International Workshop on the Ecological Impacts of Transgenic Crops♣
(March 2-4 ,2000)

Attended by 21 scientists from Universities (Berkeley, Santa Cruz, Cornell, Guelph, Iowa State, Minnesota, Swiss Federal Institute of Technology, Elmhurst College and Open University) , International Agricultural Research Centers (CIMMYT, CIP)♣♣ , NGOs (Union of Concerned Scientists, Food First, Consumers Union, AS-PTA Brasil) and Private Organizations (Dynamac Corp.)

EXECUTIVE SUMMARY

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Transgenic crops are increasingly becoming a dominant feature of the agricultural landscapes of the USA and other countries such as China, Argentina, Mexico and Canada. Worldwide, the areas planted to transgenic crops jumped more than twenty-fold in the past four seasons, from 3 million hectares in 1996 to nearly 40 million hectares in 1999. In the USA, Argentina and Canada, over half of the average for major crops such as soybean, corn and canola are planted in transgenic varieties. Herbicide resistant crops (HRC) and insect resistant crops (Bt crops) accounted respectively for 54 and 31 percent of the total global area of all crops in 1997. The rapid deployment and widespread commercialization of such crops in large monocultures raises questions regarding the potential of genetically modified crops (GMCs) to cause unacceptable impacts on the environment. Besides the widely acknowledged drawbacks of GMCs: a) the spread of transgenes to related weeds or conspecifics via crop-weed hybridization and, b)the rapid evolution of resistance of insect pests such as Lepidoptera to Bt, the workshop was concerned about the overall ecological implications of other more subtle effects that research is now starting to unravel:

- accumulation of the insecticidal Bt toxin, which remains active in the soil after the crop is ploughed under and binds tightly to clays and humic acids;

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♣♣ CIP and CIMMYT appreciate having invited to attend. The statements made in the summary are not those of CIP and CIMMYT. We fully agree that as with any new products (conventional or biotech) an assessment of potential risks and benefits must be done, that one must look at technology from a risk/benefit perspective and not simply a no-risk one. We believe that to properly address food security, especially for the resource-poor farmers, we must have a toolbox that includes all available tools, conventional and biotechnology. In addition we find that biotechnology approaches including GMCs do have potential benefits that can outweigh the possible risks.

- disruption of natural control of insect pests through intertrophic-level effects of the Bt toxin on predators;
- unanticipated effects on non-target herbivorous insects (i.e. monarch butterflies) through deposition of transgenic pollen on foliage of surrounding wild vegetation;
- vector-mediated horizontal gene transfer and recombination to create new pathogenic organisms, and
- reduction of the fitness of non-target organisms through the acquisition of transgenic traits via hybridization.

By examining specific studies that describe such effects, the group was able to assess the scale, magnitude and ecological significance of such findings.

Approach

The workshop was attended by a group of 21 scientists working on the ecological aspects of transgenic crops in research organizations located in the US, Europe, and Latin America. During the first day and a half of the workshop, participants were able to examine through formal presentations and group discussions, data on the environmental impacts of HRCs, Bt and other pesticide-producing crops and virus resistant crops. Discussions focused on known documented impacts and their overall implications, although emphasis was placed on research questions directed at dealing with potential unknown, hard to detect or low magnitude impacts, but which ecological theory predicts as probable and likely to scale up. Issues of gene flow, fitness effects of introduced transgenics on wild relatives, effects across multiple trophic levels and effects on soil ecosystems were all analyzed from an integrated and multidisciplinary perspective.

The second half of the workshop was spent evaluating the level of knowledge available on the ecological impacts of transgenic crops and the severity and scope of risks that their field deployment represents. Following is a summary of the main conclusion s and recommendations that emerged after two and a half days of intense multidisciplinary deliberation.

