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

Session 3A—Transgenic Crops in the Environment

A Scientific Framework for Assessing Transgenic Organisms in the Environment

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Abstract

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

Background—Implications of Living Modified Organisms for Biodiversity

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

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

Typically, the ecological impacts from LMOs that have so far been most studied have tended to be those felt at the population level, or onsite (table 1), such as the following:

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

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

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

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

Ecological level	On-site	Off-site
Individuals/populations	Decreasing number of studies →	
Communities	↓	↘
Ecosystems		

An objective of the Australian National Biotechnology Strategy (Commonwealth of Australia 2000) is to ensure that the potential risks from the introduction of genetically modified organisms (GMOs) are accurately assessed and are managed effectively. Some of the strategies under this objective are the following :

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

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

The program consists of two **key research areas** (KRAs).

KRA 1—Robust Risk Assessment Tools for LMOs

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

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

KRA2 - Pathfinder Studies for the Risks of New LMOs

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

The case studies will involve the following:

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

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

(See http://www.biodiversity.csiro.au/2nd_level/3rd_level/plan_gmos.htm for more information.)

Table 2: List of some GMO risk assessment models. See Hayes (1997) for a brief comparison of each.

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

Risk Analysis Context

Four Pillars of Risk Analysis

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

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

To these four terms I would add the following:

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

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

Ecological Risk Assessment

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

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

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

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

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

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

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

As ecologists, we must take into account the distribution and abundance of organisms in nature, as well as scale effects, and the possibility that cascade effects and feedback loops will occur. Indeed, the simple model above, useful though it was in highlighting the deficiencies of the Monarch studies, is itself inadequate for ecological risk assessments when we are interested in ecosystem effects from living organisms. The "pollutant" can self-

replicate, and it might affect many possible species, not just humans. One model that attempts to consider these factors is that of the U.S. Environmental Protection Agency (EPA) (US EPA 1992). Others specifically developed for GMO risk assessment are cited in table 2.

Community perceptions of risk

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

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

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

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

Non LMO Introductions as Model Systems

Impacts

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

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

- Cascades in ecosystems—food webs, and so forth amplify or damp effects;
- Scale effects are paramount in ecology—what is true in field plots at 1 ha is unlikely to be true at 10^4 km^2;
- Lag phases—It may take 150 years for trees, for example, to become invasive (Kowarik 1995);
- Base-rate effect (see section on Decision theory).

Because technologies are not always taken up and used successfully, the rate at which introduced organisms become useful is also probably quite small. Therefore, predictability of both harm and benefit is generally low.

Are LMOs Less Predictable than Non-LMOs?

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

*Organism * "similar" environment elsewhere → organism * Australian environment.*

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

*Parent organism * Australian environment → novel organism * Australian environment*

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

*Parent organism * "similar" environment elsewhere → novel organism * Australian environment.*

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

Decision Theory

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

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

This simple but powerful analysis deserves further exploration. It may turn out to be too simplistic, but it certainly suggests aspects of the risk–benefit research agenda. **We should**

be carrying out economic evaluations of the costs of different types of pests and weeds and the benefits of differing types of agricultural animals and plants, conducting metaanalyses of the rate at which different kinds of organisms become pests or weeds, and aiming to increase the accuracy of our predictions of harm. Note that the estimate of harm could also embody social perceptions (outrage, etc,) and policy initiatives such as the Precautionary Principle.

Concluding Remarks

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

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Appendix—Points for Discussion

The points below emerged during Session 3 discussions.

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

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

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

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

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

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

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

- Global food politics—Are GMOs the way to feed the world? On the one hand, losses to pests, weeds, and diseases are huge across the developing world. On the other hand, hunger in the developing world is rarely simply a consequence of food shortage but more of inequalities of wealth and food distribution.
- What constitutes an adverse effect? Even biodiversity scientists might consider the loss of individuals of one species acceptable because of concomitant gains for other

species elsewhere, but such a rationalization might not be acceptable in the wider community.

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

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

- For some countries, such as the United Kingdom, so much of the landscape is agricultural that the agricultural landscape *is* the natural environment. In Australia, by contrast, large swathes of the landmass are under conservation. For example, World Heritage-listed Kakadu National Park in northern Australia is roughly the size of Israel.

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

Session 3A: Transgenic Crops in the Environment

The Farm-Scale Evaluations of Herbicide-Tolerant Geneticially-Modified Crops in Great Britain

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Abstract

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

Introduction

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

To test these possible effects on biodiversity, the Farm-Scale Evaluations were established (Firbank et al. 1999). This study began in April 1999 and was immediately one of the most controversial agroecological studies ever undertaken because of the background of public concern about genetic modification (Krebs 2000). The study has become the focus of intense media attention and public debate as well as the target for direct action by groups opposed to growing GM crops. The last field season begins in 2002, and the first results due to be reported in 2003.

The study is designed to assess the effects of the agricultural management of field-scale releases of GMHT maize, beets, and winter and spring oilseed rapes (canola) on farmland wildlife abundance and diversity in Great Britain. The project is therefore concerned primarily with comparing the indirect effects of managing GMHT and non-GMHT crops on species diversity, abundance, and trophic relationships (Walker and Lonsdale 2000); it does not constitute a complete environmental risk assessment. The project does not focus on any effects of these specific crops on biodiversity arising from gene flow in Great Britain, as has already been addressed by a growing body of research (e.g. ACRE 1999), although gene-flow monitoring is taking place at the field sites.

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

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

Project Design

Selecting Biodiversity Indicators

It is impossible to assess the range of biological variation in all living creatures in and around GMHT crops. Therefore, indicators are required to represent larger groups of organisms and to elucidate processes that may lead to significant ecological shifts not detectable directly given the time and spatial scales available for the study. The experiment focuses on the effects of weed management on weed populations and hence on higher trophic levels. We assume that the major ecological effects of GMHT crops result from the direct and indirect effects of the different herbicide regimes on the arable weeds (Firbank et al. 1999; see also Watkinson et al. 2000). The regimes differ in timing and specificity; the herbicides glyphosate and glufosinate ammonium are broad spectrum and can be applied later in the development of tolerant crops than can those herbicides applied to nontolerant crops. The potential advantages to the farmer are the simplification of weed management (because the timing is less critical, and the number of applications required may be reduced) and the option of an additional method of bringing more severe weed infestations under control (Firbank and Forcella 2000). The weeds are important for farmland biodiversity, partly in their own right (Firbank 1999), and partly for their contributions to food resources, cover, and microclimate for other organisms (Potts 1997). The indicators of these weed populations must be sensitive to the differences in weed management and be capable of providing data that can be related to resources for higher trophic levels. These include data on the weed seedbank, seedlings (before and after postemergence herbicide application), adult plants, seed set, and dissemination. The biomass of mature arable plants is also recorded, for this is considered a potential measure of food resources available to animals within the crop towards the end of the season.

To quantify effects on different trophic levels, a broad range of invertebrate groups must be sampled at a variety of habitats in the field, including the soil surface, on the weeds, and on crop plants. The sampled taxa include carabids, collembola, and other soil-surface arthropods; arthropods on vegetation; gastropods; and crop pests. Birds, small mammals, and some insects involved in the food webs have territories and foraging areas that are too large for changes in populations to be detected readily at the scale of the experiment. Bees and butterflies are being monitored, but this work quantifies foraging behavior rather more than effects on populations. In general, potential effects on wideranging species will have to be inferred from changes further down the food webs, using data on biomass as well as abundance of species (see Watkinson et al. 2000).

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

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

Experimental Design

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

The pilot trials included halved and paired field sites, and from these it was concluded that halved fields were preferable as the experimental unit largely because of the reduced variability between treatments. The fields are split to try to keep biodiversity resources as similar as possible between the two halves (e.g., both halves should have roughly the same

amount of hedgerow or woodland adjacent to them). The allocation of GM crop to field halves is strictly at random and cannot be influenced by the farmer or field surveyor. The number of blocks corresponds to the number of sites at which the crop is grown.

Choice of Study Sites

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

Crop Management

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

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

Program of Field Sampling

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

Table 1. Summary of the Field-Assessment Program

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

Statistical Analysis of the Data

At its simplest, the analysis consists of a statistical test for each biodiversity indicator assessment for each crop with a wide range of potential covariates, including location, crop growth stage, year, and management variables. The tests are paired, because we are looking at the differences between the two halves of the field, and are two-tailed in as much as we are looking for both increases and decreases for biodiversity indicators on the GMHT crops. These tests will need further interpretation, however, because both positive and negative results may occur by chance. We therefore need to distinguish between results that represent signals of ecological processes and random patterns of significant and nonsignificant results.

Such work will require an understanding of the ecological system as a whole, which may be expressed at different levels of complexity from a decision tree to a formal mathematical model of the dynamics of the ecological components of the system.

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

The Division of Responsibilities within the Project

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

The Role of Government

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

The Scientific Steering Committee

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

The Project Consortium

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

SCIMAC

The GM seeds are supplied by companies under the umbrella organization SCIMAC (Supply Chain Initiative for Modified Agricultural Crops), which is legally responsible for ensuring that the crops are grown within the regulations. Farmers apply to SCIMAC to take part in the project, but their acceptance is up to the research team, which has to provide an

adequate sample for approval by the SSC. The farmer is then contracted by SCIMAC to grow the crop. Although SCIMAC provides the seeds and can give some advice concerning crop management, this can only be done for the herbicide regime of the GMHT crop (this is appropriate because so few farmers or advisers have experience of growing these crops). This advice is audited. The SCIMAC is also responsible for appropriate disposal of the crop and has also undertaken a 3-year voluntary agreement with Government not to plant GM crops commercially to allow time for the FSE study.

The Farmer

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

Discussion

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

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

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

The FSE study has other important lessons for studies of living modified organisms (LMOs) in general. The first is the importance of partnership between Government, scientists, the biotechnological industry, and the farmers, but this partnership must be created in ways that are transparent to the general public so that they can have confidence in the quality and integrity of the results. The second is that the study of the impacts of LMOs need to take into account the behavior of people; indeed, the role of people deciding how LMOs are taken up and managed may prove the most important variable in many risk assessments. Moreover, no single study can, or should, be expected to provide all the answers concerning risks and benefits of LMOs.

Finally, important limits to our knowledge remain that restrict our ability to forecast the ecological effects of LMOs, or indeed of many other forms of ecosystem disturbance. Thus, work on LMO risk assessment will help inform fundamental research just as fundamental research will help inform risk assessments. However, this can only be done if the results of the many experiments and surveys around the world can feed into metadatabases and ideally have some common structure to allow effective data mining.

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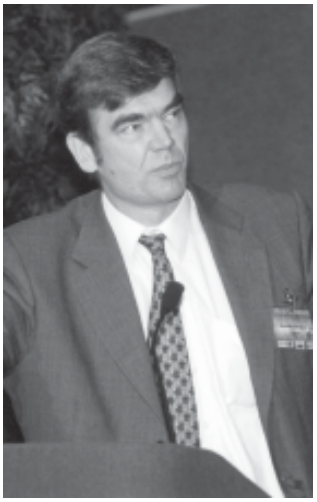
Monitoring Case Report: Impact of Transgenic Plants Within Cropping Systems



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Abstract

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

Introduction

After about 15 years of biotechnology research carried out by public research teams as well as by private companies, the first marketing releases of living modified organisms (LMOs) occurred in North America in 1995, and they are now planted on a significant part of the arable land. Meanwhile, Europe has strictly limited the commercial releases of LMOs and, apart from Spain, where Bt corn has been cultivated to some extent in 1998, only cultivation for experimental purposes is, in practice, carried out. A moratorium has been decided in different European countries and, owing to environmental and food safety concerns, new regulation rules are reinforcing the premarketing evaluation and traceability of novel products.

More generally, with the development of LMOs, new concerns have been raised and, even if most of them are not specific to recombinant DNA techniques, LMOs are now at the heart of major debates and processes as manifested by the following:

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

Towards a More Systemic Approach

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

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

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

- Evaluating the impact of different genetically modified (GM) crops and different traits within cropping systems,
- Assessing cumulative and long-term effects of GM cropping systems,
- Detecting potential and unexpected adverse effects,

- Constructing crop management guidelines for farmers, and
- Providing regulatory bodies with a framework and tools for postmarketing monitoring—the so-called biovigilance or biosurveillance.

Different methods for such a systemic analysis

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

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

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

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

A multicrop and multiyear study for a systemic analysis

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

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

2. European corn borer tolerance (using the Bt system) for corn.

A 500-m area around the field where LMOs are cultivated is monitored to assess the spatial and temporal impact of transgenic crops. This multiyear experiment aims at

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

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

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

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

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

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

A Tentative Cost–benefit Analysis for Herbicide-Tolerant Rapeseed

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

Weed control

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

From current weed control practices observed in France, it has been estimated that the direct costs for weed control could be reduced by an average 30 percent (from 75 euros/ha to 52 euros/ha). However, this estimate does not take into account either the cost of the technology (seed costs and fees) or additional costs for controlling tolerant volunteers in the

rotation and for other specific management measures. Again with these additional measures excluded from consideration, the amount of active measures used for weed control would decrease from 20 up to 85 percent according to the situation.

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

Volunteers

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

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

Seed Dispersal Outside Fields

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

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

Crop to Crop Gene Flow Between Fields

Dispersion of pollen by wind and insects was mainly studied in continuous field conditions, both in specific experiments and in the platforms previously described. Results show that most of the pollen released remains within several meters of the emitting plant. At 30 m, less than 1 seed out of 100 bears this trait. At 120 m, less than 5 seeds out of 1,000 bear this trait.

Observations of dispersal over long distances (between 400 and 1,000 m) indicate that the dispersion of pollen becomes highly irregular but that tolerant seeds can be detected in most of the commercial fields that have been surveyed (from 0 to 1 seed out of 1,000 bearing this trait). Rates depend on the size of the emitting and receiving fields as well as the pattern of the landscape. No distance could be determined beyond which there would be no dispersion of pollen at all.

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

Crop to Wild Relatives Gene Flow

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

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

Constructing Crop Management Guidelines

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

Undesirable effects related to gene flow result mainly in **agronomic** considerations (persistence of resistant volunteers, creation of new weeds, multiple resistance) and **in commercial** considerations (unintended presence of LMOs in conventional rapeseed production affecting the plant's competitiveness in the marketplace).

Many results are now available for these concerns, but more research is required on the impact of larger field size for pollen dispersal and on gene flow from the crop to wild relatives under natural conditions.

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

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

2. The avoidance of selection pressure on these undesirable plants.

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

1. Favor the immediate emergence of seeds remaining on the soil after harvesting in order to withdraw them from the seed bank: no tillage until the first rain and then repeated minimum soil tillage to avoid seed dormancy (Lutman and Sweet 2000).

2. Increase control of rapeseed volunteers within the subsequent crops. The requirement is not only made to avoid competitiveness of weeds but also to reduce the seed bank.

3. Avoid other crops resistant to the same herbicide within the rotation to make the control of tolerant volunteers easier in the subsequent crops.

4. Organize the spatial location of crops through adequate isolation distances, through regional specialization, or both.

5. Dedicate the use of broad-spectrum herbicides with their active material used alone (glyphosate or glufosinate) to tolerant crops and associate another active material with these for their nonselective uses (preharvest applications, fallow land management).

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

Tools for Postmarketing Monitoring

Even if the premarketing evaluation process must be improved by taking into account systemic effects on the environment, it will never ensure that no unintended event

will occur. A postmarketing monitoring system (or "biovigilance") must be implemented in order to

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

2. Detect as soon as possible the unintended or unexpected effects that have not been identified during the premarketing process

The general framework for such a monitoring system is widely agreed upon, but the precise threshold between the premarketing process and the postrelease monitoring system is one major issue on which countries certainly diverge. But, in fact, the premarketing evaluation, mitigation measures, and postmarketing monitoring are parts of a continuous process aiming at ensuring an efficient and sustainable development of new technologies.

Conclusions

Results from various risk assessment studies suggest that there is no direct major ecological risk in the case of herbicide-resistant oilseed rape, because the presence of a gene for tolerance to an herbicide in oilseed rape (or a related species) does not appear to increase its fitness in natural ecosystems (CGB 2000). However, various agronomic and commercial concerns have been raised, and specific crop management guidelines are required. Mitigation measures have been defined and are being evaluated in terms of efficacy to keep under control the unexpected or undesirable events and in terms of feasibility and acceptability.

Furthermore, even if North American experience provides us with data that, after evaluation, may be of equal value for European agriculture and these data are implemented to some extent, large-scale experiments are required under European agro-ecosystems to assess the effect of scaling-up as well as to establish an overall environmental balance, to design guidelines for crop management, and to build methods and tools for monitoring. Furthermore, a global consideration has to be given to the impacts of large-scale use of those two nonselective herbicides whose resistance is being introduced in various crops.

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Fitness Effects and Importance of Baselines—The Sugar Beet Example of Virus-Resistant Traits

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Abstract

Biosafety studies typically show no difference in hybridization between genetically modified plants (GMPs) or non-GMPs with related wild species. Because risk is a product of both exposure and hazard, biosafety research should clearly not only target gene-flow exposure but specifically concentrate on expected hazards emerging from successful transgene flow to wild relatives of GMPs. The conventional sugar beet (*Beta vulgaris* ssp. *vulgaris*) has now been cultivated for 200 years. This cultivar has not shown unwanted ecological effects despite the introduction and spread of this European species to the New World. The only realistic way of assessing the environmental effect of transgenic beets is a comparison with classically bred cultivars. In particular, we compared the ecological performance of rhizomania-resistant genotypes under various environmental conditions with regard to parameters such as winter hardiness, seed production, and ecological relevance of virus resistance.

Introduction

The number of biosafety-related publications concerning transgenic organisms has increased within a decade (1990 to 2002) more than 3,700 citations according to one of the most comprehensive databases (http://www.icgeb.trieste.it/~bsafesrv/). Because risk is a product of both exposure and hazard (Sharples 1991), it is clear that biosafety research on environmental effects should not only target the probability of gene flow but must also focus on the consequences (and potential hazards) of successful transgene flow to relatives of transgenic crops. This means that biosafety research should address the phenotype (especially the fitness phenotype) of the transgenic hybrid versus that of nontransgenic controls.

In their outstanding review Ellstrand et al. (1999) demonstrated that 12 of the 13 most important crops worldwide hybridize with wild relatives somewhere within their cultivation area. These events could be defined as baselines in the sense of evolutionary references for crops with transgenes. However, with the exception of worst-case laboratory studies, no adverse effect has been reported for transgenic plants so far; but hazard is based on anthropocentric assessment and value judgement

that is very often a sociological–political compromise made by different stakeholders. Scientific biosafety research can therefore not answer a question such as Can we observe an unwanted spread of transgenes? Instead, biosafety research has a better chance of providing answers to questions such as Is there a difference in the spread and environmental effect of transgenic in comparison conventional plants?

The definition of "unwanted" is a political one, and nature conservation has more to do with public perception than with scientific observation. Today, unwanted ecological effects are commonly manifested by the decline of rare species or loss of genetic diversity. These phenomena are not new for they are a well-known aspect of human activity that has increased within agricultural practice over the last 10,000 years. Today, the situation requires a case-by-case and step-by-step assessment of GMPs. Here, we present the example of transgenic sugar beets resistant to rhizomania virus.

Conventional sugar beet (*Beta vulgaris* ssp. *vulgaris* L.) has now been cultivated for 200 years. This cultivar has not shown unwanted ecological effects despite the introduction and spread of this European species to the New World (Bartsch and Ellstrand 1999). The only realistic way of assessing the environmental effect of transgenic beets is a comparison with classically bred cultivars. In particular, we compared the ecological performance of rhizomania-resistant genotypes under various environmental conditions with regard to parameters such as competitiveness, winter hardiness, and seed production. Our results are summarized below.

Results

Winter Hardiness

The biennial sugar beet needs to survive cold winter temperatures in order to produce offspring. Winter hardiness is an important ecological factor for the geographical distribution of cultivated and wild beets in Europe. The natural distribution range is limited to mild areas at the seacoast in the Northern Hemisphere. Some of our experiments focused on overwintering of transgenic and nontransgenic sugar beets at different locations in Europe experiencing mild to cold winters in the years 1994–99. We found no survival differences even under virus infestation conditions (Pohl-Orf et al. 1999; fig. 1A).

Sexual Reproduction

Transgenic attributes are transmitted by natural reproduction and by gene flow to all sexually compatible relatives. One prerequisite is sympatric growth of cultivars and their hybridization partners. Only a few plants can cross with the sugar beet: the Swiss chard, fodder beet, table beet, and wild *Beta* species belonging to the Section Vulgaris. No difference was found in the hybridization ability of transgenic in comparison with non-transgenic controls (Bartsch and Pohl-Orf 1996, Dietz-Pfeilstetter and Kirchner 1998). No significant differences among the three plant genotypes were found at a given virus infestation level in terms of seed production (fig. 1B).

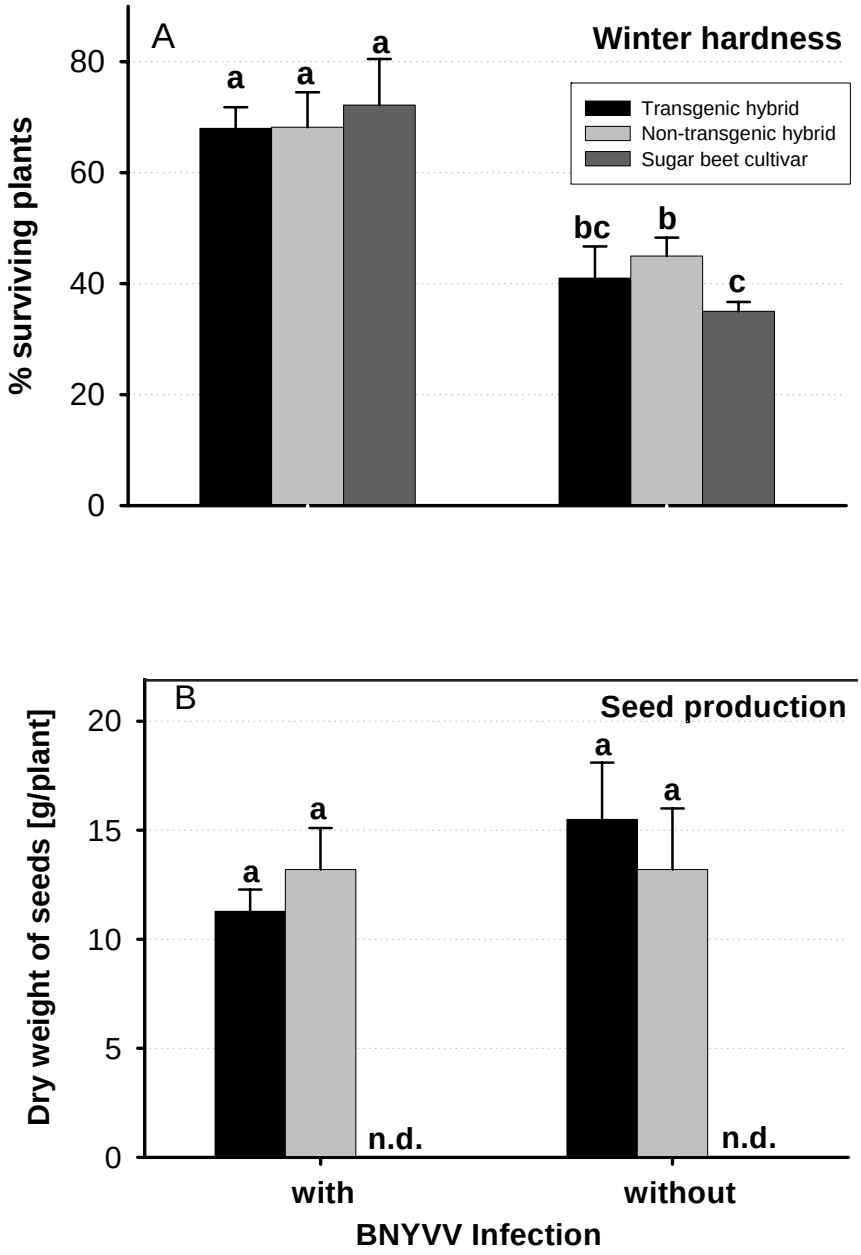


Fig. 1: Ecological parameters (A) winter hardness and (B) seed production of two wild beet hybrids and sugar beet cultivar grown with or without virus infection. Mean levels of characters with the same indicator letter are not significantly different by 2-way Anova /Tukey Test. Mean and Standard Error are given, n.d. = not detected)

Development of Weediness Due to Early Bolting

Weeds are simply plants in the wrong place in either agricultural or nature conservation areas. Interestingly, the same species can be protected as a plant genetic resource in one country and eradicated as a weed in another.

Beet seed bolters pose problems for mechanical harvest machinery and reduce yields; therefore they are regarded as weeds in sugar beet fields. In contrast early bolting and seed production in the first vegetation period is an important attribute for the ecological distribution of beets, because freezing temperatures can be better tolerated by seed in the Northern Hemisphere. In addition, the development of an annual habit is also important for the weediness of beets in disturbed habitats such as agricultural fields.

The unwanted annual habit can evolve in two ways: random introgression of genetically dominant genes from wild beets or selective reevolution towards wild characters (genetic drawback). The latter phenomenon was targeted by one of our field experiments. We found that the transgenic genotype had a much "safer" performance, owing to its higher resistance to early prebolting, than the isogenic control (Bartsch et al. 2001). Because the physiological background is still unknown, this pleiotropic effect should be carefully considered and cannot be related to transformation events *per se*.

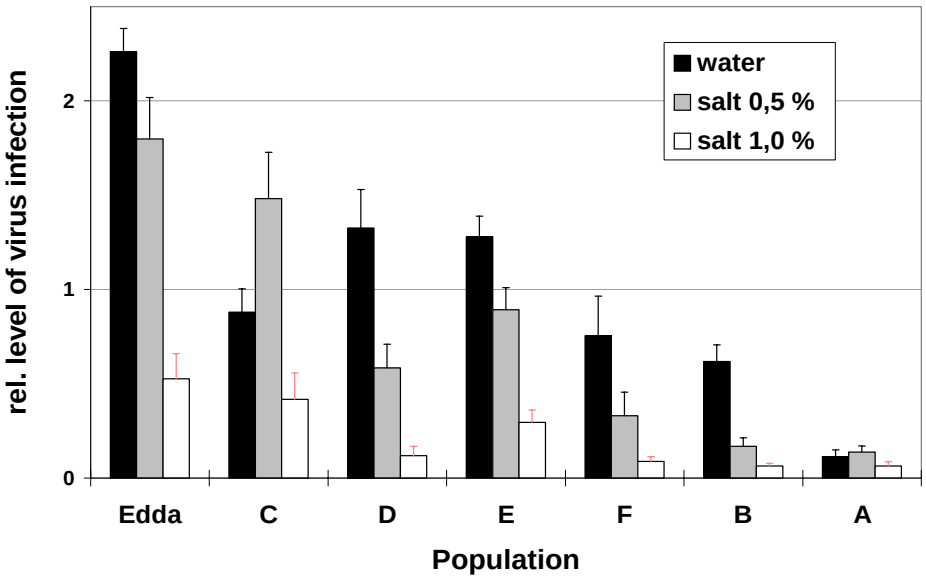
Ecological Relevance of Virus Resistance

If virus resistance is ecologically significant, we should detect rhizomania virus in sea beets. We checked 40 populations of sea beet and could not detect virus infested plants. Therefore, there is no ecological advantage for transgenic hybrids in sea beet habitats. The reason for this is that the habitat of sea beets is found in coastal areas. There the plants are regularly exposed to showers of salty water and temporary flooding. Indeed, the level of virus infection is influenced by salt concentration. In our experiment we irrigated a highly susceptible sugar beet variety and sea beets from six different populations from Italy with water (both 0.5 percent and 1 percent salt solutions). Plants were grown in virus-contaminated soil. Our results showed that in every population the level of virus infection depended upon salt concentration. With increasing salt concentration virus infection decreased. But this picture is not consistent, population C was more susceptible when irrigated with 0.5 percent solution than with the 1 percent solution, and population A showed no susceptibility to rhizomania virus infection (fig.2) most likely due to an inherent natural resistance.

Discussion

Generally, the ecological behavior of transgenic sugar beet plants is similar to that of non-GMPs if the modified trait confers a neutral advantage under environmental or experimental conditions. However, GMPs perform better than non-GMPs if the new phenotype is challenged by conditions ecologically advantageous for the modified trait. So far, we have no evidence that the use of GMPs has an adverse impact on sustainable agriculture and nature conservation *per se.*

Fig. 2: Relative level of virus infection depending on salt concentration of sugar beet variety Edda and six different wild beet populations from Italy. Mean and Standard Error are given.



There is still a gap in basic long-term knowledge about how conventional pest management strategies influence nontarget species in agricultural systems. We also have little knowledge of how past gene flow from cultivars may have influenced the genetic diversity of related wild species (Bartsch et al. 1999). Biosafety research and monitoring may become the driving force for comprehensive studies encompassing traditional and modern agricultural systems. Overall, new extensive field studies support the view that harmful effects on nontarget organisms in the laboratory are rarely detected in the environment—at least not so far (Bartsch and Schuphan 2002). Biosafety studies have typically demonstrated that there is no difference in the hybridization ability of GMPs compared with non-GMPs with crossbreeding wild populations. Indeed, numerous reports describe cultivars that have escaped into natural ecosystems (Bartsch and Ellstrand 1999). In this respect transgenic plants will be no exception, and we have no evidence that the use of GMPs is contrary to sustainable agriculture and nature conservation *per se*. No field study has reported a severe effect caused directly by transgenic plants, but this could be based on the poor ecological relevance of traits such as herbicide, virus, and insect resistance (Saeglitz and Bartsch 2002). In the future, more significant implications may arise with the introduction of ecologically more important traits such as drought and salt tolerance. Because biosafety research is a time-consuming and resource-intensive process, we will have to concentrate on well-thought-out and thorough experiments as well as on targeting the ecologically "riskier" organisms.

Biosafety research cannot solve every open and basic question of general ecology (Kareiva et al. 1997). After the best pragmatic use of the case–by–case and step–by–step approach, a well-designed monitoring program is necessary following commercialization. This monitoring must prove, on a larger scale, the prognostic assumptions made by former biosafety research and assessment (Marvier et al. 1999). We know for certain that containment strategies do not work properly and provide no justification to avoid monitoring (Sukopp and Sukopp 1993;

Saeglitz et al. 2000). Monitoring must be flexible enough to recognize unpredictable phenomena such as pleiotropic effects. Currently, we have no evidence that transgenic plants systematically express more pleiotropic effects than plants from classical breeding programs (Bartsch and Schuphan 2002).

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Nontarget Food Chain Effects and the "GMO-Guidelines Project"

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Abstract

Current biosafety procedures are inadequate to test the impacts of transgenic crops on their environment. For instance, current U.S. models rely on pesticide methodologies and consider novel transgenic products, such as the Bt toxin, as equivalent to the toxin when used as a pesticide. However, toxin exposure for prey and their natural enemies from transgenic plants is very different from pesticide exposure as shown by the effects of Bt toxin on *Chrysoperla carnea* (the green lacewing) when it ingests the toxin via Bt maize fed to prey species rather than an artificial Bt-containing diet. The example illustrates the need for new biosafety methodologies that go further than pesticide-based approaches. This paper launches an international initiative of public sector scientists that aims to develop comprehensive scientific guidelines for prerelease biosafety testing of transgenic plants. Public sector scientists from developing and developed countries are invited to join the group and participate in this unique effort.

Introduction

Currently, commercially produced transgenic plants are mainly cultivars of corn, cotton, soybeans, and canola that are tolerant to herbicides, insect resistant, or both (James 2002). Insect resistance is mostly due to a novel, highly bioactive compound, the *Bacillus thuringiensis* (Bt) toxin. This toxin is produced in high concentrations throughout most parts of the transgenic plant and throughout most of the growing season. Consequently, most if not all organisms feeding and living on these transgenic plants are likely to be exposed to the expressed novel toxin for a prolonged period, potentially leading to so-called nontarget effects. Nontarget effects are any unintended side effect of the transgenic plant on organisms other than the target pest species. These include detritivorous organisms, pollinators, other herbivores, and higher trophic level organisms such as insect natural enemies. Nontarget effects are well known from the use of pesticides. These have led to serious agroecological problems (e.g., the development of pest resistance and disruption of naturally occurring regulation mechanisms, leading to secondary pests and pest resurgences) and have resulted in an ever increasing dependency on synthetic chemicals in industrial agriculture. On the basis of this experience, nontarget effects of novel insecticidal transgenic plants need to be carefully investigated.

