Biosafety for Sustainable Agriculture:

Sharing Biotechnology Regulatory Experiences of the Western Hemisphere

Biosafety for Sustainable Agriculture:

Sharing Biotechnology Regulatory Experiences of the Western Hemisphere

Anatole F. Krattiger
International Academy of the Environment

Arno Rosemarin
Stockholm Environment Institute

Editors

With a Foreword by Gary Toenniessen
International Program on Rice Biotechnology
The Rockefeller Foundation

and a Preface by Clive James
Chair, International Service for the Acquisition of Agri-biotech Applications (ISAAA)

ISAAA-International Service for the Acquisition of Agri-biotech Applications
SEI-Stockholm Environment Institute
1994

The designations of geographical entities in this book and the presentation of the material do not imply the expression of any opinion whatsoever on the part of the International Service for the Acquisition of Agri-biotech Applications (ISAAA) or of the Stockholm Environment Institute (SEI) concerning the legal status of any country, territory, or area, or of its authorities, or the delimitation of its frontiers or boundaries.

The views of the authors expressed in this publication do not necessarily reflect those of the International Service for the Acquisition of Agri-biotech Applications (ISAAA) or of the Stockholm Environment Institute (SEI).

Published by: The International Service for the Acquisition of Agri-biotech Applications (ISAAA), Ithaca, USA and the Stockholm Environment Institute (SEI), Stockholm, Sweden.

Citation: Krattiger, A.F. and A. Rosemarin. 1994. Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere. International Service for the Acquisition of Agri-biotech Applications (ISAAA): Ithaca and Stockholm Environment Institute (SEI): Stockholm. *xiv* & 278 pp.

ISBN: 91-88116-972

Design: A.F. Krattiger

Text Layout: A. Rosemarin/A.F. Krattiger/H. Pohjolainen

Cover: A.F. Krattiger/E. Rens

Cover photo: Agricultural Field: PHOTO OIKOUMENE (Courtesy World Council of Churches, Geneva); Wheat Field: MARTIN BOND (Science Photo Library, London).

Printed by: The Burlington Press Ltd., Cambridge, UK.

Available from: The International Academy of the Environment
4 chemin de Conches
CH-1231 Conches/Geneva
Switzerland.

The Text of this Book is Printed on Fineblade Environmental Pulp Paper.

Contents

Section 2: Regulatory Philosophies and Government Experiences in Developing and Enacting Biosafety Regulations

Section 3: Public and Private Sector Experiences in Conducting Field Trials with Transgenic Crops

Section 4: Developing Biosafety Regulations to Enable Technology Transfer

Section 5: Afterword

Section 6: Appendix

Foreword

Over the next half century agriculture will face formidable challenges. The number of people living on our small planet is increasing rapidly with at least a doubling inevitable before population stabilization is finally achieved. Most of this increase will occur in developing countries, some of which are already struggling with frequent food shortages.

Agriculture, in the not too distant future, must provide adequate nutrition for between 10 and 15 billion people, contribute to their continued economic development so that there will be an increasing desire to limit family size, and accomplish this without jeopardizing the capacity of the natural resource base to meet the needs of future generations. At present agriculture does not have the technologies needed to double or triple food production in developing countries, and there is a real threat, already materializing in some locations, that farmers will irreparably damage the resource base as they seek to feed more and more people. Success in meeting these challenges will depend on the discovery of new knowledge and the development and wise use of new technologies, which combined with broader application and better adaptation of existing technologies will allow for greater intensification of crop production on a sustainable basis.

The genetic manipulation of plants, initially undertaken by farmers and more recently by plant breeders, made an important contribution to past increases in agricultural productivity. Fortunately, just when needed, plant breeding is on the verge of receiving a substantial further boost in its technological potential. A new set of genetic monitoring and manipulation tools, in aggregate referred to as biotechnology, is

becoming available as a result of advances in molecular and cellular biology. Genetic engineering is perhaps the most powerful of these tools and includes the precise transfer of genes from one species to another. Combined with breeding, biotechnology has the potential for producing new crop varieties enabling farmers to produce more abundant supplies of nutritious food while causing less environmental damage.

As with any powerful new technology, plant genetic engineering needs to be employed carefully. To ensure this, government agencies in most developed countries have effected biosafety regulations that cover laboratory and greenhouse experimentation, field evaluation and commercialization. In the United States of America alone, over one thousand permits have been approved for field testing genetically engineered plants. In Canada, as well as the United States the first approvals were recently obtained for commercial production and marketing of such plants.

If the developing countries are to share fully in the benefits of this new technology while minimizing risks and guarding against misuse, they too must develop and implement biosafety systems which are workable, effective, and based on rigorous scientific evaluation. The Convention on Biological Diversity encourages harmonization of regulations across countries and there is much to be gained by such an approach. However, there are also disadvantages which need to be further examined and debated. In the Western Hemisphere some developing countries such as Mexico and Costa Rica have already taken major steps toward implementing biosafety regulations and international agencies are providing assistance throughout the region.

This book, based on a workshop organized by the International Service for the Acquisition of Agri-biotech Applications (ISAAA) and held in Costa Rica, provides a review of the Western Hemisphere experience thus far with biosafety regulations for genetically engineered crop plants. While each country should develop a biosafety system based on its own needs and priorities, much can be learned from this experience that is relevant elsewhere. The key biosafety issues that should be addressed, and regulatory options considered and selected by various countries are described in a clear and balanced series of commentaries which anyone with responsibilities or interest in this field will find useful. By helping to ensure that crop biotechnologies are used effectively and appropriately in developing countries, this book can make a contribution toward achieving future food security for our world.

Gary Toenniessen
The Rockefeller Foundation
New York, New York, July 1994.

Preface

The International Service for the Acquisition of Agri-biotech Applications (ISAAA) was founded in 1991 as a not-for-profit organization, co-sponsored by public and private sector institutions, with the aim of facilitating the acquisition and transfer of agricultural biotechnology applications from the industrial countries, particularly proprietary technology from the private sector, for the benefit of the developing world. From the outset ISAAA made a commitment to assist developing countries to build institutional capacity in regulatory oversight to ensure that transgenic products in the Third World were developed, tested and adopted in a responsible and effective way. Accordingly, ISAAA planned a series of workshops for Latin America, Asia and Africa; at the time of publication of this book three have been completed, two in Latin America and one in Asia, and a fourth is being planned for Africa in early 1995. The first workshop was organized in Costa Rica in February 1992 and this publication documents the experience of the workshop so that others can share and benefit from the exchange of information and accumulation of knowledge about biosafety.

The principal objective of the Costa Rica Workshop was to share the experience of several developed countries, who have pursued different biosafety policies, with scientists, policy makers and special interest groups from two of ISAAA's target developing countries in Latin America, Costa Rica and Mexico. More specifically, the objective of the workshop was to provide "hands on" experience to selected personnel from Costa Rica and Mexico who would be involved in regulating the development and testing of transgenics in their respective countries. The "hands on"

experience was provided by supplementing plenary presentations with actual case studies so that participants could be walked through a decision making process and the rational underpinning those decisions. Representations were made by promulgators of biosafety legislation as well as by users of the legislation from both the public and private sector in the industrial countries. Workshop participants also worked in small groups to critique simulated applications for testing transgenic crops and the decisions of the various groups were reviewed and discussed in plenary sessions to examine the rational that led groups to make different decisions. Thus the whole emphasis of the workshop was on the "practitioner approach" which complements the significant effort undertaken by many international organizations to develop codes of conduct for the development, testing and release of transgenic products.

The workshop has undoubtedly contributed to the sharing of experiences from the North with the South, and facilitated harmonization of regulations on a global basis, without in any way suggesting a compliance requirement on Third World countries which must always preserve their sovereign right to promulgate legislation which meets their own needs and requirements. The challenge for the developing countries is to promulgate and enact appropriate legislation with much less resources than are available to industrial countries.

It is a pleasure to acknowledge the support of the Inter-American Institute for Cooperation in Agriculture (IICA) in Costa Rica, where the workshop was held, and the sponsorship of the Rockefeller Foundation and the Stockholm Environment Institute (SEI). ISAAA has worked closely with SEI in the establishment of the Biotechnology Advisory Commission (BAC) which is now in a position to provide, on request, impartial guidance to developing countries on the testing, and release of transgenic products.

Clive James
Chair, International Service for the Acquisition of Agri-biotech Applications (ISAAA)
Cayman Islands, July 1994.

Editors' Introduction

From Biosafety to Sustainable Agriculture

We are clearly at a time when we need concrete achievements in a world with a burgeoning population and increasing environmental problems. It is axiomatic that we must be concerned with concrete action to mitigate the dilemma inherent in the need for increased food, feed and fiber, and the need for safeguarding the environment.

Biotechnology, it is hoped, will contribute towards agricultural sustainability. Of course, real impact in the future will have to come not only through genetic advances—brought about by conventional breeding, by biotechnology and by biodiversity—that reduce the need for external inputs and that increase production, but also through a better management of resources and production systems. It is in this context that we have a role to play in the safe and effective application of biotechnology for the benefit of agriculture and the environment at large. This is the ultimate objective of this book: to outline how to build upon existing experience in order to assist the countries of Africa, Asia, the Americas, and Eastern Europe to further develop biosafety regulatory mechanisms appropriate to their own needs, circumstances, objectives and priorities in agriculture, biotechnology and the environment.

Modern agri-biotechnology applications have emerged only in the last ten years. The topic has made its way into discussions not only in science and technology, in ethics and sociology, but also in other domains such as industrial development, environmental management and international relations. Issues have been analyzed from different perspectives: the need

for a balance between control and promotion of biotechnology, and differences between and similarities with past technologies. This increased interest in biotechnology often centers around its great promises in fighting disease, improving food quantity and quality and dealing with environmental problems. It is precisely these high expectations, however, that also create fears about the potential perils of the new biotechnologies.

Indeed, the *newness* of the technology is an essential element that influences the relationship between science, technology and policy. The production of transgenic material is new to humankind and familiarity first needs to be established. Without doubt further developments will require further discussion and open new areas for debate. It is through dialogue, particularly between the public, the scientific community, and policy makers, that a better understanding, more objectivity and a closer *rapprochement* may be achieved as the process of biotechnological development continues. Policies in the long term must not only reflect technological and scientific realities, but also people's concerns and aspirations, and, last but not least, international realities. It is hoped that this book makes a constructive contribution to this dialogue.

Many countries at present have not sufficiently developed their capabilities for taking advantage of the new opportunities in biotechnology applications, nor for building the regulatory framework that is essential for its safe application. A basic preoccupation of many countries and regions is still how to gain access to the new applications of biotechnology. Unfortunately, this will not be a simple task since such access needs to be built upon appropriate biosafety regulatory mechanisms. Paradoxically, regulations cannot be developed in a vacuum without the technology that brings about experience and creates stewardship, commitment and motivation through ownership.

A further complication is that in the past, agricultural technologies were largely in the public domain, whereas today, fewer and fewer technologies that developing countries may want to transfer, adapt and adopt are public, and this is likely to decline even further in the future. As a result, another new dimension to biotechnology transfer and biosafety has emerged, namely international relations. This latter aspect has partly been brought about by the entering into force of the Convention on Biological Diversity.

Paradoxically, the attention to biosafety within the Convention on Biological Diversity is a reflection of existing concerns rather than the emergence of a new issue. Although there are two Articles in the Convention that deal specifically with biosafety (8[g] and 19.3), biosafety really enters into the process of the Convention as a result of the agreements stipulated in Article 16 for facilitating access to the methods

and products of biotechnology (technology transfer). Thus the connection of biosafety with biodiversity is two-fold. First, for appropriate biotechnology applications to be transferred in a safe and effective way presupposes—and should presuppose—that biosafety regulatory mechanisms are in place. Second, the saving and protection of biodiversity is a complex endeavor that requires, on the one hand, protecting natural habitats (for example from the invasion of alien species), and, on the other hand, alleviating pressure on land extension into natural habitats. It is this latter aspect that is directly related to the sustainability issue and agricultural production and productivity.

The challenge now is to understand the implications of biotechnology transfer for existing and emerging institutional structures; to develop viable options that will speed up the implementation of biosafety regulatory mechanisms around the world; and to design mechanisms within the technology transfer process that meet new scientific, ecological and political requirements.

All the above aspects distinguish this book from others dealing with the issue of biosafety: information and experience are exchanged in order to facilitate decision making on the technology transfer process. The experience shared in this book underscore the importance of adopting flexible mechanisms that allow for the incorporation of new learning experiences as the process evolves. This is an important point because the tendency has been to establish rigid mechanisms that very shortly become obsolete. What is needed are institutional structures that have the flexibility to evolve in a changing world of science, societies and policies.

This book has been produced to assist developing countries in their development of an independent capacity in biosafety regulatory oversight to strengthen the technology transfer process. If biotechnology is to have an impact on future global sustainability, then it has to be available world-wide. Biotechnology is but one principal technology that allows investment in this future: it enables an increase in the understanding of ecosystems, and potentially allows us to interact with the environment in non-damaging ways. The future of biotechnology certainly holds such promise. This is the ultimate dimension that should make us work together to develop effective biosafety regulations and achieve concrete results towards a more sustainable agriculture.

Anatole F. Krattiger
International Academy of the Environment
Geneva, July 1994.

Arno Rosemarin
Stockholm Environment Institute
Stockholm, July 1994.

Acknowledgments

We would first of all like to acknowledge our debt and gratitude to all of the authors who contributed to this book, particularly their collaboration, cooperation, persistence and willingness in meeting our deadlines in the editorial process.

Special thanks go to a host of people without whose help and dedication this book would not have been possible. We would like to thank Brian Johnston for unstinting help in editing and throughout the production process, and for much of the preparation of the index; Heli Pohjolainen for her contributions to the layout and correspondence with the many authors; and Claudia Sapsezian for dedicated and skillful secretarial help.

The Rockefeller Foundation made special contributions and warrants particular acknowledgment. Firstly, we are very appreciative of the Rockefeller Foundation for funding the production of this book together with support from the International Academy of the Environment in Geneva. Secondly, the Rockefeller Foundation is thanked for sponsoring the first ISAAA Biosafety Workshop in Costa Rica in February 1992 from which the papers in this book were developed. The workshop was organized in conjunction with the Rockefeller Foundation's support for a collaborative project brokered by the International Service for the Acquisition of Agri-biotech Applications (ISAAA) between Mexico (CINVESTAV) and Monsanto.

We gratefully acknowledge the organizers of the meeting for their vision and inspiration in contributing to furthering expertise and the development of appropriate biosafety regulations. The ISAAA Biosafety

Workshop took place at the Inter-American Institute for Collaboration in Agriculture (IICA) in San José. The workshop facilities, translation services and secretarial support during the workshop were provided by IICA and we would like to thank the Institute and its staff for their assistance. The contribution by Ciba-Geigy AG, Switzerland, towards various costs, including to this book, is also gratefully acknowledged. In addition, in-kind support was donated by many participants from industrial countries through travel funds provided by their agency or company; these generous contributions, as well as the time commitment for preparing presentations and helping to moderate sessions, enabled the ISAAA Biosafety Workshop to take place.

We are particularly indebted to the International Academy of the Environment, ISAAA and SEI for offering institutional support that made this book possible. Particular thanks go to Clive James (ISAAA), Bill Lesser (Academy and ISAAA), Mike Chadwick (Stockholm Environment Institute [SEI]), Bob Frederick (SEI-BAC), and Jean-Pierre Revéret and Bernard Giovannini (Academy) for many constructive suggestions and for their encouragement throughout the undertaking.

Anatole F. Krattiger
International Academy of the Environment
Geneva, July 1994.

Arno Rosemarin
Stockholm Environment Institute
Stockholm, July 1994.

Section 1

From Biotechnology to Biodiversity

Chapter 1.1

The Contributions of Plant Biotechnology to Agriculture in the Coming Decades

Robert T. Fraley
Group Vice President and General Manager
New Products Division, Monsanto - The Agricultural Group
800 North Lindbergh Boulevard
St. Louis, MO 63167, USA.

Introduction

The application of recombinant DNA technology to plant biology and crop production has the potential to exert a tremendous impact on world agriculture in coming years. Central to plant biotechnology is the ability to isolate genes, direct their expression, monitor inheritance and re-introduce them into plants; this represents one of the most significant developments in the entire history of plant breeding and crop improvement. Plant biotechnology complements plant breeding efforts by increasing the diversity of genes and germplasm available for

Fraley, R.T. 1994. The Contributions of Plant Biotechnology to Agriculture in the Coming Decades. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 3-28.

incorporation into crops and by significantly shortening the time required for the production of new cultivars, varieties and hybrids.

From an economic perspective, plant biotechnology offers significant potential for the seed, agrochemical, food processing, specialty chemical and pharmaceutical industries to develop new products and manufacturing processes. Perhaps the most compelling attribute of the application of plant biotechnology to agriculture is its relevance both to helping ensure the availability of environmentally sustainable supplies of safe, nutritious and affordable food for developed countries; and to providing a readily accessible, economically viable technology for addressing primary food production needs in the developing world.

Before discussing potential applications of plant biotechnology, it is important to address two key questions: Why do we need new agricultural technologies, particularly plant biotechnology? Can plant biotechnology really have the impact that many have speculated it will?

The need for new agricultural technologies, in general, is driven by two distinct, and at times contradictory societal requirements—ensuring a safe, nutritious, and affordable food supply for the planet, and at the same time, minimizing the negative environmental impacts of food production itself. It is estimated that world population will double in the next 40 years, to exceed 10 billion. The combination of population increase, the decline in the availability of arable land, and the need for improvements in the quality of dietary intake in many developing countries means that agricultural production will have to be doubled, or even tripled, on a per acre basis to meet this need. Simply put, farmers will have to produce more calories during the next 40 years than they have done in the entire history of agriculture!

At the same time, societal concerns over the environmental impact of certain agricultural practices will increasingly restrict the types of tools that can be used in crop production. How will agricultural systems evolve by the year 2030 to meet these needs? How do we increase the productive efficiency of existing cultivated land without irreversibly damaging the planet? The answer is deceptively straightforward: investment in, and development of, new agricultural technologies is absolutely critical for a sustainable agriculture for the future. Current agricultural technologies such as plant breeding and agrochemical research and development (R&D) will continue to play a major role in assuring a plentiful and safe food supply; environmentally sensitive and economic farm management practices will also play an important role. Advances in all these areas will be required to meet world food production needs. Plant biotechnology is uniquely important in this regard because it is:

- a new tool which can significantly impact crop productivity;
- compatible with sustainable, environmentally sound agricultural practices;
- a non-capital intensive approach that will benefit agriculture in developing countries; and
- a source of value-added genes and traits that will increase farmer productivity and profitability.

Analysis of the current status of plant biotechnology methods and tools addresses the question of whether it's impact on agriculture will be limited by obvious technical barriers, as outlined below.

Analysis of the Status of Plant Biotechnology

Plant Transformation

Remarkable progress has been made in the development and application of gene transfer systems to crops since the first demonstration of transgenic plant production just over ten years ago. Today, over 80 species of crop plants can be genetically manipulated using available *Agrobacterium tumefaciens* or a variety of free DNA delivery transformation systems (Table 1). This list includes nearly all major dicotyledonous crops and a rapidly increasing number of monocotyledonous crops, including wheat, rice and maize. It is highly likely that routine gene transfer systems will exist for nearly all crops within the next two or three years. While technical improvements will lead to further increases in transformation efficiency, extend transformation to elite commercial germplasm and lower transgenic plant production cost, there is no significant barrier, even today, to the application of plant transformation to crop improvement.

Gene Expression

Plant genetic engineers currently have in hand a large battery of regulatory sequences that provide for both constitutive expression as well as highly accurate targeting of gene expression to specific tissues within transgenic plants (Goldberg, 1988; Benfey and Chua, 1989). Moreover, established differential screening methods allow for ready isolation of regulatory sequences that may be required for even more sophisticated expression requirements (Terryn *et al.*, 1993; Shewmaker *et al.*, 1994). The ability to decrease endogenous gene expression in plants represents a remarkably powerful tool, and striking phenotypic alterations have been

Table 1: Species in which Transgenic Plants have been Produced

Alfalfa	Foxglove	Potato
Apple	Grape	Rapeseed
Arabidopsis	*H. albus*	Rice
Asparagus	Horse radish	Rose
B. carinata	Kiwi	Rye
B. rapa	Lettuce	*S. integrifolium*
Brown Sarson	Licorice	Snap Dragon
Cabbage	Lotus	Soybean
Canola	*M. truncatula*	Strawberry
Carnation	Maize	Sugarbeet
Carrot	Morning glory	Sunflower
Cauliflower	Muskmelon	Sweet potato
Celery	Orchard Grass	Tobacco
Chicory	Orchid	Tomato
Chrysanthemum	Papaya	Tulip
Cotton	Pear	*V. officinalis*
Cranberry	Peas	Walnut
Cucumber	Petunia	Wheat
Eggplant	Poplar	White spruce
Flax	Poppy	Yam

observed by selective inactivation of genes using antisense technology (Smith *et al.*, 1990; van der Krol *et al.*, 1988; Sheehy *et al.*, 1988; Hamilton *et al.*, 1990; Oeller *et al.*, 1991). Achieving even higher levels of gene expression in selected plant organs would increase opportunities for more economic specialty chemical or pharmaceutical production in plants, and site-specific insertion could minimize the variability of gene expression among transformants. However, current expression systems appear sufficient for meeting immediate crop improvement needs.

Gene Discovery Methods

Advances in methods for the identification and isolation of new gene coding sequences are of great importance to the engineering of improved

plants. The interspecies-specific use of transposons and T-DNA insertion has permitted the tagging and isolation of novel genes from several plant sources (Feldmann *et al.*, 1989; Haring *et al.*, 1991; Chuck *et al.*, 1993). The availability of high resolution physical maps in tomato and Arabidopsis has already led to mapping of several novel loci and new methods will allow direct testing of the isolated DNA for its ability to complement the mutation of interest at each step of the walking process. Advances in the redesign of coding sequences for plant expression allow for predictable, high-level expression of a variety of nonplant genes in crop plants (Perlak *et al.*, 1991, 1993; Sutton *et al.*, 1992; Adang, 1993). Again, while ongoing research efforts will predictably and dramatically increase the probability and efficiency of gene discovery and isolation, it would appear that even with today's methods most genes can be identified and isolated. Even assuming only modest advances in gene discovery methods and progress on the sequence analysis of the Arabidopsis genome in the next ten years, one could infer that gene discovery will not be a limiting element for very long.

Gene Stability / Germplasm Access

By the end of 1991, nearly five hundred field test experiments evaluating the performance of genetically engineered plants had been carried out in the USA and Europe alone. The overwhelming conclusions from these extensive studies are that newly introduced genes are stable, inherited and are expressed like any other plant gene (MacKenzie and Henry, 1991). This includes a variety of new genes which provide for control of insects, weeds, and plant diseases as well as for quality improvement. Such traits have already been successfully introduced into several important crop species and genetically engineered soybean, cotton, rice, rapeseed, sugarbeet, tomato, alfalfa, potato and maize crops are expected to enter the marketplace between the years 1995 and 2000. Broad germplasm access will likely require extensive backcrossing or micropropagation efforts. These established methods, although cumbersome, appear adequate for ensuring large germplasm access in most annual and perennial crops.

Regulation, Society Input and Acceptance

From this brief analysis, it seems almost certain that plant biology is entering a unique period where both basic research and commercial applications will be limited only by the creativity of the researcher and by funding levels. While there is an obvious need for substantial expansion of our understanding of basic plant biochemistry and physiology in order to

fully exploit scientific advances, there are no significant technical hurdles remaining. We face an unparalleled opportunity to modify and improve crop plants.

The First Products

The initial wave of research in plant biotechnology has been driven by the seed and agrochemical industries and has appropriately concentrated on the engineering of "agronomic traits" that relate directly to the traditional roles of these industries in farming, such as the control of insects, weeds, and plant diseases. Progress in this area has been exceedingly rapid, and genes conferring these new traits have already been successfully introduced into several important crop species. The status of some of these product candidates is discussed below.

Insect Resistance

The production of plants that naturally control insects has obvious, important implications for crop improvement, and for both the seed and agrochemical industries. Progress in developing insect control in transgenic plants has been initially achieved through the expression in plants of the insect control protein genes of *Bacillus thuringiensis* (*B.t.*). *B. t.* is a naturally-occurring soil bacterium that produces an insect control protein which is lethal to selected insect pests (Dulmage, 1981; Aronson *et al.*, 1986; Hofte and Whitely, 1989). Most strains of *B.t.* are toxic to lepidopteran (caterpillar) larvae, although some strains with toxicity to coleopteran (beetle) or dipteran (fly) larvae have also been described. The insect toxicity of *B.t.* resides in a large protein; this protein has no toxicity to beneficial insects, other animals or humans. The mode-of-action of the *B.t.* insect control protein involves disruption of K^+ ion transport across brush border membranes of susceptible insects.

Transgenic tomato, tobacco, cotton and maize plants containing the *B.t.* gene have exhibited tolerance to caterpillar pests in laboratory tests (Fischhoff *et al.*, 1989; Vaeck *et al.*, 1987; Fujimoto *et al.*, 1993; Koziel *et al.*, 1993).

A novel approach for increasing expression of *B.t.* genes in plants, which involves restructuring of the DNA coding sequence without altering the encoded amino acid sequence, has led to substantial enhancement in insect control (Perlak *et al.*, 1991). Cotton plants with a high level of resistance to boll damage by caterpillars have been developed (Perlak *et al.*, 1990), and commercial levels of control have

been achieved with both potato and cotton. Both products are being reviewed by the EPA.

Field tests have confirmed excellent protection (equivalent to weekly insecticide spraying) from bollworm, budworm and pink bollworm. Excellent protection from defoliation by Colorado potato beetle has also been observed in greenhouse and field experiments with potato plants containing the novel coleopteran-active *B. t. tenebrionis* gene (Figure 1; Mosher, 1991; Perlak *et al.*, 1993). The insect resistant plants sustained no damage from Colorado beetles throughout the growing season under conditions of high insect pressure. Other types of insecticidal molecules are clearly necessary to extend biotechnology approaches for controlling additional insect pests in these and other target crops. Extensive efforts are under way to identify other microbial and plant insecticidal proteins. It has been demonstrated that plants genetically engineered to express a proteinase inhibitor gene demonstrate enhanced resistance to a range of insect pests (Boulter *et al.*, 1989); *in vitro* studies indicate the alpha-amylase inhibitor protein has broad-spectrum insecticidal activity (Huesing *et al.*, 1991). It is highly likely that a large percentage of insect control in annual crops such as cotton, maize and vegetables will be provided by introduced genes in the next 10-20 years. These developments will provide new solutions for insect control and allow for significant reduction in insecticide usage. An important focus of seed companies introducing these new crops will be ensuring that appropriate agronomic and farm management practices are utilized to minimize any possibility of insects developing resistance to the plants (Gibbons, 1991; Shelton *et al.*, 1992; Mallet and Porter, 1992; Tabashnik, 1994).

Weed Control

Engineering tolerance to a specific herbicide into a crop plant represents a new alternative for conferring selectivity and enhancing the crop safety of herbicides. While laboratory experiments have shown that it is possible to achieve resistance to nearly a dozen different herbicides, R&D efforts by private companies have concentrated only on those herbicides with minimal environmental impact, with emphasis on properties such as high unit activity, low toxicity and rapid biodegradation (CAST, 1991). Care has also been taken to ensure that herbicide-tolerant genes will not be introduced into crops which could become ”volunteer” weeds in subsequent crop rotations or which outcross readily with weed species.

The development of crop plants which are tolerant to such herbicides would provide for more effective, less costly and more

Figure 1: Performance of Potato Plants (Russet Burbank) Containing the Novel *B. t. tenebrionis* Insect Control Protein Gene (Center Row) compared to Control Plants (Left and Right) in a 1989 Laboratory Test

environmentally attractive weed control options than exist today. The commercial strategy behind engineering herbicide tolerance is to gain market share through a shift in herbicide use, not to increase the overall use of herbicides as is popularly held by critics (Goldburg *et al.*, 1990).

Two general approaches have been pursued in engineering herbicide tolerance:

1) altering the level and sensitivity of the target enzyme for the herbicide; and
2) incorporating a gene encoding an enzyme which can inactivate the herbicide.

As an example of the first approach, Roundup® herbicide acts by specifically inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; Steinrücken and Amrhein, 1980). Roundup® herbicide is active against annual and perennial broad leaf and grassy weeds, it has very low animal toxicity, and it is rapidly inactivated and degraded in all soils.

Tolerance to Roundup® herbicide has been engineered into canola, soybean, cotton and maize by introducing genetic constructions for the overproduction of herbicide resistant EPSPS enzymes (Shah *et al.*, 1986). Similarly, resistance to sulfonylurea compounds, the active ingredients in Glean®, and Oust® herbicides, has been produced by the introduction of mutant acetolactate synthase (ALS) genes into canola and cotton (Haughn *et al.*, 1988; Hinchee *et al.*, 1993). Sulfonylureas are broad spectrum herbicides that are effective at very low application rates. Resistance to gluphosinate, the active ingredient in Basta®, and bromoxynil has been achieved by the alternative approach of introducing bacterial genes encoding enzymes that inactivate the herbicides by acetylation (de Block *et al.*, 1987) or nitrile hydrolysis (Stalker *et al.*, 1988) respectively. In field tests, gluphosinate-tolerant canola, soybean and maize have shown excellent tolerance to the herbicide. Similarly, bromoxynil tolerant cotton has been extensively evaluated and shown to provide excellent control of broadleaf weeds.

The current crop targets for engineered herbicide tolerance include soybean, cotton, maize, rapeseed and sugarbeet. For the farmer, many factors such as weed spectrum, herbicide performance, environmental impact, seed and chemical cost, application timing and flexibility have to be considered when choosing a particular weed control system. The availability of herbicide tolerance in annual crops over the next decade will give farmers more flexibility in choosing effective and less costly options for weed control.

Herbicide-tolerant plants will have the positive impact of shifting overall herbicide usage through substitution of more effective and

environmentally acceptable products. Such improvements in chemical weed control will also allow for higher adoption of minimum tillage practices, and encourage crop rotations which will further reduce soil erosion (CAST, 1991).

Disease Resistance

Significant resistance to a variety of plant viral diseases has been achieved by coat protein-mediated protection, which involves expressing the coat protein gene of a particular virus in transgenic plants (Powell-Abel *et al.*, 1986). The mechanism for coat protein-mediated cross protection is likely to involve interference with the uncoating of virus particles in cells prior to translation and replication. Using this approach, results have been obtained for transgenic tomato, alfalfa, tobacco, potato, melon and rice against a broad spectrum of plant viruses, including alfalfa mosaic virus, cucumber mosaic virus, potato virus X (PVX), potato virus Y (PVY) and potato leaf roll virus (Beachy *et al.*, 1990). Excellent tolerance has been observed in field tests of Russet Burbank potatoes containing coat protein genes to both PVY and PVX (Kaniewski *et al.*, 1990; Kaniewski and Thomas, 1993). Recently, very significant resistance to tobacco mosaic virus in tobacco plants has also been obtained by an alternative method which involves expression of a subgenomic viral replicase component (Golemboski *et al.*, 1990; Hemenway and Braun, 1992).

Rapid progress is also being made in engineering resistance to bacterial and fungal pathogens by several groups around the world. Resistance to the bacterial pathogen *Pseudomonas syringae*, which causes wildfire in tobacco, has been introduced in transgenic tobacco by expressing a tabtoxin resistance gene that codes for an acetyltransferase (Anzai *et al.*, 1989). This result demonstrates a successful approach to engineering disease resistance in plants by detoxification of pathogenic toxins (Carmona *et al.*, 1993; During *et al.*, 1993). Some success in engineering resistance to fungal diseases has also been reported. A chitinase gene from the soil bacterium *Serratia marcescens* was stably expressed in transgenic tobacco (Jones *et al.*, 1988). The preliminary results indicated that the expression of the bacterial chitinase in transgenic tobacco leaves resulted in significantly reduced severity of disease caused by a brown-spot pathogen, *Alternaria longipes*. The plants were reported to have significantly reduced fungal lesions as well as delayed susceptibility to the pathogen. A bean chitinase gene driven by a high level, constitutive promoter has been expressed in tobacco plants (Broglie *et al.*, 1991). These plants exhibit increased resistance to the pathogenic fungus *Rhizoctonia solani,* resulting in significantly reduced

root damage and enhanced ability to survive in infested soil. Genes conferring fungal resistance based on the plant's own defense response are being cloned by a number of research groups; one of these proteins, termed osmotin, has been shown to have potent *in vitro* activity against *Phytophthora infestans*, the causal agent of late blight disease in potato (Woloshuk *et al.*, 1991). Manipulating existing defense mechanisms may also prove to be useful (Ryals *et al.*, 1994; Alexander *et al.*, 1993; Hain *et al.*, 1993).

Stress Resistance

A variety of abiotic stresses including water, temperature and soil composition are known to impact crop productivity. Although the complexity of plant stress responses has eluded early demonstration of improved phenotypes using plant biotechnology methods, these tools are being applied to dissect and understand the molecular basis for plant response. A number of plant genes induced by exposure to heat, cold, salt, heavy metals, phytohormones, nitrogen etc., have been identified (see Goldberg, 1988; Benfey and Chua, 1989). Additionally, rapid progress is being made in identifying ion transport pumps and proteins which regulate transport of molecules through channels and plasmodesmata. Metabolites such as proline and betaines have been implicated in stress tolerance in both bacteria and plants—experiments are in progress in a number of laboratories to evaluate the potential of these metabolites to alleviate stress in engineered plants and understand their mode of action (see McCue and Hanson, 1990; Vernon *et al.*, 1993; Tarczynski *et al.*, 1993; Van Camp *et al.*, 1994). As these advances accelerate, it is highly likely that there will be demonstrations of heat, cold, drought and salt tolerance in the near future.

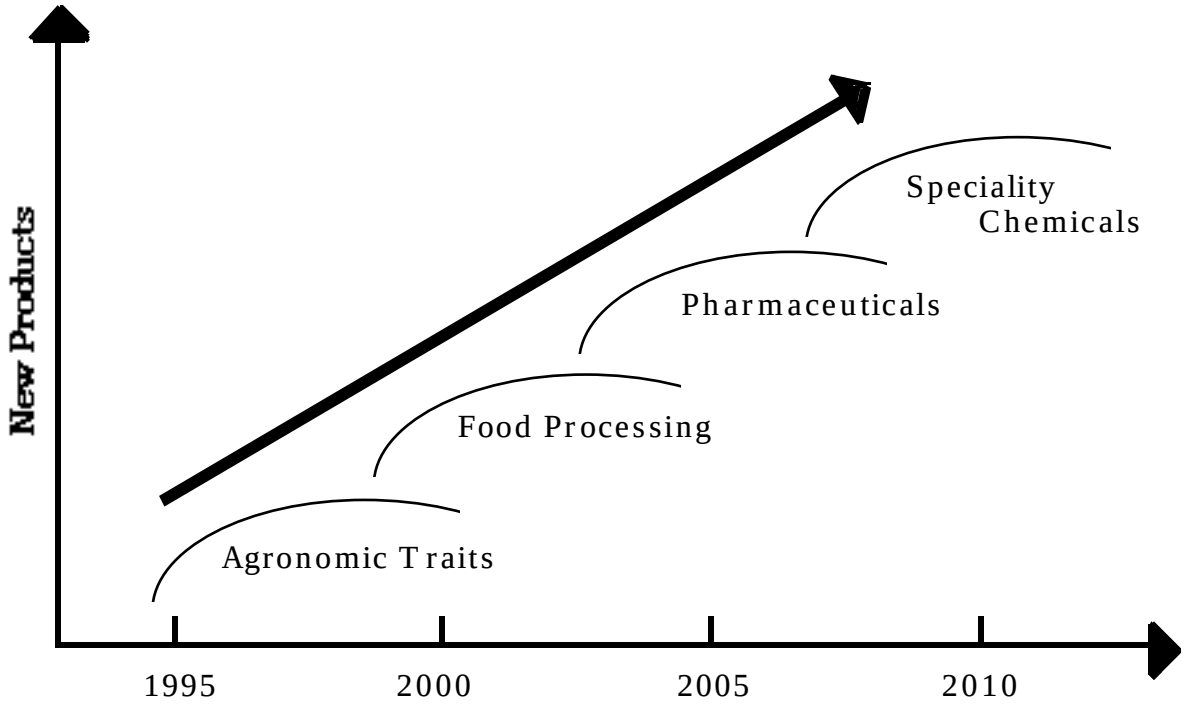
The Next Wave of Products

The world-wide seed and agrochemical industries have been the leading commercial sponsors of agricultural biotechnology research. However, the technical advances and the success of the first products has increased interest by the food processing, specialty chemical and pharmaceutical industries (Figure 2).

Food Processing

Plant biotechnology offers exciting opportunities for the food processing industry to develop new and more nutritious food products and cost

Figure 2: Plant Biotechnology Promises to Deliver Many New Products in Coming Decades



effective processes. Examples of such applications include production of higher quantities of sugars, starch or specialized starches with various degrees of branching and chain length to improve texture, storage and cooking properties. Starch levels have been increased by 20-40% in potato by expression in tubers of a bacterial ADP glucose pyrophospho-rylase gene (ADPGPPase), which has been shown to be the rate-limiting step in starch production in plants (Stark *et al.*, 1991). Expression of the gene encoding sucrose phosphate synthase in transgenic tomato plants has been shown to elevate sucrose levels and reduce starch (Worrell *et al.*, 1991). The opportunity to specifically create specialty oils or to eliminate particular fatty acids in seed crops is quickly becoming a reality (Ohlrogge, 1994; Cahoon and Ohlrogge, 1994).

The identification and cloning of several key enzymes in fatty acid biosynthesis has allowed for antisense inhibition of key steps in the pathway; by expressing antisense constructs to stearyl ACP desaturase in canola, significant increases in stearic acid levels have been observed (Kridl *et al.*, 1991; Dorman *et al.*, 1994; Ohlrogge, 1994). The production

of proteins with nutritionally balanced amino acid compositions has been shown to result in significant changes in seed amino acid composition (Altenbach and Simpson, 1990). In tomato, it has been possible to increase bruise resistance by expression of antisense RNA to polygalacturonase and to delay fruit ripening by expression of antisense RNA to enzymes involved in ethylene production (Sheehy *et al.*, 1988; Hamilton *et al.*, 1990; Oeller *et al.*, 1991). Recently, the expression of ACC deaminase, an enzyme that degrades the immediate precursor to ethylene, has also been shown to confer controlled ripening (Klee *et al.*, 1991). The enzymes and genes involved in biosynthesis of coloring materials and flavors are also important to the food industry and to the consumer; it has been possible to manipulate color of flowers by sense and antisense expression of the flavonoid biosynthetic genes (van der Krol *et al.*, 1988).

Specialty Chemicals

Enormous opportunity lies in the successful exploitation of crops for both commodity and specialty chemical products. Plants have traditionally been a source of a wide range of monomeric and polymeric materials. These range from sugars and fatty acids to polymers such as starch and celluloses, which are carbohydrate based, and to polyhydrocarbons such as rubber and waxes. Many of these polymers have been replaced in the last two to three decades by synthetic materials derived from petroleum-based products. However, the cost, supply and waste-stream problems often associated with petroleum-based products are issues which have focused new attention on the use of renewable, biological materials. Genetic engineering will significantly enlarge the spectrum and composition of available plant monomers and polymers. Expression of an *Escherichia coli* mannitol dehydrogenase gene in tobacco has allowed for increased levels of mannitol in plants (Tarczynski *et al.*, 1991). Cyclodextrins are interesting specialty starches which have application in catalysis, formulations, and food processing; introduction of a bacterial cyclodextrin glucosyl transferase has been reported to result in low but detectable levels of cyclodextrins in potato tubers (Oakes *et al.*, 1991). Recently, researchers have reported the expression of acetoacetyl CoA synthetase and the acetoacetyl CoA reductase in transgenic plants—these two enzymes constitute the two steps leading to the production of poly-hydroxybutyrate, an interesting thermoplastic polymer (Poirier *et al.*, 1992). It has been proposed that expression of a novel fatty esterase could result in production of C-12 fatty acids in temperate crops like soybean and canola (Maelor *et al.*, 1991; Voelker *et al.*, 1992).

Pharmaceuticals

Plants also offer the potential for production of foreign proteins with a variety of health-care applications. Proteins such as neuropeptides, blood factors and growth hormones could be produced in plant seeds and this may ultimately prove to be an attractive economical means of production. Several mammalian proteins have already been produced in genetically engineered plants, including pharmaceutically-active peptides, enkephalins, in rapeseed (Vandekerckhove *et al.*, 1989) and human serum albumin in potato (Sijmons *et al.*, 1990). Plant virus vectors containing desired genes may represent a particularly interesting route for producing large quantities of proteins in plants, due to their high copy number. In the longer term, there is little doubt that biotechnology will be used to improve the nature of both the micro- and macro-ingredients in plant-derived foods. The relationship between diet and disease is slowly emerging, and the consumer demand for more healthy foods is expected to grow.

Plant Biotechnology in Developing Countries

From the above examples, it is clear that plant biotechnology will have an enormous impact on the seed, agrochemical, food processing, specialty chemical and pharmaceutical industries of the industrialized world. However, the majority of the five billion additional people that will live on our planet in 2030 will be in the less developed or developing countries. Can biotechnology really help? Most experts agree that biotechnology will, and in fact must, have a positive impact on agriculture in the developing world (World Bank, 1991). It will not be a “quick fix” to the problems of food production and world hunger, and it will not substitute for conventional agricultural applications, economic and political reform, education, solutions to rural landlessness, international debt relief or population control, among others. But biotechnology can make big contributions in helping to ensure a sustainable supply of adequate food. One of the key reasons plant biotechnology will have this impact is because of its inherently low capital cost for delivery and implementation. Biotechnology enables delivery of the latest technology to a rural farmer in the package he is most familiar with—the seed. In fact, “plant biotechnology” has been identified by the World Bank, the United Nations Educational, Scientific and Cultural Organization (UNESCO), the French Institute for Scientific Research for Development and Cooperation (ORSTOM), the United States Agency for International

Development (USAID), the Rockefeller Foundation, the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) and numerous other international aid and scientific groups as a high priority in order to meet the future agricultural production needs of developing countries. Because of the unique challenges associated with regional population density, food distribution, and dietary preference, agricultural biotechnology must be developed locally in developing countries. Some of the issues which currently must be addressed to facilitate biotechnology in developing countries are:

- identification of near-term crop/trait targets;
- mobilization of resources and funding;
- establishment of technology transfer mechanisms with private/public organizations;
- development of a regulatory oversight framework; and
- addressing of property rights and trade issues.

Success will clearly require a coordinated effort between international agencies and governments as well as private companies and public institutions. Several private companies have taken a leadership role in technology transfer to the developing world by establishing relationships which bring these important constituent groups together. Monsanto Company, for example, has projects with Mexico and Africa, which are sponsored by the Rockefeller Foundation and USAID respectively, to develop virus resistant potatoes and other root crops. The benefits of such interactions are both short and long term. Obvious and most important, in the short term, is the opportunity to help alleviate suffering and poverty; in the long term, it is hoped these countries will become important trading partners.

Barriers to Commercialization

Despite the phenomenal scientific progress that has underscored the development of the first generation of plant biotechnology products, significant and serious challenges remain which could delay or even prevent their successful introduction and acceptance in the marketplace.

Regulatory Approval

Field testing and commercial introduction of genetically engineered products come under the statutory jurisdiction of three federal agencies

in the USA: the United States Department of Agriculture (USDA), the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA). In Europe, regulation is based on individual country guidelines, but efforts are progressing to develop a policy on field release to cover the entire European Union (formerly the European Community). The field testing of genetically engineered crops has proceeded remarkably smoothly; since 1987 there have been thousands of tests of engineered crops in diverse locations across the USA and Europe. These field tests, along with thousands of laboratory experiments, have produced a remarkable degree of consensus internationally that engineered crops present virtually no risk to the environment or to human or animal health (MacKenzie and Henry, 1991).

The regulatory emphasis has now shifted from laboratory and field testing to commercialization approval. In the USA, progress has been made to develop a coordinated framework between USDA, FDA and EPA for oversight of the commercialization of engineered crops and food products. Timing is particularly critical since the defining of regulatory approval requirements sets both projected regulatory costs and commercialization timelines for new products; this is a significant issue for the universities, institutes and companies currently in the process of developing improved genetically engineered crops for introduction in the mid-1990s.

It is essential that regulatory requirements for transgenic plants continue to be based on scientific principles that protect the safety of consumers and the food supply (Fuchs *et al.*, 1993), and recognize the inherent low risk of gene transfer technology and the benefits afforded by genetically engineered crops to growers, food processors and consumers. International harmonization of regulatory approval standards is necessary to prevent development of non-tariff trade barriers between countries that could ban shipments of genetically engineered seeds or food products without sound scientific basis (PCC, 1991). Other imposed political barriers could include labeling and inspection “requirements” that would effectively limit access and distribution of these products.

Proprietary Protection

Patent protection for genetically-engineered plants is essential to offset the cost of developing crops with significant new traits, and to encourage more overall investment and research in this field. Patent protection provides a broader proprietary right than is currently provided under either the International Convention for the Protection of New Varieties of Plants (UPOV) or the Plant Variety Protection Acts (PVPA) in the USA.

While few dispute that companies that have invested heavily in R&D to isolate, test and commercialize genes are entitled to protection for their inventions, there is considerable debate within the seed industry concerning how much protection is deserved and how patents will impact the cooperative nature of the seed industry itself (Johnson, 1987). Much of this concern results from confusion surrounding the restrictions imposed by patent rights *versus* the incentive they provide for competitive research and product development which stimulates innovation. Many of the conciliatory proposals, including patenting of genes (but not plants) as well as compulsory licensing in the event that plant patenting is permitted, if implemented, could significantly reduce the incentive for private industry funding. Lack of proprietary protection for genetically engineered plants in many countries outside the USA remains a very serious limitation; plant and animal varieties are largely excluded from patent protection by the European countries that signed the 1973 European Patent Convention.

The availability, scope and enforceability of plant patents in other countries, including Japan, China and Eastern European countries, remains questionable; perhaps more than any other issue, proprietary rights protection may limit plant biotechnology R&D and commercialization in these regions.

Public Perception

Questions have been raised, discussions have begun and opinions are forming on the subject of biotechnology, which is no longer the exclusive domain of scientists. Several groups have, by their past actions and expressed interest in the subject, established themselves as participants in the formation of what ultimately will become the public perception of biotechnology. Representatives of industry and organized environmental groups are easily identified. Both messengers have a distinctive voice, representing specific points of view. However, one group is not easily seen, yet their voice is being heard. Members of the *status quo* generally oppose any change.

This type of opposition must not be confused with the questions being raised by other groups. It is incumbent on everyone to identify and recognize the specific point of view that is being presented for public review. That is a difficult task for many members of the general public who do not understand science.

As a group, scientists have high credibility with the general public. Respecting and safe-guarding this trust, scientists must continue to speak out so that the public will gain familiarity with the benefits of

biotechnology products such as those used in human health: growth hormone, for the treatment of congenital dwarfism and miraculous recovery of burn victims; tissue plasminogen activator (TPA), with it's life saving role for heart attack victims; erythropoietin (EPO), which substantially reduces the need for blood dialysis for kidney patients; diagnostic kits for everything from Strep throat to identifying criminals. Open dialogue with politicians, consumer advocacy groups, religious organizations and consumers will have the effect of filling the information vacuum. The knowledge of the benefits of biotechnology will play a key role in determining how the public comes to grip with this science.

Anti-Science Advocates

In today's era of global communications, radical groups opposed to technological creativity have demonstrated an increasing ability to influence public and political opinion. As opposed to the more mainstream public-interest groups with whom dialogue is possible, these groups exploit the public's lack of understanding of biotechnology and interweave political, societal and emotional issues (population control, animal rights, religion, concerns over the environment, etc.) for purposes of gaining visibility, credibility, membership, financial support and political presence. These critics represent various positions across a spectrum of thought; in most cases, they rarely attack science and technology directly, but instead focus on peripheral, but more vulnerable issues, such as food safety, proprietary rights, religious views, etc. (Caulder, 1988; Fraley, 1990). Popular strategies involve reducing the commercial incentive for R&D through actions which delay product introduction and increase commercialization costs. For example, proposals for mandatory licensing of patented plants are often supported because this would reduce the incentive for companies to invest in the development of proprietary new crops. By attempting to block critical and needed components of plant biotechnology such as the use of marker genes in plants through political, nonscience-based regulation, some of these groups hope to effectively delay commercialization or create opportunities for eventual trade sanctions. Similarly, by falsely raising concerns over food safety, they hope to increase regulatory testing costs to prohibitive levels. Others are demanding labeling of foods to discourage consumer purchase and also to facilitate subsequent boycotting activities against particular brands or companies who introduce, distribute or sell these products. These groups represent a serious threat; they are just as motivated, and often as well-funded, as the companies and organizations who are trying to commercialize plant

biotechnology products. Their misrepresentations of issues and concerns are dangerously effective at further confusing a skeptical public and undermining the science-based regulation that is in place to protect the food system.

Conclusion

It is clear that technical advances in plant biotechnology are now translating into commercial products that will provide significant advantages and benefits to growers, processors and consumers in the next few years. The inevitable growth in world population and demand for food, and the clear sensitivity expressed by consumers for environmentally sustainable agricultural production methods, are key factors that underline the important role these new products will have in assuring a safe and affordable food supply for the future. Plant biotechnology can and must be encouraged to play an important role in addressing the food production needs of developing countries.

Plant biotechnology is moving forward everywhere and will soon reach the marketplace. How soon, how effectively, and with what degree of public consensus, will depend on the constructive participation of all those who stand to benefit from the technology. The first plant biotechnology products, tomatoes with improved taste and shelf life and cotton plants which control insect pests without chemical sprays, will be the focus of intense scrutiny and interest; their successful introduction will pave the way for many other, as yet unimagined, improvements in crop plants in the decades to follow.

Acknowledgments

I would like to thank Ganesh Kishore, Jim Altemus, Dilip Shah, Nilgun Tumer, Fred Perlak, Dennis Hoerner, Frank Serdy, John Hebblethwaite and David Fischhoff for their special help in the preparation of this paper.

References

Adang, M. J., M. S. Brody, G. Cardineau, N. Eagan, R. T. Roush, C. K. Shewmaker, A. Jones, J. V. Oakes and K. E. McBride. 1993. The re-construction and expression of a *Bacillus thuringiensis* cryIIIA gene in protoplasts and potato plants. *Plant Molecular Biology* 21:1131-1145.

Alexander, D., R. M. Goodman, M. Gut-Rella, C. Glascock, K. Weymann, L. Friedrich, D. Maddox, P. Ahl-Goy, T. Luntz, E. Ward and J. Ryals. 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. *Proceedings of the National Academy of Sciences* (USA) 90:7327-7331.

Altenbach, S. and R. Simpson. 1990. Manipulation of methione-rich protein genes in plant seeds. *Trends in Biotechnology* 8:156-160.

Anzai, H., K. Yoneyama and I. Yamaguchi. 1989. Transgenic tobacco resistant to a bacterial disease by detoxification of a pathogenic toxin. *Molecular and General Genetics* 219:492-494.

Aronson A. I., W. Beckman and P. Dunn. 1986. *Bacillus thuringiensis* and related insect pathogens. *Microbiological Review* 50:1-24.

Beachy, R. N., S. Loesch-Fries and N. E. Tumer. 1990. Coat protein mediated resistance against virus infection. *Annual Review of Phytopathology* 28:451-474.

Benfey, P. and N.-H. Chua. 1989. Regulated genes in transgenic plants. *Science* 244:174-181.

Boulter, D., A. Gatehouse and V. Hilder. 1989. Use of cowpea trypsin inhibitor (CpTi) to protect plants against insect predation. *Biotechnology Advance* 7:489-497

Broglie, K., L. Chet, M. Holliday, R. Cressman, P. Biddle, S. Mureatta, J. Mauvis and R. Broglie. 1991. Transgenic tobacco plants with induced resistance to the fungal pathogen *Rhizoctonia solani. Science* 254: 1194-1197.

Cahoon, E. B. and J. B. Ohlrogge. 1994. Metabolic evidence for the involvement of a D4-palmitoyl-acyl carrier protein desaturase in petroselinic acid synthesis in coriander endosperm and transgenic tobacco cells. *Plant Physiology* 104:827-837.

Carmona, M. J., A. Molina, J. A. Fernandez, J. J. Lopez-Fando and F. Garcia-Olmedo. 1993. Expression of the alpha-thionin gene from barley in tobacco confers enhanced resistance to bacterial pathogens. *The Plant Journal* 3:457- 462.

CAST. 1991. Herbicide-Resistant Crops. ISSN 0194-4096, No. 1991-1. Council for Agricultural Science and Technology (CAST): Ames.

Caulder, J. 1988. Biotechnology's first turning point—and its next. *Bio/Technology* 6:984.

