companies—sometimes by the seed dealer, the commodity group, and often by the U.S. Department of Agriculture’s Cooperative Extension Service. Information is also provided through the Internet and via newsletters and other media. Once the farmer is educated to the requirements, it is his or her responsibility to plant and manage the refuge. It must be the correct size (such as 20-percent non-Bt corn to 80-percent Bt corn in the Corn Belt), it must be placed at the correct distance so that any Bt-resistant insects coming from the Bt crop will easily find mates from the Bt-susceptible insects coming from the refuge, and the farmer must plant a refuge using a crop variety compatible with the Bt crop in the time that adults would emerge from the refuge and the Bt crop.

Farmers also play an important role in supplementing monitoring by reporting any failure of the Bt crop to control the target pest. Because farmers pay an extra fee when they buy the Bt seeds, they have an incentive to complain to the company that sold them the seed if it is not preforming correctly. We have made it a requirement of the registrations that the companies must report to EPA any valid complaints from farmers of failures of Bt crops to control a target pest. An important farmer action in the overall IRM program, is cooperation with and accurate response to, questionnaires and surveys about actions the farmer has actually taken. Another interesting role from a regulator’s perspective is that at least some farmers in the U.S. will tell the company or its representatives if another farmer is not abiding by the refuge requirements. We have made followup on tips and complaints from other farmers, a part of our new compliance-monitoring program.

Compliance Monitoring Program
EPA recognizes that compliance is a complex issue for \textit{Bt} crops and IRM; therefore, a balance must be achieved between refuge size and deployment with grower compliance. Currently, the financial burden of implementing refuge requirements is borne primarily by the growers. Increasing refuge size, limiting refuge deployment, or both to reduce the risk of resistance will likely increase costs to growers and result in a higher rate of grower noncompliance.

Our recent reassessment has greatly strengthened the compliance monitoring to increase the likelihood of IRM adoption, to measure the level of compliance, and to institute penalties should noncompliance become a significant problem (see terms and conditions of registrations (EPA 2001b). Until recently, monitoring for farmer compliance with the IRM program has been largely voluntary, but now it is mandatory. Key to this program is the grower agreement between the company and the farmer promising the farmer will abide by the IRM requirements. Although it varies somewhat by crop, the program is basically a tiered approach of actions reflecting the results of a grower survey. The survey is conducted by a third party, an independent organization using funds provided by the companies. The survey questions are developed in consultation with academic and government researchers knowledgeable on the subject, and there is also an EPA review of the survey. The survey focuses on areas of highest risk, which are typically those areas of highest adoption. If the survey indicates that an area of the country is not fully complying with the requirements, increased education and more intense surveying will be implemented in that area. The degree of increased effort may be directly related to the type of problem. For example, if bad weather conditions cause farmers to plant refuges late, the situation is quite different than farmers in an area deciding it is unimportant to plant the refuge at all. In addition, typical on-farm visits conducted by the companies, their representatives, or both will report on farmers who are or are not complying with the requirements and the followup actions that are taken. Any farmer determined to be out of compliance will automatically receive an on-farm inspection the following year. If that farmer is still found to be significantly out of compliance, that grower will be denied the use of the \textit{Bt} crop the following year. Although that farmer may be able to buy the technology the third year, he or she would again automatically receive an on-farm visit during that growing season. If that farmer was again out of compliance, he or she would be denied the use of the technology permanently. Some of the details of the penalty phases of the compliance monitoring program have not been fully worked out. The companies must submit plans for these to EPA early in 2002 for review and approval. Of course, tips supplied to the company regarding a farmer out of compliance, especially one refusing to plant a refuge for field corn and cotton, would require on-farm visits and might necessitate penalties.

**Insect Resistance Monitoring**

Monitoring has been part of the requirements of the \textit{Bt} crop products registration from the beginning (USEPA, 2001b). The ambitious goal is to detect insect resistance before it occurs in the field or before it spreads and, if possible, to prevent the development of resistance by detecting increased pest susceptibility. Our program includes monitoring for the important target pests. The effort has been evolving over the last 6 years. To be effective, the plan requires sensitive tools be in place to detect changes in resistance allele frequency to the particular \textit{Bt} protein and to be able to differentiate between natural variation in the population and a trend indicating resistance is likely to happen soon or may have already happened. As one of its early steps in developing this program, EPA established a working definition for
resistance versus natural tolerance variation and the analysis was reviewed by our scientific advisory panel for confirmation. In addition, EPA, working with the pesticide companies, has established definitions for suspected versus confirmed resistance. An additional consideration is the time required to “confirm” resistance.

The basic resistance monitoring program entails gathering target insects in an adequate sample size from an appropriate number of locations and testing for susceptibility to the $Bt$ protein. Samples can be collected from various life stages. Adults might be collected from light or pheromone traps that attract the moths or larvae, eggs masses, or both might be collected either from $Bt$ fields or other crop or noncrop areas. Depending on the life stage collected, the insects might have to be reared to a stage at which they could be fed the appropriate $Bt$ protein to determine their level of susceptibility to the insect toxin.

Resistance monitoring is a difficult and imprecise task. The chances of finding resistant larvae in a $Bt$ crop depend on the level of pest pressure, the frequency of resistant individuals, the location and number of samples collected, and the sensitivity of the detection technique. Therefore, as the frequency of resistant individuals in the insect population increases or the number of collected samples increases, the likelihood of locating a resistant individual becomes greater. The likelihood of resistance is dependent on the genetics and mechanism of resistance for a particular pest.

A resistance monitoring program is more important when models predict resistance is imminent rather than when resistance is expected to be delayed for a very long time. On the basis of predictive models, level of adoption, and compliance for European corn borers, resistance to $Bt$ proteins expressed in field corn would not be likely to develop for 75 years or more, but for cotton bollworms, tobacco budworms, and pink bollworms, the predicted number of years to resistance to $Bt$ proteins expressed in cotton is much shorter (EPA, 2001b).

The resistance monitoring program needs to consider the pest biology and ecology, population dynamics, genetics of resistance, mechanism of resistance, sampling methodology, bioassay methodology, standardization procedures, detection technique and sensitivity, and the statistical analysis of the probability of detecting resistance. To determine if refuges or any other resistance management tactics are working, one must track the frequency of resistance in field populations. With typical bioassays used for resistance monitoring, resistance cannot be detected readily when the allele is recessive (as often is the case) and rare. For example, if the frequency of a recessive resistance allele is 0.001, only one in a million individuals is expected to be a resistant homozygote (carrying two resistance alleles) capable of surviving exposure to a high concentration of the $Bt$ protein.