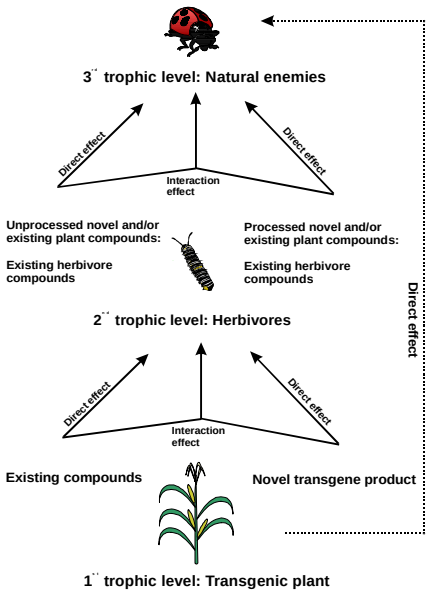
The constitutive expression of an insecticidal gene, such as the Bt toxin product, can be expected to have highly complex impacts on the insect community associated with the introgressed transgenic plant in the region where it is grown such as changes in community composition and structure (fig. 1) (Obrycki et al. 2001, Hilbeck 2001, 2002). Although Bt toxins are typically most effective against the larval stages of particular herbivore species, they may have subtle sublethal effects on other herbivores. Ecologically, these can be as disruptive for population dynamic processes and trophic interactions as lethal effects.

Natural enemies will be exposed to novel plant compounds in transgenic plants predominantly via their prey or hosts, although some natural enemies also feed to a limited degree directly on the host plants as a source of water and certain additional nutrients. Thus, our major concern is so-called tritrophic effects. These are effects which the first trophic level (the host plant, here the transgenic plant) exerts on the third trophic level (the natural enemies of the plant-feeding herbivores) mediated via the second trophic level (herbivores feeding on the transgenic plant). Tritrophic interactions are more than the sum of two bitrophic interactions, that is, host plant–herbivore and herbivore–natural enemy interactions (Kareiva and Sahakian 1990, Malcolm 1992). Although the primary concern in agricultural ecosystems is that such tritrophic effects may offset the benefits from biological control if natural enemies are adversely affected by the Bt toxin (or any other insecticidal compound for that matter), an additional concern in unmanaged natural ecosystems is a decline in biodiversity due to adversely affected insect species at whatever trophic level.

Exposure of, and impact on, natural enemies could be highly complicated and composed of several overlaying factors, as outlined in figure 1. When herbivores ingest the novel insecticidal compound, natural enemies can be affected in various ways as follows:

1. The insecticidal compound, or any metabolite of it, may affect the natural enemy directly;
2. The insecticidal compound, or any metabolite of it, exerts an interaction effect in concert with other secondary or primary compound(s) of the plant (e.g. 2,4-dihydroxy-7-methoxy-(2H)-1,4-benoxazin-3(4H)-one (DIMBOA) in corn);
3. The insecticidal compound affects the nutritional quality of the sublethally affected prey or host herbivore and thus affects the natural enemy indirectly;
4. The natural enemy may be affected by any combination or all of the above. It will be very difficult to distinguish between these different levels of impact, and the limited resources for research in this field may simply render it unfeasible.

Figure 1. Outline of potential food chain effects of transgenic, insecticidal plants.



Current Biosafety Testing Procedures

Current prerelease biosafety testing procedures draw heavily on the testing protocols for pesticides, that is, short-term testing of a few indicator species for acute, direct (bitrophic) effects. In the U.S., an additive two-part model for regulating transgenic Bt plants is applied consisting of the conventional crop plant and the Bt-toxin. The conventional crop plant is considered safe, and consequently no additional testing is required; thus the "added" expressed Bt toxin is considered simply as a pesticide outside its plant context and tested as such. This means that prerelease, nontarget testing is conducted with either microbially produced purified toxins or with highly processed, lyophilized, ground-plant protein typically in a bitrophic experimental setup (i.e., the toxins are administered directly to the natural enemy and not via a prey or host species).

This pesticide paradigm is deficient in several aspects. First, pesticide release is controlled by the applicator, who determines timing, point location, concentration, frequency, and much more. Spray coverage of the crop plant is rarely ever complete, and thus unsprayed refuges remain where nontarget and target organisms can survive. Second, pesticide degradation begins immediately after application. And third, the mode of action for most synthetic pesticides is typically acute and immediate also for nontarget organisms. In contrast, transgenic Bt-plants release the Bt toxin continuously and in almost all plant parts. The tissue-specific toxin production varies over time and in different environments, and the mode of action is not immediate (it takes 2 days or longer before even the target pest dies) and not necessarily acute. Sublethal, chronic effects become more important for nontarget organisms. The resulting dynamics and types of nontarget effects therefore differ from those caused by pesticides.

These deficiencies of the additive concept for prerelease testing and environmental impact assessment can lead to under- or overestimation of the real impact. Any position, pleiotropic, or epistatic effects as well as any interaction effects are also ignored. An illustrative case example is the impact of Bt proteins and transgenic Bt-corn on *Chrysoperla carnea* (1998 a,b; 1999; for an overview see Hilbeck 2002).

The *Chrysoperla carnea* Example

The effects of transgenic Bt-expressing maize and microbially produced Bt-proteins on an important, very polyphagous natural enemy species, *Chrysoperla carnea* (the green lacewing), were studied in a tri- and bitrophic model system approach. Three series of no-choice experiments were carried out using different Bt-delivery systems, transgenic Bt-maize, and Bt-incorporated diets. Prey-mediated effects of Bt-containing diets for herbivorous prey and direct effects of a Bt toxin on *C. carnea* larvae were investigated. The results of all three series of experiments consistently demonstrated the susceptibility of immature *C. carnea* to Bt proteins (Cry1Ab toxin and protoxin, Cry2A protoxin) either provided via prey or directly (Hilbeck et al. 1998a, b; Hilbeck et al. 1999). The degree of mortality varied depending on the Bt-delivery system, and an increase in toxicity of the Bt protein through the food chain was observed. Prey-mediated mortality of immature *C. carnea* was highest when the prey food source was transgenic Bt-maize (59-66 percent) relative to the concentration of the Bt toxin Cry1Ab, which was the lowest in plants (<5 ug/g fresh weight [Fearing et al. 1997]) compared with all other concentrations in the other diets. When feeding the Bt toxin (100 mg Cry1Ab/mL artificial diet) directly to chrysopid larvae at a concentration approximately 10–20–fold higher than in the transgenic plants, the induced mean total immature mortality of *C. carnea* was significantly higher than in the respective control but lower than expected, and similar to the prey-mediated mortality induced by transgenic Bt maize expressing lower Bt concentrations. But when the comparable Bt toxin concentration was incorporated into a meridic diet (100 mg Cry1Ab toxin/g meridic diet) and provided via lepidopteran prey to *C. carnea*, total immature mortality of *C. carnea* was 21 percent higher (78 percent) than when feeding this concentration directly to *C. carnea* larvae (57 percent). At this high concentration, *C. carnea* mortality may also have been confounded by increased intoxication of *Spodoptera littoralis* (42 percent) that was observed at that concentration only. But similar effects were observed when incorporating Cry1Ab toxin at lower concentrations (50 and 25 g into meridic diet), where *Spodoptera littoralis* was not lethally affected by these Bt concentrations. However, they did exhibit a sublethal effect: stunting of growth (for more details see Hilbeck et al. 1999).

Also Bt protoxin-incorporated diets (Cry1Ab and Cry2A) caused significantly higher prey-mediated mortality in immature *C. carnea* than in the untreated control, although to a lower degree than the Cry1Ab-toxin-incorporated diet. *S. littoralis* was not lethally affected by the protoxins, regardless of the concentrations applied, but exhibited similar sub lethal effects as in the toxin-treatment. Further, when comparing control mortalities of all studies, prey-mediated *C. carnea* mortality in the trials using transgenic Bt maize plants was approximately 11 percent higher (37 percent for both *S. littoralis* and *Ostrinia nubilalis* fed predator larvae) than when using the meridic diet (26 percent), suggesting that plant-fed prey larvae were less suitable for optimal nutrition of immature *C. carnea* than meridic diet-fed prey.

The need for new biosafety methodologies to accommodate the differences between transgenic plants and pesticides and that consider the novelty of the technology is evident. In the following section, a new initiative of an international group of public sector scientists aiming to develop such new guidelines will be presented.

The GMO Guidelines Project of the IOBC Global Working Group

The guidelines project "Development of International Scientific Biosafety Testing Guidelines for Transgenic Plants" is an international initiative of public sector scientists organized within a global working group on "Transgenic Organisms in Integrated Pest Management and Biological Control" under the umbrella of the International Organization of Biological Control (IOBC). This project is funded by the Swiss Agency for Development and Cooperation.

The project aims to accomplish the following:

1. Develop comprehensive, transparent scientific guidelines for prerelease biosafety testing of transgenic plants such as could serve as an international standard.
2. Facilitate the development of scientific capacity in the contributing countries that can guide the implementation of the guidelines.
3. Test the application of the guidelines in real policy contexts to assist in the evaluation of particular transgenic crops.
4. Publish the guidelines and periodically revise them in response to new developments, thereby keeping them up-to-date and providing for their long-time use.
5. Extend the guidelines for possible use in postrelease monitoring.

The guidelines will give a series of questions and corresponding methodologies by which any particular genetically modified organisms (GMO) issue can be evaluated scientifically. The questions will start by addressing broader issues and will become progressively more specific and be structured as a series of interlinking modules. The guidelines will have no regulatory legitimacy themselves, but regulatory authorities can choose to implement parts or all of the guidelines as they desire or need with confidence in the scientific soundness behind the evaluations. The guidelines will also be tested in real policy contexts using case studies from countries in Africa, Asia, and South America. This will take place in 3 workshops over the next 2 years.

The development of the guidelines will be an open process that incorporates scientific and technical capacity building and communication between scientists and policy-makers in developed and developing countries. The project is coordinated by a steering committee of public scientists and invites contributions from public sector scientists from all countries, who will form the core group of the project. Figure 2 illustrates the organization of the first year of the project. An advisory committee comprising of representatives from various international and national organizations will accompany the process to critique the drafts constructively and advise on their improvement.

Scientific Approach to the Guidelines

The scientific work is divided into five sections: needs analysis or good agricultural practices, transgenic plant characterization, nontarget and biodiversity effects, pest-resistance management, and gene flow and its effects (figure 1).

Needs Analysis/Good Agricultural Practices

This section sets the context for the rest of the analysis. It will provide a framework for evaluating the need for the transgenic plant in specific crop production contexts. This includes providing an approach to evaluating projected changes in crop production practices such as tillage systems or insecticide use.

Transgenic Plant Characterization

This section will assess (1) how a transgene should be described to enable evaluation of its stability and inheritance; and (2) how the phenotypic effects of the transgene in the plant should be specified to facilitate assessment and management of environmental effects (what, how, what plant parts, and when product concentrations should be measured in transgenic plants).

Typically, transgenes are comprised of integrating elements, a marker gene and its promoter, a target gene and its promoter, and possibly other genetic elements. Questions arising here are, How many copies of the transgene elements are incorporated into the plant? Where is the transgene integrated in the genome? Where might the transgene break into parts (by recombination or other genetic mechanisms)? How is stability evaluated or proved?

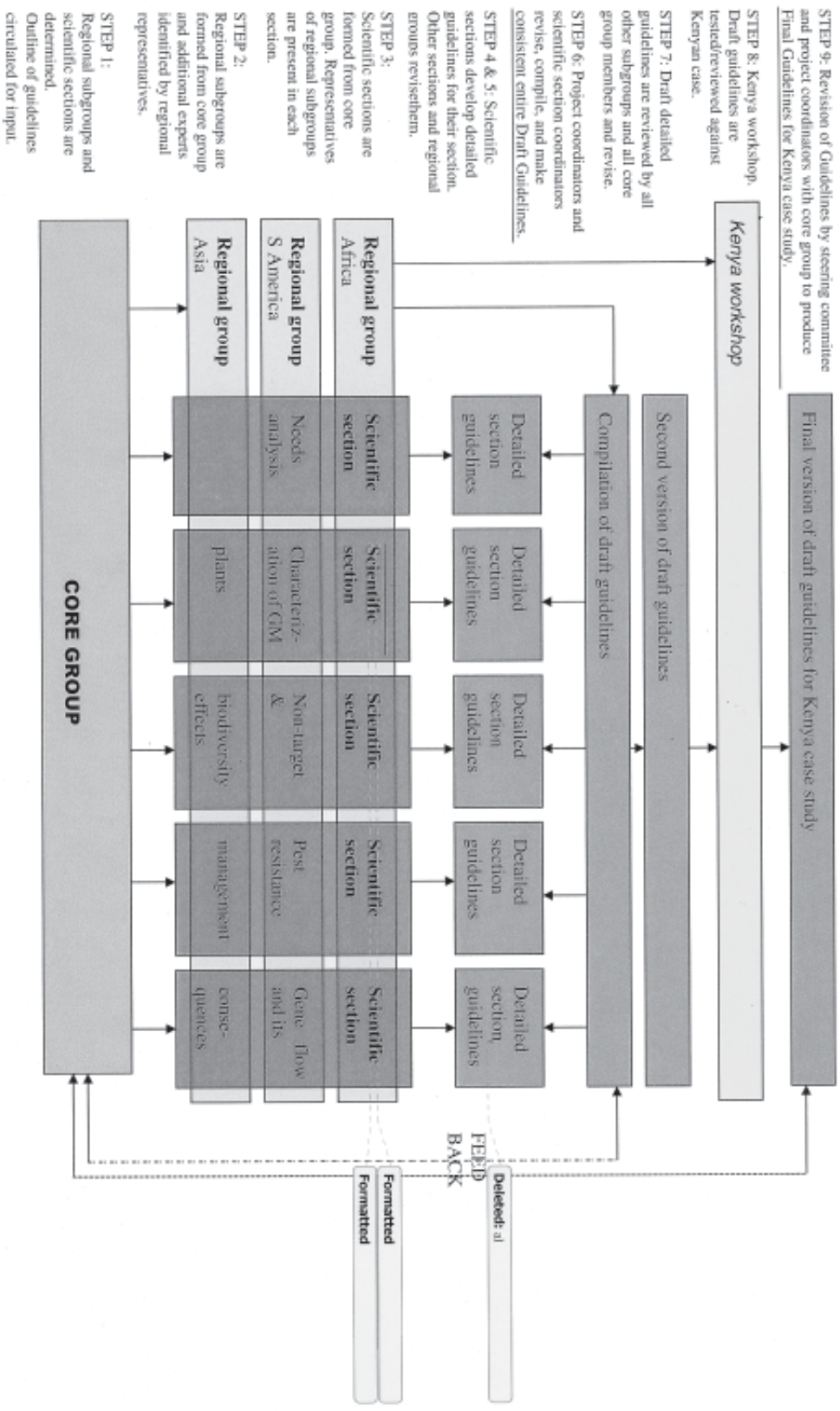
Non-target Effects and Biodiversity

In this section, there are two main tasks to accomplish for each of the identified categories of organisms: (1) Specify a procedure to determine the nontarget species or function or processes that should be tested (= selection procedures). (2) Specify scientific procedures for testing these species, functions, or processes (= testing procedures). Seven categories of organisms that need to be addressed have been identified: (a) natural enemies, (b) pollinators, (c) soil organisms, (d) species of conservation concern, (e) species of cultural significance, (f) nontarget pests, (g) other non-target species. Routes of exposure need to be identified. Exposed organisms are determined through suspected causal chains of impact. On the basis of this information, protocols and methodologies for appropriate testing can be developed.

Pest Resistance Management

To determine the resistance risk and management responses needed to reduce this risk, it will be important to address the feasibility of implementation. In addition, approaches for developing a practical monitoring and response system to detect resistance and to adapt management appropriately should be considered. Although this primarily addresses resistance development in pests, resistance development of weeds as a result of commercial production of transgenic herbicide-tolerant crops will also be considered.

Figure 2: Organisation and product Development in the first year of the GMO Guidelines Project



Gene Flow and Its Effects

Gene flow is the route along which transgenes can spread genetically into populations of related species and geographically into other regions, including protected areas of sensitive ecological value. Gene flow is considered a risk because of the great uncertainties associated with the possible consequences in the recipient ecosystems. Successful transgene flow will simultaneously affect both recipient plants and their associated organisms. Protocols need to be developed for establishing (a) the likelihood of intra- and interspecific gene flow, (b) the possibility of subsequent geographic and genetic spread of transgenes, (c) the potential ecological effects resulting from gene flow, and (d) the effectiveness of sterility mechanisms, their breakdown, and management

Launch of the GMO Guidelines Project and Invitation to Participate

Current biosafety procedures are inadequate to test the impacts of transgenic crops on their environment. Current U.S. models rely on pesticide methodologies and consider novel transgenic products, such as the Bt toxin, as equivalent to the toxin when used as a pesticide. However exposure is very different when the product is continually expressed in the plant tissues as against when it is periodically applied as a pesticide. This is illustrated by the effects of Bt toxin on *C. carnea* (the green lacewing) when it ingests the toxin via Bt maize fed prey species as compared with an artificial Bt-containing diet. When feeding the Bt toxin directly to chrysopid larvae, the induced mean total immature mortality of *C. carnea* was similar to the mortality from prey food fed Bt maize that contained Bt concentrations approximately 10- to 20- fold lower than in the artificial diet. In addition, the chrysopid larvae exhibited a sublethal effect of the Bt toxin: stunted growth. Such tritrophic effects on natural enemies could be composed several of interacting factors that are difficult to distinguish, and they can be as disruptive for population dynamic processes and trophic interactions (leading to serious agroecological problems) as the direct lethal effects of the Bt toxin. This example illustrates the need for new biosafety methodologies that go further than pesticide-based approaches and that consider the novelty of the technology.

This paper presents the launch of an international initiative of public sector scientists that aims to develop comprehensive scientific guidelines for prerelease biosafety testing of transgenic plants such as could serve as an international standard. The guidelines will be tested in real policy contexts using transgenic-crop case studies from countries in Africa, Asia and South America over the next 2 1/2 years. The guidelines will present a series of questions and corresponding methodologies by which any particular GMO issue can be evaluated scientifically. The guidelines will be published in the open literature and on the project Web site and will be revised regularly to ensure their continual relevance.

For further information on the GMO Guidelines Project, please contact Angelika Hilbeck (hilbeck@geobot.umnw.ethz.ch) or Evelyn Underwood at the project secretariat (underwood@geobot.umnw.ethz.ch), or go to our Web site (http://www.gmo-guidelines.info). Public sector scientists from developing and developed countries are invited to join the group and participate in this unique effort.

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

Session 3B—Transgenic LMOs in the Environment: Additional Considerations

Effect of Living Modified Organisms on the Soil

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Abstract

Methods to determine baseline perturbations of soil microbial communities and functions are illustrated briefly. The impact of living modified organisms (LMOs), whether genetic modification is in the plant or rhizosphere micro-organisms, is usually small compared with changes induced by conventional agricultural practices. Notwithstanding, plant nutrition can be improved by LMOs and the ACC deaminase gene can decrease plant stress and facilitate bioremediation. Modification of organisms by insertion of marker genes can decrease biological fitness of the organism, and therefore the environmental impact of microbial LMOs is likely to be less than those produced by wild types.

Introduction

Micro-organisms have been used extensively in crop protection as well as bioremediation and are seen as primary targets for genetic modification to improve performance. However, it has also been quickly realized that the useful genes could also be incorporated into plants and that this might provide a more predictable and economically viable route for delivery. Thus, the vast majority of release requests received by most national regulatory committees have been for living modified organisms (LMO) plants rather than micro-organisms. However, the principles of determining any perturbations on the baseline soil ecology are essentially the same irrespective of the type of LMO being introduced. The rhizosphere is a site of particular interest because 40 percent of the plant's captured photosynthate is released as rhizodeposition products and is therefore available to the soil biota (Lynch and Whipps 1990). This is therefore the energy powerhouse of the soil ecosystem and is also the site at which introduced genes, plant or microbial, may exert greatest influence.

Horizontal Gene Movement

In the early investigations, the primary concern was whether there would be horizontal gene transfer from the introduced living modified organisms (LMOs). To investigate LMO effects, marker genes were used as the initial targets. In our own studies we focused on two common soil–rhizosphere organisms,

Enterobacter cloacae and *Pseudomonas cepacia*. The donor strains had tetracycline and trimethoprim resistance on plasmids, and the recipients had nalidixic acid resistance. Continuous-flow columns packed with coarse sand or glass beads, with or without a hollow fiber down the center pumped with glucose to stimulate rhizodeposition from a root were used (Sun et al. 1993, 1999; Pearce et al. 1997, 2000, 2001).

For *E. cloacae* one transconjugant was produced per 10^4 and 10^6 donors or recipients, but the level was heavily dependent on the flow rate down the column and the distance from the hollow fiber (Pearce et al. 2001). This rate is comparable with the plasmid exchange frequencies that have been observed for other soil bacteria. Gene exchange has generally not been observed from chromosomally borne genes. Plasmid fluidity between wild types is a natural occurrence, but antibiotic marker or functional gene marking would have uncertain consequences; therefore, it seems reasonable that chromosomal genetic modification is the safest option.

Need for New Technologies for Baseline Population Studies

Traditionally, monitoring of microbial populations in soil has centered on the enumeration of specific populations. However, for a significant perturbation to be measured, changes of between 100 and 300 percent (0.3 and 0.5 on a log scale) are necessary. Moreover, there is the problem of nonculturability. In one study (Troxler et al. 1997), only 0.08 percent of *Pseudomonas fluorescens* CHAO–Rif cells were culturable after 200 days, 4.77 percent were viable but nonculturable, and 95.2 percent were dormant or nonviable of the methods relying on culturability. We have found the most effective way to assess culturables quantitatively is to determine the *r* and *K* strategists from different habitats and to formulate an ecophysiological index (EPI) (De Leij et al. 1993), which is a technique finding increasingly wide acceptance (e.g., Van Elsas et al. 2002). A range of nonculture techniques that do not rely on culturability of micro-organisms such as fatty acid, methyl ester organic phyrophosphate content with ms pyrolysis, immunofluorescence, and cellular protein profiles have been used, but these seldom give a perspective on the perturbations that affect the ecosystem. There is a similar problem with the nucleic-acid-based methods such as restriction fragment length polymorphism (RFLP), DNA fingerprinting, amplified ribosomal DNA-restriction analysis (ARDRA), analysis of randomly amplified polymorphic DNA markers (RAPD), polymerase chain reaction (PCR), DNA–RNA sequence analysis, or hybridization probes and community hybridization using the reannealing of DNA samples.

Methods that target ecosystem functions or gene products can be very useful. Commonly these can involve enzymes as the gene products or nutrient cycle analysis. Ultimately the impact on the plants themselves can be most readily determined by a plant bioassay. Similarly, soil faunal assays can be carried out. It is important in this respect to use several methods to determine the ecosystem baseline and the way in which it is perturbed. What follows are some examples of a few studies in which this has been done.

Marker Genes

The first release of a free-living LMO bacterium in the United Kingdom (UK) was carried out during 1993 and 1994 on spring wheat in a silt loam at Littlehampton, West Sussex, and on sugar beet in a heavy clay soil in Oxford. The bacterium *Pseudomonas*

fluorescens SBW 25 was isolated from the phylloplane of sugar beet, but it was also shown to colonize the rhizosphere of the sugar beets readily as well as the phylloplane and rhizosphere of wheat. The marker genes (*lacZY* and *kan^{r}xylE*) were chosen to facilitate identification and detection of the LMO by simple culture methods and positioned 1 Mb apart on the 6.5Mb chromosome to ensure genotypic and phenotypic stability as well as to facilitate any gene exchange between microbial populations associated with the two crops. At the Ee site, lacZY (4.0kb) was inserted, and at the 6 site, *kan^r–xylE* (7.2kb) was inserted (Rainey and Bailey 1996).

Prerelease studies were carried out to determine the natural ecology of the phylloplane of the sugar beet (Thompson et al. 1993) and wheat (Legard et al. 1994). Before the release, studies were conducted to determine any perturbation effects under contained glasshouse conditions (De Leij et al. 1994 a,b). Subsequently, the release trials were carried out with the consent of the UK Advisory Committee for Releases into the Environment and reported (De Leij et al. 1995 a, b; Thompson et al. 1995). The full account of the studies has been reviewed (De Leij et al. 1998). In terms of impact, the conclusions drawn are summarized in table 1. The principal results were that the organism became established and disseminated, but gene transfer to other organisms was not detected. Subsequent studies (De Leij et al. 1998) investigated the potential metabolic burden of the inserted genes on the ecological competence of a variety of constructs modified with the marker genes used in the release-study strain of the bacterium (table 2). Whereas the kanamycin resistance did not seem to affect the fitness of the organism, both of the other marker inserts did reduce ecological competence. The table shows that the X-gene (in combination with Kr) caused a decline in SBW 25 population. The conclusion, therefore, is that even though the modified bacterium was competent in the field, the wildtype is even more competent. The marking was essential for monitoring purposes, but because the marker inserts added no beneficial function, it would not be sensible to use them in any exploitation of the bacterium.

Table 1. Ecological Effects of Pseudomonas fluorescens SBW 25 EeZY-KX

Effect	Wheat	Sugar Beet
Survival and establishment	$>10^6$cfu^{-1}g root during season and 7 months after harvest	Up to 5 x 10^6cfu^{-1}g senscent leaves
Dissemination	Vertical > 45cm Lateral > 2m Colonised volunteer and resown plants and weeds	Vertical < 10 cm Lateral < 10cm Colonised volunteer and resown plants and weeds
Gene transfer	None of markers	None of markers but on mercury resistance plasmids exchange
Community analysis	Small and transient, no effects on plant health	Small and transient, no effects on plant health

Table 2. Ecological competence of Pseudomonas fluorescens SBW 25 variants. (De Leij et al. 1998)

SBW 25 variant	Percentage of total introduced *P. fluorescens* SBW25 population Time (days) 0	14	28
SBW 25 – 6K	30.0[a]	52.4[b]	55.3[b]
SBW 25 – 6KX	44.8[b]	25.5[a]	20.0[a]
SBW 25 – EeZY-6KX	25.3[a]	25.1[a]	24.8[a]

Antifungal Genes

The DAPG gene

In 1993 the European Commission funded a major program on Biotechnology and Ecology of Microbial Inoculants (IMPACT) followed 3 years later with another titled Harnessing the Potential of Genetically Modified Microorganisms and Plants (IMPACT 2). The partnership has involved CSIC Spain, Irish Sugar, Agronomica (Italy), S & G Seeds (The Netherlands), TUV (Germany), and the Universities of Cork, Turin, ETH Zurich, Lausanne, Leiden, Surrey, Padua, Pisa, Bielefeld, Madrid Polytechnic, Leuven, and York. One target was to determine the impact of *Pseudomonas fluorescens* F113, which had been isolated from sugar beets and found to produce the antibiotic 2, 4 diacetylphloroglucinol (DAPG) (Shanahan et al. 1992). Besides being active against *Pythium* damping-off, DAPG was also active against the potato soft-rot pathogen *Erwinia carotovora* ssp. *atroseptica* (Cronin et al. 1997) and the potato cyst nematode *Globodera rostochiensis* (Cronin et al. 1997b). For comparative purposes, strain F113 G22 was constructed, which is a Tn5::lacZY DAPG-negative derivative of F113 that does not have the ability to inhibit the growth of plant pathogenic fungi (Shanahan et al. 1992).

The impact of the *Pseudomonas* strains on the rhizosphere was carried out primarily at Surrey. One of the main approaches was to determine the effect on the rhizosphere–soil enzymes N-acetyl glucosaminidase, chitobiosidase, acid and alkaline phosphatase, phosphodiesterase, aryl sulfatase, and urease, which are representative enzymes in the carbon, nitrogen, phosphorus, and sulfur cycles in soil (Naseby and Lynch 1997). The results were published in a series of papers and are summarized in table 3 (Naseby and Lynch 1997, 1998, 1999, 2001; Naseby et al. 2000, 2001 a, b).

Table 3. Effect of Pseudomonas fluorescens on F113 producing the antibiotic DAPG on rhizosphere enzymes.

Plant	**Increases**	**Decreases**
Pea	alkaline phosphatase aryl sulphatase urease	B-glucosidase NAGase
Wheat	alkaline phosphatase	chitobiosidase aryl sulfatase urease

A further series of studies addressed the mineralization and uptake of ^{15}N-enriched wheat residues (Brimecombe et al. 1998, 1999, 2000). Inoculation of pea seeds with *P. fluorescens* F113 or F113G22 increased mineralization and uptake of organic nitrogen in the rhizosphere. In contrast, the inoculation of the same strains onto wheat seeds reduced mineralization and uptake (table 4). The explanation seems to be that inoculation of pea resulted in an increase in the number of nematodes and protozoa in the rhizosphere, but for wheat there was a decrease in the microfauna, which stimulated the mineralization of organic nitrogen. The inoculants, when provided to peas could catabolize nematicidal compounds, produce a nematocide, or both. This is therefore a clear benefit of the inoculants but took place irrespective of whether they had been modified.

As a further aspect of inoculation effects on nitrogen cycling in the rhizosphere, the impact on nodulation of peas was studied (table 5). Nodulation was increased, but only with the DAPG-producing strain of the bacterium (Aldrade et al. 1998). Thus, this beneficial effect was canceled by the genetic modification of the wild type.

Table 4. Effect of *Pseudomonas fluorescens* on nematodes and nitrogen uptake (Brimecombe et al, 2000).

Plant/ Treatment	Nematodes g^{-1} Soil	% N in shoot derived from organic residue
Pea		
Control	2.9^{a}	8.3^{a}
F113 G22	4.9^{ab}	20.3^{b}
F113	6.2^{b}	25.7^{b}
Wheat		
Control	4.5^{a}	29.4^{b}
F113 G22	3.3^{b}	20.7^{a}
F113	2.6^{b}	22.7^{a}

P. fluorescens F113 is the wild type which produces the antibiotic DAPG. *P. fluorescens* F113G22 has been modified to delete DAPG production. Values not followed by the same letter are significantly different at $P = 0.05$. Plants were grown for 17 days.

Table 5. Effect of *Pseudomonas fluorescens* on nodulation of peas by *Rhizobium leguminosarum* (Andrade et al, 1998).

Treatment	Shoot dry weight (g)	Number of *Rhizobium* nodules/g root
Control	1.41[d]	5.1[a]
Rhizobium	1.24[cd]	7.9[ab]
Pseudomonas F113	1.02[abc]	9.9[b]
Rhizobium + *Pseudomonas* F113	0.89[ab]	20.3[c]
Pseudomonas G22	0.81[a]	7.9[ab]
Rhizobium + *Pseudomonas* G22	1.22[bcd]	6.0[ab]

P. fluorscens F113 is the wild type which produces the antibiotic DAPG. *P. fluorescens* F113G22 has been modified to delete DAPG production. Values not followed by the same letter are significantly different at $P = 0.05$.

Table 6. Effect of Brassica napus (oil seed rape or canola) cultivar variation and transgenesis with antifungal proteins on rhizosphere nitrate and alkaline phosphatase before and after rainfall.

Treatment	ppm nitrate/g dry soil		alkaline phosphatase (mg released/hr)	
	before	after	before	after
Border variety	63[a]	77[a]	5.7[a]	9.2[a]
Wild type (Westor)	43[b]	150[b]	13.2[b]	8.8[a]
Null	43[b]	173[b]	13.5[b]	8.9[a]
Acc AMPl	57[a]	123[b]	12.1[b]	8.4[a]
Null	80[a]	123[b]	16.7[b]	9.9[a]
Dm AMPl	77[a]	143[b]	16.0[b]	9.2[a]

All, except the border are Westor wild types modified with antifungal genes Acc AMPl from *Allium cepa* or Dm AMPl from *Dahlia merckii*. The divergent null lines do not have chromosome conjugation. Values not followed by the same letter are significantly different in column, but all values significantly different across column ($P = 0.05$).

Table 7. The ethylene effect on plants and alleviation by ACC deaminase.

Effects of ethylene in the rhizosphere

- Root initiation
- Root length inhibition
- Promotes seed germination
- Inhibits modulation and mycorrhiza

Ethylene producing stresses

- Phytopathogens

- Low/high temperature
- High salt
- Flooding/drought
- Heavy metals/organic contaminants
- Insect predation

ACC deaminase

- Converts ACC to ammonia and ±-Ketobutyrate
- Requires pyridoxal phosphate
- Native form is a 105 Kda trimer
- Km 1 – 15mM
- Temperature optimum ~ 30°C
- pH optimum ~ 8.5
- Bacteria growing on ACC with ACC deaminase promotes root elongation

Transgenic oil seed rape

The soil enzyme methodology has been used to assess the impact of genetically modified plants on soil biochemistry (Naseby, D.C., Greenland A., and Lynch, J.M., unpublished data). Two modified oilseed rape lines were used (*Brassica napus*, var Westar), which produced small cysteine-rich proteins with antifungal activity specifically expressing either the DmAMPl gene from *Dahlia merckii* or the AceAMPl gene from *Allium cepa*. The null lines only have the genetic modification on one chromosome, whereas breeding following modification yields the genes on both chromosomes (chromosome conjugation). The field trial consisted of these transgenic lines compared with several controls, their divergent null lines, a wild type control (Westar), and a different variety of oilseed rape. Sampling of this trial for enzymatic analysis consisted of taking the rhizosphere soil of 10 replicated plants from each treatment. Sampling occurred over 2 days (i.e., five replicates from each treatment were taken on the first day and five on the second day of sampling). A range of soil enzyme activities were measured, and the available soil nutrients were analyzed.