Chuck, G., T. Robbins, C. Nijjar, E. Ralston, N. Courtney-Gutterson and H. K. Dooner. 1993. Tagging and cloning of a petunia flower color gene with the maize transposable element activator. *The Plant Cell* 5:371-378.

de Block, M., J. Botterman, M. Vanderweile, J. Dockx and C. Thoen. 1987. Engineering herbicide resistance in plants by expression of a

detoxifying enzyme. *EMBO Journal* 6:2513-2518.

Dorman, P., M. Frentzen and J. B. Ohlrogge. 1994. Specificities of the acyl-acyl carrier protein (ACP) thioesterase and glycerol-3-phosphate acyltransferase for octadecenoyl-ACP isomers. *Plant Physiology* 104:839-844.

Dulmage, H. T. 1981. Insecticidal activity of isolates of *Bacillus thuringiensis* and their potential for pest control. In *Microbial Control of Pests*. Burgess, H. D. (ed.). Academic Press, New York.

During, K., P. Porsch, M. Fladung and H. Lörz. 1993. Transgenic potato plants resistant to the phytopathogenic bacterium *Erwinia carotovora. The Plant Journal* 3:587-598.

Feldmann, K. A., M. D. Marks, M. L. Christianson and R. S. Quatrano. 1989. A dwarf mutant of Arabidopsis generated by T-DNA insertion mutagenesis. *Science* 243:1351-1354.

Fischhoff, D., K. Bowdish, F. Perlak, P. Marrone, S. McCormick, J. Niedermeyer, D. Dean, K. Kusano-Kretzmer, E. Mayer, D. Rochester, S. Rogers and R. Fraley. 1987. Insect tolerant trans-genic tomato plants. *Bio/Technology* 5:807-813.

Fraley, R. 1990. Regulatory and public perception issues related to agricultural biotechnology. *AgBiotech News and Information* 2:631-633.

Fuchs, R. L., S. A. Berberich and F. S. Serdy. 1993. Safety evaluation of genetically engineered plants and plant products: insect-resistant cotton. In *Biotechnology and Safety Assessment.* Thomas, J. A. (ed.). Raven Press, New York.

Fujimoto, H., K. Itoh, M. Yamamoto, J. Kyozuka and K. Shimamoto. 1993. Insect resistant rice generated by introduction of a modified d-endotoxin gene of *Bacillus thuringiensis*. *Bio/Technology* 11:1151-1155.

Gasser. C. and R. Fraley. 1989. Genetically engineering plants for crop improvement. *Science* 244:1293-1299.

Gibbons, A. 1991. Moths take the field against biopesticide. *Science* 254:646.

Goldberg, R. B. 1988. Plants: Novel developmental processes. *Science* 240:1460-1467.

Goldberg, R., J. Rissler, H. Shand and C. Hassebrook. 1990. *Biotechnology's Bitter Harvest.* Biotechnology Working Group, Washington DC.

Golemboski, D., G. Lomonossoff and M. Zaitlin. 1990. Plants transformed with a Tobacco Mosaic Virus nonstructural gene sequence are resistant to the virus. *Proceedings of the National Academy of Sciences* (USA) 87: 6311- 6315.

Hain, R., H.-J. Reif, E. Krause, R. Langebartels, H. Kindl, B. Vornam, W. Wiese, E. Schelzer, P. H. Schreier, R. H. Stocker and K. Stenzel. 1993. Disease resistance results from foreign phytolexin expression in a novel plant. *Nature* 361:153-156.

Hamilton, A. J., G. W. Lycett and D. Grierson. 1990. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. *Nature* 346:284-287.

Haring, M., C. Rommens, H. Nijkamp and J. Hille. 1991. The use of transgenic plants to understand transposition mechanisms and to develop transposon tagging strategies. *Plant Molecular Biology* 16:449-461.

Haughn, G. W., J. Smith, B. Mazur and C. Somerville. 1988. Transformation with a mutant Arabidopsis acetolactate synthase genes renders tobacco resistant to sulfonylurea herbicides. *Molecular and General Genetics* 211:266-271.

Hemenway, C. L., C. J. Braun. 1992. Expression of amino terminal portion or full length viral replicase genes in transgenic plants confers resistance to potato virus X infection. *Plant Cell* 4:735-744.

Hinchee, M. A. W., S. R. Padgette, G. M. Kishore, X. Delannay and R. T. Fraley. 1993. Transgenic Plants. In *Herbicide-Tolerant Crops*. pp. 243-263.

Hofte, H. and H. Whitely. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Review* 53:242-255.

Huesing, J. E., R. E. Shade, J. M. Chrispeels and L. L. Murdock. 1991. Alpha-Amylase, not phytohemagglutinin, explains resistance of common bean seeds to cowpea weevil. *Plant Physiology* 96:993-996.

Johnson, J. 1987. Genetic patents spark global industry debate. *Seedsmen's Digest* 38:6-8.

Jones, J., C. Dean, D. Gidoni, D. Gilbert, D. Bond-Nutter, R. Lee, J. Bedbrook and P. Dunsmuir. 1988. Expression of bacterial chitinase protein in tobacco leaves using two photosynthetic gene promoters. *Molecular and General Genetics* 212:536-542.

Kaniewski, W. and P. E. Thomas. 1993. Field testing of virus resistant transgenic plants. *Semin Virology* 4:389-396.

Kaniewski, W., C. Lawson, B. Sammons, L. Haley, J. Hart, X. Delannay and N. Tumer. 1990. Field resistance of transgenic Russet Burbank potato to effects of infection by potato virus X and potato virus Y. *Bio/Technology* 8:750-754.

Klee, H., M. Hayford, K. Kretzmer, G. Barry and G. Kishore. 1991. Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. *Plant Cell* 3:1187-1193.

Koziel, M. G., G. L. Beland, C. Bowman, N. B. Carozzi, R. Crenshaw, L.

Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Madox, K. McPherson, M. R. Meghji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright and S. V. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Bio/Technology* 11:194-200.

Kridl, J., A. Knutzon, W. Johnson, G. Thompson, S. Radke, J. Turner and V. Knauf. 1991. Modulation of stearoyl-ACP desaturase levels in transgenic rapeseed. Abstract 723 presented at the *Third International Congress of Plant Molecular Biology*, Tucson.

MacKenzie, D. R. and S. Henry (eds.). 1991. International Symposium: The Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms. Agricultural Research Institute, Bethesda.

Maelor D. H., L. Anderson, C. Fan and D. Hawkins. 1991. Developmental induction, purification and further characterization of 12:0 ACP thioesterase from immature cotyledons of Umbellularia California. *Archives of Biochemistry and Biophysics* 290:37-45.

Mallet, J. and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? *The Royal Society* 250:165-169.

McCue, K. and A. Hanson. 1990. Drought and salt tolerance: towards understanding and application. *Trends in Biotechnology* 8:358-362.

Mosher, P. 1991. Super plants: *B.t.*-based plants stand up to the troublesome Colorado potato beetle. *The Grower* 11:14-15.

Oakes, J., C. Shewmaker and D. Stalker. 1991. Production of cyclodextrins, a novel carbohydrate in the tubers of transgenic potato plants. *Bio/Technology* 9:982-986.

Oeller, P., L. Min-Wong, L. Taylor, D. Pike and A. Theologis. 1991. Reversible inhibition of tomato fruit senescence by antisense RNA. *Science* 254:437-439.

Ohlrogge, J. B. 1994. Design of new plant products: engineering of fatty acid metabolism. *Plant Physiology* 104:821-826.

PCC. 1991. *Report on National Biotechnology Policy*. President's Council on Competitiveness (PCC), Washington DC.

Perlak, F. J., R. L. Fuchs, D. A. Dean, S. L. McPherson and D. A. Fischhoff. 1991. Modification of the coding sequence enhances plant expression of insect control protein genes. *Proceedings of the National Academy of Sciences* (USA) 88:3324-3328.

Perlak, F. J., T. B. Stone, Y. M. Muskopf, L. J. Petersen, G. B. Parker, S. A. McPherson, J. Wyman, S. Love, G. Reed, D. Biever and D. A. Fischhoff. 1993. Genetically improved potatoes: protection from damage by Colorado potato beetles. *Plant Molecular Biology* 22:313-321.

Perlak, F., R. Deaton, T. Armstrong, R. Fuchs, S. Sims, J. Greenplate and

D. Fischhoff. 1990. Insect resistant cotton plants. *Bio/Technology* 8:939-943.

Poirier, Y., D. E. Dennis, K. Klomparens, C. Somerville. 1992. Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants. *Science* 256:520-523.

Powell-Abel, P., R. S. Nelson, B. De, N. Hoffmann, S. G. Rogers, R. T. Fraley and R. N. Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* 232:738-743.

Ryals, J., S. Uknes and E. Ward. 1994. Systemic Acquired Resistance. *Plant Physiol*ogy 104:1109-1112.

Shah, D., R. Horsch, H. Klee, G. Kishore, J. Winter, N. Tumer, C. Hironaka, P. Sanders, C. Gasser, S. Aykent, N. Siegel, S. Rogers and R. Fraley. 1986. Engineering herbicide tolerance in transgenic plants. *Science* 233:478-481.

Sheehy, R. E., M. Kramer and W. R. Hiatt. 1988. Reduction of polygalacturonase in tomato fruit by antisense RNA. *Proceedings of the National Academy of Sciences* (USA) 85:8805-8809.

Shelton, A. M., J. L. Robertson, J. D. Tang, C. Perez, S. D. Eigenbrode, H. K. Preisler, W. T. Wilsen and R. J. Cooley. 1992. Resistance of diamondback moth (*Lepidoptera: Plutellidae*) to *Bacillus thuringiensis* subspecies in the field. *Insecticide Resistance and Resistance Management* 86:697-705.

Shewmaker, C. K., C. D. Boyer, D. P. Wiesenborn, D. B. Thompson, M. R. Boersig, J. V. Oakes and D. M. Stalker. 1994. Expression of *Escherichia coli* glycogen synthase in the tubers of transgenic potatoes (*Solanum tuberosum*) results in a highly branched starch. *Plant Physiology* 104:1159-1166.

Sijmons, P., B. Dekker, B. Schrammeijer, T. Verwerd, P. van den Elzen and A. Hoekema. 1990. Production of correctly processed human serum albumin in transgenic plants. *Bio/Technology* 8:217- 221.

Smith, C. J. S., C. F. Watson, P. C. Morris, C. R. Bird, G. B. Seymour, J. E Gray, C. Arnold, G. S. Tucker, W. Schuch and D. Grierson. 1990. Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. *Plant Molecular Biology* 14:369-379.

Stalker, D., K. McBride and L. Malyj. 1988. Herbicide resistance in transgenic plants expressing a bacterial detoxification gene. *Science* 242:419-422.

Stark, D., G. Barry, Y. Muskopf, K. Timmerman and G. Kishore. 1991. Abstract 714 presented at the *Third International Congress of Plant Molecular Biology*, Tucson.

Steinrücken, H. and N. Amrhein. 1980. The herbicide glyphosate is a

potent inhibitor of 5-enolpyruvyl- shikimic acid-3-phosphate synthase. *Biochemistry and Biophysics Research Communications* 94:1207-1212.

Sutton, D. W., P. K. Havstad and J. D. Kemp. 1992. Synthetic cryIIIA gene from *Bacillus thuringiensis* improved for high expression in plants. *Transgenic Research* 1:228-236.

Tabashnik, B. E. 1994. Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered. *The Royal Society* 255:7-12.

Tarczynski, M. C., R. G. Jensen, H. J. Bohnert. 1993. Stress protection of transgenic tobacco by production of the osmolyte mannitol. *Science* 259:508-510.

Tarczynski, M., R. Jensen and H. Bohnert. 1991. Abstract 1517 presented at the *Third International Congress of Plant Molecular Biology*, Tucson.

Terryn, N., M. B. Arias, G. Engler, C. Tire, R. Villarroel, M. van Montagu and D. Inze. 1993. *rha1*, a gene encoding a small GTP binding protein from Arabidopsis, is expressed primarily in developing guard cells. *The Plant Cell* 5:1761-1769.

Vaeck, M., A. Reynaerts, H. Hofte, S. Jansens, M. M. de Beuckeleer, C. Dean, M. Zabeau, M. Van Montagu and J. Leemans. 1987. Transgenic plants protected from insect attack. *Nature* 328:33- 37.

van Camp, W., H. Willekens, C. Bowler, M. Van Montagu, D. Inze, P. Reupold-Popp, H. Sandermann, Jr. and C. Langebartels. 1994. Elevated levels of superoxide dismutase protect transgenic plants against ozone damage. *Bio/Technology* 12:165-168.

van der Krol, A. R., P. E. Lenting, J. Veenstra, I. M. Van der Meer, R. E. Koes, A. G. M. Gerats, J. N. M. Mol and A. R. Stuitje. 1988. An anti-sense chalcone synthase gene in transgenic plants inhibits flower pigmentation. *Nature* 333:866-869.

Vandekerckhove, J., J. van Damme, M. van Lijsebettens, J. Botterman, M. De Block, M. Vanderweile, A. de Clerq, J. Leemans, M. Van Montagu and E. Krebbers. 1989. Enkephalins produced in transgenic plants using modified 2s seed storage proteins. *Bio/Technology* 7:929-936.

Vernon, Daniel M., M. C. Tarczynski, R. G. Jensen and H. J. Bohnert. 1993. Cyclitol production in transgenic tobacco. *The Plant Journal* 4(1):199-205.

Voelker, T. A., A. C. Worrell, L. Anderson, J. Bleibaum, C. Fan, D. J. Hawkins, S. E. Radke, H. M. Davies. 1992. Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plant. *Science* 257:72-74.

Woloshuk, C., J. Meulenhoff, M. Sela-Buurlage, P. van den Elzen and B. Cornelissen. 1991. Pathogen-induced proteins with inhibitory activity

toward *Phytophthora infestans*. *The Plant Cell* 3:619-628.

World Bank. 1991. Agricultural Biotechnology: The Next "Green Revolution"? World Bank Technical Paper, No. 133. The World Bank, Washington DC.

Worrell, A., J-M. Bruneau, K. Summerfelt, M. Boersig. M. and T. A. Volker. 1991. Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning. *The Plant Cell* 3:1121-1130.

Chapter 1.2

The Importance of Biotechnology to Sustainable Agriculture

Wally D. Beversdorf
Head, Seeds Research and Biotechnology
Ciba Limited
Postfach, SE2, CH-4002 Basle, Switzerland.

Introduction

Biotechnology may contribute to sustainable agriculture in the future, or it may diminish sustainability of our agri-food sector. It is fair to say that our agri-food systems are not currently sustainable in the long term. Demands for agri-food outputs have increased 400% over the last century due to real population growth (high birth rates and increasing longevity). Demographers estimate that the global population could reach an equilibrium at between 10 and 16 billion by the year 2060. Can the planet's agri-food systems provide the continued nutritional requirements of such a population?

Beversdorf, W.D. 1994. The Importance of Biotechnology to Sustainable Agriculture. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 29-32.

Limitations and Uncertainties of Agricultural Production

Agricultural production systems have real resource limitations. There is limited land area suitable for agricultural production, which land resource scientists believe will see a net increase of 5% over the next century. Other limitations include fossil fuels—most temperature zone crops require more fossil fuel for production than the plants capture as harvestable photosynthate. Based on current sources and rates of consumption, we also have limited supplies of phosphorous and potassium.

A second, perhaps equally important concern is the instability of agricultural production environments. Predictions of climatic change suggest that global mean temperatures could change by either plus or minus 2-4°C. While it is unlikely that we will experience such radical mean temperature changes over the next decade, it is realistic to expect some temperature and associated precipitation changes over the next 20 to 50 years. Net changes in mean temperature over the next 50 years will probably reflect the balance between increasing greenhouse gases and changes in atmospheric particulates from volcanic activity and combustion. Desertification processes appear active in both Africa and Asia. Clearly, climate is unstable, and may have an impact on the stability of agricultural production over the next century.

If we examine total world agricultural production over the past 30 years, two dramatic changes are perceptible. First, agricultural production has increased dramatically (on par with population growth). Secondly, there is increasing annual variation in total production around that trend line. Variation has increased substantially in the past few decades, perhaps as a result of the natural interplay between variable climatic conditions and increasingly intense agricultural output.

A third major source of uncertainty is associated with variable economic and trade policies, including uncertainties associated with the implementation and effect of the recently concluded negotiations of the General Agreement on Tariffs and Trade (GATT). Mr. Arthur Dunkel, former Director General of GATT, proposed the process in the hope that GATT would eliminate agricultural production subsidies, and there will certainly be an impact, whether positive or negative. All three areas of uncertainty (resources, climatic change, and trade policy) will determine when—or if—the global agri-food system will be sustainable.

Biotechnology and Sustainability

What impact can biotechnology have on agricultural sustainability or global food security? Biotechnology may help in global agricultural sustainability if it accelerates genetic advances in productivity. There has been much talk about biotechnology in a general way, in some very specific applications. Increases in productivity in the past 100 years—which have been huge and have accommodated a 400% increase in human population—have relied on two major components: genetic advances and increases in resource inputs. Many of our agricultural resources are limited, so in the long term it is very important to use resources wisely. Future sustainability will probably also come about through genetic advance. The maize industry in North America and Europe has increased productivity at a rate of two bushels per acre per year in recent decades, mostly through improved hybrids. Similar relative gains through genetic advances have been made in cereals, soybeans and most other major grain crops. Gains in vegetable crop productivity have been somewhat slower over that period.

If biotechnology can help us advance the genetics of our food crops (greater output per unit of finite resource input), then it can contribute towards agricultural sustainability. If biotechnology can help preserve environmental resources, and by that I mean both essential resources (air, water and nutrients) and also aesthetic resources (green space, parks, diversity, etc.), then biotechnology may help us move towards agricultural sustainability.

If biotechnology (and plant breeding and genetics) fails to help improve resource utilization efficiency, then agriculture will probably not be sustainable, and the size of the global community will decline as resources are exhausted. If biotechnology fails to help preserve aesthetic components of the environment, then there will also be a decline in the quality of life for the global population. Environmental considerations are critical to sustainable agriculture, and biotechnology may contribute to enhancing or conserving environmental resources, if properly managed. If not properly managed, biotechnology could accelerate environmental degradation. Most of the discussions in this book focus on capturing the opportunities, while avoiding the risks, associated with this new technology.

If biotechnology is really to have an impact on sustainability, real sustainability, then it has to be available to those who need it. Part of this book discusses how to provide technologies that can contribute to sustainability to those who need them. Certainly, the pilot programs of the International Service for the Acquisition of Agri-biotech Applications

(ISAAA) are an effort towards that end. But there are many additional efforts that are required.

One area often ignored, in the context of technology transfer, is human resource development in those regions that can best benefit from biotechnology, in order to have the expertise available and apply it in the most appropriate ways to answer regional needs. Technology transfer is an essential requirement if biotechnology is going to have a real impact on global agricultural sustainability.

The real challenge has been an evolution of appropriate regulatory processes that ensure biotechnology impacts positively on the major factors related to agricultural sustainability, while protecting environmental resources and human health and welfare. If, through regulatory processes, we can move towards positive benefits from biotechnology, sustainability will be one step closer—that is, sustainability may become a real possibility, rather than the major uncertainty it is today.

Conclusion

Sustainability of the planet's agri-food production system reflects finite resources (land, fuel, nutrients, etc.), uncertainties about variable climatic patterns, and uncertainties about variable regional and global economic and trade policies. Biotechnology may contribute to agri-food sustainability by contributing to the genetic advance (food output per unit of resource input) of most of our food crops. If properly managed, biotechnology will also help protect both the essential and aesthetic components of our environment, in part by reducing exploitation of non-agricultural land (e.g. rainforests and recreational habitats) for food production. Appropriate regulatory processes for managing the application of biotechnology to agricultural sustainability and environmental protection are an essential early step towards development of a truly sustainable global agri-food production system.

Chapter 1.3

Potential Ecological and Evolutionary Problems of Introducing Transgenic Crops into the Environment

Robert K. Colwell
Professor of Ecology and Evolutionary Biology
Department of Ecology and Evolutionary Biology
U-42, University of Connecticut
Storrs, CT 06269-3042, USA.

Introduction: History of Involvement of the Ecological Society of America

The Ecological Society of America (ESA) is an organization of some 6500 academic and professional ecologists in the USA, Canada, Mexico, and 62 other nations, more than two-thirds of whom are researchers with doctorates. Historically, the ESA has steered clear of institutional involvement in public policy issues. Beginning in 1984, however, through its Public Affairs Committee, the ESA became cautiously involved in the

Colwell, R.K. 1994. Potential Ecological and Evolutionary Problems of Introducing Transgenic Crops into the Environment. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 33-46.

scientific debate over the environmental testing and use of transgenic organisms (Brown *et al.*, 1984). The first official involvement was a formal response, in 1987, to the Coordinated Framework for the Regulation of Biotechnology—still the basis of regulatory policy in the USA. In that response (Colwell *et al.*, 1987), we endorsed the development and wise use of environmentally safe, genetically engineered products, including plants. But we strongly questioned the rationale behind certain classes of proposed exemption from close regulatory scrutiny, and we urged a broader view of potential ecological and evolutionary risks. Our response concluded that "...we offer the abilities and experience of our profession to help improve the criteria and protocols for risk assessment in the environmental use of the products of biotechnology, to help make sound and timely judgments on particular cases, and to aid in the design of effective products of biotechnology that will benefit society."

The following year, at the request of the Public Affairs Committee of the ESA, a critique of the report of the National Academy of Science of the USA (NAS, 1987) was prepared and published (Colwell, 1988). This critique applauded the *body* of the National Academy of Science report, which made much progress "toward integrating the experience of biologists of diverse expertise"—that is, ecologists as well as molecular biologists—while strongly criticizing as "oversimplified and remarkably sanguine" the widely-cited executive summary of the report.

In 1989, on behalf of the ESA, seven ecologists and evolutionary biologists with a broad range of special expertise tackled the job of producing an in-depth, scholarly analysis of all aspects of ecological risk assessment for the release of genetically engineered organisms (Tiedje *et al.*, 1989). The process took nearly a year, and the document went through 19 drafts.

The objective of the ESA document was to provide a rigorous basis for a "science-based regulatory policy that encourages innovation without compromising sound environmental management." We did our best to position professional ecologists as partners—and not simply as critics—in the enterprise of biotechnology by insisting that "understanding ecological traits and requirements of transgenic organisms is critical not only to managing risk, but to ensuring successful implementation." Or, as I once put it metaphorically, "If biotechnology is the cutting edge of biology, perhaps ecology is the whetstone" (Colwell, 1986).

While providing an overview of the ESA document as a whole, I will concentrate on those issues that particularly concern transgenic plants, and I will use examples from plants. Although the title of this book suggests we confine our discussions to field crops, including forage species, I am eager to broaden the discussion to include one additional,

important category of transgenic plants: forest trees. This additional category is not only important in the domestic and international economies of both Costa Rica and Mexico, but it presents particular kinds of risks that may be less important for field crops, and it promises to be an important focus of development in biotechnology.

Conceptual Background

I like to say that civilization was founded on the evolutionary "inventions" of wild species (Colwell, 1989). The domestication of food plants simply improved on the existing storage tissues of plants—seed endosperm, roots, tubers. With fiber plants, humans simply improved and extracted the support tissues (linen, sisal, hemp, jute) or fibers involved in seed dispersal (cotton, kapok). The effective principles of drug plants, spices, herbs, and natural dyes rely heavily on compounds evolved by plants in protective response to the depredations of insects, mites, and diseases (Simpson and Conner-Ogorzaly, 1986). Hundreds of generations of farm women and men have contributed to this process through traditional systems of genetic innovation, producing a geographically and culturally complex mosaic of genetic variation in land races and traditional varieties of cultivated plants.

In the past century, scientific breeders of plants and animals have utilized the rich genetic resources of cultivars and land races in crop improvement programs (Witt, 1985). They have also reached back into the evolutionary history of domesticated species to recapture useful genetic traits from their wild relatives—sometimes from the true ancestral species, sometimes from evolutionary cousins. Resistance to disease, pests, or stress, nutrient balance, growth form and fruit shape or quality have been developed in crops through hybridization with wild relatives, followed by complex breeding programs to combine desired traits in a single strain (Goodman *et al.*, 1987; Iltes, 1988).

This rich genetic heritage for many crop species is now in serious peril, as commercial varieties and changing land use patterns eliminate land races of crops (Keystone Center, 1991) and as the transformation of wildland habitats pushes wild relatives to extinction (Williams, 1988).

There is widespread agreement that genetic resources that have direct relevance to cultivated plant species deserve active protection and conservation (Council of Europe, 1990; Keystone Center, 1991). Molecular and cellular biotechnologies, even more than traditional breeding techniques (organismal biotechnology), stand to benefit from these genetic libraries of plant adaptations (Janzen, 1987). In fact, if we

are careful to avoid serious ecological mistakes, agricultural biotechnology may play a key role in slowing the rate of loss of species and gene diversity of plant genetic resources.

The genetic improvement of cultivated plants through the use of recombinant DNA techniques promises to enhance the quality of food and fiber, to increase yield through better protection from diseases and pests, and to reduce the need for chemical inputs—both toxins and fertilizers (Levin and Strauss, 1991). Because many of these advances allow more efficient use of land already in agricultural use, they promise not only to feed and clothe people but to help alleviate the pressure for conversion of wildlands into croplands and pastures. The development of improved tree varieties for reforestation of degraded tropical agricultural lands, through rDNA techniques, could provide a sustainable supply of forest products that would further relieve pressure on tropical forests.

Potential Ecological and Evolutionary Problems with Field Testing and Commercializing Transgenic Plants

What, then, are the conceivable ecological hazards associated with the testing and use of transgenic plants? The ESA report lists six categories of environmental concern, each of which will be discussed in more depth below (see also Ginzburg 1989; Levin and Strauss, 1991):

1) the creation of new weeds;
2) the amplification of the effects of existing weeds;
3) harm to nontarget species;
4) disruptive effects on biotic communities;
5) adverse effects on ecosystem processes; and
6) squandering of valuable biological resources.

Creation of New Weeds or Amplification of the Effects of Existing Weeds

Many crops have been domesticated to such a degree that they are entirely dependent on human activities. Maize, wheat and bananas are good examples. Such crops, whatever the improvements made through biotechnology, are unlikely to become self-propagating weeds in any ecological context. Some other cultivated plants are weeds in some contexts and crops in others—certain kinds of millets (*Pennisetum* spp.) and sorghums (*Sorghum* spp.) fall into this category. The introduction of a gene that increases plant fitness (such as resistance to disease or to pests) to a crop in this category might shift the balance toward

weediness in areas where they are now safely grown as crops, or promote weediness in varieties now considered safe.

Other crops have close weedy relatives—including sugarcane (*Saccharum*), rice (*Oryza*), potatoes (*Solanum*), sweet potato (*Ipomoea*), vegetable and oil seed *(Brassica),* sunflower (*Helianthus*), and oats (*Avena*). For some of these crops, which still share many characteristics with their weedy ancestors, certain kinds of genetic alterations might create weed problems with the crop itself (Keeler, 1989). For example, a transgenic, highly salt-tolerant variety of paddy rice might itself invade estuaries (Tiedje *et al.*, 1989). Generally, however, the likelihood of most crops themselves becoming serious weeds is small.

Considerably more likely is the development of increased problems with already-weedy relatives, by the acquisition of fitness-conferring genes from a transgenic crop through hybridization and introgression. Ecologists consider this scenario to be the primary ecological risk from transgenic plants (Tiedje *et al.* 1989; Ellstrand and Hoffman, 1990; Hoffman, 1990; Klinger *et al.*, 1991; 1992). It is often wrongly discounted, however, due to a common confusion between the concepts of introgression and hybridization.

Some pairs or groups of closely-related plant species (for example, orchids or cucurbits) hybridize freely in cultivation (Wilson, 1990), free from the constraints of the co-evolved pollinator systems of their ancestral habitats. For other groups of plants, hybridization is infrequent, rare, or completely impossible. If formed, first-generation (50/50) hybrids may be fully fertile, but more often they have reduced fertility, or are completely sterile.

Introgression is the incorporation of genes from one species into the gene pool of another. The process must begin with hybridization, but it is more complex. Introgression in nature is precisely analogous to a common technique of plant varietal improvement. The plant breeder may start with a variety that has many desirable commercial characteristics, but is (let us say) susceptible to some pathogen. The breeder crosses this variety with a wild relative that lacks the commercial traits, but is resistant to the pathogen. Often, the first-generation hybrids that have the resistance trait will have lost many of the desirable commercial traits of the cultivated parent. These must be recaptured by repeated backcrossing and selection.

The process of hybridization plus backcrossing and selection is an exact parallel, under artificial selection, to the process of introgression under natural selection. Just as the breeder's first generation hybrid lacks the combination of traits that makes it either a fit wild plant or a desirable cultivated plant, the first generation hybrid between a

transgenic crop and a weedy wild relative at the edge of a test plot or a farmer's field would likely be rejected both by the farmer and by nature—its fitness relative to the wild plant would be low. But if it flowers and passes pollen to its wild cousins, the next generation will have 3/4 of the genes of the wild relative, the next 7/8, and so on: this is the process of introgression. If the hybrid included a transgenic trait that would, by itself, confer high fitness on the weedy relative (Manasse and Kareiva, 1989; Klinger *et al.*, 1991; 1992)—such as disease or pest resistance—then natural selection will rapidly promote the reproductive success of these successively "wilder" genotypes, just as the plant breeder regains the commercial qualities of the new variety by backcrossing.

Two important points should be made from this discussion. First, hybridization need not be a common event for introgression to proceed, carrying a transgenic trait from crop to weedy relative. That a crop and a weedy relative are known to hybridize only "rarely" is sufficient for the escape of a transgenic trait into the population of a weedy relative. If the gene does as much for the weed as the genetic engineer hopes it will do for the crop, then, at the limit, hybridization need only occur once for escape; natural selection will take care of the spread of the gene. Likewise, even crops such as sugarcane (*Saccharum officinarum*) and sweet potato (*Ipomoea batatas*), which are generally considered infertile, occasionally produce normal pollen; if hybrids form, once may be enough (Keeler and Turner, 1991). (Given the significance of wild *Ipomoea* species and of *Saccharum spontaneum* as agricultural weeds, the example is not an idle one.)

The second point is that hybrids need not be particularly fit in *themselves*, as long as they are competent to backcross with the weedy relative. Maize (*Zea mays*) and its wild relatives, the teosintes of Mexico and Guatemala, provide a good example. Teosintes include three wild subspecies of maize itself (*Zea mays mexicana, Z. mays parviglumis,* and *Z. mays huehuetenangensis*) and three closely related species of *Zea* (*Z. dipolperennis, Z. perennis* and *Z. luxurians*) (Doebley, 1990). Some of these taxa are agricultural weeds and some are wildland species. Hybrids between teosintes and cultivars of maize are known for several of these taxa.

Supposedly, some Mexican farmers even encourage some hybridization by allowing weedy teosintes to grow at the edge of their fields to make the maize "stronger," but genes may flow from maize to teosinte in such cases, too. In all cases, the hybrid is of low fitness, as evaluated by both natural selection (the seeds do not disperse easily) and artificial selection (to the farmer, the grain is inferior in quality and is not as easily harvested as maize, so is not kept for seed).

Nonetheless, introgression from cultivated maize into wild teosinte populations is known to occur, as shown by Doebley (1990) using electrophoretic techniques. Such introgression simply indicates that, however low the fitness of first-generation hybrids, they are nonetheless competent to backcross with teosinte. Because of the low fitness of intermediates, maize and the teosintes have kept their distinctive identities; an evolutionary biologist might call it a case of disruptive selection. Some cite the fact that maize and teosinte have coexisted for centuries without losing their identities as evidence against the danger of creating a seriously weedy teosinte through acquisition of a transgenic trait, and against the danger of genetic contamination of wild teosinte gene pools with novel genes of distant origin (such as the *Bacillus thuringiensis gene—B.t.*).

These reassurances, based on a misunderstanding of the nature of introgression, are entirely misguided. It is also well to remember that when low-probability events (or hazards) are multiplied by large exposures, they become virtually inevitable. Even if the outcrossing rate of a crop plant is only 0.1%, if there are a million flowers in field, then we must expect 10,000 outcrossing events (Manasse and Kareiva, 1989; Keeler and Turner, 1991).

A final point for this topic is the irrelevance of traditional plant quarantine regulations and practices, with respect to guarding against introgression of transgenic traits into wild or weedy relatives of cultivated plants. Because a gene is not a disease or a pest, quarantine is pointless. The danger point is the open field somewhere in the middle of the countryside, not a port of entry. Unfortunately, small numbers of familiar local weeds that contain a newly acquired, high-fitness, transgenic trait are likely to go unnoticed, simply because they are familiar, until they become a significant problem. Thus, awareness of the potential for introgression and vigilance regarding potential recipients of such genes are called for not at the port of entry, but around test plots and, later, in the first commercial fields (Keeler and Turner, 1991). The exception would be the very difficult task of controlling, at ports of entry, the illegal importation of small quantities of transgenic seed by individuals, even well-meaning farmers or amateur gardeners. Again, as we know from countless cases of “informal” importation of weeds and other undesirable species by individuals, one mistake can be costly.

Harm to Non-Target Species

In the case of transgenic plants, the nontarget species of concern include both animals and other plants. When genes producing compounds

intended to deter or kill pest insects or mites are incorporated into crop plants, the ideal result is that only the target pests are affected. We know from decades of unhappy experience that this ideal result is all too often not the case with chemical pesticides. Of course, our great hope is that transgenic plants with highly specific secondary chemicals are the answer. Precautions are needed, however. An insect-pollinated transgenic plant that has been engineered to make an insecticidal compound intended to deter leaf or root-eating pests had better not produce the same compound in its pollen or nectar. A fiber plant that produces an insect toxin in its leaves had better not poison the farmer's cattle that wander into the field. A plant that produces a mite toxin would likely affect beneficial predatory mites as well as phytophagous mites.

Even *B.t.* toxin, of which some strains affect only lepidoptera insects, could potentially harm beneficial—or at least desirable—butterflies and moths. For example, the striking red-, orange-, or yellow-and-black heliconine butterflies of tropical forests not only help attract many natural-history tourists to tropical countries, but are considered to be "keystone species" in most natural communities in the New World tropics (Gilbert, 1979, 1980). The larvae of most of this diverse group of butterflies feed on wild species of *Passiflora* or passionfruit vines (*maracuyá*). Because certain lepidopteran larvae are also pests of cultivated passionfruit, incorporation of the *B.t.* gene would seem a natural approach to improving this crop. The wildland butterfly species would be severely affected, however, by the acquisition of the *B.t.* gene by these wild plants through introgression with a cultivated transgenic passionfruit carrying the gene. Thus, unless hybridization can be prevented, it would seem dangerous to field test or commercialize passionfruit cultivars with the *B.t.* gene anywhere near wild *Passiflora* spp.

Harm to other plants caused by transgenic crops includes several potential problems, all of them consequences of the acquisition of transgenic traits by wild relatives through introgression. Because these problems are more appropriate to later sections of this paper, I will not discuss them here.

Disruptive Effects on Biotic Communities and Ecosystem Processes

The composition and relative abundance of species, and the spatial structure of natural plant communities, depends upon a complex balance between plant-plant competition, the effects of herbivores and seed predators, and interaction with pollinators, seed dispersers, and soil

mutualists. The acquisition of a high-fitness trait, such as protection against herbivorous insects, by a wildland plant species through introgression with a related transgenic crop could have several disruptive consequences.

We know from agricultural experience that pests are capable of causing massive reductions in crop reproductive fitness and yield. Experiments in natural communities and the record of successful biological control of weeds by imported insects and pathogens testify to the importance of reproductive control of plants by enemies in both agricultural and natural communities (Keeler and Turner, 1991). A wildland plant species, released from significant natural control, would become a better competitor, likely to reduce the density of competing plant species. Secondary effects could include declines in animal populations dependent on these species, and even changes in vegetation structure.

Ecologists have expressed concern about the effects of transgenic plants, or wild plants that acquire transgenic traits through introgression, on the functioning of ecosystems. Of course, transgenic trees used in reforestation projects may have very beneficial effects on ecosystem processes by increasing the per-area carbon fixation rate, by stabilizing or enriching soil, and by buffering climate. But some caution is called for regarding transgenic (or other) plants that significantly affect soil nutrient balance. For example, if and when nitrogen fixation gene complexes are successfully introduced to forest trees, careful testing should precede widespread introduction in order to determine the effects on the supply and demand for other soil nutrients, particularly phosphorus (Janzen, 1987).

Squandering of Valuable Biological Resources

The principle biological resources that may be at risk from the testing and use of transgenic plants are plant genetic resources—in this case, not so much the genetic diversity of land races and cultivars, but diversity of the gene pools of wild species. Once again, the principal risk arises from introgression of cultivated crops with wild plants.

Table 1 lists examples of significant cultivated plants of Costa Rica that have congeners (members of the same genus) among the indigenous flora of the country. (Because I am more familiar with the flora of Costa Rica than of Mexico, I have listed Costa Rican examples, but an equal or longer list could be made for Mexico.) Some, such as African oil palm and cacao, are important components of the Costa Rican agricultural economy. In the case, at least, of oil palm, Costa Rican wild relatives have

Table 1: Selected Examples of Costa Rican Economic Plants that have Indigenous Wild Populations or Congeners

Common English Name	Common Costa Rican Name	Latin Name	Congeners
Cashew	Marañon	*Anacardium occidentale*	*A. excelsium*
Peach palm	Pejivalle	*Bactris gasipaes*	several
Papaya	Papaya	*Carica papaya*	several
Melon, cucumber, squash	Melón, pepino, ayote	*Cucurbita* spp.	several
Yam	ñame	*Dioscorea alata*	several
African oil palm	Palma de aceite	*Elaeis guineensis*	*E. oleifera*
Sweet potato	Camote	*Ipomoea batatas*	many
Cocoa palm	Cocoa	*Theobroma cacao*	*T. mammosum, T. simarum*
Passionfruit	Maracuyá	*Passiflora* sp.	many
Avocado	Aguacate	*Persea americana*	*P. rigens*
Bean	Frijol, vainica	*Phaseolis vulgaris*	several
Black pepper	Pimienta negra	*Piper nigrum*	nearly 100
Potato	Papa	*Solanum tuberosum*	many

already been the source of genetic variation for past crop improvement (Ewel, personal communication).

The genetic diversity—and the evolutionary inventions—of the wild relatives listed in Table 1 represent not only an irreplaceable natural resource for the improvement of the corresponding crops, but part of the national patrimony of Costa Rica. To permit the contamination of the gene pools of these native species by high-fitness genes through introgression with transgenic crops is an outcome to be avoided, not only for practical reasons, but for ethical ones.

Indeed, an ethical argument can be made against allowing introduction of genes from unrelated organisms to wild organisms of any

species. For example, we know that genes from cultivated maize have entered the gene pools of wild teosintes. This can be considered a natural process that is part of the evolution of crops from wild relatives. But the introduction of, for example, a gene from bacteria (*B.t.* toxin), or from a distantly related plant group, seems to some of us to be a different matter—a process I have referred to as the "conduit effect" (Figure 1) (Colwell, 1989).

The argument can be made that, even if there is no adverse ecological effect, such genetic contamination devalues the genetic resource and evolutionary integrity of wild species. This ethical argument, of course, rests on the distinction between domesticated species, which have long evolved within the evolutionary domain of humans, and wild species, which have historically been comparatively free from our influence (Colwell, 1989).

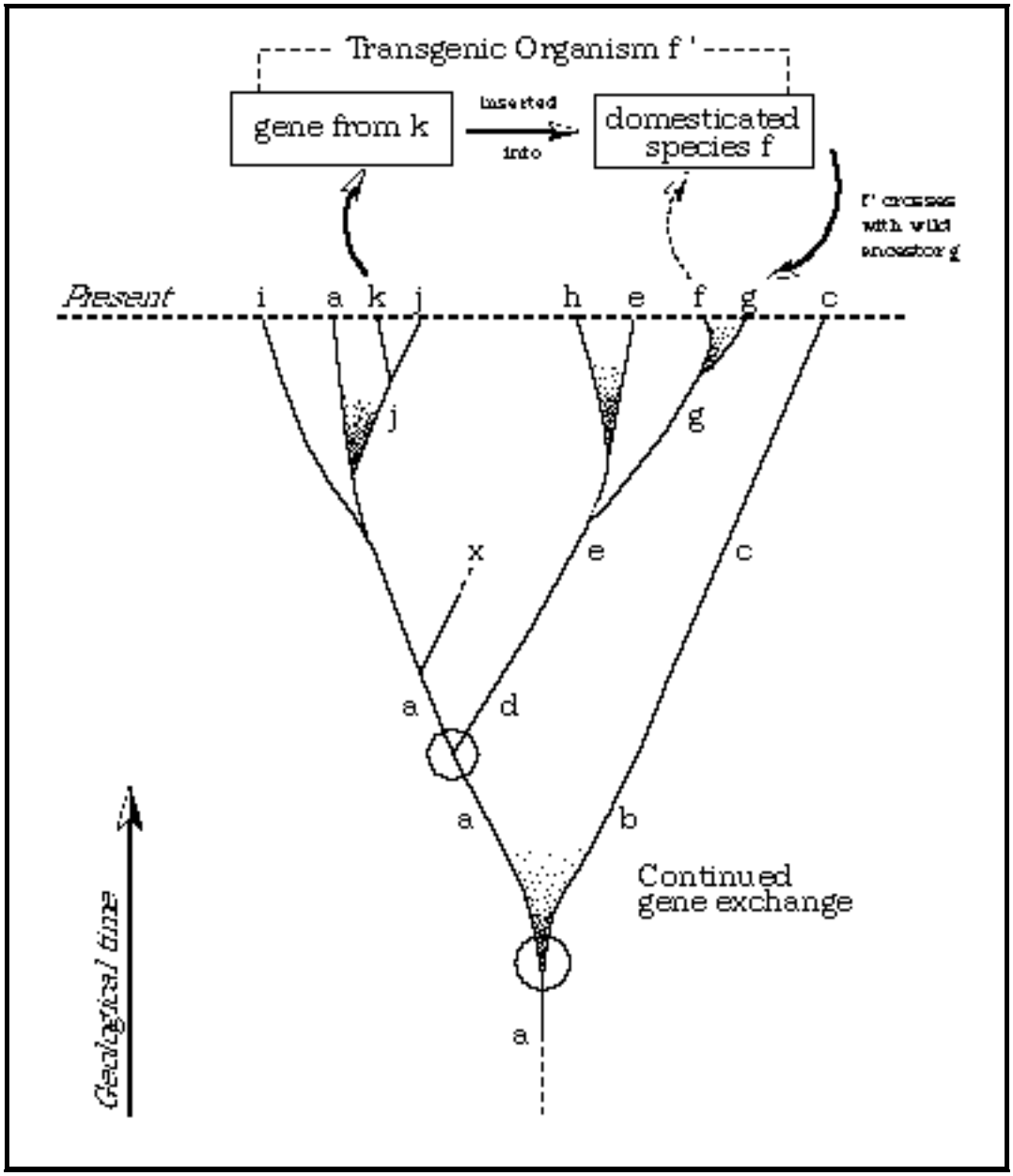
I have not conducted library research to learn how many of the cultivated plants in Table 1 are known or suspected to form fertile hybrids (even occasionally) with their indigenous relatives; this information is probably not even available for some of these cases. Such congeneric matches are simply the most likely suspects for introgression—suspects that a wise regulatory policy should insist on knowing more about before granting permission for even an initial field trial, in my opinion.

One final resource that transgenic plants may threaten, if used unwisely, is the very small library of highly specific genes for the control of insect pests; the most obvious example is *B.t.* As for any other pesticide, resistance can evolve quickly if pest populations are continuously exposed to it over large areas, eliminating the pockets of susceptible genotypes whose resurgence is the key to continued effectiveness (Gould, 1988). The ESA report (and earlier publications [Colwell *et al.*, 1985]) called this problem to the attention of the regulatory agencies and the biotechnology industry, which had initially belittled the problem.

At present, a special cooperative effort between ecologists, evolutionary biologists and molecular biologists is underway, funded by the biotechnology industry, to find ways to prolong the useful life of the *B.t.* gene by minimizing exposure and pinpointing expression to vulnerable tissues, instead of relying on constitutive expression of the trait.

This cooperative project is a fitting example to end with, showing the potential role that collaboration between ecologists and evolutionary biologists may play in the safe and effective implementation of biotechnology.

Figure 1: The "Conduit" Effect



References

Brown, J. H., R. K. Colwell, R. E. Lenski, B. R. Levin, M. Lloyd, P. J. Regal and D. Simberloff. 1984. Report on workshop on possible ecological and evolutionary impacts of bioengineered organisms released into the environment. *Bulletin of the Ecological Society of America* 65:436-438.

Colwell, R. K. 1986. Biological responses to perturbation: Genome to ecosystem. In *Engineered Organisms in the Environment: Scientific Issues*. Halvorson, H. O., D. Pramer and M. Rogul (eds.). pp. 320-232. American Society for Microbiology, Washington DC.

Colwell, R. K. 1988. Another reading of the NAS gene report. *BioScience* 38:421-423.

Colwell, R. K. 1989. Natural and unnatural history: Biological diversity and genetic engineering. In *Scientists and their Responsibility*. Shea, W. 1R. and B. Sitter, (eds.). pp. 1-40. Watson Int. Publishing, Canton.

Colwell, R. K., E. A. Norse, D. Pimentel, F. E. Sharples and D. Simberloff. 1985. Genetic engineering in agriculture. *Science* 229:111-112.

Colwell, R. K., L. W. Barnthouse, A. Dobson, F. Taub and W. R. Shea. 1987. Response to the Office of Science and Technology Policy Notice "Coordinated framework for regulation of biotechnology". *Bulletin of the Ecological Society of America* 68:16-23.

Council of Europe. 1990. Recommendation No. R(90)16 of the Committee of Ministers to Member States on the conservation of wild progenitors of cultivated plants. Council of Europe, Strasbourg.

Doebley, J. 1990. Molecular evidence for gene flow among *Zea* species. *BioScience* 40:443-448.

Ellstrand, N. C., and C. A. Hoffman. 1990. Hybridization as an avenue of escape for engineered genes. *BioScience* 40:438-442.

Gilbert, L. E. 1979. Development of theory in the analysis of insect-plant interactions. In *Analysis of Ecological Systems*. Horn, D., R. Mitchell and P. H. Raven (eds.). pp. 117-154. Ohio State Univ. Press, Columbus.

Gilbert, L. E. 1980. Food web organization and the conservation of Neotropical diversity. In *Conservation Biology*. Soulé, M. E. and B. A. Wilcox (eds.). pp. 11-33. Sinauer Associates, Sunderland.

Ginzburg, L. (ed.). 1989. *Risk Assessment in Biotechnology*. Wiley, New York.

Goodman, R. M., H. Hauptli, A. Crossway and V. C. Knauf. 1987. Gene transfer in crop improvement. *Science* 236:48-54.

Gould, F. 1988. Evolutionary biology and genetically engineered crops. *BioScience* 38:26-33.

Hoffman, C. A. 1990. Ecological risks of genetic engineering in crop

plants. *BioScience*. 40:434-437.

Iltes, H. H. 1988. Serendipity in the exploration of biodiversity: What good are weedy tomatoes? In *Biodiversity*. Wilson, E. O. (ed.) pp. 98-105. National Academy Press, Washington DC.

Janzen, D. H. 1987. Conservation and agricultural economics. *Science* 236:1159.

Keeler, K. H. 1989. Can genetically engineered crops become weeds? *Bio/Technology* 7:1134-1139.

Keeler, K. H. and C. E. Turner. 1991. Management of transgenic plants in the environment. In *Risk Assessment in Genetic Engineering*. Levin, M. A. and H. S. Strauss (eds.). pp. 189-218. McGraw-Hill, New York.

Keystone Center, 1991. *Final Consensus Report: Global Initiative for the Security and Sustainable Use of Plant Genetic Resources*. Keystone International Dialogue Series on Plant Genetic Resources. The Keystone Center, Keystone.

Klinger, T., D. R. Elam and N. C. Ellstrand. 1991. Radish as a model system for the study of engineered gene escape rates via crop-weed mating. *Conservation Biology* 5:531-535.

Klinger, T., P. E. Arriola and N. C. Ellstrand. 1992. Crop-weed hybridization in radish (*Raphanus sativus*): Effects of distance and population size. *American Journal of Botany* 79:1431-1435.

Levin, M. A., and H. S. Strauss, (eds.). 1991. *Risk Assessment in Genetic Engineering*. McGraw-Hill, New York.

Manasse, R., and P. Kareiva. 1989. Quantitative approaches to questions about the spread of recombinant genes or recombinant organisms. In *Risk Assessment in Biotechnology*. L. Ginzburg (ed.). Wiley, New York.

NAS. 1987. *Introduction of Recombinant DNA-engineered Organisms into the Environment: Key Issues*. National Academy of Science (NAS). National Academy Press, Washington DC.

Simpson, B. B. and M. Conner-Ogorzaly. 1986. *Economic Botany: Plants in our World*. McGraw Hill, New York.

Tiedje, J. M., R. K. Colwell, Y. L. Grossman, R. E. Hodson, R. E. Lenski, R. N. Mack and P. J. Regal. 1989. The planned introduction of genetically engineered organisms: ecological considerations and recommendations. *Ecology* 70:298-315.

Williams, J. T. 1988. Identifying and protecting the origins of our food plants. In *Biodiversity*. E. O. Wilson, (ed.). pp. 240-247. National Academy Press, Washington DC.

Wilson, H. D. 1990. Gene flow in squash species. *BioScience* 40-449-455.

Witt, S. C. 1985. *Biotechnology and Genetic Diversity*. California Agricultural Lands Project, San Francisco.

Chapter 1.4

The Testing and Release of Transgenic Potatoes in the North American Center of Diversity

Robert E. Hanneman, Jr.
Research Geneticist
Agricultural Research Service (ARS)
United States Department of Agriculture (USDA)
Department of Horticulture, University of Wisconsin
Madison WI 53706, USA.

Summary

The question of what will happen if transgenic potato plants are released into the North American center of diversity is examined through known cases of release of other plants into a new environment where there are, and/or are not, relatives present, and through evaluating the consequences of those actions. In most cases, the release of new

Hanneman, R.E. Jr. 1994. The Testing and Release of Transgenic Potatoes in the North American Center of Diversity. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 47-67.

introductions has caused no significant consequences; in some instances they have been beneficial, and in a few instances the releases become weeds, such as kudzu in the southeastern USA "shattercane", produced from the hybridization of grain sorghum with its wild relatives, and an example of a threat that can occur through hybridization.

When considering the introduction of transgenic potatoes into centers of diversity, one must take into account the effect of the presence or absence of 2n gametes, stylar barriers, and Endosperm Balance Numbers (EBNs). One also needs to consider the reproductive characteristics of related species in the area, as well as the presence of pollinators (insect vectors). Taking these into consideration, it is concluded that there is little threat of introduction of genes from transgenic 4x(4EBN) *Solanum tuberosum* ssp. *tuberosum* plants to wild species in Costa Rica, but that in Mexico the chance of movement of genes to native species is possible, particularly to the 6x(4EBN) species and the cultivated and commercial 4x(4EBN) varieties.

Introduction

The field testing of transgenic plants occurs rather routinely in the USA, and procedures seem to have been gradually worked out to the apparent satisfaction of most parties involved (McCammon and Medley, 1990). As time has gone on, the stringency of the rules has been progressively relaxed, as most of the presumed risks seem less severe than once thought. However, these releases are generally not being made in areas where there are related species that could hybridize with the transgenic plants.

Part of the problem in considering the release of transgenic plants lies in consideration of the origin of the gene incorporated. A gene brought in from the same or a related species seems acceptable, as long as it is introduced through natural means of hybridization. But the situation is categorized differently when the gene is from a more distantly related organism, and the more distantly related, the greater the problem with the concept. Yet, a gene is a gene, so why should it make any difference whether it came from another plant, a fungus, a virus, a bacteria, or an animal?

Our concerns do not center on the known, but rather on the unknown. If, when searching through a plant species and its relatives, one finds a unique gene for resistance, that gene is incorporated into breeding materials, but if a gene for that resistance is brought in from a bacteria, then concerns are raised because it is not a naturally occurring

gene in the population under consideration. The question is one more of philosophy than of science. This type of transfer has occurred in nature generally unnoticed as lower life forms infect higher life forms, yet when manipulated in the laboratory it comes into question. The use of *Agrobacterium* as a vector and as a disease agent is one such example (Nester *et al.*, 1984; Furner *et al.*, 1986).

It would seem that the process by which an "improved" organism is produced, whether derived by traditional or molecular means, is of little importance in considering what controls and guidelines need to be set up. It simply comes down to one's perspective on biology and how large a gene pool one is willing to consider. There are many means to genetically alter plants. One can use hybridization, mutagenesis, anther and ovule culture, embryo rescue, somaclonal variation, cell fusion, vectored and non-vectored modifications, etc. These are the means to provide genetic change, but they have little to do with the change itself, other than providing the technique.