Several issues are associated with this program. The first is sample size. The number of samples and number of locations that need to be sampled are dependent on the pest biology and ecology and population dynamics. If the genetic variation in an insect is known, then sampling strategies can be constructed with a greater probability of detection and a low probability of nondetection. Both factors must be considered to reduce the likelihood of Type 1 (false positive) and Type 2 (false negative) errors. Sampling should also be done uniformly. Uniformity and standardization in the bioassays are also critical to the interpretation of monitoring information. Finding enough insects to test is related to sample size. Sampling insects exposed to the $Bt$ crop is preferred, but if sampling is primarily in the $Bt$ crop, then few, if any, larvae of the target insect will be found in most $Bt$ fields. This means that
sampling methods need to be adapted either to collect adults or egg masses to generate the volume of individuals needed to increase the probability of detecting resistance or samples need to be taken from non-Bt fields.

Current resistance monitoring plans in the United States have a goal to collect at least 250 individuals from any one location with a target of at least 20 locations for tobacco budworms and cotton bollworms, pink bollworms, and European corn borers. Additional sampling for the southwestern corn borer is focused in those areas of the Corn Belt in which this pest is an economic problem. The greater the number of samples and locations, the greater the probability that resistant individuals will be collected.

Another issue is the sensitivity of the detection methods. If resistance is recessive (rather than dominant or codominant), it is less likely to develop, but it is more difficult to detect. It is useful to know the frequency of the resistance allele in the natural population. Estimates of the frequency of resistance alleles have been determined based on laboratory selection experiments (surrogates for what might happen but not necessarily what will happen in the field). Field verification of resistance allele frequency requires reliable and sensitive detection methods. However, if extremely sensitive detection methods (especially if resistance is recessive) are available and economically feasible, changes in resistance allele frequency (and verification of estimates) can be detected before any signs of field failure, thus creating opportunities for proactive, adaptive IRM.

EPA has evaluated the advantages and disadvantages of various detection methodologies and will continue to watch for a highly effective and economically viable test as the detection methodology improves and is accepted. New testing requirements will then be implemented. The currently required basic test method has been a discriminating dose/diagnostic dose bioassay system that distinguishes between resistant and susceptible phenotypes, but such tests have been criticized as being too insensitive to be able to provide early detection before resistance develops or can spread very far—especially if the alleles for resistance are rare in the insect population. Discriminating dose bioassays are most useful when resistance is common or conferred by a dominant allele (resistance allele frequency >0.01%) (Andow and Alstad 1998). This method is currently one of the central components of any monitoring plan, but other monitoring methods may have value in conjunction with the discriminating concentration assay.

A second detection technique is the F₂ screen (Andow and Alstad 1998). The F₂ screen may be the best method for detecting rare, recessive resistant alleles. The F₂ screen is conducted by taking mated females and sibmating the F₁ progeny, producing the F₂ progeny that are tested using an appropriate screening procedure, such as a discriminating concentration assay or Bt crop, and performing statistical analysis. The technique also requires fewer samples be collected to detect potential susceptibility shifts than the discriminating dose assay. The F₂ screen may be most useful to analyze populations that are expected to be at high risk for developing resistance. Each isofemale line allows for characterization of four genomes, thus improving the sensitivity over the discriminating dose assay. The technique is an effective method for detecting changes in the allele frequency of a recessive or partially recessive allele and can be used to verify some of the assumptions underlying high dose–refuge resistance management. If resistance alleles are found, they can be characterized to estimate the fitness of the genotypes, to determine whether there is a cost of resistance, and to predict the evolution of resistance. A potential obstacle to the F₂ screen is that it may be too
expensive because it is highly labor intensive and may not be suitable for routine screening purposes—especially if there is replication at each site. In general, the $F_2$ screen is more expensive than other methods for detecting dominant resistant alleles when the resistance allele frequency is $>0.01$. However, for recessive alleles, the $F_2$ screen is the least expensive method and can estimate resistance allele frequencies to a high level of precision ($<0.005$) for under $5,000$ per location.

Additional tests include grower reports of unexpected damage, sentinel plots or the use of both in-field screening procedures, to screen against resistant test stocks (allelic recovery method) and in-field detection (using DNA markers) kits.

In a first step toward more efficient DNA-based monitoring, Gahan et al. (2001) in Science described using a DNA-based screening system for detecting a Cry1Ac-resistant tobacco budworm that has developed resistance through a specific mutation in the cadherin gene (characterized by the mechanism of $Bt$ resistance found in the YHD2 strain [see Gould et al. 1997]). This mutation results in a truncated cadherin that lacks the toxin binding region and thus cannot bind Cry1Ac. The power of DNA based screening depends on the diversity of resistance conferred mutations. Tobacco budworm field populations might harbor this same mutation, other mutations of the same gene, or other genes and mechanisms of resistance. The Gahan et al. findings are the first to identify a DNA-based screening for $Bt$-resistant tobacco budworm heterozygotes by directly detecting the recessive allele. The Gahan et al. DNA marker is being evaluated in the field, and other DNA markers are being screened.

Gould et al. (1997) used a series of genetic crosses with test stocks of highly resistant tobacco budworm (YHD2) selected on Cry1Ac in the laboratory to estimate the resistance allele frequency in a natural population of tobacco budworms. This method can identify recessive or incompletely dominant resistance alleles from field-collected males. By using an assay that discriminates between heterozygotes, Gould et al. could establish which wild males carried a resistance allele. Using this allelic recovery method, Gould et al. estimated the resistance allele frequency to be $1.5 \times 10^{-3}$. This method is only useful when there are previously identified resistance alleles. As noted in the preceeding paragraph, Gahan et al. (2001) were able to identify the mechanism of resistance in this YHD2 line and were the first to develop a DNA marker that might be used in the field to screen for resistance.

Venette et al. (2000) proposed the use of an in-field screen to examine resistance allele frequency. This method uses $Bt$ sweet corn to screen for European corn borers and corn earworms resistant to the $Bt$ protein. That is, the $Bt$ crop is the discriminatory screen for resistant individuals. By sampling large numbers of $Bt$-expressing plants for live corn borer larvae, the frequency of resistance can be estimated and resistant individuals collected for documentation of resistance. A high number of false positives can reduce the efficiency and accuracy of resistance allele measurement. One source of false positives is the occurrence of weakly or nonexpressing “off-type” plants among the sampled plants. Another source might be surviving susceptible larvae that are incorrectly scored as resistant larvae because of larval movement between $Bt$ and non-$Bt$ off-types or weeds. Another problem is that there might not be sweet corn varieties contain the same $Bt$ genes as the field corn varieties. This would reduce the efficiency of sampling.