The results (table 6) showed large differences between the two sampling days in soil enzyme activities (e.g., alkaline phosphatase) and available soil nutrients (e.g., nitrate). Differences were found in most soil enzymes measured and the available soil nutrients such as nitrate. Differences were also detected between the various oilseed rape varieties assessed. However, there was little difference between the enzyme activities in the rhizosphere of the genetically modified (GM) and non-GM plants. However, AMP-1 before the rainfall did differ from its null analog result. The major factor influencing the enzyme activities and soil nutrients between the two sampling days was the soil moisture content, which was increased by overnight rain. Therefore, in this field trial, the differences between soil enzyme activities were not attributable to plant genetic modification but to environmental variation and to differences in plant variety.

ACC deaminase

Ethylene has a range of effects on plants. It is produced endogenously in the plant and exogenously by soil micro-organisms, and both sources affect plant growth regulation (table 7). Most notably ethylene is the classical inhibitor of root growth in flooded soils, either from endogenous root production, causing arenchyma (air spaces) to form in roots, or from exogenous

microbial sources. The substance 1-aminocyclopropane-1-carboxylate (ACC) is synthesized in roots and transported to plant shoots where it is converted to ethylene by ACC oxidase. The synthesis of ethylene can be inhibited by the enzyme ACC deaminase. The ACC deaminase has been found in a range of strains of rhizosphere bacteria (*Enterobacter cloacae*, *Pseudomonas* spp. *Kluyvera ascorbata*) that appear to promote plant growth by inhibiting ethylene stress (Burd et al. 1998, 2000; Grichko and Glick 2000, 2001; Li and Glick 2000; Ma et al. 2001; Shah et al. 1998; Wang et al. 2000). The plants not only become flood tolerant, but the destressing effect enables them to accumulate heavy metals and therefore become potential agents of bioremediation. Transgenic tomato plants have been produced with the bacterial gene under the transcriptional control of either two tandem 35S cauliflower mosaic virus promoters (constitutive expression), the *rolD* promoter from *Agrobacterium rhizogenes* (root-specific expression) or the pathogenesis-related *PRB-lb* promoter from tobacco to generate the flooding tolerance and ability of the plants to accumulate cadmium, cobalt, copper, nickel, lead, and zinc (Grichko et al. 2000; Grichko and Glick 2001). Thus, microbial inoculants or transgenic plants expressing novel products and with abilities to enhance bioremediation might become a very exciting new initiative to improve the soil environment.

Conclusions

Clearly, baseline ecology needs to be established to determine perturbation effects. Some conclusions that can be drawn from our studies and those of others thus far can be summarized as follows:

- Gene products are better indicators of population change than monitoring populations directly.
- Gene exchange is mainly mediated by plasmids.
- Field impacts of LMOs are generally smaller than impacts of meteorological conditions and agricultural practices such as ploughing, which is not discussed here.
- Living modified organisms can carry metabolic loads that reduce ecological fitness.
- Living modified organisms may influence microbe–faunal interactions that indirectly regulate plant nutrition.
- The enzyme ACC deaminase may decrease plant stress and facilitate bioremediation.

The focus of this very brief summary of research has been on *Pseudomonas* strains. One of the major issues not covered here is the significance of the *Bt* toxin gene to soils, but this product of a soil microbe has received attention in very recent publications from Guenther Stozky's laboratory in New York (Saxena and Stozky 2000; Saxena et al. 2002 a,b).

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Transgenic Insects: Programs, Technology, Benefits, and Risks

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Abstract

The use of genetics as an insect control tool emerged from the broader tradition of biological control more than 50 years ago. The limited use of genetic control strategies is due, in part, to the limited abilities of entomologists to create the necessary genotypes required for successful program implementation. Transgenic insect technology provides entomologists with new opportunities to execute genetic control programs. Transgenic insect technology is being considered in three types of applications: beneficial insect augmentation, population suppression and eradication, and pest-status modification. At least four broad host–range transformation systems are currently available for insects. Most of the safety issues associated with transgenic insects are analogous to those identified for transgenic plants. The limited ability to manage transgenic insects following their release presents unique challenges. Transgenic insects will permit the expanded use of genetics in biological control strategies, which is consistent with the growing demand to reduce chemical inputs into the environment and the development of sustainable agricultural systems.

Introduction

The intense interest in transgenic technology in agriculture has focused largely on crop improvement. In particular, efforts to confer on crops properties that result in their being more insect resistant and herbicide tolerant have dominated early development efforts. Pest management remains an important emphasis of transgenic crop development but future efforts will also likely focus on yield improvements. It is not surprising, therefore, because insect pest management remains a priority in agriculture, that strategies to exploit the power of transgenic technologies are being explored by entomologists. It should also be noted that the interest by entomologists in transgenic technologies is not focused solely on insects of agricultural importance. The emergence of insecticide-resistant insect vectors of disease, among other things, is leading to a resurgence in insect-borne diseases such as malaria and dengue fever. Currently there are over 1 million deaths from malaria and over 300 million acute cases of the disease annually. No less than 40 percent of the world's population is now at risk of contracting malaria. Ninety

percent of the mortality associated with malaria presently is confined to sub-Saharan Africa (WHO/OMS 1998). Although the severity of this problem is reflected by its impact on human health, note that the economic impact of malaria is similarly severe. It has been estimated that the economic growth of Africa is reduced up to 1.3 percent annually as a result of this disease. The short-term economic benefits of controlling malaria are estimated to be between $3 and $12 billion per year (WHO/OMS 1998). Consequently, transgenic insect technologies are also being explored as tools for solving public health problems.

Current interest in the application of transgenic technologies to insects represents a new phase in an area of insect pest management that began 50 years ago and has focused on genetics as a tool for biological control (Whitten 1985). Despite the proven success of certain insect genetic control strategies such as the control of the new world screwworm (*Cochliomyia hominivorax*) following the large-scale release of radiation-sterilized insects (the sterile insect technique), the widespread application of genetic control strategies has been limited largely by our inabilities to genetically manipulate pest species in ways that are compatible with certain programatic requirements. There was great enthusiasm for insect genetic control in the 1960s and 1970s which resulted in theoretical and conceptual advances but few programatic successes (Whitten 1985). Again, the limited abilities of entomologists to create the necessary genotypes and the fitness costs associated with these genotypes and their production were largely responsible for preventing the successful implementation of these programs. There have been significant advances in insect molecular genetics during the last decade, including the development of robust transgenic technologies for insects of agricultural and public health importance (Handler and James 2000). These advances have renewed an interest in insect genetic control strategies, and, although such strategies have many advantages over conventional chemical-based control methods, they can also pose some unique risks. Whether these risks can be minimized and managed to the extent required to make these insect control strategies acceptable and attractive remains to be seen.

Applications of Transgenic Insect Technologies

Transgenic insect technologies are being considered for a variety of applications, including beneficial insect augmentation, population suppression and eradication, and pest-status modification.

Beneficial Insect Augmentation—

Silkworms and honeybees have been the targets of genetic improvement for thousands of years. Current advances in our abilities to create transgenic insects are likely to provide new opportunities for silkworm and honeybee breeders to create strains with useful characteristics such as resistance to certain diseases and pests. The use of transgenic technologies in these cases is analogous to the application of transgenic technologies to crop and livestock improvement. In addition to the genetic manipulation of domesticated insects, there has been an interest in genetically manipulating the natural enemies of insects such as parasitoids and predators (Beckendorf and Hoy 1985). Improvement efforts have focused on insecticide resistance with the hope that these insects could be deployed as part of an Insect Pest Management (IPM) program. The feasibility of beneficial arthropod augmentation has been well documented for the predatory mite *Metaseiulus occidentalis* (Hoy 1985). A strain of *M. occidentalis* was developed using conventional breeding and selection strategies

that was resistant to organophosphorus insecticides under field conditions. This strain was successfully deployed in California almond orchards and resulted in decreasing production costs by reducing the number of pesticide applications for spider mite control (Headley and Hoy 1987). Transgenic technologies are expected to provide more opportunities to employ beneficial insect augmentation strategies because the number of genotypes that can be created is almost without limit and the time required to produce these genotypes can be quite short.

Population Suppression and Eradication—

Population suppression and eradication remains the primary objective of most insect pest control strategies. Clearly, insecticides have played the major role in these efforts, but insecticide use is becoming increasingly difficult because of the emergence of resistant pests, the difficulty in developing new chemical control agents, and the growing levels of societal intolerance to their use in the environment. Consequently, alternative strategies for pest control need to be developed, and among those "alternative" strategies that hold great promise are genetic control methods. These methods can be divided into two categories: the sterile insect technique (SIT) and genetic load control (GLC) (Waterhouse et al. 1976). In both cases the pest species with an appropriate genotype (sterility in the case of SIT, deleterious genes or conditional lethal genes in the case of GLC) is reared en masse and released into the pest population in the field. Mating between wild and released insects either results in no progeny (as in the case of the SIT), fewer progeny, or progeny that will die prematurely (as in the case of GLC), depending on the genotype of the parents. As with strategies for augmenting beneficial insects, producing insects with the necessary genotypes fit enough to compete successfully when released into the environment is a major problem; however, the use of transgenic technologies affords entomologists the opportunity to better solve and manage these problems.

Pest Status Modification—

Perhaps the most ambitious proposal for the application of transgenic insect technology to pest management is to use it to modify the pest status of individuals within a population (Curtis and Graves 1988). This approach does not prescribe the eradication of the pest insect population but instead involves its conversion via the incorporation of a pest phenotype-altering transgene. For example, genetically altering the mosquito *Anopheles gambiae* such that it can no longer serve as a host for the human malaria parasite *Plasmodium falciparum* might serve to reduce malaria transmission and the incidence of disease. The successful implementation of this strategy not only requires the creation of a genotype that will ultimately prevent parasite development in the mosquito but also the spread of this transgene through natural populations of the insect, resulting in a stable genetic transformation of an insect population in nature. This application of transgenic technology, which might be called "environmental gene therapy," is perhaps one of the most ambitious applications of transgenic insect technology and will present us with some rather unique risk issues.

The Technology

Interest in transgenic insect technology has a long history extending back to the 1960s (Handler 2000). In the 1970s the first report of the stable genetic transformation of the laboratory fruitfly *Drosophila melanogaster* appeared, but unfortunately the methods employed

did not constitute a "system." In the early 1980s a method for systematically and repeatedly creating transgenic *D. melanogaster* was developed based on a gene vector constructed from a transposable element (the P-element). Unfortunately, the P-element has a very restricted host range and is unable to function as a gene vector in insect species outside the family Drosophilidae. Consequently, none of the major insect pests could be transformed with the *Drosophila* system, although it did provide a useful paradigm for subsequent insect gene vector development efforts. During the 1990s there were widespread efforts to discover, analyze, and test other insect transposable elements for their abilities to serve as gene vectors analogous to the *Drosophila* P-element. Several promising candidate elements were found, and the first reports of genetic transformation of insects of economic and public health significance appeared (Handler and James 2000). As of today there are four major insect gene vector systems other than the P-element system. All are constructed from different transposable elements and have broad host ranges. More than 15 different species of insects have now been genetically transformed using at least one of these vectors (Atkinson et al. 2001). It is fair to say that the technology for creating genetically transformed insects is widely available.

Molecular Biology—

Transposable elements comprise a large and diverse collection of genetic elements that share (either now or in the past) the ability to move within the genome. Certain types of elements such as Class II elements move through a process of element excision followed by element insertion (Berg and Howe 1989). This excision and insertion process is exactly what the genetic engineer attempts to do when creating a transgenic organism. Therefore, the inherent mobility properties of transposable elements make them attractive genetic platforms upon which to construct integrative gene vectors. All of the gene vector systems currently available for insects are constructed from Class II transposable elements (P, hobo, Hermes, mariner, Minos, piggyBac) isolated originally from insects (P and hobo—*D. melanogaster*; Hermes—*Musca domestica;* mariner—*D. simulans*; Minos—*D. hydei*; piggyBac—*Trichoplusia ni*). Each system consists of an integration vector comprised of a pair of element-specific, terminal-inverted repeat sequences that are essential for integration. These repeat sequences flank the transgenes to be integrated, conferring on them the same mobility properties as the native transposable element. The system also consists of an element-specific transposase-coding region under an appropriate promoter control system. The transposase-coding region encodes for system-specific proteins that perform the integration reaction.

Biology—

The two components of the system, the inverted repeat-containing vector and transposase-producing "helper" plasmid, are coinjected into the posterior pole of preblastoderm insect embryos, where integration will occur in primordial germ cells. The resulting adult insect is a chimera of transgenic and nontransgenic tissue. If the germ-line is transgenic, the insect will produce fully transgenic progeny from which stable lines can be established (Spradling 1986). Dominant visible markers such as the Green Fluorescent Protein are commonly employed to aid in the identification of transgenic individuals. The microinjection methods and subsequent animal husbandry and genetic manipulations can be extremely challenging and severely limit the applicability of existing gene vector systems.

Benefits

Genetic control methods, whether or not they employ transgenic insects, have a number of remarkable benefits when compared with traditional chemical-based control methods. By their very nature genetic control methods are species specific and minimize chemical use. In addition, genetic control methods tend to be most efficient when the target insect population is at low density owing largely to the ability of the released insects to locate and find conspecific individuals. Genetic control strategies that involve population replacement and pest status modification have the potential to be highly sustainable, requiring little or no input after the initial implementation of the program. The benefits of using transgenic technologies within the context of genetic control programs are the ability to construct insects with the desired genotypes rapidly, the possibility of constructing a much greater repertoire of genotypes, the ability to minimize secondary genetic alterations associated with conventional breeding practices, and the ability to apply genetic control strategies to a wide variety of insect species. In short, transgenic technologies now provide entomologists with the opportunity to customize the insects being used in a genetic control strategy to meet their programmatic requirements more precisely. Not only can insects be constructed with appropriate "effector" genes, but they can also be modified in ways that facilitate mass rearing and competitiveness.

Hazards and Risks

Hazards are associated with genetic control methods, some of which are rather general and common to many insect control programs. Insect eradication programs of any type result in the elimination of a species from an ecosystem and consequently can disrupt the ecology in ways that are undesirable. For example, the emergence of secondary pests may result in a pest problem as severe or worse than the original problem. Genetic control programs involving the massive release of a pest species (SIT and GLC) pose the potential hazard of increasing the size or range of the pest population. The probability of such an event in the case of SIT is directly proportional to the efficacy of the sterilization methods employed in the program. Consequently, the risk of increasing the size or range of the pest population can be reliably assessed. Programs designed to modify pest-status, whether or not they use transgenic technologies, also pose hazards that are similar to those encountered in conventional biological control programs such as unanticipated and unwanted invasion of new habitats. Because the released insects are fertile and, by design, competitive—as in the case of pest-status modification programs—the exposure component of any risk calculation becomes rather large. Consequently, programs involving the release of fertile and fit individuals must minimize hazards to keep the overall risk to an acceptable level. Consequently, pest status modification programs will be extremely challenging. Such programs will need to consider carefully the possibility of changing the pest status of an insect in an unintentional way, leading to an enhancement in pest status rather than a reduction. In the case of programs designed to modify the transmission capabilities of disease vectors such as mosquitoes, changes in vectoral capacity, host range, life history characteristics, and parasite–pathogen biology will need to be carefully considered (Hoy 2000).

The use of transgenic insect technologies in insect genetic control programs adds some additional hazards, many of which are similar to those identified for transgenic crops. The six safety issues (gene transfer, expression of genetic material from pathogens, weediness, trait effects, genetic and phenotypic variability, and worker safety) identified by the Organization

for Economic Cooperation and Development (OECD) as being of major significance to the release of transgenic plants are generally applicable to transgenic insect releases (OECD 1993). However, transgenic insects also can present us with some rather unique challenges.

Gene transfer and the expression of genetic material from pathogens are hazards arising as a result of a loss or movement of the transgene and a breakdown in biological containment. These hazards exist when deploying transgenic insects as they do when deploying transgenic plants and can be managed generally by using gene–vector systems that permit postintegration stability of the transgene to be maximized. For some applications of transgenic insect technology this will not be desirable or possible and represents a unique feature of some transgenic insects. For example, pest modification strategies rely on the introduction and spread of a phenotype-altering transgene in a wild population. One method of spread being considered is to link the transgene to a self-mobilizing transposable element. Transposable elements by virtue of their mobility characteristics under some conditions can sweep through natural populations. Linking a transgene to a "sweeping" transposable element is envisioned as a way of spreading the transgene through a natural population. Hence, stability is minimized and intraspecific spread is desirable. This represents a rather unique feature of transgenic insect technology.

The tendency of plants to spread beyond the fields where they were planted has been referred to as "weediness." Containing or limiting the distribution of transgenic insects is an analogous issue, but the dispersal characteristics of insects make this particular hazard more complicated than that for plants. For all insect genetic control programs dispersal is a critical requirement for achieving success. The objective of all insect genetic control programs is to release insects and have them disperse and mate with conspecifics in the environment. In programs where released insects are sterile (e.g., SIT) biological containment will be maximized and weediness tends to be minimized. Genetic load control programs, although releasing fertile insects, are designed ultimately to kill them or negatively impact insect reproduction again, tending to maximize containment and minimize weediness. Pest status modification programs are likely to maximize the weediness threat because of the use of fertile and fit transgenic insects.

Trait effects derive from transgenic traits that are harmful to nontarget organisms. Effects on nontarget organisms are an issue for all insect control strategies and that will also be an issue to be considered in the release of transgenic insects. Transgenic insects present a unique situation for insect control specialists and risk assessors because the stability of the transgene will influence the likelihood of its being transferred to another species. For most applications of transgenic insect technology, stability will be a highly desirable trait, and efforts to engineer the transgenic insect and the vectors used to create it will tend to maximize stability. Only in programs in which the transgene is being spread through a natural population using an autonomous transposable element will stability be minimal—at least initially. The presence of a highly unstable transgene (as a result of linkage to an actively transposing transposable element) will increase concerns for nontarget organisms as well as transfer of transgenes to pathogens and symbionts. Perhaps the most prudent approach to the use of actively transposing transposable elements as genetic engineering tools is to maximize their species-specificity so that, should they be transferred to other organisms, they would be incapable of integrating. Thus the risks of trait effects and expression of transgenic material from pathogens due to horizontal transfer would be minimized.

Genetic and phenotypic variability can lead to unpredictable outcomes in the field and, as with transgenic plants, transgenic insects will need to be characterized carefully with respect to phenotype. Our abilities to describe and understand the phenotypes of transgenic organisms until recently had been fairly limited. Consequently, critics of releases of transgenic organisms into the environment point to this limitation and the ability of genes to affect more than one trait (pleiotropy) as reasons for justifying bans on such releases. Testing claims that transgenes are pleiotropic can be difficult. The advent of transcriptome profiling using microarrays now provides geneticists with more powerful tools to assess the degree of pleiotropy associated with transgenic organisms. Current genomics efforts will increase our potential to determine the effects of transgene integration and expression effects in transgenic organisms and to make decisions concerning deployment based on a sophisticated and more thorough understanding of phenotypes.

Conclusions

The environmental safety issues associated with the release of trangenic insects parallel those identified for transgenic plants. Most of these same issues arise during conventional (nontransgenic) approaches to genetic and biological control. What makes insect genetic and biological control programs challenging and somewhat different from the release of transgenic crop plants is the degree of management that is possible. Insect genetic and biological control programs attempt to release fit and sometimes fertile insects into the environment. Dispersal of released organisms is essential for these programs to be successful and as a result cannot be managed to the degree that plants in monoculture can be. Hazard identification and risk assessment of transgenic insects are likely to place a high demand on understanding the phenotype of the transgenic insect being released. In addition, a thorough understanding of the mobility characteristics of the gene vector being employed, the use of "suicide" vectors to maximize stability, and the construction of gene vectors with a very high degree of species-specificity will help mitigate many of the risks associated with transgenic insects, most of which stem directly or indirectly from potential transgene instability. Although the challenges associated with the development of safe and effective transgenic insects are significant, this technology may lead to the expanded use of genetic and biological control strategies in pest management programs. This would satisfy the growing demands to reduce chemical inputs into the environment and the development of sustainable agricultural systems.

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Ecological Risk from Aquatic Living Modified Organisms

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Abstract

For more than one billion people, fish is the primary source of animal protein. Demand for seafood is now much greater than the yields from already threatened wild fisheries. Aquaculture and aquatic living modified organisms may greatly reduce pressure on ocean ecosystems. Some propose that aquatic living modified organisms (LMOs) may increase in the wild and cause irreversible damage—even extinction—and that case-by-case contained testing is necessary to screen these organisms. This paper does not recommend case-by-case contained testing because genotype * environment interactions limit prediction of fitness in the wild. Moreover, this case-by-case approach fails to generate generic predictions for a range of LMOs. This paper does suggest that a generic and falsifiable prediction of (negligible) risk can be drawn from genetic theory. What is unknown is how long it will take for the negligible risk hypothesis, without falsification, to be accepted as dogma by regulators and the public.

Introduction

Drivers of Aquatic LMOs

Most of the world's capture fisheries are fully or over-exploited[1,2] (Wijkstrom et al. 2000). Wild fisheries plateaued in the late 1980s at around 90 million tons per annum, but world seafood consumption is 140 million tons per annum. The failure of wild fisheries to meet world demand[3] for seafood is a major reason for the recent expansion of aquaculture[4]. Today, aquaculture is one of the fastest growing agriculture sectors worldwide. Per annum growth and value of aquaculture is 10 percent and US$ 50 billion, respectively (Rana and Immink 1984–1996). Against this backdrop of finite wild fisheries and increasing demand for seafood, aquaculture production will need to expand rapidly and continually for the foreseeable future. With only 1 percent of present aquaculture using genetically improved stocks (Gjedrem 1997), there is some prospect that genetics can accelerate aquaculture production to meet demand and do so in a sustainable way (i.e., with increased efficiency and reduced pollution and diseases). In particular, present marine aquaculture requires substantial fishmeal and thus adds to the pressure on

wild fisheries. There is some urgency to reduce this dependency. Options include the modification of terrestrial plants for fish feed or modification of fish (Knibb et al. 1998) to consume transgenic meal. Together, these factors translate into some interest to apply genetic engineering technologies in aquaculture.

There is also interest to use fish, particularly zebra fish (*Danio rerio*) and medaka *(Oryzias latipes)* as models for vertebrate gene discovery and function (Hackett and Alvarez 2000).

Previously I set out hypotheses (Knibb 1994, 1997) that predict negligible ecological risk from transgenic fish, and these predictions apply for living modified organisms (LMOs) in general:

> Despite concerns to the contrary, the following hypothesis remains to be falsified: "laboratory induced allele frequency/genotype changes and novel alleles or genes have a negligible probability of being selectively favoured in wild populations under natural selection, and accordingly, without sustained large scale releases, have little potential for ecological impact."

Now, some years later, I will review how the hypothesis stands—has it been falsified? In this way, I will address some of the stated objectives for this meeting—in particular, to consider hypotheses underlying the assessment of LMOs in the environment.

Discussion

What Aquatic LMOs Are There?

To date, most research on LMOs in aquaculture concerns the acceleration of growth in salmonids (Atlantic salmon [*Salmo salar*], Du et al. 1992; coho salmon [*Oncorhynchus kisutch*], Devlin et al. 1994; channel catfish [*Ictalurus punctatus*], Dunham et al. 1992; tilapia [*Oreochromis niloticus*], Martínez et al. 1996; and carp [*Cyprinus carpio*], Chen, et al. 1990) using heterologous growth hormone constructs. Resulting growth acceleration in salmonids is often dramatic (order of magnitude increase) but is less for other species. More preliminary work concerns cold (Wang et al. 1995) and salinity tolerance, disease resistance (Hew et al. 1995), metabolic modification, and fishmeal replacement (Pitkanen et al. 1999, Knibb et al. 1998)). In model fish (medaka, zebra fish), most LMOs are generated to elaborate vertebrate gene function. Hence, model fish LMOs are more numerous and varied than those from aquaculture (Hackett and Alvarez 2000). Methodologies for gene transfer vary, although microinjection of transgenes into eggs and random incorporation of DNA is the most common technique.

Special Concerns for Aquatic LMOs

Should aquatic LMOs survive in the wild, then their recovery from oceans, lakes, and rivers no doubt would be challenging. Indeed, a special concern for fish and aquatic LMOs is that release may be irreversible. This concern is tempered by the likelihood that LMOs will increase in proportion in wild populations, and the consequences of such an increase. There are various selective and stochastic processes whereby transgenes could increase in frequency in the wild (Knibb 1997) such as the following:

- Continual or large-scale releases of LMOs with reduced "Darwinian" fitness;
- Drift (even for deleterious alleles) (Crow and Kimura 1970);
- Genetic drive (even for deleterious alleles) (Morita et al. 1992);
- Selective advantage either as (intraspecific) polymorphism in a species, or due to speciation.

It is selective advantage that attracts most interest and debate (Tiedje et al. 1989, Kapuscinski and Hallerman 1990, Kapuscinski and Hallerman 1991, Regal 1994), for two reasons. First, selective advantage is suspected of being more probable for transgenics than spontaneous changes, although all of the preceding processes can apply for engineered and classical changes. Second, the release of just a few LMOs with transgenes of increased Darwinian fitness may lead to a wide–scale spread.

Mesocosms and Risk Assessment

Following on from concerns that release may be irreversible, there is some interest to describe the relative wild adaptive values of transgenes before release. Some advocate contained mesocosms as the preferred vehicle for fitness assessment (Levin et al. 1987, Hallerman and Kapuscinski 1995, Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish[5]). Intuitively, assessment by mesocosm is logical, for it presents opportunities for containment and empirical assessment. Indeed, research funds are now being channeled into contained testing, and the first data for fish are emerging (see section titled Experience and Data). But is intuitive reasoning sufficient? Does fitness in a mesocosm predict relative fitness in the wild? Are mesocosms adequate from a genetics perspective in view of unpredictable Genotype * Environment (G*E) interactions (Knibb 1999)?

As contained facilities, mesocosms are less complex than wild environments. Mesocosm testing should (but does not) encompass the following:

- A range of environments because a transgene with a high adaptive value in one environment may have a low value in another. Also, testing should consider environmental variation in space and time, including seasonal and long-term changes.
- A range of populations, strains, and genotypes, for relative fitness of the transgene in a population may depend on background genotypes (Dobzhansky 1970, Griffing 1967).
- This range should consider the different genotypes present in the wild (allele type and frequency) and, perhaps, the evolution of modifiers via multigeneration testing.

Fitness varies with further parameters, and thus mesocosm testing should also include the following:

- A range of frequencies of the transgene (fitness may change if the transgene is rare or common should there be frequency-dependent selection).
- Different transgenic lines because each line may represent unique insertion events (and unique chromosome locations), unique copy numbers, and unique genetic backgrounds of transgenic lines.

- Different life stages in as much as relative fitness for survival, reproduction, and so forth may vary ontogenetically (ideally, cross generation zygote to zygote data should be obtained).
  For fitness assessment we may also need knowledge of the following:

- The role of drift (Crow and Kimura 1970).
- Closely linked elements near insertion sites to distinguish between selection on transgene and linked elements.

Hence, fitness is context specific. Any one mesocosm will represent just a small subset of environments and genotypes of the wild but not necessarily in the proportion they exist in the wild. Mesocosms are unique situations confounded with unique factors relevant to fitness. Thus, finding lack of evidence of risk in one situation, no matter how many times the observation is repeated, will not prove the absence of risk in the wild. Nor will finding evidence for risk necessarily predict risk in the wild because mesocosms may have selective forces not present in the wild (e.g., selection for stress and disease tolerance and other novel factors in containment; Knibb et al. 1987).

An inability to predict fitness or performance is not a new concept and is well described in the animal genetics literature. For example, unpredictable G*E interactions exist for carp, whereas European varieties outperform their Asian counterparts when fed rich but not poor diets (Moav et al. 1975). In lay terms, we can refer to this phenomenon as "horses for courses." Also, when measured in two different environments, the same phenotypic character (e.g., growth) in a given population can be determined by different sets of alleles and genes. So when dealing with different environments, it may be prudent to consider a measured character not as one but as many different characters (Falconer and Mackay 1996).

Risk Assessment—Better Options?

The preceding discussion on empirical testing leads to the proposition that only release into the wild may be sufficient to assess ecological risk of transgenics. Perhaps this is so, but even for research purposes, wild release would not easily meet with public or regulatory acceptance—at least not without some knowledge or prediction that the chance for an irreversible event of significant consequence is not credible. Moreover, it is doubtful that empirical testing, even releases into the wild, will yield generic predictions for a range of LMOs.

Genetic Theory

Previously, Knibb (1994, 1997) suggested that genetic theory should be explored to predict the (generic) probability fitness distributions for transgenic LMOs. To recapitulate and summarize, we note that major mutations are usually pleiotropic (Dobzhansky and Holz 1943) (i.e., to some degree they influence many different characters and cellular and biochemical process). Hence, spontaneous mutations or transgenes that change one character will tend to alter different aspects of the phenotype. Because many aspects of the phenotype are adaptations for survival and reproduction in the wild, changes to them typically reduce overall fitness. Hence, we expect almost all genetic changes (that affect function) will be selected

against in the wild, although there are various ways beyond major or continuous release that deleterious alleles can increase in frequency (drifting, hitchhiking, driving). Presently, we have insufficient scientific knowledge of the genetic architecture of fitness to know a priori what constitutes an adaptive change or how to make one deliberately. Without this blueprint, genetic changes, classical or engineered are accidental with respect to fitness. In lay terms, this may be equivalent to tuning a piano without hearing. Hence, genetically engineered changes (that change a character) are expected almost inevitably to reduce fitness (or approximate genetic changes already produced in nature). From a lay perspective, this reasoning may seem counter-intuitive. Certainly, it is contradictory to present public perception that genetic engineering is planned, deliberate, and likely to increase fitness.

Concerning the continuing debate whether genetic engineering is qualitatively different from natural processes, it is often suggested that genetic engineering results in large and novel phenotypic changes, novel gene arrangements and combinations, lateral DNA transfer, and so forth that translate into a reasonable likelihood for fitness increase (Tiedje et al. 1989, Kapuscinski and Hallerman 1990, Kapuscinski and Hallerman 1991, Regal 1994). This is contentious (Knibb 1997), but argument whether or not nature can produce functionally complementary phenotypes may be quite secondary to the question of fitness equivalence. For genetic changes producing significant phenotypic, physiological, and biochemical change, theory and experience predict the probability for detriment to fitness will increase, not decrease, with the magnitude of the change (Fisher 1930, Endler 1986). Also secondary to the debate are the position effects and insertion mutagenesis which can accompany gene insertion and reduce fitness, for this will not apply for all LMOs. Similar processes apply for classical mutations.

Overall, there is no suggestion here that adaptive mutations did not occur in nature, nor that some laboratory genetic changes, classical or engineered, will not have increased fitness in the changed and novel selective environments of the laboratory, farm, or factory. It may well be a question of probability why genetic changes generated in the laboratory are not adaptive in the wild. The low intrinsic probability of generating an adaptive mutation is compounded by the low probability of making the mutation in the laboratory before a selectively equivalent change is produced in nature. And perhaps we should not underestimate the capacity of natural populations to "experiment" with new mutations. For example, a single brood of a single female scallop producing 10^6 to 10^7 eggs may have mutations in a large number of loci in the genome if mutation rates on the order of 10^{-5} to 10^{-6} per loci per generation are assumed (Voelker et al. 1980). There will be multiple mutations per quantitative trait on the assumption of a mutation rate of 10^{-2} per character (Barton and Turelli 1989). Nature seems to have much more time, opportunity, and numbers to produce mutations before we can make them in the laboratory. Moreover, consideration of the various natural mechanisms of gene shuffling has led to the question, With all the mechanisms that exist for moving the genes around, why is the genome so stable? (Crow 1984). Similar argument applies to the probability of speciation from "laboratory" genetic changes ahead of natural process.

In summary, considering fitness rather than the specific type of change and the unplanned and accidental nature of genetic changes with respect to fitness, pleiotropism, and the dynamic nature of genomes in natural populations, we reach a general prediction for new genetic changes, that is, a testable hypothesis of negligible probability that transgenes will be selected in the wild.