General Ecological Concerns

The rushed commercialization of existing GMCs is unwarranted from an ecological point of view as many scientists had already raised concerns about obvious potential impacts of GMCs. Given this fact, HRCs and Bt crops have been a poor choice of traits to feature the technology given predicted environmental problems and the issue of resistance evolution. In fact, there is enough evidence to suggest that both these types of crops are not really needed to address the problems they were designed to solve. On the contrary, they tend to reduce the pest management options available to farmers. There are many alternative approaches, (i.e. rotations, strip-cropping, biological control, etc.) that farmers can use to effectively regulate the insect and weed populations that are being targeted by the biotechnology industry. To the extent that transgenic crops further entrench the current monocultural system, they impede farmers from using a plethora of alternative methods.

At issue is the potential for transgene insertions to cause expression of not simply the target trait, but also unintended secondary outcomes that could pose environmental risks. Risk assessments of transgenic crops focus narrowly on only the intended outcomes, virtually ignoring the possibility of unintended outcomes or side-effects. The resulting phenotypes carry novel hazards, and regulatory agencies must address these in appropriate manners. From an ecological perspective, transgenic crops can not be considered substantially equivalent to conventional crops, as the effects of trangenes are often broader than expected, with pleiotropic or gene insertion site effects common. Workshop participants were also concerned about the genetic constructs used to transform plants and the possible ecological impacts from promoter and marker gene sequences.

Gene flow

The general consensus of the group is that just as it occurs between tradtionally improved crops and wild relatives, pollen mediated gene flow will occur between GMCs and wild relatives or conspecifics despite all possible efforts to reduce it. Little is known about the long-term persistence of crop genes in wild populations or about the impact of fitness-related crop genes on the population dynamics of weedy relatives. The main concern with trangenes that confer significant biological advantages that may transform wild/weed plants into new or worse weeds. In the cases of hybridization of HRCs with populations of free living relatives will make these plants increasingly difficult to control, especially if they are already recognized as agricultural weeds and if they acquire resistance to widely used herbicides. A case discussed suggested that transgenic resistance to glufosinate is capable of introgressing from *Brassica napus* into populations of weedy *Brassica napa*, and to persist under natural conditions.

Another case discussed suggested that introgression with genetically modified oats, *Avena sativa*, resistant to barley yellow dwarf virus (BYVD) would confer virus resistance to wild oats, *Avena fatua*. This would release wild oats, which are more susceptible to BYVD, from natural suppression, thus potentially triggering a more severe weed problem.

In addition to having high levels of agrobiodiversity, many developing countries constitute centers of genetic diversity, and in such environments the transfer of coding traits from transgenic crops to wild or weedy populations of these taxa and their close relatives is expected to be high. Genetic exchange between crops and their wild relatives is common in traditional agroecosystems and transgenic crops are bound to frequently encounter sexually compatible plant relatives, therefore the potential for gene exchange via pollen transfer in traditional agroecosystems is worrisome.

Until recently, gene flow risk assessment research has centered on addressing four basic questions:

1) Can crops and their wild relatives produce viable offspring?
2) What is the likelihood of progeny formation under field conditions?
3) Do the progeny survive to reproductive maturity?
4) What is the relative fitness of the F_1 compared to the parents under field conditions?

A problem with these questions is that they center largely on *probability* of gene flow events. The data have been collected using simple genetic markers and model systems that are in most cases of too small scale, and involve only traditionally improved crop varieties. One must question the usefulness of continuing to collect data that may be of limited value in truly estimating the risk of wide-scale GM crop release. Therefore, it is suggested that risk assessment research begin to move away from these probabilistic models and begin to address questions relating specifically to consequences of gene flow events in agroecosystems at scales that are comparable to modern agricultural settings.