In addition to sampling and detection sensitivity, other equally complex issues are related
to cost and feasibility. It would be virtually impossible and economically prohibitive to sample every farm in which Bt crops are used. For example, there are approximately 14,000 Bt cotton producers (out of approximately 25,000 cotton producers). These producers planted about 4.5 million acres of Bt cotton in the 2000 growing season. Current resistance monitoring programs have focused sampling in areas of highest adoption of the Bt crops as the areas in which resistance risk is greatest. About 20 million acres of Bt corn were planted in the 2000, growing season. The cost of the U.S. monitoring program is borne chiefly by the companies although academic institutions and the U.S. Department of Agriculture researchers who carry out the bioassays probably bear some costs (i.e., University of Nebraska for European corn borer, University of Arizona for pink bollworm, University of Missouri for southwestern corn borer, and USDA/Agricultural Research Service at Stoneville, MS for tobacco budworm and cotton bollworm).

Related to who will pay for resistance monitoring programs is the issue of cost-effectiveness. If money is not a limiting factor, will the resistance monitoring programs be more proactive, more expansive, and more sensitive? What is the best test to be used on the basis of how much information is found for the money involved? Cost-effectiveness is related to the perceived and real value of the technology and the likelihood of resistance. Those who believe there is little likelihood of resistance development are less enthusiastic about a rigorous monitoring program.

**Remedial Action Plan**

EPA requires that a remedial action plan be available in the unfortunate situation of suspected or actual resistance (USEPA, 2001b). Again, as for resistance monitoring plans, remedial action plans are specific for the crop and pest. For example, because the pink bollworm is primarily a pest of cotton in the Western U.S. and differs biologically from the other two target pests of Bt cotton, the remedial action plan for pink bollworm is quite different from those for cotton bollworm and tobacco budworm in the Southeastern U.S. These plans define not only suspected and confirmed resistance but the key steps and actions needed if resistance develops. Generally, if resistance is confirmed, the farmers involved will treat their Bt crop with alternative pest control measures. This might be a chemical pesticide known to be highly effective against the insect or it might mean measures such as crop destruction. In addition, the sales and distribution of the Bt crop would be suspended in the affected area and its environs until it could be determined that insects in that area had regained their susceptibility to the Bt protein. Increased monitoring would also be needed to define the remedial action area(s). Other remedial action strategies include increasing refuge size, changing dispersal properties, using sterile insects, or other modes of pesticidal activity. Geospatial surveys would help define the scale of remedial action and the locations requiring intensified monitoring.

Because no field resistance has yet been found to any of the Bt crops, all of these tactics are untested. However, EPA believes that a key attribute of these plans is involvement in their development by the local farmers who would be affected most by the loss of this technology. So far there is only a regional remedial action plan for the Arizona area in which the pink bollworm is the chief pest controlled by Bt cotton. An interim remedial action plan is required and is being revised to address tobacco budworm and cotton bollworm resistance to Bt cotton, for they are the key economic pests of cotton in the mid-South and the Southeastern U.S. There is also a general remedial action plan to address resistance to
European corn borer, southwestern corn borer, and corn earworm.

**Conclusion: Balancing the Four IRM Activities**

The four IRM activities described above (farmer actions, compliance monitoring, insect resistance monitoring, and remedial action plans) need to be balanced. To some extent, they are at least partial substitutes for each other. In other words, if the refuge is extremely large (95-percent), there is virtually no need to monitor for insect resistance because resistance is so unlikely to occur. However, having a 95-percent refuge would eliminate many of the benefits to growers as well as the environment for cotton growers. Monitoring every Bt field for insect resistance reduces the need for a compliance program, but such an intensive effort is infeasible and extremely costly. In its regulation of these Bt products, EPA has attempted to balance these activities. EPA believes that the increased quality and substance of the compliance monitoring and resistance monitoring programs required through our just-completed reassessment can compensate to some extent for the small refuge size for Bt cotton. In addition, EPA has required additional data on the effect of alternate plant hosts and alternative modes of actions on delaying cotton bollworm resistance to Bt cotton. EPA believes that technological improvements to detect resistance earlier in the field will result in scientifically valid methods that will be cost-effective for insect resistance management in the future. Our faith in future improvements comes from knowing that academic, company, and Government research continues to be strong in the area of IRM for Bt crops.

**Endnotes**

1. See for the reports of the Scientific Advisory Panel and for the meeting notes from the Office of Pesticide Programs Pesticide Program Dialog Committee. Scientific Advisory Panel meetings related to biotechnology can also be found through links from the Biopesticides web page at http://www.epa.gov/pesticides/biopesticides.

2. Sections III and V of the Bt Crops BRAD at http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm

**References**


Future Needs: Unique Challenges and Opportunities for Environmental Assessment (Abstract)

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The public’s general understanding of living modified organisms (LMOs) places particular emphasis on improving agricultural production efficiencies and product quality as well as on environmental conditions and human well-being. Further development of these areas should be supported by research progress in the scientific assessment of environmental issues. This may involve the following three levels of biological interactions.

The first is the level of genomes. Rapid progress in genomics with cereals—particularly rice—and in transformation technologies will increase the frequency of so-called gene-stacking products in which a wide range of transgenes may be combined using conventional breeding or sequential transformation. Scientific reports suggest that combining homologous DNA sequences with transgenes can lead to transgene instability and silencing. Further, more complicated gene manipulations in metabolic pathways such as in functional foods, industrial processing, and pharmaceuticals yield new products expressing unique characteristics that are different from the traditional categories. Determining if these changes at the genome level alter the environment will require cautious future research.

The second level is at the one of plant populations. Experience reveals that distinguishing environmental impacts of a particular genetic modification in isolation are difficult in some outcrossing species such as perennial grasses and many coniferous trees. Continuous changes in genetic variation are occurring even in the original mother population, leading to a lack of an appropriate stable baseline. The impact of specific genetically modified (GM) crops on wildlife biodiversity is more complex because there are almost no data analyzing the impact of particular conventionally bred crop varieties on wildlife biodiversity. Thus, few baseline data studies exist against which to compare specific GM crops. All of these problems indicate the need for research to establish relevant baselines for conducting environmental assessments.
The third level is the one of the less-developed agricultural systems—particularly in developing countries. Statistics show that more than 50 percent of major abiotic limitation for agriculture exists because of drought and mineral stress. Research on molecular responses to drought, cold, heat, and salt stress in higher plants is in progress worldwide. For example, development of GM *arabidopsis*, tobacco, and rice tolerant to these stresses has been planned in Japan. These GM crops, once introduced, will certainly contribute greatly to increased production in extensive areas of the less-developed agricultural systems in developing countries. However, information on baselines is very scarce, because no conventionally bred varieties of such stress-tolerant crops have yet been widely cultivated. We need to research new approaches to the environmental assessment of the impact of GM crops, which possess great future potential.