Some idea of what we need to be concerned about can be derived from the study of the introduction of new materials into an environment that holds no natural relatives, compared to one that does (Mooney and Drake, 1990). Generally when new introductions have been made, they seldom result in significant environmental harm (Simberloff, 1985); some have benefited us greatly, but a few have become weeds. Kudzu has become an important example of the latter in the southeastern part of the USA (Miller and Edwards, 1983).

Gene Exchange with Related Species

Maize provides us with an opportunity to look at gene exchange with related species. Smith *et al.*, (1981) noted gene flow from commercial maize to teosinte. Maize cannot persist in the wild because of its unprotected seeds and lack of a dispersal mechanism. If these traits were incorporated into teosinte, it would put teosinte at a disadvantage in the wild; nevertheless, this has not happened (Doebley, 1984).

When thinking about other domesticated crops, generally we find that the traits necessary for survival in nature have been eliminated. Ears are born for ease of harvesting, shattering of seed is selected against, production of fruit over time has been eliminated, stolons are reduced or eliminated, etc. The processes of breeding and domestication have, at their core, the elimination of weediness.

One of the main concerns about the introduction of genetically modified plants is that it may result in a new weed or form of an existing

weed (Tiedje *et al.*, 1989; Keeler, 1989). Serious weediness is usually related to 10-12 traits (Keeler, 1989). Transgenic plants likely to be introduced in the near future are most likely to involve only a few changes—maybe only one—and then most likely for pest or stress resistance, or single changes concerned with processing characteristics. It is unlikely that quantitative traits will be significantly modified, because presently the molecular means to effect gene transfer with multigenic traits does not exist. This does not mean that the initial changes will be less threatening, but it does mean that they will be easier to follow and assess, unless they are pleiotropic. There is evidence that suggests that a change in a few characteristics can make a plant a weed. Sometimes these changes are noted in pest resistance or fecundity in a new environment.

A report of the National Research Council of the USA (1989) states: "Two closely related ecological questions that may be important to the introduction of genetically modified plants are:

1) Does hybridization between crops and their wild relatives result in transfer of traits from the cultivated form to the wild relative?
2) Does such gene flow increase the weediness of wild relatives?

If the opportunity exists for the transfer of genetic traits from a genetically modified organism to a wild (and potentially weedy) relative, a potential problem exists. The problem poses three relevant questions:

1) Does the genetically modified crop have extant relatives?
2) What is the extent of hybridization between crop and relatives in nature?
3) What is the current ecological role of the relative in natural ecosystems?"

These are the very questions to be dealt with. Certainly the introduction of genetically modified plants into an environment where they have natural relatives offers the possibility for the incorporation of genes from the transgenic plant into that community of plants. If the gene originated from species of that pool, the questions are somewhat different. Even in such an environment, safety still may exist, if the related species are not in proximity to the transgenic plants, or if barriers to gene flow exist, such as lack of an insect vector for pollination, or strong barriers to hybridization such as pre- or post-fertilization barriers, ploidy differences, flowering time, etc. (Simmonds, 1979).

Gene transfer from cultivated to wild relatives has been documented for several crops. It has been noted in the amaranths (Sauer, 1967; Tucker and Sauer, 1958), where the hybrids were thought to out-

compete the weedy relatives. Jain (1977) and Suneson *et al.* (1969) noted that gene flow occurred from rye (open pollinated) to its wild relatives, with the weedy form becoming more crop-like. Similar evidence also exists for African rice (Second, 1982), teosinte to maize (Doebley, 1984), and the squash family and Texas gourd (Decker and Wilson, 1987). In none of these cases were the hybrids more aggressive, nor did they have an enhanced range, but they were more crop-like.

A more threatening example is provided by the production of shattercane when cultivated sorghum hybridizes with its wild relatives, in particular *Sorghum halepense* (Johnson grass), which confers a perennial habit, and *Sorghum sudanense*, which contributes self-sowing seed. The hybrids with *S. halepense* are particularly difficult to eradicate (Holm *et al.*, 1977; Warwick and Black, 1983). The Johnson grass hybrids provide an example of the type of risk associated with gene flow from crops to weedy relatives (Warwick *et al.*, 1984).

Those genes which have been introduced to produce transgenic plants that are likely to be of importance to agriculture in the near future are the gene from *Bacillus thuringiensis* producing endotoxins, coat protein viral genes, herbicide resistance, altered flower color, fruit firmness, and protein or oil composition (NRC, 1989). It should be noted that so far, all genes introduced through molecular means that have become integrated into the host's genomes behave similarly to those introduced by traditional means (Christou *et al.*, 1989; Fraley *et al.*, 1986; Spencer *et al.*, 1992).

The attributes of weediness generally are considered to be discontinuous germination, great seed longevity, germination in diverse environments, rapid vegetative growth, self-compatibility, ease of cross pollination, continuous seed production, high seed output, high seed dispersal, some seed production in a wide range of environments, strong competitiveness, and if perennial, vigorous vegetative reproduction/ regeneration (Baker, 1974). There is a concern about herbicide-tolerant plants becoming weeds or spreading the gene to weedy species, but generally it is thought that these can be dealt with through the use of crop rotation and alternative herbicides.

The Potato

With this as background, let us turn to the question of the introduction of transgenic potato plants into a center of diversity. It is appropriate that thought be given to this subject, for most of the testing of such transformed crops has occurred outside these major centers of diversity.

Central Mexico is one of the centers of diversity for potatoes with Peru, Bolivia and northwest Argentina being the other (Hawkes, 1990).

In Mexico, 42 species or subspecies have been described as tuber-bearing *Solanums* (Table 1). These include both wild and cultivated species, and they are a rich source of genes for resistance and quality (Hanneman and Bamberg, 1986). They consist of diploid, tetraploid, and hexaploid species found principally in mountainous areas. Figure 1 indicates their distribution by series, as described by Hawkes (1990).

Costa Rica has only three species described, with one being a cultivated species, and again they are found in the mountains. This country is on the southern edge of the Mexican center of diversity and appears to be a relatively species-poor region for potatoes.

The species have been delineated from one another by morphological characteristics, ploidy level and geographical location. They have been placed into taxonomic series according to their common features—a "series" being a subdivision of the subgenus *Potatoe*. Thus, in the tuber-bearing Solanums the taxonomic description is as follows:

Family	Solanaceae
Genus	*Solanum* L.
Subgenus	*Potatoe* (G. Don) D'Arcy
Section	*Petota* Dumortier
Subsection	*Potatoe* G. Don
Series	
Species	

However, the taxonomy only helps name the species, and does not help when considering hybridization. To understand this one needs to know something about the presence or absence of 2n gametes, stylar barriers and EBNs. 2n gametes are known to be present for most of the species listed in Table 1 (den Nijs and Peloquin, 1977; Novy and Hanneman, 1991; Watanabe, 1988). The presence of 2n gametes offers the possibility for a species not only to hybridize with others of its own ploidy level, but also with those of higher ploidy levels. This opens the door to the potential for considerable gene exchange between members of different ploidy levels (den Nijs and Peloquin, 1977).

Stylar barriers occur in both intra- and inter-specific crosses, preventing the growth of pollen tubes from a donor through the style of the recipient (Fritz and Hanneman, 1989). This inhibition can be due to several factors including the "S" alleles of gametophytic self-incompatibility, incongruity, and/or undescribed interspecific barrier/isolating systems (Abdalla and Hermsen, 1972; Camadro and Peloquin, 1981; Dionne, 1961; Grun and Aubertin, 1966; Hermsen,

Table 1: Series and Species of Wild and Cultivated Potatoes Native to Mexico and Costa Rica (based on Hawkes, 1990)

Species	Ploidy	EBN	Country	Habitat
Series Morelliformia				
S. morelliforme	2x	?	C-S Mexico, Guatemala	epiphyte, wet mountain forests
Series Bulbocastana				
S. bulbocastanum				
ssp. *bulbocastanum*	2x	1	C-S Mexico	woods, grassland
ssp. *dolichophyllum*	2x	1	C Mexico	rocks and field
ssp. *partitum*	2x	?	S Mexico, Guatemala	borders
S. clarum	2x	?	S Mexico, Guatemala	high mountain forests
Series Pinnatisecta				
S. brachistotrichum	2x	1	NW Mexico	dry pinon scrub vegetation
S. *cardiophyllum*				
ssp. *cardiophyllum*	2x	1	C Mexico	weeds of cultivation
ssp. *ehrenbergii*	2x	1	C-NW Mexico	dry scrub vegetation, fields
ssp. *lanceolatum*	2x	1	C-S Mexico	borders, old lava fields
S. hintonii	?	?	C Mexico	by stonewalls, among low shrubs under trees
S. jamesii	2x	?	NW Mexico, SW USA	dry scrub vegetation
S. x *michoacanum*	2x	1	C Mexico	damp grassy fields, among rocks
S. nayaritense	2x	?	W Mexico	maize fields; probably normally distributed in natural vegetation
S. pinnatisectum	2x	1	C Mexico	cultivated fields, waste places and field borders
S. x *sambucinum*	2x	?	C Mexico	weed of fields and field borders
S. stenophyllidium	2x	?	W-C Mexico	dry hilly rangeland
S. tarnii	2x	1	C Mexico	open vegetation of small shrubs and herbs, among rocks with pine or oak
S. trifidum	2x	1	W Mexico	oak and pine forests, maize fields, roadsides

Species	Ploidy	EBN	Country	Habitat
Series Polyadenia				
S. lesteri	2x	?	C Mexico	damp mountain forests
S. polyadenium	2x	?	C Mexico	dry stony hillsides, by old walls, on old lava, among trees and shrubs
Series Longipedicellata				
S. fendleri				
ssp. *arizonicum*	4x	2	N Mexico, SW USA	pine forest clearings and roadsides
ssp. *fendleri*	4x	2	N Mexico, SW USA	dry pine-oak forests
S. hjertingii	4x	2	NE Mexico	dry pinon scrub
var. *physaloides*	?	?	NE Mexico	among *Agave* and herbs
S. matehualae	4x	?	NE Mexico	field borders
S. papita	4x	2	NW Mexico	open juniper, oak and pine woodland and scrub among rocks and herbs
S. polytrichon	4x	2	N-C Mexico	*Opuntia* scrub, in wastelands, as field weed
S. stoloniferum				
ssp. *stoloniferum*	4x	2	C Mexico	dry plateaus, valleys and hillsides
ssp. *moreliae*	4x	?	C Mexico	dry plateaus, valleys and hillsides
S. x *vallis-mexici*	3x	?	C Mexico	woods, fields and waysides
Series Demissa				
S. brachycarpum	6x	4	C Mexico	pines and *Abies* forests
S. demissum	6x	4	N-C and C Mexico, Guatemala	pine and *Abies* forests, weed of fields and field borders
S. x *edinense*				
ssp. *edinense*	5x	?	no natural distribution	no natural distribution
ssp. *salamanii*	5x	?	C Mexico	weed of potato fields
S. guerreroense	6x	4	W-C Mexico	pine-oak forests
S. hougasii	6x	4	W-C Mexico	pine-*Abies* forests
S. iopetalum	6x	4	E-C Mexico	pine-oak forests
S. schenckii	6x	?	S-C Mexico	pine forests, among bushes
S. x *semidemissum*	5x	?	C Mexico	field weed, along hedges and waysides, sometimes pine forests

Species	Ploidy	EBN	Country	Habitat
Series Conicibaccata				
S. agrimonifolium	4x	2	S Mexico, Guatemala, Honduras	cloud forests
S. longiconicum	4x	?	Costa Rica, Panama	humid forests, clearings, roadsides
S. oxycarpum	4x	2	S-C Mexico	humid pine forests and clearings
S. woodsonii	?	?	Costa Rica, Panama, Venezuela	high mountain meadows
Series Tuberosa (wild)				
S. verrucosum	2x	2	S-N-C Mexico	pine, fir and oak forests
Series Tuberosa (cultivated)				
S. tuberosum				
ssp. *andigena*	4x	4	Argentina, Bolivia, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Peru, Venezuela	cultivated in mountains
ssp. *tuberosum*	4x	4	S-C Chile, worldwide	cultivated at lower altitudes

E, W, S, N and C refer to geographical areas within the countries (i.e. east, west, south, north and central, respectively).

1978; Pandy, 1962). In any case, they typically lead to a stoppage of pollen tube growth usually in the upper portion of the style, thus preventing fertilization of the egg.

Also, the successful development of the endosperm, the tissue nurturing the embryo, is another means of control in seed development. In potato, an EBN hypothesis (Johnston *et al.*, 1980) has been put forth which states that maternal and paternal EBNs must be in a 2:1 ratio in the endosperm for seed development to occur. EBNs are independent of ploidy but can be thought of as the “effective ploidy” of the parent. EBNs have been assigned to most of the species and can be used to group the

Figure 1: Distribution of Wild Potato Series in North and Central America
(Reproduced with permission from Hawkes, 1990)

species into crossing groups (Johnston and Hanneman, 1980; 1982). For Mexico and Central America there are ten 2x(1EBN), one 2x(2EBN), eight 4x(2EBN), one 4x(4EBN), and five 6x(4EBN) species that have been identified. Costa Rica has one 4x(2EBN) and one 4x(4EBN) species within its boundaries.

EBN theory says that when the EBNs match, those species are potential partners for hybridization, barring the occurrence of stylar barriers (Figure 2). It also means that if 2n gametes are present they can also match with a higher ploidy level, since EBNs are additive. For example, a 2x(1EBN) species can only cross with other 2x(1EBN) species, but if it has 2n gametes, then it has the potential to behave like and cross with 4x(2EBN) species as well.

Why should time be spent discussing crossing barriers, 2n gametes and EBNs? Because these are the keys to understanding and predicting the potential natural hybridization that may occur between transgenic plants and native species in their vicinity. It must be recognized that there are some details which are not known, and sometimes one is faced with making the best estimate possible, based on information at hand. This should encourage caution.

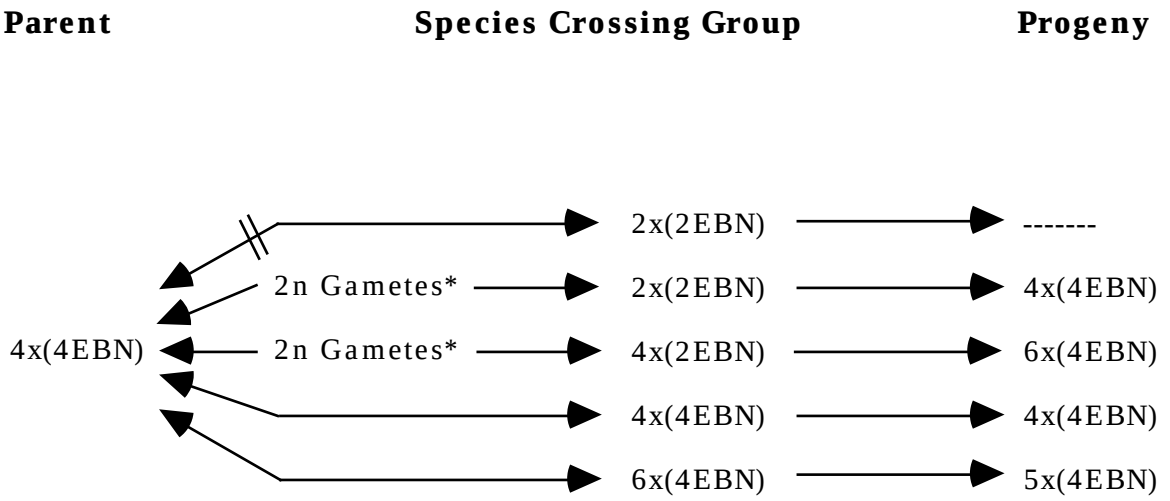
Figures 3.1-3.4 from a report of the National Research Council (NRC, 1989) provide us with a framework for decision making with regard to risk assessment. They deal with the questions of familiarity, confinement and environmental effects. In general, one can answer most of the questions dealing with familiarity quite readily, but questions concerning confinement and environmental effects are areas where answers are less definitive and where one may not have the necessary data.

With these questions in mind, let us examine the possibility of gene exchange for the species in Mexico and Costa Rica (see Table 1). The commonly cultivated potato, the likely transgenic candidate, is 4x(4EBN). This means that it can cross directly with a 6x(4EBN) and 4x(4EBN) species as well as with those 2x(2EBN) and 4x(2EBN) species that form 2n gametes, provided there are no stylar barriers. It cannot cross with 2x(1EBN) species because of EBN and ploidy differences.

Potential for Gene Exchange in Costa Rica

In Costa Rica, the two wild species known are both in series *Conicibaccata* (Table 1). *S. longiconicum* is 4x, but neither the chromosome number nor EBN of *S. woodsonii* is known. Based on the fact that all of the other 4x wild species in North America are 2EBN,

Figure 2: Potential Crosses and Progeny from Hybridizations of 4x(4EBN) Potato Plants with Various Crossing Groups based on 2n Gametes and Endosperm Balance Number (EBN)



* Only possible with 2n Gametes from 2EBN parent.

S. longiconicum and *S. woodsonii* are likely to be 2EBN as well. A form of the cultivated species 4x(4EBN) *S. tuberosum* ssp. *andigena* has been collected in Costa Rica as well.

The wild species are found in humid forests, clearings, roadsides, or high mountain meadows. These habitats do not suggest that they are weeds of cultivation, but one could speculate that they may be found in proximity to crop fields. Nevertheless, since the likely EBN of *S. longiconicum*, and probably that of *S. woodsonii,* does not match that of cultivated ssp. *tuberosum*, it is unlikely that hybridization will occur unless a 2n gamete would function. It is unknown whether 2n gametes occur among these species, but they do occur in closely related *S. oxycarpum* (den Nijs and Peloquin, 1977).

The transgenic 4x(4EBN) ssp. *tuberosum* could hybridize with the native ssp. *andigena*, as it could with other varieties of ssp. *tuberosum*.

Figure 3.1: Framework to Assess Field Testing of Genetically Modified Plants (from NRC, 1989)

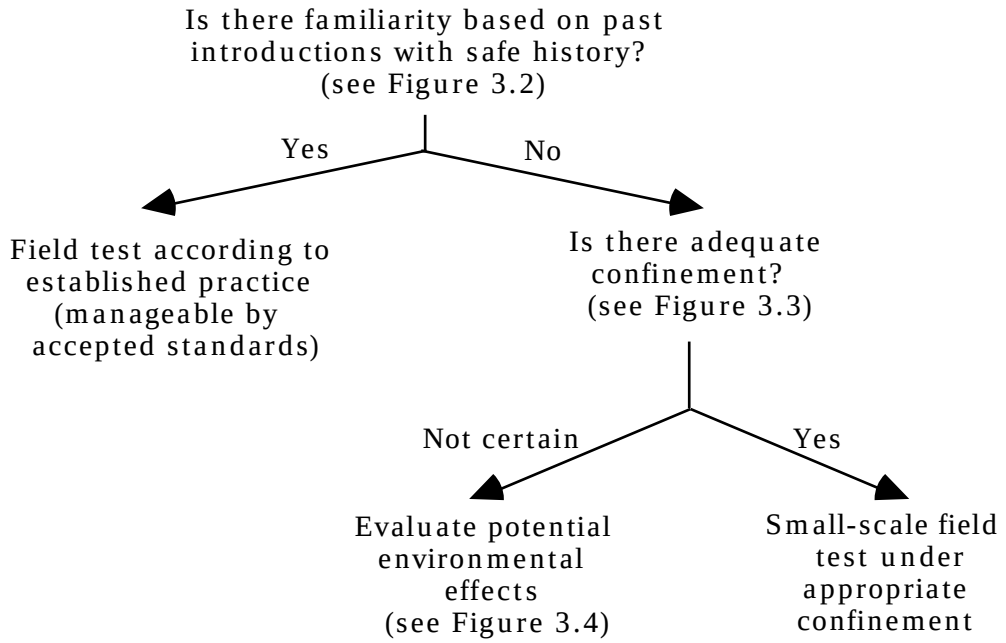


Figure 3.2: Familiarity (from NRC, 1989)

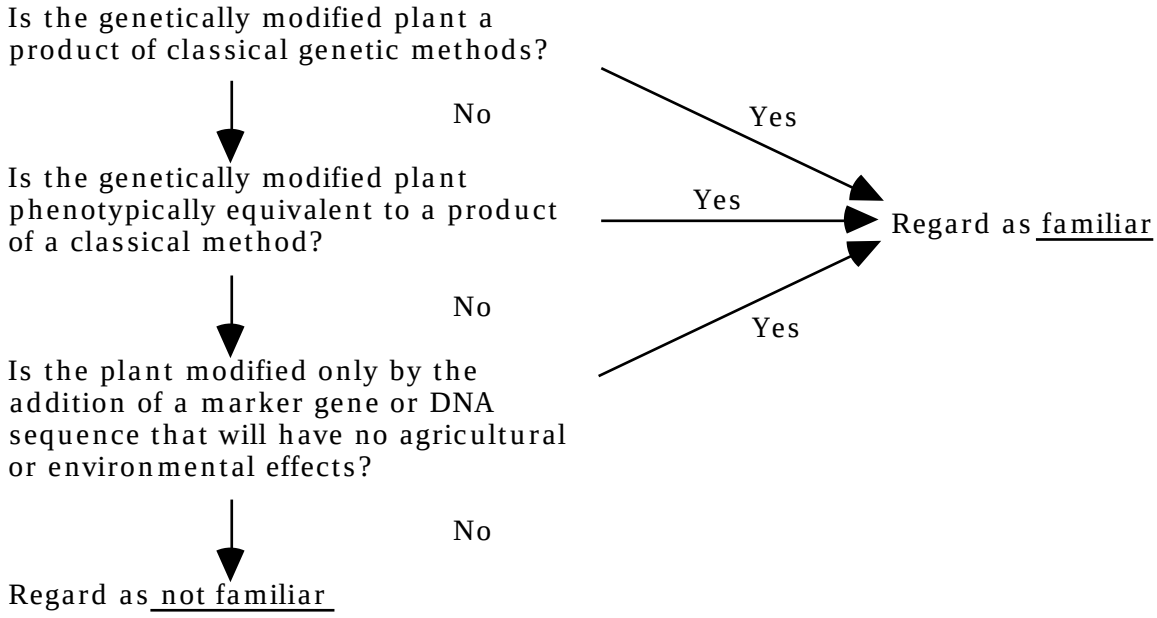


Figure 3.3: Confinement (From NRC, 1989)

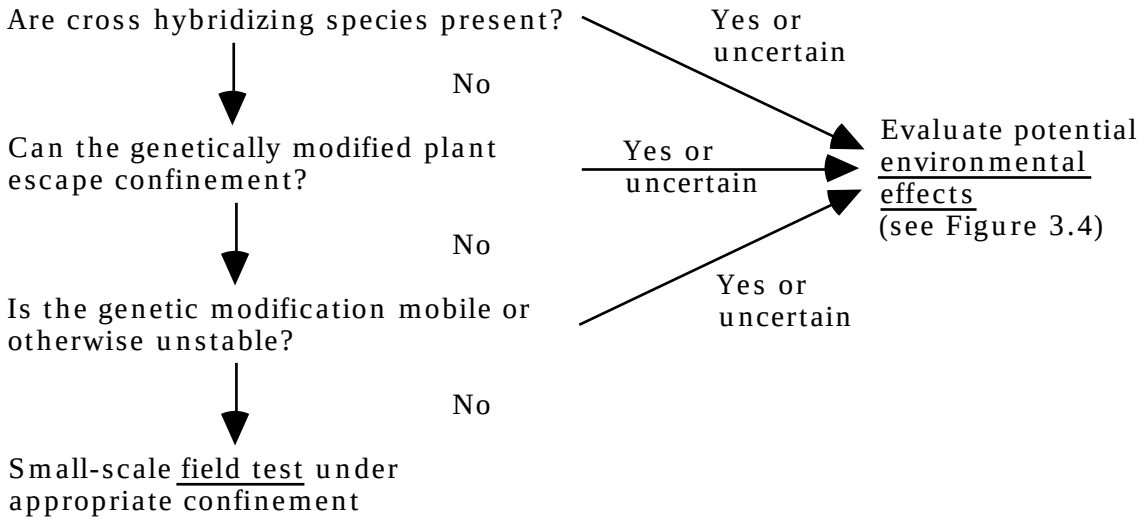
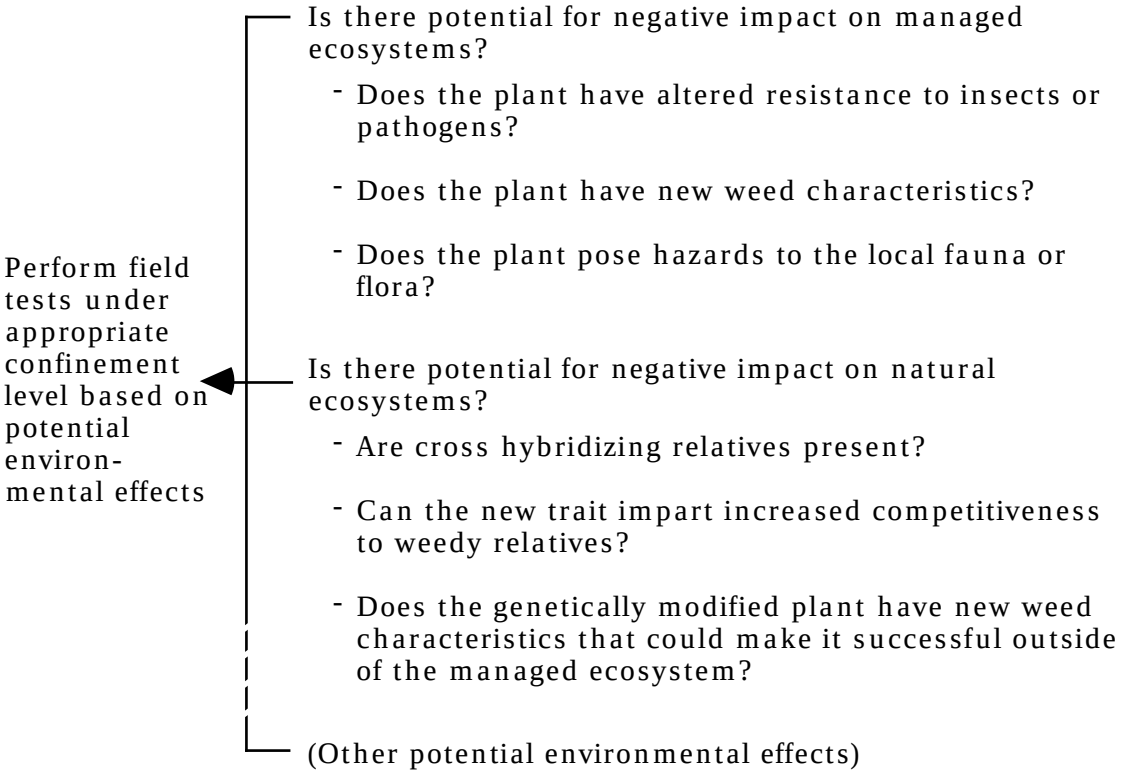


Figure 3.4: Potential Environmental Effects: Appropriate Questions for Specific Applications to be added by Users of the Framework (From NRC, 1989)



But since the ssp. *andigena* gene pool is small, chances of this occurring would seem remote and certainly placement of plots away from the areas where *andigena* cultivars are grown could significantly reduce this possibility.

In summary, the potential for the introduction of genes from transgenic 4x(4EBN) ssp. *tuberosum* plants into the wild species of Costa Rica seems minimal. If there are concerns, the areas where the native cultivated species are grown or where wild species are known to grow could be avoided.

Potential for Gene Exchange in Mexico

Mexico is home to the greatest number of wild species known in North America. It is a major center of diversity for potatoes (Hawkes, 1990). It has 2x(1EBN), 2x(2EBN), 4x(2EBN), and 6x(4EBN) wild species and 4x(4EBN) cultivated species. The 2x(1EBN) species are unlikely candidates for hybridization because of the disparity in EBN and ploidy differences. The possibility exists for 4x(4EBN) transgenic plants to hybridize directly with 6x(4EBN) wild species and 4x(4EBN) cultivated species, and with 4x(2EBN) and 2x(2EBN) wild species through 2n gametes. Of these, only *S. demissum, S. matehualae, S. polytrichon, S.* x *vallis-mexici* (3x), *S.* x *edinense* ssp. *salamanii* (5x), and *S.* x *semidemissum* (5x) have been found in or on borders of potato fields. Others probably encroach upon or grow in the vicinity of these fields. The native 4x(4EBN) cultivated form ssp. *andigena* is grown here, and could cross directly with transgenic 4x(4EBN) ssp. *tuberosum* plants, as could commonly grown 4x(4EBN) ssp. *tuberosum* cultivars. Hybridization between the hexaploid species and cultivated 4x(4EBN) varieties is known to occur as is evidenced by the two naturally occurring pentaploid hybrids, S. *edinense* ssp. *salamanii* and S. *semidemissum*. (Hawkes, 1990; Ugent, 1967; 1968).

2n gametes are known to occur at a relatively low frequency for the 4x(2EBN) species and for the one 2x(2EBN) species (den Nijs and Peloquin, 1977; Watanabe, 1988). So while hybrids are possible with these, chances for hybridization are remarkably low. If it did occur, crosses with 4x(2EBN) species would yield 6x(4EBN) hybrids and they would not be able to cross with the 4x(2EBN) species because of EBN differences, unless 2n gametes functioned. Hybridization with 6x(4EBN) species is likely if they are in proximity, and this would be a major concern.

The progeny would be 5x(4EBN) and could cross to the hexaploid species or to the commonly cultivated 4x(4EBN) native or commercial

varieties. The 2x(2EBN) species could cross with the 4x(4EBN) transgenic plants via 2n gametes yielding 4x(4EBN) hybrids capable of crossing to other 4x(4EBN) or 6x(4EBN) species. A major stylar barrier is known to occur between 2x(1EBN) species and Tuberosum haploids when the latter are used as females (Novy and Hanneman, 1991). It is not known if such a barrier exists for the 4x(2EBN) species or for the 6x(4EBN) species. If it does, it probably is not as stringent as at the 2x level.

The potential of introduction of genes from transgenic 4x(4EBN) ssp. *tuberosum* plants to native species in Mexico is possible, particularly into the 6x(4EBN) species and the 4x(4EBN) cultivated native and commercial varieties.

Possible Containment Measures

When there is a threat of introduction of "foreign" genes into native populations, one must consider the environmental risk and then decide if containment measures should be taken. If necessary, what should they be? Let us assume that the possibility of introduction of 'foreign' genes is judged to be significant. What can be done? In Mexico, one could avoid areas where the threat is greatest, where 6x(4EBN) species are endemic (Figure 1) and/or where native cultivated forms are grown. This would be the simplest means of control.

Secondly, one could determine if insect vectors are present that could transfer pollen in the proposed test areas. The common pollinators of potato are bees (usually bumble bees) that are capable of vibrating the flowers to collect the pollen. Unlike honey bees, bumble bees live in small groups, and are very hard workers. Typically they will be found to have their nests in the field or immediate surrounding area. They generally forage close to their nests, but will fly several kilometers to forage if necessary (Heinrich, 1979). Knowing the distance they commonly fly would help in setting up safe distances from other cultivated and wild species in the area. Their absence would substantially reduce the chance for natural cross pollination.

There may be other pollinators of potato, but little work has been done to identify them. If others are known, their habits and behavior should be taken into account.

In addition to physical barriers, biological barriers such as male sterility could be used. Often US and Canadian potato varieties are male sterile or have reduced fertility. If one chose a male sterile variety for transformation, this would significantly reduce the chance of gene

exchange, even if it were female fertile, since bees tend not to visit male sterile flowers (Arndt *et al.*, 1990; Sanford and Hanneman, 1981). This would be a 'safe' answer even in areas where cross compatible species exist. If one knew the stylar barrier relationship between the species and the varieties, one might also be able to use stylar barriers to reduce the chances of crossing with native species.

Conclusions

In summary, the chance of genes being introduced into native populations of potato species seems to be relatively small in Costa Rica. The only concern is with its native varieties of ssp. *andigena,* and if it were judged to be of concern this could be dealt with by avoiding having plots in these areas. In Mexico, there is a possibility for the introduction of genes from transgenic 4x(4EBN) ssp. *tuberosum* plants into the hexaploid wild species and the cultivated native tetraploid species if precautions are not taken. If this were judged important, it could be dealt with through containment measures such as avoiding areas where native species grow, selecting areas where the natural insect vectors are not present, or through the use of male sterile transgenic plants. These precautions would greatly reduce the risk of gene transfer.

The decision as to the significance of the risk has scientific as well as social and political implications. One can make judgments based on science as has been done in this chapter. One can pass the information through the scheme (Fig. 3.1-3.4) suggested by the National Research Council report (NRC, 1989) to help determine if containment measures need to be implemented. These may serve as helpful guidelines and can, of course, be adjusted to meet local/national needs.

Finally, whether or not the scientific community agrees that there is risk, there is the matter of social and political concern—both national and local (Siddhanti, 1991; Tait, 1988). There may be a strong aversion to the introduction and/or use of any plant with a foreign gene. There may be no market for the product if it is of transgenic origin, just because it is transgenic, and there may be fear or concerns about how else this gene may affect the product.

These are questions that express the reality of present day concerns and public awareness. These questions may be more important to the testing and introduction of a new product than the science that has been behind its development and evaluation, and the efforts to make its testing in the environment as safe as possible. The fear of the unknown is an overpowering fear innate to mankind, even in this modern world. So the

risks—scientific, social and political—must be weighed as one considers the introduction of transgenic plants into centers of diversity, with the decision falling on the shoulders of those scientists and politicians of the countries concerned. It is hoped that this discussion will help form a framework which can be modified to fit their needs as they wrestle with this difficult question.

References

Abdalla, M. M. F. and J. G. Hermsen. 1972. Unilateral incompatibility: hypothetical debate and its implications for plant breeding. *Euphytica* 21:32-47.

Arndt, G. C., J. L. Rueda, H. M. Kidane-Mariam and S. J. Peloquin. 1990. Pollen fertility in relation to open pollinated true seed production in potatoes. *American Potato Journal* 67:499-505.

Baker, H. G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5:1-24.

Camadro, E. L. and S. J. Peloquin. 1981. Cross incompatibility between the sympatric polyploid *Solanum* species. *Theoretical and Applied Genetics* 60:65-70.

Christou, P., W. F. Swain, N. S. Yang and D. E. McCabe. 1989. Inheritance and expression of foreign genes in transgenic soybean plants. *Proceedings of the National Academy of Sciences* (USA) 86:7500-7504.

Decker, D. S. and H. D. Wilson. 1987. Allozyme variation in the *Cucurbita pepo* complex: *C. pepo* var. *ovifera* vs. *C. texana*. *Systematic Botany* 12:263-273.

den Nijs, T. P. M. and S. J. Peloquin. 1977. 2n gametes in potato species and their function in sexual polyploidization. *Euphytica* 26:585-600.

Dionne, L. A. 1961. Mechanism of interspecific incompatibility in tuber-bearing *Solanum* species. *American Potato Journal* 38:73-77.

Doebley, J. F. 1984. Maize introgression into teosinte—a reappraisal. *Annals of Molecular Botany Gard*. 71:1100-1113.

Fraley, R. T., S. G. Rogers and R. B. Horsch. 1986. Genetic transformation in higher plants. *Critical Reviews in Plant Sciences* 4:1-46.

Fritz, N. K. and R. E. Hanneman, Jr. 1989. Interspecific incompatibility due to stylar barriers in tuber-bearing and closely related non-tuber-bearing Solanums. *Sex, Plant and Reproduction* 2:184-192.

Furner, I. J., G. A. Huffman, R. A. Amasino, D. J. Garfinkel, M. P. Gordon and E. W. Nester. 1986. An *Agrobacterium* transformation in the evolution of the genus *Nicotiana*. *Nature* 319:422-427.

Grun, P. and M. Aubertin. 1966. The inheritance and expression of unilateral incompatibility in *Solanum*. *Heredity* 21:131-138.

Hanneman, R. E. Jr. and J. B. Bamberg. 1986. Inventory of tuber-bearing *Solanum* species. *Agricultural Experimental Station Bulletin* 533: 216.

Hawkes, J. G. 1990. *The Potato: Evolution, Biodiversity and Genetic Resources*. Belhaven Press, London and Smithsonian Institute Press, Washington DC. 259 p.

Heinrich, B. 1979. *Bumblebee Economics*. Harvard University Press, Cambridge. 245 p.

Hermsen, J. G. T. 1978. Factors controlling interspecific crossability and their bearing on the strategy for breaking barriers to intercrossing of tuber-bearing *Solanum* species. pp. 311-318 In *Proceedings of the Conference on Broadening the Genetic Base of Crops*. Zeven, A. C. and A.M. Harten (eds.). Wageningen.

Holm, L. G., D. L. Plucknett, J. V. Pancho and J. P. Herberger. 1977. *Sorghum halepense* (L.) Pers. pp. 54-61. In *The World's Worst Weeds*. The East-West Center, University of Hawaii, Honolulu.

Jain, S. K. 1977. Genetic diversity of weedy rye populations in California. *Crop Science* 17:480-482.

Johnston, S. A. and R. E. Hanneman, Jr. 1980. Support of the Endosperm Balance Number hypothesis utilizing some tuber-bearing *Solanum* species. *American Potato Journal* 57:7-14.

Johnston, S. A. and R. E. Hanneman, Jr. 1982. Manipulations of Endosperm Balance Number overcome crossing barriers between diploid *Solanum* species. *Science* 217:446-448.

Johnston, S. A., T. P. N. den Nijs, S. J. Peloquin and R. E. Hanneman, Jr. 1980. The significance of genic balance to endosperm development in interspecific crosses. *Theoretical and Applied Genetics* 52:5-9.

Keeler, K. H. 1989. Can genetically engineered crops become weeds? *Biotechnology* 7:1134-1139.

McCammon, S. L. and T .L. Medley. 1990. Certification for the planned introduction of transgenic plants into the environment. pp. 233-250. In *The Molecular and Cellular Biology of the Potato.* Vayda, M. E. and W. D. Park (eds.). C.A.B. International, Wallingford.

Miller, J. H. and B. Edwards. 1983. Kudzu: Where did it come from? And how do we stop it? *Southern Journal of Applied Forestry* 7:165-169.

Mooney, H. A. and J. A. Drake. 1990. The release of genetically designed organisms in the environment: Lessons from the study of the ecology of biological invasions. pp. 117-129. In *Introduction of Genetically Modified Organisms into the Environment.* Mooney, H. A. and G. Bernardi (eds.). John Wiley and Sons, New York.

Nester, E. W., M. P. Gordon, R. M. Amasino and M. F. Yanofsky. 1984. Crown gall: A molecular and physiological analysis. *Annual Reviews in Plant Physiology* 35:387-413.

Novy, R. G. and R. E. Hanneman, Jr. 1991. Hybridization between Gp Tuberosum haploids and 1EBN wild potato species. *American Potato Journal* 63:151-169.

NRC. 1989. *Field Testing Genetically Modified Organisms-Framework for decisions*. National Research Council (NRC). National Academy Press, Washington DC. 170 p.

Pandy, K. K. 1962. Interspecific incompatibility in *Solanum* species. *American Journal of Botany* 49:874-882.

Sanford, J. C. and R. E. Hanneman, Jr. 1981. The use of bees for the purpose of intermatings in potato. *American Potato Journal* 58:481-485.

Sauer, J. D. 1967. The grain amaranths and their relatives: A revised taxonomic and geographic survey. *Annals of Molecular Botany Gard*. 54:103-137.

Second, G. 1982. Origin of the genetic diversity of cultivated rice (*Oryza* spp.): study of the polymorphism scored at 40 isozyme loci. *Japanese Journal of Genetics* 57:25-57.

Siddhanti, S. K. 1991. *Multiple Perspectives on Risk and Regulation*. Garland Publishing Co., New York. 339 p.

Simberloff, D. 1985. Predicting ecological effects of novel entities: Evidence from higher organisms. pp. 152-161. In *Engineered Organisms in the Environment: Scientific Issues*. Halvorson, H. O., D. Pramer, and M. Rogul (eds.). American Society for Microbiology, Washington DC.

Simmonds, N. W. (ed.). 1979. *Evolution of Crop Plants*. Longman, New York. 339 p.

Smith, J. S. C., M. M. Goodman and R. N. Lester. 1981. Variation within teosinte. I. Numerical analysis of morphological data. *Economic Botany* 35:187-203.

Spencer, T. M., J. V. O'Brien, W. G. Start, T. R. Adams, W. S. Gordon-Kamm and P. G. Lemaux. 1992. Segregation of transgenes in maize. *Plant Molecular Biology* 18:201-210.

Suneson, C. A., K. O. Rachie and G. S. Khush. 1969. A dynamic population of weedy rye. *Crop Science* 9:121-124.

Tait, J. 1988. Public perception of biotechnology hazards. pp. 119-128. In *Hazards of Biotechnology: Real or Imaginary?* Dayan, A. D., P. N. Campbell and T. H. Jukes (eds.). Elsevier Applied Science, New York.

Tiedje, J. M., R. K. Colwell, Y. L. Grossman, R. E. Hodson, R. E. Lenski, R. N. Mack and P. J. Regal. 1989. The planned introduction of

genetically engineered organisms: Ecological considerations and recommendations. *Ecology* 70:298-315.

Tucker, J. M. and J. D. Sauer. 1958. Aberrant *Amaranthus* populations of the Sacramento-San Joaquin delta, California. *Madrono* 14:252-261.

Ugent, D. 1967. Morphological variation in *Solanum* x *edinense,* a hybrid of the common potato. *Evolution* 21:696-712.

Ugent, D. 1968. The potato in Mexico: Geography and primitive culture. *Economic Botany* 22:108-123.

Warwick, S. I. and L. D. Black. 1983. The biology of Canadian weeds. 61. *Sorghum halepense* (L.) Pers. *Canadian Journal of Plant Sciences* 63:997-1014.

Warwick, S. I., B. K. Thompson and L. D. Black. 1984. Population variation in *Sorghum halepense,* Johnson grass, at the northern limits of its range. *Canadian Journal of Botany* 62:1781-1790.

Watanabe, K. 1988. Occurrence, frequency, cytology and genetics of 2n pollen: and sexual polyploidization in tuber-bearing Solanums. 267 p. Ph.D. Thesis. University of Wisconsin-Madison, Madison.

Section 2

Regulatory Philosophies and Government Experiences in Developing and Enacting Biosafety Regulations

Chapter 2.1

A Regulatory Perspective on Harmonization of Regulations and Public Perception[1]

Terry L. Medley
Acting Administrator, Food Safety Inspection Service (FSIS)
Animal and Plant Health Inspection Service (APHIS)
United States Department of Agriculture (USDA)
Administration Building, 14th and Independence Avenue SW
Washington DC, 20250 USA.

Harmony is to be valued and avoidance of wanton opposition to be honored. When those above are harmonious and those below well disposed towards one another, there is concord in the discussion of business. Right views of things spontaneously gain acceptance, then what is there which cannot be accomplished?

≈ Confucius ≈

Medley, T.L. 1994. A Regulatory Perspective on Harmonization of Regulations and Public Perception. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 71-78.

Introduction

Biotechnology is an enabling technology with broad application to many different areas of industry and commerce. For agriculture, biotechnology has the potential to increase productivity, enhance the environment, and improve food safety and quality. The challenge, however, is whether we will be able to strike the proper balance of direction and oversight to allow this technology to be safely applied (OTA, 1992). To meet this challenge we will need to effectuate changes and paradigm shifts.

When dealing with the concept of effectuating change, or having new paradigms, one needs effective change-agents. I believe that everyone reading this book is potentially an effective change-agent. One has to possess the ability to go out and discuss the importance of this technology—biotechnology—to humankind and to lay a foundation for technology development and transfer. We should all view this as a very difficult task but one which, fortunately, we have an opportunity to carry out.

It is not very often that one has this opportunity to be on the cutting edge of a new scientific revolution whose safe application holds great potential benefit to humankind. But for humankind as a whole to be able to benefit, particularly the less-privileged friends in many developing countries, we need to be able to facilitate the safe transfer of this technology and the harmonization of the regulatory review process as key elements.

Principles of the Regulatory Review Process

Achieving the desired goals of expanded development, safe technology transfer and commercialization of agricultural biotechnology products requires that high priority be placed on utilization of appropriate "oversight" structures. "Oversight" refers to the application of appropriate laws, regulations, guidelines, or accepted standards of practice to control the use of a product based on the degree of risk or uncertainty associated with it. In the area of regulations and the implementation of mandatory review requirements, it is of paramount importance that these requirements be balanced and commensurate with risk (Miller *et al.,* 1991).

If structured and administered properly, regulations can facilitate rather than impede expanded development, safe technology transfer, and commercialization of agricultural biotechnology products. Regulations should prevent or at least mitigate risks and not inhibit innovation and

product development. Development of regulations which neither over-regulate nor under-regulate is a most formidable task for any national authority (McCammon and Medley, 1990).

Specifically, the national authority must ensure that the regulatory structure adequately considers health and environmental safety standards as biotechnology applications are transferred from laboratory to field to marketplace. The exact nature of regulatory structures will have a direct impact on the potential contribution of agriculture to the country's economy. It will also directly impact the competitiveness of the country's agricultural production in both domestic and world markets (Office of Science and Technical Policy, 1984; Schiffbauer, 1985). For agricultural biotechnology, as in many other high technology industries, national regulatory structures are a critical determinant of the time and of the cost of bringing a product to the market. The cost of testing to meet regulatory requirements, the potential for delay in regulatory approval, and the uncertainty associated with possible imposition of extensive restrictions or outright disapproval of new agricultural biotechnology research or production could present substantial barriers to product development.

In the USA, national authorities have sought to eliminate unneeded regulatory burdens from all phases of the development of new biotechnology products. This includes laboratory and field experiments, product development, and eventual sale and use. To provide guidance in determining the level and type of necessary oversight or regulatory review, the following "Four Principles of Regulatory Review" were developed (PCC, 1991):

1) federal government regulatory oversight should focus on the characteristics and risks of the biotechnology product and not the process by which it is created;
2) for biotechnology products that require review, regulatory review should be designed to minimize regulatory burden while assuring protection of public health and welfare;
3) regulatory programs should be designed to accommodate the rapid advances in biotechnology; and
4) in order to create opportunities for the application of innovative new biotechnology products, all regulation in environmental and health areas should use performance standards rather than specifying rigid controls or specific designs for compliance.

In the establishment of risk based regulations, the technique or process by which an agricultural product is modified should not be the sole litmus test for determination of risk. Although knowledge about the

process used is useful in assessing the characteristics of the modified organism, use of new molecular techniques does not establish *a priori* risk. The goal of the above principles is to ensure that regulations and guidelines affecting biotechnology are based solely on the potential risks and are carefully constructed and monitored to avoid excessive restrictions that curtail the benefits of biotechnology to society.

Harmonization of Regulatory Requirements

Harmonization and collaboration are essential in our global society and global village. When we say harmonization, or harmonization of regulatory requirements, this does not mean everyone should have the *same* regulatory requirements, but that they should have *equal* or *equivalent* standards. Such equivalent standards provide a base on which we can cooperate. At the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA), we are committed to the policy of the USA in promoting both national and international harmony and cooperation as it relates to biotechnology.

At APHIS, we have divided the concept of international harmonization into a three-pronged proposition. First, when we talk about international harmonization, we are talking not about the same or identical national approaches, but national approaches that are consistent and compatible. These are key elements: *consistency* and *compatibility*.

Secondly, when we look at how we are going to achieve our goal, *coordination* becomes the key; we must have effective coordination of those national approaches. Such coordination can occur in a number of different ways (i.e. bilateral country discussions, participation in international organizations, or meetings and agreements).

Lastly, the third and main tenet of our international harmonization efforts and any appropriate oversight structure, must be looking at scientific principles for evaluation of organisms. I am not talking about various policies, priorities or strategies, or socioeconomic factors which vary from country to country, but established biologically sound scientific principles for the evaluation of organisms.

We should realize that technology transfer is based on a cultural, attitudinal and institutional process that generally cannot be regulated or directed by legal mandate. Therefore, our roles as change-agents become even more important because we have to effectuate positive public perception. It is axiomatic that positive public perception leads to public acceptance, but the latter is, in turn, the necessary basis for efficient technology transfer.

Public Acceptance through Effective Communication

Public acceptance of applications of biotechnology is a prerequisite for technology transfer, commercialization, and utilization of the products of agricultural biotechnology. Regulatory systems must include procedures that ensure an opportunity for meaningful public participation in decision making (Shapiro, 1990). Participation is essential to public acceptance of biotechnology. The general public can learn enough about biotechnology to be comfortable or uncomfortable with it and make informal decisions about it. When we feed the public appetite with exotic tales of our technological future, we tend to arouse equally exotic fears (Bendix, 1987). We must continue to feed the appetite and not arouse the fears; we must make the public comfortable, not uncomfortable. There is an array of ways to accomplish this task. One of the most essential is effective communication.

There are two principles that should underlie the approaches chosen for communication. First, when writing about biotechnology, one should clearly identify the audience as well as the goal of the specific communication. Secondly, risk communication is an interactive process of exchange of information and opinion.

There is something magical about the written word. Thoughts that appear in print are deemed true or at least more credible. Accompanying this awesome power is an even greater responsibility: accountability. Francis Crick offered the following advice on responsible scientific writing for the lay public: “Anyone writing on scientific matters for the lay public must try to avoid a number of hazards. He must not use excessive technical jargon or dwell too much on the many scientific details, or his readers will desert him. Especially, he must avoid oversimplification, or the science will become vacuous. In addition, he must not try to side-step difficult political, religious, and ethical problems, otherwise his writing will smack too much of the ivory tower. And yet he will do no one a service if, in an attempt to grab readers, he sensationalizes the issues involved” (*cit.* Zimmerman, 1984).

In 1989, the National Research Council of the USA published a report entitled *Improving Risk Communication*. The report was intended to “significantly improve the understanding of what the problems are in risk communication, particularly the risk communication activities of government and industry” (NRC, 1989).

The report provided some extremely helpful recommendations to significantly improve the risk communication process. The report concluded that “risk messages can be controversial for many reasons. The hazards they describe are often themselves centers of controversy.

Frequently, there is enough uncertainty in the underlying knowledge to allow different experts to draw contradictory conclusions. Experts are frequently accused of hiding their subjective preferences behind technical jargon and complex, so-called objective analyses. Often a message that is precise and accurate must be so complex that only an expert can understand it. Messages that non-experts can understand necessarily present selected information and are thus subject to challenge as being inaccurate, incomplete, or manipulative" (NRC, 1989).

We should also pay attention to the fact that there is a crucial distinction between risk messages and the risk communication process. Risk communication is an interactive process of exchange of information and opinion among individuals, groups, and institutions. However, one should not be misled by this principle of risk communication. Improved risk communication will not always reduce conflict (NRC, 1989).

Regulatory requirements for agricultural biotechnology products must be scientifically defensible, and public perception must also be adequately addressed for efficient technology transfer and commercialization. The national authority must determine and balance the appropriate form and extent of meaningful public participation. For example, how should the public interest in reviewing the scientific data underlying a decision be balanced against industry's interest in protecting confidential information? What role, if any, should technical advisory committees play in the decision-making process? Can technical advisory committees, on a part-time basis, keep up with the rapidly expanding numbers of new products? Or is this best left to national authorities which use such committees for novel or complex submissions?

Conclusion

Approval for the commercialization of the first transgenic crop, the FLAVRSAVR™ tomato, occurred in the USA in May 1994. Many of these new plant varieties will be available in the 1990s. But their introduction will be under circumstances unlike any met by other new plant varieties because of the technology used to develop them. "Uncertainties over these new technologies raise questions of potential impacts on food safety and the environment, and possible economic and social costs. Nevertheless, there will be a push for ... biotechnology ... to be used commercially, adopted by industry, and accepted by the public ... The *challenge*, however, will be whether government, industry, and the public can strike the proper balance of direction, oversight, and allow these technologies to flourish." (OTA, 1992; emphasis added).

Strategic regulations have the greatest potential for creating a framework or process to meet this challenge. For agricultural biotechnology, strategic regulations are regulations which provide a framework or process for actions that lead to consistent and planned results. Therefore, they are regulations that are *developed* and *applied* in a strategic manner. Strategic regulations are developed and applied in a comprehensive manner to avoid being one-dimensional or limited to a single issue focus. Although consideration of risk *versus* safety is of paramount importance, the regulations should also consider their impact on other concerns such as product verification and utilization, safe technology transfer, economic competitiveness, international harmonization, and global needs and acceptance. Consequently, strategic regulations have a multi-dimensional focus.

Biosafety reviews must focus on the scientific questions and the most efficient way of undertaking the review in the context of facilitating the safe application of the technology. Science must be the basis of the decisions that address the concerns associated with the application of biotechnology to agriculture. A necessary role for regulatory officials is to frame the questions and issues that science must answer.

Science is the foundation upon which regulatory officials can assure and build upon credibility, remain up-to-date, and assure a rational basis for decision making. Science and process are inextricably linked for strategic regulations that evaluate biological programs and products.