The problem facing the ecological community centers on the identification of the right sets of questions that will allow a direct rebuttal to the USDA policy that "there is not problem until a problem is identified". The data that have been collected for most crop-wild complexes are very clear, the likelihood of gene transfer is high when the proper conditions for such events are met (i.e. range overlap, sexual compatibility, and flowering time synchrony). It follows that the potential for an "identifiable problem" should be high. Thus, more energy and resources must be directed towards articulating the possible consequences of gene flow events (vertical and horizontal) in agroecosystems so that appropriate experiments can be proposed, and gaps in the data pool be filled. One way to address the deficiencies in our data set is to assess the long-term effects of pollen flow from widely used traditionally improved crops to their sympatric, compatible relatives. Greater understanding of the historical consequences of gene flow will allow us to better evaluate the possible effects of introgression of a transgene into the wild populations. Secondly, data addressing biotic and abiotic factors that limit the distributions and abundance of the wild relatives needs to be collected, or more likely, retrieved from the weed science literature. Again, these data will provide us with the means to seriously consider the potential consequence of transgene persistence and spread.

Ecological, economic and agronomic implications of HRCs

World-wide in 1999, transgenic herbicide resistant crops were planted on 28 million hectares. In North America, there are now commercially available transgenic glufosinate resistant cultivars of canola and corn, and transgenic glyphosate resistant cultivars of soybean, corn, cotton, and canola. Bromoxynil resistant transgenic cotton has also been developed. Published research indicates that herbicide resistance has been transferred successfully to many other crops using genetic engineering techniques.

Transgenic herbicide resistance in crop plants simplifies chemically based weed management because it typically involves compounds that are active on a very broad spectrum of weed species, yet which do not damage the crop. Post-emergence application timing for these materials fits well with reduced or zero-tillage production methods, which can conserve soil and reduce fuel and tillage costs. This is why in 1998 about 44 percent of Midwestern soybean was glyphosate resistant (Roundup Ready).

However, HRCs also have significant problems. Reliance on HRCs perpetuates the weed resistance problems and species shifts that are common to conventional herbicide based approaches. The use of HRCs in areas where weedy relatives of crops are present creates the additional possibility of crop-to-weed resistance gene transfer. Herbicide resistance becomes more of a problem as the number of herbicide modes of action to which weeds are exposed becomes fewer and fewer, a trend that HRCs may exacerbate due to the dictates of the marketplace and the limits of synthetic chemistry. Pleiotropic effects may affect the performance of HRCs, as indicated by recent evidence of stem cracking in glyphosate tolerant soybean under high temperature conditions. Lower yields in transgenic glyphosate resistant soybean varieties, compared with conventional non-transgenic cultivars, may represent pleiotropic effects or a lack of attention from plant breeders. If consumers reject GMOs in the marketplace, use of HRCs will further decrease the prices farmers receive for low-value commodities.

Perhaps the greatest problem of using HRCs to solve weed problems is that they steer efforts away from crop diversification and help to maintain cropping systems dominated by one or two annual species. Crop diversification can not only reduce the need for herbicides, but also improve soil and water quality, minimize requirements for synthetic nitrogen fertilizer, regulate insect pest and pathogen populations, increase crop yields, and reduce yield variance. Thus, to the extent that transgenic HRCs inhibit the adoption of diversified cropping systems that include perennial crops, cover crops and green manure, they hinder the development of sustainable agriculture.

Ecological risks of Bt crops

Based on the fact that more than 500 species of pests have already evolved resistance to conventional insecticides, pests can also evolve resistance to Bt toxins present in transgenic crops. No one questions if Bt resistance will develop, the question is now how fast it will develop. Susceptibility to Bt toxins can therefore be viewed as a natural resource that could be quickly depleted by inappropriate use of Bt crops. However, cautiously restricted use of these crops should substantially delay the evolution of resistance. The question is whether cautious use of Bt crops is possible given commercial pressures that have resulted in a rapid roll-out of Bt crops reaching 9 million acres in the USA in 1997. Will the refuge strategy of setting aside 20-30 percent of the land to non-Bt crops work? Can such regional plans be enforced and will it be commercially viable for farmers? If instead 20-30 percent of the land was devoted to growing soybeans and corn in a strip cropping design, would similar pest control advantages emerge from such mixed and rotational cropping systems? Data from the Midwest shows that Bt corn saves on some insecticide use and yields are 2.4 bu/acre higher than conventional corn but only under high European corn borer infestations. On the other hand organic corn growers use no insecticides and obtain yields (4.8-9 t/ha) similar or slightly higher than conventional farmers (5.0-7.l t/ha) .