In conclusion, further research on environmental issues—particularly on baselines—is needed, not on the basis of a science-absent fictional stringency but founded on sound, science-based, and broad perspectives for future development.
Abstract

Biotechnology provides new opportunities for achieving productivity gains in agriculture. However, mobilizing modern biotechnology to address food and agricultural needs in developing countries implies increased responsibilities for determining benefits and risks. This paper examines capacity and efficiencies of national regulatory systems through research studies conducted in Egypt and Argentina. These studies were designed to elicit managerial and policy recommendations to strengthen regulatory systems, stimulate scientific risk assessment, and advance efforts in the areas of public acceptance, technology transfer, and harmonization. Following this analysis, one tool for building comprehensive regulatory capacity is presented. This is a conceptual framework for implementing biosafety and thus supports regulatory needs related to the Cartagena Protocol on Biosafety. One element of the framework, scientific knowledge, skills and capacity base, is examined in terms of policy options and decisions for locating scientific expertise. Final conclusions are provided emphasizing the need for comprehensive capacity building, including expertise in policy and managerial skills as well as for risk assessment.

Introduction

Developing countries face complex decisions regarding the introduction, testing, and use of products from modern biotechnology. These responsibilities include determining the benefits and risks of agricultural biotechnology applications. Although potential applications are varied and growing (Pew Initiative on Food and Biotechnology 2001), there are still relatively few events
or products of widespread use in modern agriculture. Products from multinational companies include herbicide-resistant soybeans, insect-resistant cotton, maize, or canola. Although these products are primarily produced for temperate regions, investments in research conducted by and with agricultural and scientific research organizations of developing countries are producing innovations to meet local market or food security needs, or both (Cohen 2001, Komen 2001, Morris and Hoisington 2000).

These transgenic events, whether from public or private research, raise concerns and requirements regarding their use, regulation, and assessment. Products derived from genetic modification technologies for use in developing countries are subject to extensive product development cycles and a lengthy review for potential environmental and health risks. The changing dynamics of political and international debate regarding risk and public perception also affect acceptability (Paarlberg 2001). Addressing these concerns and responsibilities demands that capacity be present at the political–international, regulatory, and scientific level in developing countries.

In the international arena, national systems for risk assessment and national biosafety frameworks emerged as a priority in chapter 16 of Agenda 21 and Articles 8(g) and 19 of the Convention on Biological Diversity. These articles instigated a global initiative to reach agreement on measures to ensure the safe handling and use of living modified organisms (LMOs) that may have an adverse effect on biodiversity while taking into account human health. The resultant Cartagena Protocol on Biosafety was adopted in January 2000. In addition to specific articles dealing with transport and use of LMOs, significant emphasis was placed on capacity building. Article 22 states that parties should cooperate in the development and strengthening of human resources and institutional capacity in biosafety.

At the political level, regulatory concerns can arise because of campaigns undertaken to curtail the use of products of genetic engineering such as those calling for a moratorium on the commercial use of genetically modified organisms (WWF 2001) or trade-related directives that ban genetically modified products from import. These concerns, moratoriums, and trade embargoes create difficulties in developing countries for those responsible for setting clear policies and agendas for biotechnology research and product regulation. At the scientific and regulatory management level, capacity is severely strained and competent individuals serve many pressing responsibilities.

To study the interrelated dimensions of regulatory responsibilities, compliance with international agreements, and related approaches for capacity building, the International Service for National Agricultural Research (ISNAR) initiated ongoing research studies. The first of these explored regulatory efficiencies and needs through research partnerships with selected developing countries, beginning in Egypt and Argentina. The second effort resulted in a conceptual framework for biosafety implementation based on a synthesis of contributions from an international expert consultation, *A Framework for Biosafety Implementation: A Tool for Capacity Building*. Together, these studies highlight regulatory systems, resources, and capacity needed to respond to transgenic events.
Regulatory Systems in the Developing World—Two Country Studies

To help assess the efficacy of national biosafety systems, a collaborative research project with Virginia Polytechnic Institute and State University and partner institutions in Egypt and Argentina was undertaken. The studies were designed to review policies and procedures associated with the introduction of genetically engineered crops in developing countries. The specific objectives of the studies are to accomplish the following:

1. Assess the efficacy of biosafety policies and procedures associated with the introduction of biotechnology products;
2. Develop recommendations for enhancing the operation of each country’s biosafety system and minimizing potential constraints to technology transfer; and
3. Identify areas where international organizations can provide further assistance.

The studies (Madkour et al. 2000, Burachik and Traynor 2002) examine four common elements of biosafety systems: guidelines, people, the review process, and mechanisms for feedback (Traynor 1999). Information is collected regarding the following:

- The organization, membership, and operations of national biosafety committees;
- The nature and availability of information on biosafety procedures and requirements;
- The regulatory review paths and necessary approvals leading to commercial release;
- The extent of public involvement in biosafety matters; and,
- The personal experiences of applicants and reviewers in dealing with the biosafety system.

Country Studies—Synthesis and Findings

Argentina and Egypt are among the more advanced developing countries in terms of current and intended uses of genetically engineered crops and products derived from them. Egypt has approved several dozen confined field trials. Argentina has been exporting commercial genetically modified organisms (GMO) commodities since 1996. Several common characteristics are found between the two countries regarding their handling of biosafety matters. For both countries, the first step in establishing a biosafety system was the drafting of guidelines for ensuring the environmental safety of GMO releases. National guidelines were formulated after a thorough examination of regulatory documents from Canada, Australia, the United States and other countries with appropriate adaptations to national agricultural parameters. In contrast, application, review, and approval procedures for food safety and seed registration, which typically are subsequent steps in the path to commercialization, were built on a framework of preexisting laws and authorities.

For both countries, mechanisms for evaluation and approval evolved over time. As the first few GMO products reached each stage leading to commercial production—field testing, food safety review, seed registration, and commercial sale—the necessary guidelines, committees, and processes for each stage were implemented on an as-needed basis. In this way, successive regulatory procedures could be functionally coordinated with previous steps and with other ministries and regulatory authorities. The drawback to this approach is that it
tends to create delays; applications may be put on hold until procedures for the next step are worked out.