In the area of biotechnology regulation, science without a process to frame the issues becomes overwhelming and misleading; while process without science is reduced to being bureaucratic, self-serving, and ineffective.

Neither science or regulation can afford to be in an all-or-nothing category. To adapt to new information and new needs, both types of systems need to work together to frame and address the concerns and requirements of the many components of our society.

Strategic regulations can assist in achieving these goals by i) having identifiable science based triggers that are consistent, easily understood, and transparent; ii) by being effective and responsive as well as flexible and dynamic; and iii) by meeting domestic and international needs.

Disclaimer

The views expressed in this article are those of the author and do not necessarily represent those of the United States Government.

Note

1. The text of this chapter is based a manuscript being prepared by Medley, T.L. and S.A. McCammon for Vol. 12: Modern Biotechnology: Legal, Economic and Social Dimensions (in preparation).

References

Bendix, P. 1987. Strategies for Building Public Understanding of Biotechnology: The Talbot Group—Public Perceptions of Biotechnology. ARI.

McCammon, S. L. and T. L. Medley. 1990. Certification for the Planned Field Introduction into the Environment of Transgenic Plants and Micro-organisms. In *Biological Monitoring of Genetically Engineered Plants and Microbes*. D. R. MacKenzie and S. C. Henry (eds.). Purdue University, West Lafayette.

Miller, H. I., R. H. Burris, A. H. Vidaver and N. A. Wivel. 1991. Risk-Based Oversight of Experiments in the Environment. *Science* 250:490-491.

NRC. 1989. Improving Risk Communication, National Research Council (NRC). National Academy Press, Washington DC.

Office of Science and Technical Policy. 1984. Proposal for a Coordinated Framework for Regulation of Biotechnology. 49 Fed. Reg. 50856 (December 31, 1984). U.S. Government Printing Office, Washington, DC.

OTA. 1992. A New Technological Era for American Agriculture. U.S. Congress, Office of Technology Assessment (OTA), OTA-F-474. U.S. Government Printing Office, Washington DC.

PCC. 1991. Report on National Biotechnology Policy. President's Council on Competitiveness (PCC). Government Printing Office, Washington DC.

Schiffbauer, W. G. 1985. Regulating Genetically Engineered Microbial Products Under the Toxic Substances Control Act. *Environmental Law Reporter* 10279.

Shapiro, S. A. 1990. Biotechnology and the Design of Regulation. *Ecology Law Quarterly* 17.

Zimmerman, B. K. 1984. Biofuture Confronting the Genetic Era. Plenum Press, London & New York.

Chapter 2.2

Canadian Approaches to Biotechnology Regulation

Jean E. Hollebone
Director, Biotechnology Strategies and Coordination Office
Agriculture Canada
Nepean K1A 0Y9, Canada.

Louise Duke
Chief Variety Registration Officer
Plant Products Division, Agriculture Canada
Nepean K1A 0Y9, Canada.

Introduction

Canada manages biotechnology under a federal framework called the National Biotechnology Strategy, which reports to the Prime Minister of Canada through the Department of Industry. The strategy has five main components (Figure 1):

Hollebone, J.E. and L. Duke. 1994. Canadian Approaches to Biotechnology Regulation. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 79-90.

1) Research and Development (R&D) Networks. These comprise national supporting R&D Networks which bring together scientists, industry, government and academia to promote cooperation and communication. These are for nitrogen fixation; plant strain development; human and animal health care; cellulose utilization; waste treatment and mineral leaching; metals recovery; forestry; and aquaculture. Each Network is administered by a federal department, and membership is open to industry, university and government communities.
2) R&D incentives for cost-shared programs with industry and government through the Industrial Research Assistance Program (IRAP) (C$5 million/year allocation of federal funds).
3) Annual funding to federal departments for research and regulatory activities (C$6 million/year). This fund includes a large training component.
4) An Interdepartmental Committee on Biotechnology that coordinates federal activities, supported by a subgroup consisting of all regulatory agencies in government, with working committees on special issues such as communications, Organization for Economic Cooperation and Development (OECD) support, intellectual property, ethics, etc.
5) A multi-stakeholder National Biotechnology Advisory Committee (NBAC) drawn from academia, the private sector, industry and government. NBAC advises on new developments and policy requirements, and makes recommendations in annual and other reports. These are well respected and usually acted on; for example, the 1987-88 NBAC Report provided recommendations for a national regulatory framework, which were largely adopted in the federal regulatory framework announced in January 1993.

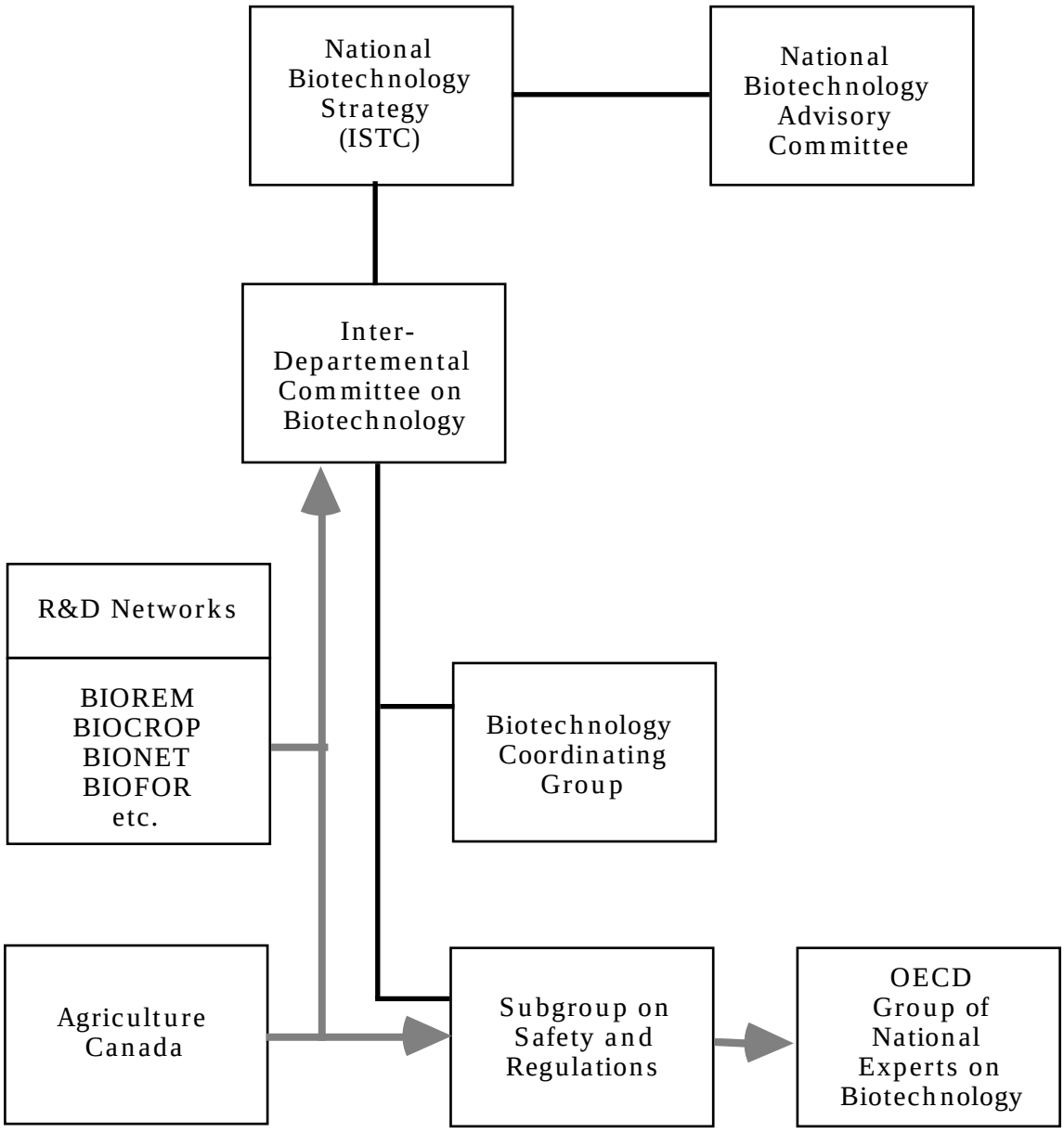
Departmental Mandates

Although over nine departments are involved in some aspect of biotechnology regulation, three departments in particular have specific mandates—the departments of Environment, Health and Agriculture.

Environment Canada, under the Canadian Environmental Protection Act and Regulations, regulates biotechnology products used in pollution control, mineral leaching, chemical residue destruction, waste disposal and novel uses not covered under other acts. In addition, enzymes, complex lipids, and other chemicals produced by biotechnology processes are regulated by the department.

Health and Welfare Canada regulates drugs and cosmetics, medical devices including *in vitro* diagnostic kits, and food products of

Figure 1: Organization of the Federal Biotechnology Framework



biotechnology, and sets allowable residue limits for biotechnological food additives and pest control agents, under the Food and Drugs Act and Regulations. The department also reviews health and safety considerations for products submitted under the Pest Control Products Act and Regulations and the Canadian Environmental Protection Act.

Agriculture Canada is the third major department, and regulates biotechnology products (Table 1).

Regulation of Transgenic Plants

Organizational Mechanisms

The Plant Products Division of Agriculture Canada is the lead agency for the review and assessment of the safety of genetically modified plants. However, it may call on other regulatory agencies to provide advice when and as needed. For example, the Pesticides Directorate provides evaluations for genetically modified plant material containing novel pest resistance properties, such as the delta endotoxin from *Bacillus thuringiensis*, and for herbicide tolerant plant combinations. All applications are reviewed by the Plant Protection Division for Plant Risk Assessment.

The Plant Advisory Committee provides advice on scientific and policy issues and meets on an annual basis. This multi-stakeholder group has representation from the seed trade, researchers, the biotechnology industry, academia and various levels and disciplines of government.

Regulatory Authority

Since 1987, the Plant Products Division has been authorizing field trials of transgenic crop plants, as well as plants with altered potential risk produced by mutagenesis. However, a review of legislative authorities and legal advice completed in 1993 indicated that powers for environmental assessment were weak and needed to be strengthened. New regulations are currently being adapted, and should be in place as early as 1995.

Table 1: Regulation of Agricultural Products of Biotechnology

Act	Areas/Products Regulated
Health of Animals Act	veterinary biologics
Feeds Act	livestock feeds and feed additives
Fertilizers Act	fertilizers and supplements
Pest Control Products	pesticides
Plant Protection Act	introduction and spread of plant pests
Seeds Act	seeds and other plant propagules
Various Acts related to food	food products inspection procedures

Genetically engineered plant materials are regulated for several reasons, namely because:

- the plants produced do not occur naturally;
- there is to date little familiarity with the new constructs and no great experience with these new organisms;
- the potential hazards to human and animal health and the environment are poorly characterized; and
- the potential impact of these plants on other organisms in the surrounding environment and ecosystems is poorly understood.

Scope of Regulations

Currently, the Plant Products Division regulates both plants which are genetically engineered and those which are developed by conventional breeding. In the USA, the scope definition exempts products produced by mutagenesis from regulatory oversight. The rationale for exemption is based on familiarity with such products with experience, and the knowledge that these products are of inherently low risk to health and the environment. Similar steps are being taken in Canada.

Regulatory Approaches

All agricultural products of biotechnology are regulated under the following general principles:

- the end-use product is regulated rather than the process, since this is the product that is used, sold, moved in commerce, released into the environment and to which humans are exposed;
- assessments are based on scientific evaluation of the risks, with a tiered system of increasing oversight with probability of increasing risk;
- a flexible approach building on existing as opposed to new legislation is adopted, and guidelines developed with provision for waivers based on an appropriate and accepted rationale;
- there is case-by-case assessment until experience is gained; and
- there is gradual reduction of oversight for categories of determined low risk.

Stages of Risk Assessment

Regulatory assessment of genetically modified plant material follows a stepped approach which parallels the stages in development and testing,

and provides for increasing scrutiny as the potential for environmental impact increases.

1) Laboratory/greenhouse—import permit may be required.
   - No environmental review required.
2) Confined field release—permit required.
   - Small-scale;
   - no food uses allowed;
   - no feed uses allowed; and
   - criteria set for containment.
3) Unconfined field trials—no permit for field testing.
   - Environmental assessment completed;
   - food safety review underway; and
   - no food use unless authorized.

Stage 1: Contained Research

Research in the laboratory and the greenhouse is considered to be contained, and is not currently regulated. Researchers are advised to follow the *Medical Research Council Guidelines* which are mandatory for all research sponsored by government funds, and local biosafety committees may establish additional operational criteria. Contained research may also be required to meet provincial labor laws which govern health and safety in the workplace.

Stage 2: Confined Research Trials

This stage involves trials involving genetically modified materials which are released into the environment. Trials are generally small in size, e.g. under 1 hectare. Following environmental assessments, authorization to conduct trials under specified conditions is granted if it has been determined that the trial will not result in adverse human and animal health, or environmental, problems.

Before proceeding to unconfined releases, assessment must provide assurance that larger-scale trials may proceed without significant adverse effects to the environment.

Stage 3: Unconfined Research Trials

The third stage involves precommercial trials, such as varietal testing which must occur in many different sites and under the supervision of a researcher with cooperators who may not be trained scientists. Hence at this stage, questions about effects on humans, animals and the environment must be answered.

At stage 3, data will be developed and assessed to support the safety of the transgenic plant product as human and/or animal food. The product, however, will not yet be released into the food chain but will be destroyed or used as seed in order to meet food safety requirements.

Stage 4: Commercial Release

To enter the stage of commercial use of a product, both the environmental and human safety reviews must be complete, and the use of the plant variety deemed to pose no unacceptable risk in use. No genetically modified plants have yet reached this stage (i.e. as of June 1994). Agricultural crop varieties must be registered for sale in Canada. Registration is based on genetic identity and agronomic merit.

Importation

Unregistered varieties that are imported into Canada must obtain import permits as well as authorization for use in field trials.

Data Requirements

Several types of data are required to support field trial applications:

- product identification;
- human or animal health safety;
- performance, including merit and value;
- environmental safety; and
- information on post-harvest land use.

1. Product Identification

The host species is examined for the gene that has been selected as well as its donor organism, in order to predict the possibility of transfer of toxic or weedy traits. The transfer of genes from the same species or from agricultural crops requires less scrutiny than genes being inserted from another species or genus. Possible effects of the inserted gene are examined, such as the biochemical pathway that is affected by the gene, the desired effect of the gene, and possible nontarget effects including potential production of toxic secondary metabolites. The mechanism of transformation and vector are also examined.

The species of the vector and its level of stability of insertion is examined. Since some vectors are plant pathogens, the vectors are

examined to determine whether or not they have been disarmed, and if so, what genes have been removed in order to disarm them and whether they may integrate into the host chromosome. Southern blot analysis is not currently required for *Agrobacterium tumefaciens* mediated transformation since it is known that genes are stably integrated into the nucleus. However, it is required for other forms of transformation. The regulatory sequences that have been attached to the desired genes are reviewed. Again, since regulatory sequences may be introduced from plant pathogens, the source of regulatory sequences is reviewed carefully.

Finally, consideration is made of the whole plant and plant genes that have been introduced from wild or weedy species. Where herbicide resistance has been inserted, greenhouse data is requested on any appropriate components of weediness, such as shattering and dormancy.

2. Human and Animal Safety

Potential impact on human health will depend on:

i) the nature of the test sites (e.g. laboratory or field);
ii) whether the donor plant is generally considered to be edible and whether the trial is going to produce potentially edible plant material;
iii) whether the trial is going to be treated with unregistered pesticides that have not been evaluated for human safety factors in the plant crop used;
iv) the nature of the gene product and the biochemical pathway affected by the gene product (e.g. the potential for production of toxic secondary metabolites); and
v) the fate of the harvested material, crop residues and residues from laboratory analysis.

At the present time, harvested transgenic plant material is not allowed to enter the human or animal food chain. Plant material may be fed to animals as part of toxicology studies required for the registration of a pesticide, but residue from laboratory analysis is not yet allowed to be used as animal feed.

3. Environmental Safety

The host species is examined with regard to its relative ability to compete in the environment. Wild or weedy relatives which may be present in the area of the test site are required to be identified, and the ability of the modified crop to cross with wild relatives is determined.

The stability of the vector and the nature of the genetic modification of the material that is going to be introduced to the environment are

examined in order to determine whether or not there may be any negative environmental impact. Here, possible transfer of a genetic modification of a pest species, and possible nontarget effects of the introduction of this plant material into the environment (such as plant pathogenicity) are examined. When looking at biopesticides in particular, the possibility of environmental dissemination and effects on nontargets is considered.

4. Performance Date

The impact of the actual field trial on the environment adjacent to the trial is examined. The protocol of the field test is reviewed to determine what provisions have been made to ensure reproductive isolation. Historically, this would be isolation distances, where the plant material is outside the crossing range of plant material of the same species or closely related species. Isolation distances are those used for the production of high generations of pedigreed seed. Isolation distances do not have to be kept fallow, but they have to be kept free of weedy relatives with which the transgenics could cross.

Other forms of reproductive isolation include pre-flowering harvest, removal or bagging of flowers, use of isolation cages or male sterile plant material.

5. Post-Harvest Land Use

Post-harvest land use is considered in the review of an application for field testing. The Plant Products Division requires that the land not be planted with a crop of the same species or similar species as the transgenic trial for a period of one to three years, depending on the species. In this way, the land can be monitored for volunteers in the subsequent growing seasons. The proposed post-harvest land treatment is also reviewed to ensure some method of destruction or disposal of the crop and seed, if harvested, has been identified. This ensures, once again, that volunteers will not be found in subsequent years.

Harvested seed may be analyzed for agronomic or quality characteristics or for pesticide residues. Seed may also be retained for future field trials. All seed must be disposed of as previously authorized or must be destroyed by an accepted method. Records must be kept as to the material handled and its disposal. As part of the post-harvest land treatment, the applicant must outline an appropriate program for the monitoring and destruction of volunteer plants and closely related weed species.

To the end of 1990-91, 102 field trials have been authorized for alfalfa, maize, flax, potato, rapeseed, tobacco and tomato which have been engineered to show novel herbicide tolerance, altered fertility/sterility, altered storage proteins, genetic markers (especially antibiotic resistance), stress tolerance and insect or disease resistance. By 1994, this had increased to over 678 approved trials involving eleven different types of genetic construct, such as herbicide tolerance, nutritional changes, virus and stress resistance, and marker genes (Tables 2.1 to 2.3).

Ongoing Challenges

The biggest challenge facing regulatory agencies is how to provide regulatory oversight that is effective in meeting the following concerns:

Table 2: Summary of Trials Conducted in Canada

2.1 By Crop

Crop	Number of Trials
Alfalfa	7
Canola/napus	696
Canola/rapa	34
Maize	13
Flax	17
Potato	42
Soybean	10
Tobacco	3
Tomato	0
Wheat	21
Other	5
Total trials applied for	**848**
Trials canceled	72
Not Authorized	98
Authorized to proceed	678

2.2 By Breeding Objective

Objective[1]	Number of Trials
Novel herbicide tolerance	684
Male sterility/restoration	139
Insect resistance	47
Nutritional change	5
Modified oil composition	71
Virus resistance	11
Stress tolerance	12
Fungal resistance	0
Pharmaceutical	1
Genetic research	1
Generation of mutants	2
Other	0
Markers	
No marker	256
Marker genes only	1
Marker and other traits	591

1 From trials applied for; some submissions have more than one breeding objective.

2.3 By Development Method

Transformation Method	Number of Trials
Biologistics	34
Mutagenesis	2
Direct DNA uptake	0
Agrobacterium-mediated transformation	771
Somatic hybridization of transformants	0
Backcross to genetically modified plant	33
Other development method	0

- the information needs of the public and special interest groups who are genuinely concerned about human and animal safety and effects on the environment;
- industry's need to meet acceptable standards of safety in a timely, equitable manner;
- the government's concern regarding the costs of regulation and the need to develop partnerships with the provincial authorities to reduce duplication; and
- special interest groups who wish to participate fully in the development of new regulations.

Our immediate workplan places priority upon developing mechanisms to publish risk assessments of authorized field trials, to fill the identified regulatory gaps after consultations with the public, affected industry and interested parties, and to look at options for reducing regulatory oversight for those categories of genetic engineering which are considered low risk.

Chapter 2.3

Field Testing Genetically Modified Plants: Guidelines for Applications to the Plant Products Division of Agriculture Canada

Jean E. Hollebone
Director, Biotechnology Strategies and Coordination Office
Agriculture Canada
Nepean K1A 0Y9, Canada.

Louise Duke
Chief Variety Registration Officer
Plant Products Division, Agriculture Canada
Nepean K1A 0Y9, Canada.

Introduction

By the end of 1990/91, the Plant Products Division of Agriculture Canada had authorized over 100 field tests of genetically modified plant material. This had risen to over 678 trials by 1994. Guidelines have been designed

Hollebone, J.E. and L. Duke. 1994. Field Testing Genetically Modified Plants: Guidelines for Applications to the Plant Products Division of Agriculture Canada. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 91-99.

designed to aid applicants to submit a complete application for authorization to either import and/or field test genetically modified plant material (see Chapter 2.2: Hollebone and Duke).

Applications to Field Test Genetically Modified Plants

Where to Apply

Applications to field test genetically modified plant material should be submitted to: The Director, Plant Products Division, Plant Industry Directorates, Agriculture Canada, 59 Camelot Drive, Ottawa, CANADA K1A 0Y9.

Genetically modified material to be imported, for whatever purpose, will require an import permit (form AGR 1154) which must be obtained prior to import from the same address.

When to Apply

Applications for field testing and importation should be made a minimum of eight weeks prior to the proposed initiation of the importation/field test.

Processing of Applications

Applications will be received by the lead agency as described above. The lead agency will, if applicable, send duplicate copies to secondary agencies for their assessment. For instance, the Plant Products Division would send copies of applications to the already nominated primary provincial contact(s) in the provinces where proposed trials are to be conducted; and, if the material involves unregistered pesticide use or altered pesticidal tolerance or activity, to the Pesticide Directorate.

Reviews from the secondary agencies are returned to the lead agency, and a final assessment performed. From this a decision is made whether to authorize the field test. Any mitigation procedures required will be determined before authorization.

All applications are marked “CONFIDENTIAL” and are treated as such by the agencies reviewing them.

Applications to Field Test Genetically Modified Plants

i) The Plant Products Division requires an application for a field test for plant species modified to show a specific trait (e.g. tolerance to a

specific herbicide resulting from a specific gene) to be tested at a specific location in a specific year. For instance:

- A species, such as canola (*B. napus*), modified to show tolerance to a specific herbicide resulting from the insertion of one specific gene, and another canola modified to show tolerance to certain insects by the insertion of the delta endotoxin gene from *Bacillus thuringiensis*, both of which will be tested in a small-scale field trial at *one* location in *one* year will be considered as *two* field tests. A separate assessment is made on each of the two different genetic constructs.
- A canola modified to be resistant to a specific herbicide as a result of one specific gene, to be tested for agronomic performance in small-scale field trials at *six* locations in the *same* year, will be considered as *six* field tests.
- The same modified canola to be tested at the same six sites over *two* growing seasons will constitute *twelve* field trials.

ii) The guidelines have been arranged in a "modular" fashion, and this, together with the definition of a field trial above, will allow the applicant to complete only those sections that are relevant. For instance, considering the *second* example above:

- Section I of the Application (see below) would list all personnel involved, including contact persons for each trial site;
- Section II need be completed only if the purpose of the six trials is the same;
- Sections III, IV, and V need be completed once only;
- Section VI would be completed six times, once for each of the six trial locations;
- Sections VII, VIII and IX would be completed once only, unless the proposed trial protocol, monitoring and public consultation and notification procedures differed for the different locations.

iii) To facilitate the assessment of field trial applications, information should be supplied in the same order and with the same numbering system as in the guidelines.

iv) Repeat applications. Providing there has been *no* change in a specific aspect of a trial (for instance, the genetic construct), and providing the Plant Products Division has received this information previously in the format described in these guidelines, then the relevant request for information may be referenced to the earlier application.

Information Considered to be Confidential

In the application, applicants are required to indicate information which is confidential, such as exact trial sites, plasmid maps, exact genetic

change, or others to be specified. Although other information may be initially retained as being confidential, the retention of this information as confidential is subject to the provisions contained in the Access to Information and Privacy Act.

Specific Requirements for Applications

I. Personnel Involved in the Application

A. Applicant
    1. Name
    2. Address
    3. Phone number
    4. Fax number
B. Field manager (person conducting trial)
    1. Name
    2. Address
    3. Phone number
    4. Fax number
C. Other personnel involved (if applicable)
    1. Name
    2. Address
    3. Phone number
    4. Fax number

II. Purpose of Trial

Briefly describe the purpose of the field trial(s).

III. Unmodified Plant

A. Scientific name.
B. Common name.
C. Describe the life cycle of the plant with special emphasis on outcrossing *versus* self-pollination, and wind *versus* insect pollination.
D. Is it fertile?
E. Describe:
    1. habitats where populations exist:
        a. managed
        b. unmanaged;

2. locations where it is a known pest, if any;
3. mechanisms resulting in:
    a. tendency to weediness
    b. allelopathy
    c. dormancy;
4. mechanisms the plant possesses:
    a. for pollen dispersal
    b. for seed dispersal
    c. for dispersal through vegetative means; and
5. mechanisms and frequency of outcrossing with:
    a. members of its own species
    b. genus and species relatives.

F. Does the species produce known toxins (including natural defensive compounds)?
    1. What compounds are produced?
    2. At what levels do these compounds induce toxicity?
    3. What species are affected by these toxins?

IV. Modification

A. What trait(s) are conferred to the recipient plant?
B. Was the modification achieved through mutagenesis or recombinant DNA transformation?
    1. Mutagenic:
        a. method used to induce mutation(s);
        b. trait(s) selected;
        c. mode of action of trait(s):
            (1) gene product
            (2) metabolic pathway.
    2. Transformed:
        a. Supply a map of *each* construct
        b. For each gene construct, list:
            (1) promotor(s), including source(s);
            (2) gene(s) conferring desired trait(s), including source(s);
        c. Is the gene construct(s) described stable?
        d. Method of transformation:
            (1) vectorless method—describe;
            (2) method using natural vector:
                (i) vector name
                (ii) is vector naturally pathogenic?
                (iii) was the vector disarmed?
                (iv) how was the vector disarmed?

(v) is there expression of the gene in the vector?

e. For non-*Agrobacterium tumefaciens* mediated transforma-tions, please supply Southern blot analysis.

f. What procedures were used to select transformed material?

g. Mode of action of the trait(s):
   (1) gene product
   (2) metabolic pathway.

V. Modified Plant Material

A. Is the desired trait(s) expressed?
   1. Is the expressed trait tissue specific?
   2. Is the trait expressed during a specific developmental stage?

B. Once inserted into the plant, has the genetic modification(s) been shown to be stable? What data is available demonstrating stability?

C. Are the plant characteristics previously described in Section III altered? If applicable, confirm that there are no changes with respect to the following:
   1. weediness;
   2. allelopathy;
   3. dormancy;
   4. other trait that might give the modified material an ecological advantage/disadvantage; and
   5. known toxin production.

D. Is the modified material known to produce toxins that were not produced by the unmodified material?

E. Provide available data showing the fate of the gene products listed in IV. B. 2. b(5) when ingested by:
   1. humans and livestock; and
   2. native faunal populations (i.e. mammals, birds, reptiles and insects).

F. If no data is available, describe the probable fate of the gene products listed in IV. B. 2. b(5) when ingested by:
   1. humans and livestock and;
   2. native faunal populations (i.e. mammals, birds, reptiles and insects).

VI. The Trial Site

A. Supply a map of the trial site showing general geographic location, specific (legal) description, and exact location of trial plots.

B. Is the trial site part of a managed or natural ecosystem?

C. If managed, how close is the nearest natural ecosystem?
D. List related wild species present at the trial site.
E. List related managed species present at the trial site.
F. How near is/are the closest planted stand(s) of the tested species? If within 400 m:
    1. Is/are the crop(s) for commercial food production?
    2. Is/are the crop(s) for seed production? State the pedigree status
    3. Is/are the planting(s) a breeding nursery?
    4. Is/are the planting(s) for other experimental purpose?
G. How near is/are the closest managed commercial crop(s) of related species? If within 400 m:
    1. Is/are the crop(s) for commercial food production?
    2. Is/are the crop(s) for seed production?
    3. Is/are the planting(s) a breeding nursery?
    4. Is/are the planting(s) for other experimental purpose?
H. Is the local fauna likely to remove transgenic material from the site?

VII. Trial Protocol

A. What is the proposed date of the field trial?
B. What quantity of material is to be field tested?
C. Is the test considered a Small-Scale Test, a Varietal Registration Test, or a Large-Scale Test?
D. Experimental design:
    1. What design is to be used?
    2. How many replicates?
    3. How many plots in total?
E. What are the dimensions and area that the trial will occupy? (Do not include border and guard rows of material not genetically modified.)
F. What reproductive isolation measures are proposed? Describe fully:
    1. isolation distances;
    2. the use, size and arrangement of border/guard rows;
    3. the use of any physical methods to prevent pollen movement (e.g. cages).
G. What data will be collected from, and observations made during, the trial?
H. Seeding:
    1. How will the trial material be transported to the trial site?
    2. How much seed is to be seeded?
    3. Will the material be seeded by hand or machine?
    4. If by machine, what precautions will be taken to avoid dissemination of seed from the trial site?

5. Is it proposed to seed any unmodified plants of the same species, or related species, to determine, for instance, herbicide efficacy in plants modified to show herbicide tolerance?

I. Spraying:
   1. Will the trial material be sprayed with pesticide?
   2. If so, is the pesticide(s) a registered product, and is it registered for use on the trial material?

J. Harvesting:
   1. Will the material be allowed to set seed?
   2. If so will the seed be harvested by hand or machine?
   3. If by machine, what precautions will be taken to avoid dissemination of seed from the trial site?
   4. How will remaining plant matter be disposed?

K. Post-trial land use:
   1. Who has long term (minimum three years) control over the trial site?
   2. Who has long term (minimum three years) control over the site within the isolation area?
   3. For what will the land be used following the field trial (minimum three years—neither the same crop as the transgenic nor a relative should be grown in the field in order to distinguish volunteer growth).

L. How will the harvested seed be stored?

M. What procedures for recording quantities of seed left over from seeding, and seed progeny produced, are proposed?

N. How will the following be disposed:
   1. surplus seed?
   2. seed progeny?
   3. plant residue?
   4. living plant material?

O. How will the boundaries of the trial site be marked so that inspections may be made in subsequent years?

P. What contingency plans have been developed in the case of accidental release of transformed seed?

Q. What contingency plans have been developed in the case of unexpected spread of the genetically modified plant material?

VIII. Monitoring Capabilities and Intentions

A. What monitoring procedures are proposed during the trial period?

B. What monitoring procedures are proposed during the post-trial period?

C. Please supply a monitoring timetable showing frequency of monitoring during the trial and post-trial periods.
D. Are any controlled monitoring procedures proposed for this trial, e.g. plantings of unmodified plant material or related species or genera to determine the possibility/frequency of gene flow from the modified material?
E. Is monitoring proposed to determine whether there is gene flow of the modified trait into surrounding plants of the same species or of related species or genera?
F. How will a record of monitoring results/actions taken be recorded?

IX. Public Consultation/Notification

A. Has there been any public consultation or notification of the proposed field trial(s)?
B. Is the public in the locality of the test site(s) aware of the proposed field trial(s)?
C. Has there been any concern expressed by the public, or other persons, concerning the proposed field trial(s)?
D. Is notification of the public and or press/media proposed once the trial(s) is authorized?

Chapter 2.4

Certification Review Process for the Planned Field Introduction of Transgenic Plants in the USA

Sally L. McCammon
Science Advisor
Animal and Plant Health Inspection Service (APHIS)
United States Department of Agriculture (USDA)
Administration Building, Washington DC 20250, USA.

Introduction

The review process of the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA), for field testing of transgenic plants certifies that there is no significant plant pest risk even if the organism being released is derived from a plant pest. As of 14 March, 1994, APHIS has issued 474 field test permits and acknowledged 471 notifications for plants containing various genes for

McCammon, S.L. 1994. Certification Review Process for the Planned Field Introduction of Transgenic Plants in the USA. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 101-111.

insect, virus, fungal and herbicide tolerance, nutritional and value factors, heavy metal sequestration, pharmaceutical products, and selective markers. An environmental assessment is carried out in conjunction with the issuing of a permit for two reasons: to document and verify that plant pest potential has been removed, and that there is no significant impact on the environment or human health (McCammon and Medley, 1990).

Certification and Plant Pathogens

The procedures developed at APHIS for issuing permits are to ensure the environmental safety and elimination of plant pest risk in the release of transgenic plants for field testing. The organism being considered for release is a "regulated article" if it is a plant pest, or if the plant has been genetically engineered to contain nucleic acid sequences derived from a plant pest (7 C.F.R. § 340; refers to Federal Register Number). Thus, an analysis of the molecular biology of the donor of the genetic material, the plant, and of the vector for moving the genes into the plant is carried out. APHIS ensures that the genetic material is well-characterized, that gene insertion is irreversible and stable, and that gene expression is as predicted.

Vectors are evaluated because plant pathogens are used to move genetic material into the plant. In addition, genetic material from viruses and pathogens are used as promoters, terminators, polyadenylation signals, and enhancers; and genes from plant pathogens are inserted to obtain resistance to these pathogens. For example, there are many different constructs of the Ti plasmid of the pathogenic bacterium *Agrobacterium tumefaciens* that are used to insert genetic material into plants. (APHIS has even received applications where the strain of *Agrobacterium* could not be identified by the researcher.) Evaluation includes the verification of the removal of the plant pathogenic potential of biological vectors so that pathogenic properties do not become part of the inheritable characteristics of new crop varieties. Strictly interpreted, concern is not over the use of viral promoters or disarmed Ti plasmids but over the elimination of genes for pathogenesis or unknown/non-characterized DNA. APHIS regulations do not assert that genetic traits would cause a plant to exhibit plant pest characteristics unless the plant itself is already a pest to other plants. However, they do allow verification that a disarmed plasmid was used, and that genes implicated in plant pathogenesis do not become part of the inheritable characteristics of the plant.

APHIS verifies that the pathogenic potential contained in the construction of the organism or performance of the field test has been

removed or will be contained. This is done through an evaluation of the biology of the donor and recipient organisms and of the molecular biology of the gene which has been taken from the donor organism and genetically engineered into the recipient to be field tested. Analysis of the molecular biology includes an analysis of all newly acquired sequences, including regulatory sequences, engineered genes, marker or antibiotic resistance genes and other non-coding sequences. The APHIS review process provides independent verification of assertions of environmental safety.

Permit and Submission Requirements

Evaluation of any safety issues is most appropriate at the initial field testing stage of research and variety development. The purpose of these tests is either to make decisions on the development of the new plant into an accepted agricultural variety, or to gain answers to basic research questions. Plants that do not perform well, or that show unexpected abnormalities, are discarded.

Although the permit applicant undertakes the field test primarily to test the efficacy of the trait, the initial stage of testing also gives the most valuable information on the interaction of a gene in a new organism. The plant itself is the most sensitive test for the effects of a new gene in a new organism. In assessing the environmental effects of a field test, APHIS evaluates the interaction of the trait, the plant and the environment.

APHIS permit application data requirements form a logical sequence in which the necessary data is present to allow a review of a permit request for environmental effects. A permit application addresses the fourteen points stated within the Federal Register (7 C.F.R. § 340). These points cover the who, what, why, how and where of the field test (see Chapter 2.4: Kubicek). APHIS has prepared a *User's Guide for Genetically Engineered Plants and Microorganisms* (USDA, 1991) that explains and gives examples of permit applications.

The permit information includes the biology and molecular biology of the organism to be field tested; the processes, procedures and safeguards used to prevent contamination, release or dissemination; and the purpose and proposed experimental design of the field test(s). Detailed descriptions of how the organism will be prevented from disseminating into the environment, both during transport and during the field test, are required. Monitoring procedures are included. This information is submitted by the applicant and is used to conduct an environmental

analysis of the proposed field test. APHIS has *not* developed or implemented procedures for extensive confinement, monitoring and disposal. All the procedures followed depend upon the individual test itself and are applicant driven, in that the applicant submits the proposal and APHIS reviews it.

When the permit is issued it contains special conditions in addition to the standard conditions which must be met. One of these involves the collection and submission of test data to APHIS which is used to verify conclusions and build a database upon which to make future assessments. Four basic questions are addressed in the submission of field test data:

1) Was any spread of the recombinant organism from the test plot detected?
2) Were any symptoms of *Agrobacterium tumefaciens* infection observed on plants at the test site, if applicable?
3) Was there any evidence of test organism survival at the test plot during the year following termination of the field test (e.g. volunteer plants)?
4) Were any unpredicted differences between the recombinant and non-modified control organisms detected, such as morphological or growth habit effects?

The data that APHIS requires at the completion of any test relates directly to either pathogenic potential or negative effects on the crop plant, such as changes in survival ability. These questions require at minimum a “yes” or “no” answer.

In addition, any publications resulting from the field trial should be submitted to APHIS. Site inspections by APHIS personnel may accompany the issuance of a permit. Inspections are to verify that the site is as described in the application, and to ensure that containment conditions are adequate.

Environmental Analysis

Before a permit for field testing is issued, an environmental assessment (EA) is conducted in accordance with the provisions of the National Environmental Policy Act (NEPA) of 1969 (US Federal Government Publications, 1970). Under NEPA, there is a requirement for the production of an assessment of the risk to human health and the environment. Such assessments examine the alternatives to a given action and evaluate data on the potential risks accompanying each of the favored alternatives. From the analysis in the assessment, the best informed decision is made using logical reasoning.

This type of environmental review contrasts with the traditional quantitative chemical environmental risk analysis that emphasizes numerical data, in that there is no *a priori* assumption of the presence of a hazard or risk (risk = hazard X exposure), especially to human health. Traditional risk assessments are done to ensure safety from identified risks to human health and the environment such as pathogens and specific pollutants or toxic agents. EAs do not *prove* safety. Instead, the NEPA environmental review process provides the necessary structure for identifying concerns and making decisions.

The consequences of field testing of transgenic plants that would be of concern to APHIS fall into general categories: weed pests, effects on endangered species, effects on beneficial species, effects at centers of diversity or genetic resources, and pathogens or epidemics. Many of these concerns are recognized general concerns in the USA as indicated by the presence of the Federal Noxious Weed Act of 1974, the Endangered Species Act of 1973, the Honey Bee Act of 1922 and the Federal Plant Pest Act of 1957.

To date, 29 different kinds of plants have been tested in the USA, namely alfalfa, apple, barley, beet, cantaloupe, carrot, chrysanthemum, cotton, cucumber, grass, lettuce, maize, papaya, pea, peanut, petunia, plum, poplar, potato, oil rapeseed, rice, soybean, spruce, squash, sunflower, tobacco, tomato, walnut, and wheat. Most of these plants are highly domesticated and would have difficulty surviving without human intervention. The biology of these plants is well-known, as they are not new crop species being introduced into a given area for the first time. Thus, the exotic species model for invasiveness of a new species is not valid in evaluating the environmental impacts of well-known and understood crop plants. Comparison for environmental evaluation must be made with the parental crop plant (NAS, 1989).

An EA is prepared before each of the permits for field testing is issued. When the EA results in a “Finding Of No Significant Impact” (FONSI), the permit is issued. An EA has no requirement as to length. The EA is the document which is the basis for making an informed decision. Components of the EA include the purpose of the EA, USDA Regulations, the conditions under which a permit is granted or denied, procedural and physical precautions against environmental risks, an analysis of the molecular biology and biological background of the inserted genetic material as well as that of the recipient organism to be field tested, and the environmental consequences of the field test (McCammon and Medley, 1991).

To ensure environmental safety, the environment that will be affected by the field test and the precautions for protecting that

environment are analyzed. Points that are considered in this environmental evaluation include containment, potential for gene transfer, potential for dissemination, final disposition, field inspection, mitigation measures, and the consequences to the environment if the organism becomes established. The EA itself can also include descriptions of the field plot design, monitoring procedures, and the security precautions at the field test site.

An evaluation of the biological effects of the genetic modification looks at the gene expression of the inserted gene, including gene expression compared with the non-modified organism, plant pathogenic genes remaining in the system, the mechanism of gene transferral, and the potential for gene transfer by reversal of the method for gene introduction. Evaluation of morphological or structural characteristics, physiological processes, products and secretions, growth characteristics, and the number of copies and location of inserted material may be undertaken. Additionally, the origin of the vector or vector agent used to transfer the gene from the donor to the recipient is also scrutinized.

Several parameters affect the necessity for recommending appropriate containment measures, including the possible impact on nontarget organisms, potential affect on genetic resources, the consequences of the modified organism becoming established in the environment, the rate of survival of the organism, and agricultural production and practices in the area. The possibility of risk to nontarget native floral and faunal (vertebrate and invertebrate) communities is examined, with emphasis on endangered species present in the area. The possibility of altering the susceptibility of the crop species to pathogens or of effects on agriculture are also examined, as are impacts on human health. Genetic resources are looked at from the point of view of effect on susceptibility of economically important species to pathogens or the availability of herbicide or pesticide resistant economically important varieties.

An analysis of the potential for gene transfer in plants, whether the plant is self-pollinating, cross pollinating or sterile, along with the determination of the presence of wild members of the same species or relatives or other plantings of the same crop, will determine whether flowering is allowed or the kinds of safeguards to be instigated if flowering is allowed during the field test. Most crop plants grown in the USA do not have wild relatives and therefore the emphasis in environmental analysis is on the effect of the trait.

The fitness of a gene in a plant can be assigned general values (McCammon and Dwyer, 1990). Thus, a gene that might confer high fitness would be one that would endow some broad-based defensive

qualities, such as for disease or insect tolerance, and would persist for a long time in the gene pool. A gene for moderate fitness would endow a plant with a quality or value in a special setting, such as those for herbicide tolerance or changed biochemical composition. A gene of low fitness, such as a male sterility gene, could handicap a plant, and would have low persistence in the gene pool.

The potential for plant dissemination is evaluated primarily for effects on agricultural productivity in the field test site and surrounding area. Thus, the presence or potential of both the modified and unmodified plant for weediness is evaluated. This can include the ability for seeds to overwinter or for volunteers to form. Species and cultivar characteristics, including reproduction rates in test areas, dissemination potential and dispersal mechanisms, are researched.

Final disposition methods can include the use of herbicides, mechanical incorporation of test material into the soil, soil sterilization, and collection and autoclaving of test material. Evaluation of mitigation plans includes a determination of the effectiveness of the methods proposed to control or eliminate the organism from the site and surrounding area, if appropriate.

Safeguards to prevent introduction into the environment are developed from analysis of the biology of the donor, recipient, and vector or vector agent, and an evaluation of the potential for contamination, release and dissemination of these components into the environment. The risk to the environment can be limited either by the nature of the organism or by the specific safeguards that have been designed into the protocol.

Safeguards can be biological, temporal or physical. Examples of biological safeguards to prevent gene transfer and dissemination include prevention of flowering, use of self-compatible varieties, and the use of male sterile varieties. Temporal safeguards include manipulation of time of planting or time of flowering. Physical safeguards include the use of fallow ground surrounding the field test site, fences, isolation distances from other compatible crops or related plants, plot design, chemicals, and border rows/trap plants to attract insects and pollen. Isolation distances of plants are normally taken from those recommended by the Association of Official Seed Certifying Agencies (AOSCA, 1971). These distances were developed to assure seed purity. A certain percentage of outcrossing is presumed in these published isolation distances, so supporting data is recommended and normally twice the recommended distance is used in field testing.

Environmental analysis is a key component in the permit review process. It is an environmental evaluation, rather than a risk-benefit

analysis, and it is the public's assurance that APHIS has thoroughly considered the possible consequences of releasing the regulated article into the human environment. APHIS ensures that environmental reviews are being undertaken by a responsible, informed and objective body at the initial stages of testing of transgenic plants. Thus, prevention of a predictable, high consequence event is also assured.

Conclusions

APHIS regulations have several underlying principles, including:

1) allowing "informed decisions" to be made, based on analysis and consideration of the available alternatives that are necessary for environmental concerns to be identified, managed and evaluated; and
2) coordinating state and federal government agencies within the USA to eliminate duplication, and internationally to facilitate similar or equivalent regulatory oversight based upon scientific principles.

In biotechnology regulation APHIS is committed to the following goals:

1) the development of a balanced regulatory framework;
2) assurance that the regulatory structure is scientifically based to assure credibility and voluntary compliance;
3) the presence of a regulatory structure which protects agriculture as well as facilitating technology transfer; and
4) the maintenance of a regulatory structure based on risk rather than process (Medley, 1990).

APHIS is committed to reasonable and risk-based procedures for conducting its reviews and analyses both from the perspective of the applicant and the concerned public. The information gained from the first field test and product approvals is of vital importance to the future development of safe and beneficial products.

As products are developed and successfully marketed, and as information about the environment is gained from field testing, public acceptance will grow. These are critical years in the development of a new generation of transgenic plants. Admirable progress has been made, and, with cooperation, the small-scale field test stage has proceeded to product-testing and commercialization. The first transgenic food plant, the Calgene FLAVRSAVR™ tomato with delayed ripening characteristics, was marketed in the USA in May of this year.

The field trials that have been permitted proceeded unimpeded after the permits were issued. Many generations of particular plants have

commenced with eventual commercialization in view. Many field tests done by industry often are now larger in scale and occur at multiple sites in multiple states in the USA. In addition, the flow of sophisticated applications from academic institutions is increasing in the same manner as did applications from private industry several years ago (McCammon and Medley, 1990).

Many field tests to date have been small-scale, and the determination of biosafety has revolved around the ability to contain or eliminate the organisms from the field test site. With plants this has primarily meant the prevention of pollen dispersal and/or survival and dissemination of seeds or plant material. Evaluations have been case-by-case and have included an analysis of the organism, field test site (including the surrounding environment), and the agricultural and experimental practices employed. In most current cases in the USA, environmental review at the initial stages of testing shows that consequences either cannot occur or that consequences can be handled by standard agricultural practices.

As small-scale field tests have given way to developmental research, evaluation of the same components and the same issues has occurred. However, the emphasis has shifted for certain issues. Thus, the potential for weediness and probability of its occurrence has become more important to evaluate, as it is more difficult to assure containment, if necessary, in larger field tests. The nature and stability of the inserted gene, the probability of gene transfer, and the consequences of gene transfer into wild species, weedy species, or into related crop varieties, are important biosafety factors.

After almost six years evaluating permit applications and considering the results of field trials under permit, APHIS has been able to verify and document data that these plants behave as other plants that undergo strenuous development. In some cases, environmental review by APHIS does not need to continue after initial reviews and the plant can be released into the traditional variety development and certification systems for any new plant variety. Such traditional systems include ensuring plant breeder's rights.

Release from APHIS review is based on experience that indicated that categories could be defined for certain field tests that do not present plant pest risks, uncertainty or significant agricultural safety issues. The type of analysis described above for the issuance of a permit was demonstrated to be unnecessary for such categories. Therefore, on March 31, 1993, APHIS put in place, in addition to the existing permit process, two other regulatory options, notification and petition, which became effective April 30, 1993 (58 F.R.; US Government Printing Office, 1993).

Field testing under notification (section 340.3) requires that the test meets specified eligibility criteria and performance standards. If the eligibility criteria are met, APHIS acknowledges the receipt of the notification, without doing an EA or issuing a permit. The eligibility criteria impose limitations on the types of genetic modification that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. The notification option is presently restricted to field tests of new varieties of six crops: maize, potato, cotton, tomato, soybean, and tobacco. Before notification was allowed, 85% of the field test permits were for these six crops. The notification option is further limited by precluding certain types of modifications (e.g., those encoding genes for pharmaceutical compounds or from human or animal pathogens) and requiring field tests under notification to be conducted under specified performance standards that amount to genetic containment (Medley and McCammon, 1994). Field tests not falling within these constraints may still be conducted under permit, with and EA being prepared by APHIS as before.

The petition option allows an applicant to request that APHIS decide whether a given transgenic plant should continue to be regulated. The determination is, as with a permit, based on information provided by the applicant, based on previous field tests and other experimental evidence, which is considered with other information collected by APHIS. Once a petition is approved by APHIS there is no longer any need for APHIS review or approval for introductions of the article into agriculture or commerce of the USA (Medley and McCammon, 1994). Food safety, pesticide or other regulatory questions may still be addressed by the relevant regulatory agencies. APHIS has determined that three particular transgenic plants do not present a plant pest risk and, therefore, no longer need to be regulated; the FLAVRSAVR™ tomato, Calgene's bromoxynil-tolerant cotton, and Monsanto's glyphosate-tolerant soybean. The Food and Drug Administration (FDA) of the USA also approved the safety of the tomato before it was marketed.

In summary, the USA has been able to maintain its competitive advantage through field testing because there have been no legal challenges or public outcry due to inadequate oversight. These environmental assessments have been found adequate by public interest groups and have not been challenged in court.

Disclaimer

The views expressed in this article are those of the author and do not necessarily represent those of the United States Government.

References

AOSCA. 1971. AOSCA Certification Handbook. Publication Number 23. Association of Official Seed Certifying Agencies (AOSCA), Raleigh.

McCammon S. L. and S. G. Dwyer (eds.). 1990. Workshop on Safeguards for Planned Introduction of Transgenic Oilseed Crucifers (Proc.). 33 p. Cornell University, Ithaca.

McCammon, S. L. and T. L. Medley. 1990. Certification of field release of genetically engineered plants. In *Molecular and Cellular Biology of the Potato,* M. E. Vayda and W. Park, (eds.). pp. 233-250. CAB International, Oxford.

McCammon, S. L. and T. L. Medley. 1991. Certification for the planned field introduction into the environment of transgenic plants and microorganisms. In *Biological Monitoring of Genetically Engineered Plants and Microbes,* D. R. MacKenzie and S. C. Henry (eds.). pp. 107-117. Agricultural Research Institute, Bethesda.

Medley, T. L. 1990. APHIS seeks to aid tech transfer while ensuring environmental safety. *Genetic Engineering News* 10:14-15.

Medley, T. L. and S. L. McCammon. 1994. Strategic regulations for safe development of transgenic plants. In *Vol. 12: Modern Biotechnology: Legal, Economic and Social Dimensions,* D. Brauer, (ed.), (in press).

NAS. 1989. *Field Testing Genetically Modified Organisms: Framework for Decisions*. National Academy of Sciences (NAS), National Academy Press, Washington DC.

USDA. 1991. *User's Guide for Introducing Genetically Engineered Plants and Microorganisms*. 1991. Technical Bulletin No. 1783. United States Department of Agriculture (USDA), Hyattsville.

US Federal Government Publications. 1970. 7 C.F.R. § 340; 42 U.S.C. § 4332 (1970); F.R. 58: 17044-17059. United States Federal Government, Washington DC.

Chapter 2.5

Requirements for Applications for Field Trials of Transgenic Plants in the USA

Quentin B. Kubicek
Trade Support Team
Animal and Plant Health Inspection Service (APHIS)
United States Department of Agriculture (USDA)
Room 1128, South Building, Washington DC 20250, USA.

This chapter reviews the information required for the evaluation of a sample application for a field trial of transgenic plants in the USA. Regulations of the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) can be found in 7 C.F.R. Part 340 (1987) entitled *Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests.*

Genetic engineering is broadly defined as the genetic modification of organisms by recombinant DNA techniques. In the USA, it is a requirement that an applicant who wishes to conduct a field test of certain transgenic plants completes APHIS Form 2000. Form 2000 may

Kubicek, Q.B. 1994. Requirements for Applications for Field Trials of Transgenic Plants in the USA. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 113-125.

also be used to permit movement of transgenic organisms within the USA, from one state to another, and also to import transgenic organisms.

A permit for a field test of certain transgenic plants is required if any of the following three conditions exist:

1) the plant has been modified using recombinant DNA techniques;
2) the plant has been modified with genetic material from an organism that is on the list of regulated organisms (known plant pests) and conforms with the definition of a plant pest—an organism which can injure or cause disease to any plant or plant part; and
3) the plant will be imported into the USA, moved to another state, or openly released into the environment. Testing in a greenhouse or laboratory is not considered a release into the environment.

Many researchers believe that the USDA regulations will inhibit laboratory research and that laboratory experiments will be regulated. This perception is most unfortunate, and is simply not true. An applicant may have an organism exempted from the regulations. APHIS regulations (7 C.F.R. 340.4) contain a petition process whereby an applicant may submit data and information to show that the organism in question is not a plant pest.

An applicant who meets these three conditions must forward a completed APHIS Form 2000 to the APHIS office located in Hyattsville, Maryland. Financial or commercial information that an applicant does not want disclosed for competitive reasons may be claimed as confidential business information (CBI). This information must be commercially valuable, used in the applicant's business, and maintained in secrecy. An applicant must submit a written justification to support each claim. Data that is considered CBI must be clearly indicated in the application. Because these are publicly available documents through the Freedom of Information Act (5 U.S.C. 552), an applicant must forward a copy of the application in which the confidential data has been removed (CBI-deleted). The CBI-deleted copy must be a facsimile of the CBI copy. APHIS will not disclose CBI.