Experience and Data

In contained facilities, Atlantic salmon, transgenic for Chinook salmon (*Oncorhynchus tshawytscha*) GH, tended to feed in the presence of predators at a rate much greater than nontransgenic controls, which suggests less predator avoidance of the transgenics (Abrahams and Sutterlin 1999). Contained testing of channel catfish, transgenic for salmonid GH constructs, indicated less predator avoidance (less survival) of the LMOs compared with the nontransgenic controls ((Dunham et al. 1999). Others think their data indicate fitness reduction in transgenic or GH-treated fish (Guillen et al. 1999, Jonsson et al. 1996, Farrell et al. 1997)). Cranial deformities, opercula overgrowth, and reduced viability are evident for coho salmon engineered with GH constructs (Devlin et al. 1995, Ostenfeld 1998). The caveat with these data is that they derive from contained facility testing and do not include cross-generation fitness assessment. For future empirical risk assessment, it would be interesting to assess the feasibility of releasing model fish (zebra fish or medaka) transgenic for a great range of different constructs into natural (but isolated) environments and then monitor transgene frequency changes over generations (see Barker and East 1980).

In direct contrast to the preceding claims, Muir and Howard (1999 and 2001) argued that their experimental data from medaka transgenic for GH, and subsequent modeling, indicate credible risk. Specifically, a transgene causing growth acceleration could lead to population, even species, extinction on the assumption that transgenic males have a substantial mating advantage due to large size but their offspring have low viability ("Trojan gene hypothesis; Muir and Howard 1999). Some (Maclean and Laight 2000) have criticised this work (Muir and Howard 1999) on the grounds that the experimental data did not show adult size differences. Nor were any mating preferences for the transgenic medaka reported. One can add to this list a failure to assess fitness empirically across generations (zygote-to-zygote viabilities) let alone assess fitness outside the laboratory. With such short generation times, it is surprising and noteworthy that cross-generation competition data were not presented. More important, all models were based on many assumptions, including the absence of selection for modifiers and hence of genetic variance and mutations for modifiers, as well as the absence of G*E interactions. Indeed, some question whether modeling can predict fitness and evolution (Barton and Turelli 1989). But these criticisms are of a somewhat minor technical matter, for, in principle, a deleterious gene can increase in frequency, at least initially, under a range of conditions. This situation is well documented elsewhere for a category of rare classical mutations (Sandler et al. 1959, Braden 1958). So it is not entirely clear that the "Trojan gene hypothesis" scopes a principle peculiar to transgenics or something already discussed elsewhere (Lande 1980, Barton 1990). If not conceptually novel, this work (Muir and Howard 1999) could make a new contribution should GH transgenes display selective characteristics not evident from natural processes, that is, a probability of risk different from that of natural processes. However, Knibb (1997) pointed out that natural processes readily mimic the growth acceleration of GH transgenes in vertebrates (i.e., gigantism from mutations in major genes or selection for existing additive polygenic genetic variance). Indeed, Knibb (Knibb et al. 1998, Knibb 2000) reported a spontaneous major locus variant in sea bream (*Sparus aurata*) that accelerates growth and that classical selection increases growth. Devlin et al. (2001) showed that trout from stocks with a prior history of classical selection grow about as fast as unselected fish transgenic for GH, indicating the presence of additive genetic variance in wild populations sufficient for dramatic growth increases (should selection occur).

Altogether, this work (Muir and Howard 1999, 2000) would generate less argument if the use of the word transgene had been generalized to transgene and other genetic changes. But then, the novelty would be lost, and these "Trojan gene" arguments would devolve to one of the preceding sections (i.e., the probability of generating selectively equivalent mutations before nature).

For the lay reader to assess the credible likelihood of risks implied by Muir and Howard (1999 and 2000) we can consider the implications of their suggestions (1), that existing species are at a credible short-term extinction risk from natural mutations causing meiotic drive, mating advantage, and so forth, and (2), that genetic engineering offers a credible prospect for pest eradication through the release of just a few animals. Certainly there would be great interest, public and financial, in eradicating noxious exotic carp and tilapia in Australia. Unhappily for these purposes, this type of genetic control, albeit using classical chromosome mutations, has been attempted before and has failed (Cantelo and Childress 1974, MacKenzie 1976)). Do we propose that by accident we will achieve something not possible by design?

Likelihood of Risk and Consequences from an Adaptive LMO

Knibb (1997) suggested a potential risk might exist whenever a transgene has a nonnegligible probability of increasing in the wild. Negligible probability events can include issues we do not regulate or insure against such as genetic damage from products of classical selection or damage from a meteorite strike. One lens through which we can glimpse the probably of adaptive mutations in nature is the rate of amino acid sequence divergence, which for coding regions is on the order of 1–2 percent per million years. This change represents an astonishingly small subset of possible mutations when considered in the context of population sizes, mutation rates, number of generations, and the probability that some divergence arises without selection. A requirement that transgenic changes happen before selectively equivalent "natural" changes would suggest that sequence divergence rates overestimate the likelihood of this type of risk.

Consequences of new adaptive intraspecific genetic change will vary and follow a probability distribution. Prima facie evidence (from the natural world today) is that new adaptive polymorphism almost invariably is not associated with significant change (e.g., extinction). Quite the contrary, new polymorphism may contribute to genetic variation important for long-term selection response. The probability for significant ecosystem change is the negligible probability of generating an adaptive genetic change before nature multiplied by the remote probability that a genetic change can alter the community. The likelihood that the change will be perceived as a benefit or cost is not considered here, for this requires value judgments. There is some probability that specific changes will be perceived as beneficial by all.

From experience, the probability of a given genetic change's leading to speciation (reproductive isolation) is even more remote than that leading to adaptive polymorphism. The model used to predict environmental consequences of speciation is the one of introducing exotic species (Knibb 1997) into new environments (e.g., carp, tilapia, rabbits, cactuses or cane toads into Australia). Again the likelihood for change will follow a probability distribution. Only a small minority of introduced fish species cause significant community change (Welcomme 1988). Why do even the minority of species spread? Communities are not coevolved with the exotic, and some exotics can find absence of effective controls (predators, parasites, and competitors). Indeed, the cactus (*Opuntia* spp.) in Australia was controlled

once the moth *Cactoblastis cactorum* was imported and released. Accordingly, it is questionable whether the exotic model is entirely appropriate for cases of speciation within ecosystems or sympatric speciation. If so, then the probability of change from sympatric speciation will be less than that predicted using the exotic model and will be conditional on the probability of producing speciation before nature.

Inasmuch as we explore the hypothetical environmental risks from LMOs, so should we explore potential environmental gains and the consequences and opportunity costs from a philosophical rejection of transgenesis. There is an acute need to reduce pressure on wild fisheries (14 of our 16 major fisheries are overexploited). Aquaculture may reduce pressure, especially when we can find substitutes for the marine fish meal used in aquaculture. Engineering terrestrial plants as fish food, or engineering fish (Knibb et al. 1998) to consume terrestrial plants, may lead to major environmental dividends, as would the engineering of disease resistance, reduced feed conversion rates (FCRs) and so forth.

Conclusion

In part, the background leading to the hypothesis of Knibb (1997) was a perceived need, a gap, to describe a generic testable or falsifiable hypothesis and to do so in the traditional Popperian (Popper 1935) scientific fashion. That is, a falsifiable hypothesis should draw on existing theory and describe available data rather than start with a particular position. To illustrate, we have little evidence for Martians on Earth (albeit arguably more than for adaptive LMOs in the wild; Friedmann et al. 2001), and we do not set the null hypothesis as "there are Martians on Earth." To date, empirical data for a range of species fail to disprove the hypothesis of negligible ecological risk (without large or sustained releases). What is unknown is how long it will take for this hypothesis, without falsification, to be accepted as dogma by regulators and the public.

This paper does not recommend the continuation of expensive -case -by case empirical testing in contained facilities because of potential GxE interactions. If the generic predictions based on theory and cumulative experience are considered inadequate for regulators, then testing should encompass multi-generation fitness assessment of large numbers of different transgenes in wild environments, possibly using model species.

A countervailing philosophy advocates the use of the so-called precautionary principle, which at its most extreme requires proof of universal safety. In this form, and following the canons of Popperian logic, the precautionary principle with its requirement of proof rather than disproof is inherently untestable and hence unscientific. Certainly, the issue of G*E effects makes the problem of comprehensive empirical testing intractable without wild releases. Of more consequence is the understanding that, far from being safe, the precautionary principle may be inherently risky through opportunity costs and failure to replace agricultural practices harmful to human health and the environment (Morris 2000).

Finally, a failure to separate or distinguish between the adaptive profiles of spontaneous and transgenic changes, as suggested here, may mean that science is not the appropriate forum for the transgenic debate, and we should turn to religious (Genesis I:26,28), cultural, or other values for guidance. This sentiment will be of little joy for regulators, but this recognition should advance our understanding of the very roots and nature of the debate. Here, perhaps

we may find consensus, though more likely, contradictions—and I conclude with two. First, do we preserve genomes and environments as static or look to an uncertain future and equate an increase in genetic variance with an increase in fitness (Fisher 1958), an increased ability to respond to selection and survive even without apparent phenotypic change? For the latter, will some view genetic engineering as a potential environmental management tool, redeeming small inbred demes or rescuing others from global climate change? Second, do we view "natural" genetic process as all benign to the environment? If so, how then do we describe spontaneous mutations leading to fitness reduction and possibly extinction? Is evolution dangerous?

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Endnotes

[1] One billion people worldwide rely on fish as their primary source of animal protein
[2] Fish represent 16 percent of animal protein consumed worldwide
[3] From population growth. Also from increasing per capita consumption due to increasing affluence and recognition of sea food as a healthful food.
[4] The Food and Agriculture Organization (FAO) defines aquaculture as "the culture of aquatic organisms, including fish, mollusks, crustaceans, and aquatic plants."
[5] "Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish" (1995) prepared by the Agricultural Biotechnology Research Advisory Committee of the U.S. Department of Agriculture (Documents 95–04 and 95–05).

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Transgenic Living Modified Organisms in Forestry—A Canadian Perspective

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Abstract

Intensive forestry and allocation of forest land for different levels of management are receiving increased attention in Canada. Advances in tree genetic engineering could thus provide unprecedented opportunities. At the same time, we are very conscious that the environmental benefits of transgenic trees may be difficult to maximize because of the complexity of social, public opinion, and regulatory issues. Although the knowledge generated for crop species can to some extent be applied to forest trees on a case-by-case basis, some issues remain unique to the forestry context. The technology for the production of transgenic trees is developing faster than the knowledge required for thorough environmental risk assessments, and there are social acceptance issues. Several discussions have taken place in Canada and internationally on the issues surrounding the environmental impacts of transgenic trees and on research required to address them. These are discussed in this paper.

Introduction

Increasing movement has occurred worldwide towards tree farming or plantation forestry in recognition that this strategy may be necessary to meet future global demands for wood and wood products while addressing the global commitment to sustainable development of our forests (Sedjo 2001). Genetically modified (GM) trees will primarily be used in the tree farm or plantation setting to create high-yield, high-quality products in a sustainable manner. At the same time, if the proper policies are in place, protected areas would be expanded and conservation of natural ecosystems increased.

There are several categories of living modified organisms (LMOs) for use in forestry. These include genetically modified trees, pest control products, and the micro-organisms used to produce enzymes in pulp and paper processing and effluent treatment. This discussion will focus specifically on the potential ecosystem impacts of the release of genetically modified trees into the environment and a comparison with those of GM agricultural crops.

Discussion

Some of the traits of interest for genetic modification of forest trees are similar to those that have successfully been introduced into agricultural crops (e.g., insect and disease resistance, herbicide tolerance and stress tolerance). Other traits are of unique interest to forestry such as lignin modification, improved wood quality, and modification of tree size, form, and performance. It is important to consider not only the theoretical potential of trait modification but also the unintended effects. For example, modification of lignin is of importance to the pulping process for improving the ease of pulping and reducing the use of toxic chemicals, thereby reducing pollution. However, modification of this trait could change critical fitness properties of the forest tree, including tolerance to cold, rendering trees with modified lignin composition impractical for planting in countries like Canada.

Many species of trees have been genetically modified, the most common being poplar species and hybrids. The first report on poplar transformation was published over 15 years ago. Other forest tree species that have undergone genetic modification include pine, spruce, elm, walnut, chestnut, maple, eucalyptus, and birch. Several fruit tree species have been genetically modified, including apple, pear, citrus species, persimmon, plum, apricot, and papaya (Pena and Séguin 2001). The success story of papaya genetically modified for virus resistance, which saved the papaya industry in Hawaii, is well known. Papaya is the only case of a commercialized genetically engineered tree to date.

It is important to realize that the technology for the production of transgenic trees is developing faster than social acceptance and the generation of knowledge required for thorough environmental risk assessments. An integrated approach is provided in Canada through the Canadian Biotechnology Strategy, an active horizontal framework involving several Federal government departments and regulatory agencies that incorporates social, ethical, health, environmental, and regulatory considerations. The Canadian Forest Service is advancing the policy agenda towards the responsible deployment of forest biotechnology products by carrying out research and facilitating Federal, provincial, and ad-hoc expert committee discussions towards the development of sound, science-based regulatory frameworks. The Canadian Food Inspection Agency is responsible for the regulation of importation and environmental release of plants with novel traits, including trees. Several international discussions have taken place on the issues surrounding the environmental impacts of transgenic trees. The Organization for Economic Cooperation and Development (OECD) hosted an international workshop on the environmental impacts of transgenic trees in Trondheim, Norway, in 1999. The objectives and outcomes from this meeting are further described in the Proceedings of the OECD Workshop, 13-15 September, 1999, Environmental Considerations—Genetically Modified Trees. Both environmental and socioeconomic issues were recently discussed at an IUFRO (International Union of Forestry Research Organizations) meeting in Stevenson, Washington, July 22–27, 2001 (proceedings available on line at http://www.fsl.orst.edu/tgerc/iufro2001/eprocd.htm). The issues were further investigated at the workshop of the Pew Initiative on Food and Biotechnology entitled "Biotech Branches Out: A Look at the Opportunities and Impacts of Forest Biotechnology" which was held December 4–5, 2001 in Atlanta, Georgia (proceedings available on line at http://pewagbiotech.org/events/1204/).

Forest trees differ in many ways from agricultural crop plants. There are unique features of the forest trees, forest ecosystems, and forest tree breeding. Trees are large, long-lived perennials that are essentially undomesticated. Forest tree populations have tremendous genetic diversity, can adapt to seasonal environmental stresses, and are highly ecologically competent within complex ecosystems. Forest ecosystems have high species level biodiversity and complex interactions among the forest species. Forest tree breeding differs significantly from breeding of agricultural crops because of the long timeframes involved (i.e. decades) in genetic improvement programs. A rich pool of genes is available in the natural populations of forest trees that have not been tapped, and large gains can still be made by transferring genes within species and among close relatives (Mullin and Bertrand 1998).

Although forest trees differ significantly from agricultural crop plants, the major biosafety issues are quite similar, but with larger and more complex effects. These issues include the horizontal and vertical spread of transgenes, ecosystem interactions, species integrity, and biodiversity. Each of these issues will be considered in turn.

Vertical gene flow, that is gene flow from the genetically modified tree to non-modified relatives, is complicated by wind-borne pollen that travels hundreds of kilometers. Trees are perennial and long-lived, shedding pollen and seed repeatedly. Gene flow from genetically modified trees will occur unless they are completely unable to reproduce in any manner, i.e. through sexual or vegetative propagation. Horizontal gene flow, that is gene flow from the genetically modified tree to unrelated organisms, is also possible. Although there is scientific evidence for gene flow among soil microorganisms and from soil microorganisms to trees—as is the case for *Agrobacterium* infection—there is no scientific evidence for genes to flow in the opposite direction from trees to other organisms.

Control of flowering in transgenic forest tree species is being considered to prevent gene flow from pollen and seed. However, there is concern about the stability of the gene expression that controls flowering. Current regulations and the long life cycle of trees prohibit full scientific evaluation of stability of transgenes over a tree's lifetime. There is some potential for increased yield as a result of energy diversion from flowering into wood production; on the other hand, lack of flowers, pollen, and seeds will have an impact on forest species that depend on these structures for food and other uses.

Forest ecosystems are complex, and the introduction of traits that have no coevolutionary history within the ecosystem, for example insect resistance, may have some unpredicted consequences. Increased fitness could cause changes in adaptive range, and species displacement, and changing competitiveness. However, trees have been evolving for millions of years, optimizing their genetic makeup for fitness, therefore genetic modification is more likely to result in reduced rather than increased fitness. Pest-resistant trees may encourage the development of new pests by opening new niches for previously innocuous organisms. Moreover, as insect pests become resistant to the transgenic tree, any biological control products based on the same product will lose efficacy or become ineffective.

Environmental safety assessment requires a multidisciplinary approach. It requires a baseline understanding of species and ecosystem interactions, a thorough characterization of the novel practices or products, a rigorous analysis of potential environmental consequences, and the development of appropriate tools, protocols, and criteria for risk assessments.

Environmental safety assessment research carried out at Natural Resources Canada's Canadian Forest Service (CFS) in relation to genetically modified forest trees addresses issues such as gene flow from transgenic trees to natural populations, long-term stability of introduced genes, and the potential long-term effects of genetically enhanced trees in the ecosystem. Tools are being sought to analyze the impact of transgenic trees in intensively managed plantations in order to develop sound deployment strategies. Examples of the techniques used include laboratory bioassays, DNA monitoring techniques (DNA markers and microarrays), toxicity assays, modeling, and so forth. Small-scale field trials involving transgenic poplar, white spruce, and black spruce are carried out by the CFS under strict confinement conditions and monitoring protocols under the regulatory authority of the Canadian Food Inspection Agency. These trials are used to develop protocols for tracking the fate of genetically modified DNA in forest soil and litter, to monitor changes in soil microbial populations, and to enhance scientific understanding of the performance of the experimental trees (Bonfils 2001).

Conclusion

Social acceptance of transgenic trees will be highly dependent on our capacity to show that environmental and sustainable management issues have been properly addressed. The issues are more acute for products of genetic engineering. Over the past several years there have been numerous incidents of serious vandalism by radical environmental activists. The World Wildlife Federation has called for a global moratorium on research and development of transgenic trees. There are also international trade issues with the growing demand for certified wood products that are affecting acceptance of forestry LMOs. Several strategic questions arise. Firstly, do we have the tools and methods to predict and assess the potential impacts? Secondly, what is the magnitude and scope of potential environmental impacts of forest management practices and products of biotechnology? Finally, what research areas should be the focus of a sound, coordinated, strategic approach to the use of LMOs in forestry? These will have to be addressed in a well-integrated strategic research and policy framework in order to benefit fully from forest biotechnology opportunities while minimizing the risks. Further information on issues surrounding forest biotechnology, visit the Natural Resources Canada website at http://www.nrcan.gc.ca/biotechnology/english/discuss.htm.

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Summary of the Workshop on Genetically Modified Trees; Aim of the Norwegian Gene Technology Act

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Abstract

The presentation included two parts. First I gave a short summary of some of the main elements from the OECD workshop on genetically modified trees that was held in Trondheim 13th to 15th September 1999. Then there was a short presentation of the aim of the Norwegian Gene Technology Act.

OECD Workshop on Trees

At the OECD workshop on genetically modified trees that was arranged in Trondheim in 1999 presentations were given by 21 experts from 11 countries. The presentations were divided into three different sessions: 1) Present status and future possibilities in gene technology concerning GM-trees, 2) Contribution from forestry practice and silviculture in risk assessment and 3) Systems and challenges in assessing environmental considerations. Some of the main topics discussed and raised by the working groups were:

- Trees include both forest trees and fruit trees. Both can be used in different types of plantations. Their use is often dependent on level of domestication. Many tree species are keystone species in ecosystems, and some forest trees develop their own ecosystems. Basic knowledge of the species biology, interaction and function in the environment was considered very important for the risk assessments.
- Their longevity and size is different from other plants. Some trees (e.g. spruce and pine) can have generation times from 10-20 years and become 100-300 years old. Other tree species can live more than 3000 years (e.g. giant sequoias). Gene flow and spread of pollen and seeds usually occur over longer distances and longer time frames than for most other plants. Transfer into natural populations and introgression with possible effects on fitness was considered important. Possible long-term effects on non-target species can be among the consequences. The ecological significance of gene flow has to be evaluated in the context of the specific trait, rotation and management practices. Monitoring of effects will be difficult in connection with many tree species due to the long life span.
- The long term stability of the genetic modification was considered as a difficult and an important issue to take into account in connection with risk assessments.

- It was considered possible to have different approaches regarding risk assessments for trees in plantations (fruit trees) in comparison with trees under natural conditions or used in traditional forestry.

One important conclusion was to use the "old" OECD principles "case by case" and "step by step" when considering deliberate releases of genetically modified trees.

Norwegian Regulations

Due to many questions regarding socio-economical considerations raised in the discussion the previous day, I decided to present the aim of the Norwegian Gene Technology Act. The Norwegian act differs from most other regulations with respect to considering ethical, social and sustainable questions in connection with applications for commercial and deliberate releases of genetically modified organisms. In section one of the act the purpose is stated as:

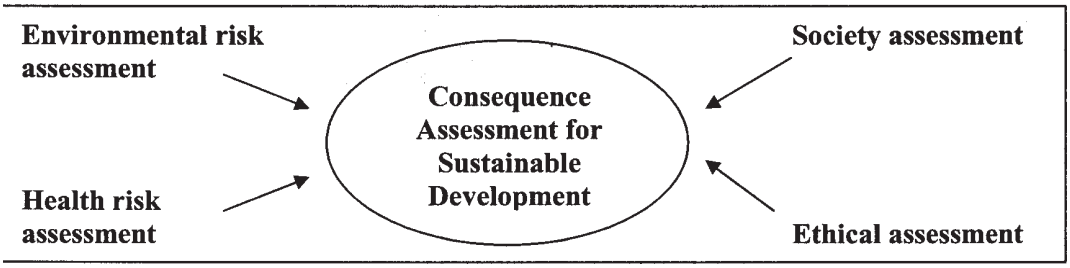
"The purpose of this Act is to ensure that the production and use of genetically modified organisms takes place in an ethically and socially justifiable way, in accordance with the principles of sustainable development and without detrimental effects on health and the environment".

Section 10 the Approval, States That:

"In deciding whether or not to grant the application, significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development".

One attempt to transfer the aim of the act to practical management when treating application for releases of GMOs, has been done by the Norwegian Biotechnology Advisory Board (NBAB) in guidelines released last year. The NBAB has the opinion that the Norwegian Gene Technology Act should be understood in a way that the demand for sustainable development, socially utilitarian value and other ethical and socially considerations, are requirements for a decision that alone can give conclusive weight against approval of an application. However this shall also be considered in proportion to the risk for harmful effects, when this is low.

One way to interpret the broad aim of the act, and which is the goal of the consequence assessment, is as the following figure shows:



The left side represents input from the assessments considering risks in connection with a release, while the right side considers the social justification and the ethical part of the assessment. There can be linkages both between the right and left side and the top and bottom of the diagram. Together the different assessments will merge into the overall assessment of the consequences for sustainable development.

Plants in Uses Other than Food Production: A Summary of Session 3B

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The speakers in Session 3B on plants in uses other than food production presented papers on the environmental use of living modified organisms (LMOs) about which we are not yet very familiar. The examples the participants presented were very interesting, not only for their own merit but also because they lead to more general considerations that are of interest for the discussion of LMOs and the environment. In my response to the session I pointed out these considerations, and several other issues that came to mind while listening to the presentations.

David Heron gave an overview of the regulatory situation around "plant pharming". The regulations aim mainly at preserving the safety and identity of the LMOs during the entire process—from planting to pharmaceutical processing—and at preventing these living modified organism (LMO) plants from entering the food supply in some way, such as by outcrossing. Interestingly, it is anticipated that these plants will not be deregulated.

In the early 1990s the Organization for Economic Cooperation and Development (OECD) Group of National Experts on Safety in Biotechnology discussed safety issues for the scaleup of LMOs in the environment—from field trials to full-scale production. The results of the discussion were published, and one of the points stipulated in this document was that scaleup of "pharmaceutical plants" would require more discussion of the specific risk assessment issues for this application. Over the past decade we have seen that many of the earlier claims about the technique of genetic modification turned out to be untrue, such as the once-asserted precision of insertion using the *Agrobacterium tumefaciens* T-borders or the predictability of expression of cloned sequences. One consequence of risk assessment might be that requirements are imposed that these pharmaceurical GMOs be constructed with special techniques that restrict the possibility of imprecise genetic transformations.

For certain types of LMOs, we might want to require that more reliable strategies be taken for their construction. One emerging strategy is the targeted integration of the modifying sequences at previously selected sites in the genome. Some of the advantages of this method are the avoidance of unexpected gene disruption at the site of integration and "location effects" at the level of expression. The location of the modifying sequence in the genome may influence the rate of outcrossing and the stability of the trait in the population. This restriction of gene location will allow

more stability when different traits are integrated at the same genomic location. These techniques may offer many advantages for production of LMOs having predictable traits, but, as always, we should carefully consider whether new techniques are "nice to apply" or are a "need to apply".

In his presentation on transgenic fish, Wayne Knibb raised the point that transgenic fish may not be that different from fish generated by more traditional genetic techniques. This is a very important observation that is true for LMOs in general. It is often forgotten that some traditional genetic techniques used in plant breeding had an enormous impact on the genome, causing massive rearrangements. Against this background, the so-called pleiotropic effects of genetic modification, (e.g., integrative disruption of genes and mutations caused by random insertion of fragments of the modifying DNA) cannot have many effects that we have not already seen in traditional plant breeding. On the other hand it is clear that genetic modification offers much wider and better focused possibilities for gene exchange. This should be the emphasis of LMO risk assessment and not the pleiotropic effects.

One item on the original program for the meeting but that unfortunately had to be eliminated was bioremediation. This application of LM micro-organisms is important, for it teaches us several important lessons. It turns out to be very hard to get micro-organisms, whether modified or not, to work the way we want them to after their introduction in the environment. And, once we know how to do this at one site, it is difficult to transport that knowledge to another site. If anything, all the research efforts on bioremediation have shown us the versatility of the microbial environment and the genetic plasticity of microbial communities that appear to "share" their gene pool. It is illustrative that bioaugmentation—that is direct injection of a DNA sequence into the soil—works; the sequence apparently is taken up by micro-organisms in the environment that can put them to use. The main problem, however, appears to be how to enhance the activity of the micro-organisms in the environment when it is limited by the supply of energy-rich substrates. An interesting possibility is to use plants for delivery of these substrates into the soil.

I pointed out the impact that genomics is likely to have on the risk assessment of LMOs. Genomics is already causing a revolution in our way of thinking about genomes. That one-third of the genes identified in each new prokaryotic genome sequence are totally new, one-third are only similar to known genes, and only one-third of the genes can be readily recognized as "previously known" shows how limited our genetic knowledge really is. For eukaryotic genomes the situation is similar, but an added difficulty is the recognition of what sequences make up a gene in the first place.

How will the functions of these novel genes be identified? When these genes will be used as donor genes for genetic modification, the paradigms for risk assessment as we have used them until now will no longer be valid. Risk assessment requires extensive knowledge about the role of the donor gene in the physiology of the donor; from that knowledge the role of the gene in the physiology of the host organism can then be predicted, which leads to prediction of the interaction of the resulting LMO with its environment.

We will probably found our knowledge about the properties of these new genes on different types of data derived from the new science of bioinformatics. This is the time for risk assessors to start thinking about what type of data is an acceptable basis for risk assessment and what type of validation of data is needed.

This advance recognition is important just because of the tremendous impact that genomics will have on the possibilities of applied genetics. Our new knowledge may cause revolutions in genetic modification but may also do so in traditional breeding. Linda De Verno was certainly right in pointing out the very extended time scale that tree breeders face in developing a new cultivar. But I have no doubt that genomics information will be applied to shorten that time span substantially so that within the breeder's lifetime he or she may see the product of his or her efforts and maybe even in several rounds of breeding.

The LMOs treated in this session require rigid analysis of their environmental impact. That certainly goes for the transgenic insects that David O'Brochta talked about. In general, however, the call for prediction of environmental impacts of LMOs only points out a very basic lack of knowledge about ecological processes in general. The ecological behavior of LMOs is sometimes easier to study just because of their special characteristics that make them, or their genes, easier to trace. However, we cannot interpret the results of such studies, if we do not know the baseline: what is happening in the environment in the present context before LMOs are extensively planted. We have the feeling that there is great need for fundamental ecological research to supply the tools needed to measure the ecological impact of LMOs as well as to produce the background information needed to interpret the results of the experimentally measured impacts.

Special Session:

Maize at the Center of Origin and Diversity

Transgenic Maize in the Center of Origin and Diversity of the Crop

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Abstract

The need for research on the consequences of releasing transgenic maize in Mexico, located within the region considered as the crop's center of origin, had been identified in the years before the commercial availability of that biotechnological product in the United States. For many years, there was no funding to initiate the scientific investigation required to answer critical questions on the risks and benefits of transgenic maize in its center of origin and diversity. Up to this date, and after the first report on the possible presence of transgenic maize in Mexico, the question of whether or not unique risks are posed by novel crops in a center of origin and diversity remains unanswered.

Introduction

As defined by Vavilov (Harlan 1992), a center of origin and diversity of crops is a biogeographic region where the crop has its largest diversity and a close relationship exists with its wild relatives. Mexico is located within the Mesoamerican region, which has been identified not only as a center of origin but also of domestication of crops (figure 1). Thus, there is also a close relationship with ancient civilizations flourishing in this area of the Americas and the domestication of crops. Likewise, the main centers of primary agricultural development in Asia and the Middle East are associated with the presence of cultures and consequently with domestication of crops.

Through millenia, human populations have intensively and extensively selected and managed plants that have formed the basis of agriculture. Together with the biological forces that shape the evolution of crop plants, human intervention has been a factor of considerable importance that drives diversity of these crop plants.

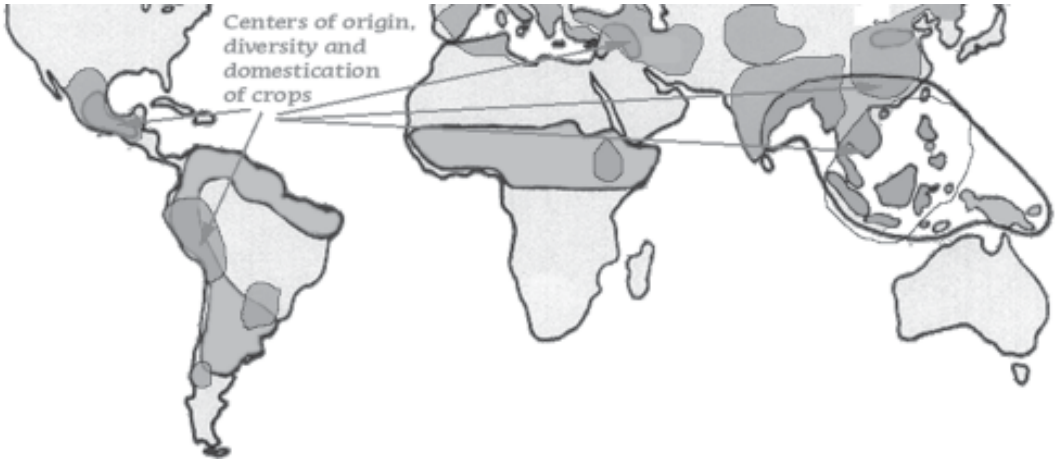

Figure 1. Centers of origin and diversity of crops in the world.

To this date, surviving practices in traditional agriculture in some regions of the world still generate and produce diversity in many crops. In Mexico traditional agriculture can be found in many regions—mainly in the south and southeastern parts of the country.