In Egypt and Argentina, the Ministry of Agriculture is the lead government entity overseeing agricultural biotechnology. It is within this ministry that environmental safety evaluations are conducted; the Ministry of Environment has a lesser role, if any. Food safety evaluations are conducted through the Ministry of Health. Both countries have constituted advisory committees that conduct technical reviews and make recommendations for approval of individual release applications. The national biosafety committees are empowered to deny a request or to hold it pending receipt of additional information from the applicant. Final decisionmaking authority to allow field tests or commercial releases, however, rests with the Minister of Agriculture. All evidence would suggest that ministry officials in both countries respect the work of their biosafety committees, for there have been no cases in which advisory committee recommendations were ignored nor instances in which ministerial approval was granted in the absence of a proper biosafety review and recommendation.

Both countries have advanced research institutes where Ph.D.-level scientists assisted by highly competent staff conduct state-of-the-art biotech research. Thus, there are pools of qualified individuals who may serve on national biosafety committees or as ad hoc technical advisors.

Nonetheless, the biosafety systems in Argentina and Egypt are very close to exhausting available expertise with competence in biosafety. This is evident in the degree of redundancy among members of the various review committees in Egypt and in the difficulty in identifying additional independent experts in Argentina. One of the most consistent messages heard throughout both studies was the immediate need for biosafety training that would build technical competence in risk assessment and risk management.

Biosafety evaluations in Argentina and Egypt, as in almost every other country, focus on risk in a proposed release. The task is to identify any potential risk and explore potential means for managing identified risks. Ostensibly, evaluations compare predicted impacts of the GMO with those of the equivalent non-GMO variety. Genetically modified varieties that present no greater risk than the referenced conventional variety are deemed acceptable for testing and eventual commercial release. As elsewhere, however, neither country includes a benefit assessment (nor assessment of the risks of not proceeding with the GMO) in the equation. Benefit assessments are a crucial part of the information needed for a comprehensive and balanced review and generate important information needed by the public.

Membership on a biosafety committee typically is an unpaid position added to each person’s regular duties. Whether university faculty, public or private sector scientist, government agency representative or research administrator, all have to adjust their schedules to accommodate the extra workload. In spite of this, none of those interviewed in the two studies expressed any sense of being burdened with an unwanted responsibility. Rather, they took pride in the scientific rigor and fairness of their reviews and felt that their biosafety work was important and valuable.

The 30-member Egyptian national biosafety committee comprises 7 representatives of the Ministries of Agriculture, Health, Environment, Industry, and Commerce; a representative of the Egyptian Academy of Science and Technology; 12 members from academic institutions
Even with such a large committee, some questions of risk may not adequately be addressed in the review process. For example, applications to commercialize Bt maize varieties have successfully passed environmental biosafety review, yet the risk of accelerated emergence of Bt-resistant pest populations and possible management strategies to reduce the risk to an acceptable level were not addressed during the discussions. In the future, experience and more forward thinking may help reviewers anticipate longer term risk problems and options for suitable management solutions.

The 19-member Argentinean biosafety commission includes people from private sector organizations (though not individual companies) as well as Government agencies and academic institutions. The major consideration for membership is the candidate’s qualifications in the desired area of expertise. Institutions represented on the commission submit the curricula of three candidates, two of whom are selected for consideration and eventual approval by the Secretary of Agriculture. Conceivably these factors contribute to the more technical nature of the Argentine review committee. When combined with years of accumulated experience, differences noted here may also contribute in part to the more comprehensive review achieved in the Argentine system.

The potential for conflict of interest is an inherent part of Argentina’s biosafety system. Nearly all biosafety reviewers conduct applied research at public institutions (leading to field tests and possibly commercial products), work collaboratively with biotechnology companies, or belong to industry organizations. Even those in the first group often have ties to private sector companies. The prevalence of these relationships makes it common for a Commission member to excuse himself or herself from taking part in a decision. Such connections also make it difficult to find independent, disinterested members to review applications containing confidential business information.

Although not a priority in Egypt, Argentina is giving serious consideration to drafting biosafety legislation that would include stringent measures to ensure compliance. Although such a step would likely make future revisions much more difficult, the loss of flexibility in the biosafety system is considered less important than the gain in legal authority and increased public visibility of a vigilant biosafety system.

A Conceptual Framework for Implementing Biosafety

As seen from the studies and findings summarized above, the design and implementation of any national biosafety system involve balancing public policy goals with economic, political, and technical realities. However, over the past two decades in developing countries, national biosafety frameworks and guidelines have often been implemented in a fragmented manner, owing to particular needs and pressures at the time. Consequently, a comprehensive, conceptual framework for biosafety implementation has often been lacking. For this reason, ISNAR convened an international expert consultation to develop such a framework.
In this section, the resultant framework is considered. The objective is to address national needs, particularly of those countries that are Parties to the Cartagena Protocol, regarding regulatory implementation and capacity building. The framework provides guidance on the design and implementation of regulatory frameworks and related capacity-building initiatives. It seeks to clarify critical decision points in the development of a national biosafety framework and choices among policy options and to delineate some of the scientific and social dimensions of these options (McLean et al. 2002).

The framework addresses five elements as fundamental to the development and implementation of a national biosafety system. The first two—(1) national policies, strategies, and research agendas regarding biotechnology and biosafety and (2) a national inventory and evaluation—provide the foundation for subsequent regulatory implementation. The next element—requisite knowledge, skills, and capacity base—is the resource environment within which the final two elements occur: development of regulations and implementation of regulations. This framework expands on the conceptual basis used for ISNAR’s national biosafety system studies and on concepts and lessons derived from other national, regional, and international experiences analyzed during the consultation. Implications of the framework are considered in relation to more recent expectations following the adoption of the Cartagena Protocol on Biosafety (CBD Secretariat 2000).

**Scientific Knowledge, Skills and Capacity Base**

Building a strong base of scientific knowledge in support of the regulatory system and developing core competencies in biotechnology product evaluation are fundamental to any national biosafety system. These activities allow an improved scientific basis for assessments of potential risks and benefits, and they strengthen the scientific capabilities for risk management, inspection, and monitoring. A thin, weak, or limited knowledge and skills base tends to produce regulations that are highly protective at the expense of innovation, poorly defined or inconsistent, comparatively rigid, or narrowly interpreted. A deep and broad knowledge, skills, and capacity base tends to foster more latitude in regulatory development and more flexibility in regulatory implementation.

The expert consultation identified two key decision points and subsequent policy options for building scientific knowledge, skills, and capacity. The key decision points are as follows:

1. Providing a coordinated approach to incorporating scientific advice into biosafety decisionmaking and
2. Locating the science evaluation function within the regulatory system.

These two points and their subsequent policy options are discussed below.