When Biotechnology, Biologics and Environmental Protection (BBEP) receives an application, it is reviewed for completeness and acceptability. This review is meant to determine if the application meets the administrative, legal and technical requirements needed to accept the application and begin its review. As soon as an application is deemed to be complete, its receipt and deposit by APHIS is published in the Federal Register. Certain applications may be under the jurisdictional review of other federal agencies such as the Environmental Protection Agency or the Food and Drug Administration. Copies of these applications are sent to these agencies. Applications that fall under this joint jurisdictional

review are separately reviewed and authorized by the agencies. Further information may be found in the Coordinated Framework for Regulation of Biotechnology (7 C.F.R. 340 23302-23350; 26 June 1986).

There are 16 general-type questions or points in APHIS Form 2000 that must be answered (Table 1).

Point 1

Name and address of the applicant. This person may not necessarily be the person responsible as indicated in Point 14. The person responsible ensures that upon issuance of the permit all permit conditions are met. The permit will be mailed to the person indicated in Point 1.

Point 2

Purpose of the permit. A researcher who wishes to conduct a field test of transgenic maize would mark the box for "Release into the Environment". The review of an application for a "Release into the Environment" is issued within 120 days. A researcher in the state of Minnesota who wishes to send transgenic seed to his colleague in the state of Oklahoma would mark the box for "Limited Interstate Movement". If a researcher would like to obtain transgenic tubers from a colleague in Peru, he would then mark the box for "Limited Importation". For the latter two cases, a permit is issued within 60 days. A courtesy permit is a permit which facilitates the movement or the release into the environment of a transgenic organism that is not a regulated article.

Point 3

This point refers to whether the application is being submitted for the first time, being renewed, or whether supplementary information is being submitted. An application that is being submitted for the first time is reviewed within 120 days. An application that is a renewal is reviewed within 60 days. These review periods assume that all of the necessary information to complete the review is present in the application. Applications that have considerable and/or significant information missing may necessitate a delay in the review period until the missing information is made available.

Point 4

Telephone number to reach the applicant. This number is useful when APHIS needs to contact the applicant to resolve a pressing issue.

Table 1: APHIS Form 2000
(Application for Permit or Courtesy Permit under 7 C.F.R. 340)

Point 5

This point refers to the way(s) the transgenic material will be transported to the destined field site or laboratory. An applicant should indicate how the transgenic material will be packaged for transportation. Container requirements can be found in Part 340.6 of APHIS regulations. Generally, transgenic seed is shipped by air mail to the research farm where the test site is located. The applicant must ensure that all packing material, shipping containers, and any other material accompanying the regulated article be treated or disposed of in such a manner as to prevent the dissemination and establishment of a plant pest.

Point 6

Nomenclature description of the transgenic plant and the organism(s) that donated genetic material. The organism(s) that donated genetic material should all be listed, even those that have donated as little as relatively short DNA sequences need to be reported. To date, the recipient organism has been one species, but several transgenic cultivars of this same species may be evaluated in the field test. Recipient organisms are described in their non-modified state and this description resembles that of a cultivar registration. The vector or vector agent refers to the method of transformation. In plant molecular biology, the commonly used vector is *Agrobacterium tumefaciens* and the vector agent is the Ti plasmid. The molecular biology of *A. tumefaciens* is well known and is the workhorse of plant molecular biology. A disarmed Ti plasmid carries the gene(s) which will confer the desired trait(s). These plasmids should be described in detail and plasmid maps included which contain the nucleotide sequences for all the coding and noncoding regulatory sequences which confer the desired trait(s). These plasmid maps allow APHIS to know what DNA sequences are integrated into the plant genome. Another commonly used vector, albeit not biotic, is the biolistic or microprojectile system of transformation. Plasmids used in this latter system are smaller and simpler. Consequently, their detailed description is shorter. To date, the regulated organism has always been the recipient organism and no product has been a regulated article.

Point 7

The number of transgenic plants that will be evaluated in the field test. An applicant may prefer to choose a range of plants rather than a fixed

number and may, during or after the review process, increase or decrease the number of plants. An applicant should be aware that APHIS will take into consideration the size of the field test when they determine the significance of its impact on the environment. The number or range of plants may be indicated as the absolute total or as the total for each of several scheduled plantings. APHIS must be notified if any change in the number of plants will be made.

Point 8

This refers to the date the transgenic plants or plant material will be imported into the USA, moved to another location, or planted. Again, this point, like Point 7, is flexible. The applicant may choose to indicate a range of dates when the field test will begin. For example, an applicant may indicate that the field test will begin during the last week of the month of April or later, depending on site-specific weather and field conditions. An applicant may conduct a long-term experiment with several plantings. In this case, an applicant must indicate a date or range of dates for each planting of a multiple planting field test.

Point 9

Address where the transgenic plant originated. This address may or may not be the same as that of the applicant or the responsible person. The address should be complete such that correspondence will reach this location. It is the responsibility of the applicant to ensure the accuracy of this address. There has to date been no instance where APHIS has been in need of contacting anyone at the country/point of origin of a regulated article.

Point 10

Location of the field release or port of arrival of the transgenic material. Transgenic material arriving in the USA must do so at specific ports of entry that are staffed by APHIS Plant Protection & Quarantine officers. These officers verify that the arriving transgenic material corresponds to that indicated in their cataloged APHIS Form 2000. The transgenic material is then mailed to the address of the recipient. For a field release, the location of the test must be indicated. This information may be claimed as CBI but must minimally indicate the county where the field release will occur. Many applications include state, local and farm maps which disclose the field site. Site-specific information, such as the name

of the owner of the research station or plot location, may be claimed as confidential.

Point 11

This point refers to possible biological material that may accompany the transgenic material. The reason for asking this question is that some biological material may have biotic contaminants that require quarantine. Moving live plants from one state to another may not be permissible because certain pathogens or pests, such as nematodes, may be carried in the potting soil and these are not permitted to enter the destined state. The movement of seed avoids biotic contaminants because seed is cleaned prior to storing and does not require accompanying soil. Stored seed is generally treated with fungicides and/or insecticides.

Point 12

Addresses those applicants who are petitioning for a courtesy permit. A courtesy permit facilitates the movement or release into the environment of a transgenic organism that is not a regulated article. A transgenic plant that was developed without any genetic material from a plant pest, or without the use of a plant pest, would not be a regulated article. An APHIS courtesy permit is, in fact, not a permit but rather a statement affirming that the organism is not a regulated article. It is possible for a transgenic plant not to be a regulated article.

Point 13

This refers to the detailed description of the transformation process and mechanics of the field test. An applicant must spend considerable effort in ensuring that the information presented will be sufficient for APHIS to review and conclude that the petitioned field release will not have a significant impact on the environment. As indicated earlier, the 120 day review period may be delayed because considerable and/or significant information requested in Point 13 is absent from the application. Information requested in Point 13 may be supported by published scientific articles, CBI, letters by recognized authorities, data from previous field releases, non-published in-house company data, etc. Considerable details of the organism(s) used to donate coding or noncoding (i.e. regulatory sequences) genetic material, method(s) of transformation, botanical, physiological and agronomic information of the recipient organism, and experimental and field plot design(s) are

required. Most of the information found in an application refers to the information requested in Point 13.

13a) refers to the names and addresses of those persons involved in the development of the transgenic plant. During the review process it may be necessary to clarify doubts about the transgenic plant.

13b) refers to the anticipated expression of the gene(s) of interest. The expression of each gene must be described. For example, an application for a gene which confers herbicide resistance would contain an enzymatic description, indicating the biochemical and enzymatic pathways involved which lead to the gene conferring resistance to the herbicide. Greenhouse and/or laboratory data may be presented in the application, but APHIS recognizes that this data may not be indicative of field performance. A field test may be the only way to obtain much of the information being requested in Point 13. It is important for an applicant to provide APHIS with as much pertinent information as is available on the gene being evaluated.

13c) refers to the description of the molecular biology used to obtain the transgenic plant. Point 13c contains a description of the genes and noncoding regulatory sequences, such as the promoters and transcription enhancing and termination sequences, obtained from each donor organism. Point 13c also contains a description of the vector(s) and vector agent(s) involved in the process.

13d) is self-explanatory and is for information purposes. It does not impact the review process.

13e) refers to the description of the field test and its design. A field test does not have to be well-designed and APHIS will not judge the validity of the data obtained from a field test. Generally, the number of rows and plots, plants per row, proximity to other fields and crops, planting and harvesting schedules, application schedules of fertilizers or pesticides, pollination schedules and techniques are described in 13e. These descriptions resemble traditional agronomic field plots and their associated activities.

13f) is important because the transgenic plants are regulated articles and APHIS must ensure that these plants do not escape into the environment. A proper review requires knowledge of the safeguards taken to prevent possible escape of a transgenic organism. A geographic description of the field test in relation to sexually compatible species is important. Many field tests take place in agricultural lands where sexually compatible species are present. These can be avoided by planting at a date when the transgenic plants will not be compatible with their neighboring relatives or by locating the field test sufficiently distant from neighboring relative

plants. APHIS strongly favors the former technique because pollen dissemination by wind (i.e. outcrossing) may occur over large distances. An applicant must describe the procedures that will be used to ensure that all harvested seed or other propagating plant material is accounted for. As indicated in 13i, plant material remaining in the field can be destroyed to prevent any unintentional release.

13g) refers to the outcome of the seed harvested from the field test. Some seed is hybrid seed and will be used in future plantings. Where this seed will be stored and who will be responsible for it are important considerations. The seed or progeny of a regulated article are themselves regulated articles and thus must not become commercially available or unknowingly become food or feed. Some harvested seed may be shipped to future field sites or to laboratories for analysis. These shipments require an APHIS permit and must not occur prior to the granting of such a permit.

13h) is similar to 13f. There are well known agronomic procedures that minimize the possibility of plants becoming established at the test site. Many transgenic plants behave as annuals and thus have little chance of becoming permanently established at the test site. Many tests take place in research stations where commercial crop production does not occur. A commonly used practice is to leave the test site fallow for a period sufficiently long to permit the germination and emergence of transgenic plants. These are then destroyed manually or mechanically. There are other procedures applicable to the plant species being tested or to the site that may be used to minimize an unintentional release.

13i) in combination with 13f and 13h, provides the proposed method of final disposition of the transgenic plants. By following established agronomic procedures, a researcher may easily prevent the unintentional release of a transgenic plant. APHIS will closely review the proposed methods of disposing the transgenic material because it is very important that no plant pest be released or established in the environment as a result of a field test of a transgenic organism.

Points 14, 15, and 16

These points are self-explanatory and are clearly for administrative purposes. Again, the “Responsible Person” denoted in Point 14 does not necessarily need to be the applicant as stated in Point 1. The responsible person need not reside in the USA. One very important point is that the

application received by APHIS must bear the original signature (i.e. not photocopied or stamped) of the responsible person.

The boxes located below Point 16 are for administrative purposes by APHIS. If an application is approved by APHIS, the applicant will receive a copy of the original application, but have the boxes located below Point 16 furnished with answers. The permit is valid for one year from the date of issue and the applicant may begin the test at any time during that one year. In addition to the permit, an applicant also receives a list of Standard Permit Conditions that must be obeyed. These are clearly stated and will not be discussed here. As indicated earlier, a permit may be renewed. If a permit is denied or revoked, an applicant may appeal this decision, as indicated in the APHIS regulations.

Chapter 2.6

The Role of Risk Assessment in Developing Statutes and Regulations

Morris Levin
Research Professor
Maryland Biotechnology Institute
University of Maryland
College Park, Baltimore, MD 21228, USA.

Introduction

Assessing the risk associated with a product in a manner suitable for regulatory action is a difficult and complex endeavor. The needs of the regulator govern the activities. The objective of this chapter is to provide an understanding of what is needed to devise and support a regulatory system which is not too onerous yet provides assurance of safety. Not too onerous means speedy, easy to understand and comply with, and easy to enforce. Logistics are important, as is the cost per case and the availability of needed expertise. Achieving this balance requires an

Levin, M. 1994. The Role of Risk Assessment in Developing Statutes and Regulations. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 127-137.

understanding of risk assessment, of what is needed to turn the law into enforceable reality, and of risk management—i.e. the decision to act, choice of action, and its impact on the industry and the economy of the country.

Development of Regulations

This book, designed to discuss the contents of *to be enacted* statutes and regulations, is an exceptional situation. Regulations, of course, are based on statutes. The top level is the statute, the law. If we ask how and why the various statutes used in the USA were enacted, we may be surprised to find that most safety related statutes are born out of crisis situations (Table 1).

This is the top level, born in a crisis situation, supposedly but not really well thought out, usually a compromise and often deliberately vague. Yet the statutes are extremely important because they define each regulatory agency's activities. An example could be the Environmental Protection Agency (EPA) requirement to make water "swimmable" and "fishable".

The next level involves scientific input. At the top of the science level we have input from organizations like the National Academy of Science (NAS) which is composed of scientists who are asked to report to the government about how to deal with or regulate particular problems. Some agencies have their own standing advisory committees; some form them as needed. The NAS impacts statutes and heavily impacts regulatory agencies.

At the next level, agency administrators and scientists in cooperation with academic scientists actually look at the statutes and regulations in terms of information needs, and turn those needs into research programs.

The objective is to develop protocols responding to safety concerns as defined by the statute and further developed by NAS or other "blue ribbon committees". An explanation of the basics of risk assessment is necessary to understand its relation to the topic of developing regulations for transgenic plants.

NAS is an example of science guiding government thinking or policy. NAS has published two documents dealing with risk assessment of transgenic plants (NAS, 1987; 1989). Before that, however, NAS published a well received volume on risk assessment (NAS, 1983). The study was oriented toward human health. Table 2, taken from the study, has been modified slightly to emphasize the environmental component.

Table 1: Major Environmental Statutes

Year	Event	Result
1901	contaminated diphtheria toxin	PHS Act; Virus/Biologic/ Serum Act (1902)
1913	hog vaccine failure	Virus/Serum/Toxin Act (1913)
1937	contaminated sulfa drug	Food, Drug and Cosmetics Act (1938)
1938	steamboat explosion	Steamboat Act (1938)
1962	Silent Spring	Federal Fungicide, Insecticide, and Rodenticide Act (1972)

Table 2: Elements of the Risk Assessment and Risk Management Process (modified from NAS, 1983)

Risk Assessment	Research
Hazard Identification: Does the agent cause the adverse effect?	Laboratory and field observations of adverse health and environmental effects and exposures to particular agents
Dose-Response Assessment: What is the relationship between dose and incidence in humans or environmental effects?	Information on extrapolation methods for high to low doses and animals to humans
Exposure Assessment: What exposures are currently experienced or anticipated under different conditions?	Field measurements, estimated exposures, characterization of populations and environmental effects
Risk management	
Development of regulatory options	*Risk Characterization:*
Evaluation of public health, environmental, economic, social, and political consequences of regulatory options	What is the estimated incidence of the adverse effect in a given population or on the environment?
Agency decisions and actions	

Risk Assessment Concepts

First, it must be pointed out that on a conceptual level risk assessment is very simple, consisting of the bringing together of two components, hazard and exposure.

Each activity or product has a certain degree of hazard associated with it. This is the first element of risk assessment. Crossing the street, driving a car, ironing a shirt, using any product from a lawn mower to a pesticide, all have a probability of adverse effect. Some activities—crossing a street—are more common than others, constituting the exposure factor. Exposure factors are based on how often one engages in the particular activity and thus is exposed to the particular hazard.

Risk assessment brings together the probabilities of hazard and exposure to produce an understanding of the likelihood of an adverse outcome. Decision making involves three components (Table 2): research to estimate the hazard and exposure; assessment and characterization to combine the factors; and a management decision based on the scientific and legal options available.

Research means measuring or somehow determining what happens when a product is used in the field. Perhaps a better title for this aspect would be data acquisition, reserving the word research for developing specific methods to acquire the information. Estimates of effects on target populations—the insect to be controlled in the case of pesticidal plants—are always made by the investigator, the producer of the test product.

The risk assessor, however, is interested in effects on nontarget populations. The nontarget populations and the way the data is gathered are defined by the law under which the government agency is operating.

The information obtained in field studies may be direct effects (e.g. how many honeybees or other beneficial insects are killed) or more general (i.e. how much of the product is actually present at a given time and place). This type of concentration (or dose) data leads to the need for laboratory data to permit extrapolation from dose to effect.

Often data is only available on the effect of a product on one or a few test species. After field measurements provide exposure levels and identify the specific species which would be exposed, this data must be extrapolated to other species and to other exposure levels.

Research involves developing methods to accurately and rapidly obtain the required data. Models required for extrapolating between species and for predicting exposure must be developed. Identification of the end point—the harm or adverse effect—and ways to measure it are needed. This research is carried out or funded by the specific agencies.

Risk Characterization and Management

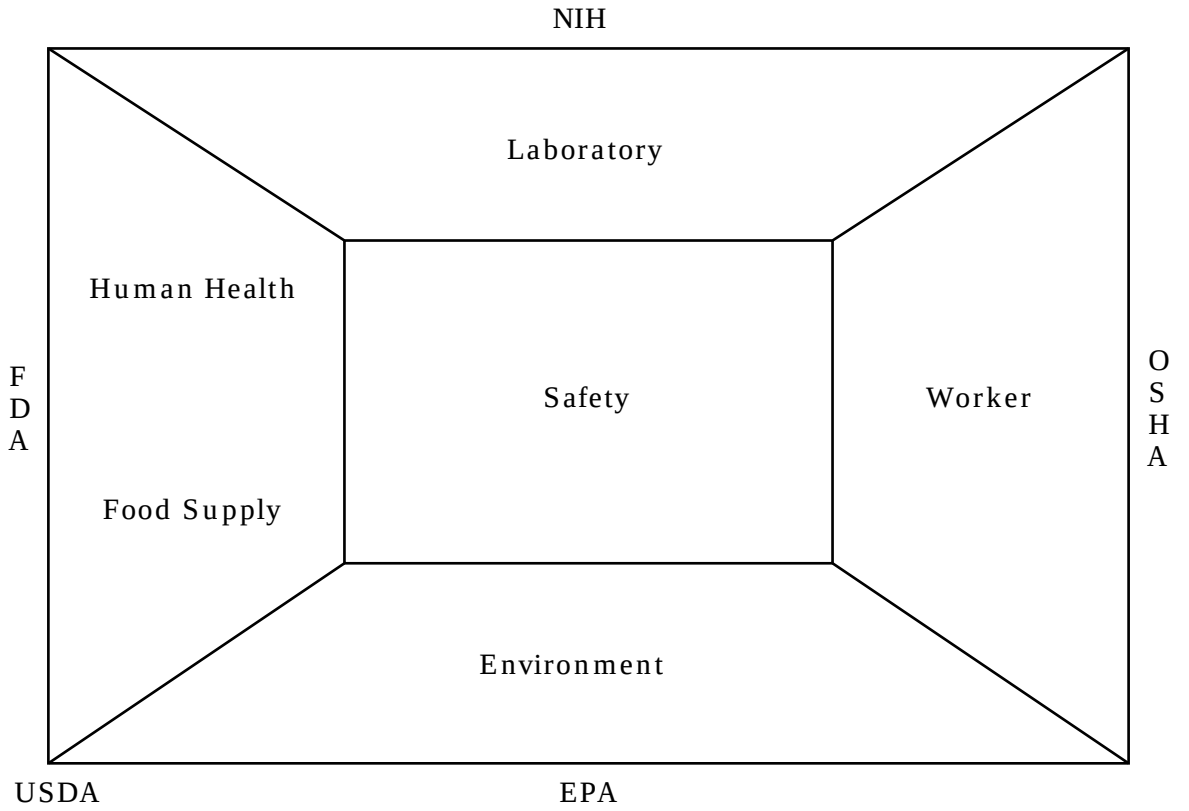
Once the methods are in place the risk assessor is able to understand what hazard is present and its severity. The essential information—how much product is present, how many species are exposed and for how long, and what effects are involved—has been obtained from laboratory and field measurements. The dose response and exposure assessments can then be combined to produce the likelihood and magnitude of the effect—the risk characterization.

Development of regulatory options (Table 2) means consideration of the actions which can be taken under specific laws. The Canadian laws and systems (Chapters 2.2 and 2.3: Hollebone and Duke), the United States Department of Agriculture (USDA) law and system (Chapter 2.4: McCammon) and the EPA law and system (Chapter 2.7: Zeph) are quite different in scope and practice. Government administrators in respective agencies must evaluate the risk assessment, and the effects and the consequences of those effects, and make a decision as to permitted use. The action taken depends on the law under which they operate. EPA can totally ban or permit restricted use, while USDA can allow or halt introduction or shipment of a regulated article; each regulator has different options. There have been long debates in the USA over the advisability of a new law for biotechnology products *versus* using a patchwork of existing laws.

Application of Regulations

All of this sounds straightforward on the surface, but there are many problems. Credibility of risk assessment rests on the data gathered, so the quality of science is critical. However, emphasis placed on different elements of data—what data to gather, how accurate must the method be, which are the most important elements when gathering and evaluating—is a function of the way the assessors see their mission. In the USA the responsibilities are divided between a number of agencies. All are looking at the same general topic: safety. Their individual perspectives nevertheless affect the way they collect data, what data they collect and how they treat what is collected. Relationships between the major agencies in the USA involved in safety regulation of biotechnology products are outlined in Figure 1. The National Institute of Health (NIH) is the only agency without regulatory authority: compliance with its guidelines is voluntary. As one might expect there is some overlap. For example, the Occupational Health and Safety Administration (OSHA) is charged with worker safety and is interested in the effects of products on

Figure 1: Relationship between Safety and Agency Responsibility



those producing them. These are, of course, health effects issues. The Food and Drug Administration (FDA) and EPA are both interested in the same question from the perspective of the user of a product and from the perspective of environmental exposure. USDA is charged with the production of food and fiber. Environmental issues are involved but are not paramount: there is overlap with EPA. EPA is somewhat unique. It has a broader scope and is required to be more general than other agencies.

What does an agency do when faced with a need to enforce a statute, especially in what seems to be a new area? Agency science and policy managers try to scope the problem and to develop the basis for guidelines or rules assuring compliance; that is, for assuring that the products or issues involved will be properly examined. This development usually means taking stock of things like NAS publications and assembling panels of experts to help apply the general statements to the particular problem. Scoping involves defining the industry. In the case of

genetically engineered microbes, this takes the form of panels and workgroups. In terms of what an agency might be faced with, a summary of patents which might lead to EPA covered products was prepared covering 1980-1986. The number of possible products to be reviewed was large, with over 3000 patents issued during the time period examined for products which might come under EPA jurisdiction. As a result, a fairly large effort was initiated to develop scientific methods to obtain data required to make regulatory decisions. In the case of transgenic plants, the process is younger but there has been a rapid increase in the number of species and in the total number of tests.

Specific Problems

Panels of ecologists were involved in defining what could be considered an adverse effect or what could result in an adverse effect. Some were specific for microbes. Some could be applicable to plants. An issue raised by evolutionary biologists at the macro- and micro-levels involves concern over the possibility of drastically altering the direction, path and rate of evolution by altering the raw material (the gene pool) at a given time and place. No real solution or approach to evaluate this issue has been suggested. Quite clearly, however, gene products in new niches, stability of introduced genetic material and the possibility of gene flow could create problems, clearly specific to gene and location and applicable to both plants and microbes. These and other considerations at the managerial level led to identification of information and possible research needs. These are stated in general terms and are applicable to plant issues as well as microbes, methods to *identify the product*; to *assess the potential for survival and dispersal*; and to *assess the impact on man and the environment*. These needs are similar to those developed by Canadian regulators (Chapter 2.3: Hollebone and Duke) and by USDA's review staff (Chapter 2.4: McCammon).

Identification is important because of the need to know the genealogy of the plant. Identification is more straightforward in the plant kingdom than in the microbial world, but identification of all possible relatives with an understanding of the likelihood of cross fertilization is not as easy as simply identifying the plant. Some of the questions which must be answered to support a regulatory decision involving survival and dispersal are: For any released product, will the plant (organism) survive over the winter? Can the seed be transported long distances ? What are the common routes?

Concern about gene transfer can easily be understood. A gene in a new species can be spread to related plants. This is less of a problem in

the USA and more of a problem where diversity among nonagricultural plants is much greater. In some areas—Costa Rica in particular—this is a local issue of major importance. Gene stability and expression issues are, of course, closely related to gene transfer questions.

Identification of hazards is very case-specific. However, the major issue discussed in most cases was the effect of gene products on nontarget insects, target insects and on humans if ingested. Other examples include concerns about research involving genes controlling production of insect neurotoxins; the degree of similarity between the product produced when the gene is functioning in a plant and the natural product; the possibility of resistance to the biopesticide developing as a result of prolonged exposure; and the trend towards producing biopesticides which are less specific and faster acting. This generalization of information/research needs was then related to the risk assessment process with which a given agency was familiar. When the biotechnology risk assessment research program for microbes was established in 1983, there was some resistance, primarily because funds needed to support the program had to come from other programs. Plant issues received an even lower priority because they were felt to be unlikely products in the near future.

These three elements were then turned over to the regulatory specialists and laboratory personnel to develop the needed guidelines and protocols for enforcement. The research staff interpreted the administrative guidance from their understanding of what information was needed and what methods were currently available. This understanding was based on experiences with other—chemical and biological (non-engineered)—products which the agency was required to evaluate. The basic questions remained the same:

- What will be released?
- What does/will it do?
- Will it survive? For how long?
- How about stability and transport?
- Can other effects be anticipated?

For plants the issues are to clearly identify the type of plant and the identity and location of relatives in the release area, how and when and where the release will occur, data concerning the resistance of the modified plant to environmental conditions, and the potential for effects on the environment.

The survival, colonization and monitoring questions for engineered microbes have been examined in detail and these have been reviewed (Levin *et al.* 1987; 1992). The agency is now trying to develop a

standardized, inexpensive and fairly rapid procedure to provide data describing their competitive and survival characteristics. This research is being conducted at the Maryland Biotechnology Institute of the University of Maryland.

Long after the microbiology research program had been established, many groups became interested in risk assessment relative to engineered organisms. In 1989 the Ecological Society of America (Chapter 1.3: Colwell) produced a scholarly, well accepted version of the data needs for risk assessment of engineered organisms. Table 3 lists the parameters they identified as essential for consideration in risk assessment of macro- or micro-organisms which have been engineered for release to the environment. All have been included in the EPA risk assessment research program designed in 1983.

The program at its annual research review in 1992, with its methods development phase having ended, focused on environmental exposure, environmental and human health effects, and risk control (EPA, 1992). Categories one and two have been described above. Risk control is related to the third aspect of risk assessment—risk management (Table 2). It is an attempt to provide containment and mitigation procedures that can be used to conduct tests more safely and to provide some assurance that the product can be controlled, contained or eradicated. This provides the regulator with more options.

Recently the term confinement has been suggested in lieu of containment because you cannot really contain a commercial scale activity, one dealing with millions of acres. Standard control procedures for microbes have been reviewed (Vidaver and Stotzky, 1992) and high technology procedures—such as insertion of suicide genes—have been proposed and are being tested in microbes (Cuskey, 1992). The ESA group also examined this question and provided a list of factors important in the survival of any organism (Table 4). The manipulation of these factors will provide at least a limited ability to control releases.

Conclusion

The major point to be made is that the basic information needed to assess risk from a scientific perspective is the same no matter what the perspective. In order to arrive at a credible assessment, one must assess the hazard and exposure. This is the basic data from which impact on environment or health can be determined. The scientific methods and protocols do not change as a function of type of assessment. The risk manager may elect to weigh the information obtained from the

Table 3: Assessment Problems and Information Needs

Problem	Evaluation Requirements	Resources
1. Establishment of population *in situ*	- enumeration methods - stability determination	culture and DNA techniques
2. Transportation: - physical - genetic	- identification of significant parameters	microcosm data
3. Effect: - positive - neutral - adverse	- predictive ability	predictive models

Table 4: Summary of Information Needed to Confine Engineered Organisms

Containment/Mitigating Factors	
Mitigating	**Enhancing**
adverse conditions	acceptable conditions
starvation	adaptation
competition	competitiveness
predation	avoidance
parasitism	resistance
Outcome	
death	revitalization
debilitation	establishment
	dispersal

perspective of the particular statute in order to make an appropriate decision.

A final point to consider is one which is generally well accepted. Risk assessors need access to data. This can be met by electronic means or through the use of consultants. However, here is an equally great need for in-house expertise to interpret information. This need can only be filled by means of training and by maintaining personal contacts.

The procedure looks and is complex. Taking advantage of the thinking and mistakes of others will permit shortcuts to successful development of regulatory structures and risk assessment.

References

Cuskey, S. M. 1992. Biological containment of engineered micro-organisms. In *Microbial Ecology: Principles, Methods and Applications*. Levin, M. A., R. A. Seidler and M. Rogul (eds.). pp. 911-918, McGraw Hill, New York.

EPA. 1992. Review of progress in the biotechnology - biological control agent program. Environmental Protection Agency (EPA) Environmental Research Laboratory, Gulf Breeze.

Levin, M. A., R. Seidler, R. Bourquin, A. Fowle III and T. Barkay. 1987. EPA developing methods to assess environmental release. *Bio/technology* 5:38-45.

Levin, M. A., R. Seidler and M. Rogul (eds.). 1992. *Microbial Ecology: Principles, Methods and Applications*. McGraw Hill, New York.

NAS. 1983. *Risk Assessment*. National Academy of Sciences (NAS). National Academy Press, Washington DC.

NAS. 1987. *Introduction of Recombinant DNA Engineered Organisms into the Environment: Key Issues*. National Academy of Sciences (NAS). National Academy Press, Washington DC.

NAS. 1989. *Field Testing Genetically Modified Organisms: Framework for Decisions*. National Academy of Sciences (NAS). National Academy Press, Washington DC.

Vidaver A. and G. Stotzky. 1992. Overview: Containment, decontamination and mitigation. In *Microbial Ecology: Principles, Methods and Applications*. Levin, M. A., R. A. Seidler and M. Rogul (eds.). pp. 781-797, McGraw Hill, New York.

Chapter 2.7

The Role of the Environmental Protection Agency in the Introduction of Biologically-Based Pesticides in the USA

Larry Zeph
Science and Policy Staff, Biotechnology
Office of Prevention, Pesticides and Toxic Substances
United States Environmental Protection Agency (EPA)
401 M Street SW, Washington DC, USA.

Introduction

This chapter describes the Environmental Protection Agency's (EPA) experience in the review of small-scale field tests of transgenic plants with pesticidal properties. It first address two aspects of EPA's policy on biologically-based pesticides which are relevant to biosafety.

Zeph, L. 1994. The Role of the Environmental Protection Agency in the Introduction of Biologically-Based Pesticides in the USA. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 139-145.

EPA's Policy on Biologically-Based Pesticides

In the USA, the agency currently most involved in the review of plants produced through biotechnology is the United States Department of Agriculture (USDA). EPA is examining its role in the regulation of specifically those plants which have pesticidal properties under the laws which govern the use of pesticides in the USA: the Federal Fungicide, Insecticide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetics Act (FFDCA).

FIFRA creates a statutory framework under which EPA, through a registration process, regulates the development, sale, distribution and use of pesticides, regardless of how these pesticides are made or their mode of action. Pesticide residues in foods are regulated under FFDCA. EPA expects to have a proposed policy on plants containing pesticides (termed "plant pesticides") later this year.

Under an interagency agreement currently in place between USDA and EPA, transgenic plants with pesticidal properties have been informally reviewed by EPA staff in the Office of Pesticide Programs, and comments have been forwarded to USDA for consideration in their regulatory decisions. The experience that EPA has gained in informal review of small-scale field tests is helping in the development of a final policy on plant pesticides.

The following are a few words about EPA's policy on biologically-based pesticides. Biologically-based pesticides include microbial pest control agents, such as the bacterium *Bacillus thuringiensis*; biologicals such as growth regulators and attractants; and pesticides produced in higher organisms. In this third grouping are pesticidal substances produced in plants, particularly the transgenic plant pesticides which EPA's Office of Pesticide Programs has reviewed over the past several years. EPA's policy is to encourage the development and use of pesticides that are environmentally sound and conducive to sustainable agriculture. In general, EPA's past experience indicates that pesticides that are biologically based, such as the products of biotechnology, are of this type.

For example, EPA believes that plant pesticides will continue to have several properties that allow them to be considered, in general, environmentally sound. Why is this so? EPA's experience over the years with microbial pest control agents and biologicals has certainly contributed to this perception. In general, it has been found that microbial pest control agents are relatively specific in the pest agents that they effect in comparison to certain chemical pesticides. Similarly, biologicals used as attractants and growth regulators are generally highly specific to the target pest.

Moreover, the continual increase in our knowledge of biological pesticides from basic research—for example, in their modes of action and specificity—has helped to reinforce this belief. We expect the same to be true for plant pesticides, particularly when genes and gene products are used in plants for which we have previous experience with uses in microorganisms.

As mentioned previously, EPA has not yet issued a policy statement on how it intends to regulate plant pesticides. Current thinking is that use of EPA's authorities under the FIFRA and the FFDCA is an appropriate vehicle for addressing the potential risk related to plant pesticides.

Over the years EPA has attempted to encourage the development of some of these biologically-based pesticides in a number of ways (EPA, 1988; 1989a; 1989b):

- biologicals have a separate, reduced set of data requirements;
- petitions for registrations of biologicals are given expedited reviews; and
- the review process for microbial pest control agents has been streamlined for those microbials that pose a low risk.

EPA's Experience in the Review of Transgenic Plant Pesticides

The following is an overview of our experience in the review of transgenic plants which express pesticidal properties. EPA has conducted informal reviews of small-scale tests of certain plants that produce pesticides in conjunction with the Animal and Plant Health Inspection Service (APHIS) of USDA. To date, EPA has participated in the review of over 200 small-scale field tests involving transgenic plants that have been submitted for review to USDA. What issues have been raised in EPA's review of these tests?

As these are small-scale research and development tests, human health issues associated with consumption of food crops are generally not an issue. As a result, environmental considerations have been the primary focus of EPA's informal reviews. I will briefly discuss two of the major issues related to risks to the environment.

First, in the review of plant pesticides, one logically wants to consider possible effects on organisms other than the intended pest. Non-target effects are exemplified by unwanted toxicity to beneficial insects or animals, and possible effects on populations of endangered species.

The Office of Pesticides Programs works closely with the Department of Interior to ensure that the use of pesticides will not affect any

endangered species. One would also consider the two general routes of exposure of the pesticide product to nontarget organisms, including exposure due to production of the pesticide product in the living plant or possible exposure through plant material after harvest or seed collection—for example, in plant material incorporated into the soil after completion of the field test.

For the small-scale field tests that EPA has reviewed to date, EPA has identified few concerns for nontarget exposure or effects. The reason for this is that exposure has been limited both by the relatively small-scale of the tests and the fact that the plants that have been used in these tests are adequately contained, i.e. they are not likely to disseminate in the environment under the conditions of the test.

A second issue concerns possible transfer of genetic material to closely-related plant species. That is, under the conditions of the small-scale test, is there any potential for significant outcrossing to weedy relatives? Of course, certain crops with which EPA has trial experience in the USA have no weedy relatives or, in many cases, the field tests are carried out in areas where no weedy relatives exist. Thus outcrossing has not presented a significant concern, in EPA's experience, with small-scale tests of plants with pesticidal properties.

Review Information

The following briefly discusses some of the general types of information and data that EPA believes to be relevant to the assessment of small-scale field trials of plant pesticides. This summary is not intended to be exhaustive, but to serve as a focal point for discussion.

The relevant information can be divided into three categories. The first is information on the identity of the organism. That is, what is the plant and pesticide trait involved in the experimental use? This information would specifically include the identity, characterization, and mode of action of the pesticidal gene product encoded by the inserted genetic material. In addition, the identity of the introduced genetic material, including a description of the genetic material encoding the pesticidal product and the vector system employed in its introduction into the plant genome, is useful. Finally, a description of the relevant characteristics of the recipient plant can be helpful. In our experience at EPA, in some cases the submissions have also included information on the level of expression of the inserted gene sequence.

A second category of information is environmental fate. One issue to be addressed is the biological fate of the inserted genes through

consideration of the potential for movement of the genes to wild relatives. Information relevant to this question includes the level of domestication of the crop plant, the barriers to germplasm transfer, the method by which the crop pollinates, and the occurrence of closely related wild species in the area of the test.

This information may well be obtained from scientific literature or, in some instances, through laboratory experimentation. A second consideration under the heading of environmental fate is the fate of the pesticidal gene product itself. Relevant information here includes the nature of the pesticide (e.g. proteinaceous *versus* non-proteinaceous), whether the pesticide is exuded from the plants through roots or other plant parts, and the rate of degradation of the pesticide in the environment.

A third category is ecological effects information. This includes identification of potential effects of the pesticidal substance on terrestrial or aquatic nontarget organisms, including endangered species. These concerns, of course, depend on the crop and use pattern as to whether effects on beneficial insects, avian toxicity, or effects on nontarget aquatic organisms are relevant considerations.

There are different ways to gain information on these issues if it is not contained in scientific literature. Toxicology testing is not necessarily the only mechanism for assessing nontarget effects. For example, one can have information on levels of the gene product in pollen such that concerns for exposure due to insect pollination are limited or minimal.

These points can be briefly illustrated by discussing a field test of cotton plants modified with the gene for *Bacillus thuringiensis* (*B.t.*) delta-endotoxin. This field test is part of an ongoing series of tests carried out by Monsanto Company in several states in the USA. EPA's risk assessment for this field test addressed many of the issues outlined above. This review for eleven different states covered field tests, ranging in size from a small two-acre test in Hawaii for seed increase up to tests in Mississippi on two 20-acre breeding nurseries.

In terms of the exposure assessment, the issue addressed was whether the *B.t.* gene might spread from any of the test sites, either to wild cotton or to adjacent commercial cotton growing in nearby fields. It is known that wild cotton can only overwinter in tropical areas in the USA, such as southern Florida and Hawaii. As one small-scale test was being carried out in Hawaii, assessors looked at the issue of spread to wild cotton.

Their conclusion was that the test site was sufficiently well-contained to prevent cross pollination with wild relatives. A more likely scenario would be the spread of *B.t.* genes to adjacent commercial cotton. The

preliminary risk assessment estimated outcrossing frequencies to commercial cotton would be at most three percent. In addition, as the gene would be expressed only in the seed of a few of these plants, actual exposure would be very low.

The ecological effects assessment focused primarily on nontarget insects because of the use of the *B.t.* toxin. A second issue considered was the development of insect resistant cotton that could have weedy characteristics. The preliminary risk assessment concluded that these field tests were sufficiently well-contained at all sites such that exposure to nontargets would be very limited and of minimal concern. Moreover, the likelihood of this particular crop being turned into a potential weed was determined to be very low.

In the consideration of potential human health effects, concerns for direct human exposure during the field test itself were also very limited because of the small-scale nature of the test and the type of pesticide product in the *B.t.* toxin. As the crops were not to be used for human consumption, dietary exposure was not considered.

Conclusion

EPA has a policy to encourage the development and use of pesticides that are environmentally sound. We believe that biotechnology may be successfully used to address some of the critical environmental issues that are facing us today. In the area of plant biotechnology this includes the important issue of replacing some of the more problematic chemical pesticides with more benign products.

The advent of newer techniques in molecular biology and genetics for use in biotechnology has presented government agencies at all levels with a number of issues. First, there is the need to evaluate the adequacy of their own procedures for reviewing a variety of products for food, agriculture and environmental uses. Within the USA, we are at varying stages in the establishment of regulatory procedures for biotechnology products, and I believe workshops are helpful to all of us in deciding on the appropriate level of oversight. Second, there is a strong need to develop harmonization activities with other government organizations with the aim of ensuring scientific consistency in the review of these products.

References

EPA. 1988. 40 C.F.R. Parts 152, 153, 156, 158 and 162 Pesticide Registration Procedures; Pesticide Data Requirements; Final Rule.

Fed. Reg. 53:15952. Environmental Protection Agency (EPA), Springfield.

EPA. 1989a. Pesticide Assessment Guidelines: Subdivision M—Biorational Pesticides. National Technical Information Service, Springfield. No. PB89-211676. Environmental Protection Agency (EPA), Springfield.

EPA. 1989b. Pesticide Assessment Guidelines: Subdivision M—Microbial and Biochemical Pest Control Agents (Parts A9). No. PB89-211676. National Technical Information Service. Environmental Protection Agency (EPA), Springfield.

Section 3

Public and Private Sector Experiences in Conducting Field Trials with Transgenic Crops

Chapter 3.1

Field Releases of Transgenic Plants in Practice

Willy de Greef
Consultant to the International Center for the Acquisition of Agri-biotech Applications (ISAAA)
*ISAAA Euro*Center, *John Innes Center*
Colney Lane, Norwich NR4 7UH, UK.

Introduction

Field experiments with genetically engineered plants are not a goal in themselves but a means to obtain information which should allow the performer of the experiment to make some progress in his or her project. It is therefore the nature of the project in the context of which a field release is carried out that will determine the way in which a trial is organized. The variety of projects that include field trials with transgenic plants has grown substantially, and covers very diverse areas of fundamental and applied research in biology and agronomy.

de Greef, W. 1994. Field Releases of Transgenic Plants in Practice. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 149-161.

The ultimate goals of such projects are also very different. Some projects aim at the production of new crop varieties incorporating new genes introduced by genetic engineering. These are the most widely publicized: insect, virus or herbicide resistant plants, plants with altered protein or lipid composition, etc. Most of these projects are executed by industrial corporations, sometimes in collaboration with public research institutes. They also include many of the earliest achievements in plant genetic engineering.

Another rapidly growing group of projects involves the creation of transgenic plants, and the testing of them in some environments is only a step on the road to better understanding the processes governing the development of plants and their interaction with the outside world.

In short, genetically engineered plants are generated with one of two broad goals in mind: the production of material products, or the production of knowledge.

The Purpose of Field Trials in Plant Biotechnology

Testing in the field is usually part of a more extensive sequence of tests performed on plants. The relative importance of field tests in such a sequence is determined by the nature of the trait which has been introduced, by the plant species under investigation, and by the ultimate goal of the project.

If the goal of the project is to test the phenotypic expression of a gene involved in some aspect of the plant metabolism, most of the work may actually be done in the laboratory, in growth chambers, or in a greenhouse. This often has the advantage of allowing better control over the environmental conditions in which the plant is tested, resulting in more clear-cut experimental results. In such projects, scientists may actually avoid bringing plants outdoors, since it does not help them in their work. Hence, an important part of *in vivo* experimentation in plant physiology, phytopathology and even ecology takes place in a controlled environment. However, a controlled environment only allows the testing of those interactions between plants and their environment which are allowed to vary.

That is why no serious model experiment can be interpreted satisfactorily without reference to those conditions which are kept constant, and without considering possible other interactions in an open field situation, regardless of the nature of the plants being studied. It explains why, after nearly a century of research in controlled environments, nearly all the research in plant breeding and agronomy is

still done in the field. There is simply no other effective way to integrate all the variable components of the environment when testing a trait expressed in a plant line for its genetic or agronomic potential. The same applies for genetically engineered plants, even for "simple" characteristics such as monogenic resistance to pests, diseases or herbicides.

The detailed reasons for the necessity of testing transgenic lines in the field will be further discussed later, with a number of practical examples. Now we will turn to the practical implications of the existing regulatory framework for field testing genetically engineered plants, as compared with other plants.

Regulatory Constraints on Transgenic Field Tests

It is important to be aware of the fact that agronomic or genetic field testing is a matter of statistics, of working with quantitatively changing parameters. A plant is not either disease resistant or susceptible: it is scored on a continuous scale from completely devastated to (sometimes) almost completely unaffected. The amount of damage varies depending on a wide range of factors which at first sight may seem to have little to do with either the plant or the pest attacking it. Therefore any plant breeding program which includes selection for characters such as disease resistance requires very extensive testing programs under a wide range of natural environments, usually over several years. The same is true of resistances built into the plant by genetic engineering. However, there are major differences in the way experiments with genetically engineered plants are set up, compared with non-engineered plants.

1) Field tests with genetically engineered plants require a permit. Trivial as this may seem, it is a very major new situation for scientists working in agronomy and plant breeding, and it takes time for the research community to adjust. The process of obtaining such a permit is complicated and time-consuming. Moreover, the data required in the application for permits is often quite difficult to find, making the preparation of the application a significant part of the experimental work as a whole.
2) Regulations usually require that experiments with transgenic plants be set up in conditions of isolation from related plants, in order to minimize the chance that the engineered genes "escape" into uncontrolled plant populations. Isolation distances can be quite substantial, especially for species which are considered to be efficient cross pollinators.

3) It is also usually required that all the seeds harvested from the trial should be destroyed (including material from nontransgenic controls), and the site of the trial monitored for several years with particular reference, for example, to the development of offspring from buried volunteer seed.

As a result of these special requirements, it is usually impossible to compare results from experiments with transgenic plants directly with results obtained from more traditional trials. One way to overcome this problem is to include all the non-engineered plant lines in the "transgenic" trials, and to treat the whole trial as a transgenic one. Although this is perfectly feasible for some traits, it creates impossible work loads if undertaken in a conventional breeding trial, where a breeder may well want to compare several tens of lines, only a few of which are engineered.

An additional effect is that it is very difficult to comply with the regulatory requirements for companies or institutions working with engineered lines of their main crop. This apparent paradox is best explained with a practical example. Consider a company specialized in sugarbeet breeding, which has developed transformation technology for its crop. Field trials with the transgenic sugarbeet would be quite difficult to conduct at the main experimental stations of that company, since these stations will have a considerable number of other sugarbeet trials on those sites, and would therefore usually not meet isolation requirements. This is why so many field releases of transgenic plants have been carried out in environments which are somewhat unusual for the crop under investigation.

A final restriction on work with engineered plants *versus* traditional material is that regulations differ between countries. Procedures differ even more, and criteria for approval or rejection of an application are sometimes contradictory. This poses serious constraints to multi-site agronomic trials undertaken in several countries simultaneously—a very common type of trial in most breeding programs with major crops in Europe.

Incorporating Field Tests in Project Designs

From the above, it can be deduced that any project in plant biotechnology which includes the prospect of field trials should be designed from day one with the restrictions on these trials in mind. To explain why this is so, it is again best to consider a number of practical examples, and to define which early choices in the project have an impact on the acceptability of the resulting plants for field introduction.

Vector Construction

The DNA sequence that carries the desired gene into the plant is often a quite complex construct. Apart from the target gene and its promoter and termination sequence (both of which, like the gene itself, can originate from very different donor organisms), the construct usually contains a gene coding for a selectable marker (with its own promoter) operating during the *in vitro* culture phase of the project. Often a different marker gene is used to follow the transgenic line in the greenhouse and in the field. If transformation is achieved with the use of *Agrobacterium tumefaciens* (the most common method) all the pieces of DNA mentioned above are embedded in two border sequences, originating from the *Agrobacterium* genome. It is therefore quite common that the DNA inserted in the host plant contains sequences from three to seven totally different organisms. If one of these sequences originates from an organism which is classified as a human pathogen, this can lead to delays in the approval of the recombinant plant for field release, or even to refusal of the application.

Another aspect of vector construction which can create serious problems is the presence of "junk DNA". This is a rather unfortunate name for non-coding sequences of DNA, usually leftover pieces of sequences flanking one or more of the genes of interest in the donor organism. Often, scientists do not go to the trouble of eliminating all the non-essential DNA in the early stages of vector construction, partly because these flanking sequences often provide useful sites for linking different genes together. Another type of junk DNA is a total DNA preparation from a commercial source (e.g. calf thymus DNA) which is used to protect the DNA of interest during direct transformation experiments. Some of this DNA may become inserted in the genome of the host plant. One of the recent evolutions in the application of regulations on field releases has been the insistence on "clean vectors", containing only DNA of which the function is well understood.

The time lag between the start of vector construction and the first field trial of the transgenic plant is usually two or more years. The cost of starting with vectors unacceptable for field trials is therefore very high, in terms of time and effort wasted. It is important that information on conditions for field releases is widely distributed in the research community. Most industrial corporations entering a research area are aware of these restrictions, or have procedures in place whereby their scientists check up, at the start of a project, on the regulatory framework in which they are asked to operate. The same is not true for most academic institutions, or for many bodies funding biotechnology research.

Choice of Host Plant

The selection of the right recipient for the genes to be engineered is crucial in determining the chances of success of later field evaluation of the transgenic plants. Until recently, transformation methods were not sufficiently advanced to allow the scientist a wide choice of host species and/or varieties. Most of the pioneering work in plant genetic engineering was carried out with tobacco, simply because it was the only species for which the actual production of transgenic plants was not a limiting factor in the research projects. Somewhat later, scientists started using certain tomato and potato lines as alternative "model species". When those early projects reached the stage where plants were ready for testing under open field conditions, it became clear that a lot of the work had been carried out with varieties of these plants that were not suitable for field evaluation. Tobacco *as a species* actually turned out to be an unfortunate choice, because it was difficult to grow to maturity in the climate of the regions where much early genetic engineering work was done. This forced scientists to make complex arrangements to conduct field trials sometimes thousands of kilometers away from their laboratories.

A problem of a different nature arose with early potato work, much of which was done with a very old variety called Berolina. The only reason for choosing this variety was that the transformation results were quite good. Unfortunately, it turned out to be a variety that was very difficult to transfer from the test tube to the field. Therefore it was impossible to test the performance of the introduced genes (in this case insect resistance) in the field. Another much used potato variety (Désirée) has none of these problems, but flowers profusely. Since many of the early field experiments were approved on condition that the plants would not be allowed to flower (to avoid spreading of the recombinant genes), trial fields had to be patrolled daily and flower buds removed during the entire flowering period.

Troublesome as these early problems were at the time, they served the useful function of stressing the need to consider very carefully the choice of recipient plant in any new genetic engineering project. Today, it is a priority in most applied projects to establish sufficiently reliable transformation procedures for elite breeding material of the project's target crop. This is often a major factor in hindering research. Research teams have repeatedly found, to their distress, that solving transformation for one particular line of a given species offers no guarantee that this will be automatically applicable to other varieties. Nevertheless, the list of plant species of major scientific and/or

economic interest for which transformation is now routinely possible using elite germplasm is growing rapidly (Table 1).

Data Collection on Newly Produced Plants

Transformation experiments, and the plants produced, are constantly monitored for parameters indicating success of the experiments. One transformation experiment will usually generate several (sometimes hundreds) of independent transgenic plants. The main goal of the monitoring process is to find the line(s) that gave the best expression of the desired trait in the absence of any side effects. Many of these observations are also used in the preparation of requests for field releases. This early work is done in growth cabinets or, more usually, in a greenhouse. Parameters tested in almost all projects are outlined below.

Expression of the Target Gene

The primary interest of the scientist is, of course, to see if the transformed plants express the new traits. This can be done biochemically, by detection of the protein encoded by the engineered gene, or by a measure of its activity. If the new gene also gives the plant a recognizable new phenotype (e.g. insect resistance), bioassays will also be performed. In the case of insect resistance these assays will include target and nontarget insect species. It is quite common to find differences in expression levels of a factor hundred between independent transformants. This is due to the effect on expression of the place where the gene is inserted in the plant genome.

Expression of the Marker Genes

The expression of the marker (or reporter) genes can be very important for later genetic work on the transgenic lines, especially if the target trait of the project is not readily observable (e.g. improved protein composition of seeds). The most widely used marker genes are the resistance genes to the antibiotic Kanamycin and to the herbicides phosphinothricin and sulfonylurea, and the gene coding for the enzyme glucuronidase.

Gene Copy Number

In most cases it is preferable to obtain the desired effect with only a single copy of the new gene, since this greatly facilitates the subsequent

Table 1: Routinely Engineered Crops

Major field crops:	tobacco	potato
	tomato	rapeseed
	cotton	alfalfa
	sugarbeet	maize
	soybean	melon
Vegetable crops:	cauliflower	lettuce
	carrot	chicory

genetic work of transferring the trait in other germplasm by conventional breeding techniques. Plants are usually screened for copy number by Southern blot analysis on the original transformants, followed by a segregation study on the first generation progeny. In those cases where no single copy plants with all the desirable traits are found, multiple copy plants are separated into single copy plants by outcrossing.

Plant Morphology

All plants are carefully observed for visible malformations. These can result from mutations induced by the *in vitro* growth conditions of the plants, or from the fact that the engineered genes have by chance been inserted in a locus of the plant genome which is important for normal plant development. These observations are usually continued into the second greenhouse-grown generation.

Upscaling of Material for Field Testing

This is a critical part of the greenhouse work. Field tests usually require substantial amounts of seed. More importantly, for most crops the quality of the seed produced in the greenhouse is not as uniform as field-produced seed. For some crops, even the best seed batches produced in the greenhouse will perform less than elite field-grown seed in a yield trial. To make the best of the first field trials in a project, it is essential that utmost care is taken to use uniform seed from the onset of the work.