At least 300 landraces have been identified in Latin America (Goodman et al., 1988). In Mexico more than 40 landraces (figure 2) have been collected from the 1940s to the 1970s, and most of these collections are preserved *ex situ* in gene banks of international public institutions such as International Maize and Wheat Center (CIMMYT) in collaboration with

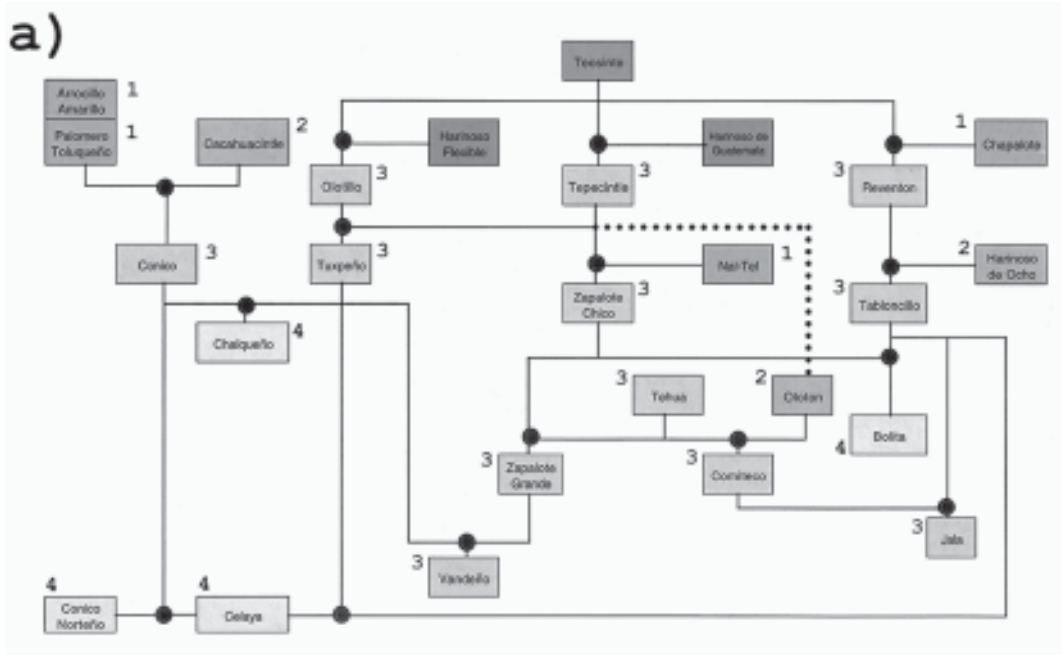

Figure 2.
a) Races of maize in Mexico and their relationships according to Wellhausen et al. (1952): 1) Ancient Indigenous Group; 2) Pre-Columbian Exotic Group; 3) Pre-Historic Mestizos; 4) Modern Incipient Group.

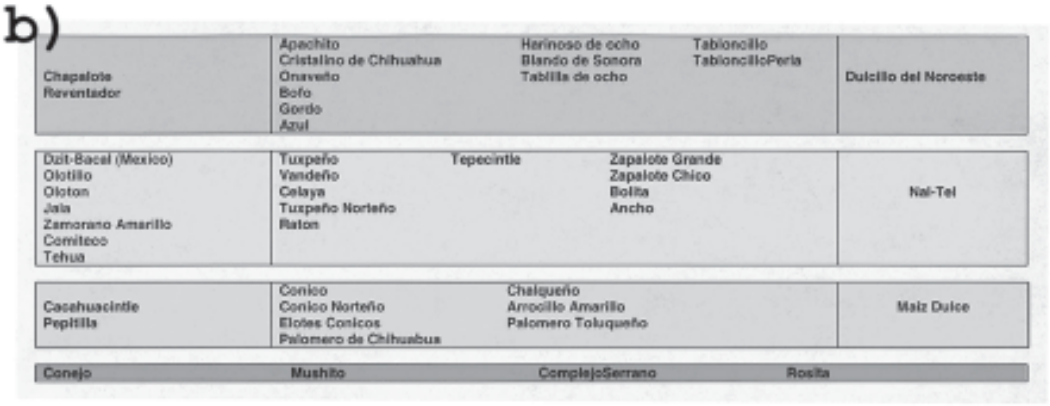

Figure 2.
b) Grouping affinities of the races of maize in Mexico according to Goodman et al. (1988).

Mexican national agricultural research programs. At the same time, projects related to *in situ* conservation of maize have been established, and the dynamics of maize diversity is actually being observed, described, and analyzed.

With the advancement of biotechnology and the production of novel genetically engineered crops, the Mexican Secretariat of Agriculture in 1988 initiated an *ad hoc* committee to cope with the first requests of permits for testing of these biotechnology developments. Soon after these first requests, it was evident that a more formal committee was needed.

With the submission of the request for testing of transgenic maize material in the field, the discussion and assessment of risks for the maize crop in Mexico began. The highly social, cultural, economic, and agricultural importance of maize prompted the interest of the National Agricultural Biosafety Committee (NABC), which started a close consultation with experts on maize crop from many different scientific disciplines. In 1994, the NABC had been consolidated and an official national standard for the release of genetically modified organisms to the environment had been discussed. In 1995, a forum was organized to analyze the implications for maize diversity in Mexico in view of the imminent release of transgenic maize in the United States (Serratos et al. 1997).

Many unresolved matters relating to biosafety and regulation of transgenic maize triggered a new workshop in 1997 organized with the support of the North American Plant Protection Organization (Serratos et al. 2000). After this workshop, several discussions within the NABC and the Secretariat of Agriculture led to the decision in 1998 of a *de facto* moratorium of transgenic maize testing in Mexico.

In 1999, an *ad hoc* committee was organized with the aim of elaborating a report on the status and update of biosafety and regulation of genetically modified organisms, with a particular emphasis on the assessment of transgenic maize. Deriving from that report, the Intersecretarial Commission of Biosafety and Genetically Modified Organisms (CIBIOGEM, in Spanish) was created by Presidential Decree. This Commission overtook the duties of the NABC and expanded its regulatory and policymaking mandates to the environmental and health sectors. With regard to maize, the CIBIOGEM initiated a new consultation with experts from different scientific disciplines in 2001 to elaborate the terms of reference for the introduction, release, and management of transgenic maize in Mexico considered as the center of origin of the crop.

The biosafety and implications of the release of transgenic maize in Mexico, as the center of origin and diversity of maize, had been discussed for many years but only at the academic level. In the next section, some of the ideas that were discussed during these years are presented.

Discussion

The main conclusions and recommendations from the workshop organized at CIMMYT in 1995 (Serratos et al. 1997) were grouped in three sections as described in the following paragraphs.

Gene Flow from Transgenic Maize to Teosinte and Maize Landraces

1. Bidirectional introgression between maize and teosinte should be considered as present in the field even though this may occur at low frequency.
2. Always consider that the probability of gene flow between transgenic maize and landraces is much higher than that between transgenic maize and teosinte.
3. Design and conduct studies to obtain precise quantitative information on gene flow between *Zea* species and varieties to elucidate any possible effects from interactions between transgenes and "native" genes before releasing transgenic maize for commercial use in Mexico.
4. Assign different risk levels in the Mexican territory for field testing with transgenic maize.
5. Place the existing *ex situ* collection of the National Agricultural System (INIFAP) in proper long-term storage and build a national plant germplasm bank to preserve native species.
6. Before commercial release of transgenic maize, collection of the approximately 20 percent of teosinte diversity that was presumably not being collected in Mexico was recommended.
7. Establish a collaborative program to monitor teosinte populations and to salvage the knowledge of communities associated with the management of this germplasm.
8. First target landraces and then teosinte in deciding research and conservation priorities given that the transgenic flow will presumably occur in this order.
9. Begin conservation and characterization of maize and teosinte in zones close to settlements with high demographic growth and in areas with significant ecological changes.
10. Conduct a risk–benefit analysis.

Research in the Area of Risk, Impact and Biosafety

1. The effect of transgenes on teosinte cannot be anticipated or inferred until these transgenes are incorporated into its genome. Therefore, research on maize–teosinte introgression focusing on currently available transgenes should be established in two lines of investigation: (a) insecticidal protein in Bt maize to determine if introgression of these genes contributes to the development of insect populations resistant to the toxin; (b) resistance to herbicides, which could imply two different situations for teosinte populations. In one scenario teosinte could be at danger of extinction because of the application of herbicides that would accompany the

herbicide-resistant maize, and in the second scenario teosinte would develop a greater fitness or increase its potential as a weed because of the introgression of the transgenes.

2. Set up experiments to determine the frequency of migration (m) of maize pollen to fertilize teosinte, the fitness or selective coefficient (s) of maize–teosinte hybrids independent of the selective coefficient of the transgene, and the selective coefficient of the transgene in the hybrid. These parameters could be used in population genetic models and risk analysis. Some of these experiments can be done *in situ*.

Regulation and Safety Measures in Transgenic Maize Tests

1. Field tests with transgenic maize could be carried out in Mexico as long as proper measures were adopted to prevent gene flow to other *Zea* species.
2. Critical questions for transgenic maize in Mexico are not at the laboratory or experimental level, where conditions can be controlled, but at the stage of deciding whether to permit commercial release when there cannot be containment. Therefore, careful analysis of the consequences of deregulation is recommended.
3. The workshop recommended establishing an education and communication program to inform the public about the introduction of transgenic maize and to clarify the decisionmaking process regarding deregulation.
4. The workshop recommended that research on gene flow and the analysis of biological risks derived from the use and release of transgenic plants be a coordinated multi institutional task. This would involve the participation of biotechnologists, ecologists, plant breeders, and other scientists from diverse disciplines.

After this workshop and for some time thereafter some proposals for projects aimed to study the lines of research that were discussed in this forum were advanced. Unfortunately, there was a lack of interest from possible donors, and the necessary funds to finance this research were not available to follow up the recommendations given at the workshop. The only project that was developed after the meeting was financed by the Mexican Seed Association, and the main objective was to determine the frequencies of pollination from maize to teosinte; however, no publications resulted from that investigation nor a report to the Ministry of Agriculture.

Between 1995 and 1997, most of the field testing of transgenic maize was carried out in very small plots within public research institutions under the supervision of the General Directorate of Plant Health. Questions about the impact of transgenic maize on maize diversity were not specifically discussed. Predominantly, the issues related to the analysis of scenarios on the impact of Bt maize and herbicide-tolerant maize in the agroecosystem were discussed at length in different fora.

The workshop organized in 1997, which had a broader scope involving the regulatory systems of the three countries within NAFTA, had the main objective of reviewing the status of transgenic maize in Mexico. The recommendations from this meeting were as follows:

1. That NABC together with a panel of experts establish a working plan for specific research in the area of biosafety.
2. That the framework of a Mexican risk assessment model be elaborated.
3. That maize genetic resources be preserved *in situ*, not only with the view of transgenic

maize impact, but to identify the factors involved in the genetic erosion of the crop.

During the meeting, the opinion of the participants was that not much had been advanced in terms of research after the workshop in 1995 and that, consequently, the main concern was how to get the necessary funding from different sources. Actually, the situation regarding the research on biosafety, genetically modified organisms and genetic diversity, and risk assessment and management did not change that much in all these years from 1994 to 1997.

For several years, however, it had been noted within the NABC that the deregulation of transgenic maize in the United States could be a significant source of grain-containing transgenic material. Also, in the two workshops devoted to transgenic maize in Mexico, the possibility for this mechanism as a port of entrance of transgenic maize into the country was foreseen, but banning the importation of transgenic maize from the United States was not formally proposed. In any case, through NAFTA quotas for grain importation from the United States had been imposed on Mexico.

Again, within NABC several discussions took place among the members to define a position on risk assessment, management, and research related to transgenic maize in Mexico. The NABC sent a report containing different scenarios of transgenic maize regulation to the General Directorate of Plant Health (GDPH). The GDPH, after analyzing the report, stopped receiving submissions for field testing of transgenic maize. With the support of the Under Secretary of Agriculture, a moratorium on the release to the environment of transgenic maize in Mexico was established in 1998.

Reports in the newspapers about the presence of transgenic maize seed in commodities from the United States prompted the creation in 1999 of an *ad hoc* committee that produced a document on the status of the Mexican biosafety system for the president (Sarukhan-Kermez and Larson-Guerra 1999). By the end of that year, the Intersecretarial Committee on Biosafety and Genetically Modified Organisms (CIBIOGEM) was created by presidential decree.

Once again, countless fora were organized to analyze the issues of transgenic organisms, transgenic food, risk assessment, risk management, and policies involving LMOs and products of biotechnology in Mexico. At the same time, the controversy was filled with just opinions from different angles of the problem (Martinez-Soriano and Leal-Klevezas 2000). However, the hard data from research on fundamental questions of gene flow, pest resistance to transgenic maize, gene–transgene interactions, diversity, and human intervention in maize agricultural systems in Mexico were almost completely absent. Only a handful of reports related to basic questions posed since the workshop in 1995 were published (Garcia et al. 1998, Kato and Sanchez 2002, Louette et al. 1997, Luna et al. 2001, Ruiz et al. 2001, Sanchez et al. 1998, Serratos et al. 2001).

In 2001 there were confidential reports stating the possibility that transgenic maize crops were present in Mexico. The National Institute of Ecology and the National Commission for the Conservation and Use of Biodiversity started a survey to analyze these reports (Ezcurra and Soberon 2002, this proceedings). Almost at the same time, the CIBIOGEM organized a seminar to establish the terms of reference for transgenic maize in Mexico. To date, this document has not been published.

Despite the controversy around the work of Quist and Chapela (2001) on the validity of their results, undoubtedly this work pointed out a fundamental issue for the Mexican biosafety

system, which is the assessment, management, and monitoring of transgenic maize in Mexico. All these activities need strong support from scientists and the research system in Mexico. Despite the time that has elapsed it is still possible to address this situation.

Conclusion

The important issues such as uncertainty, risk assessment, risk management, gene flow, prediction, diversity and evolution, environmental complexity, and biotechnology in the maize center of origin have not been thoroughly investigated. Therefore, one question will remain for some time: do novel plants in a center of origin and diversity of crops pose unique risks?

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Evidence of Gene Flow from Transgenic Maize to Local Varieties in Mexico

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Abstract

Maize, originated and domesticated Mexico, is the basis of many food and feed products. Its development is correlated with the development of Mesoamerican civilizations. To date, traditional agricultural practices in Mexico promote and maintain maize diversity. Following a communication related to the presence of transgenic material in Mexican maize landraces, the National Institute of Ecology and the National Commission on Biodiversity started an investigation in collaboration with two national institutions. Here we present preliminary results of the first of a series of tests in progress. We obtained polymerase chain reaction amplifications of the CaMV 35S promoter and the NOS terminator from DNA extracted from maize seedlings grown from seeds collected in different localities at Oaxaca and Puebla in Mexico. Our preliminary data suggest that the frequency of transgenic constructs in the field might be low, although the geographic dispersion seems to be widespread. Further analyses will help to corroborate this pattern.

Introduction

Even though there is still some controversy on the origin and early history of maize, in general agreement exists that the domestication of *Zea mays* occurred in Central Mexico (Kato 1976, Mangelsdorf 1974, Dobley and Goodman 1984, Doebley et al. 1987, Doebley 1990). The development and improvement of maize are correlated with the development of cultural complexity

and the rise of highly organized civilizations in pre-hispanic Mesoamerica. A recent analysis (Piperno and Flannery 2001) using accelerator mass spectrometry to date maize cobs from the Guilá Naquitz cave in the mountainous eastern part of the Valley of Oaxaca established that this sample from about 6,250 calendar years ago represents the oldest maize cobs known to date. Because of the absence of *Zea* macrofossils in earlier sediments of the cave, these phytoliths, unlike pollen grains, provide evidence of an early domestication process somewhere else before settlement in this region.

A high diversity of maize populations is still present in many regions of the Mexican territory, where more than 40 landraces of maize have been described (Ortega 1980, Benz 1986, Sánchez 1989, Wellhausen 1987, Hernández Xolocotzi 1998). Traditional agricultural practices in many parts of the country promote and maintain maize diversity. In places with high biological and landrace diversity, most of the land planted with maize occurs in relatively small units and often in combination with beans and squash. The small farmer and peasant communities are highly open to seed exchange, and it can be observed that the traditional management of varieties leads to a constant flow of genetic material among communities over large areas (Louette 1997). Farmers continually maintain cultivars through seed selection. Through the years Mexican races of maize have been used by Mexican farmers to generate new varietal mixtures as well as creolized materials, which are crosses between modern improved varieties and hybrids with traditional landraces.

The wild relatives of maize, the teosintes, are present in many areas of maize production. Because maize is primarily a wind cross-pollinating species, the possibility of a low-frequency introgression cannot be completely discounted. Maize and teosinte coexist sympatrically and form fertile hybrids (originated from maize plants fertilized by teosinte pollen) in many regions (Kato 1997). Although there are genetic barriers that hinder the fertilization of teosintes by maize pollen (Evans and Kermicle 2001), the risk of gene flow from the cultivated species into its wild relatives cannot be totally ruled out.

In late 2000, researchers from the Zapotec–Chinantec Union (UZACHI) and the University of California at Berkeley initiated a program to document the absence of transgenic markers in traditional maize in the Sierra de Juárez, Oaxaca, with the aim of opening a market for "transgenic-free gourmet corn." However, during the process of setting up their experimental protocols they found that some ears from criollo samples gave positive results for the transgenic 35S promoter. Ignacio Chapela from the University of California at Berkeley, the coordinator of this research program, communicated his findings to the environmental authorities in Mexico. On the basis of this communication, the National Institute of Ecology (INE) from the Ministry of Environment and Natural Resources (SEMARNAT) and the National Commission on Biodiversity (CONABIO) started an investigation to corroborate the results and to evaluate and quantify the levels of the gene flow from transgenic corn to landraces from Oaxaca. The research performed by Chapela was published last year (Quist and Chapela 2001).

Methods

We sampled 21 locations as well as two grain distribution centers. Sampling of maize consisted of both complete ears and harvested seeds. Most locations were small rural communities in the Sierra de Juárez in the State of Oaxaca. Two localities were sampled in the State of Puebla in Mexico (table 1).

Two random subsamples were taken and sent to two independent laboratories: the Center of Research and Advanced Studies (CINVESTAV) from the National Polytechnic Institute at Irapuato and the Institute of Ecology at the National University of México (UNAM). The samples were blind-coded, and the whole procedure was notarized by a Mexican public notary. When the sample consisted of complete ears (i.e., seeds left attached to the cobs), the seeds arising from each different ear were tagged to preserve the maternal identity.

The work at each laboratory followed similar research protocols for better comparison of results. Seeds were treated with a fungicide and planted in controlled conditions. After germination the first leaf was used for DNA extraction. Subsequent polymerase chain reaction (PCR) analyses followed standard protocols.

The DNA was extracted and purified from a total of 1,876 seedlings, which included between 30 and 275 for each location. PCR analyses were performed with primers for the 35S promoter from the cauliflower mosaic virus (CMV) and the nopaline synthase terminator (T–NOS) sequence from *Agrobacterium tumefasciens*. Two series of primers for each DNA sequence were tested. T–NOS 118bp—GCA TGA CGT TAT TTA TGA GAT GGG; T–NOS 118bp—GAC ACC GCG CGC GAT AAT TTA TCC; 35S 195pb—GCT CCT ACA AAT GCC ATC A; and 35S 195pb—GAT AGT GGG ATT GTG CGT CA. We also amplified the 16S nuclear ribosomal gene to test DNA quality. Positive and negative controls were included in each PCR run.

Once both laboratories finished their initial analyses we compared their results for potential discrepancies and pooled them if they did not differ significantly. In the few cases in which significant differences were found in two subsamples, the analyses were repeated to discard contamination and other technical artifacts.

Table 1. Localities sampled in the States of Oaxaca and Puebla.

Loc. #	Locality	State	Municipality	altitude	latitude	longitude
1	Carr. Palmarito-Tehuacán	Puebla	Palmar de Bravo	1419	18°52’	97°38’
2	Jesus Nazareno	Puebla	Palmar de Bravo	2183	18°52’	97°37’
3	Santa Maria Yahuiche	Oaxaca	Ixtlán de Juárez	1806	17°17’	96°28’
4	Santiago Comaltepec	Oaxaca	Santiago Comaltepec	2028	17°33’	96°32’
5	San Pablo Macuiltianguis	Oaxaca	San Pablo Macuiltianguis	2153	17°32’	96°33’
6	San Juan Analco	Oaxaca	San Juan Analco	2138	17°24’	96°32’
7	Santa Maria Jaltianguis	Oaxaca	Santa Maria Jaltianguis	2074	17°21’	96°31’
8	Rancho Tejas	Oaxaca	Ixtlán de Juárez	2075	17°19’	96°28’
9	Ixtlán de Juárez	Oaxaca	Ixtlán de Juárez	2076	17°19’	96°29’
10	Calpulalpan	Oaxaca	Calpulalpan	2242	17°18’	96°26’
11	Santiago Xiacui	Oaxaca	Santiago Xiacui	2041	17°17’	96°26’
12	Santiago Xiacui	Oaxaca	Santiago Xiacui	2041	17°17’	96°26’
13	La Trinidad	Oaxaca	Santiago Xiacui	2035	17°15’	96°25’
14	San Andrés Yatuni	Oaxaca	Santiago Xiacui	2285	17°15’	96°24’
15	Ixtlán de Juárez	Oaxaca	Ixtlán de Juárez DICONSA			
16	Ixtlán de Juárez	Oaxaca	Ixtlán de Juárez Local market			
17	San Juan Chicomezuchit	Oaxaca	Ixtlán de Juárez	1806	17°17’	96°29’
18	San Miguel Amatlán	Oaxaca	Ixtlán de Juárez	2028	17°16’	96°28’
19	Lachatao	Oaxaca	Pueblos Mancomunados	2113	17°16’	96°28’
20	El Punto	Oaxaca	Ixtepeji	2422	17°13’	96°35’
21	Las Presas	Oaxaca	Tlalistac	1653	17°05’	96°39’
22	Nochixtlán	Oaxaca	Nochixtlán	1660	17°27’	97°13’
23	Santo Tomás Teipan	Oaxaca	Santa Maria Ecatepec	2380	16°15’	95°59’

Results

We found PCR evidence for the presence of the 35S promoter in 95-percent of the localities sampled. For all different localities a total of 142 (7.6-percent) seedlings gave positive results for this sequence. All (100-percent) seedlings gave positive results for ribosomal gene 16S. Amplifications indicating the presence of the T–NOS sequences showed consistently lower frequencies (see table 2). A small sample of the PCR amplifications obtained with the 35S primers was cloned and sequenced and compared with the sequence of the 35S CMV promoter. Most of them showed sequence identity whereas one showed a single base pair difference.

In 15 localities we found that less than 10-percent of the seeds showed evidence of transgenic markers. However there was considerable variation in the frequencies found (from 1 to 35-percent). In the sample taken in a grain store at Ixtlán de Juárez, where maize grains for tortillas imported from outside the region are sold, 17-percent of the grains showed amplifications of the 35S promoter, whereas the sample from the local market, where we sampled locally grown *pozole* (stewed maize) grains, showed no evidence of the presence of either marker used.

In five localities (mostly outside the core of the Sierra de Juárez, Oaxaca) we found higher frequencies of transgenic introgression ranging between 10 and 35-percent. These localities are found in the Central Valleys of Oaxaca: in the Mixtec Region, in the southern portion of the Sierra de Juárez, and in the Tehuacán Valley in Puebla. However, the high frequencies observed in these last sites could also be caused by a sample artifact: our sampling involved only a few, randomly selected maize ears on which we arbitrarily sampled individual grains. Thus, there is a fixed experimental maternal effect (what statisticians call "plants-nested-within-sites") that could be driving these results. A logistic analysis (Crawley 1993) of the data did detect significant maternal effects, but these effects were always detected at low-frequency sites. Thus, we can conclude (with some caution) that the sites showing high frequencies are probably places where the frequency of transgenic constructs is significantly higher.

Table 2. Observed frequency of PCR amplification products

LOCALITY	SEEDLINGS	FREQUENCY	
		35S	nos
1	45	0.044	-
2	76	0.039	-
3	94	0.149	-
4	97	0.072	0.04
5	89	0.022	-
6	91	0.011	-
7	128	0.062	0.03
8	105	0.038	-
9	84	0.107	0.05
10	275	0.036	-
11	37	0.351	0.05
12	32	0.062	-
13	163	0.098	-
14	65	0.020	0.02
15	30	0.167	-
16	30	-	-
17	60	-	-
18	60	0.067	-
19	75	0.040	-
20	60	0.033	-
21	60	0.100	-
22	45	0.111	-
23	75	0.173	0.01

Discussion

These preliminary results present provocative evidence suggesting that the amplification of the 35S sequence and the T–NOS are due to the introgression of transgenic sequences into Mexican traditional maize populations. However, because our analysis was done through PCR amplification, the possibility of false positive results cannot be totally ruled out. If these results are corroborated by a series of other analyses currently in progress, the presence of transgenic elements planted in Mexico will be definitely confirmed in spite of a national policy that has put into place a standby moratorium on the planting and cultivation of transgenic maize in the country.

The ecological consequences of the possible flow of transgenic constructs into traditional varieties are not well known, and more research is clearly needed on the subject. Among other consequences, the possible introduction of transgenic constructs into populations of the different species and subspecies of teosintes (corresponding to all the wild species of the genus *Zea*, including all the wild subspecies of *Zea mays*; see Buckler and Holtsford 1996) needs to be studied in detail. Two of the possible consequences that need to be addressed are (a) the potential genetic erosion of the traditional landraces (e.g., Ortega Paczka 1999) and (b) the possible increased weediness of teosinte plants if insect-resistant or herbicide-tolerant transgenes were allowed to drift into the wild populations. Effects on biodiversity in general should also be evaluated.

Our preliminary data suggest that the frequency of transgenic constructs in the field might be low, although the geographic dispersion of the presence of the transgenes seems to be widespread. Further analyses in other parts of the country as well as monitoring and the sampling of additional localities will provide a clearer picture of the situation. However, we still need to know if enzyme-linked immunosorbent assay (ELISA) tests, as well as BASTA resistance experiments and Southern blot hybridization will further confirm this distribution pattern and rule out the possibility of false positives in the PCR analysis.

More extensive sampling—including *milpas* (traditional maize fields) in many parts of Mexico as well as wild populations of teosintes in successive planting seasons—will allow us to define in a more precise manner the trends and the risks involved for biodiversity.

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In Situ Conservation of Maize Diversity, Gene Flow, and Transgenes in Mexico

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Abstract

Mexico is within the primary center of domestication and diversity of maize (*Zea mays* L.). The knowledge, preferences, and farm management practices of small-scale Mexican farmers have played a key role in the evolution of maize and its diversity in the country—a role that is still present and widespread. This paper argues that these same conditions—farmers' knowledge, preferences, and farm management practices—that promoted and maintained maize diversity in Mexico would be conducive to the diffusion of transgenes into maize landraces if they were introduced in Mexico. To assess the potential diffusion and impact of transgenes into maize landraces in Mexico, it is therefore fundamental to take farmers' conditions and management into consideration. The paper describes the way Mexican small-scale farmers manage their maize populations, particularly landraces. It relates this management to the maintenance and evolution of maize diversity and in turn to its conservation in situ and explores the implications of farmers' management for the potential diffusion of transgenes into farmers' maize populations. A key message is that the parameters used in developed countries to assess the environmental impacts of transgenic maize varieties may not be appropriate for the situation in Mexico and Central America where these parameters may be different.

Introduction

Mexico is within the primary center of domestication and diversity of maize (*Zea mays* L.). This diversity is confirmed by the presence in Mexico of most maize races reported for Mesoamerica (Bretting and Goodman 1989). A maize race is the basic taxonomic unit used to describe the diversity of maize landraces[1]. A maize "race" has been defined as "a group of related maize plants with enough to be recognized as a group" (Anderson and Cutler 1942:71). In Mexico 49 maize "races" have been identified (Sanchez and Goodman 1992). Both isozyme analysis (Doebley et al. 1985) and analysis of morphological characteristics (Sanchez and Goodman 1992) indicate that the variability between races is significant. A long history of coevolution connects maize and human populations in Mesoamerica

(Hernandez 1985, Wellhausen et al. 1952). Small-scale Mexican farmers' knowledge, preferences, and management practices have played a key role in the evolution of maize and maize diversity—a role that is still present and widespread. The cultural significance of the crop, its multiple uses by rural communities, and specialized tastes and preferences for foods prepared from the crop are expressed in farmers' selection criteria and the diversity present among the maize populations they grow.

Maize is the staple food of Mexicans—particularly the rural poor. During the rainy season of 2000, about 7.5 million hectares were planted to maize out of a total of 12.5 million hectares planted to annual crops (SAGARPA 2001). About three million small-scale farmers plant maize. Despite the availability of improved maize varieties over the last 40 years and repeated Government programs to encourage their use, today improved varieties are planted in only about one-fifth of the total maize area of the country. Most of this area is located in the commercial production zones of central and northwestern Mexico (Morris and Lopez-Pereira 1999). Hence, about four fifths of the area under maize is planted to landraces or recycled improved varieties (*creolized* varieties). Mexican small-scale farmers are not only heirs to the diversity of maize landraces but continue to maintain it.

This paper argues that small-scale maize farmers in Mexico play a key role in the maintenance and evolution of maize diversity and that the same farmers' conditions and practices that have helped maintain and promote maize diversity in their fields will also be conducive to the diffusion of maize transgenes if they are introduced into Mexico. To assess the potential diffusion and impact of transgenes into maize landraces, it is therefore fundamental to take these farmers' conditions and management into consideration.

The goals of this paper are as follows:

1. To describe the way Mexican small-scale farmers manage their maize populations, particularly landraces
2. To relate this management to the maintenance and evolution of maize diversity and therefore to its conservation in situ
3. To explore the implications of farmers' management for the potential diffusion of transgenes into farmers' maize populations

The rest of paper is divided into four sections. The first describes the way small-scale Mexican farmers manage their maize populations. This is followed by a discussion of on farm (in situ) conservation of maize diversity as a component of global strategy to conserve genetic resources. The third section discusses the implications of farmers' conditions and management for the potential diffusion of transgenes into farmers' landraces. Finally, the conclusions are presented.

Small-Scale Farmers and Maize Diversity in Mexico

Small-scale Mexican farmers' knowledge, preferences, and management practices have played a key role in the evolution of maize and its diversity in Mexico, which is a role that is still present and widespread. Key maize management practices of small-scale Mexican farmers include planting numerous maize "varieties"[2] within a small area or under management by a single farmer, seed recycling, seed flows, mixing seed of different origins, and creolization. Furthermore, farmers' activities have a direct impact on teosinte—the wild relative of maize—"cultivation" and on regulating gene flow between maize and teosinte. A

discussion of the relationship between farmers' practices and teosinte, however, is beyond the scope of this paper.

Landscapes with Multiple Maize Populations.

Many small-scale maize farmers simultaneously plant more than one "variety" to meet different needs and preferences (Bellon 1996). This is particularly important because most farmers consume what they produce, which means that their decisions of what to plant are not only influenced by the agronomic performance of a variety but also by the quality of the end-products such as tortillas, tamales, or atole[3].

Because even within a community farmers are not homogenous they may plant different varieties, which leads to a landscape in which numerous different maize populations coexist side by side (Bellon and Brush 1994, Louette et al. 1997, Perales 1999). Furthermore, because these farmers usually own several small plots scattered throughout the landscape, they are unable to prevent the exchange of pollen between varieties (Bellon and Brush 1994). This condition creates a landscape in which numerous different maize populations are planted side-by-side and an environment conducive to pollen flow among different maize populations.

Seed Recycling

Saving seed from one season to the next (also known as seed recycling) is an almost universal practice among Mexican small-scale farmers. Farmers usually follow strict procedures to choose what they keep as seed for the next season. Saving seed is not only a practice associated with landraces, and saving seed from hybrids is much more prevalent than generally believed (Morris et al. 1999). Seed selection has important genetic implications. First of all, it defines which individuals, and therefore which traits and alleles, go to the next generation and which do not, therefore affecting the genetic structure of the population. Farmers exert direct selection pressures on ear characteristics but only indirect pressures on related plant characteristics such as plant height given that seed is selected in the household and not in the field; hence plant characteristics are rarely taken into account (Louette and Smale 2000, Smale, et al. 1999). It may also be fundamental to maintain the integrity of a variety (at least from the point of view of the farmers), which can easily be lost owing to hybridization (Bellon and Brush 1994, Louette et al. 1997).

Seed Flows

Besides maintaining seed from their own stocks, Mexican farmers commonly acquire it from other farmers or sources in their own community or far away from it. For example, in our work we discovered the introduction of *Zapalote chico* (a tropical race found at sea level) of the Istmo de Tehuantepec into communities of the Central Valleys of Oaxaca 200 km away and at 1,800 meters above sea level. There are several reasons for seed flows. The risk of losing seed of an appreciated variety is a constant threat owing to pests, disease, drought, or frost. Farmers may plant small areas due to socioeconomic constraints, or in the case of particular varieties, such as black or red maize types, therefore easily finding themselves without enough seed to plant the next season (Aguirre Gómez 1999; Louette et al. 1997). There is a common belief among farmers that they must change seed regularly to maintain the productivity of the variety, as Louette et al (1997:31-2) recount "sow the same maize type but

from new seed". As they report, the frequency of seed renewal varies from several cycles to several years.

Seed flows are important to understand the diversity in a given location because they are the basis of incorporating new varieties and obtaining materials that have been lost but are desirable. These flows may have important genetic implications because they may be an important mechanism for the migration of genes and may counter genetic drift and mutation accumulation in varieties planted over very small areas (Louette et al. 1997).

Mixing Seed of Different Origins

It is not uncommon for farmers to get seed from other farmers to plant alongside their own either because they do not have enough seed or with the expressed idea of modifying their maize population. Aguirre Gómez (1999) has described this practice as "partial seed exchange." The modification may involve combining desirable characteristics of a foreign variety with one's own, or it may be done to counter the loss of vigor in one's variety. Many farmers said that after planting a variety for many consecutive seasons, it "gets tired" (*se cansa*), and therefore one needs to add seed from a foreign variety to it. For example, in our work with landraces collected in the central valleys of Oaxaca, when they are selfed they exhibit a high proportion of deleterious mutations probably owing to endogamy. An influx of foreign genes may enhance heterozygosity and hence avoid expression of these mutations.