**Key Decision Point One: Coordinating Scientific Expertise**—

As the science involved in the creation of LMOs advances and the products themselves become more complex, there is an increasing need to strengthen the science base supporting risk assessment and regulation. Developing skills required for biotechnology product evaluation and maintaining parity between risk assessors and their counterparts involved in
developing new products is of fundamental importance. This requires ongoing training about new scientific advances without which a regulator’s knowledge base has a limited life expectancy.

The policy options as regards coordination are whether development of national capacity for scientific risk assessment should be given exclusive priority or whether it is possible to coordinate risk assessment at a regional or subregional level. The second policy option is to determine if a country will rely on international experts versus domestic self-sufficiency and capability. Each of these policy options is being explored in various ways by developing countries and with respect to expectations for adequate risk assessment of LMOs in relation to the Cartagena Protocol on Biosafety.

Adequate scientific capacity provides an improved scientific basis for assessments of potential risks and benefits and can improve the quality of risk management decisions and inspection and monitoring capabilities. Limitations in national scientific and technical capacity identified during the inventory and evaluation can be addressed through a co-coordinated approach. This would aim to enhance domestic expertise through training but also would rely on subregional, regional, or international cooperation, or all of these in performing risk assessments and using outside experts and the international academic community.

Key Decision Point Two: Locating the Science Evaluation Function—

Maintaining access to scientific expertise is an issue for developed as well as developing countries. Structurally, different approaches to locating and securing scientific advice within the regulatory framework can be taken. In considering the risk assessment of biotechnology products, some countries have implemented a system of expert advisory committees whereas others have relied primarily on scientists and professionals working within government agencies. In the latter approach, the mandate for risk assessment may be vested within a single agency exclusively tasked with regulating products of biotechnology (e.g., a gene technology regulator) or it may be distributed between agencies in accordance with their existing responsibilities (e.g., departments of health, agriculture or environment).

The first policy option identified, as related to location of scientific expertise, is how to include the development of core competence for risk assessment within government departments and agencies versus a combination of both inhouse and external scientific expertise. The second policy issue is whether a country concentrates the risk assessment function within a single indefinable body versus distribution of this function among different government departments and ministries.

Generally, independent advisory committees have more transparent accountability frameworks than government departments and agencies in which the range of expertise and academic credentials of risk assessors is rarely published.

However, advisory bodies can suffer because committee members are part-time volunteers who cannot devote their full energies to risk assessments. Out of necessity, committee meetings occur only a few times per year, thus limiting efficiency; moreover, the selection process for committee members may not result in the right combination of scientific expertise and regulatory experience. Product evaluations performed by competent scientists within a regulatory agency or agencies, supplemented by the use of issue-specific expert panel consultations, is an approach to LMO regulation that may combine the best of both worlds.
Discussion: Implications for Capacity Building, Funding and International Support

The Cartagena Protocol has focused attention on the needs for broad-based efforts regarding capacity development. To respond effectively to political debates, campaign-related moratoriums, and the need to build regulatory expertise, it is essential that comprehensive and credible expertise be built among scientists, institutional managers and directors, and key policy- and decision-makers. Such commitments by and for developing countries will more adequately respond to public sector needs regarding their role in the governance of new technologies.

Increased knowledge of the costs of regulating new products, particularly pest-protected crops, will become essential. Although most commercial providers will meet regulatory costs, it is still unclear how public sector research organizations in developing countries will meet the costs of regulation and risk assessment. The need for environmental assessments of locally produced events can be expected to increase as research capacity and competency increases. As noted in the NRC report (National Research Council 2000), regulatory testing can be expensive in terms of management time and money (Lichtenberg 2000). Consequently, testing barriers can become barriers to entry for small companies or national agricultural research organizations.

Thus, providing comprehensive capacity will be crucial for implementing biotechnology research and regulatory structures and for acquiring abilities to comply and participate with international forums. The least advanced countries will be hard-pressed to assemble more than a few qualified professionals with competence in risk assessment procedures. Studies from Egypt and Argentina have noted that both of these countries are functioning near the limits of available expertise, which raises questions about the capacity available for future reviews and how to circumvent the possibility of conflicts of interest. In addition, regulatory systems need to address risk factors adequately through research and to gather or supply relevant data, as recognized by the Biosafety Protocol.

Unfortunately, developing countries will continue to face limited funding and investment opportunities regarding biotechnology research and regulatory support (Cohen 2001). They use professionals to address regulatory requirements and are not able to compensate them. Such harsh economic realities are not going to change in the immediate future, nor will the needed human capacity become suddenly available to address the policy and scientific challenges that surround biotechnology. Therefore, further consideration by donors, international bodies, and national policymakers must be given to implementing biosafety guidelines and regulatory systems in the context of developing, not developed, countries. These considerations should draw on opportunities for creating increased efficiencies, economies of scale, regional cooperation, and the means by which these countries can economically and scientifically comply with the increased calls for safety and environmental risk assessments. In this regard, the conceptual framework introduced in this paper should prove most useful (McLean et al. 2002).

To supplement national funding, new mechanisms to ensure environmental assessments for events arising from public research will be needed. Consortia grouped by crop and event, working across countries and regions, can be effective in this regard. Significant international
attention and funding to projects such as those listed below would significantly augment the limited opportunities and funding available to developing countries seeking to conduct fundamental biotechnology research as well as develop suitable safety and environmental testing capacity.

One example of such an initiative comes from the U.S. Agency for International Development though its competitive granting program called the Biotechnology and Biodiversity Interface (BBI). These grants address the interface between the use of agricultural biotechnology and natural biodiversity. This program develops data and builds capacity to assist developing countries in the use of biotechnology in an environmentally responsible manner. To date, five grants have been awarded (Pathak 2001) covering diverse target organism and ecological settings such as transgenic fish and biodiversity in Thailand, transgenic rice and potential for outcrossing in Vietnam and gene flow in Thailand, effects of transgenic maize on nontarget soil organisms in Colombia, and ecological impacts of introducing transgenic crops in Africa.

A second opportunity comes from a proposed international initiative of public sector scientists organized through the working group on Transgenic Organisms in Integrated Pest Management and Biological Control, working under the International Organization of Biological Control. This initiative is planning to develop scientific principles and detailed scientific guidelines for international biosafety testing of transgenic plants (Hilbeck 2001). Developing country participation in this proposed initiative would greatly facilitate their understanding and compliance with comprehensive, transparent scientific guidelines for prerelease biosafety testing of transgenic plants.