Execution of the Field Trial

Pre-Release Activities

The work on a field trial starts about one year before anything is planted, with the prospection of possible sites. This includes checking whether the expected isolation requirements can be met. It may also include preliminary experiments with control plants to verify that the site is suitable for study of the target gene (e.g. presence of insect pests), especially if the trial has to be conducted in a place where the crop is not usually cultivated.

Trial Approval

Most countries require about three months to process an application file. In practice, and especially if a previously untested gene or crop is proposed, it is better to introduce the file five to six months before the expected field planting date, to allow for additional questions from the authorities.

Execution of the Field Experiment

The central goal of most field experiments is the continued observation of the new traits of the engineered plants. Methods used, and trial layout itself, will be determined by this. In experiments where the main goal is to compare yield, a conventional statistical design will be used. In disease resistance trials it may be necessary to provide for good inoculation conditions for the disease. There is a large body of experience with this type of trial in plant pathology.

One of the specific features of early trials with engineered plants is the emphasis on further screening of different transformed lines for conformity to the non-transformed control. Although most off-type plants will have been eliminated in the greenhouse stage, subtle side effects of the transformation procedure or of the introduced genes can only be visualized in a replicated growth and yield trial in the field, where the full complexity of a variable environment influences the development of the plants, and where plot size and number is sufficient to allow the often very small effects on growth and/or yield parameters to become statistically measurable.

Another aspect of the observations in the field is related to the requirements for monitoring put forward by the authorities in the approval. The requirements depend on the crop and trait under

investigation. Monitoring extends to the period after termination of the experiment, and to the surrounding area of the trial.

Termination Activities

After harvesting the trial, the plant material in the field has to be disposed of. It should be kept in mind that in many trials the "harvest" consists of observations and measurements in different stages of development, and many trials are terminated well before the plants have grown to maturity or set seed. Depending on the developmental stage of the plants at termination, different effective methods for destruction are used. If the plants are still green and growing actively, by far the most effective destruction method is by spraying them with a systemic herbicide, which ensures that the roots are destroyed along with the above-ground parts. If the crop is mature and dry at harvest, it is often burned, or rotovated into the soil.

Case Studies

In this section some examples of implementation of the above principles are discussed. The goal is to illustrate how different in nature the field trials in a large program can be, and to illustrate some of the limitations imposed by the present regulatory frameworks.

Testing Insect Resistance

The first field experiments done by Plant Genetic Systems Ltd. (PGS) were on tobacco plants which had been engineered to express a protein of bacterial origin *(Bacillus thuringiensis)* which kills the caterpillars and other larvae of certain major tobacco insect pests. However, the trials had to be done in the USA (North Carolina) because these pests are not significant in Europe, and therefore no meaningful data on field resistance could be obtained in Europe. The most remarkable result of these trials was that insect control was much better in the field than in previous growth chamber tests.

This quite unexpected observation was studied further by scientists of North Carolina State University. They discovered that the insect resistant plants, which had not been treated with chemical insecticides, carried a much larger population of predator insects and parasites of the pest than control plots that were maintained insect free with conventional insecticides.

Testing Promoter Activity

One of the most important qualities demanded from any genetic system before it can be considered for commercialization is stability of performance under different field conditions. In genetically engineered crops, two conditions can cause failure to perform: the instability of the gene product (enzyme), or erratic expression of the gene. The latter is caused by the sensitivity of the promoter to changes in environmental conditions. It is possible to obtain some preliminary information on the stability of enzymes by extrapolating results from *in vitro* tests (although these should be verified *in vivo*), but the stability of promoter activity can only be tested reliably *in vivo*, preferably under the highly variable conditions found in the field.

In two separate field tests, on tobacco and alfalfa, it was demonstrated that two promoters, both of which controlled expression of a herbicide resistance gene, and both of which gave adequate resistance in greenhouse tests, behaved very differently under field conditions, one promoter giving good resistance under all conditions, while the other gave much lower levels of resistance in the field than in the greenhouse.

Predicting Behavior of Genes and Promoters

One of the most appealing qualities of genetic engineering is that, in principle, the same gene will work in the same way in any organism, provided that the signals controlling its expression function equally well in different receptor species. This is why much of the early transformation work on monocotyledonous crops (at present mainly maize) is again using the old and very thoroughly studied gene coding for antibiotic resistance and/or herbicide resistance to evaluate the performance of promoters specifically isolated for work in monocotyledonous species, and to find out if the “old” dicotyledonous and non-plant promoters can be relied on in these new crops. The answer to this last question is important: it could mean years of work to find suitable analogous promoters to the trusted workhorses developed in dicotyledonous plants. Now that greenhouse evaluation has been completed, the planting season will deliver the critical data to finalize the analysis, in the form of a series of maize trials in Europe and the USA.

Multi-Site Yield Component Trials

Yield is notoriously difficult to measure, because it is an aggregate index of all the influences of the genotype, the environmental conditions and

the interactions between these two on the development of plant communities. To separate all these influences, and to single out the effects of differences in genotype, requires testing over a range of environments. Few such multi-site trials have been attempted with transgenic plants, partly because of lack of suitable transformed plant varieties, and partly because of the complexity of undertaking replicated trials in different countries, which impose different confinement and/or monitoring requirements on the replicas, thereby defeating the basic feature of the trial.

An example of such a trial was a two-year, multi-site study of rapeseed expressing an engineered seed storage protein. The material was tested in 1989 in Belgium, Sweden and Canada, and in 1990 again in these three countries and in the UK. The interpretation of the results was complicated by the fact that the variety originally chosen for the transformation was genetically heterogeneous, and therefore gave problems with the choice of suitable control lines for performance evaluation. One of the positive results of this project was that in this case it had been possible to obtain reasonably similar conditions for the release in the different countries.

Testing Engineered Male Sterility Genes

The use of conventional male sterile carrier plants for field testing of genetically engineered traits has often been proposed as a good method for eliminating the risk of gene spread through pollen. Recently, male sterility has also been obtained in plants by recombinant DNA technology. The evaluation of this material requires even more multi-site testing than yield testing, since the first requirement of such a system is stability under all the environmental conditions where it may eventually be used. Field work was begun with tobacco in 1989, rapeseed in 1990 and chicory in 1991. It is anticipated that the present range of trial countries (Belgium, France, Sweden, Canada) for rapeseed testing will be further expanded, as trial results accumulate, to include the full range of climatic conditions where the trait may be used in rapeseed breeding.

Concluding Remarks

Field testing of genetically engineered plants has come a long way since the first attempts of 1985-1986. It has come to be seen as a fully integrated part of all the projects that aim at eventual commercial introduction of such plants, and as an important component of many

fundamental research projects in plant biology. The early trials have done much to make scientists in other fields aware of the potential of plant molecular biology in general as a tool in their own research or industrial development projects. At the same time these trials have sparked off a vigorous debate about the safety aspects of such work, leading to important efforts in risk management in a newly emerging technology. As more data from the monitoring of experiments is accumulated, this debate will probably become more focused, making a distinction between perceived and potential real risks. The start of several research programs specifically targeting these issues will be instrumental in providing the basic facts needed for the further evolution of the entire field.

Beversdorf, W.D.. 1994. A Canadian Experience in Conducting Field Trials. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 163-166.

Chapter 3.2

A Canadian Experience in Conducting Field Trials[1]

Wally D. Beversdorf
Head, Seeds Research and Biotechnology
Ciba Limited
Postfach, SE2, CH-4002 Basle, Switzerland.

Introduction

Canada has a very large agri-food sector. The 67 million hectares of improved farmland in Canada are divided into 280,000 farms, 98% of which are family-operated production units. The majority of Canada's major field crops have been introduced during the past 200 years (wheat, alfalfa, barley, rapeseed, canola, and soybean). Most of Canada's crops have undergone significant genetic modification through classical plant breeding during the past 100 years.

Due in part to Canada's excellent history in variety improvement (yield improvements, quality improvements, and improvements in

pest/stress tolerance), producers are not only receptive to genetic modifications but, in many instances, financially support, through producer associations, the research, development and plant breeding efforts associated with genetic improvement of major crop species.

The evolution of recombinant DNA technologies since the mid-1970s, and the emergence of plant transformation technologies in the 1980s, are providing opportunities in Canada for crop improvement through a combination of genetic engineering and classical plant breeding. These opportunities are currently being exploited by a number of public institutions as well as the private sector.

Transgenic Plant Research

Although Canada had significant experience with plant cell and tissue culture, there was relatively little activity in plant genetic engineering prior to the breakthrough by Jeff Schell, Marc van Montagu and Mary dell Chilton in developing and utilizing disarmed *Agrobacterium tumefaciens* in the early 1980s. At about the same time, the National Research Council's Prairie Regional Laboratory at Saskatoon was transformed into the Plant Biotechnology Institute. Numerous researchers in public institutions and a few private companies initiated transgenic plant research in Canada. Many public sector faculty positions in plant biology or plant science departments were filled with recruitment trained abroad in molecular biology. Many faculty members also used sabbatical leaves to retool in plant genetic engineering technologies during the mid-1980s. Several small private sector plant biotechnology companies emerged during the early and mid-1980s in Canada, recruiting molecular biologists from the USA and Europe. Canadian plant scientists worked in networks (both domestic and international) to access technologies and transgenic germplasm for continuing development and utilization. By the mid-1980s, many public and private sector laboratories were handling transgenic plant material, associated transformation vectors, and genes of potential relevance to Canada's field crop sector.

Field Evaluations of Transgenic Plants

By 1987, it was clear that transgenic plants under evaluation in several laboratories in Canada would eventually need evaluation in simulated production environments under field conditions. In that year Canada's federal Ministry of State for Science and Technology commissioned a

background report on regulatory policy options for biotechnology. In the same year, Agriculture Canada reviewed international regulations for genetically engineered organisms and drafted a regulatory process for products of plant biotechnology. Their reports recommended that "open environment" testing of plant material altered to contain additional genetic material from other genera or kingdoms should be controlled. Further, they recommended that applications for environmental release of genetically altered plant materials be submitted, and that such applications be reviewed on a case-by-case basis by a review committee prior to approval. Agriculture Canada, through consultation with the federal Department of the Environment and the Department of Health and Welfare, and through participation in a series of regulatory workshops and consultation with a large number of Canadian public and private sector plant geneticists and breeders, developed a preliminary application procedure in early 1988 for controlled field testing of transgenic plants. The first field evaluation of transgenic plants was conducted later that year by Agriculture Canada. Since then, the evaluation of transgenic plants in Canada has grown steadily and dramatically. Agriculture Canada reviewed more than 100 applications for field evaluations of transgenic plants in 1991, 302 in 1992, 503 in 1993 and 848 during the first half of 1994 (up to 16th May).

The University of Guelph initiated field evaluations of transgenic canola in 1989 and transgenic alfalfa in 1990. To date, transgenic canola and alfalfa families that have been field-evaluated include a variety of alien gene constructs involving DNA from other plant species, microbial species, fungal species and the coat protein of cauliflower mosaic virus. Characteristics associated with these "transgenes" include antibiotic resistance, herbicide tolerance, modified amino acid composition, male sterility, male fertility restoration, and tolerance to abiotic (physical) and biotic stresses.

In all cases, field evaluations of transgenic plants have followed application to, and approval from, Agriculture Canada. Reviews of individual applications have taken from two to four months and have required fairly specific information on the nature of the genetic modifications of transgenes, the experimental procedures that will be employed for the field evaluation, a description (physical and biological) of the field trial locations, and a description of post trial procedures to prevent entry of transgenic plant material into the food chain or agricultural production ecosystem.

The University of Guelph has also provided public notices of intent to evaluate transgenic plants in field environments. These notices have included letters to local, regional and national politicians representing

the area of the intended field trial and notices through general media press releases. Prior to the University's first transgenic field trial, the University hosted a public discussion of transgenic field evaluations for neighboring producers, the general public and the media. This public discussion focused on the purpose of the trials and the procedures employed to minimize escape of transgenic material from the trial location. Local farmers and the media appeared satisfied that the research was necessary, and in the interests of the Canadian agri-food system generally.

Although no subsequent public discussions have been held, trial-specific information is released publicly each year. To date, no trial-specific concerns have been directed to the University as a result of these information releases.

In spite of the regulatory framework developed by Agriculture Canada, at least one environmental activist group has criticized the government for permitting field evaluation of transgenic plants. Both the government and the research community (public and private sector) have been criticized for secrecy and irresponsibility associated with field evaluations of transgenic crops. Criticism has been of a general rather than specific nature, intended more to elicit public fear or outrage rather than to address specific concerns regarding human health, environmental protection or sustainable agricultural ecosystems.

Conclusions

The University of Guelph has now had five years experience in transgenic field trials under Canada's regulatory framework for transgenic plants. To date, our experience has been satisfactory, but I should caution that the current regulatory framework deals primarily with small-scale field trials. The regulatory process for scale-up and commercialization of transgenic crop varieties is being developed but is, as yet, far from clear.

Based on previous field trials, there is certainly potential for the emergence of improved transgenic varieties of important Canadian crops within the next few years. A lack of regulatory processes which permit commercialization may delay the availability of improved transgenic crop varieties in Canada.

Note

1. This paper is based on the author's experience while working as the Director of the Department of Biotechnological Research at the University of Guelph, Ontario N1G 2WI, Canada.

Quemada, H.D. 1994. The Asgrow Seed Company's Experience with Vegetable Biotechnology. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 167-173.

Chapter 3.3

The Asgrow Seed Company's Experience with Vegetable Biotechnology

Hector D. Quemada
Associate Director, Experimental Plant Genetics
The Upjohn Company
Kalamazoo, Michigan 49001, USA.

Introduction

The goal of Asgrow Seed Company's vegetable biotechnology program is to develop virus resistant cantaloupe and squash, using the coat protein-mediated protection strategy pioneered by Roger Beachy and the Monsanto Company (Powell *et al.*, 1986). The first work on this project began in 1986 with the cloning, characterization and engineering of viral coat protein genes and the development of transformation and regeneration procedures for cantaloupe and squash. However, the major work on this project began in August 1989. By that time, we had two major elements in place which gave this project a good chance of

success. First, we had been able to clone and engineer the coat protein genes from the four most destructive viruses of cucurbits: cucumber mosaic virus (CMV), papaya ringspot virus (PRSV), watermelon mosaic virus 2 (WMV2), and zucchini yellow mosaic virus (ZYMV). Not only did we have these clones in hand, but we had demonstrated in tobacco that the phenomenon worked to provide protection against at least one virus: cauliflower mosaic virus. The phenomenon had already been demonstrated for cauliflower mosaic virus in tobacco by Monsanto, but the fact that we were able to duplicate those results gave us added confidence in the phenomenon. This was not a reflection of our opinion of the work done by Monsanto, but was, rather, a reflection of the state of the research at the time. Second, we had established protocols for the transformation and regeneration of cantaloupe and squash tissue. With these elements in place, we were able to undertake the project of developing commercial transgenic virus resistant plants.

Field Trials

In the fall and winter of 1989-90, scaled up transformation efforts were conducted to produce a number of transformed lines which could be screened first in the greenhouse, then subsequently in the field. This required not only the application of existing transformation protocols, but also the development of protocols for transforming new genotypes. At the same time, we also developed the means by which the transgenic plants could be analyzed, such as developing DNA probes and determining diagnostic restriction patterns to confirm the occurrence and number of integration events. We also developed the means by which to detect the proteins coded by the integrated genes.

In addition, we knew that in order to be commercially viable, the transformed plants we developed had to be resistant to more than one of the above-mentioned viruses. Consequently, we increased the effort to produce multiple coat protein vectors. As soon as these vectors were constructed, they were used in transformations, which eventually produced plants for field testing in the late summer of 1990.

The initial trials, however, were held in the late spring and early summer of 1990, and involved lines we had already produced. The field trials were—and are—conducted at four locations in the USA:

1) in Kalamazoo, Michigan, at the headquarters of Asgrow (the location of our biotechnology laboratories);
2) at Asgrow's Southeast Breeding Station, Tifton, Georgia;
3) at Asgrow's Pacific Coast Breeding Station, San Juan Bautista, California; and

4) at Asgrow's San Joaquin Breeding Station, Arvin, California.

These first trials yielded little valuable information on the ability of lines to resist virus infections. However, they provided valuable experience in the actual mechanics of conducting a field trial to those of us whose previous experience had been entirely in the laboratory. For example, we learned the proper timing of planting and proper inoculation conditions if we wanted optimal infections of each virus. We also established a standard design to be used in all our subsequent tests. Our breeders and other cooperating Asgrow researchers learned to incorporate isolation measures as standard operating procedures when dealing with transgenic plants.

Containment

We also established the regulatory parameters within which we were to work. Because cantaloupe and squash are insect-pollinated (primarily by bees), isolation measures which were acceptable to the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) had to be set in place. First, border rows of nontransgenic plants were planted to serve as pollen traps; these were typically 30 feet wide. The border width was based on published information about pollen flow in cucurbits and other insect-pollinated crops, in addition to consultation with a university researcher who was familiar with cucurbit pollination biology. Second, the planting sites were isolated by distance (at least half a mile, but usually greater) from other non-Asgrow cantaloupe and squash. The minimal distance was based on published standards for genetic isolation of fields used in seed production, as well as existing company standards.

To deal with the question of possible wild relatives which could serve as recipients of transgenic pollen, we consulted published flora of the regions where the field tests were conducted and confirmed the absence of compatible wild relatives. Third, to genetically isolate transgenic plants from other nontransgenic Asgrow breeding lines which were planted in the same field, we relied on standard breeding practices. For example, in the case of squash, breeding nurseries are regularly inspected when flowering, and female flowers are removed. Only those females to be used in a controlled cross are left on the plant, and these female flowers are bagged the day before they open. When the females open, newly opened male flowers are used in the cross. Similar measures are used in cantaloupe crosses as well. I have taken the time to detail this

procedure in order to illustrate the idea that certain standard breeding practices for preventing cross contamination of nontransgenic genotypes can be applied to transgenic plants in order to contain the spread of engineered genes.

Parenthetically, it should be mentioned that although measures were taken to limit the spread of transgenic pollen, we were confident that no health, plant pest, or ecological concerns were presented by our transgenic plants. We were able to do this primarily because of our thorough molecular knowledge of the specific genes we were inserting, and also because we knew that existing examples of virus resistance genes in cultivated plants presented no ecological problems of which we were aware. We have therefore viewed containment measures, such as the first two mentioned above, to be largely unnecessary for our field trials. Nevertheless, we have been sensitive to the perceived need at this time to genetically isolate these plants.

Protection Against Infection

The early 1990 trials were followed by later trials which incorporated new transformed lines, primarily of cantaloupe, many of which were crossed to combine coat protein genes. Although the resulting lines were still segregated for individual coat protein genes and were therefore difficult to analyze thoroughly, these tests demonstrated that plants expressing multiple coat proteins could be effectively protected from simultaneous infection by two or more viruses—the situation which occurs in most cucurbit fields. This gave us an additional basis for subsequent efforts, which have been concentrated on testing individual lines expressing single coat protein genes or multiple coat protein genes introduced as a unit. Tests were first done with a segregating generation, then subsequently with homozygous lines.

Work in Guatemala

The fall and winter of 1990-1991 were devoted to generating more transgenic lines, especially those which were transformed with multiple coat protein vectors. Greenhouse testing of lines which produced seed too late to be included in field trials was also conducted. Promising lines identified by field and greenhouse tests were then increased. For those increases, we made use of a valuable resource: Asgrow's Caribbean Basin Support Station in Guatemala, which was established to conduct off-season increases and breeding of nontransgenic lines, but which was also suitably equipped to handle transgenic plants.

Since Guatemala did not have (and as far as we know, still does not have) an established regulatory framework for dealing with transgenic plants, we met with that country's Minister of Agriculture to obtain permission to bring these plants to Guatemala and grow them in glasshouses. Having obtained that permission, we grew our plants in screenhouses (i.e. finely-screened houses with double-entry doors) which were adequately equipped to prevent escape of the transgenes either by pollen or by seed. Because Guatemala is in an area which is a center of diversity of the genus *Cucurbita*, we were sensitive to issues of containment (although the statement above about health, plant pest, or ecological concerns should still apply in this situation). Nevertheless, the literature on crossing relationships between *Cucurbita* species which were cultivated in the region, as well as between cultivated and wild species, indicated that there was no opportunity for escape of our transgenes into wild relatives. Despite these assurances of safety, no field trials were conducted by us in Guatemala.

As a result of increase in seed in Guatemala, we were able to generate a large amount of seed for field trials. The seed produced was subsequently imported to the USA, and was first used in 1991 field trials. Since that time, we have regularly used our site in Guatemala for winter seed increases.

Production of Commercial Hybrids

During the 1991 field season, we built upon the experience obtained in 1990. Applications for field trials became more routine, although still not trivial; all of our field work in 1991 was done under renewals of the 1990 permits. These trials were more successful from a scientific point of view. The operational lessons learned in the previous year allowed us to design and execute tests which provided solid data on the performance of certain lines. We were able to test our more promising lines repeatedly, especially those expressing multiple coat proteins. We were able to test those lines in separate trials for their ability to resist infection by more than one virus.

As a result of these tests, parental lines were identified which were used in the production of our first commercial hybrids. In particular, we identified squash with showed good resistance against ZYMV, WMV, and CMV. These and other lines were advanced, and also used as starting points for extensive backcrossing into other genotypes. We also were able to plant a large 10-acre trial of cantaloupe to further evaluate selected lines on a large scale. These were single as well as multiple coat protein

expressing cantaloupe lines. This trial was possible because of the previous round of seed increase in Guatemala.

In 1992, in addition to the primary field trial sites, horticultural evaluations at several locations throughout the normal growing range of cantaloupe and squash were conducted with our advanced transgenic lines as well as commercial hybrids produced from those lines. At two locations, pilot production increases were conducted for our most promising line of squash. Similar tests and multiplication followed in 1993 and 1994.

Practical Aspects of Regulation

Throughout the execution of these field trials, we enjoyed a good relationship with APHIS, and we commend that agency for their approach in regulating these trials. It has balanced a concern for proper regulation with a reasonable scientific assessment of the concerns (or lack thereof) arising from the field testing of our plants.

In looking forward to the next few years and the road to commercialization, I believe that we have a reasonably good grasp of what needs to be accomplished from a research and development perspective. On May 23, 1994, APHIS announced a provisional finding of non-significant impact for one squash line, ZW20. After providing opportunity for public comment, Asgrow is optimistic that this provisional finding will become final. This would be the first step toward commercialization. For the other agencies, we are relying on open consultation as their policies evolve. In November of 1993, Asgrow submitted a petition for an exemption as a result of our discussions with the Environmental Protection Agency (EPA). Consultation with the Food and Drug Administration (FDA) in accordance with their published policy of 1992 has also taken place.

Our greatest concern is that excessive regulation—in the form of either the number of agencies which may decide to regulate these plants, or the actual regulations which they may establish—may render the development of transformed plants too costly for seed companies the size of Asgrow. If the benefits of transgenic plants are to be widely accessible, especially to developing countries, it is necessary to allow a wide range of companies, not just the very large, to have the opportunity to develop these plants. Because business goals differ from one company to another, the types of transgenic plants each chooses to develop (species and traits) will differ.

For example, some companies will choose to develop transgenic crops exhibiting certain quality traits. Others will be more interested in

developing plants with disease resistance. For some companies, devoting a large effort to developing transgenic cucurbits will not be a sensible option; for Asgrow, it is. Therefore, the greater the number of companies that are able to develop transgenic plants, the greater will be the variety of needs which will be met. On the other hand, costly registration processes will result in a number of seed companies being unable to afford the cost of development. This will limit not only the number of industrial laboratories engaging in this research and the number of species/trait combinations developed, but also the number of paths to the market for this technology. Regulatory agencies should realize that even requirements which would be considered minimal for the registration of a chemical pesticide will most likely be prohibitive for a seed company.

Because of this, the case-by-case approach of APHIS appears to be a good one. It provides flexibility in dealing with specific engineered traits in specific crop species. Certain species/trait combinations may legitimately require extensive and expensive premarket risk assessment studies, while others may not. This will reduce the cost of developing certain transgenic crops and allow companies with limited resources to bring these crops to market, and this process appears to be underway.

For Asgrow, notwithstanding the possible regulatory expense, the question is not whether these and other transgenic plants should be introduced, but when and under what conditions and limitations. I hope that whatever regulations are finally established by the regulatory agencies of our respective countries, these regulations do not unreasonably delay such introductions. This could unnecessarily deprive us of the benefits derived from our ability to genetically engineer plants.

Reference

Powell, P. A., R. S. Nelson, B. De, N. Hoffmann, S. G. Rogers, R. T. Fraley and R. N. Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* 232:738-743.

Debus, E.E. 1994. Monsanto's Experience in Developing Biotechnology Products: The Importance of Communication with the Public. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 175-180.

Chapter 3.4

Monsanto's Experience in Developing Plant Biotechnology Products: The Importance of Communication with the Public

Edward E. Debus
Director, Public and Regulatory Affairs, Plant Biotechnology
Monsanto Agricultural Company
700 Chesterfield Village Parkway, St. Louis, MO 63198, USA.

Introduction

The Monsanto Agricultural Group has had years of experience in conducting field trials with a number of crops improved through plant biotechnology. The early experience gained from field testing of insect-protected cotton plants in the USA pointed towards the importance of Monsanto's policy of being open with respect to communication with the public.

Through modern tools of plant breeding and biotechnology, Monsanto has successfully incorporated a gene from *Bacillus thuringiensis* (*B.t.*), a naturally occurring soil microorganism, into cotton so that the cotton plant effectively controls the larvae of caterpillar insects. Monsanto researchers worked for many years before they were ready to take this cotton to the field for its first tests in 1991. During the first year of field testing, 20 small-scale tests covering a total of three hectares were conducted.

An essential element in gaining regulatory approval and successfully conducting the tests was open communication with many of the audiences that were interested in this work. These audiences included Monsanto employees, governmental regulators, cooperators, members of the community near the test sites, and members of interest groups.

Openness Is Better

When Monsanto first entered the modern plant biotechnology arena over a decade ago, we instituted a policy of openness in our scientific developments and field studies. Early on we worked with regulatory agencies in keeping them aware of our progress. We were able to strike a reasonable balance between information that truly provided us with a competitive advantage in the marketplace, which we protected as confidential, and information that was important for an understanding of the scientific and product attributes of our work. We have been able to gain protection for our discoveries within the patent system while communicating very openly about our work.

Openness in many aspects of developments in plant biotechnology has been very important in gaining freedom to operate throughout the earliest stages of work. General public awareness of science and new scientific principles in the USA is not well advanced. However, there is a predominant view in the public arena that science is neither intrinsically good or bad, but is dependent on the people who are undertaking scientific research. Given this situation, public confidence can be enhanced when those involved with the work communicate about it. Early discussions with key community leaders, government officials, educators and others have been well received and have proven invaluable. Respectful relationships have resulted with many individuals and organizations, even though they may not always agree with our opinions.

Communication and Planning Go Hand in Hand

Monsanto's earliest field trials were designed to evaluate the level of gene expression. Subsequent research-oriented field studies have evaluated a

variety of biological, agronomic and economic performance factors (see Chapter 1.1: Fraley). In addition to these parameters, successful outcomes have then led to traditional breeding work and basic seed production.

Monsanto's earliest field trial experiences in the late 1980s confirmed the importance of having clearly defined objectives and methods. Protocols for studies must ensure that the information collected meets our needs as well as the needs of regulatory bodies and our cooperators. The key to a successful study therefore also lies in effective communication and planning.

In the USA, a number of regulatory agencies in addition to the United States Department of Agriculture (USDA) are interested in field studies, depending on the crop and objective of the study. If the new plants contain pesticide traits the Environmental Protection Agency (EPA) will be involved, and varying requirements are necessary depending on the size of the test and the disposition of the crop products. If the crop products will be used for food and/or feed, the Food and Drug Administration (FDA) may be interested. At the state level, several states have their own requirements which should be considered prior to field testing. Monsanto provided information to all of the appropriate regulatory agencies very early on in the development of policies and in the conduct of specific studies. One of the consequences of early and regular open communication with these organizations has been the establishment of mutually respectful relationships. We have also found it very helpful in dealing effectively with new questions that arise.

Monsanto has found particular value in working closely with its partners and cooperators in the early planning stages of its field trials. Every year we engage new cooperators as we expand our trials to cover all important crop growing areas. Site selection is important as it involves both agronomic criteria and non-agronomic criteria such as convenience to facilities and security.

In plant biotechnology research, the design of this year's trials often depends on the previous year's test results. Unfortunately, the time between the collection and analysis of last year's data and the regulatory deadline for the submission of the paperwork requesting the next round of tests is very short. We have a very narrow time-frame in which to meet the research, planning and administrative requirements. Again, good communication is a key to successful completion of these requirements.

Following test approval, we have a second very narrow time-frame in which to communicate with all of the individuals and organizations that require specific information about the test. We must ensure that all test requirements are met, from shipping, receiving and storing seed to

planting conditions. Compliance with all requirements is a very important part of our program, and effective communication is therefore vital.

Where Do We Stand?

All of Monsanto's effort on both the science and the communication front has proven invaluable. The results of our research and field studies over the past five years speak for themselves, as we have proven significant crop benefits. In the case of insect-protected cotton, we have achieved commercial, season-long control of caterpillars while meeting agronomic needs such as yield and quality. We have also confirmed the overall environmental safety of the crop system.

In future we envision successfully expanding field trials to provide more data and information on product performance and to achieve commercial approval for the first improved plant varieties. Our procedural and philosophical frameworks have served us well from the first trial to our current expanded studies. Good planning, flexibility in responding to new developments and requests, and openness all remain important aspects of our program.

Communication with the Public

The ultimate acceptability of any new product depends in part on the large and varied group of people we casually label the "general public". In the USA, saying this group is varied in an understatement!

In early discussions of public acceptance of plant biotechnology we tended to treat this group as a single entity and debate whether the language we used to communicate about the science impacts how "they" felt about it. Next we would identify and discuss various subgroups such as the media, environmental advocates, politicians and community leaders. While these are useful groupings in some ways, this segmentation really doesn't help understand how individuals actually respond to new products developed through biotechnology.

To find out more about how people will respond obviously requires that we listen to them. Monsanto has taken the time to do this in a number of issues over the past several years. In the general area of environmental issues, several studies within and outside Monsanto have been conducted, seeking greater insight into individual values, beliefs, attitudes and opinions.

Studies over the past few years have found several well-defined groups, each with a different set of values and beliefs. Not surprisingly

there is a certain percent of the public who are very happy with scientific discoveries and new applications, and see tremendous benefit and value in biotechnology for the future. In the USA, this group is not in the majority. In contrast, there is also a group that has very strong views against biotechnology, perhaps driven by certain religious beliefs; to question their feelings about biotechnology is therefore to question their basic values and beliefs. They are not supportive of new products developed through biotechnology. This is a very small group in the USA.

As one would expect, the majority of the public is made up of people with less rigid values and beliefs. Within this group there are those who are always willing to learn, those that are only willing to learn when they have a decision to make that impacts them, and those who are not willing to learn. In short, there are those who are willing to engage in discussion about important issues and those who are not.

Why is it important to consider the public in this way? It is important because of the value of communication concerning biotechnology developments and acceptability. We must appreciate that when we talk to a group of people, we will be talking to people who have different values and beliefs. This points to the challenge of listening and learning how to more effectively communicate about the matters that are important to us. The better we understand the audience, the better we can do this.

Viewed in another way, we also know that people learn in different ways. Some learn by reading and listening. This is probably true for most scientists and for the segment of the general public that is comfortable with developing fields like biotechnology. Others learn by experience. They learn by seeing, experiencing and feeling. We expect that some of the people concerned about biotechnology learn in this manner. For these people, speeches and articles are not going to be the most effective way to communicate with them. This points again to the importance of being open about what we do. For example, laboratory and field visits are an important part of Monsanto's program and have been effective in encouraging a better understanding among many individuals and groups.

Conclusion

Monsanto has accumulated considerable experience in the scientific and regulatory aspects of plant biotechnology and has worked hard in the area of public acceptance. Open and effective communication is a very important key to success in both arenas. The challenge has been and will continue to be great. It will take courage and hard work to continue the progress that has been made so that beneficial new products can achieve commercial acceptance.

In the USA, we are confident that the majority of the public will accept new plant biotechnology products. The better they understand who we are and what benefits our products will provide, the more reassured they will be. Our actions must remain grounded in responsible science and we must be active and open in our communication with all interested audiences.

Townsend, R. 1994. Pioneer Hi-Bred's Perspective on Field Testing Transgenic Maize. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 181-190.

Chapter 3.5

Pioneer Hi-Bred's Perspective on Field Testing of Transgenic Maize

Rod Townsend
Director of Regulatory Affairs
Pioneer Hi-Bred International Inc.
Plant Breeding Division, Johnston, Iowa, USA.

Biotechnology and Pioneer Hi-Bred

Pioneer Hi-Bred International Inc. is one of the world's largest independent seed companies. Its goal is to deliver improved genetics to the world's farmers. To achieve this goal, the company has strategically placed research stations around the world, including several in South America.

Pioneer Hi-Bred's major products are hybrid maize, sorghum, soybean, sunflower, canola, alfalfa and wheat. We expect biotechnology to play a major role in the continued improvement of all these crops, through the technologies of gene mapping and genetic engineering.

Pioneer does not see biotechnology as a unique technology producing a unique stream of products, but rather as another tool to be used by its plant breeders in their continuing search for improved germplasm.

Biotechnology in Plant Breeding

In order to be useful to the plant breeder, germplasm derived through biotechnology must be readily available for use in breeding programs. The scale of breeding programs for Pioneer's major crops dictates that plant breeders make selections in the field, not in the greenhouse, so the ability to plant genetically engineered material in the field is vital to future crop improvement programs.

The development of new crop varieties is a finely tuned process. Hundreds of thousands of new genetic combinations are tested each year in the USA alone. Maximum use is made of breeding nurseries in locations such as Hawaii, Puerto Rico and Mexico, so that more than one generation of a crop may be grown in a single year. Potential products are subjected to wide-scale performance testing in areas where they may be sold, including thousands of side by side comparisons in growers' fields. Finally, there is a rapid scale-up to commercial seed production of those few lines that are finally selected for commercialization.

If genetically engineered materials cannot be integrated into this system, but are subject to unique barriers, whether raised by technical problems, unnecessarily restrictive regulations, bureaucratic delays or unreasonable costs, then the use of biotechnology in the seed industry will be curtailed, ultimately depriving farmers, processors and consumers of the benefits of crops that use fewer chemical inputs and have improved agronomic performance.

Regulations Should Be Risk-Based

Pioneer supports sound science-based regulatory oversight of biotechnology research and product development, for the public confidence that such regulations provide and the high standards they promote. Clear delineation of the regulatory review process for such products will promote further development of the technology. However, regulations designed to safeguard the environment and human health must also be flexible in order to facilitate the conduct of safe research activities.

Pioneer does not feel that biotechnology poses an entirely new set of safety issues. There is a continuum of concern that should be taken into account when establishing a system of regulatory oversight.

In some instances, biotechnology derived products will be directly comparable with traditionally derived products. For example, Pioneer has developed herbicide resistant maize hybrids derived from plants carrying a natural mutation in the acetolactate synthase gene. An equivalent product can be derived through genetically engineering the same mutation into the acetolactate synthase gene and transforming the gene back into maize. The genetically engineered version does not seem to raise any unique safety concerns, so why should it be subjected to additional regulatory oversight?

Regulatory oversight should be based on the risks, if any, that are posed by the product. Plant breeders have always sought to introduce new characteristics into domesticated plants, often employing wide outcrosses to wild relatives to access those characteristics.

Confidence in the safety of the resulting material is based on the breeders degree of familiarity with the germplasm, focusing particularly on any known toxins associated with the plant, and on the extent to which the genetic contribution of the wild species has been diluted by backcrossing. Typically it might take at least six generations of backcrossing to recover the recurrent parent with the new trait. During this process the genetic contribution of the wild species has been reduced from 50% to less than 1%, representing perhaps 500-1000 genes. By these standards, changes brought about by the genetic engineer, who introduces only two or three genes, are amazingly focused.

Many “new” genes will actually be somewhat familiar, because they are derived from plants with a long history of consumption as food or feed, but which are being moved into different crops. Examples might include the methionine-rich seed storage protein gene from Brazil nuts (*Bertholetia excelsa*) that Pioneer has introduced into soybeans in order to enhance the levels of essential amino acids; or wheat germ agglutinin, a plant lectin that inhibits the growth of European corn borer, an important pest of maize. Pioneer sees little inherent risk in field testing such genes in these crops, but agrees that it should proceed with caution until questions about the possible adverse effects of altered patterns of consumption of these proteins (e.g. potential food allergies) or impact on nontarget species have been resolved.

Products may also be derived from uncharacterized genes or genes from sources that are not consumed as food or feed. In these cases, greater caution is warranted. Some researchers have proposed deploying genes encoding potent invertebrate toxins and snake venoms, which are

effective against insect pests. Clearly, tight regulatory controls should be exerted over such material until all issues of safety and nontarget impact have been completely addressed.

The North American Experience

Pioneer has developed the capacity to genetically engineer most of its key product crops. It has field tested transgenic alfalfa, maize, canola, soybean and sunflower in North America. All these trials have been reviewed by the United States Department of Agriculture (USDA) or by Agriculture Canada, as well as officials from the state or province in which the trials were conducted. The test protocol also has to be approved by the Institutional Biosafety Committee, a group of Pioneer scientists and managers with experience in biotechnology, agriculture and risk management, and two outside experts in related disciplines. Pioneer has also taken steps to inform local opinion leaders and the press of its activities.

Undoubtedly, much of the success of the field testing program in the USA is due to the scheme of regulatory oversight, outlined in the 1986 Coordinated Framework for the Regulation of Biotechnology, as implemented by the Biotechnology, Biologics and Environmental Protection unit of USDA's Animal and Plant Health Inspection Service (APHIS). By focusing on scientific principals of risk assessment and avoiding judgments based on speculation, they have earned the respect of the regulated community and discouraged the proliferation of local regulations. Local regulations, while often well intentioned, are seldom consistent and frequently duplicative of national regulations. From the applicants standpoint, the advantages of dealing with a single regulatory entity are considerable.

The USDA system is certainly a model worthy of serious consideration by any country seeking to implement regulatory oversight of transgenic crops. However, it is not perfect, and some of the problems will be discussed in the context of Pioneer's experiences in field testing genetically engineered maize.

Field Testing Genetically Engineered Maize

Maize represents the prime target for most major seed companies but has proved a demanding technical challenge. Within the last two years, Pioneer and several other companies have finally achieved stable transformation of maize. This has been possible through use of the

particle gun to accelerate microprojectiles, bearing recombinant DNA, into plant cells, coupled with tissue culture responsive cell lines.

Initially, regulators expressed some concern as to the stability of recombinant genes introduced using this technology. This resulted in demands to see molecular and genetic evidence, in the form of Southern blots and segregation data, confirming stable integration. Recombinant DNA introduced in this way has not proved unstable and does not replicate independently without a plant origin of replication.

Pioneer does not have the resources to conduct molecular or genetic characterization of every construct that may be field tested. After all, it may take 100 independently transformed lines to identify one that has adequate expression of the recombinant gene under field conditions. If molecular and genetic characterization is required for regulatory purposes, then it should be done on those lines that are finally selected for commercial advancement.

The genes that Pioneer has introduced into maize and has (or will shortly) field test include two herbicide resistance marker genes, two marker genes controlling pigmentation in maize, two dominant inhibitors of gene expression both derived from maize genes, three different viral coat protein genes that may confer resistance to important maize viruses, and wheat germ agglutinin.

Pioneer does not consider that any of the genes described represent a high risk, either in terms of enhancing the weedy properties of wild relatives of maize or in terms of human or animal health. However, Pioneer is conscious that some of these genes, and the control sequences used to achieve expression, do not occur in domesticated maize, and care should be taken to limit dissemination of these genes through seed or pollen until their properties have been adequately evaluated.

Experience Helps Evaluate Risk

In working with maize, biotechnologists are fortunate to have a wealth of published information on the biology of the crop together with nearly 75 years experience of hybrid development and seed production.

Maize itself is a highly domesticated crop and there are no feral populations. It would take fundamental changes in the biology of the plant to make it an invasive weed. There are no wild or weedy sexually compatible relatives of maize in North America, so there is no genetic bridge by which recombinant genes could find their way into wild populations.

Wild teosinte relatives do occur in Mexico and Guatemala. Maize (*Zea mays* L spp. *mays*) and teosinte (*Z. mays* spp. *parviglumis*) are sexually

compatible and can produce fertile hybrids (Wilkes, 1977). Indeed, an examination of the literature prior to 1980 would lead to the conclusion that there is constant gene flow between maize and teosinte, and that the weedy teosinte (*Z. mays* spp. *mexicana*) is a hybrid of the two sub-species, and functions as a genetic bridge between the two (de Wet and Harlan, 1972; de Wet, 1975; Galinet, 1973).

However, this assumption has been re-evaluated using techniques of gene mapping, which failed to show any evidence of recent introgression between maize and teosinte (Smith *et al.*, 1985). Moreover, weedy teosinte is not a hybrid of the wild and cultivated forms of *Zea* and therefore does not serve as a genetic bridge; physical similarities are due to parallel adaptation to the same habitat (Doebley, 1984). There is evidence of highly restricted gene flow between *Zea* spp. but it apparently occurs from teosinte into maize (Doebley *et al.*, 1987). Introgression into wild teosinte populations is still a risk that needs to be evaluated, but evidence from genetic mapping suggests that introgression of recombinant genes into teosinte is not inevitable.

In considering how to conduct its field trials in the USA, Pioneer was adamant that its genetically engineered material should be planted in normal breeding and disease screening nurseries, where crosses could be made conveniently. Maize is wind pollinated and pollen can travel some distance before losing viability. Industry standards exist for the production of high purity seed so that contamination of seed stocks can be avoided by physical isolation and the use of border rows.

Naturally there was some concern on the regulators behalf that isolation could not be maintained under such conditions, and that pollen from the transgenic material would contaminate other breeding material. Pioneer answered such questions with a combination of knowledge gained from experience and by experiment. For successful plant breeding it is essential to maintain genetic integrity of germplasm. Maize breeders have conclusively demonstrated that this can be achieved, even when different lines are grown side by side, providing certain simple precautions are taken.

The receptive ear silks of the female inbred must be protected with an ear shoot bag from contaminating pollen. All breeding material in a nursery is protected from unwanted pollination in this way. Pollen from the male inbred must be collected in a bag as it is shed from the tassel. Controlled pollinations can then be made by removing the ear shoot bag and replacing it with the tassel bag containing pollen.

Pollen Flow Experiments

During the above activity there is potential for a little pollen to escape, either from the tassel before the bag is put on or during pollination. Because of this possibility, Pioneer decided additional isolation, in the form of border rows, would be appropriate at this stage of research. But as nursery space is at a premium, Pioneer cannot plant extensive areas to border rows. To determine appropriate minimum border requirements, we conducted pollen flow studies using kernel color markers to track gene flow under conditions prevailing in a nursery.

Pioneer employed a standard hand pollination procedure except that the tassels were cut from the plants while still inside the bags. Each tassel was removed and placed in a plastic sac before the bag was placed over the ear. By employing this procedure, pollen dissemination was limited to a radius of less than ten feet from any self-pollinated plant. In practical terms, adequate isolation for small plots can be achieved by bordering transgenic rows with four rows planted on 30 inch centers, of nontransgenic material on either side and one range of 15 foot long rows, at each end of the transgenic material. To maximize space utilization, these border rows are used as male or female parents in crosses with the transgenic material.

Unusually high winds could cause pollen to be disseminated over much greater distances. However, the greater the distance, the greater the dilution and loss of viability. Any pollen that found its way to a commercial crop would be hugely diluted by the pollen shed by that crop and, if there was expression of a recombinant gene in the developing seed, the gene products would be insignificant in terms of the whole crop.

Harvest

Shattering is not a problem with maize: it is easy to harvest ears by hand and account for all transgenic seed. Plot harvesting by machine will be needed in the future, which may lead to some loss of seed, but maize seeds show poor dormancy and volunteer plants are easily rouged from the field.

At the conclusion of the experiments, plants are allowed to dry down in the field and then cultivated into the soil to decay naturally. In some soils, decay is very slow so we may elect to burn the trash.

Transgenic seed from these experiments cannot be fed to livestock. Small quantities of unwanted seed can be destroyed by autoclaving but larger quantities have to be incinerated, or buried below cultivation

depth. Requirements to destroy experimental crops represent a major problem for large-scale trials or those that might be conducted in farmers' fields.

Conclusions

Regulatory Requirements and Industry Priorities

Timing is of enormous importance in developing new maize hybrids. Missing a single planting in the early stages of product development may delay commercial introduction by a year. Such delays are costly. The average yield of the maize crop in the USA increases by two bushels each year, and much of this is genetic gain achieved by plant breeders. Therefore, every year's delay in hybrid introduction to the market translates into a potential loss of two bushels per acre of yield advantage for that hybrid, relative to competitive products. This is a major incentive for efficiency.

Seed may be harvested from a winter nursery one week and planted in Iowa a couple of weeks later. Given this sort of turnaround, it is apparent that the 120-day review period required by USDA represents a significant problem. It is often impossible to predict the quantity of seed that will be available for planting, when they may be planted and exactly where in any particular nursery they will be planted. The problem is also apparent in the early stages of product development.

In order to have sufficient time to prepare and submit an application, and give USDA 120 days for the review, Pioneer may have to draft applications when our transgenic plants are just emerging from culture. At this stage we know what genetic construct we put into the cells and assume that they are probably transgenic because they are growing on selective media. We certainly do not have detailed information on gene expression or molecular characterization.

Regulations Must Be Flexible

For most small-scale releases of most transgenic crop plants, an adequate review of the potential risk can be made based on knowledge of the transformed species, the genetic construct introduced, the location and approximate size of the trial, and precautions to prevent gene dissemination. If additional information is required, then regulators must appreciate our predicament and work with Pioneer to update applications, without stopping the clock on the review process.

While Pioneer tries its best to predict how a trial will be performed, circumstances such as seed supply, disease or weather can change those plans in the course of an experiment. Pioneer is reluctant to dig up a trial and start again when a few seeds can represent hundreds of thousands of dollars in research expense alone. Wherever possible, Pioneer seeks to amend the agreed protocol. USDA has been most accommodating in this respect, and their understanding and timely action is appreciated. Any regulatory system has to be flexible enough to give priority review to protocol modifications.

Regulations Must Be Compatible

Expedited movement of research material is critical for an international seed company. Mutual recognition of regulatory standards will greatly facilitate the international exchange of germplasm. Where countries require inspection and/or permits for imported seed, it is essential that there is good communication between the group responsible for implementing quarantine measures and the group reviewing field trial applications. We would also encourage a flexible interpretation of what is regulated. Recombinant DNA in common cloning vectors and tissue samples from transgenic plants should not be subject to additional oversight as long as they comply with normal quarantine and shipping requirements.

Entry of regulated material into public registration trials presents a unique problem if the variety is still subject to regulatory review and cultural restrictions. From a practical standpoint, material should be exempted from oversight prior to entry in such trials.

Outlook

Pioneer encourages any government that is drafting new regulations to ensure they look beyond small-scale research applications of biotechnology, and consider the needs of the plant breeding community that seeks to apply the technology to commercial crop development. The regulatory system in the USA provides a valuable model with much to commend it. However, it was developed to provide oversight for research trials.

Pioneer is optimistic that the system will prove flexible enough to meet the demands that will be made upon it as genetically engineered materials enter commercial breeding programs for major crops such as maize. Only time will tell.

References

de Wet, J. N. J. 1975. Evolutionary dynamics of cereal domestication. *Bulletin of the Torrey Botany Club* 102:307-312.

de Wet, J. N. J. and J. R. Harlan. 1972. The origin of maize: the tripartite hypothesis. *Euphytica* 21:271-279.

Doebley, J. F. 1984. Maize introgression into teosinte—a reappraisal. *Annals of the Missouri Botanical Garden* 71:1100-1112.

Doebley, J., M. M. Goodman and C. W. Struber. 1987. Patterns of isozyme variation between maize and Mexican annual teosinte. *Economic Botany* 41: 234-246.

Galinet, W. C. 1973. Preserve Guatemalan teosinte, a recent link in corn's evolution. *Science* 180:323.

Smith, J. S. C., M. M. Goodman and C. W. Struber. 1985. Relationships between maize and teosinte of Mexico and Guatemala: numerical data analysis of allozyme data. *Economic Botany* 39:12-24.

Wilkes, H. G. 1977. Hybridization of maize and teosinte in Mexico and Guatemala and the improvement of maize. *Economic Botany* 31:254-293.

Meeusen, R. 1994. Sandoz Seeds' Experience in Conducting Field Trials in the USA. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 191-196.

Chapter 3.6

Sandoz Seeds' Experience in Conducting Field Trials in the USA

Ronald Meeusen
Director, Biotechnology Activities
Northrup King Co.
317 330th Street, Stanton MN 55018, USA

Introduction

This chapter reviews Sandoz Seeds' experience with field testing of transgenic crops in the USA. To put the proposed uses of this new technology into an historical context, this chapter offers a perspective on the use of technology in agriculture which attempts to persuade that the net effect of such technology has been advantageous to the environment in the USA.

Although not as widely recognized as the names of its component companies, Sandoz Seeds is, in fact, the second largest, and most diversified, seed company in the world. Annual sales exceed US$750 million, of which about 10% is devoted to research. We breed and

conduct research in over 20 major crops and many more minor crop species, and carry on operations in over 125 countries. Biotechnology research is pursued by each of the four major operating divisions: Northrup King of Minneapolis (where the author is currently employed), S&G Seeds (formerly Zaadunie B.V.), Hilleshög NK of Sweden and Rogers NK Seeds of Idaho. Four major research sites house these activities, one each in Holland and Sweden, and two in the USA. The author directs Northrup King Company's biotechnology activities and has been involved in field testing transgenic crops since 1986, the first year of such trials.

Sandoz Seeds' Experience

Sandoz Seed companies have conducted various field trials in the USA and Europe (see Table 1) but their initial experience was with tobacco plants, the "white rats" of plant engineering, which had been engineered to produce small quantities of the crystal protein from *Bacillus thuringiensis* (*B.t.*). Protein from this common soil bacterium is a natural insecticide for caterpillars, and the bacterium itself has been used as a safe, biological alternative to chemical pesticides for over three decades. In a 1987 Northrup King field test in North Carolina, transgenic tobacco plants with the engineered *B.t.* gene grew without damage through the worst infestation of tomato hornworms to have hit the area in nine years. Commercial growers on the adjacent farm sprayed chemical insecticides five times that season, yet still suffered losses. The *B.t.* plants remained uninjured despite no pesticide application, demonstrating the power of this approach to provide the alternatives to chemical controls which environmentalists have so long desired.

Time and Cost of Regulation

The need for permits adds both time and cost to field testing transgenic crops. However, for initial small-scale tests our experience has been that these are not overly burdensome. Field tests must be planned at least six months ahead, since time must be allowed to gather the needed data for permit applications, prepare documents, and for review by the United States Department of Agriculture (USDA). USDA has committed itself to a 120-day review period. At the time of the Biosafety Workshop held by the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) in 1992, we knew of no cases in which the Animal and Plant Health Inspection Service (APHIS) of USDA had missed this target in over 100 permits issued. By June 1994, the number of field test permits

Table 1: List of Transgenic Crops and Genes Tested in the USA and Europe by Sandoz Seed Companies through 1993

Crop	Location	Trait/Gene
Tomato	California, Florida	Insect resistance (*B.t.*) Improved shelf life
Tomato	California	Virus resistance
Petunia	Florida	Altered color
Alfalfa	Minnesota	Herbicide resistance
Sweet Corn	Minnesota	Insect resistance (B.*t.*) Endophyte engineered with *B.t.*
Sugarbeet	France, Spain, Sweden	Herbicide resistance Virus resistances
Field Maize	Multiple states in the USA, Canada	Insect resistance (*B.t.*)
Field Maize	Multiple states in the USA	Virus resistance Herbicide resistance
Lettuce	France, Spain	Multiple virus resistances

(and notifications) reviewed by the agency had risen to over 300 per year, yet average review time is now only 80 to 90 days.

The time to gather data typically requires from two to four weeks of effort by one of our scientists. We have found that the bulk of information needed for a permit is available either from the laboratory notebooks of researchers developing the plants, from the scientific literature, or from prior permit applications which USDA makes available under the Freedom of Information Act (with the exception of confidential business information). This presupposes that one is not the first to conduct such trials in a particular state, and that the crop is not one for which data on its biology (specifically ability to cross with wild relatives) is lacking. The time to actually prepare documents is another two to four weeks, this time by a regulatory affairs manager.