Creolization

Although the adoption of improved maize varieties has been limited in Mexico, there is increasing evidence that small scale subsistence farmers have incorporated improved varieties into their farming systems, planting them alongside their landraces and, once adopted, managing them the same way as their landraces. These farmers, willingly or accidentally, have promoted the hybridization of improved varieties and their landraces. This process, through which materials produced by the formal plant breeding programs change when placed in the hands of farmers, has been termed "creolization" or "rustication" (Bellon and Risopoulos 2001, Wood and Lenné 1997). Farmers recognize the products of this process as "creolized" varieties (*variedades acriolladas*). They are appreciated because they are perceived to combine the advantages of improved varieties and landraces.

Farmers' conditions and management practices described above can be summarized in the following factors:

- Multiple maize populations coexisting in the same landscape
- Fragmented landholdings (small plots and large border effects)
- Seed recycling
- Short- and long-distance seed flows among farmers
- Creolization
- Partial seed exchanges

These practices and conditions generate important gene flows among distinct, and sometimes distant, maize populations. These flows are fundamental to maintain the viability of these maize populations. Table 1 presents examples of farmers' practices and management conditions that are conducive to gene flow from case studies carried out in Mexico.

Table 1. Examples of Farmers' Practices and Management Conditions

	Chiapas [a]	Oaxaca [a]	Guanajuato [b]
Year	1997	1997	1996
Number of households	98	240	160
Varieties/household			
average	2.4	1.5	1.95
min-max	1-5	1-5	1-4
Fields/household			
average	2.6	3.4	2.2
min-max	1-7	1-9	1-6
Field size (ha)			
average	3.4	0.92	4.22
min-max	.05-13	.062-6	.5-26
Partial exchange households (%)[c]	7.1	30.4	49.3
Total exchange households (%)[d]	43.9	19.6	39.8
Seed flows			
flows (% households)	36.7	37.5	nd
local (% households with flows)	50.0	97.8	nd
non local (% households with flows)	61.1	13.3	nd
Seed recycling (% households)	92.9	96.3	nd

Sources:
[a] Unpublished data CIMMYT;
[b] Aguirre Gómez 1999
[c] Planting a mix of seed from different origins including seed from one's previous harvest
[d] Planting exclusively seed obtained outside the household.

Maize diversity in farmers' fields is not a static condition but rather a dynamic process. Gene flow and farmer selection are the basis of this diversity. Furthermore, gene flow counters endogamy in maize populations planted in small areas. The introduction of "foreign" germplasm can be a source of morphological and agronomic diversity rather than genetic erosion (Louette et al. 1997). Gene flow can occur over long distances with very diverse materials, and even though some may not be appropriate for the environments where they are introduced, they may constitute a source of new alleles for local populations.

Maize landraces are not static and are continuously evolving owing to the gene flow that farmers favor and their selection of maize characteristics for changing conditions and preferences. Maize landraces are open genetic systems that continuously incorporate traits from exotic germplasm, including improved varieties. For example, morphological and genetic analyses of maize landraces collected in the central valleys of Oaxaca, Mexico, have shown that there is a strong selection for morphological traits of importance to farmers, mainly ear and kernel traits, which can be (or are perceived to be) related to culinary qualities. However, when the analysis was done with neutral molecular markers, no clear structure was detected among the landraces from different farmers—that is all the landraces shared the same genetic

neutral diversity, which can be explained by a very strong migration effect (either through seed or pollen, G. Pressoir, pers. comm.)

In Situ Conservation of Maize Diversity

There is a worldwide recognition of the importance of conserving crop genetic diversity. This has led to public investment in the creation and maintenance of gene banks around the world for many different crops (i.e., ex situ conservation; Plucknett et al. 1987). More recently, on farm (in situ) conservation has emerged as an important complement to ex situ conservation (Altieri and Merrick 1987, Maxted et al. 1997) and as part of a global strategy to conserve genetic resources (Brush 1999, IPGRI 1993, Maxted et al.1997, Wood and Lenné 1999).

On-farm conservation involves farmers' continued cultivation and management of a diverse set of landraces in the agroecosystem where they were developed (Bellon et al. 1997). This approach depends on farmers' active participation because it only succeeds to the extent that farmers find it in their interest to maintain diversity (Brush 1991). On-farm conservation seeks to maintain the evolution of crop populations in response to natural and human selection. In the case of maize, conservation aims at maintaining farmer management practices and conditions associated with maize diversity and not necessarily any specific maize population. Hence, the practices and conditions described above are at the core of the conservation of maize genetic resources on-farm in Mexico.

Maize Diversity, Farmers, and Transgenic Varieties

The recent discovery of transgenic products in maize landraces planted by small-scale Mexican farmers has caused great concern (Quist and Chapela 2001). Although these results have been questioned (Christou 2002), they point out the need to look into the potential spread of transgenes into maize landraces in Mexico[4]—the center of origin and domestication of maize—and their potential impact on the environment, biodiversity, and the livelihoods of small-scale maize farmers.

The management of maize germplasm by Mexican small-scale farmers is very different from that of their counterparts in the USA, Canada, and Western Europe where hybrids dominate maize farming, farmers purchase seed from commercial sources and have large landholdings (particularly compared with small-scale Mexican farmers). Clearly these conditions contrast with the conditions of Mexican farmers presented above. Therefore, the parameters used in developed countries to assess the environmental impacts of transgenic maize varieties may not be appropriate for Mexico. In this country, it is fundamental not only to take into account biological factors, but also the practices and conditions of small-scale farmers that inadvertently or even willingly may or could introduce transgenes into their agroecosystems. For example, a simple approach to assess the potential diffusion of transgenes may be to measure the distance at which pollen can flow and remain viable (e.g. Luna et al. 2001).[5] However as illustrated above, the conditions and practices of Mexican farmers foster pollen and therefore gene flow, which is basic for the maintenance of the diversity and viability of their landraces.

One can hypothesize that if small-scale Mexican farmers have access to transgenic varieties,

and if these varieties are perceived as valuable by them, they will foster their diffusion wittingly or unwittingly into their local maize populations. The same farmers' conditions and practices that maintain and promote diversity in their fields and farms may lead to the diffusion of transgenes into their landraces if transgenes are introduced. Clearly, this is a complex process that merits much research since there are many unknowns. For example the diffusion of transgenes may depend on the scale of introduction of transgenic varieties, on the genetics of the associated transgenes, and the fitness that those transgenes may confer to the populations they enter. However, this fitness cannot be assessed purely on biophysical factors, but also needs to be evaluated in terms of farmers' management practices and their cultural preferences.

There are many questions that have to be addressed if the spread of transgenes to maize landraces happens. Some of those questions are: Is this diffusion positive or negative? For whom is it positive and for whom is it negative and why? Does the spread of transgenes jeopardize genetic diversity, and if so, how? What may be the impact of the diffusion of transgenes on the livelihood of small-farmers who depend on maize for their sustenance? How would the owners of these transgenes react to their diffusion into non-target maize populations? How would maize consumers react to this?

The answers to some of these questions should be addressed through scientific research, while others have to do with values and preferences of different members of society. This in turn requires a broad debate on the potential benefits and costs of introducing transgenes in areas such as Mexico. For all these reasons, it is important to recognize the complexity and uncertainty faced by scientists, policy makers and society in general in trying to assess the potential diffusion and impacts of transgenes in a center of maize diversity and domestication.

Conclusions

Small-scale Mexican farmers' knowledge, preferences, and management practices continue to play a key role in the evolution of maize and its diversity. This role is fundamental for the conservation of this diversity on-farm. However, the same farmers' conditions and practices that maintain and promote diversity may lead to the diffusion of transgenes into their landraces if transgenes are introduced. To assess the potential diffusion and impact of transgenes into maize landraces in Mexico, it is fundamental to take farmers' conditions and management into consideration. These conditions are different from those of farmers in developed countries and the parameters used in developed countries to assess environmental impacts of transgenic maize varieties may not be appropriate for Mexico. Furthermore, recognizing the importance of farmers and their management for these issues, points out the complexity and uncertainty faced by scientists, policy makers and society in general in trying to assess the potential diffusion and impacts of transgenes in a center of maize diversity and domestication.

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Endnotes

1. The concept of a landrace is complex (Zeven 1998), and here we used this term for a locally grown maize population that a farmer cultivates and manages as a seed lot. A seed lot is defined as "…all kernels of a specific type of maize selected by a farmer and sown during a cropping season to reproduce that particular maize type" (Louette et al. 1997:24).

2. Here we use the term "variety" to refer to farmer varieties: crop populations that a group of farmers recognize as distinct units, regardless of whether they are landraces, improved, or creolized varieties. This definition contrast with the one used in the context of developed country agriculture, where a variety is defined as a plant grouping within a single botanical taxon of the lowest rank, which grouping can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes. Additionally for a commercial variety it should be new, distinct, uniform, and stable (UPOV, 1991).

3. These are traditional maize preparations common in Mexico.

4. Many of the issues described in this paper for maize farmers in Mexico are pertinent for many parts of Central America, which are also within the center of domestication and diversity of maize.

5. To be fair to Luna et al. (2001) they just focus their research for research scale plantings in the context of maize research activities.

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Potential Consequences from Contamination of Maize Landraces and Teosintes by a *Bt* Transgene

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Abstract

Scientists have recently found evidence of transgene contamination of Mexican maize landraces. Most likely the contamination is from a gene encoding a *Bt*-endotoxin. In this article we discuss the potential for further spread of the gene to other landrace and teosinte populations. We review the scientific literature on theoretical consequences of gene flow for rare populations. We also consider broader ecological impacts of the introgression of this specific transgene, which codes for an insecticidal protein. To prevent harm, we argue that steps must be taken similar to those of the U.S. Environmental Protection Agency in its efforts to prevent *Bt*-transgene contamination of a wild relative of cotton, *Gossypium tomentosum*. The genetic resources found in the Mexican maize and teosinte center of diversity are at least as valuable as those of *G. tomentosum*; similar provisions to prevent gene flow should be taken to safeguard this global heritage.

Introduction

In September 2001, the Mexican Government announced the discovery of transgenic sequences in maize landraces in Mexico; research published 3 months later in the journal *Nature* confirmed the findings (Quist and Chapela 2001). Environmental groups had long argued that uncontrolled release into the environment of engineered crops would lead to contamination of centers of diversity (see, for example, Rissler and Mellon 1996). Industry and Government officials alike made pronouncements about the undesirability of such an event. In 1998, the Mexican Government took the unprecedented step of placing a moratorium on all planting of transgenic maize within the country to prevent contamination of an important center of diversity for one of the world's most essential staple crops.

Now that contamination has taken place, much scientific revisionism is occurring. Industry representatives who once swore never to sell transgenic crops in their centers of origin are now claiming that no harm will result from the introgression of the *Bt* transgene into maize landraces and teosintes.

Early on in the debate over the ecological consequences of engineered crops, scientists argued that most transgenes would have associated fitness costs and would quickly disappear from recipient populations (Tiedje et al. 1989; but see also Bergelson and Purrington 1996). Results from empirical research on this topic call into question this original assumption. Several studies conducted during the 1990's show persistence and spread of monitored genes—even those that might have a fitness cost associated with them. It has also been argued that F1 hybrid sterility, in many cases, would serve as an effective barrier to gene introgression. However, many crop plants produce fertile hybrids when mating with wild relatives.

In this paper, we review the published results of empirical research that challenges these assumptions. We also review recent contributions to evolutionary theory regarding the consequences of gene flow and introgression for small and rare populations such as those of maize landraces. Finally, we consider the impact of the introgression of a particular transgene, the *Bt* gene, with regards to broader environmental consequences it may pose.

Hybridization

Introduced maize varieties can hybridize with local cultivated varieties, landraces, or teosintes. The only outstanding question regards the degree of hybridization that might occur. How much hybridization will take place depends on the proximity of the transgenic crop to landrace or teosinte populations. In the case of teosinte, it will also depend upon the frequency with which outcrossing occurs between the species. Outcrossing rates vary depending on the teosinte species (Wilkes 1972; Doebley 1990; Castillo, González and Goodman 1997).

Gene Flow and Introgression

"***Gene flow can be a potent evolutionary force.***" (Ellstrand et al. 1999)

There is little dispute over whether gene flow and introgression will result from hybridization between transgenic maize, landraces, and teosintes, though some scientists challenge whether introgression will be anything more than a transient phenomenon. Three main theoretical concerns over gene flow between transgenic varieties and landraces or teosintes—each with implications for maize diversity—have been identified: outbreeding depression, swamping, and permanent introgression of the transgene.

Swamping and outbreeding depression

Outbreeding depression is a reduction in fitness due to hybridization (Ellstrand 1997); swamping is also known as genetic assimilation.

> Outbreeding depression from detrimental gene flow will reduce the fitness of a locally rare species that is mating with a locally common one. An alternate route to extinction is by swamping, which occurs when a locally rare species loses its genetic integrity and becomes assimilated into a locally common species as a result of repeated bouts of hybridization and introgression. We would expect swamping to result from gene flow that is largely neutral or beneficial.

> Both outbreeding depression and swamping are frequency-dependent phenomena and show positive feedback. With each succeeding generation of hybridization and backcrossing, genetically pure individuals of the locally rare species become increasingly rare until extinction occurs. *Both phenomena can lead to extinction rapidly* (Ellstrand et al. 1999, emphasis added).

Outbreeding depression in maize may be manifested as a general decrease in yield or as other agronomic effects on landrace populations. If a farmer is unhappy with the agronomic characteristics of seed from landrace–transgenic crosses, he or she is unlikely to maintain that lineage. When that happens, the genetic information contained in those seeds will no longer be reproduced year after year and will be lost (Rhymer and Simberloff 1996). Diversity may also be lost without selection by the farmer. When reproductive effort is spent on hybridization that results in less fit offspring, there can be consequent loss of diversity (Rhymer and Simberloff 1996).

Swamping can be expected if farmers save seed from the transgenic–landrace hybrid for replanting or if there is a continual influx of transgenic seed into the farming community.

Both landraces and teosintes are found in small populations and may be at risk from these processes. As noted by Arriola (1997), "this potential loss of genetic variation can be argued to be the most pressing biological threat to the populations of teosinte and maize land races at present."

Introgression

Introgression of genes between crops and wild relatives is a well-documented phenomenon, including introgression between maize and teosinte (Doebley 1990, Kato 1997). Numerous researchers have considered the questions of hybridization and introgression as related to transgenes over the past decade (see, for example, Langevin 1990; Klinger and Ellstrand 1994; Mikkelsen et al. 1996; Arriola and Ellstrand 1997; Whitton et al. 1997; Linder et al. 1998; Ellstrand et al. 1999; Snow et al. 1999, 2001; Klinger 2002; and Bergelson and Purrington 2002). In particular, these laboratories have investigated the fitness of hybrids between crops and their wild relatives and questions regarding the persistence and spread of an introgressed transgene.

A significant factor determining whether introgression will occur is the fitness of the first-generation hybrids. For a gene to make its way into a wild population, it first must pass through the F1 hybrid generation, which is often of much lower fitness than either the crop or the wild relative. Research examining fitness of crop–weed hybrids in *Raphanus sativus* and between *Sorghum bicolor* and *Sorghum halepense* found hybrids with fitness equivalent to, or exceeding, that of wild siblings (Klinger and Ellstrand 1994; Arriola and Ellstrand 1997). In reporting results of their research on sunflowers (*Helianthus annuus*), Snow and colleagues described the F1 barrier to introgression as "quite permeable" (Snow et al.1998). Linder et al. (1998) came to similar conclusions from their work on sunflowers: that there was a "lack of a strong correlation between hybrid fitness and potential for gene dispersal."

Scientific advisors to the U.S. Environmental Protection Agency (EPA) (United States Environmental Protection Agency 2001a) have commented on the F1 hybrid barrier and the cological significance of gene flow:

> First generation hybrids may pose minimal threat if they have low vigor or are infertile. However, even infertile hybrids could pose a threat if they are able to reproduce asexually. The production of fertile F1 hybrids would create a genetic bridge between lineages that would promote introgression. Introgressive hybridization can occur when transfer of transgenes from one lineage to another requires the establishment of fertile F1s and backcross hybrids. Finally, there is a chance for polyploid speciation. The production of fertile F1 hybrids between normally incompatible lineages is possible via chromosome duplication after fertilization. Such polyploid species are fully fertile

> In most cases where wild relatives co-occur with transgenic crops, some gene flow would be expected even at substantial distances… Even if gene flow is low (<1%), it may result in evolutionary changes in recipient species if selection favors the new trait ***Rare hybridization events can be ecologically important—even a single event***. (emphasis added)

In general, genes can be detrimental to the recipient population, they may be neutral, or they may be beneficial. As noted in the introduction, many scientists had originally assumed that transgenes were inherently problematic for a plant and would likely eventually be lost, that is, they considered that permanent introgression of a transgene was unlikely to occur. Linder et al. (1998) concluded otherwise:

> A transgene will be prevented from introgressing into a sympatric wild population only if it lowers fitness or is tightly linked to a gene that lowers fitness. Advantageous or neutral transgenes will quickly spread into wild populations.

Whitton et al. (1997), also working in sunflower, agree:

> We conclude that neutral or favorable transgenes have the potential to escape and persist in wild sunflower populations...cultivar genes are capable of persistence in weedy populations, and thus even low levels of hybridization may result in transgene establishment in weedy sunflower populations.

Even crop genes that reduce the fitness of a crop–weed hybrid have been shown to be maintained in weed populations over time (Snow et al. 1999; Snow et al. 2001).

What empirical evidence shows, and what researchers have predicted based on population genetics theory, is that genes associated with increased fitness, such as resistance to herbivores (including insects), herbicides, or environmental stress, may easily spread in recipient populations. An insect resistance gene such as the *Bt* gene is expected to confer a benefit on the recipient plant.

Some authors (Martinez-Soriano et al. 2002) have asserted that in nature there are no pests that limit teosinte in the wild. They argue that because the *Bt* gene will not confer any benefit on teosinte it will not persist in wild populations. The research cited above challenges this conclusion. Moreover, as Power (2002) has shown, initial assumptions about the pest resistance of crop wild relatives can be incorrect. Such assertions by Martinez-Soriano et al. (2002) regarding teosinte–pest dynamics are inappropriate without corresponding empirical evidence. Indeed, if teosintes are limited by insects that would be killed by the Bt toxin, the introgressed gene could prove advantageous to recipient populations. Those populations may pose increasing problems for farmers.

If the gene introgresses and persists in landrace or teosinte populations, the gene product may eventually be widely distributed in the environment across space and time. This geographic and temporal spread is cause for concern because of the numerous other ecological problems that could result from introgression of a transgene, including impacts of the transgene product on nontarget organisms (Obrycki et al. 2001, Letourneau et al. 2002). These potential impacts are detailed in the following section.

Environmental Effects of the Bt Gene

According to a recent U.S. EPA scientific advisory panel (U.S. Environmental Protection Agency 2001a), potential consequences of *Bt* transgenes include "increased fitness, increased invasiveness and weediness." Such an insect resistance gene is considered by scientists to be a fitness-enhancing gene and thus be likely to increase in frequency and spread throughout local populations. Following introgression into landraces and teosinte, the *Bt* gene could have broader ecological impacts, through

- persistence of the *Bt* protein in the soil with toxicity to soil organisms
- toxicity to nontarget herbivores, predators, and parasites (natural enemies of affected pests)
- the development of resistance to *Bt* in affected pests

Impact on Soil Organisms

Because of the crucial role that soil organisms play in soil health, it is necessary to understand how different agricultural practices affect them. *Bt* crops may be problematic for long-term soil health, because they express proteins known to be toxic to certain insects such as lepidopterans (moths and butterflies) and coleopterans (beetles) and are suspected of being toxic to a range of nontarget organisms as well, including earthworms (Marvier 2001). An unknown number of species make up the soil food web and could be affected by *Bt*, yet, tests have been conducted on very few, in very few soil types and ecosystems.

If the *Bt* deposited in the soil by these crops has an impact on soil organisms—bacteria, fungi, insects, worms—there will necessarily be downstream effects. If you kill or otherwise reduce the activity of any of these soil organisms, you disturb the web of relationships necessary for carrying out essential ecosystem functions such as decomposition and nutrient cycling.

According to the U.S. EPA's scientific advisory panel, Cry proteins "are likely to be present in the rhizosphere soil not only throughout the growth of the crop, but perhaps long after the crop is harvested" (U.S. Environmental Protection Agency 2001a). Therefore researchers and regulators must assume "that continuous exposure to Cry proteins is likely within the soil system." The panel concluded that "it would be prudent to determine under operational field conditions in different geographical regions and soil types, the extent to which Cry proteins accumulate in soil" (U.S. Environmental Protection Agency 2001a). They drew attention to studies that showed *Bt* could persist in certain soil types for up to 234 days (Koskella and Stotzky 1997, Tapp and Stotzky 1998) and recognized that further studies needed to be done to determine whether the persistence of *Bt* would cause problems for nontarget organisms and the health of the soil ecosystem.

As noted by Benbrook (1999), in addition to long-term research on impacts of *Bt* in soils,

> Research is needed on the short-term soil microbial community impacts of a big dose of *Bt* as corn trash and other crop residues break down in the spring and early summer. One might hypothesize that under some circumstances, *Bt* entering the soil will impact soil microbial communities in ways that lead to complex, multi-tier impacts on microbial and soil insect biocontrol, pathogen pressure, immune response and nutrient cycling. Even if the impacts last only 4 to 8 weeks, that is ample time to leave a lasting mark on the performance of the cropping system, both in one season and over many years as microbial communities evolve to a new steady state.

Impacts on Non-target Organisms

As noted in the previous section, genetically engineered crops can have impacts on organisms other than those they are intended to kill. The impact of *Bt* corn pollen on Monarch butterflies is the most well-known example of this phenomenon (Losey et al. 1999, Hansen Jesse and Obrycki 2000, Sears et al. 2001, Losey et al. 2002). Other organisms that have been shown to be affected by *Bt* crops are lacewings, which are beneficial insects that play an important role in the natural control of crop pests (Hilbeck et al. 1998a, 1999). Both earthworms and collembola (other small soil-dwelling invertebrates) have been shown to be affected by *Bt* crops (EcoStrat 2000, Marvier 2001).

Changes in populations of both other pests and of natural enemies have been documented in *Bt* cotton. Data from China show that use of *Bt* crops can exacerbate populations of other secondary pests, including aphids, lygus bugs, whiteflies, Carmine spider mites and thrips (Cui and Xia 1998). Cui and Xia (1999) have shown significant reductions in populations of the parasites *Microplitis* sp. (88.9-percent reduction) and *Campoletis chloridae* (79.2-percent reduction) in *Bt* cotton fields. Data being collected in India indicate higher levels of aphids and jassids in *Bt* cotton fields (Ghosh 2001). Wold et al. (2001) recently demonstrated impacts of *Bt* corn on field populations of *Coleomegilla maculata*, a predatory coccinellid commonly found in corn fields.

Insect Resistance

A large literature exists on the ability of pests to develop resistance to pesticides, including pesticides such as *Bt* that are engineered into the plant. Local lepidopteran pests of maize susceptible to *Bt* would initially be controlled by *Bt* expressed in transgenic maize or landraces or teosintes that were recipients of the transgene. However, owing to the continuous selection pressure exerted by the transgenic plants, populations of pests are likely to develop resistance to the *Bt* protein. Resistant pest populations would cause problems for those farmers deliberately using *Bt* as a topical insecticide.

Defining Harm

Muir and Howard (2002) define the potential harm of a transgenic organism as a composite measurement between the risk of transgene introgression and hazards posed by the transgene if permanent introgression occurs. They observe that a

single outcrossing event could pave the way for transgene introgression. They also point out that

> "long-term hazards to the ecosystem are difficult to predict because not all non-target organisms may be identified, species can evolve in response to the hazard, and a nearly infinite number of direct and indirect biotic interactions can occur in nature" (Muir and Howard 2002).

This understanding leads the authors to conclude that the only way to ensure the environment will not be harmed is to release only those transgenic organisms whose fitness is such that the transgene will not spread. In the case we are considering here, *Bt* maize, it is clear that the only way to prevent the spread of the gene through landrace and teosinte populations, and to prevent harm, is to prevent the introduction of *Bt* maize into Mexico. Indeed, this has been the policy of the Mexican government to date.

Preventing Harm

The EPA recently published revised restrictions on the cultivation of *Bt* crops. Contained in the EPA decision document (U.S. EPA 2001b) are provisions to prevent gene flow from *Bt* cotton to wild and feral relatives of cultivated cotton. These provisions, and EPA's justification for the restrictions, are detailed below:

> *Gene flow containment provisions*
>
> The most obvious concern is the development of weediness, but also concerns of biodiversity and loss of genes that might provide value in plant breeding have been considered.
>
> Adequate data do not exist to complete a full risk assessment on the effects of the *Bt* Cry1Ac protein in wild cotton. Until thorough research on the impacts of gene flow can be completed, restriction [sic] on where *Bt* cotton can be planted are being implemented.
>
> In light of the lack of basic biological data (e.g., pollinator ecology, compatibility/sterility factors, potential impact of *Bt* on herbivores, distribution of native populations) on *G. tomentosum*, the wild Hawaiian cotton, conservative measures are needed to mitigate hybridization with cultivated cotton on these islands. Similarly, the paucity of data on the distribution of feral cotton in the U.S. Virgin Islands and Puerto Rico indicates the following terms and conditions must be instituted to mitigate gene flow concerns:
>
> a. No planting of *Bt*-cotton south of Route 60 (near Tampa) in Florida,
> b. Commerical culture of *Bt*-cotton is prohibited in the state of Hawaii,
> c. Test plots or breeding nurseries established in Hawaii must be surrounded by 24 border rows of a suitable pollinator trap crop regardless of the plot size and must not be planted within 3 miles of *Gossypium tomentosum*,
> d. Commercial culture, experimental plots and breeding nurseries of *Bt*-cotton are prohibited in the U.S. Virgin Islands, and
> e. Commercial culture of Bollgard cotton is prohibited in Puerto Rico. Test plots or breeding nurseries established on the island of Puerto Rico must be surrounded by 24 border rows of a suitable pollinator trap crop regardless of the plot size and must not be planted within 3 miles of feral cotton plants.

Certainly the genetic resources found in the maize and teosinte diversity of the Mexican center of origin are at least as valuable as *G. tomentosum*; nothing short of similar provisions to prevent gene flow should be in place in centers of origin of all of our most valuable crop plants.

Conclusions

1. **"Plant genetic resources for food and agriculture are a common concern of all countries"** (International Treaty on Plant Genetic Resources 2001).

2. **"Recognizing that states have sovereign rights over their plant genetic resources for food and agriculture, we also confirm our common and individual responsibilities in respect of these resources"** (Leipzig Declaration on Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture 1996).

Clearly questions remain regarding the potential impacts of *Bt* introgression into maize landraces. Population genetic theory predicts that the transgene is likely to spread throughout the landrace population. There may be consequences for those small populations from swamping or outbreeding depression. Additionally, the evidence for environmental effects of the *Bt* gene in the environment is wide-ranging, including impacts on nontarget organisms and alterations in populations of secondary pests, natural enemies, and parasites.

The international community, in numerous international agreements and declarations, has repeatedly emphasized the crucial importance of centers of diversity to future food security. The maize landraces and teosintes of Mexico are an essential component of this valuable diversity. To allow an open-air experiment on the impacts of transgene introgression to continue in a center of diversity is to abdicate the responsibility of the world community to protect this precious heritage for future generations.

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Session 4: Future Needs: Unique Challenges and Opportunities for Environmental Assessment

Research and Monitoring in the Industrialized World: European Commission Policy and Experience

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Abstract

The European Commission has been active in supporting genetically modified organism safety research for some 15 years. This paper examines how the Commission has gone about research on genetically modified organisms in the environment, considers the governance of this research, and identifies suggestions for future research priorities. Although no particular safety or environmental problems have been revealed, the multinational consortium approach is considered particularly valuable for this type of work. Communication of safety research results appears to be a particular bottleneck—especially from the perspective of public perception.

Introduction

It has been European Commission (EC) policy from the beginning to accompany its research programs in biotechnology with research on safety aspects. In the last 15 years, over 80 projects involving over 400 research teams and a European Community financial contribution of over €70 million have been supported in the area of genetically modified organism (GMO) safety. The research has covered investigations of plants, plant microbes, biocontrol, food, bioremediation, fish, and vaccines. A review of this work is given by Kessler and Economidis (2001).

Most of this work has dealt with the environment—even the food safety research. This is because many of the food issues actually derive from traits that had been introduced for environmental objectives such as pest control. Many consumers are now demanding choice, not only in the characteristics of the products they buy but in the characteristics of the production chain that produced them and, in particular, the environmental impact of that production chain.

In discussing GMO use, a message frequently repeated is that more research is needed or, worse, that no research has been done at all. Although further research may be justified, there is obviously a corresponding need for communicating research results that are already available, and this conference is particularly significant in this respect. This paper will examine how the EC has gone about research on GMOs in the environment, consider the governance of this research, and conclude by looking at some suggestions of future priorities for research.

The EC's risk assessment research

The first question asked in the early 1980s was, Does genetic engineering make an organism riskier? Tackling this question revealed gaps in underlying knowledge in disciplines such as ecology, population genetics, soil science, and biodiversity. Research rapidly built up to a peak in the mid-1990s. However, by the later 1990s a public backlash to the technology set in, and new areas for concern were raised (e.g., virus resistance and nontarget effects); this gave further impetus for going into more detail in the research program.

In supporting this research the EC took no preconceived position and considered that genetically modified organisms (GMOs) are neither inherently risky nor inherently safe. Research followed the classical pattern of examining the organism, the insert, and the environment; taking a precautionary approach; and identifying the two phases in risk assessment: hazard identification and frequency of occurrence.

Several features make the EC approach to research funding particularly appropriate to GMO safety research. First, it is undertaken by multinational consortia of teams from any entity with an appropriate research capacity, it can include other organizations like nongovernmental organizations (NGOs), consumer groups, or farmers' organizations that can contribute to exploitation or communication of results; and it is open to participation from third countries. Because this is publicly funded research, there is an obligation to exploit results. Second, in addition to standard peer-review criteria, considerable emphasis is placed on societal and policy criteria, which for modern life sciences are becoming increasingly important. This research is termed prenormative, that is, it precedes the establishment of regulations and embodies the idea that science underpins regulation. This idea is sketched in figure 1, which illustrates research as a dynamic entity driving regulation and management and leading to outputs in the form of accumulating experience and best practice as well as determination of long-term effects.

The European Union (EU) research activities complement national activities and the research undertaken in preparation for submitting dossiers to fulfill regulatory requirements. These research activities are an example of regional scientific cooperation that is implementable in other parts of the world, and through which groups of countries with common problems or common ecological conditions can benefit enormously through complementation, sharing of skills, and achieving a critical mass of activity, which are attainments smaller countries might not be able to realize on their own.

Figure 1

GMO safety research, regulation and practice: synergy and feedback

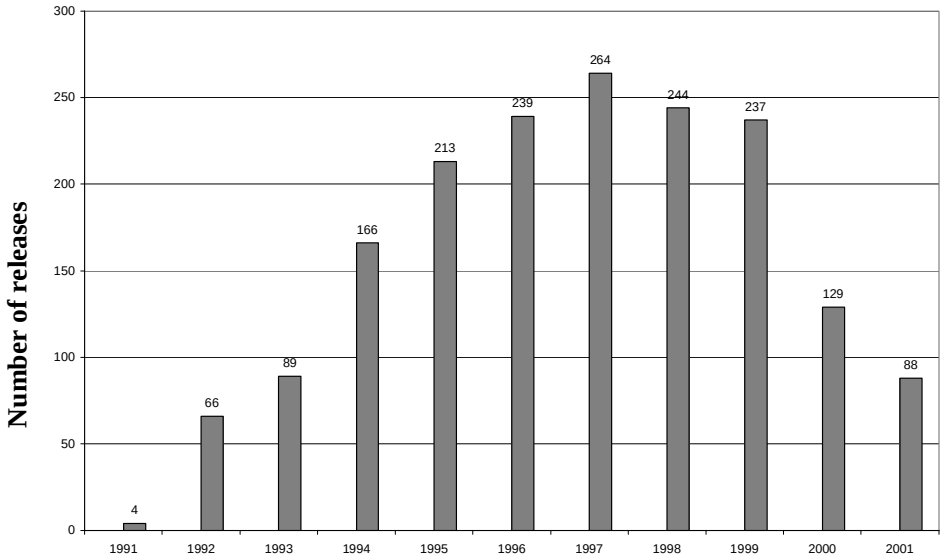
Examples of recent research include ecological effect of gene flow in virus-resistant plants, effects of *Bt* transgenes on nontarget biodiversity, the impact of biotechnological approaches to potato pathogen control on soil microbiota, evaluation of gene flow from transgenic (chloroplast transformation) plants, and biosafety assessment of novel plant growth promoting micro-organisms.