Finally, greater focus is needed as regards public sector biotechnology arising from research conducted by, and with, developing countries. In addition to understanding the nature of transgenic events being researched, such information will be crucial to assuming a more predictive and proactive stance towards the needs of both environmental and health assessments. Therefore, ISNAR is now collecting data on such events, including agronomic and regulatory steps, through its Next Harvest project in collaboration with 15 developing countries. It is proposed to link this information with global scientific expertise so that additional consortia can be developed to further support both regulatory and biotechnology research needs. The final step will be to relate this regulatory and research information to economic and costing studies as per expected costs required to undertake assessments and prepare for scale up and commercial trials.

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Capacity Building for Research and Monitoring in the Developing World: Unique Challenges and Opportunities

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Abstract

Capacity building is a recurring concept within the framework of sustainable development; however, the term’s meaning, applicability, and perspectives can change considerably depending upon the context in which it is used. In this presentation I will therefore try first to analyze the general concept of capacity building and then concentrate thereafter on its implications for the field of biosafety. In this general analysis a few questions and remarks on issues relating to biosafety and the developing countries will be considered followed by an overview of the experience gained by our organization on biosafety in the last several years.

Introduction

In looking for a definition of “capacity building,” one will encounter several meanings; many would be pertinent to the theme of biosafety, others would not. The following four definitions certainly address the topic of this presentation:

1. “Capacity Building is not defined through the instruments used, but through its goal to enhance the capability of people and institutions to improve their competence and problem-solving capacities” (GTZ 1999).

2. “Capacity Building refers to investment in people, institutions and practices that will, together, enable countries in the region to achieve their development objectives” (World Bank 1997).

3. “Capacity Building is the process by which individuals, groups, organizations, institutions and societies increase their abilities to understand and deal with their development needs in a broad context and in a sustainable manner” (UNDP 1997).

4. “Capacity Building may be defined as the actions needed to create or enhance the capability of a country or an institution (or an individual) to carry out its allotted functions and achieve its objectives” (UNDP 1993).
It is interesting to note that, in each of these four definitions, there are two elements that keep recurring, namely the need to enhance the capacities of people and institutions. This should in fact dispel one incorrect concept that equates capacity building (or considers it a synonym) with the training of individuals only. Capacity building is rather the insertion of training activities into an institution as a whole to enhance its own capacities, with the possibility that the concept of “institution” can be extrapolated to the level of a society.

Just after the 1992 adoption of the Convention on Biological Diversity (CBD), the United Nations Environment Program (UNEP), analyzed the meaning of capacity building to be applied to biosafety issues as follows:

“...capacity-building means the strengthening and/or development of both human resources and institutional and infrastructural capacities which ensure that, in the wake of the emerging biotechnology revolution, countries (in particular developing countries) are able to cope with new developments and applications of biotechnology as they arise, and to achieve safety in biotechnology, through effective implementation of existing or planned biosafety guidelines, directives or regulations, and of any future international agreement on biosafety”. (UNEP, Capacity Building for Biosafety: Option for Action, 1992)

Once again, the development of human resources and institutions is the main goal of capacity building activities. The focus of these activities is to permit the developing world access to, and benefit from, the biotechnology revolution. At the end of this last definition, there is a reference to the Cartagena Protocol on Biosafety; Article 22 deals with the capacity building concept and contains the main issues that are at stake:

“The Parties shall cooperate in the development and/or strengthening of human resources and institutional capacities in biosafety, including biotechnology to the extent that it is required for biosafety, for the purpose of the effective implementation of this Protocol...through existing global, regional, subregional and national institutions and organizations and, as appropriate... Cooperation in capacity-building shall include scientific and technical training in the proper and safe management for biosafety, and the enhancement of institutional capacities in biosafety.”

Once more, there is a reference to enhancing capacities of human resources and institutions as well as the need to cooperate with the existing regional and national institutions and organizations as appropriate. For the first time also the concepts of research and technical training make their appearance; this is one of the key elements with which I will deal at the end of this presentation.

The final document of the conference entitled “New Biotechnology Food and Crops: Science, Safety and Society”, organized by the Organization for Economic Cooperation and Development (OECD) in Bangkok in July 2001, underlined how capacity building in biosafety should be addressed towards (a) developing research capacity; (b) ensuring the capacity to make decisions on biosafety, risk assessment and management, monitoring, certification and labeling; (c) answering specific needs of stakeholders (including researchers, regulators, consumers, and producers); and (d) ensuring access to reliable information (Internet access). A few months later, the Intergovernmental Committee for the Cartagena Protocol meeting in Nairobi at the beginning of October adopted by consensus a report detailing an action plan for capacity building that provided for the following key elements:
• Institutional capacity building
• Human resources development and training
• Risk assessment and other scientific and technical expertise
• Risk management
• Awareness, participation, and education at all levels, including decisionmakers, stakeholders, and the general public
• Information exchange and data management, including full participation in the Biosafety Clearing-House
• Scientific, technical, and institutional collaboration at subregional, regional, and international levels
• Technology transfer
• Identification.

Summarizing what comes out of these definitions and the different discussions that have been going on in the international arena, we can identify the main issues at stake as follows:

1. The diffusion of biotechnology;
2. The need to establish adequate mechanisms for the exchanges of information;
3. The establishment of specific programs aimed at enhancing institutional capabilities;
4. The safe research, development, and application of biotechnology products;
5. The transfer of know-how, especially to the developing world;
6. The stimulation of scientific training; and
7. The initiation of specific programs aimed at risk assessment and in risk-management.

Note that these elements were already recognized in 1992 and were present in the agenda of the UNEP on the eve of the Rio U.N. Conference on the Environment and Development. In other words, after almost 10 years of debate on these issues, we returned to the original problems present at the very beginning of the debate on the safety of genetically modified organisms (GMOs).

In the context of these identified issues, what should the international community provide? In particular, what kind of assistance can an international organization like the International Centre for Genetic Engineering and Biotechnology (ICGEB) offer to the developing world, for it to meet the requirements cited above? A very brief description on the origins, the mandate, and the activities of the ICGEB follows focusing on the experience it has developed in the last several years in the field of biosafety.

The ICGEB is an intergovernmental organization that started its operations in 1987 as a special program of UNIDO, the United Nations Industrial Development Organization, to become a center of excellence for research and training in genetic engineering and biotechnology. Special attention was to be placed on the needs of the developing countries. In 1994, after its statutes (i.e., the international treaty establishing the organization) entered into force, ICGEB became a fully autonomous intergovernmental organization that is still closely related to the U.N. system. Since 1997, the ICGEB operated a Biosafety Unit provide its member states with specific activities of interest in the field of biosafety with special emphasis on dissemination of information, development of training programs and international cooperation.
Looking at the membership of the organization, one immediately notes two aspects of interest: its complementarity to OECD membership (because most of the ICGEB member States are developing countries or countries in transition) and the participation of all those countries (like China, Argentina, and South Africa) that, although belonging to the developing world, already have important activities in the use, production and commercialization of GMOS.