A concern of the group was the spill over effects resulting from the massive use of Bt toxin in cotton or other crops occupying a larger area of the agricultural landscape, onto neighboring farmers who grow crops other than cotton, but that share similar pest complexes. Such farmers may end up with resistant insect populations colonizing their fields. As Lepidopteran pests that develop resistance to Bt cotton move to adjacent fields where farmers use Bt as a microbial insecticide, this may render farmers defenseless against such pest, as the biopesticide becomes ineffective thus farmers stand losing an important biological control tool. Among those most affected would be organic farmers who rely on Bt based microbial insecticides for their pest management programs.

In the case of cotton there is not demonstrated need to introduce the Bt toxin into the crop at all, as the Lepidopteran pests of this crop are pesticide-induced secondary pests. Therefore, the best way to deal with such pests is not to spray insecticides, but instead to use cultural and biocontrol techniques. In the Southeast, the key pest is the boll weevil, a beetle immune to the Bt toxin. To fully assess the need for Bt cotton to control Lepidoterans in the Southeastern USA, experimental tests need to be conducted in areas not disrupted by insecticide misuse to determine the real pest status of each species before the need for biotechnology or any particular technology can be assessed.

Bacillus thuringiensis proteins are becoming ubiquitous, highly bioactive substances in agroecosystems present for many months. Most, if not all, non-target herbivores colonizing Bt crops in the field, although not lethally affected, ingest plant tissue containing Bt protein which they can pass on to their natural enemies in a more or less processed form. Polyphagous natural enemies that move between crop cultures are found to frequently encounter Bt containing non-target herbivorous prey in more that one crop during the entire season. This is a major ecological concern given previous studies that documented that Cry1 Ab adversely affected *Chrysoperla carnea* reared on Bt corn-fed prey larvae. These effects are not unique to Bt crops, as researchers in Scotland found that predaceous Coccinellidae feeding on aphids reared on GNA potatoes (containing snowdrop lectin) had lowered fecundity than ladybugs fed on control potato aphids. Such ladybugs lived twice as long as females fed on aphids from GNA potatoes.

These findings are problematic for small farmers in developing countries who rely for insect pest control, on the rich complex of predators and parasites associated with their mixed cropping systems. Research results showing that natural enemies can be affected directly through inter-trophic level effects of the toxin present in Bt crops raises serious concerns about the potential disruption of natural pest control, as polyphagous predators that move within and between crop cultivars will encounter Bt-containing, non-target prey throughout the crop season. Disrupted biocontrol mechanisms will likely result in increased crop losses due to pests or to the increased use of pesticides by farmers with consequent health and environmental hazards. There is a clear need for tri-trophic level studies to assess the long-term interactions of transgenic, insecticidal plants with natural enemies.

Effects of Bt crops on the soil ecosystem

There are many transgenic plants that are being considered, developed or released which produce insecticidal, nematicidal, or anti-microbial products. Due to natural wounding, senescence, root exudates, and sloughing-off of root cells, along with tillage of plants into the soil, soil biota will be exposed to these transgenic products. Because of the importance of soil biota in mineralization and immobilization of nutrients, physical and biochemical degradation of organic matter, biological control of plant pests, and as food sources for other organisms, it is crucial to evaluate the potential impacts of transgenic plants on soil ecosystems. Research in this area has been quite limited but the little research conducted has already demonstrated long term persistence of insecticidal products (Bt and proteinase inhibitors) in soil. The insecticidal toxin produced by *Bacillus thuringiensis* subsp. *kurskatki* remain active in the soil, where it binds rapidly and tightly to clays and humic acids. The bound toxin retains its insecticidal properties and is protected against microbial degradation by being bound to soil particles, persisting in various soils for at least 234 days. In another study researchers confirmed the presence of the toxin in exudates from Bt corn and verified that it was active in an insecticidal bioassay using larvae of the tobacco hornworm. Given the persistence and the possible presence of exudates, there is potential for prolonged exposure of the microbial and invertebrate community to such toxins, and therefore studies should evaluate the effects of transgenic plants on both microbial and invertebrate communities and the ecological processes they mediate.