Three general comments can be made about the results of this research. First, no particular safety or environmental problem of the technology has been revealed. Second, analysis of the role of GMOs in agriculture has raised many questions about the environmental impact of conventional agriculture in Europe, and in this way attention has been drawn to certain environmental issues. Third, as a positive spinoff new knowledge has been generated in many different disciplines.

Governance of Research

The perception that science has run ahead of public opinion raises the question of governance. This is an issue that is given some prominence in the Commission's recent Communication on Life Sciences and Biotechnology—A Strategy for Europe (Commission of the European Communities 2002) produced after a public consultation process. The use of GMOs in the environment is governed in the EU by Directive 90/220/EEC, which is now updated as Directive 2001/18/EC. The numbers of field trials authorized under the directive are shown in figure 2. Following the introduction of the directive, numbers of trials built up steadily, but there has been a sharp decline in recent years to the level of the early 1990s.

Figure 2



Number of Part B (Research) releases authorized under Directive 90/220/EEC (JRC database)

In an attempt to raise the voice of science in the debate on the use of GMOs, the EC has initiated a round table on GMO safety research. This aims to achieve a balanced discussion among all stakeholders of the results of safety research and the areas of uncertainty or concern. To avoid mixing issues and confusing the argument, each session focuses on a single topic. The first meeting examined the benefits and risks associated with *Bt* maize and was structured around environmental, animal feed, and human food issues. Results are published on a Web site (http://biosociety.cordis.lu/).

Recently Identified Research Priorities

As a result of various consultation processes and discussion forums the following priorities, concerning both the content of the research and the way it is carried out, have emerged. Many of the general issues are also mentioned in the Commission's Communication on Life Sciences and Biotechnology—A Strategy for Europe (Commission of the European Communities, 2002), especially Actions 13, 17, and 23.

First, because there is concern over the speed of change in agriculture in Europe, baseline studies to define agro-ecosystems are needed to provide a known starting point from which to measure changes. Such studies would evaluate new systems of any sort—conventional, low-input, organic, and so forth as well as systems using GMOs. In particular, field experiments are needed to determine the benefits or risks of new components. For different systems to co-exist and be validated, separation distances and other buffering techniques need further

investigation. In the case of GM crops, soil impacts, particularly on nontarget organisms and in the long-term, and effects of gene stacking are a cause of concern. In this work, consensus on research methodology, including monitoring, is clearly desirable from scientific and public perception viewpoints.

A second approach relates to the inherent safety of products. Fewer concerns will be raised if safety issues and public perception issues can be tackled at the design stage by building in specific features. In particular, strategies for prediction will become essential if risk assessment measures are to cope with many new products in the future.

Finally, in order that research not be too far removed from the public and for communication purposes, stakeholders should be involved as much as possible.

Conclusions

The EC has been active in supporting GMO safety research for many years, and the multinational consortium approach is considered particularly valuable for this type of work. Although no particular safety or environmental problems have been revealed, further items for research and areas of concern have been identified. Communication of research results appears to be a particular bottleneck, especially because of a continuing public perception that little or no GMO safety research has been carried out.

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Monitoring Case Study: Insect Resistance Management in *Bt* Crops: (*Bt* Crop IRM)

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Abstract

The U.S. Environmental Protection Agency (EPA) has imposed an unprecedented insect resistance management (IRM) program for *Bt* crop products to delay or prevent the target insects from becoming resistant to the *Bt* proteins. Maintaining this IRM program requires the effective actions of farmers, pesticide companies, researchers, and Government regulators. The science of insect resistance management and insect resistance monitoring is complex and is continuing to develop. Insect resistance monitoring is expensive, and the extremely high costs can be offset by more reliance on farmer actions to carry out robust IRM plans, on compliance monitoring, and through remedial action plans. EPA will continue to monitor all of these activities closely for the *Bt* crop products.

Introduction

The development of pesticide resistance in insects, fungi, and weeds is well documented in agriculture. As resistance begins to develop, more pesticide is needed to achieve control until total failure of that pesticide occurs. Integrated pest management or IPM grew out of insect resistance to insecticides, and pesticide resistance management remains a common component of IPM programs today. Monitoring for the increased pesticide tolerance of the pest is a valuable asset in an IPM program, but it is rarely done proactively.

Insect resistance management (IRM) is the term used to describe practices aimed at reducing the potential for insect pests to become resistant to a pesticide. Bacillus thuringiensis *Bt* IRM is important because insect resistance poses a threat to future use of microbial *Bt* pesticides and *Bt* technology as a whole. Academic scientists, public interest groups, and organic and other farmers have expressed concern that the widespread planting of these genetically transformed plants will hasten the development of resistance to pesticidal *Bt* endotoxins. Effective insect resistance management can reduce the risk of resistance development.

An IRM plan is not specifically required under the U.S. pesticide laws or regulations. Rather, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the U.S. Environmental Protection Agency (EPA) is mandated to ensure that there will be no unreasonable adverse effects from the use of a pesticide when economic factors are taken into account. In this specific case, EPA has stated that we are working to prevent potential adverse effects if *Bt* could not be used and more toxic compounds were used to control the insect pests.

The goal of IRM is to have the target pest continue to be susceptible to the pesticide. Each IRM program consists of strategies to reduce the likelihood that insect resistance will develop and strategies to manage insect resistance once it occurs. At the EPA, IRM is an important tool in protecting against the loss of safer pesticide products. In 1992 we began to consider what would be an appropriate resistance management approach but one not limited to *Bt* crops. EPA has implemented an unprecedented IRM program for the *Bt* crops; however, we have not forgotten the conventional and microbial pesticides. We have recently published a final policy notice regarding labeling statements for most pesticides as part of a project under the North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (USEPA 2001a). This notice provides a numerical system to identify the pesticide's mode of action and label statements to encourage users to rotate between pesticides with different modes of action.

History of IRM for *Bt* Crops in the United States

In contrast to all other pesticides, the IRM plan(s) for the *Bt* crops are truly unprecedented in detail, scope, and implementation. The program has been enhanced early on when EPA held public meetings on biotechnology products where public interest groups voiced concern that *Bt* crops under development could lead to the insect pests' developing cross-resistance to microbial *Bt* products. EPA shared this concern and has always made IRM a key element in its regulation of *Bt* crop products. EPA has repeatedly consulted with our outside Scientific Advisory Panel and our public policy advisory group, the Pesticide Program Dialog Committee, regarding our IRM program for *Bt* crop products. In addition to excellent advice, EPA has received strong support for its work at each of these meetings.

At a Scientific Advisory Panel meeting in March 1995, the EPA laid out a multifaceted program we considered appropriate for *Bt* crop products. The elements are as follows:

- knowledge of pest biology and ecology, dose (level of toxin expressed in the *Bt* crop),
- refuge design and deployment (non-*Bt* plants producing *Bt*-susceptible insects),
- cross-resistance between different *Bt* proteins
- effective field monitoring for insect resistance
- remedial action if resistance occurs
- integrated pest management
- development of alternate modes of action
- grower education

Repeatedly, the Science Advisory Panel has agreed with EPA that an appropriate resistance management strategy is necessary to mitigate the development of insect resistance to *Bt* proteins expressed in transgenic crop plants. Resistance management programs should be based on the use of both a high dose of *Bt* endotoxin and structured refuges designed to provide sufficient numbers of susceptible adult insects. This so-called high-dose/structured refuge strategy assumes that resistance to *Bt* is recessive and is conferred by a single locus with two alleles resulting in three genotypes: susceptible homozygotes (SS), heterozygotes (RS), and resistant homozygotes (RR). It is also assumed in this strategy that there will be a low initial resistance allele frequency and that there will be extensive random mating between resistant and susceptible adults. Ideally, only rare RR individuals will survive a high dose produced by the *Bt* crop. Both SS and RS individuals will be susceptible to the *Bt* toxin. A structured refuge sets aside some percentage of the crop land for non-*Bt* varieties of that crop. The refuge provides for the production of susceptible (SS) insects that may randomly mate with rare resistant (RR) insects surviving the *Bt* crop to produce susceptible RS heterozygotes that will be killed by the *Bt* crop. This will remove resistant (R) alleles from the insect populations and delay the evolution of resistance. The scientific advisory panels held in 1998 and 2000 noted that insect resistance management strategies should also be sustainable, and to the extent possible, strongly consider grower acceptance and logistical feasibility.

Scientific Basis of IRM

To be effective, an IRM plan must be specific to the target pest, the crop, and to the class of pesticide being used. The use of a high-dose strategy is often not the best approach for chemical pesticides sprayed on a crop, and pesticide rotation is one of the preferred approaches. Annually rotating between *Bt* cotton and non-*Bt* cotton treated with conventional insecticides is likely to wipe away many of the environmental benefits of increased nontarget organisms we are seeing in areas where *Bt* cotton is frequently grown. As an IRM program is developed and implemented, each pest's unique biology must be factored into the plan. For example, how far the larvae move within the field and how far the adults move affects the distance between the refuge and the *Bt* crop. The susceptible insects from the non-*Bt* refuge need to be in close enough proximity to randomly mate with the resistant insects that emerge from the *Bt* fields to produce heterozygous offspring that are fully susceptible to the *Bt* protein. Additional important issues that need to be addressed are the number of insect generations produced each year, the mating behavior and the egg-laying or oviposition behavior, the host range of the insect, population dynamics, pest ecology, and, if possible, the genetics and mechanism of resistance and the frequency of resistance alleles in the insect population. In addition, how the crop is grown, including other pest management practices, when it matures, the extent of the acreage, and the overlap in distribution with other *Bt* crops are all important in the development of an appropriate program.

Mathematical models to predict the potential for resistance development have helped EPA make decisions regarding the requirements for *Bt*-crop IRM. In general, the predictive models allow the EPA to compare the relative efficacy of different IRM strategies to mitigate the development of insect resistance. For example, these models allow for a qualitative comparison of different refuge sizes, the impact on efficacy of the refuge if chemical insecticides are used, and differences in having the refuge be within the field or external to the field.

EPA has just completed an extensive reevaluation of the *Bt* crop products (*Bt* plant-

incorporated protectants) registered in the U.S., including IRM (USEPA 2001b). The EPA's IRM requirements are discussed in the next section.

The New IRM Requirements for *Bt* Crops in the United States

The EPA has determined that the 20-percent non-*Bt* field corn refuge requirements for *Bt* field corn grown in the Corn Belt and the 50-percent non-*Bt* corn field refuge requirements for *Bt* field corn grown in cotton-producing areas are scientifically sound, protective, feasible, sustainable, and practical to growers. EPA believes that the use of predictive models provides confidence that resistance will not evolve to any of the target pests (i.e., European corn borer, corn earworm, southwestern corn borer, fall armyworm, and other stalk-boring pests) under the time frame of the registrations.

For *Bt* sweet corn, no specific refuge requirements are necessary because sweet corn is typically harvested much earlier than field corn (18–21 days after silking) and before most lepidopteran larvae complete development (USEPA, 2001). However, to mitigate the development of resistance, EPA has determined that crop residue destruction is necessary within 30 days. This practice will likely destroy any live larvae left in *Bt* sweet corn stalks and prevent overwintering of any resistant insects.

At this time, EPA believes that available empirical data substantiate the success of the 5-percent external unsprayed, 20-percent external sprayed, and 5-percent embedded structured refuge options to delay insect resistance to *Bt* cotton. However, EPA believes that it is imprudent to allow the 5-percent external, unsprayed refuge option for more than a limited time because current data indicate that it has a significantly greater likelihood of insect resistance than either of the other refuge options. The 2000 Scientific Advisory Panel stated that the external, unsprayed option poses the highest risk to resistance evolution—especially for cotton bollworms. Because of the greater risk of resistance development, the external, unsprayed option will expire after three growing seasons (30 September 2004). During the next years, the registrant is required to develop considerable new data on alternative host plants as possible effective refuges.

In addition, the Agency is mandating additional improvements to the current *Bt* corn (field and sweet) and *Bt* cotton IRM programs that will require the following (USEPA, 2001).

1. Anyone purchasing *Bt* corn and *Bt* cotton must sign a grower agreement contractually binding the grower to comply with the IRM program and ensuring that there will be a mechanism by the year 2003 by which every grower will affirm his or her contractual obligations to comply with the IRM program,
2. An ongoing IRM education program will be implemented,
3. An ongoing IRM compliance monitoring program, including a third-party compliance survey and mechanisms to address noncompliance will be implemented,
4. An ongoing insect resistance monitoring program for each target insect pest will be designed,
5. Remedial action plans will be implemented if resistance does develop,
6. The IRM (and other) activities are to be reported annually. No other pesticide products besides the *Bt* crop products have such extensive IRM requirements.

The U.S. IRM Strategies for *Bt* Plant-Incorporated Protectants

In planning the new IRM regulatory program for these *Bt* plant-incorporated protectants (PIPs), EPA considered not only the science but also factors such as grower costs and compliance, resistance monitoring, and remedial action if resistance should occur. EPA considered four important areas: farmer actions, compliance monitoring, insect resistance monitoring, and remedial action plans. Each of these areas was considered in making the decision to continue the registrations of the *Bt* PIPs.

Farmer Actions

Farmer adoption of IRM requirements is critical to the long–term, sustainability of IRM strategies for *Bt* crops. Probably the first essential farmer action is to become familiar with the IRM requirements for a *Bt* crop. Without farmer implementation of appropriate IRM strategies, pest resistance cannot be mitigated. Each farmer must sign a contract or grower agreement indicating that he or she will abide by the IRM requirements, and the farmer receives a technical bulletin describing the latest requirements. Education also includes making sure that the farmer is aware of any changes that have occurred since he or she last grew the *Bt* crop—whether it was last year or 2 or more years ago. Educational materials are provided by each company, and education sessions are held by the companies—sometimes by the seed dealer, the commodity group, and often by the U.S. Department of Agriculture's Cooperative Extension Service. Information is also provided through the Internet and via newsletters and other media. Once the farmer is educated to the requirements, it is his or her responsibility to plant and manage the refuge. It must be the correct size (such as 20-percent non-*Bt* corn to 80-percent *Bt* corn in the Corn Belt), it must be placed at the correct distance so that any *Bt*-resistant insects coming from the *Bt* crop will easily find mates from the *Bt*-susceptible insects coming from the refuge, and the farmer must plant a refuge using a crop variety compatible with the *Bt* crop in the time that adults would emerge from the refuge and the *Bt* crop.

Farmers also play an important role in supplementing monitoring by reporting any failure of the *Bt* crop to control the target pest. Because farmers pay an extra fee when they buy the *Bt* seeds, they have an incentive to complain to the company that sold them the seed if it is not preforming correctly. We have made it a requirement of the registrations that the companies must report to EPA any valid complaints from farmers of failures of *Bt* crops to control a target pest. An important farmer action in the overall IRM program, is cooperation with and accurate response to, questionaires and surveys about actions the farmer has actually taken. Another interesting role from a regulator's perspective is that at least some farmers in the U.S. will tell the company or its representatives if another farmer is not abiding by the refuge requirements. We have made followup on tips and complaints from other farmers, a part of our new compliance-monitoring program.

Compliance Monitoring Program

EPA recognizes that compliance is a complex issue for *Bt* crops and IRM; therefore, a balance must be achieved between refuge size and deployment with grower compliance. Currently, the financial burden of implementing refuge requirements is borne primarily by the growers. Increasing refuge size, limiting refuge deployment, or both to reduce the risk of resistance will likely increase costs to growers and result in a higher rate of grower noncompliance.

Our recent reassessment has greatly strengthened the compliance monitoring to increase the likelihood of IRM adoption, to measure the level of compliance, and to institute penalties should noncompliance become a significant problem (see terms and conditions of registrations (EPA 2001b). Until recently, monitoring for farmer compliance with the IRM program has been largely voluntary, but now it is mandatory. Key to this program is the grower agreement between the company and the farmer promising the farmer will abide by the IRM requirements. Although it varies somewhat by crop, the program is basically a tiered approach of actions reflecting the results of a grower survey. The survey is conducted by a third party, an independent organization using funds provided by the companies. The survey questions are developed in consultation with academic and government researchers knowledgeable on the subject, and there is also an EPA review of the survey. The survey focuses on areas of highest risk, which are typically those areas of highest adoption. If the survey indicates that an area of the country is not fully complying with the requirements, increased education and more intense surveying will be implemented in that area. The degree of increased effort may be directly related to the type of problem. For example, if bad weather conditions cause farmers to plant refuges late, the situation is quite different than farmers in an area deciding it is unimportant to plant the refuge at all. In addition, typical on-farm visits conducted by the companies, their representatives, or both will report on farmers who are or are not complying with the requirements and the followup actions that are taken. Any farmer determined to be out of compliance will automatically receive an on-farm inspection the following year. If that farmer is still found to be significantly out of compliance, that grower will be denied the use of the *Bt* crop the following year. Although that farmer may be able to buy the technology the third year, he or she would again automatically receive an on-farm visit during that growing season. If that farmer was again out of compliance, he or she would be denied the use of the technology permanently. Some of the details of the penalty phases of the compliance monitoring program have not been fully worked out. The companies must submit plans for these to EPA early in 2002 for review and approval. Of course, tips supplied to the company regarding a farmer out of compliance, especially one refusing to plant a refuge for field corn and cotton, would require on-farm visits and might necessitate penalties.

Insect Resistance Monitoring

Monitoring has been part of the requirements of the *Bt* crop products registration from the beginning (USEPA, 2001b). The ambitious goal is to detect insect resistance before it occurs in the field or before it spreads and, if possible, to prevent the development of resistance by detecting increased pest susceptibility. Our program includes monitoring for the important target pests. The effort has been evolving over the last 6 years. To be effective, the plan requires sensitive tools be in place to detect changes in resistance allele frequency to the particular *Bt* protein and to be able to differentiate between natural variation in the population and a trend indicating resistance is likely to happen soon or may have already happened. As one of its early steps in developing this program, EPA established a working definition for

resistance versus natural tolerance variation and the analysis was reviewed by our scientific advisory panel for confirmation. In addition, EPA, working with the pesticide companies, has established definitions for suspected verus confirmed resistance. An additional consideration is the time required to "confirm" resistance.

The basic resistance monitoring program entails gathering target insects in an adequate sample size from an appropriate number of locations and testing for susceptibility to the *Bt* protein. Samples can be collected from various live stages. Adults might be collected from light or pheromone traps that attract the moths or larvae, eggs masses, or both might be collected either from *Bt* fields or other crop or noncrop areas. Depending on the life stage collected, the insects might have to be reared to a stage at which they could be fed the appropriate *Bt* protein to determine their level of susceptibility to the insect toxin.

Resistance monitoring is a difficult and imprecise task. The chances of finding resistant larvae in a *Bt* crop depend on the level of pest pressure, the frequency of resistant individuals, the location and number of samples collected, and the sensitivity of the detection technique. Therefore, as the frequency of resistant individuals in the insect population increases or the number of collected samples increases, the likelihood of locating a resistant individual becomes greater. The likelihood of resistance is dependent on the genetics and mechanism of resistance for a particular pest.

A resistance monitoring program is more important when models predict resistance is imminent rather than when resistance is expected to be delayed for a very long time. On the basis of predictive models, level of adoption, and compliance for European corn borers, resistance to *Bt* proteins expressed in field corn would not be likely to develop for 75 years or more, but for cotton bollworms, tobacco budworms, and pink bollworms, the predicted number of years to resistance to *Bt* proteins expressed in cotton is much shorter (EPA, 2001b).

The resistance monitoring program needs to consider the pest biology and ecology, population dynamics, genetics of resistance, mechanism of resistance, sampling methodology, bioassay methodology, standardization procedures, detection technique and sensitivity, and the statistical analysis of the probability of detecting resistance. To determine if refuges or any other resistance management tactics are working, one must track the frequency of resistance in field populations. With typical bioassays used for resistance monitoring, resistance cannot be detected readily when the allele is recessive (as often is the case) and rare. For example, if the frequency of a recessive resistance allele is 0.001, only one in a million individuals is expected to be a resistant homozygote (carrying two resistance alleles) capable of surviving exposure to a high concentration of the *Bt* protein.

Several issues are associated with this program. The first is sample size. The number of samples and number of locations that need to be sampled are dependent on the pest biology and ecology and population dynamics. If the genetic variation in an insect is known, then sampling strategies can be constructed with a greater probability of detection and a low probability of nondetection. Both factors must be considered to reduce the likelihood of Type 1 (false positive) and Type 2 (false negative) errors. Sampling should also be done uniformly. Uniformity and standardization in the bioassays are also critical to the interpretation of monitoring information. Finding enough insects to test is related to sample size. Sampling insects exposed to the *Bt* crop is preferred, but if sampling is primarily in the *Bt* crop, then few, if any, larvae of the target insect will be found in most *Bt* fields. This means that

sampling methods need to be adapted either to collect adults or egg masses to generate the volume of individuals needed to increase the probability of detecting resistance or samples need to be taken from non-*Bt* fields.

Current resistance monitoring plans in the United States have a goal to collect at least 250 individuals from any one location with a target of least 20 locations for tobacco budworms and cotton bollworms, pink bollworms, and European corn borers. Additional sampling for the southwestern corn borer is focused in those areas of the Corn Belt in which this pest is an economic problem. The greater the number of samples and locations, the greater the probability that resistant individuals will be collected.

Another issue is the sensitivity of the detection methods. If resistance is recessive (rather than dominant or codominant), it is less likely to develop, but it is more difficult to detect. It is useful to know the frequency of the resistance allele in the natural population. Estimates of the frequency of resistance alleles have been determined based on laboratory selection experiments (surrogates for what might happen but not necessarily what will happen in the field). Field verification of resistance allele frequency requires reliable and sensitive detection methods. However, if extremely sensitive detection methods (especially if resistance is recessive) are available and economically feasible, changes in resistance allele frequency (and verification of estimates) can be detected before any signs of field failure, thus creating opportunities for proactive, adaptive IRM.

EPA has evaluated the advantages and disadvantages of various detection methodologies and will continue to watch for a highly effective and economically viable test as the detection methodology improves and is accepted. New testing requirements will then be implemented. The currently required basic test method has been a discriminating dose/diagnostic dose bioassay system that distinguishes between resistant and susceptible phenotypes, but such tests have been criticized as being too insensitive to be able to provide early detection before resistance develops or can spread very far—especially if the alleles for resistance are rare in the insect population. Discriminating dose bioassays are most useful when resistance is common or conferred by a dominant allele (resistance allele frequency >0.01%) (Andow and Alstad 1998). This method is currently one of the central components of any monitoring plan, but other monitoring methods may have value in conjunction with the discriminating concentration assay.

A second detection technique is the F_2 screen (Andow and Alstad 1998). The F_2 screen may be the best method for detecting rare, recessive resistant alleles. The F_2 screen is conducted by taking mated females and sibmating the F_1 progeny, producing the F_2 progeny that are tested using an appropriate screening procedure, such as a discriminating concentration assay or *Bt* crop, and performing statistical analysis. The technique also requires fewer samples be collected to detect potential susceptibility shifts than the discriminating dose assay. The F_2 screen may be most useful to analyze populations that are expected to be at high risk for developing resistance. Each isofemale line allows for characterization of four genomes, thus improving the sensitivity over the discriminating dose assay. The technique is an effective method for detecting changes in the allele frequency of a recessive or partially recessive allele and can be used to verify some of the assumptions underlying high dose–refuge resistance management. If resistance alleles are found, they can be characterized to estimate the fitness of the genotypes, to determine whether there is a cost of resistance, and to predict the evolution of resistance. A potential obstacle to the F_2 screen is that it may be too

expensive because it is highly labor intensive and may not be suitable for routine screening purposes—especially if there is replication at each site. In general, the F_2 screen is more expensive than other methods for detecting dominant resistant alleles when the resistance allele frequency is >0.01. However, for recessive alleles, the F_2 screen is the least expensive method and can estimate resistance allele frequencies to a high level of precision (<0.005) for under $5,000 per location.

Additional tests include grower reports of unexpected damage, sentinel plots or the use of both in-field screening procedures, to screen against resistant test stocks (allelic recovery method) and in-field detection (using DNA markers) kits.

In a first step toward more efficient DNA-based monitoring, Gahan et al. (2001) in *Science* described using a DNA-based screening system for detecting a Cry1Ac-resistant tobacco budworm that has developed resistance through a specific mutation in the cadherin gene (characterized by the mechanism of *Bt* resistance found in the YHD2 strain [see Gould et al. 1997]). This mutation results in a truncated cadherin that lacks the toxin binding region and thus cannot bind Cry1Ac. The power of DNA based screening depends on the diversity of resistance conferred mutations. Tobacco budworm field populations might harbor this same mutation, other mutations of the same gene, or other genes and mechanisms of resistance. The Gahan et al. findings are the first to identify a DNA-based screening for *Bt*-resistant tobacco budworm heterozygotes by directly detecting the recessive allele. The Gahan et al. DNA marker is being evaluated in the field, and other DNA markers are being screened.

Gould et al. (1997) used a series of genetic crosses with test stocks of highly resistant tobacco budworm (YHD2) selected on Cry1Ac in the laboratory to estimate the resistance allele frequency in a natural population of tobacco budworms. This method can identify recessive or incompletely dominant resistance alleles from field-collected males. By using an assay that discriminates between heterozygotes, Gould et al. could establish which wild males carried a resistance allele. Using this allelic recovery method, Gould et al. estimated the resistance allele frequency to be 1.5×10^{-3}. This method is only useful when there are previously identified resistance alleles. As noted in the preceeding paragraph, Gahan et al. (2001) were able to identify the mechanism of resistance in this YHD2 line and were the first to develop a DNA marker that might be used in the field to screen for resistance.

Venette et al. (2000) proposed the use of an in-field screen to examine resistance allele frequency. This method uses *Bt* sweet corn to screen for European corn borers and corn earworms resistant to the *Bt* protein. That is, the *Bt* crop is the discriminatory screen for resistant individuals. By sampling large numbers of *Bt*-expressing plants for live corn borer larvae, the frequency of resistance can be estimated and resistant individuals collected for documentation of resistance. A high number of false positives can reduce the efficiency and accuracy of resistance allele measurement. One source of false positives is the occurrence of weakly or nonexpressing "off-type" plants among the sampled plants. Another source might be surviving susceptible larvae that are incorrectly scored as resistant larvae because of larval movement between *Bt* and non-*Bt* off-types or weeds. Another problem is that there might not be sweet corn varieties contain the same *Bt* genes as the field corn varieties. This would reduce the efficiency of sampling.

In addition to sampling and detection sensitivity, other equally complex issues are related

to cost and feasibility. It would be virtually impossible and economically prohibitive to sample every farm in which *Bt* crops are used. For example, there are approximately 14,000 *Bt* cotton producers (out of approximately 25,000 cotton producers). These producers planted about 4.5 million acres of *Bt* cotton in the 2000 growing season. Current resistance monitoring programs have focused sampling in areas of highest adoption of the *Bt* crops as the areas in which resistance risk is greatest. About 20 million acres of *Bt* corn were planted in the 2000, growing season. The cost of the U.S. monitoring program is borne chiefly by the companies although academic institutions and the U.S. Department of Agriculture researchers who carry out the bioassays probably bear some costs (i.e., University of Nebraska for European corn borer, University of Arizona for pink bollworm, University of Missouri for southwestern corn borer, and USDA/Agricultural Research Service at Stoneville, MS for tobacco budworm and cotton bollworm).

Related to who will pay for resistance monitoring programs is the issue of cost-effectiveness. If money is not a limiting factor, will the resistance monitoring programs be more proactive, more expansive, and more sensitive? What is the best test to be used on the basis of how much information is found for the money involved? Cost-effectiveness is related to the perceived and real value of the technology and the likelihood of resistance. Those who believe there is little likelihood of resistance development are less enthusiastic about a rigorous monitoring program.

Remedial Action Plan

EPA requires that a remedial action plan be available in the unfortunate situation of suspected or actual resistance (USEPA, 2001b). Again, as for resistance monitoring plans, remedial action plans are specific for the crop and pest. For example, because the pink bollworm is primarily a pest of cotton in the Western U.S. and differs biologically from the other two target pests of *Bt* cotton, the remedial action plan for pink bollworm is quite different from those for cotton bollworm and tobacco budworm in the Southeastern U.S. These plans define not only suspected and confirmed resistance but the key steps and actions needed if resistance develops. Generally, if resistance is confirmed, the farmers involved will treat their *Bt* crop with alternative pest control measures. This might be a chemical pesticide known to be highly effective against the insect or it might mean measures such as crop destruction. In addition, the sales and distribution of the *Bt* crop would be suspended in the affected area and its environs until it could be determined that insects in that area had regained their susceptibility to the *Bt* protein. Increased monitoring would also be needed to define the remedial action area(s). Other remedial action strategies include increasing refuge size, changing dispersal properties, using sterile insects, or other modes of pesticidal activity. Geospatial surveys would help define the scale of remedial action and the locations requiring intensified monitoring.

Because no field resistance has yet been found to any of the *Bt* crops, all of these tactics are untested. However, EPA believes that a key attribute of these plans is involvement in their development by the local farmers who would be affected most by the loss of this technology. So far there is only a regional remedial action plan for the Arizona area in which the pink bollworm is the chief pest controlled by *Bt* cotton. An interim remedial action plan is required and is being revised to address tobacco budworm and cotton bollworm resistance to *Bt* cotton, for they are the key economic pests of cotton in the mid-South and the Southeastern U.S. There is also a general remedial action plan to address resistance to

European corn borer, southwestern corn borer, and corn earworm.

Conclusion: Balancing the Four IRM Activities

The four IRM activities described above (farmer actions, compliance monitoring, insect resistance monitoring, and remedial action plans) need to be balanced. To some extent, they are at least partial substitutes for each other. In other words, if the refuge is extremely large (95-percent), there is virtually no need to monitor for insect resistance because resistance is so unlikely to occur. However, having a 95-percent refuge would eliminate many of the benefits to growers as well as the environment for cotton growers. Monitoring every *Bt* field for insect resistance reduces the need for a compliance program, but such an intensive effort is infeasible and extremely costly. In its regulation of these *Bt* products, EPA has attempted to balance these activities. EPA believes that the increased quality and substance of the compliance monitoring and resistance monitoring programs required through our just-completed reassessment can compensate to some extent for the small refuge size for *Bt* cotton. In addition, EPA has required additional data on the effect of alternate plant hosts and alternative modes of actions on delaying cotton bollworm resistance to *Bt* cotton. EPA believes that technological improvements to detect resistance earlier in the field will result in scientifically valid methods that will be cost-effective for insect resistance management in the future. Our faith in future improvements comes from knowing that academic, company, and Government research continues to be strong in the area of IRM for Bt crops.

Endnotes

1. See for the reports of the Scientific Advisory Panel and for the meeting notes from the Office of Pesticide Programs Pesticide Program Dialog Committee. Scientific Advisory Panel meetings related to biotechnology can also be found through links from the Biopesticides web page at http://www.epa.gov/pesticides/biopesticides.

2. Sections III and V of the *Bt* Crops BRAD at http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm

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Future Needs: Unique Challenges and Opportunities for Environmental Assessment (Abstract)

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The public's general understanding of living modified organisms (LMOs) places particular emphasis on improving agricultural production efficiencies and product quality as well as on environmental conditions and human well-being. Further development of these areas should be supported by research progress in the scientific assessment of environmental issues. This may involve the following three levels of biological interactions.

The first is the level of genomes. Rapid progress in genomics with cereals—particularly rice—and in transformation technologies will increase the frequency of so-called gene-stacking products in which a wide range of transgenes may be combined using conventional breeding or sequential transformation. Scientific reports suggest that combining homologous DNA sequences with transgenes can lead to transgene instability and silencing. Further, more complicated gene manipulations in metabolic pathways such as in functional foods, industrial processing, and pharmaceuticals yield new products expressing unique characteristics that are different from the traditional categories. Determining if these changes at the genome level alter the environment will require cautious future research.

The second level is at the one of plant populations. Experience reveals that distinguishing environmental impacts of a particular genetic modification in isolation are difficult in some outcrossing species such as perennial grasses and many coniferous trees. Continuous changes in genetic variation are occurring even in the original mother population, leading to a lack of an appropriate stable baseline. The impact of specific genetically modified (GM) crops on wildlife biodiversity is more complex because there are almost no data analyzing the impact of particular conventionally bred crop varieties on wildlife biodiversity. Thus, few baseline data studies exist against which to compare specific GM crops. All of these problems indicate the need for research to establish relevant baselines for conducting environmental assessments.