**Dissemination of Information**

Two major informational tools are accessible online thorough the Internet. The first one, named Biblio-Bio is a bibliographic, searchable scientific database on biosafety studies. This database (http://www.icgeb.trieste.it/biosafety/bsfdata1.htm) is updated monthly and presently contains some 3,000 scientific articles (full references and abstracts) that have been published in international, peer-reviewed scientific journals since 1990. These are selected and classified by ICGEB scientists according to the main topics of concern for the environmental release of GMOs. A list of the latest references is shown to facilitate diffusion of the main, or most recent, information.

The second informational tool developed by ICGEB is the Risk Assessment Searching Mechanism (RASM). This searchable index has been elaborated, through the funding of the Italian Government, in response to recommendations made by the Intergovernmental Committee for the Curagena Protocol-1 (ICCP1) for the setup of the Biosafety Clearing-House that included inter alia the establishment of central databases containing information from countries without an electronic infrastructure as well as the creation of searchable indexes for information to facilitate decisionmaking in accordance with article 10 of the Cartagena Protocol. The RASM aims to provide access to all the available official documents on risk assessment related to genetically modified crops in different countries and is complementary to, and interlinked with, other existing databases. The prototype of the RASM presently available online (http://www.icgeb.trieste.it/biosafety/rasm.html) contains some 180 records of risk assessment documents for 60 different transgenic events from 13 plant species issued by the official authorities from several countries. One of the future objectives for the enlargement of this index would be to collect and maintain data sent from parties without an electronic infrastructure while continuing to expand the retrieval of data available from those countries with advanced electronic networks.

**Training**

Training and technology transfer in biotechnology are among the main objectives of the ICGEB. The Centre provides its constituency with technical instruments and qualified information required in biosafety and risk assessment to allow member States and the wider international community to gain advantages from biotechnology and be informed of benefits and potential risks.

Since 1991, the ICGEB has organized annual biosafety workshops attended, to date, by close to 600 scientists from more than 60 different countries involved in related issues. In 2001, the ICGEB held two such workshops: “Biosafety 1—Introduction to Biosafety and
Risk Assessment for Environmental Release of GMOs: Theoretical Approach and Scientific Background” and “Biosafety 2—Advanced Research in Risk Assessment and Risk Management for Environmental Release of GMOs: Identification of Main Areas for Future Investigation.” This second workshop, aimed at officers of Governmental agencies and designated experts working in risk assessment of GMOs at the official level (governments, scientific institutions, private sector, etc.) and held under the auspices of the Italian Ministry for the Environment, has been organized, for the second time in collaboration with the Istituto Agronomico per l’Oltremare (IAO), Florence. A third course, that was supposed to be held in 2001 in Venezuela, had to be postponed to 2002 for logistical reasons.

After collaborating with the UNEP/Global Environmental Fund (GEF) “Pilot Biosafety Enabling Activity Project,” the ICGEB is now participating in the steering committee of a new major project implemented by the UNEP and financed by GEF aimed at building capacities in the developing countries to design National Biosafety Frameworks and help to prepare for the implementation of the Biosafety Protocol. Such a project is an excellent opportunity for the ICGEB to lend its technical and scientific support to the international effort that has been initiated in response to the Cartagena Protocol. In this respect, the GEF/UNEP project team and ICGEB are developing an agreement through which ICGEB will organize, starting in September 2002, several regional workshops on risk assessment, thus providing the participants with an overview of the current research in biosafety and different risk assessment approaches used for the environmental release of GMOs.

International Cooperation

Biosafety is an excellent area for combined actions directed to enhance the environmental standards of biotechnology management. The special relationship that links the ICGEB to UNIDO, the United Nations Educational, Scientific, and Cultural Organization (UNESCO), and other U.N. bodies as different autonomous organizations committed to cooperating on biotechnology issues of mutual interest, coupled with their long-term experience in developing co-operation programs, creates a perfect synergism with the renowned experience in advanced research and training in molecular biology and biotechnology of the ICGEB. Moreover, this specific field has been recognized as one of the main topics for collaboration between the Secretariat of the United Nations and the ICGEB; accordingly, the Cooperation Agreement entered into by the two Secretariats in March 2001 specifies that the U.N. and the ICGEB may decide to cooperate in activities related to the sustainable and safe use of genetic engineering and biotechnology as well as in the implementation of the international cooperation programs foreseen by the Convention on Biological Diversity and its Cartagena Protocol on Biosafety (Article VI.2 of the UNICGEB Cooperation Agreement).

The Centre has actively participated in the elaboration of the Voluntary Code of Conduct for the Release of Genetically Modified Organisms into the Environment (prepared by the informal UNIDO/UNEP/WHO/FAO Working Group on Biosafety in July 1991), is a party to the Inter-Agency Network for Safety in Biotechnology (IANB) chaired by the OECD and is actively involved in providing the Secretariat of the Convention on Biological Diversity with scientific and technical tools for its Biosafety-Clearing House, which is one of the most important information tools foreseen by the Cartagena Protocol.
In the course of its almost 13 years of experience, the ICGEB has also gained capabilities in techniques available at the laboratory level. In the future, the ICGEB plans to develop training curricula that may provide scientists from developing countries with hands-on training activities in specific techniques that may be necessary for the detection, assessment and management of GMOs. Moreover, the Centre is now developing a project for the establishment of a Biosafety outstation aimed at setting up an ICGEB facility for training and research in risk assessment and management relating to the environmental release of GMOs. The outstation, equipped for studies in molecular genetics, will be located close to Venice and will develop research programs for the investigation of those gray areas in scientific knowledge that concern the safe use of agricultural products derived from biotechnology. The cost of the buildings that will host the new laboratories and a guesthouse for trainees, their remodeling, and the operation of the outstation will be met by an Italian nonprofit foundation.

With its pending involvement in the Biosafety outstation foreseen by the end of 2002 or the beginning of 2003, the ICGEB will complete its spectrum of activities dedicated to biosafety. The ICGEB’s focus on enhancing the scientific capacity of individuals while strengthening institutions in developing countries through major international efforts is a unique example of two fundamental elements of capacity building at large being addressed. This is an intrinsic part of the ICGEB mandate that needs to be included in the global context among all the efforts made by individual countries, the CBD Secretariat, and other international organizations aimed at the full implementation of the Cartagena Protocol and at a safe and sustainable use of biotechnology.
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