While this is six months more than is required for testing of traditionally bred varieties, it should be noted that initial testing of a transgene usually comes after three to five years of laboratory work. Since the initial test usually is structured to look at whether the gene performs in the field the same as in the greenhouse, this delay usually can be handled. In subsequent years the transgene moves into breeding

programs and, as Dr. Townsend describes (Chapter 3.5), this requires multiple site, multiple plot testing over a wide range of soils and climates. Six month delays for this development phase could be crippling, but this is an issue USDA is aware of and which it is studying intently. Subsequent to 1992, USDA developed and implemented a much faster notification system for certain well-studied classes of transgenic crops. For these classes the notification process has decreased field test review time to 30 days.

The cost of preparing and submitting permit applications is a separate issue. If one assumes that professionals such as scientists and regulatory specialists cost a company about US$100,000 annually, then the cost to prepare and submit a typical permit application is somewhere in the range of US$16,000.

The three conclusions drawn from our experience in the USA and Europe are:

1) that for small-scale field testing, the time and cost involved in acquiring permits are significantly increased over traditional seed industry practices, but are still manageable;
2) that the review process, especially in the USA, reliably provides answers (yes or no) within the defined 120 days; and
3) that the types of data needed for these reviews are generally available either from the literature or the laboratory work. Investment in additional studies strictly for safety evaluation are not typically needed for small-scale testing.

Adopting New Technologies in Agriculture

A few years back a layman at a public forum expressed the following fear: "It seems every technology adopted by agriculture has caused environmental problems. Pesticides can contaminate groundwater, mechanized cultivating increases soil erosion. Why should we welcome another new technology?" This perspective, that technology in agriculture is injurious to the environment, is very widely held in the USA. Is this true?

Going back to the records to examine the facts presents a startlingly different story. In 1930, before technologically advanced "modern" agriculture, a population of about 100 million people in the USA were fed and clothed. To achieve this, we plowed up 379 million acres of land each year. That is, our environmental cost of supporting these 100 million people was the annual stripping of 379 million acres of land of essentially all its wild flora and fauna in order to plant monocultures. We sacrificed 3.8 acres of wilderness per person.

By 1987, the population of the USA had grown to near 240 million, a 240% increase. To support this population with the same yields per acre as in 1930 would have required committing another 900 million acres to agriculture! Instead, the USA actually farmed only 312 million acres in 1987, some 67 million fewer than in 1930. For comparison, this approximately equals the total area of all national parks set aside in the USA since the inception of national park systems. We fed and clothed 2.4 times as many people on less land, sacrificing only 1.3 acres of land per person.

How was this accomplished? Those new technologies which the public perceived as harming the environment raised crop yields dramatically. Average maize yields in the USA rose from 25 bushels per acre in 1930 to 107 in 1987. Wheat yields rose from 13 to 40 bushels per acre, soybean yields from 13 to 28 and cotton from 164 to over 600 pounds per acre. The same acre of land in 1987 gave respectively 4, 3, 2 and 3.6 times more of these crops than it had in 1930. Yields in other crops followed this trend. Other nations without access to agricultural technologies have not been so fortunate.

So what has been the net effect of new technologies in agriculture? The USA has been able to avoid converting vast tracts of wildlands to agriculture, and even retired 70 million acres from the plow, while continuing to feed and clothe a growing population.

If new technologies saved from the plow at least as much wildlands as the whole national park system of the USA, and arguably many times that, then why are new technologies in agriculture so widely viewed as harmful to the environment? I believe there are two reasons.

First, new technologies raised productivity before the conversion of wilderness to farmland become a problem in the USA. Threats which are solved before they bite are essentially invisible. Saving wilderness land was therefore a solution to a problem the public never saw. Second, the liabilities of new agricultural technologies were all too visible on the smaller number of acres that we farm. Soil erosion from ploughing and water table contamination by pesticides draw attention by the media and therefore also attention by the public.

Yet it may be here that biotechnology can play its most visible role in dealing with negative effects of current agricultural practices. For example, as maize growers in the USA try to move to minimum or zero till practices to reduce soil erosion, they often find that crop diseases increase. In reduced tillage, seeds are planted into colder, wetter soils more conducive to fungal disease. Engineering of crops for greater disease resistance thus complements these practices that approach long-term sustainability by removing barriers to their adoption.

Similarly, reduced tillage systems leave more trash on fields, which in turn harbors over-wintering insects. In many cases growers in the USA find that they then need to increase insecticide use. Crops engineered for insect resistance should reduce this barrier as well. Finally, crops engineered for insect resistance, such as maize expressing the *B.t.* endotoxin gene, do not kill beneficial insects as do most broad-spectrum chemical insecticides. Transgenic insect resistant crops should make integrated pest management both more effective and more attractive to growers and consumers.

Section 4

Developing Biosafety Regulations to Enable Technology Transfer

Macaya, G. 1994. Towards the Implementation of Biosafety Regulations in Costa Rica. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 199-206.

Chapter 4.1

Towards the Implementation of Biosafety Regulations in Costa Rica[1]

Gabriel Macaya
Director
Centro de Investigaciones en Biologica Celular y Molecular (CIBCM)
Universidad de Costa Rica
Ciudad Universitaria Rodrigo Facio
San José, Costa Rica.

Introduction

The development of biosafety regulations has to be analyzed in terms of both needs and opportunities. Even if we recognize a general need for harmonization of regulatory procedures at a regional level, local specificity imposes a series of requirements that it may or may not be possible to harmonize. Other specific needs arise when considering the risk of impact of specific developments or products on the ecosystem. Costa Rica is in a region of multiple centers of diversity, and probably its

only patrimony is its biodiversity. Thus the impact of gene transfer needs careful evaluation.

Costa Rica agrees on the importance and relevance of developments originating in the new biotechnologies, and we want to be not only beneficiaries or consumers of the new findings and products but partners and actors in their development. One of the main issues to be faced is the development of a set of regulations that, giving assurance of an adequate risk assessment, still promotes the development of the technology. An integral part of the risk assessment process is analysis of the direct benefits to the country. What we should look for is a regulatory framework that promotes the development and uses of technology in an economically, socially and ecologically responsible way.

Costa Rica, like many other Latin American countries, has not yet developed a set of biosafety regulations. However, even though specific regulations covering the development and testing of genetically modified organisms are lacking, there is ample experience in plant health and quarantine regulations and in seed certification procedures. Thus, there is an opportunity to face the issues involved in the development of biosafety regulations with a "blank slate" approach, while knowing that any development can easily be inserted into the existing and future legal framework.

Developing Biosafety Regulations in Costa Rica

The Players

A generally accepted belief is that biosafety is not a public opinion issue in Costa Rica. This belief needs reevaluation in the light of more recent experience. Early in 1992, the Ministry for Science and Technology (MICIT) and the National Biotechnology Commission organized a round table on biosafety, with participants from regulatory, research and industrial sectors. The round table attracted a broad audience that manifested an active interest in biosafety and regulatory issues. It is foreseeable that ecologists and "greens" will have an important role in any public discussion that develops on biosafety and regulatory issues. Such groups are far from extremists, and their participation is expected to be positive and valuable, as judged from their role in the development of the biodiversity conservation effort in Costa Rica. The strategy to be used to implement biosafety regulations in Costa Rica should therefore actively involve these groups.

Even if the development of a local biotechnology-based industry is very limited (Coombs and Campbell, 1990), several activities that we

think have to be adequately regulated are beginning to develop in Costa Rica. These activities involve mainly seed increase and field trials with transgenic crops. At least one permit for seed increase activities has been officially presented for consideration to the Ministry of Agriculture by a seed company based in the USA.

The regulation of such activities should be approached so as to include necessary safeguards, but also to allow for the possibility for local scientific and technical personnel and groups to gain experience from both the regulatory and technical perspective on how to correctly perform field trials and seed increase programs. It is necessary to stress here the importance of new biotechnologies to developing countries. According to the National Research Council of the USA, these technologies, "used in conjunction with conventional breeding programs, could make dramatic contributions to sustainable agriculture by producing improved crops that are more compatible with their environment" (NRC, 1990). New biotechnologies could also develop at different levels of investment, mainly because the most important input is scientific and technical knowledge. Thus, profitable enterprises with a real impact on production can develop not only through foreign implantation of transnationals, but also through individual "garage" operations in plant propagation by tissue culture (Sasson, 1986). As an instrument to promote and develop the new biotechnologies in Costa Rica, MICIT established a National Biotechnology Commission in 1991, as a steering group to establish a mid-term Biotechnology Development Plan to be integrated into the National Strategy for Sustainable Development (see Coombs and Campbell, 1990). Among the tasks of this Committee is "[t]o cooperate in the adoption and application of biosafety norms in all aspects and related fields" (MICIT, 1992).

Steps Followed

The aims of this work should reconcile two antagonistic requirements: the urgency to establish regulatory procedures on the one hand, and on the other the need to promote an ample discussion of the issues involved. As we will try to make clear below, these two antagonistic requirements can be reconciled using the existing legal base of the Dirección General de Sanidad Vegetal (DGSV, General Directorate of Plant Health), through creation of a biosafety committee advisory to DGSV and the Oficina Nacional de Semillas (ONS, National Seeds Office), the two existing bodies that deal with effectual and legal issues. This committee will work on a case by case study basis, asking for advice as needed.

It was originally proposed at the Biosafety Workshop of the International Service for the Acquisition of Agri-biotech Applications

(ISAAA) to integrate a Preparatory Committee to present a proposal for the organization of biosafety regulations to the Ministers of Agriculture and of Science and Technology. This committee was integrated in 1992, with members (or representatives) from DGSV, ONS, the University of Costa Rica (UCR), the National University (UNA, Universidad Nacional) and MICIT. A lawyer from UCR with experience in biosafety and intellectual property issues was also appointed to the committee. This committee has worked in two directions, preparing both a decree from the President of the Republic and the Ministers of Agriculture and of Science and Technology that formally establishes the Technical Advisory Committee on Biosafety (Comité Técnico Nacional Asesor de Bioseguridad) and a new version of the General Law of Plant Protection (Ley General de Protección Fitosanitaria) with a chapter on the regulation of organisms or products of plant biotechnology.

Issues to be Considered by the Preparatory Committee

Creation of a Biosafety Advisory Committee

The Preparatory Committee worked on a model for the integration of an advisory committee on biosafety to support pertinent activities of DGSV and ONS. The guiding idea was to integrate a small permanent core group, with the possibility of enlarging it with *ad hoc* members as the need arises. The integration of the committee finally proposed included one member each from ONS and DGSV; a representative from MICIT (it is proposed that this representative be a member of the National Biosafety Committee); and four scientists, at least one being an ecologist, appointed from a list of experts provided by the Costa Rican National Academy of Sciences.

Members of the Biosafety Advisory Committee

The members of the Biosafety Advisory Committee should balance a first consideration of representation from the two institutions involved (DGSV and ONS) and from an expert technical panel. Membership for the group should be evaluated with consideration to the legal and technical institutional responsibilities in the regulatory process, and the interdisciplinary scientific level necessary to tackle the issues involved. Avoiding the mistake of hierarchical institutional representation should guarantee the best technical, scientific and legal structure of the group. A “directed institutions representation” is proposed. The members are

selected on the basis of their technical performance and competence level, trying to balance an adequate representation of the two institutions involved. More than half of the members of the committee should be external to the two institutions, and should be chosen on the basis of their technical expertise.

This Advisory Committee can be enlarged by *ad hoc* members as needed, or seek advice on expert groups, either locally or abroad. The Costa Rican Academy of Sciences will play an important role in providing candidates for non-institutional membership of the core group, the *ad hoc* members and the expert groups.

Legitimacy of the Process and the Committee

To legitimize the creation of a set of biosafety regulations and the Advisory Committee, we have to address at least three issues. First, the process should have a strong political commitment, in our case from the Ministers of Agriculture and of Science and Technology. It is important that the activity not be perceived as a polemical issue, and that the private sector is supportive of, or at least accepting, those regulations.

Second, the activity should be developed under a clear legal framework, with competencies clearly defined. We are proposing not to go through the process of developing a new legal framework for these activities, but to use that provided by the Plant Health Law (Ley de Sanidad Vegetal) No. 6248. DGSV is clearly recognized and respected for its technical capability.

Third, the Biosafety Advisory Committee should look for adequate technical and scientific support. This should come from the research groups working actively in the field of biotechnology in public universities. When the local resources cannot offer the support needed, the Committee should have the mechanisms to obtain support from abroad.

At least five different aspects should be considered to successfully implement biosafety regulations in Costa Rica:

1) the regulatory function should have a basic role of responsible promotion of biotechnology, and this role should be one of oversight, and not of bureaucratic fettering;
2) the regulatory process should permanently assess the opportunity and benefits of the activity;
3) the Biosafety Advisory Committee should be permanently aware of public opinion issues, and its work must be transparent. Hence, the group should develop a permanent activity to form and inform public opinion;

4) the Biosafety Advisory Committee must seek permanent and strong support from the scientific community. This community should understand that they are a central element in the process, not only for promotional aspects for biotechnology, but as the main reservoir of the expertise needed for the review and approval process; and
5) the Committee should work in close contact with the private sector. It was a general feeling in the discussions that the private sector should not have representation on the Committee, but should be consulted and heard in the review and approval processes.

Specific Needs

The discussions held at the ISAAA Biosafety Workshop put into evidence some specific needs for the adequate implementation of biosafety regulations. The two most important were access to information and access to specialized personnel. Three basic categories of information needs were detected.

1) Access to basic data and to evaluation procedures and protocols for an adequate review and approval process. Highly specialized libraries are scarce in Costa Rica, and access to recent specialized journals is difficult.
2) A real problem to be faced is the possibility of requests for activities that have not been previously authorized in other countries. Access to information on approved assays or trials, as well as to pertinent information for permit refusals in other countries is necessary for an adequate review process in Costa Rica. We are aware of the difficulties in accessing such information, and we propose to establish working relationships with pertinent agencies in the USA and Europe as an alternative to direct access to this information.
3) Access to specialized personnel is one of the main limitations of the implementation of biosafety regulations in a country where the size and diversity of the scientific community is small. In many cases it could be difficult to find a competent scientist or specialist not linked directly or indirectly to the permit request. Our scientific community is not large, and the possibility of finding more than one specialist in a given highly specialized field is very low. Thus, as for information, we need a system that guarantees access to specialists abroad. Also, given this low availability of specialists, the regulatory process must not saturate those available. Preparation and qualification of scientific and technical personnel in fields related to the regulatory activities should be established. Adequate financial

resources should be allocated to short- and long-term fellowships to increase the installed base of specialists. A general policy for bilateral collaboration between DGSV and specialized foreign government agencies is necessary to profit from those agencies' accumulated experiences in the biosafety field.

Conclusion

We believe that, if the general considerations and guidelines outlined above are used to implement biosafety regulations in Costa Rica, the activity will be a valid mechanism to promote local biotechnology development in a responsible way. For the activity to be successful, we need important inputs from several sources. Failing to recognize this dependency will surely inhibit adequate development of the biosafety regulation system needed in Costa Rica.

The Preparatory Committee has now worked for almost two years following the general orientations discussed in this paper. Pressure to develop field tests and seed increase of transgenic plants in Costa Rica has forced the committee to go beyond its original tasks to examine and approve a few local operations involving transgenic plants, strictly following the regulations of the United States Department of Agriculture.

Note

1. This text is an elaboration of the discussions developed by the Costa Rican participants at the ISAAA Workshop on Biosafety, held in San José, Costa Rica, in February 1992. The author has made his best effort to reflect the opinions and considerations raised during the meetings, but he is solely responsible for the material as it is presented here.

Acknowledgments

The author wishes to thank the members of the Biosafety Preparatory Committee, especially its coordinator, Alex May, for helpful discussions and material important to the elaboration of this paper.

References

Coombs J. and P. N. Campbell (eds.). 1990. *Biotechnology Worldwide*. ICSU/COBIOTECH. CPL Press, Berkshire. 335 p.

MICIT, 1992. Presidencia de la República y Ministerio de Ciencia y

Tecnología, 1992. Decreto No. 21065-MICIT. *La Gaceta* 51:22-23.

NRC, 1990. *Plant Biotechnology Research for Developing Countries*. Report of a Panel of the Board on Science and Technology for International Development. National Research Council (NRC). National Academy Press, Washington DC. 44 p.

Sasson, A. 1986. *Quelles biotechnologies pour les pays en développement?* 200 p. Biofuture/UNESCO, Paris.

Carreón Zuñiga, M.A. 1994. Field Trials with Transgenic Plants: The Regulatory History and Current Situation in Mexico. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 207-213.

Chapter 4.2

Field Trials with Transgenic Plants: The Regulatory History and Current Situation in Mexico

Marco A. Carreón Zúñiga
Director General, Sanidad Vegetal
Dirección General de Sanidad Vegetal
Secretaría de Agricultura y Recursos Hidráulicos (SARH)
Guillermo Pérez Valenzuela No. 127
Colonia Viveros de Coyoacan, Mexico DF 04100, Mexico.

Introduction

The first application to the Mexican authorities for permission to import transgenic tomato seed with the *Bacillus thuringiensis* (*B.t.)* endotoxin gene with the objective of conducting laboratory research in glasshouses and the field in Guasave, Sinaloa, was received in 1988. The Mexican authorities, through the Department of Agriculture and Water Resources

(Secretaría de Agricultura y Recursos Hidráulicos; SARH), authorized both the importation and the field trials, which were conducted in late 1988 and early 1989 by Campbell, Sinalopasta in Guasave, Sinaloa. The possible risk associated with such field trials, particularly the possibility of escape of transgenic material into the wild, ensuing problems with wild species and potential disruption of ecosystems, led to the need for the development of regulatory mechanisms.

As a result, SARH established in 1988 a working group to draw up regulations on the introduction of transgenic plants into the environment. This working group also had the task of monitoring and evaluating the field trials of genetically modified organisms (GMOs) that had been authorized in Mexico (see Table 1). This group was transformed in early 1989 to become the Agricultural Biosafety Committee which included qualified experts from the following institutions:

- the Center for Research and Advanced Studies of the National Polytechnic Institute (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional; CINVESTAV);
- the Directorate General of Plant Health (Dirección General de Sanidad Vegetal; DGSV) of SARH, which also assumed the Committee's coordination;
- the Forestry, Agricultural and Fisheries Research Institute (Instituto de Investigaciones Forestales, Agrícolas y Pecuarias; INIFAP);
- the National Autonomous University of Mexico (Universidad Autónoma de México; UNAM);
- the National System for the Inspection and Certification of Seed (Sistema Nacional de Inspección y Certificación de Semíllas; SNICS); and
- the Postgraduate College for Agricultural Sciences (Colegio de Postgraduados en Ciencias Agrícolas; CP).

The fact that the Agricultural Biosafety Committee is not only composed of highly experienced and qualified scientists, but also of scientists representing those institutions with the most advanced biotechnological capacity and agricultural development in Mexico, lends added weight to its reviews and decisions. This joint participation of various institutions in the Committee guarantees complementarity and effective sharing of know-how and experience, which together allows the Committee to exercise its functions efficiently and to meet its objectives.

Although this group of experts offers a wide range of expertise which is essentially sufficient for the review of field trial applications, the Committee also reserves the right to consult outside specialists who are not members of the formal Agricultural Biosafety Committee.

Regulatory Philosophy

The scientific principles which form the basis of the Committee's review and analysis of risks and hazards related to the introduction into the environment of GMOs are essentially derived from ecology. The basic assumption or working hypothesis is that ecosystems—and more particularly biodiversity—can be altered due to the introduction of GMOs. Ecological data and know-how support the principles that are being applied in the development of norms for the field testing of transgenic plants.

The determination of a regulated article (i.e. a particular plant) is established on the assumption that a plant could present a risk to ecological stability, and that the potential effects of such a transgenic plant on non-transgenic organisms are not known. Hence GMOs are regulated to avoid deterioration of ecosystem and germplasm integrity, which are important elements in the conservation of genetic diversity.

The Legal Basis of Regulations

The Federal Law on Plant Health (Ley Federal de Sanidad Végatal; Diario Oficial de la Federación of 5 January 1994) and the Law for the Production, Certification and Commercialization of Seeds (Ley sobre Producción, Certificación y Comercio de Semíllas; Diario Oficial de la Federación of 15 July 1991) constitute the legal basis that provides authority to SARH for the establishment of regulations for transgenic material. A project for the development of an Official Regulation for the formalization of conditions for the transport and introduction of GMOs has been completed, and it is expected that this will enter into force in October 1994. The requirements under these Official Regulations are outlined below.

Procedure for an Application for the Release into the Environment of GMOs

1. The application must be submitted to SARH a minimum of 120 days before the proposed sowing or planting date. If insufficient information has been made available, then the applicant will be requested to submit additional information. The formal review process is only initiated when the application is considered complete.
2. SARH undertakes the review within the 30 days following receipt of an application and simultaneously notifies the federal agencies affected by the application.

Table 1: Field Trials of Transgenic Plants in Mexico

see attached document

3. SARH notifies the authorities under its delegation in the state(s) where the planned field trial(s) is to take place, together with a copy of the initial evaluation, and seeks the opinion of these authorities.

The applicant is required to submit the following information:

4. Name, organization, address, and telephone number of the person responsible for the field trial.
5. The person responsible for the project must at least have an academic degree at the "licenciatura" level (B.Sc. equivalent) or an equivalent postgraduate degree related to biological sciences, preferably in agronomy, ecology, genetics, molecular genetics or genetic engineering.
6. The scientific name(s), common and/or commercial name(s), and any other relevant designation(s) that permit the identification of the donor and recipient organisms and, where applicable, the vector(s) used, must be stated for each transformed product.
7. Name, address, and telephone number of the person(s) who developed or facilitated the transformed product.
8. The planned or proposed means of transportation of the regulated article within Mexico (mail, courier, truck).

Requirements of the Application

1. A description of the modification that resulted from the incorporation of foreign DNA, and how this modification differs from the non-modified organism.
2. A statement of the potential environmental impact of the release into the environment of the products.
3. Detailed description of the molecular biology that is the basis of the manipulated product.
4. The country of origin, location and description of the donor organism, of the recipient organism, and of the vector or vector agent, and where the modified product was collected, developed and produced.
5. A detailed explanation of the purpose of the release into the environment of the GMO, including a description of the proposed field trial design and production system.
6. The amount of transformed material to be released and the time-frame of the trials.
7. Explanation of the process, procedures and biosafety measures proposed to prevent escape and uncontrolled dissemination of the GMO and environmental contamination.

8. Description of the proposed location, including intermediate locations and field destination.
9. The use of the material after conclusion of the trial, including the use of the installations.
10. Description of the biosafety means and procedures for each of the proposed locations to prevent escape and dissemination of the modified product.

Future Perspectives on Biosafety Regulations in Mexico

The number of applications received annually by the Mexican authorities is fairly low (Table 1) and these can easily be handled by the Agricultural Biosafety Committee. There is no justification under current conditions to set up an institutional structure exclusively dedicated to agricultural biosafety.

In developing national biosafety regulations, Mexico made use of the experience accumulated by other countries. One of the most direct ways of sharing that experience was through personal consultation of Mexican officials with their counterparts in other governments. In addition, the experiences of specialists were assimilated through participation in regional and international biosafety fora that were dedicated to harmonization of biosafety procedures.

In this context, it is noteworthy that the North American geographic region established a mechanism for the regional harmonization of biosafety regulatory approaches. The North American Plant Protection Organization (NAPPO) has established a permanent biotechnology panel with the task, among others, of harmonizing regulatory approaches between Canada, Mexico and the USA. This forum is being increasingly used to discuss the biosafety resolutions of the three member states.

Jaffé, W.R. 1994. Biotechnology Regulatory Activities in Latin America and the Caribbean. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 215-223.

Chapter 4.3

Biotechnology Regulatory Activities in Latin America and the Caribbean

Walter R. Jaffé
Technology Generation and Transfer Specialist
Inter-American Institute for Cooperation in Agriculture (IICA)
Apartado 55-2200 Coronado
San José, Costa Rica.

Introduction

The use of the group of techniques generally known as biotechnology, both in research and development and in industry, is limited in Latin America and the Caribbean (LAC), in comparison to countries of the Organization for Economic Cooperation and Development (OECD) and particularly the USA. Nevertheless, biosafety considerations are particularly important for the further development of these incipient capabilities, and also for the importation of biotechnology products into the countries of the region. Regional agriculture and industry cannot wait

for the national generation of the new technologies needed to maintain and expand competitive advantages and to supply local markets and needs. The products and processes of biotechnological production will mostly be introduced through technology transfer. All this requires internationally accepted biosafety regulations to safeguard public health, agriculture and the environment from unforeseen negative consequences.

LAC countries need to examine the different issues involved in the development of biosafety regulations. Some of the general factors bearing on these strategies and policies will be discussed in the following sections. The available information on biotechnology regulations in LAC will be presented, followed by a discussion of the constraints and conditions for the development of these procedures in the region.

The Context of Biosafety in LAC

The regulation of biotechnology is not an important political or regulatory issue in LAC. The most important reason for this is the incipient development of biotechnology, which is emerging slowly (see Jaffé, 1994). But other more general factors are also important. Strategies to introduce biosafety regulations have to take these into account.

Cultural and Political Factors

The perception and acceptance of risk to health and the environment in LAC is different from industrialized countries because of cultural and economic factors. The majority of the population of the region lives in such economic conditions that biosafety considerations are often irrelevant. Organized public opinion is a relatively recent phenomenon in the region. The media, the scientific community and agricultural producers are the groups potentially interested in biosafety issues. Environmental groups are, as yet, relatively weak in most LAC countries.

Science and technology are generally regarded as progressive and important activities in the region. No anti-scientific, and particularly no anti-biotechnology movements, which have had such an important influence on the regulation of biotechnology in certain industrialized countries, yet exist in the LAC region.

One of the most important differences in the cultural context related to biotechnology of LAC, compared to most industrialized countries, is the lack of a legal tradition establishing private or public liability for damages. This explains the somewhat casual approach of industry and government in general towards the consequences of their activities on

public health and the environment. Even laboratory practices and safeguards are considered by certain people to be lax in LAC research institutions. Regulations for work with dangerous substances and organisms are, in general, either lacking or weakly enforced.

Perhaps the most important biosafety issue as perceived by the public is the possibility of the use of countries of the region for tests not allowed in the countries of origin.

Research and Development Weaknesses

Much of the basic research as well as links between scientific organizations (mostly public sector institutions) and the productive sector are weak in LAC. About 27 scientific organizations in the region have relatively well-established capabilities to conduct modern biotechnology research such as molecular biology, genetic engineering and cell fusion. Only a few have plant transformation capabilities, and only a handful of companies use molecular-based techniques for commercial purposes. No locally owned company developing transgenic plants was identified in the region in a survey conducted in 1990 (Jaffé, 1991).

These findings have to be placed in the context of the relatively weak plant breeding capabilities in the region. The larger countries have well established plant breeding programs which are principally in public sector institutions. The medium and small countries often have only basic capabilities, and in many cases no plant breeding at all. Few private plant breeding companies exist even in the larger LAC countries.

Development Strategy

As a result of the debt crisis of the early eighties, most countries in LAC have introduced profound changes in their development strategies. Protectionist policies, designed to support the development of local industry through import substitution, are being replaced by export promotion and the fostering of more internal competition through the opening of their economies and their integration into regional and sub-regional trade blocks. This last strategy raises the need for harmonized policies between the trade or economic partners in a wide range of sectors, and clearly also in biosafety matters.

The fiscal crisis has redefined the role of government, so that government intervention in the economy is being reduced substantially through privatization of many public sector activities and through gradual deregulation of the economy. The public sector, in general, has been reduced in size, or its further expansion restricted.

Existing Biotechnology Regulations

The available information on biotechnology regulations in LAC countries is presented in Table 1 (based on Inter-American Institute for Cooperation in Agriculture [IICA] surveys and other sources). It shows that only Brazil, Cuba and Mexico have issued guidelines or implemented norms to regulate the use of genetic engineering techniques at the laboratory level, based on the experience of the National Institute of Health of the USA. In Brazil, the National Science and Technology Council issued guidelines quite early, and Mexico included in their health legislation some considerations related to genetic engineering techniques. The most important research centers of these three countries have instituted mechanisms for biosafety oversight, such as institutional biosafety committees. However, no information on their actual effectiveness is available.

The countries which have had releases of genetically modified organisms (GMOs) into the environment are Argentina, Belize, Bolivia, Chile, Costa Rica, the Dominican Republic, Puerto Rico and Mexico. Mexico and Argentina have relatively better-developed regulatory experience in this area, having set up special commissions to deal with field releases. Procedures used are based on the United States Department of Agriculture (USDA) experience. Mexico has had direct assistance from USDA. The establishment of these mechanisms, it is argued here, were in response to requests for field trials submitted by multinational corporations.

Some regional and sub-regional activities to foster the introduction of biosafety regulations for biotechnology activities have been undertaken. IICA has been involved in biosafety issues since 1988, when, together with the PanAmerican Health Organization (PAHO), the Organization of American States and other national and international agencies, it organized the Inter-American Study Group on the New Biotechnologies with the purpose of offering advise on strategic issues in biotechnology development in the region. The Study Group chose to first develop biosafety guidelines. Two sets have been produced to date, covering laboratory research on genetic engineering techniques and release into the environment of GMOs (IICA, 1988; IICA/PAHO, 1991).

On the request of the Commission for Agricultural Cooperation of the Southern Area countries (CONASUR), a consultative mechanism of the Ministers of Agriculture of Argentina, Brazil, Chile, Paraguay and Uruguay, IICA is supporting the development of harmonized regulations for the release into the environment of GMOs in these countries. A specific proposal for a work plan and a regional consultative mechanism to this

Table 1: Legal Actions (to mid-1993) for the Regulation of Biotechnology in Latin America and the Caribbean
Source: Survey by IICA (1991) and personal information.

Country	Contained Use (year of introduction)	Field Tests of GMOs (year of introduction)
Andean Pact Countries (APCs; Bolivia, Colombia, Ecuador, Peru, Venezuela)	Comprehensive sub-regional biosafety regulations, inspired by European Union Directives, drafted (1994); to be presented for approval to the Junta of the Cartagena Accord (Andes Pact).	Comprehensive sub-regional biosafety regulations, inspired by European Union Directives, drafted (1994); to be presented for approval to the Junta of the Cartagena Accord (Andes Pact).
Argentina	None.	National Advisory Commission for Agricultural Biotechnology (CONABIA) for authorization of field tests (1991); Ministries of Health and Environment included as members (1993).
Bolivia (see also APCs)	None.	Ministry of Agriculture regulates field testing on *ad hoc* basis (1991); ministerial decree for creation of National Biosafety Commission drafted (1993) but not yet approved.
Brazil	Guidelines based on National Institute of Health (USA) norms established by the Program for Scientific and Technological Development of the Ministry for Science and Technology (1990).	Draft of new biosafety law proposed by Federal Senate (1991) and discussed by Chamber of Deputies (1992) but not approved to date.
Chile	National Biosafety Committee exists under National Biotechnology Commission (1991).	A plant and animal health authority, the Servicio Agricola y Ganadero (SAG) regulates field testing (1991) on *ad hoc* basis; National Commission for Field Releases, under SAG, created by ministerial decree (1993).
Colombia (see also APCs)	None.	None.
Costa Rica	None.	Biosafety Advisory Committee under plant health authority regulates field testing on *ad hoc* basis (1991); draft ministerial decree for creation of national agricultural biosafety committee (1992)
Cuba	Regulations established by National Biosafety Commission and Genetic Engineering Center (1987).	None, but regulations being formulated (1990).
Mexico	Universidad Nacional Autonoma de Mexico established Institutional Biosafety Committee based on National Institute of Health (USA) guidelines (1984); bylaws of General Health Law include control of genetic engineering (1987).	Secretary of Agriculture (SARH) created Agricultural Biosafety Committee for regulation of field testing of transgenic plants (1992); bylaw of the Seed Law (1991) regulates field tests of transgenic plants.

purpose was presented to the meeting of CONASUR in April 1992. A meeting jointly organized by IICA and the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) in November 1992 recommended a common set of data requirements and biosafety assessment criteria for the regulation of field releases of transgenic plants in CONASUR countries.

GMOs have been field tested in the region without either national or local authorization. Available published information indicates that there have been at least two cases of releases without a permit from any authority. A genetically modified live rabies vaccine was tested in Argentina at a time when its release was not authorized in the USA, where the vaccine was being developed. This caused an outcry within the local scientific community and the test was stopped (Fox, 1987). As a consequence, PAHO drew up biosafety guidelines for the research it sponsors (PAHO, 1987). In Mexico, according to one source, imported transgenic plants have been tested without any control by national authorities (Quintero, 1990).

Requirements for the Regulation of Biotechnology

For national and/or regional control mechanisms to be successful, some general conditions ought to be fulfilled, as outlined below.

A National or Regional Biotechnology Development Strategy

Regulations, if they are not to become mere formalities or, worse, active hindrances to research and commercial developments, cannot be considered in isolation. Regulatory mechanisms should be part of a more general strategy, with corresponding policies, for the local development of biotechnology. The design of these mechanisms should take into account the requirements and concerns of local research groups and industry, public health and the environment, and the interests of potentially affected economic or social groups. The principal objectives of such a strategy should be to facilitate access to available technologies and products, as well as to develop local technologies and productive capabilities in this field.

Relevant Infrastructure and Expertise

Biotechnology regulations will, in most cases, fall within existing legal statutes and mechanisms for the regulation of health aspects of food,

pharmaceuticals, agrochemicals and seeds and the workplace, which already exist in most LAC countries. The weakest area is the protection of the environment. The regulatory bodies suffer from the same constraints as the rest of the public sector in LAC. Because of the economic crisis of the last decade, there have been great budgetary limitations which have led to the loss of personnel and the limiting of expenditure. But even without these constraints, these bodies, it is argued here, often do not have the technical capability to assess risks associated with proposed actions in the area of biotechnology in order to make proper decisions. For example, the regulation and control of veterinary products of any kind was, in the late 1980s, carried out by the following number of people in the following countries: Colombia 5, the Dominican Republic 2, El Salvador 4, Guatemala 1, Guyana 2, Honduras 1, Jamaica none, Paraguay 3, Peru 3, Uruguay 1 and Venezuela 4 (Maryland Regional College for Veterinary Medicine and IICA, 1989).

Human Resources

This is probably the greatest limiting facing many of the smaller and less developed countries of the region. There are few scientists with relevant training and expertise in molecular biology, ecology or other required disciplines. Even in the larger, more developed countries, few people with the relevant expertise in genetic engineering and ecology, for example, are available. Little regulatory experience in the field of biotechnology exists.

The initiative for the creation of required national biotechnology regulations and mechanisms should ideally be taken by the organizations responsible for the development of biotechnology. Many countries have ministries of science and technology or national biotechnology commissions which could initiate and coordinate these activities, ensuring that a balanced approach between the safeguard of public health and the development of biotechnology capabilities is taken.

The limited experience to date shows that initiatives are being taken by agencies most directly related to specific issues, and therefore closest to the different groups interested in the development of regulations, be they industry, environmental groups, scientists or others. For example, plant protection agencies have taken up monitoring of field tests with transgenic plants. Nevertheless, even in the absence of a general regulatory framework within a biotechnology development strategy, it is important to ensure the participation of the different agencies with mandates related to biotechnology and biosafety. This will facilitate the development of adequate regulatory approaches and advance the

necessary consensus on more general issues important for the strengthening and advancement of biotechnology.

The development and administration of biosafety policies will most likely be the responsibility of existing regulatory agencies, and this new responsibility will strain scarce resources. Particularly in respect to technical expertise for risk assessment, these agencies will have to turn to research and development institutions, where national capabilities in fields such as genetic engineering and ecology are located. This could produce potential conflicts of interest, since the few scientists with expertise are probably also personally involved in the activities to be regulated. For the smaller, less developed countries, the most viable alternative is to create regional mechanisms. They should be closely related to existing cooperative structures for research, health and regulatory issues in general.

Perspectives of Biosafety Regulations in LAC

There is a growing interest in biosafety regulations in LAC, and particularly in the regulation of field tests of GMOs. Foreign companies are interested either in testing their products or in the counter season seed production of transgenic plants. Within a short time, local research groups will also be ready to test their products. To develop and strengthen sound regulatory mechanisms, the following interrelated issues have to be addressed soon.

Harmonization of Regulatory Approaches

The incipient state of existing experience in the region offers a great opportunity for the development of regionally harmonized regulatory criteria and mechanisms. This is also a consequence of the free trade initiatives between certain groups or individual countries which are being negotiated at this moment.

Support to Countries

The lack of experience and expertise in this field in LAC calls for training and technical assistance activities for regulatory agencies and research and development institutes, with the goal of supporting the establishment and administration of biotechnology regulatory mechanisms. Of particular importance is access to relevant data and information for the assessment of specific requests.

Analysis of International Trends and Experience

Biotechnology regulations are evolving in industrialized countries, and their accumulated experience has permitted them to streamline and better focus the process. New challenges, like approval of commercial use of transgenic products, are being faced. It is of vital importance for the countries of the LAC region to closely follow these trends so as to avoid costly mistakes. General guidelines for the development of biosafety regulations incorporating world experience are already available. Because of the rapid pace of change in this field, these should be constantly updated.

References

Fox, J. 1987. Three recombinant vaccine tests stir debate. *Bio/Technology* 5:13.

IICA. 1988. Guidelines for the Use and Safety of Genetic Engineering Techniques or Recombinant DNA Technology. Miscellaneous Publications Series DER/USA/88/001. Inter-American Institute for Cooperation in Agriculture (IICA), Washington DC.

IICA/PAHO. 1991. Guidelines for the Release into the Environment of Genetically Modified Organisms, Miscellaneous Publications Series AS/SC-91-13. Inter-American Institute for Cooperation in Agriculture (IICA) and PanAmerican Health Organization (PAHO), San José.

Jaffé, W. R. 1991. *La Problemática del Desarrollo de las Agrobio-tecnologías en América Latina y el Caribe*. Inter-American Institute for Cooperation in Agriculture (IICA) Program Papers Series No. 23. IICA, San José.

Jaffé, W. R. (ed.). 1994. *Estrategias Empresariales en Agrobiotechnologia: 21 Estudios de Caso*. Inter-American Institute for Cooperation in Agriculture (IICA), San José.

Maryland Regional College of Veterinary Medicine and IICA. 1989. *Inter-American Compendium of Registered Veterinary Products, Regulations and Authorities*. Second Edition, Bulletin 98-1. Inter-American Institute for Cooperation on Agriculture (IICA), San José.

PAHO. 1987. *Research Involving Recombinant DNA Technology*, PAHO Guidelines, PAHO/ACHR/ 26/87.7, August. PanAmerican Health Organization (PAHO), Washington DC.

Quintero, R. 1990. Estado de la Nueva Biotecnología y su Regulación en México. Presented at the *II Meeting of the Inter-American Study Group on the New Biotechnologies*, Brasilia.

James, C. and A.F. Krattiger. 1994. The ISAAA Biosafety Initiative: Institutional Capacity Building through Technology Transfer. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 225-237.

Chapter 4.4

The ISAAA Biosafety Initiative: Institutional Capacity Building through Technology Transfer

Clive James
Chairman, International Service for the Acquisition of Agri-biotech Applications (ISAAA)
P.O. Box 427, Grand Cayman, Cayman Islands, BWI.

Anatole F. Krattiger
Associate Director, Biodiversity/Biotechnology Program
(formerly Executive Consultant, ISAAA)
International Academy of the Environment
1231 Conches, Geneva, Switzerland

Introduction

Ensuring adequate food supply, produced in sustainable agricultural systems in developing countries, will be a forbiddingly difficult challenge in the years ahead. Rapid population growth, disease of plants, animals

and humans, and fast environmental deterioration militate against food production in many developing countries. Quantum leaps in agricultural production by the more sustainable use of natural resources are required to feed a 10 billion world population in 2040, when about 90% of the global population will be living in developing countries.

In the past, developing countries, and the institutes which have assisted them with agricultural research, have had the privilege of freely accessing non-proprietary traditional technology from the public sector. With the advent of new biotechnology applications, however, this situation is changing. The new applications are increasingly proprietary, owned primarily by private sector corporations in industrial countries, and accounting for approximately 75% of the investment in biotechnology research and development on a global basis. The benefits of this technology are generally not accessible to most developing countries due to institutional, political, and infrastructural constraints and a lack of investments.

To assist developing countries in the acquisition and application of proprietary biotechnology applications, the International Biotechnology Collaboration Program was created and initially developed by the Resources Development Foundation of New York and its supporters and by the Hitachi Foundation. The early initiative led to the creation of a new organizational structure (James, 1991; Krattiger and James, 1992, 1994) to internationalize all activities from September 1991 under the aegis of the International Service for the Acquisition of Agri-biotech Applications (ISAAA)—a not-for-profit and tax-exempt international organization.

In the absence of such organizations as ISAAA, developing countries may well be denied the opportunity to access the full potential that superior biotechnology applications of the present and future may have to offer the developing world. ISAAA's objectives involve building new partnerships which concentrate on results-oriented projects and which are built on the principle of balanced contribution across the industrial countries, the developing world, and the public and private sectors.

ISAAA's Technology Transfer Activities

ISAAA has initiated a pilot program employing a five-step strategy to provide the following services (see also James, 1991; Krattiger and James, 1994; ISAAA, 1994):

- assisting developing countries to identify biotechnology needs and priorities and to assess potential socioeconomic impacts;
- monitoring and evaluating availability of appropriate proprietary applications in industrialized countries;

- providing "honest broker" services matching needs and appropriate proprietary technologies;
- mobilizing funding from donor agencies to implement proposals; and
- counseling developing countries on the safe and responsible testing of recombinant products and assisting in implementing regulatory procedures, in planning commercialization activities, and in assessing the impact of the technology.

These activities are undertaken on a regional basis: three *Center*Nodes are located at centers of excellence in North America (*Ameri*Center at Cornell University, USA), in Europe (*Euro*Center at the Norwich Research Park, UK) and in the Asia-Pacific region (office for initiation of the *Asia*Center at Technova, Japan) to monitor and to evaluate availability of biotechnology for transfer to the developing world. Similarly, three *Network*Nodes will be set up in Africa (*Afri*Net), Asia (*Asia*Net) and Latin America (*Lati*Net) to assist national programs to identify priority needs for biotechnology applications.

The transfer of biotechnology is a significant challenge in itself and demands all the resources of any organization; ISAAA is entirely dedicating its efforts to this singular task, thereby ensuring the highest probability of success. The strategy which ISAAA pursues to assist the national programs in developing countries includes:

- Focus on near-term applications which must already have been tested in industrial countries and which have high probabilities of success. These applications will demonstrate potential benefits/constraints of the technology.
- Emphasis is on applications to increase the productivity of food crops and contribute to a safer environment, and to assign high priority to horticulture and to forestry, particularly non-commercial food crops grown by poor farmers and/or those applications that will make a contribution to income as well as the environment and sustainability through the development of alternatives to toxic conventional pesticides for crops like cotton.
- Concentration on three classes of plant biotechnology applications: tissue culture, diagnostics, and transgenic plants.
- Assigning of priority to the assessment of benefits and constraints, including biosafety considerations associated with introducing recombinant products.

ISAAA projects are aimed at demonstrating the feasibility of transferring proprietary biotechnology to developing countries. The first model project involves the donation of coat protein genes by Monsanto

Company to Mexico for the control of viruses in the "Alpha" variety of potato, funded by the Rockefeller Foundation and featuring technology transfer and the training of Mexican scientists. The first generation of transgenic potatoes, developed by Mexican scientists, was planted in a field test in Irapuato, Mexico, in March 1993. A companion project is assisting Mexico in developing the infrastructure and regulatory biosafety procedures for testing and introducing recombinant products (for details, see Krattiger and James, 1992; Altman and James, 1993).

The second project, funded by the United States Agency for International Development (USAID), involves the development of a "cold" DNA diagnostic probe by Washington State University for *Xanthomonas campestris* pv. *campestris*, the most important disease of crucifers world-wide. The probe will be made available to the Asian Vegetable Research and Development Center (AVRDC) and to its client countries in the developing world. The training is now being implemented.

The third project involves development of non-conventional virus resistance to the cucumber mosaic virus (CMV) of "criollo" melon in Costa Rica with technology being donated by the Asgrow Seed Company to the University of Costa Rica. An ISAAA training fellowship was awarded to a Costa Rican scientist to initiate the project within Asgrow in the USA, and USAID (ABSP) has pledged funds to implement the project.

The fourth project involves development of a diagnostic tool for the detection and monitoring of maize virus diseases (Maize Spiroplasma and Maize Fine Stripe) in Brazil. The Empresa Brasileira de Pesquisa Agropecuria (EMBRAPA) will test, adapt and transfer ELISA technology, developed by Pioneer Hi-Bred International Inc., through an intensive training program.

The fifth project involves the initial donation, by Cornell University to Brazil and Thailand, of technology for the control of papaya ringspot virus (PRSV). Brazil and Thailand are to become partners in a global training effort to develop resistant papaya on a worldwide basis. Partial funding was awarded by EMBRAPA to implement the Brazilian phase.

The sixth project involves the development of pest resistant cotton in Brazil and Zimbabwe. This project is a collaboration among Monsanto (USA), the National Center for Genetic Resources and Biotechnology (CENARGEN) of Brazil, and the Cotton Research Institute in Zimbabwe.

In addition, commitment has been made to the donation of technology and/or the provision of training to scientists from developing countries under the aegis of ISAAA by American Cyanamid (USA), Asgrow/Upjohn (USA), ICI (UK), Kleinwanzlebner Saatzucht A.G. (KWS; Germany), Kirin (Japan), Monsanto (USA), Pioneer Hi-Bred International (USA), Sandoz Seeds (Switzerland), and Schering (Germany).

Another activity that ISAAA was associated with involved the preservation of tropical tree species diversity in Malaysia. Collaborators included scientists from the Royal Botanic Gardens, Kew (UK) and the Forest Research Institute in Malaysia. ISAAA also monitors technologies to determine their development in terms of applicability to developing countries. One such promising technology that is being monitored consists of rattan micropropagation systems to assist conservation of tropical forest resources and biodiversity.

The ISAAA Biosafety Initiative

The Importance of Biosafety Regulations

Biotechnology is viewed by many political leaders, policy-makers and leading scientists in developing countries as a tool that can be used to lessen the technological gap between rich and poor countries. Many Latin American countries have active programs in biotechnology, and some countries, for example Mexico and Costa Rica, have assigned a national priority to biotechnology activities and investments. However, developing countries generally have not enacted regulatory policies to address the issues related to the development, testing and release of recombinant products. Consequently, this has not facilitated public or private involvement, which is a pre-requisite for addressing public policy issues related to biosafety regulation.

There is an urgent need to assist developing countries in building a regulatory infrastructure for oversight of recombinant products, particularly transgenic plants; the need is related to technology becoming available from two different sources. First, several national programs in developing countries are already generating transgenic plants or have the capability to do so, and formalized regulatory procedures are not available. Scientists in several developing countries are faced with a dilemma in making decisions about releasing transgenic products when there is no official regulatory mechanism to control release or to monitor biosafety.

The capacity of national programs in biotechnology is being strengthened by international and regional organizations. In Latin America these include the Inter-American Institute for Cooperation in Agriculture (IICA), the Research and Training Center for Tropical Agriculture (CATIE), the United Nations Development Program (UNDP), the United Nations Educational, Scientific and Cultural Organization (UNESCO), the United Nations Industrial Development Organization (UNIDO), the Corporacion Andina de Fomento (CAF), and ISAAA. In

addition the international agricultural research centers operating in Latin America, the Centro Internacional de Agricultura Tropical (CIAT) in Colombia, the International Maize and Wheat Improvement Center (CIMMYT) in Mexico and the International Potato Center (CIP) in Peru are all involved in biotechnology and in the imminent future will have generated, or will have access to, transgenic products that need to be field tested.

Indeed, CIAT has already submitted a field trial application for *Rhizobium* to the Colombian authorities, but Colombia is only now drafting a biodiversity law that contains a section on biosafety. CIMMYT also applied for a permit to import transgenic tropical maize calli in early 1994. In addition, the Agricultural Biosafety Committee of the Directorate General for Plant Health of the Secretaría de Agricultura y Recursos Hidráulicos (SARH) of Mexico granted permission to CIMMYT for glasshouse tests of genetically engineered tropical maize and wheat with a marker gene (GUS).

Second, many private sector corporations, which have generated transgenic crops that already have been tested in one or more industrial countries, have sought opportunities to test their products in the more advanced developing countries. Transgenic plants are being tested in certain developing countries where there are no official regulatory systems to review and ratify applications. Without a comprehensive biosafety initiative, potential hazards may not be adequately assessed, and beneficial use of the technology will be hampered. Developing countries generally do not want this situation to continue and several have requested ISAAA's assistance through the ISAAA Biosafety Initiative.

In addition, there has been a new awareness of the connection between biotechnology and biodiversity, largely brought about by the Convention on Biological Diversity (see Krattiger, 1994; Krattiger and Lesser, 1994). Paradoxically, the attention paid to biosafety within the Convention is a reflection of existing concerns, rather than the emergence of a new issue. Although there are two Articles in the Convention that deal specifically with biosafety (8[g] and 19.3), biosafety really enters into the process of the Convention as a result of the agreements stipulated in Article 16 for facilitating access to the methods and products of biotechnology (technology transfer), especially modern biotechnology. Thus the connection between biosafety with biodiversity is two-fold. First, the need for speeding up the implementation of appropriate biosafety regulations arises from the fact that one of the Convention's objectives is to facilitate the transfer of technology, particularly biotechnology. For appropriate biotechnology applications to be transferred in a safe and effective way presupposes—and should

presuppose—that biosafety regulatory mechanisms are in place. Second, the saving and protection of biodiversity is a complex endeavor that requires, on the one hand, protecting natural habitats (for example from the invasion of alien species) and, on the other, alleviating pressure on land extension into natural habitats. It is this latter aspect that is directly related to the sustainability issue and agricultural production and productivity (see also Krattiger, 1994).

Capacity Building in Regulatory Oversight

For projects brokered by ISAAA that involve transgenic plants, ISAAA ensures that products are tested and introduced in a safe and effective way, preferably in harmony with existing biosafety regulations in various industrial countries (Krattiger and James, 1994; Raman, 1993). To address this issue, ISAAA organized the first Biosafety Workshop in Costa Rica in February 1992 (from which the papers in this book were developed), in Argentina in November 1992, and in Indonesia in April 1993 that assisted the specific individuals who comprise the national biosafety committees that provide regulatory oversight for the testing of recombinant products in Mexico, Costa Rica, Argentina, Bolivia, Chile, Uruguay, Paraguay, Brazil, Malaysia, Thailand, the People's Republic of China, Indonesia and the Philippines; four of these countries are already recipients of ISAAA projects of donated recombinant DNA (rDNA) technology. ISAAA also collaborated with USAID through the ABSP of Michigan State University for a biosafety workshop in Egypt in January 1994, and with the Latin America/Caribbean biosafety workshop held in Jamaica during 1993. ISAAA staff also participated in other biosafety initiatives. These include a workshop on the analysis of regulatory measures with emphasis on phytosanitary methods in the development of biotechnology held in Mexico during 1993; and biosafety conferences in Thailand, the Netherlands, Zimbabwe, and China held during 1993 and in Colombia in 1994.

The workshops' objective was to build institutional capacity in biosafety regulations by sharing industrialized countries' experience in biosafety regulations for the testing of genetically modified plants in the field with scientists, policy-makers and special interest groups from developing countries. ISAAA's approach is unique in that it is aimed at practitioners and focused on actual project implementation, and involves the individuals who will be key in developing their country's regulations. The intent is not to determine a specific set of guidelines and regulations but to provide an opportunity to learn from the experiences and

successes of the countries that already have approved the over 1,000 field tests of transgenic plants that have taken place to date.

The First ISAAA Biosafety Workshop: Learning from the "Costa Rican" Experience

One of the prerequisites associated with technology transfer projects involving rDNA plants is to ensure that institutional capacity for regulatory oversight has been developed. It was within such a context—the collaborative project that involves the donation of coat protein genes by Monsanto to Mexico for control of viruses potato virus X (PVX) and potato virus Y (PVY) in potato—that ISAAA organized its first biosafety workshop. Accordingly, priority goals for ISAAA, and therefore of the workshop, were:

- to assist client countries who are recipients of donated rDNA technology in building institutional capacity in biosafety regulation;
- to share with Costa Rican and Mexican scientists and policy-makers the experience of the USA, Canada and European countries in biosafety regulations and in the testing of genetically modified plants in the field; and
- to provide hands-on experience of the procedures and issues described above (this was achieved by supplementing the presentations by actual case studies so that participants could be walked through a decision-making process and the rationale underpinning those decisions).

The aim of the Workshop was not to provide ready-made answers to Costa Rica and Mexico on a precise protocol to establish their regulatory mechanisms, but to enable policy-makers, scientists, lawyers and members of special interest groups to promulgate their own policies to regulate transgenic plants through informed decisions and to facilitate international harmonization of regulation and oversight. This approach recognizes that the most effective way to build national regulations is to share resources and experiences and to facilitate harmonization without imposing compliance requirements, which are the prerogative of sovereign national governments. This process will enable the sharing and transfer of technology that can contribute to making a positive impact for biotechnology on global food production and availability.