The third level is the one of the less-developed agricultural systems—particularly in developing countries. Statistics show that more than 50 percent of major abiotic limitation for agriculture exists because of drought and mineral stress. Research on molecular responses to drought, cold, heat, and salt stress in higher plants is in progress worldwide. For example, development of GM *arabidopsis*, tobacco, and rice tolerant to these stresses has been planned in Japan. These GM crops, once introduced, will certainly contribute greatly to increased production in extensive areas of the less-developed agricultural systems in developing countries. However, information on baselines is very scarce, because no conventionally bred varieties of such stress-tolerant crops have yet been widely cultivated. We need to research new approaches to the environmental assessment of the impact of GM crops, which possess great future potential.

In conclusion, further research on environmental issues—particularly on baselines—is needed, not on the basis of a science-absent fictional stringency but founded on sound, science-based, and broad perspectives for future development.

Building Regulatory Capacity in Developing Countries—Research Findings and Conceptual Framework

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Abstract

Biotechnology provides new opportunities for achieving productivity gains in agriculture. However, mobilizing modern biotechnology to address food and agricultural needs in developing countries implies increased responsibilities for determining benefits and risks. This paper examines capacity and efficiencies of national regulatory systems through research studies conducted in Egypt and Argentina. These studies were designed to elicit managerial and policy recommendations to strengthen regulatory systems, stimulate scientific risk assessment, and advance efforts in the areas of public acceptance, technology transfer, and harmonization. Following this analysis, one tool for building comprehensive regulatory capacity is presented. This is a conceptual framework for implementing biosafety and thus supports regulatory needs related to the Cartagena Protocol on Biosafety. One element of the framework, scientific knowledge, skills and capacity base, is examined in terms of policy options and decisions for locating scientific expertise. Final conclusions are provided emphasizing the need for comprehensive capacity building, including expertise in policy and managerial skills as well as for risk assessment.

Introduction

Developing countries face complex decisions regarding the introduction, testing, and use of products from modern biotechnology. These responsibilities include determining the benefits and risks of agricultural biotechnology applications. Although potential applications are varied and growing (Pew Initiative on Food and Biotechnology 2001), there are still relatively few events

or products of widespread use in modern agriculture. Products from multinational companies include herbicide-resistant soybeans, insect-resistant cotton, maize, or canola. Although these products are primarily produced for temperate regions, investments in research conducted by and with agricultural and scientific research organizations of developing countries are producing innovations to meet local market or food security needs, or both (Cohen 2001, Komen 2001, Morris and Hoisington 2000).

These transgenic events, whether from public or private research, raise concerns and requirements regarding their use, regulation, and assessment. Products derived from genetic modification technologies for use in developing countries are subject to extensive product development cycles and a lengthy review for potential environmental and health risks. The changing dynamics of political and international debate regarding risk and public perception also affect acceptability (Paarlberg 2001). Addressing these concerns and responsibilities demands that capacity be present at the political–international, regulatory, and scientific level in developing countries.

In the international arena, national systems for risk assessment and national biosafety frameworks emerged as a priority in chapter 16 of Agenda 21 and Articles 8(g) and 19 of the Convention on Biological Diversity. These articles instigated a global initiative to reach agreement on measures to ensure the safe handling and use of living modified organisms (LMOs) that may have an adverse effect on biodiversity while taking into account human health. The resultant Cartagena Protocol on Biosafety was adopted in January 2000. In addition to specific articles dealing with transport and use of LMOs, significant emphasis was placed on capacity building. Article 22 states that parties should cooperate in the development and strengthening of human resources and institutional capacity in biosafety.

At the political level, regulatory concerns can arise because of campaigns undertaken to curtail the use of products of genetic engineering such as those calling for a moratorium on the commercial use of genetically modified organisms (WWF 2001) or trade-related directives that ban genetically modified products from import. These concerns, moratoriums, and trade embargoes create difficulties in developing countries for those responsible for setting clear policies and agendas for biotechnology research and product regulation. At the scientific and regulatory management level, capacity is severely strained and competent individuals serve many pressing responsibilities.

To study the interrelated dimensions of regulatory responsibilities, compliance with international agreements, and related approaches for capacity building, the International Service for National Agricultural Research (ISNAR) initiated ongoing research studies. The first of these explored regulatory efficiencies and needs through research partnerships with selected developing countries, beginning in Egypt and Argentina. The second effort resulted in a conceptual framework for biosafety implementation based on a synthesis of contributions from an international expert consultation, *A Framework for Biosafety Implementation: A Tool for Capacity Building*. Together, these studies highlight regulatory systems, resources, and capacity needed to respond to transgenic events.

Regulatory Systems in the Developing World—Two Country Studies

To help assess the efficacy of national biosafety systems, a collaborative research project with Virginia Polytechnic Institute and State University and partner institutions in Egypt and Argentina was undertaken. The studies were designed to review policies and procedures associated with the introduction of genetically engineered crops in developing countries. The specific objectives of the studies are to accomplish the following:

1. Assess the efficacy of biosafety policies and procedures associated with the introduction of biotechnology products;
2. Develop recommendations for enhancing the operation of each country's biosafety system and minimizing potential constraints to technology transfer; and
3. Identify areas where international organizations can provide further assistance.

The studies (Madkour et al. 2000, Burachik and Traynor 2002) examine four common elements of biosafety systems: guidelines, people, the review process, and mechanisms for feedback (Traynor 1999). Information is collected regarding the following:

- The organization, membership, and operations of national biosafety committees;
- The nature and availability of information on biosafety procedures and requirements;
- The regulatory review paths and necessary approvals leading to commercial release;
- The extent of public involvement in biosafety matters; and,
- The personal experiences of applicants and reviewers in dealing with the biosafety system.

Country Studies—Synthesis and Findings

Argentina and Egypt are among the more advanced developing countries in terms of current and intended uses of genetically engineered crops and products derived from them. Egypt has approved several dozen confined field trials. Argentina has been exporting commercial genetically modified organisms (GMO) commodities since 1996. Several common characteristics are found between the two countries regarding their handling of biosafety matters. For both countries, the first step in establishing a biosafety system was the drafting of guidelines for ensuring the environmental safety of GMO releases. National guidelines were formulated after a thorough examination of regulatory documents from Canada, Australia, the United States and other countries with appropriate adaptations to national agricultural parameters. In contrast, application, review, and approval procedures for food safety and seed registration, which typically are subsequent steps in the path to commercialization, were built on a framework of preexisting laws and authorities.

For both countries, mechanisms for evaluation and approval evolved over time. As the first few GMO products reached each stage leading to commercial production—field testing, food safety review, seed registration, and commercial sale—the necessary guidelines, committees, and processes for each stage were implemented on an as-needed basis. In this way, successive regulatory procedures could be functionally coordinated with previous steps and with other ministries and regulatory authorities. The drawback to this approach is that it

tends to create delays; applications may be put on hold until procedures for the next step are worked out.

In Egypt and Argentina, the Ministry of Agriculture is the lead government entity overseeing agricultural biotechnology. It is within this ministry that environmental safety evaluations are conducted; the Ministry of Environment has a lesser role, if any. Food safety evaluations are conducted through the Ministry of Health. Both countries have constituted advisory committees that conduct technical reviews and make recommendations for approval of individual release applications. The national biosafety committees are empowered to deny a request or to hold it pending receipt of additional information from the applicant. Final decisionmaking authority to allow field tests or commercial releases, however, rests with the Minister of Agriculture. All evidence would suggest that ministry officials in both countries respect the work of their biosafety committees, for there have been no cases in which advisory committee recommendations were ignored nor instances in which ministerial approval was granted in the absence of a proper biosafety review and recommendation.

Both countries have advanced research institutes where Ph.D.-level scientists assisted by highly competent staff conduct state-of-the-art biotech research. Thus, there are pools of qualified individuals who may serve on national biosafety committees or as ad hoc technical advisors.

Nonetheless, the biosafety systems in Argentina and Egypt are very close to exhausting available expertise with competence in biosafety. This is evident in the degree of redundancy among members of the various review committees in Egypt and in the difficulty in identifying additional independent experts in Argentina. One of the most consistent messages heard throughout both studies was the immediate need for biosafety training that would build technical competence in risk assessment and risk management.

Biosafety evaluations in Argentina and Egypt, as in almost every other country, focus on risk in a proposed release. The task is to identify any potential risk and explore potential means for managing identified risks. Ostensibly, evaluations compare predicted impacts of the GMO with those of the equivalent non-GMO variety. Genetically modified varieties that present no greater risk than the referenced conventional variety are deemed acceptable for testing and eventual commercial release. As elsewhere, however, neither country includes a benefit assessment (nor assessment of the risks of not proceeding with the GMO) in the equation. Benefit assessments are a crucial part of the information needed for a comprehensive and balanced review and generate important information needed by the public.

Membership on a biosafety committee typically is an unpaid position added to each person's regular duties. Whether university faculty, public or private sector scientist, government agency representative or research administrator, all have to adjust their schedules to accommodate the extra workload. In spite of this, none of those interviewed in the two studies expressed any sense of being burdened with an unwanted responsibility. Rather, they took pride in the scientific rigor and fairness of their reviews and felt that their biosafety work was important and valuable.

The 30-member Egyptian national biosafety committee comprises 7 representatives of the Ministries of Agriculture, Health, Environment, Industry, and Commerce; a representative of the Egyptian Academy of Science and Technology; 12 members from academic institutions

an attorney; 8 people from Government research institutes, and a seeds expert. The Minister of Agriculture selects members; the private sector has no role in review and decisionmaking.

Even with such a large committee, some questions of risk may not adequately be addressed in the review process. For example, applications to commercialize *Bt* maize varieties have successfully passed environmental biosafety review, yet the risk of accelerated emergence of *Bt*-resistant pest populations and possible management strategies to reduce the risk to an acceptable level were not addressed during the discussions. In the future, experience and more forward thinking may help reviewers anticipate longer term risk problems and options for suitable management solutions.

The 19-member Argentinean biosafety commission includes people from private sector organizations (though not individual companies) as well as Government agencies and academic institutions. The major consideration for membership is the candidate's qualifications in the desired area of expertise. Institutions represented on the commission submit the curricula of three candidates, two of whom are selected for consideration and eventual approval by the Secretary of Agriculture. Conceivably these factors contribute to the more technical nature of the Argentine review committee. When combined with years of accumulated experience, differences noted here may also contribute in part to the more comprehensive review achieved in the Argentine system.

The potential for conflict of interest is an inherent part of Argentina's biosafety system. Nearly all biosafety reviewers conduct applied research at public institutions (leading to field tests and possibly commercial products), work collaboratively with biotechnology companies, or belong to industry organizations. Even those in the first group often have ties to private sector companies. The prevalence of these relationships makes it common for a Commission member to excuse himself or herself from taking part in a decision. Such connections also make it difficult to find independent, disinterested members to review applications containing confidential business information.

Although not a priority in Egypt, Argentina is giving serious consideration to drafting biosafety legislation that would include stringent measures to ensure compliance. Although such a step would likely make future revisions much more difficult, the loss of flexibility in the biosafety system is considered less important than the gain in legal authority and increased public visibility of a vigilant biosafety system.

A Conceptual Framework for Implementing Biosafety

As seen from the studies and findings summarized above, the design and implementation of any national biosafety system involve balancing public policy goals with economic, political, and technical realities. However, over the past two decades in developing countries, national biosafety frameworks and guidelines have often been implemented in a fragmented manner, owing to particular needs and pressures at the time. Consequently, a comprehensive, conceptual framework for biosafety implementation has often been lacking. For this reason, ISNAR convened an international expert consultation to develop such a framework.

In this section, the resultant framework is considered. The objective is to address national needs, particularly of those countries that are Parties to the Cartagena Protocol, regarding regulatory implementation and capacity building. The framework provides guidance on the design and implementation of regulatory frameworks and related capacity-building initiatives. It seeks to clarify critical decision points in the development of a national biosafety framework and choices among policy options and to delineate some of the scientific and social dimensions of these options (McLean et al. 2002).

The framework addresses five elements as fundamental to the development and implementation of a national biosafety system. The first two—(1) national policies, strategies, and research agendas regarding biotechnology and biosafety (2) and a national inventory and evaluation—provide the foundation for subsequent regulatory implementation. The next element—requisite knowledge, skills, and capacity base—is the resource environment within which the final two elements occur: development of regulations and implementation of regulations. This framework expands on the conceptual basis used for ISNAR's national biosafety system studies and on concepts and lessons derived from other national, regional, and international experiences analyzed during the consultation. Implications of the framework are considered in relation to more recent expectations following the adoption of the Cartagena Protocol on Biosafety (CBD Secretariat 2000).

Scientific Knowledge, Skills and Capacity Base

Building a strong base of scientific knowledge in support of the regulatory system and developing core competencies in biotechnology product evaluation are fundamental to any national biosafety system. These activities allow an improved scientific basis for assessments of potential risks and benefits, and they strengthen the scientific capabilities for risk management, inspection, and monitoring. A thin, weak, or limited knowledge and skills base tends to produce regulations that are highly protective at the expense of innovation, poorly defined or inconsistent, comparatively rigid, or narrowly interpreted. A deep and broad knowledge, skills, and capacity base tends to foster more latitude in regulatory development and more flexibility in regulatory implementation.

The expert consultation identified two key decision points and subsequent policy options for building scientific knowledge, skills, and capacity. The key decision points are as follows:

1. Providing a coordinated approach to incorporating scientific advice into biosafety decisionmaking and
2. Locating the science evaluation function within the regulatory system.

These two points and their subsequent policy options are discussed below.

Key Decision Point One: Coordinating Scientific Expertise—

As the science involved in the creation of LMOs advances and the products themselves become more complex, there is an increasing need to strengthen the science base supporting risk assessment and regulation. Developing skills required for biotechnology product evaluation and maintaining parity between risk assessors and their counterparts involved in

developing new products is of fundamental importance. This requires ongoing training about new scientific advances without which a regulator's knowledge base has a limited life expectancy.

The policy options as regards coordination are whether development of national capacity for scientific risk assessment should be given exclusive priority or whether it is possible to coordinate risk assessment at a regional or subregional level. The second policy option is to determine if a country will rely on international experts versus domestic self-sufficiency and capability. Each of these policy options is being explored in various ways by developing countries and with respect to expectations for adequate risk assessment of LMOs in relation to the Cartagena Protocol on Biosafety.

Adequate scientific capacity provides an improved scientific basis for assessments of potential risks and benefits and can improve the quality of risk management decisions and inspection and monitoring capabilities. Limitations in national scientific and technical capacity identified during the inventory and evaluation can be addressed through a co-coordinated approach. This would aim to enhance domestic expertise through training but also would rely on subregional, regional, or international cooperation, or all of these in performing risk assessments and using outside experts and the international academic community.

Key Decision Point Two: Locating the Science Evaluation Function—

Maintaining access to scientific expertise is an issue for developed as well as developing countries. Structurally, different approaches to locating and securing scientific advice within the regulatory framework can be taken. In considering the risk assessment of biotechnology products, some countries have implemented a system of expert advisory committees whereas others have relied primarily on scientists and professionals working within government agencies. In the latter approach, the mandate for risk assessment may be vested within a single agency exclusively tasked with regulating products of biotechnology (e.g., a gene technology regulator) or it may be distributed between agencies in accordance with their existing responsibilities (e.g., departments of health, agriculture or environment).

The first policy option identified, as related to location of scientific expertise, is how to include the development of core competence for risk assessment within government departments and agencies versus a combination of both inhouse and external scientific expertise. The second policy issue is whether a country concentrates the risk assessment function within a single indefinable body versus distribution of this function among different government departments and ministries.

Generally, independent advisory committees have more transparent accountability frameworks than government departments and agencies in which the range of expertise and academic credentials of risk assessors is rarely published.

However, advisory bodies can suffer because committee members are part-time volunteers who cannot devote their full energies to risk assessments. Out of necessity, committee meetings occur only a few times per year, thus limiting efficiency; moreover, the selection process for committee members may not result in the right combination of scientific expertise and regulatory experience. Product evaluations performed by competent scientists within a regulatory agency or agencies, supplemented by the use of issue-specific expert panel consultations, is an approach to LMO regulation that may combine the best of both worlds.

Discussion: Implications for Capacity Building, Funding and International Support

The Cartagena Protocol has focused attention on the needs for broad-based efforts regarding capacity development. To respond effectlivey to political debates, campaign-related moratoriums, and the need to build regulatory expertise, it is essential that comprehensive and credible expertise be built among scientists, institutional managers and directors, and key policy- and decision-makers. Such commitments by and for developing countries will more adequately respond to public sector needs regarding their role in the governance of new technologies.

Increased knowledge of the costs of regulating new products, particularly pest-protected crops, will become essential. Although most commercial providers will meet regulatory costs, it is still unclear how public sector research organizations in developing countries will meet the costs of regulation and risk assessment. The need for environmental assessments of locally produced events can be expected to increase as research capacity and competency increases. As noted in the NRC report (National Research Council 2000), regulatory testing can be expensive in terms of management time and money (Lichtenberg 2000). Consequently, testing barriers can become barriers to entry for small companies or national agricultural research organizations.

Thus, providing comprehensive capacity will be crucial for implementing biotechnology research and regulatory structures and for acquiring abilities to comply and participate with international forums. The least advanced countries will be hard-pressed to assemble more than a few qualified professionals with competence in risk assessment procedures. Studies from Egypt and Argentina have noted that both of these countries are functioning near the limits of available expertise, which raises questions about the capacity available for future reviews and how to circumvent the possibility of conflicts of interest. In addition, regulatory systems need to address risk factors adequately through research and to gather or supply relevant data, as recognized by the Biosafety Protocol.

Unfortunately, developing countries will continue to face limited funding and investment opportunities regarding biotechnology research and regulatory support (Cohen 2001). They use professionals to address regulatory requirements and are not able to compensate them. Such harsh economic realities are not going to change in the immediate future, nor will the needed human capacity become suddenly available to address the policy and scientific challenges that surround biotechnology. Therefore, further consideration by donors, international bodies, and national policymakers must be given to implementing biosafety guidelines and regulatory systems in the context of developing, not developed, countries. These considerations should draw on opportunities for creating increased efficiencies, economies of scale, regional cooperation, and the means by which these countries can economically and scientifically comply with the increased calls for safety and environmental risk assessments. In this regard, the conceptual framework introduced in this paper should prove most useful (McLean et al. 2002).

To supplement national funding, new mechanisms to ensure environmental assessments for events arising from public research will be needed. Consortia grouped by crop and event, working across countries and regions, can be effective in this regard. Significant international

attention and funding to projects such as those listed below would significantly augment the limited opportunities and funding available to developing countries seeking to conduct fundamental biotechnology research as well as develop suitable safety and environmental testing capacity.

One example of such an initiative comes from the U.S. Agency for International Development though its competitive granting program called the Biotechnology and Biodiversity Interface (BBI). These grants address the interface between the use of agricultural biotechnology and natural biodiversity. This program develops data and builds capacity to assist developing countries in the use of biotechnology in an environmentally responsible manner. To date, five grants have been awarded (Pathak 2001) covering diverse target organism and ecological settings such as transgenic fish and biodiversity in Thailand, transgenic rice and potential for outcrossing in Vietnam and gene flow in Thailand, effects of transgenic maize on nontarget soil organisms in Colombia, and ecological impacts of introducing transgenic crops in Africa.

A second opportunity comes from a proposed international initiative of public sector scientists organized through the working group on Transgenic Organisms in Integrated Pest Management and Biological Control, working under the International Organization of Biological Control. This initiative is planning to develop scientific principles and detailed scientific guidelines for international biosafety testing of transgenic plants (Hilbeck 2001). Developing country participation in this proposed initiative would greatly facilitate their understanding and compliance with comprehensive, transparent scientific guidelines for prerelease biosafety testing of transgenic plants.

Finally, greater focus is needed as regards public sector biotechnology arising from research conducted by, and with, developing countries. In addition to understanding the nature of transgenic events being researched, such information will be crucial to assuming a more predictive and proactive stance towards the needs of both environmental and health assessments. Therefore, ISNAR is now collecting data on such events, including agronomic and regulatory steps, through its Next Harvest project in collaboration with 15 developing countries. It is proposed to link this information with global scientific expertise so that additional consortia can be developed to further support both regulatory and biotechnology research needs. The final step will be to relate this regulatory and research information to economic and costing studies as per expected costs required to undertake assessments and prepare for scale up and commercial trials.

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- Madkour, M. A., A. S. El Nawawy, and P.L. Traynor. 2000. Analysis of a National Biosafety System: Regulatory Policies and Procedures in Egypt. ISNAR Country Report 62. The Hague: International Service for National Agricultural Research.
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Capacity Building for Research and Monitoring in the Developing World: Unique Challenges and Opportunities

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Abstract

Capacity building is a recurring concept within the framework of sustainable development; however, the term's meaning, applicability, and perspectives can change considerably depending upon the context in which it is used. In this presentation I will therefore try first to analyze the general concept of capacity building and then concentrate thereafter on its implications for the field of biosafety. In this general analysis a few questions and remarks on issues relating to biosafety and the developing countries will be considered followed by an overview of the experience gained by our organization on biosafety in the last several years.

Introduction

In looking for a definition of "capacity building," one will encounter several meanings; many would be pertinent to the theme of biosafety, others would not. The following four definitions certainly address the topic of this presentation:

1. "Capacity Building is not defined through the instruments used, but through its goal to enhance the capability of people and institutions to improve their competence and problem-solving capacities" (GTZ 1999).

2. "Capacity Building refers to investment in *people*, *institutions* and *practices* that will, together, enable countries in the region to achieve their development objectives" (World Bank 1997).

3. "Capacity Building is the process by which individuals, groups, organizations, institutions and societies increase their abilities to understand and deal with their development needs in a broad context and in a sustainable manner" (UNDP 1997).

4. "Capacity Building may be defined as the actions needed to create or enhance the capability of a country or an institution (or an individual) to carry out its allotted functions and achieve its objectives" (UNDP 1993).

It is interesting to note that, in each of these four definitions, there are two elements that keep recurring, namely the need to enhance the capacities of people and institutions. This should in fact dispel one incorrect concept that equates capacity building (or considers it a synonym) with the training of individuals only. Capacity building is rather the insertion of training activities into an institution as a whole to enhance its own capacities, with the possibility that the concept of "institution" can be extrapolated to the level of a society.

Just after the 1992 adoption of the Convention on Biological Diversity (CBD), the United Nations Environment Program (UNEP), analyzed the meaning of capacity building to be applied to biosafety issues as follows:

"...capacity-building means the strengthening and/or development of both human resources and the institutional and infrastructural capacities which ensure that, in the wake of the emerging biotechnology revolution, countries (in particular developing countries) are able to cope with new developments and applications of biotechnology as they arise, and to achieve safety in biotechnology, through effective implementation of existing or planned biosafety guidelines, directives or regulations, and of any future international agreement on biosafety", (UNEP, Capacity Building for Biosafety: Option for Action, 1992)

Once again, the development of human resources and institutions is the main goal of capacity building activities. The focus of these activities is to permit the developing world access to, and benefit from, the biotechnology revolution. At the end of this last definition, there is a reference to the Cartagena Protocol on Biosafety; Article 22 deals with the capacity building concept and contains the main issues that are at stake:

"The Parties shall cooperate in the development and/or strengthening of human resources and institutional capacities in biosafety, including biotechnology to the extent that it is required for biosafety, for the purpose of the effective implementation of this Protocol ...through existing global, regional, subregional and national institutions and organizations and, as appropriate.... Cooperation in capacity-building shall include scientific and technical training in the proper and safe management for biosafety, and the enhancement of institutional capacities in biosafety."

Once more, there is a reference to enhancing capacities of human resources and institutions as well as the need to cooperate with the existing regional and national institutions and organizations as appropriate. For the first time also the concepts of research and technical training make their appearance; this is one of the key elements with which I will deal at the end of this presentation.

The final document of the conference entitled *"New Biotechnology Food and Crops: Science, Safety and Society"*, organized by the Organization for Economic Cooperation and Development (OECD) in Bangkok in July 2001, underlined how capacity building in biosafety should be addressed towards (a) developing research capacity; (b) ensuring the capacity to make decisions on biosafety, risk assessment and management, monitoring, certification and labeling; (c) answering specific needs of stakeholders (including researchers, regulators, consumers, and producers); and (d) ensuring access to reliable information (Internet access). A few months later, the Intergovernmental Committee for the Cartagena Protocol meeting in Nairobi at the beginning of October adopted by consensus a report detailing an action plan for capacity building that provided for the following key elements:

- Institutional capacity building
- Human resources development and training
- Risk assessment and other scientific and technical expertise
- Risk management
- Awareness, participation, and education at all levels, including decisionmakers, stakeholders, and the general public
- Information exchange and data management, including full participation in the Biosafety Clearing-House
- Scientific, technical, and institutional collaboration at subregional, regional, and international levels
- Technology transfer
- Identification.

Summarizing what comes out of these definitions and the different discussions that have been going on in the international arena, we can identify the main issues at stake as follows:

1. The diffusion of biotechnology;
2. The need to establish adequate mechanisms for the exchanges of information;
3. The establishment of specific programs aimed at enhancing institutional capabilities;
4. The safe research, development, and application of biotechnology products;
5. The transfer of know-how, especially to the developing world;
6. The stimulation of scientific training; and
7. The initiation of specific programs aimed at risk assessment and in risk-management.

Note that these elements were already recognized in 1992 and were present in the agenda of the UNEP on the eve of the Rio U.N. Conference on the Environment and Development. In other words, after almost 10 years of debate on these issues, we returned to the original problems present at the very beginning of the debate on the safety of genetically modified organisms (GMOs).

In the context of these identified issues, what should the international community provide? In particular, what kind of assistance can an international organization like the International Centre for Genetic Engineering and Biotechnology (ICGEB) offer to the developing world, for it to meet the requirements cited above? A very brief description on the origins, the mandate, and the activities of the ICGEB follows focusing on the experience it has developed in the last several years in the field of biosafety.

The ICGEB is an intergovernmental organization that started its operations in 1987 as a special program of UNIDO, the United Nations Industrial Development Organization, to become a center of excellence for research and training in genetic engineering and biotechnology. Special attention was to be placed on the needs of the developing countries. In 1994, after its statutes (i.e., the international treaty establishing the organization) entered into force, ICGEB became a fully autonomous intergovernmental organization that is still closely related to the U.N. system. Since 1997, the ICGEB operated a Biosafety Unit provide its member states with specific activities of interest in the field of biosafety with special emphasis on dissemination of information, development of training programs and international cooperation.

Looking at the membership of the organization, one immediately notes two aspects of interest: its complementarity to OECD membership (because most of the ICGEB member States are developing countries or countries in transition) and the participation of all those countries (like China, Argentina, and South Africa) that, although belonging to the developing world, already have important activities in the use, production and commercialization of GMOS.

Dissemination of Information

Two major informational tools are accessible online thorough the Internet. The first one, named Biblio-Bio is a bibliographic, searchable scientific database on biosafety studies. This database (http://www.icgeb.trieste.it/biosafety/bsfdata1.htm) is updated monthly and presently contains some 3,000 scientific articles (full references and abstracts) that have been published in international, peer-reviewed scientific journals since 1990. These are selected and classified by ICGEB scientists according to the main topics of concern for the environmental release of GMOs. A list of the latest references is shown to facilitate diffusion of the main, or most recent, information.

The second informational tool developed by ICGEB is the Risk Assessment Searching Mechanism (RASM). This searchable index has been elaborated, through the funding of the Italian Government, in response to recommendations made by the Intergovernmental Committee for the Curagena Protocal-1 (ICCP1) for the setup of the Biosafety Clearing-House that included inter alia the establishment of central databases containing information from countries without an electronic infrastructure as well as the creation of searchable indexes for information to facilitate decisionmaking in accordance with article 10 of the Cartagena Protocol. The RASM aims to provide access to all the available official documents on risk assessment related to genetically modified crops in different countries and is complementary to, and interlinked with, other existing databases. The prototype of the RASM presently available online (http://www.icgeb.trieste.it/biosafety/rasm.html) contains some 180 records of risk assessment documents for 60 different transgenic events from 13 plant species issued by the official authorities from several countries. One of the future objectives for the enlargement of this index would be to collect and maintain data sent from parties without an electronic infrastructure while continuing to expand the retrieval of data available from those countries with advanced electronic networks.

Training

Training and technology transfer in biotechnology are among the main objectives of the ICGEB. The Centre provides its constituency with technical instruments and qualified information required in biosafety and risk assessment to allow member States and the wider international community to gain advantages from biotechnology and be informed of benefits and potential risks.

Since 1991, the ICGEB has organized annual biosafety workshops attended, to date, by close to 600 scientists from more than 60 different countries involved in related issues. In 2001, the ICGEB held two such workshops: “Biosafety 1—Introduction to Biosafety and

Risk Assessment for Environmental Release of GMOs: Theoretical Approach and Scientific Background" and "Biosafety 2—Advanced Research in Risk Assessment and Risk Management for Environmental Release of GMOs: Identification of Main Areas for Future Investigation." This second workshop, aimed at officers of Governmental agencies and designated experts working in risk assessment of GMOs at the official level (governments, scientific institutions, private sector, etc.) and held under the auspices of the Italian Ministry for the Environment, has been organized, for the second time in collaboration with the Istituto Agronomico per l'Oltremare (IAO), Florence. A third course, that was supposed to be held in 2001 in Venezuela, had to be postponed to 2002 for logistical reasons.

After collaborating with the UNEP/Global Environmental Fund (GEF) "Pilot Biosafety Enabling Activity Project," the ICGEB is now participating in the steering committee of a new major project implemented by the UNEP and financed by GEF aimed at building capacities in the developing countries to design National Biosafety Frameworks and help to prepare for the implementation of the Biosafety Protocol. Such a project is an excellent opportunity for the ICGEB to lend its technical and scientific support to the international effort that has been initiated in response to the Cartagena Protocol. In this respect, the GEF/UNEP project team and ICGEB are developing an agreement through which ICGEB will organize, starting in September 2002, several regional workshops on risk assessment, thus providing the participants with an overview of the current research in biosafety and different risk assessment approaches used for the environmental release of GMOs.

International Cooperation

Biosafety is an excellent area for combined actions directed to enhance the environmental standards of biotechnology management. The special relationship that links the ICGEB to UNIDO, the United Nations Educational, Scientific, and Cultural Organization (UNESCO), and other U.N. bodies as different autonomous organizations committed to cooperating on biotechnology issues of mutual interest, coupled with their long-term experience in developing co-operation programs, creates a perfect synergism with the renowned experience in advanced research and training in molecular biology and biotechnology of the ICGEB. Moreover, this specific field has been recognized as one of the main topics for collaboration between the Secretariat of the United Nations and the ICGEB; accordingly, the Cooperation Agreement entered into by the two Secretariats in March 2001 specifies that the U.N. and the ICGEB may decide to cooperate in activities related to the sustainable and safe use of genetic engineering and biotechnology as well as in the implementation of the international cooperation programs foreseen by the Convention on Biological Diversity and its Cartagena Protocol on Biosafety (Article VI.2 of the UNICGEB Cooperation Agreement).

The Centre has actively participated in the elaboration of the Voluntary Code of Conduct for the Release of Genetically Modified Organisms into the Environment (prepared by the informal UNIDO/UNEP/WHO/FAO Working Group on Biosafety in July 1991), is a party to the Inter-Agency Network for Safety in Biotechnology (IANB) chaired by the OECD and is actively involved in providing the Secretariat of the Convention on Biological Diversity with scientific and technical tools for its Biosafety-Clearing House, which is one of the most important information tools foreseen by the Cartagena Protocol.

In the course of its almost 13 years of experience, the ICGEB has also gained capabilities in techniques available at the laboratory level. In the future, the ICGEB plans to develop training curricula that may provide scientists from developing countries with hands-on training activities in specific techniques that may be necessary for the detection, assessment and management of GMOs. Moreover, the Centre is now developing a project for the establishment of a Biosafety outstation aimed at setting up an ICGEB facility for training and research in risk assessment and management relating to the environmental release of GMOs. The outstation, equipped for studies in molecular genetics, will be located close to Venice and will develop research programs for the investigation of those gray areas in scientific knowledge that concern the safe use of agricultural products derived from biotechnology. The cost of the buildings that will host the new laboratories and a guesthouse for trainees, their remodeling, and the operation of the outstation will be met by an Italian nonprofit foundation.

With its pending involvement in the Biosafety outstation foreseen by the end of 2002 or the beginning of 2003, the ICGEB will complete its spectrum of activities dedicated to biosafety. The ICGEB's focus on enhancing the scientific capacity of individuals while strengthening institutions in developing countries through major international efforts is a unique example of two fundamental elements of capacity building at large being addressed. This is an intrinsic part of the ICGEB mandate that needs to be included in the global context among all the efforts made by individual countries, the CBD Secretariat, and other international organizations aimed at the full implementation of the Cartagena Protocol and at a safe and sustainable use of biotechnology.

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