The ISAAA Biosafety Workshop was based on the Harvard case-study approach to allow nationals from developing countries to receive hands-on experience relevant to the procedures and issues. The Workshop provided a mix of plenary presentations, working group sessions and joint follow-up discussions. Outside the main presentations, participants

worked in small groups and shared their work in plenary sessions. The group settings provided a mix of various disciplines (e.g. law, science, public interest, policy-makers).

Following the opening session on day 1, during which Orlando Morales, Minister of Science and Technology of Costa Rica, emphasized the crucial importance for Costa Rica to have access to biotechnology applications, a series of presentations featured the policies and procedures that the USA, Canada and European countries follow in reviewing applications for testing and release of transgenic plants, in order to set the scene internationally. The case study of the working group focused on the preparation of applications for field trials and on the type of information that is required to prepare applications. Presenters included staff from the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA; see Chapter 2.4: McCammon; Chapter 2.5: Kubicek), the Environmental Protection Agency (EPA; see Chapter 2.7: Zeph) and from the Maryland Biotechnology Institute (see Chapter 2.6: Levin) from the USA and similar organizations from Canada (see Chapters 2.2 and 2.3: Hollebone and Duke).

The next session on day 2 concentrated on the decision making process and regulatory support activities (see Chapter 1.3: Colwell; Section 5: Beversdorf). The sessions featured presentations by public sector institutions, universities and government agencies who have successfully submitted applications in the USA, Canada and Europe. They drew on their experience in satisfying the current APHIS regulations and generating the required data. Presentations were also made to provide a view of current APHIS regulations within the context of public policy and perceptions, and the EPA risk assessment programs were reviewed. Emphasis was placed on the dual need to promote new technologies responsibly and protect the public and the environment from any potential hazards. Emphasis in working groups was also placed on regulatory support activities within the context of plant health and quarantine regulations in Costa Rica and Mexico.

The next day (day 3) dealt with field data, field trial designs and environmental evaluation. A special paper was commissioned to examine the implications of releasing a transgenic crop in a center of diversity—with Mexico and Costa Rica taken as case studies—with emphasis on modeling population dynamics, including potential gene transfer, environmental consequences of gene transfer, and the potential for, and possible consequences of, weediness (see Chapter 1.4: Hanneman). The case study was followed by a working group session where an actual decision process was analyzed. Participants placed special emphasis on

new constraints and aspects to be considered in developing countries, such as abundance of landraces and wild crop relatives, and other biodiversity considerations.

The afternoon session dealt with experience in conducting field trials and presentations were made from the public sector in the USA and Canada (see Chapter 3.2: Beversdorf) and Europe (see Chapter 3.1: de Greef). Complementary presentations from the private sector were also made, with the private sector having the most experience in submitting successful applications and implementing field tests (see Chapter 3.3: Quemada; Chapter 3.4: Debus; Chapter 3.5: Townsend; and Chapter 3.6: Meeusen).

The register in the USA that documents field tests of transgenic plants indicates that over 80% of the approved applications were from private sector corporations. The equivalent international data base maintained by the Organization for Economic Cooperation and Development (OECD) also confirms that the private sector has the most experience in field testing. The day concluded with an outlook of products that the regulatory agencies need to be prepared for field testing and that might be tested in Latin America (see Chapter 1.1: Fraley).

The fourth day was devoted to the larger issues raised by technology transfer. The day was structured around two working group sessions that evaluated actual field trial data and prepared a review of an application on the basis of available field trial data. Again, the group work was discussed in plenary sessions and placed within the context of technology transfer. The issues of biotechnology, biodiversity, biosafety and technology transfer were considered by inviting Costa Rican and Mexican national scientists to present their experience and views on technology transfer to agricultural producers in the two countries.

The workshop culminated with an overview and discussion of the major elements reviewed during the previous four days and explored the relevance of the presented material to the needs of Costa Rica and Mexico. To this effect, the morning was almost entirely spent in two working groups, focusing on Mexico and Costa Rica, to identify needs in the context of the regulatory structure and environmental constraints of the two countries.

The findings were presented during the afternoon session which also provided an overview of the regulatory activities in Latin America (see Chapter 4.3: Jaffé), concluding thoughts on international harmonization of regulatory activities (see Chapter 2.1: Medley) and an outlook on the contribution of transgenic crops to sustainable agriculture (see Chapter 1.1: Fraley; Chapter 1.2: Beversdorf).

The Benefits

There were three important benefits of, and outputs from, the Workshop. The first was the sharing of information which was done in one of the most effective ways and in a very collegiate forum, and by individuals who came from institutions with a very different sense of culture, tradition and values. Since the quality of decision-making is always dependent on the quality of the information that individuals can access, the sharing of information over the span of one week was an invaluable experience.

The second output of the workshop was the fellowship that resulted from getting together—an invisible college within a global perspective was built that will allow participants to continue sharing information; this is one of the most effective vehicles for facilitating the transfer of any technology, including biotechnology. The advent of biotechnology presents us with a unique challenge to choreograph on the world stage by building institutions for the world of tomorrow—building towards global harmonization of regulations and at the same time respecting different cultures and traditions by acknowledging that compliance of regulations is a sovereign right.

The third output was that, within the space of five days, the planning process for Costa Rica was initiated. One week after the conclusion of the Workshop, the Costa Rican participants, based on its results, convened at the invitation of Sanidad Vegetal of the Ministry of Agriculture together with the Ministry of Science and Technology; a Preparatory Biosafety Committee began drafting technical and legal guidelines for the submission, review and implementation of field trials with transgenic plants. Many developments have since taken place, and Gabriel Macaya (see Chapter 4.1) provides a comprehensive overview of recent developments and of the current biosafety regulatory situation in Costa Rica. For example, the President of Costa Rica is expected to soon formally establish a Technical Advisory Committee on Biosafety and issue a new version of the General Law of Plant Protection. This permanent committee will be comprised of representatives of various governmental institutions, universities and ecological groups. Noteworthy is that the Committee can appoint additional experts for specific review applications, and that these can either be drawn from Costa Rica or abroad.

In Mexico, the development of a regulatory framework had already been initiated through the initially informal establishment of an Agricultural Biosafety Committee. Shortly after the ISAAA capacity building workshop in Costa Rica, the Mexican authorities began to receive several field trial applications: two in late 1992, six in 1993, and

four in 1994 (to June). It is expected that a formal official regulation will enter into force in Mexico by October 1994 (see Chapter 4.2: Carreón Zúñiga).

Conclusions

The adage "reading is learning, seeing is believing, and doing is knowing" is particularly appropriate today in the context of biosafety regulatory capacity building. The challenge is to focus on the third—doing is knowing—because until one "does", one is really unable to get to know the subject. We can talk about principles, but the most important issue is to put those principles into practice.

The challenge is to build a regulatory oversight institution in Mexico, Costa Rica, Colombia, Venezuela—and elsewhere in Latin America, Africa and Asia—with the limited resources available of the developing world. Industrial countries have the privilege of more resources that are simply not available in developing countries. Therefore, the real challenge is to build a slim-line model that takes full advantage of the experience of institutions like Agriculture Canada, APHIS, OECD, as well as of European countries; and to benefit from the council and cooperation of institutions like IICA, ISAAA, the North American Plant Protection Organization (NAPPO), the Biotechnology Advisory Committee of the Stockholm Environmental Institute (SEI), UNIDO and the United Nations Environment Program (UNEP) to build models that are appropriate and that allow Latin America, Africa and Asia to have a responsible and effective system for overseeing the products of biotechnology for sustainable agriculture.

References

Altman, D. W. and C. James. 1993. Public and private sector partnership through ISAAA for the transfer of plant biotechnology applications. *Annals of the New York Academy of Science* 13/960:137-155.

James, C. 1991. The transfer of proprietary agricultural biotechnology applications from the industrial countries to the developing ones. *Rivista di Agricoltura e Tropicale* 85:5-24.

Krattiger, A.F. 1994. Biosafety in the Context of the Convention on Biological Diversity: Enabling Technology Transfer for Sustainable Agriculture. Working Paper No. 14. International Academy of the Environment: Geneva.

Krattiger, A. F. and C. James. 1992. ISAAA: A Not-For-Profit Organization

for Acquiring and Transferring Biotechnology for Development. *Advanced Technology Assessment System* Vol. 9: Biotechnology and Development. United Nations Department of Economic and Social Development, New York.

Krattiger, A. F. and C. James. 1994. International Organization Established to Transfer Proprietary Biotechnology to Developing Countries: ISAAA. *Diversity* 9(4)-10(1):36-39.

Krattiger, A. F. and W. H. Lesser. 1994. Biosafety—an environmental impact assessment tool—and the role of the Convention on Biological Diversity. In *Widening Perspectives on Biodiversity*. Krattiger, A.F., J.A. McNeely, W.H. Lesser, K. R. Miller, Y. St. Hill and R. Senanayake (eds.). IUCN: Gland and International Academy of the Environment: Geneva.

Raman, K. V. 1993. ISAAA's Biosafety Initiative. In *Proceedings of the USAID Latin America Caribbean Regional Biosafety Workshop*. Michigan State University, East Lansing.

Section 5

Afterword

Beversdorf, W.D. 1994. Proposed Step-Wise Decision-Making Process. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 241-243.

Chapter 5

Proposed Step-Wise Decision-Making Process[1]

Wally D. Beversdorf
Head, Seeds Research and Biotechnology
Ciba Limited
Postfach, SE2, CH-4002 Basle, Switzerland.

The purpose of this chapter is to briefly clarify a proposed step-wise decision-making process regarding transgenic plants. The process for dealing with applications to test transgenic plants in an open environment must progress logically to a simple "yes" or "no" decision. It is suggested here that a logical process to arrive at a decision would involve several questions which could be answered in sequence to simplify both time requirements and the amount of information that has to be made available:

Proposed Step-Wise Decision-Making Process

1) Is there a hazard associated with the transgenic modification (presented in the context of the test that is being proposed)?

If there is no hazard, there is no risk and there should be no reason why the applicant could not proceed with the project. If there is a hazard, or identification of the hazards are uncertain, then the reviewer must proceed with an assessment of exposure probability (the other component of risk). Analysis of exposure probability can be quite simply broken down into a sequence of questions:

2a) Can the transgenic plant escape, survive and multiply in the wild or as a weed in the region of the proposed trial?

2b) Are there wild or cultivated relatives in the region of the proposed trial?

If the answer to the above two questions is "no", then there is no exposure and hence no risk, and the logical decision would be approval to proceed. If the answer is "yes", additional questions become important, namely:

3a) Will the proposed experimental protocol permit the escape of transgenic plants from the evaluation site?

3b) Is pollen from proposed transgenic plants capable of moving recombinant DNA to related wild or cultivated species in the region of the trial under the proposed experimental protocol?

If the answer to these questions is "no", there is no risk and the decision should be to proceed. If the answer to either question is "yes", then we have a dilemma, because we have identified a risk (a hazard and a potential exposure). In such a case, the question is no longer technical. At this stage the decision must progress to a risk-benefit analysis rather than a simple technical analysis. Such a risk-benefit analysis would likely include social, political and economic considerations as well as identification of the hazards associated with the agricultural ecosystem, the natural environment and risks to human health and welfare (in its broadest context). Such an analysis cannot be done by a technical committee, because the issue is no longer technical; it must be done by an appropriate regulatory authority within the jurisdiction of the trial (e.g. a government agency). Such an analysis will normally lead to one of three conclusions:

- the risks do not justify the potential benefits and therefore the project should be rejected;
- the risks are so minimal relative to the benefits that the project should proceed; or

- there are such uncertainties about the risks and/or benefits that no decision is obvious. This would precipitate an additional question: Can the applicant mitigate the risks (i.e. limit them through technical, procedural or indemnification means)? Such a question would logically require additional dialogue with the applicant. Only if the risks could be adequately mitigated or indemnified would the regulatory authority choose to proceed with the trial.

Such a simple logical process is necessary to efficiently arrive at a decision concerning the approval or rejection of field test applications. Additional processes will also be required to approve scale-up and commercialization applications.

Note

1. The material presented in this chapter evolved during a workshop on biosafety organized by the International Service for the Acquisition of Agri-biotech Applications (ISAAA) in Costa Rica in February 1992. Participants included all authors of the other chapters in this book in addition to representatives of the governments of Costa Rica, Mexico and the USA, and of various NGOs and universities.

Section 6

Appendix

Krattiger, A.F. 1994. Field Releases of Genetically Modified Plants: Worldwide Data from 1986 to 1993/94. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 247-266.

Chapter 6

The Field Testing and Commercialization of Genetically Modified Plants:

A Review of Worldwide Data (1986 to 1993/94)

Anatole F. Krattiger
Associate Director, Biodiversity/Biotechnology Program
(formerly Executive Consultant, ISAAA)
International Academy of the Environment
1231 Conches, Geneva, Switzerland.

Summary

Worldwide data of field releases (testing) and commercialization of genetically modified plants—with particular reference to developing countries—have been compiled and analyzed. Results show that China and the USA are the only two countries where large-scale releases have been authorized for the purpose of commercialization. In China, genetically modified virus resistant tobacco has been used in industrial tobacco manufacturing for national consumption since 1992 and the cultivated area now stands at nearly 1 million ha or almost 5% of

tobacco plantations. In May 1994, the USA granted approval for commercial production of genetically modified tomato (the so-called FLAVRSAVR™) and sales began during the same month within the USA. Herbicide tolerant cotton and soybean were deregulated in February 1994 and a preliminary determination by USDA for virus resistant squash (WMV2 and ZYMV) was favorable in July 1994.

Field trials worldwide have been increasing steadily since the first such trials began in 1986 in the USA and France and over 1450 have been conducted worldwide to date (nearly 400 in 1993 alone). The majority are taking place in the USA, Canada and Europe (particularly France, Belgium, the United Kingdom and the Netherlands).

Field trial activities in developing countries amount to 8% of worldwide trials and are highest in Latin America and the Caribbean (5%) with few field releases in Africa (less than 1%) and Asia (2% almost exclusively in China), and are following a somewhat different pattern from that of OECD countries. The first trial took place in 1987 with an increasing number of trials to 1993. It appears that fewer trials are currently underway in developing countries with the exception of China, although many are planned for later this year for counter season planting in the Southern cone. The remaining slight reduction in field tests in Latin America in the first part of 1994 is more a result of the establishment of formal biosafety regulatory mechanisms that require a review of applications (hence a temporary delay) rather than a shift in overall policy towards biotechnology.

1. Introduction

The issue of biosafety arose shortly following the “discovery” of the possibility of genetically modifying organisms and the development of such techniques in 1971. The first regulations were prepared by the National Institutes of Health of the USA in 1976 to apply to laboratory procedures (51 Federal Regulation No. 16958). Far more complex has been the treatment of materials to be released into the environment, first for testing and now for commercial use. In this area, the USA, as an early entrant into biotechnology research, was an innovator in developing regulations, at least for plants. The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) established several of the key aspects of regulations in this area, namely the need for case by case and step by step evaluation.

This approach to legislation has been adopted by other countries, including the Philippines, but the bulk of the countries of the world are at this time lacking in biosafety regulations with the exception of many countries of the Organization for Economic Co-operation and Development (OECD). The list of countries lacking regulations includes virtually all developing countries with the exceptions of India, Mexico and the Philippines (synopsis in Maloney, 1994), with Argentina and Cuba having regulations in place but which are not incorporated into laws, and with Costa Rica, Bolivia, Brazil, Chile, China, Columbia, Indonesia, Malaysia, Nigeria, Thailand and Zimbabwe either having *ad hoc*

committees or being in the process of adopting regulations (see also Krattiger and Lesser, 1994). The absence of regulations has led to concerns that private firms will test potentially hazardous genetically modified organisms (GMOs) at will in those countries lacking regulations (see UNEP, 1993). Even when care is applied, the larger number of relatives of food and fiber crops which exist in centers of origin make possible outcrossing of greater concern than for most developed country applications.

This indicates that it is imperative for countries to adopt appropriate regulations in the very near future. Failure to do so leaves them vulnerable to improper precautions and delayed access to innovations from those firms and agencies which prohibit introductions when there is no national body to rule on their safety. Thus the issue is not whether there ought to be regulations but how best to implement them.

The purpose of this paper is to review worldwide field releases of genetically modified plants, with particular reference to developing countries, and to clarify a certain number of issues. Whereas data for Canada, Europe and the USA is readily available and has been reviewed extensively (Chasseray and Duesing, 1992; Ahl Goy and Duesing, 1993, 1994; Ahl Goy *et al.*, 1994; OECD, 1993), detailed and comprehensive data of Africa, Asia and Latin America and the Caribbean (LAC) is difficult to obtain or corroborate. An attempt was therefore made to compile worldwide release and commercialization data with particular reference to developing countries. It should be noted that the most detailed, comparable and comprehensive database is maintained by the Green Industry Biotechnology Platform (GIBiP), an association of major European plant biotechnology companies. The articles cited above present comprehensive analysis from this database.

The next section provides explanatory notes on the data and section 3 reviews commercialization activities. Section 4 briefly reviews field trial activities worldwide and analyzes differences between developing and industrialized countries, and difference among major geographic regions. Section 5 lists detailed trial data of developing countries.

2. Methodology and Definitions

2.1 Data Gathering

In countries with biosafety regulatory mechanisms established, official field trial data is readily available from the respective governmental agencies. In addition, the European Union (EU) keeps records of field releases in its member states and OECD countries are required to disclose data on their releases.

The availability of data in developing countries, however, is uneven. Certain countries with biosafety committees (e.g. Chile, Argentina, Thailand) make their data readily available, including information on the current status of applications and rejected applications. Other countries with formal biosafety or biotechnology committees (e.g. Costa Rica) treat applications in strict confidence, even those that have been rejected. In either case, the committees—understandably—only provide official information related to field trials that have officially been approved. Data on trials before such committees were constituted are only available through informal contacts and rarely from official sources. The information presented in this paper has been obtained through official channels, where applicable, and through personal contacts for most developing countries. Essential data was also obtained from the GIBiP database maintained at Ciba-Geigy in Switzerland.

2.2 The Meaning(s) of "One" Field Trial

A field trial with genetically modified plants has different definitions in different countries. It can be one crop at one site in one year, and it can be a category of a crop at a number of sites across a country. In the USA, a "Release Permit" is applicable to one precisely defined crop with a known modification and may be tested at more than one site in more than one state. Each proposed trial site must be listed in applications to APHIS and the permit obtained from APHIS indicates the sites where field trials may proceed (an exception to this are "deregulated articles" discussed below).

A field trial refers in this paper to release permits issued (US-terminology) and to submissions (Canadian terminology). In Canada, the distinction between "submissions" and "trials" is as follows: a given submission corresponds to a year, an applicant, a species, and a genotype. Each submission may be tested at various sites in one or more provinces and each site constitutes one trial.

In developing countries, the number of sites is often small (less than 10 for a given field trial) and the data presented here refers to one trial of a specific crop at one or more sites per country, where applicable.

Perennial crops (e.g. trees, strawberries, sugarcane) may be tested over a period of years. In such cases, the data presented here reflects the year when the trial was established and is not listed again in the years where the same planting of a trial simply continued.

2.3 Classification of Modified Characteristics

The various characters of modified plants were grouped into several categories, namely agronomic traits (A), bacterial resistance (BR), fungal

resistance (FR), herbicide tolerance (HT), industrial production (IP), insect resistance (IR), marker gene(s) (M), nematode resistance (NR), quality characteristics (Q), and virus resistance (VR).

Agronomic traits include characteristics such as male or female sterility, and virus resistance generally refers to the insertion of a viral coat protein. No distinction has been made between coat protein-mediated resistance, or satellite or 54kb replicase technology. Industrial production to date essentially means specific enzyme production (e.g. in soybean). Quality characteristics include slow ripening (tomato), increased protein production (e.g. high amino-acid composition in potato), decreased protein production (e.g. low gluten content for brewing rice), low allergen production (e.g. low gliadin in rice), and pigment production in flowers. Modified fatty acid composition (e.g. the bay thioesterase gene in rapeseed producing laurate) was classified as a quality trait despite the fact that it is a component in detergent and other manufactured items.

2.4 Status of Data

The status of field releases and commercialization aspects is as of July 1994, unless otherwise indicated.

3. Commercialization of Genetically Modified Plants

3.1 Tobacco in China

Virus resistant tobacco has been field tested in China since 1991 and many trials included double constructs (Cauliflower Mosaic Virus [CMV] and Tobacco Mosaic Virus [TMV]). A single construct coat protein tobacco (CMV) was sown on approximately 35 ha in 1992 for seed increase and a double construct (TMV and CMV) tobacco is now under seed increase.

A CMV resistant tobacco has been used in industrial tobacco manufacturing for national consumption since 1992. The cultivated area now stands at nearly 1 million ha corresponding to an estimated 5% of total tobacco plantations in China. The area is expected to grow to 30% by 1995 and 70% by the end of the decade. By early 1995, tobacco with resistance to two viruses (CMV and TMV) is also expected to be commercialized as seed increase is underway.

Virus resistant genetically modified tobacco yields an average of 5-7% more leaves for processing and saves 2-3 insecticide applications out of approximately 7 applications (note that aphids transmit the major viruses that infect tobacco, hence the saving is on insecticides).

3.2 Tomato in the USA

In the USA, approval for commercial sale and human consumption of genetically modified tomato (the so-called FLAVRSAVR™) was granted by the Food and Drug Administration (FDA) of the Government of the USA in May 1994. The sale of these tomato began the same month within the USA, particularly California and the mid-west and consumer acceptance has essentially been positive.

A new system under the regulations of the USA allows for applicants to request that APHIS considers whether a given transgenic plant could be deregulated (so-called "Petitions"). Approved petitions by APHIS stipulate that there is no longer any need for APHIS review or approval for introductions of the plant into agriculture and the environment. A permit is not a license to commercialize a crop since food safety, pesticide or other regulatory questions may still have to be addressed by other regulatory agencies. Three crops containing a specific gene have been deregulated by APHIS, namely the FLAVRSAVR™ tomato developed by Calgene, the BXN™ bromoxynil (herbicide) tolerant cotton also developed by Calgene, and glyphosate (herbicide) tolerant soybean developed by Monsanto. In addition, a favorable preliminary ruling in July 1994 by APHIS for virus resistant squash (coat proteins of watermelon mosaic virus 2 [WMV2] and zucchini yellow mosaic virus [ZYMV]) squash developed by Asgrow) means that this crop will be commercialized if FDA approval is obtained.

4. Field Testing of Genetically Modified Plants

4.1 Overview of Field Trial History and Current Status

The first field trials were conducted in 1986 with herbicide tolerant tobacco in France and in the USA (herbicide tolerance was then used as a marker and this is still often the case today). Belgium was the third country to authorize such releases in 1987. By the end of 1993, all countries of the OECD, with the exception of Austria, Luxembourg and Turkey, have authorized field trials although the USA, Canada, France, Belgium, the United Kingdom and the Netherlands accounted for 82% of the trials worldwide (Table 1).

To date, just over 60 plant species have been transformed and nearly half have been field tested but the great majority of tests are done with six species, namely cotton, maize, potato, soybean, tobacco, and tomato. These crops can routinely be transformed but other crops are following steadily, such as various cucurbit species, rice and sugarbeet. Yet in Canada, one single crop, rapeseed accounts for 65% of all releases. Of the

Table 1: Number of Field Trials Worldwide
(modified and extended after Ahl Goy and Duesing, 1994)

Industrialized Countries

Country/Region	1986	1987	1988	1989	1990	1991	1992	1993	1994 *	Total
North America[1]										
Canada			10	28	40	39	40	89	113	**359**
USA[2]	3	9	12	21	32	66	107	135	60	**445**
Subtotal	**3**	**9**	**22**	**49**	**72**	**105**	**147**	**224**	**173**	**804**
Europe[3]										
Belgium		1	4	9	14	14	12	19	8	**81**
Denmark[4]					2	1	3	4	1	**11**
Finland			1	1	2		3	1	2	**10**
France[4]	2	5	9	14	26	31	22	29	30	**168**
Germany[4]					1	1	1	3		**6**
Italy[4]				1	1		1	7	4	**14**
Netherlands			1	1	1	13	16	20	32	**84**
Norway							1		na**	**1**
Portugal[4]								2	2	**4**
Spain			2	4	5			3	2	**16**
Sweden				1	1	2	2	3	8	**17**
Switzerland						1	1			**2**
UK		1	1	4	11	13	13	13	22	**78**
Subtotal	**2**	**7**	**18**	**35**	**64**	**76**	**75**	**104**	**111**	**492**
Asia										
Australia						1	6	7	12	**26**
Israel						1	1	1	1	**4**
Japan						2		3	3	**8**
New Zealand			4	4	3	1	1	2		**15**
Subtotal			**4**	**4**	**3**	**5**	**8**	**13**	**16**	**53**
Total	**5**	**16**	**44**	**88**	**139**	**186**	**230**	**341**	**300**	**1349**

Developing Countries

Country/Region	1986	1987	1988	1989	1990	1991	1992	1993	1994 *	Total
Africa					1	2	1	5	1	**10**
Asia				2	2	2	5	10	11	**32**
LAC		1	1	1		10	22	27	10	**72**
Other[5]								2	2	**4**
Total		**1**	**1**	**3**	**3**	**14**	**28**	**44**	**24**	**118**

Grand Total	**5**	**17**	**45**	**91**	**142**	**200**	**258**	**385**	**324**	**1467**

* Partial data for 1994. Unless otherwise stated to July 1994.	**1** Excluding Mexico.
** na: not available.	**2** Data to June 1994. Excluding Notifications.
	3 Western Europe (EU, EFTA).
	4 Data to 15 March 1994.
	5 Hungary.

characters most widely tested, on a worldwide basis, virus resistance accounts for 37% in the USA over the last year, herbicide tolerance for 30%, and quality for 15%.

Of all trials in OECD countries over the last year, herbicide tolerance represents the highest proportion of trials (36%), followed by insect resistance (32%), and quality and virus resistance (14% each). Maize occupies the first place in the number of trials (30%), followed by cotton, soybean and tomato (approx. 15% each).

Figure 1 show the number of field trials worldwide and it should be noted that the figures are based on permits rather than locations (see also above). In Canada, for example, from 1988 to August 1994, the total number of trials was over 1,700, whereas the total number of submissions or permits was around 350. In the USA during the same period, the number of release permits was 450. Early field trials were conducted at one site only (and this continues to be the case for many trials), but the average today in the USA (excluding notifications) is 1.5 states per permit. There are nevertheless exceptions like in 1993 when a permit was issued in 1993 for cotton to be tested at 89 sites across eleven states of the USA. Permits in the Netherlands average 5-10 locations but one potato trial comprised 38 locations and another one 49 locations.

Hungary is the only country in Eastern Europe that recorded field trials with transgenic crops (1993: PVY resistant tobacco and a tobacco with a marker; 1994: same as 1993, plus a potato line with a marker). No field trials are known to have occurred in Russia or in the newly independent states of the former Soviet Union.

The data for the **developing countries** of Africa, Asia, and Latin America and the Caribbean are also evaluated separately in Figure 2. Overall, the highest activity has been recorded in Latin America but it should be noted that China probably has more individual sites tested and thus may overall have by far the highest activity. The low level of activity in Africa is related to few countries having regulatory procedures in place. Another reason is that biotechnology research activities in much of Africa is low and hence little national demand has been generated to field test genetically modified plants but with routine engineering of cassava and rice becoming increasingly possible this could change soon. Finally, seed companies are not well established in the region.

Virus resistance, insect resistance and herbicide tolerance—in decreasing order—represent nearly 90% of the characters tested in developing countries (Figure 3), and tobacco, maize, cotton and tomato—also in decreasing order—are the most often tested crops in developing countries. Maize, soybean and tomato are the crops most often tested in Latin America, whereas tobacco dominates Asia (i.e. China).

Figure 1: Number of Field Trials Worldwide

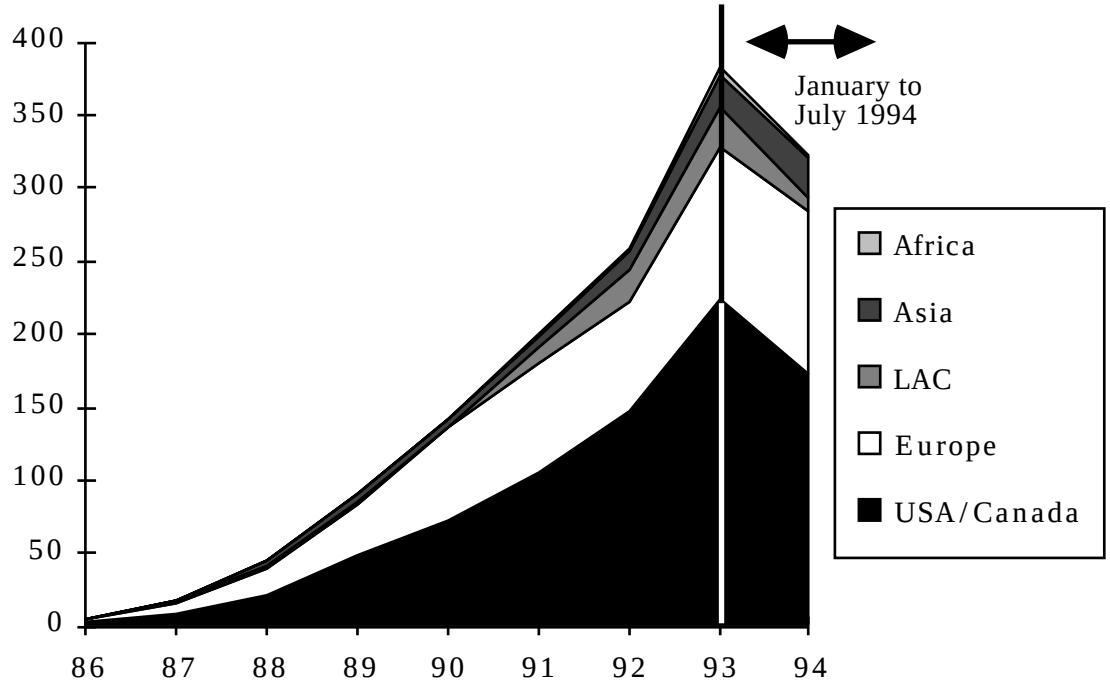
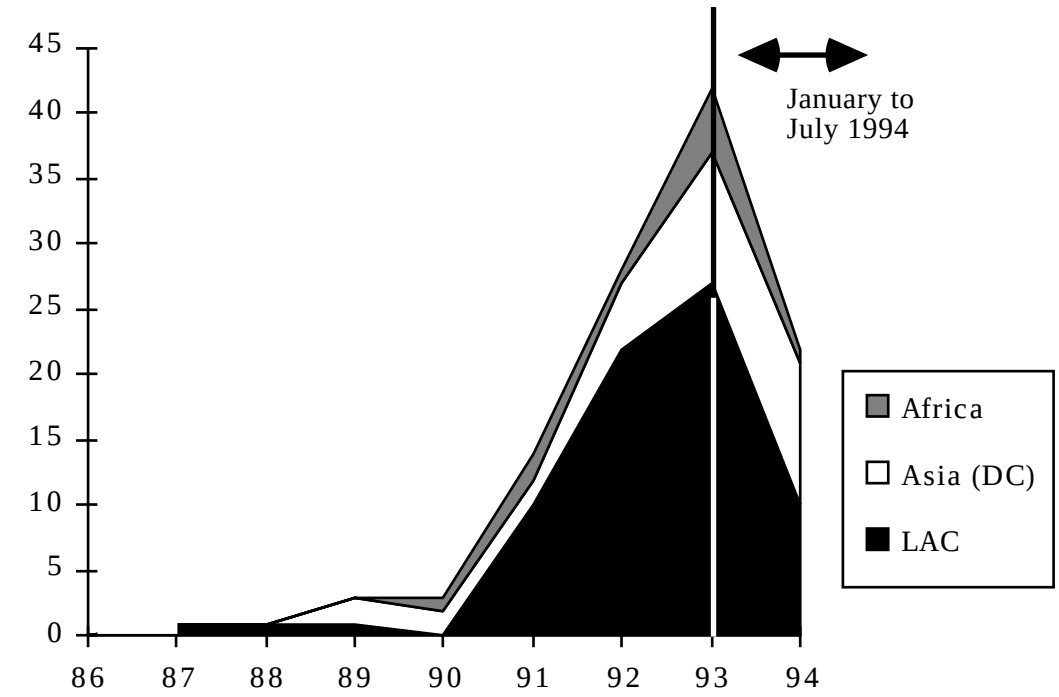
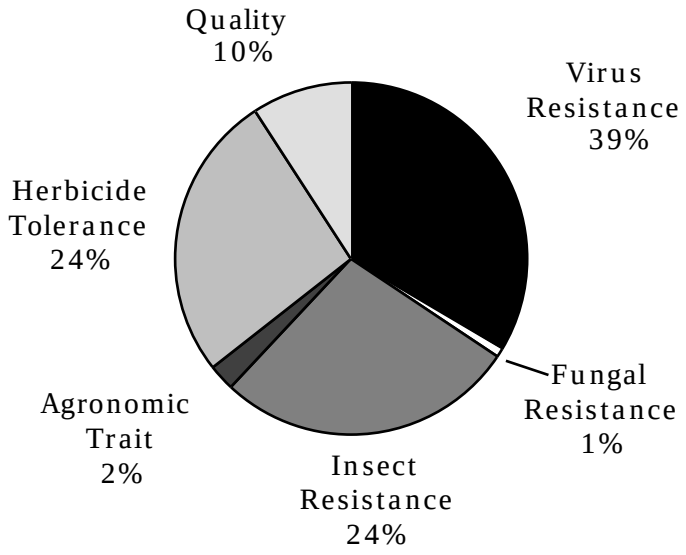


Figure 2: Number of Field Trials in Africa, Asia[1] and LAC



1 Developing countries only, including China.

Figure 3: Type of Field Trial in Africa, Asia[1] and LAC



1 Developing countries only, including China. As multiple trait field trials were counted as one or more if the traits differed, the total exceeds 100% in the original calculation. In this Figure, the numbers were scaled proportionately to 100%.

4.2 "Notifications" in the USA

APHIS recently issued a notification system (57 FR 53036; 31 March 1993) that warrants special attention. Under its scheme, applicants for subsequent, multiple trials need not seek prior approval but rather only inform the agency. The agency acknowledges such notifications if they meet the specific eligibility criteria: certain limitations on the type of genetic modification, on how introductions may be conducted, and crop species (cotton, maize, potato, soybean, tobacco, and tomato). It is noteworthy that before notification was allowed, over 80% of the field test permits were for these six crops.

Table 2 shows summary data for the six crops. Out of the 586 notifications acknowledged, cotton represents almost half with many trials for insect resistance and herbicide tolerance. Notifications for soybean and tomato represent around 15% each with many trials in soybean for herbicide tolerance and most trials in tomato for quality characteristics. Figure 4 shows the percentage of each character tested under the notification system. Note that each modification was counted as a separate trial so that the data does not differentiate between single and multiple modifications. "Other" traits include bacterial resistance, industrial products and nematode resistance.

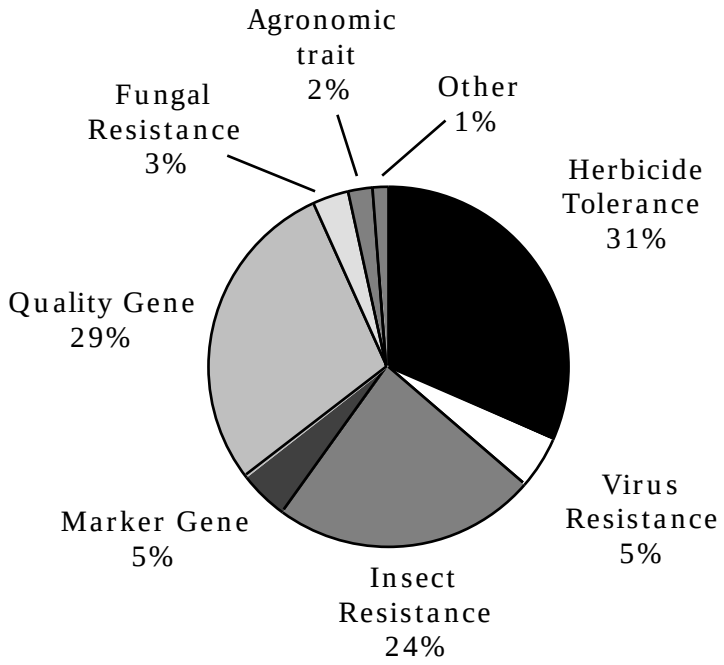
Noteworthy is that by far the largest proportion, 93%, of notifications came from the private sector. Three companies alone represent 55% of total notifications (Monsanto with 30%, Du Pont with 14% and Pioneer Hi-Bred Inc. with 11%).

Table 2: Notifications by Crop (April 1993-August 1994)

Character	**Cotton**	**Maize**	**Potato**	**Soybean**	**Tobacco**	**Tomato**	**Total**
Total for Crop							
Number of Permits	**271**	**46**	**55**	**99**	**23**	**92**	**586**
Percent of Total	46%	8%	9%	17%	4%	16%	100%
Percent of Major Trait[1]	(%)	(%)	(%)	(%)	(%)	(%)	
Herbicide Tolerance	37	63		76			
Insect Resistance	44	30	35				
Quality Gene	25		33	25		82	
Virus Resistance			24		48		

1 Trait in relation to total number of notifications per crop. The figures correspond to the number of notifications containing one or more gene(s) of the given trait in relation to the total number of notifications and the total therefore may exceed 100%.

Figure 4: Notifications by Trait[1] (April 1993-August 1994)



1 Multiple trait field trials were counted as more than one so the total exceeds 100% in the original calculation. In this Figure, the numbers were scaled proportionately to 100%.

5. Detailed Lists of Field Trials in Developing Countries

5.1 Latin America and the Caribbean

Table 3 gives a list of countries, crops and traits of field trials in the region. Argentina, Chile and Mexico are countries where the highest number of trials have taken place and overall there is a steady increase of trials to 1993.

Argentina has not established field trials so far in 1994 but many pending applications for seed increase of herbicide tolerant maize and one application to increase the seed of slow ripening transgenic tomato.

In **Belize** and the **Dominican Republic** few trials took place around 1990, and these were undertaken by private corporations maintaining winter nurseries in these countries. The trials were conducted under practices stipulated by APHIS and have been completed. None have been registered since 1992 in either of these countries. With the establishment of regulatory mechanisms in other countries of the region that lend themselves for winter nurseries (e.g. Argentina, Chile, Costa Rica, Mexico), companies now prefer to avoid countries where no formal review process is established, hence the various applications for seed production currently under review in these countries.

Chile also established a National Committee for the Protection of Agriculture (Resolution of 9 October 1993) under the Ministry of Agriculture and is currently considering several applications. Again, none have been authorized since the Committee's establishment late last year. As Table 3 shows, Chile, also having recently established a Commission, follows the same pattern as Argentina with many field trials having taken place prior to the establishment of the formal process. Both countries are considering applications for the production of seeds of transgenic tomato.

In **Columbia**, the Centro Internacional para la Agricultura Tropica (CIAT; International Center for Tropical Agriculture) also applied to the regulatory authorities to field test cassava (marker gene), rice with a marker gene and another rice variety with resistance against a virus (Oja blanca), and a flower *(Stylosanthes guinensis)* with a marker gene and other crops expected to be sown/planted later this year.

Costa Rica formally established a Biosafety Advisory Committee in 1992 (Macaya, 1994) which has reviewed a series of applications that are nevertheless treated as confidential. The Committee rejected two applications in 1993 and is currently receiving several applications. It has not authorized any field trials since the committee was constituted although genetically modified plants did get tested in the field in Costa Rica prior to the Committee's establishment (Table 3).

In **Cuba**, field testing has exclusively been done by the Centro de Ingeneria Genetica y Biotecnologia (CIGB). The center was the first to transform sugarcane (Australia followed later) and this is considered a most important achievement by Cuba. The potato trials with PVX, PVY and PLRV are planned to continue in 1995 and novel products are also expected to go to the field next year. Boniato *(Ipomoea batatas)* has been engineered for resistance to "tetuan" *(Cilas formicarius* var. *elegantulus)* and for the improvement of protein content of the tubercles. Also expected for field release in 1995 in Cuba are potato and tobacco lines with hydrolytic enzymes (e.g. glucanases, AP-20) to confer resistance to fungal infections.

Guatemala has been listed as a country where a squash trial took place in 1989. It appears, however, that the company performing this trial used its long-time winter nursery to multiply squash seed in a controlled greenhouse (net house). This trial has been listed in Table 3 although some people argue that this should not be considered a field trial.

In **Peru**, the Centro Internacional de la Papa (CIP; International Potato Center) has been the subject of many rumors regarding possible field releases in the Andean region, particularly Peru and Bolivia, and in Africa. CIP did conduct a trial in a controlled greenhouse (net house) in Bolivia in 1991 but not in other countries. Currently, applications for field trials are being considered by the Peruvian, Egyptian and Tunisian regulatory authorities and these releases may take place, if permission is granted, in early 1995. The inserted genes are for non-conventional virus resistance (coat protein of PRV, PRX and PRSV), insect resistance and bacterial resistance, although not all of the above genes are present in one single genotype.

CIAT in Columbia and the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT; International Wheat and Maize Improvement Center) in Mexico follow similar patters to CIP. Both centers—as well as certain other institutes of the Consultative Group on International Agricultural Research (CGIAR)—are currently working with transgenic material in the laboratory and confined glasshouse but it is the CGIAR's policy to only test in countries where formal authorization has been obtained. CIMMYT, for example, was seeking a permit—and obtained it—for importing transgenic maize calli for laboratory research (Carreón Zúñiga, 1994).

Table 3: Field Trials in Latin America and the Caribbean[1]

Country/Crop	1987	1988	1989	1991	1992	1993	1994*	Total
Argentina								
Cotton				HT+IR	IR HT+IR	IR		**4**
Maize				IR M	HT IR	A+HT HT+IR Q		**7**
Rapeseed					HT	A+HT HT Q		**4**
Soybean				HT	HT	HT		**3**
Sugarbeet					HT			**1**
Wheat						A+Q+M		**1**
Subtotal				**4**	**7**	**9**		**20**
Belize								
Cotton					IR			**1**
Maize					IR	HT		**2**
Soybean					HT			**1**
Subtotal					**3**	**1**		**4**
Bolivia								
Cotton				HT IR				**2**
Potato				M	Q			**2**
Subtotal				**3**	**1**			**4**
Chile								
Maize					HT Q	IR 2 HT M	2 HT	**8**
Sugarbeet						VR+HT		**1**
Tomato					Q	Q	Q	**3**
Rapeseed	HT							**1**
Subtotal	**1**				**3**	**6**	**3**	**13**
Costa Rica								
Cotton					IR	IR		**2**
Maize					IR			**1**
Soybean				HT	HT			**2**
Subtotal				**1**	**3**	**1**		**5**
Cuba								
Cabbage						IR		**1**
Potato						VR	3 VR	**4**
Rapeseed						M		**1**
Sugarcane						IR	IR	**2**
Tobacco					IR			**1**
Subtotal					**1**	**4**	**4**	**9**

continued...

Country/Crop	1987	1988	1989	1991	1992	1993	1994*	Total
Dominican Republic								
Soybean				HT				**1**
Subtotal				**1**				**1**
Guatemala								
Squash			VR					**1**
Subtotal			**1**					**1**
Mexico								
Maize						FR		**1**
Potato					VR			**1**
Squash						4 VR		**4**
Tobacco							FR	**1**
Tomato		IR		Q	IR 2 Q	IR	IR Q	**8**
Subtotal		**1**		**1**	**4**	**6**	**3**	**15**
Grand Total	**1**	**1**	**1**	**10**	**22**	**27**	**10**	**72**

***** Data to August 1994.

1 A: Agronomic Traits; BR: Bacterial Resistance; FR: Fungal Resistance; HT: Herbicide Tolerance; IP: Industrial Production; IR: Insect Resistance; M: Marker gene(s); NR: Nematode Resistance; Q: Quality characteristics; VR: Virus Resistance.

5.2 Asia and Australasia

In the **developing countries** of Asia, only China and Thailand have tested genetically modified plants (Table 4). In China, the total number of sites varies but is generally very high. The virus resistant tobacco, tomato and potato trials of 1993 and 1994, for example, were tested in 15, 4 and 2 provinces respectively, at a few dozen locations each. Thailand is currently reviewing several applications, among them two separate applications for tomato seed increase with delayed ripening characteristics. Thailand expects to test a papaya ringspot virus (PRSV) resistant papaya later in 1994.

Of the **industrialized countries** of Asia and Australasia, Australia and New Zealand registered the bulk of trials with virus resistance and herbicide tolerance accounting for 25% and 23% of the characters tested (Table 5).

Table 4: Field Trials in Asia (Developing Countries)[1]

Country/Crop	1989	1990	1991	1992	1993	1994*	Total
China							
Pepper (Sweet)					VR	VR	**2**
Potato					VR	VR	**2**
Tobacco[2]	VR VR+VR	VR VR+VR	VR VR+VR	2 VR VR+VR VR+VR	2 VR VR+VR VR+VR VR+IR	3 VR VR+VR VR+VR VR+IR	**21**
Tomato				IR	IR VR+IR	IR VR+IR	**5**
Subtotal	**2**	**2**	**2**	**5**	**9**	**10**	**30**
Thailand							
Tomato					Q	Q	**2**
Subtotal					**1**	**1**	**2**
Grand Total	**2**	**2**	**2**	**5**	**10**	**11**	**32**

* Data to August 1994.

1 A: Agronomic Traits; BR: Bacterial Resistance; FR: Fungal Resistance; HT: Herbicide Tolerance; IP: Industrial Production; IR: Insect Resistance; M: Marker gene(s); NR: Nematode Resistance; Q: Quality characteristics; VR: Virus Resistance.

2 A double construct in tobacco for virus resistance (TMV and CMV) in 1994 occupies around 35 ha for seed multiplication.

5.3 Africa

Little activity has taken place in Africa so far and the only confirmed trials have been undertaken in South Africa and Egypt (Table 6). As stated above, CIP filed field trial applications in Tunisia and Egypt. It is expected, however, that applications for cassava will soon be filed in several countries.

It is noteworthy that much of the information obtained during this study about field trials in Africa appear to be erroneous. First, when the source of the data was questioned on the institution that supposedly carried out the trial(s), this information was either "non-disclosable" or the data did not stand up to scrutiny. Second, some information even referred to field trials with crops where genetic transformation has not been successful at the time when the field trial(s) were claimed to have taken place (e.g. certain species of *Phaseolus).*

Table 5: Field Trials in Asia (Industrialized Countries)[1]

Country/Crop	1988	1989	1990	1991	1992	1993	1994*	Total
Australia								
Apple							M	**1**
Carnation					2 HT+Q		HT+Q	**3**
Chrysanthemum						Q		**1**
Clover							2 HT+Q	**2**
Cotton					IR	2 IR	4 IR HT	**8**
Potato				VR	A	A VR	A VR	**6**
Rapeseed					A			**1**
Sugarcane						M		**1**
Tea Rose							Q	**1**
Tomato					Q	Q		**2**
Subtotal				**1**	**6**	**7**	**12**	**26**
Israel								
Tomato[2]				VR	VR	VR	VR	**4**
Subtotal				**1**	**1**	**1**	**1**	**4**
Japan								
Petunia						VR		**1**
Potato							VR	**1**
Rice						2 VR	Q	**3**
Tobacco				VR			VR	**2**
Tomato				VR				**1**
Subtotal				**2**		**3**	**3**	**8**
New Zealand								
Asparagus	M							**1**
Broccoli			HT					**1**
Kiwi				HT				**1**
Maize						IR		**1**
Potato	HT 2 M	2 HT 2 M	HT VR		VR	VR		**11**
Subtotal	**4**	**4**	**3**	**1**	**1**	**2**		**15**
Grand Total	**4**	**4**	**3**	**5**	**8**	**13**	**16**	**53**

***** Data to August 1994.

1 A: Agronomic Traits; BR: Bacterial Resistance; FR: Fungal Resistance; HT: Herbicide Tolerance; IP: Industrial Production; IR: Insect Resistance; M: Marker gene(s); NR: Nematode Resistance; Q: Quality characteristics; VR: Virus Resistance.

2 1991: Field trial; 1992-1994 in net houses.

Table 6: Field Trials in Africa[1]

Crop	1990	1991	1992	1993	1994*	Total
Egypt						
Potato		M				**1**
Subtotal		**1**				**1**
South Africa						
Rapeseed				HT		**1**
Cotton	HT	HT	IR	2 IR		**5**
Forage (Lucerne)				HT		**1**
Maize				HT		**1**
Strawberry					HT	**1**
Subtotal	**1**	**1**	**1**	**5**	**1**	**9**
Grand Total	**1**	**2**	**1**	**5**	**1**	**10**

* Data to August 1994.
1 HT: Herbicide Tolerance; IR: Insect Resistance; M: Marker.

Conclusions and Outlook

Biotechnology to date has established an excellent safety record and biosafety regulations have served an important function in this regard by requiring from applicants substantial information on organisms for which environmental trials are sought. This process identifies the most likely problematic aspects of tests prior to environmental exposure. For regulators, the initial, small scale tests are easily accommodated. At that stage the emphasis is on safety, which can be achieved through a combination of separation, physical barrier to transference, or sterility.

Many systems, such as the APHIS guidelines of the USA or the Philippine regulations specify four or five levels of isolation based on potential environmental threats. For APHIS, confinement "verifies that the pathogenic potential contained in the construction of the organism or performance of the field test has been removed, or will be contained" (McCammon and Medley 1990). The resultant difficulty is the absence of safety information generated from isolation trials which can be used to structure subsequent, less restrictive ones. That limitation is compounded as products approach the commercialization stage when the multiple trials noted above are required. Isolation for multiple trials is neither feasible nor appropriate.

Initial trial protocols are sufficiently controlled to prevent most large scale problems and this process seems well established and incorporated in legislation in a range of countries. Where the system is not as well

refined is in characterizing the steps from initial, contained trials through to full commercial release. Yet this is the most critical issue for release as that level implies full exposure to the environment. Further conceptualization of this final stage is required to assure protection while not placing unnecessary delays on the adoption of appropriate biotechnology applications that may reduce the need for toxic chemical pesticides and increase production without the need for increased inputs.

Acknowledgments

I would like to extend my gratitude to a host of colleagues around the world—too numerous to mention—for supplying me with data in a short time and for openly sharing with me relevant information. Particular thanks go Peter Kearns, Patricia Ahl Goy, Chen Zhang-Liang, John H. Payne and Maria Antonia Fernandez for helpful discussions and for providing essential information.

References

Ahl Goy, P. and J. Duesing. 1993. Field Releases of Genetically Modified Plants Worldwide, with Special Emphasis on the Activity in Europe. *Plant Industrial Platform Newsletter* 3:18-20.

Ahl Goy, P. and J. Duesing. 1994. GIBiP Database on Field Trials with Genetically Modified Plants. 1. A Review of the Activity worldwide, from 1986 to 1993. *Submitted.*

Ahl Goy, P., E. Chasseray and J. Duesing. 1994. Field Trials of Transgenic Plants: An Overview. *AGRO food INDUSTRY hi tech. In press.*

Carreón Zuñiga, M.A. 1994. Field Trials with Transgenic Plants: The Regulatory History and Current Situation in Mexico. In *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere* (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 207-213.

Chasseray, E. and J. Duesing. 1992. Field Trials of Transgenic Plants: An Overview. *AGRO food INDUSTRY hi-tech* 4:5-10.

Krattiger, A.F. and W.H. Lesser. 1994. Biosafety: An Environmental Impact Assessment Tool and the Role of the Convention on Biological Diversity. In *Widening Perspectives on Biodiversity* (A.F. Krattiger *et al.* eds.). IUCN/International Academy of the Environment: Geneva. pp. 353-366.

Macaya, G. 1994. Towards the Implementation of Biosafety Regulations in Costa Rica. In *Biosafety for Sustainable Agriculture: Sharing*

Biotechnology Regulatory Experiences of the Western Hemisphere (Krattiger, A.F. and A. Rosemarin, eds.). ISAAA: Ithaca & SEI: Stockholm. pp. 199-206.

Maloney, A.P. 1994. The Deliberate Release of Genetically Engineered Organisms: Worldwide Biosafety Regulations and Guidelines. *In press*.

McCammon, S. L. and T. L. Medley. 1990. Certification of field release of genetically engineered plants. In *Molecular and Cellular Biology of the Potato,* M. E. Vayda and W. Park, (eds.). pp. 233-250. CAB International, Oxford.

OECD. 1993. Field Releases of Transgenic Plants, 1986-1992: An Analysis. Organization for Economic Co-Operation and Development (OECD): Paris.

UNEP. 1993. Expert Panels Established to Follow-up on the Convention on Biological Diversity. *Report of Panel IV*, UNEP/Bio/Panels/Int. 4. United Nations Environment Program (UNEP): Nairobi.

Index

A

B

C

D

E

F

G

H

I

L

M

N

O

P

R

S

T

U

V

W-Z