Published research has already shown that exposure of soil organisms to transgenci plants caused changes in population levels of collembola, and changes in both levels and species composition of nematodes, bacteria and fungi. Perhaps the finding of most concern from these studies is that effects were not due to the transgenic products but rather from unintentional changes in plant characteristics that resulted from the process of genetic engineering. This suggests that responsible risk assessment of transgenic plants must consider not only the engineered traits but also attempt to account for unanticipated changes in the engineered plant that may also impact the soil ecosystem.

Studies need to be performed that compare soil biota and processes in fields under sustainable agricultural practices and conventional agricultural chemical practices with fields containing transgenic plants. Levels and species composition of arthropods, nematodes, protozoa, earthworms, enchytraeids, bacteria and fungi should be monitored. When possible, multiple methodological approaches (microscopic, culturable, metabolic, and molecular) should be employed to limit the bias introduced by any one method. Efforts should be made to characterize the food webs and trophic interactions. In addition, evaluation of key soil processes (e.g. decomposition, nutrient cycling) should be included to determine if any observed difference in soil biota levels or community composition are impacting biogeochemical cycles. Finally, above-ground measurements of plant health (e.g. biomass, fitness, phenology, morphology, chemistry) are needed to assess the ecological significance of any changes occurring in the soil ecosystem resulting from the exposure to transgenic plants.

If transgenic crops substantially alter soil biota and affect processes such as soil organic matter decomposition and mineralization, this would be of serious concern to organic farmers and most poor farmers in the developing world who cannot purchase or don't want to use expensive chemical fertilizers, and that rely instead on local residues, organic matter and especially soil organisms for soil fertility (i.e. key invertebrate, fungal or bacterial species) which can be affected by the soil bound toxin. Soil fertility could be dramatically reduced if crop leachates inhibit the activity of the soil biota and slow down natural rates of decomposition and nutrient release.

Virus resistant crops

Some researchers have expressed concerns about the risks of new pathogens evolving due to transgenic viral coat proteins. In plants containing coat protein genes, there is the possibility that such genes will be taken up by unrelated viruses infecting the plant. In such situations, the foreign gene changes the coat structure of the viruses and may confer properties such as changed methods of transmission between plants. The second potential risk is that recombination between RNA virus and a viral RNA inside the transgenic crop could produce a new pathogen leading to more severe disease problems. Some researchers have shown that recombination occurs in transgenic plants and that under certain conditions it produces a new viral strain with an altered host range.

A number of studies have demonstrated that plant viruses can acquire a variety of viral genes from transgenic plants:

- Defective red clover necrotic mosaic virus lacking the gene enabling it to move from cell to cell, and hence not infectious, recombined with a copy of that gene in transgenic *Nicotiana benthamiana* plants, and regenerated infectious viruses .
- Transgenic *Brassica napus* containing gene VI, a translational activator, from the cauliflower mosaic virus (CaMV), recombined with the complementary part of the virus missing that gene , and gave infectious virus in 100 percent of the transgenic plants.
- The same experiment carried out in *Nicotiana bigelovii* gave infectious recombinants that expanded the host range of the virus.
- *Nicotiana bethamiana* plants expressing a segment of the cowpea chlorotic mottle virus (CCMV) coat-protein gene recombined with defective virus mission that gene.

As all these experiments involved recombination between defective virus and transgene, it was thought that under natural conditions, when viruses are not defective, no recombinant viruses would be generated .

Although many questions still remain, based on the available information on the potential effects of virus resistant transgenic plants, the group highlighted the importance of six points:

- The recombination of viral genetic information takes place constantly, and is a driving force in viral evolution. The literature is full of evidence of the creation of new viruses by recombination. If genetic engineering increases the potential for recombination between viruses, as many think is the case, novel viruses will be created.
- Use of *entire*, *functional* genes such as coat protein, movement protein, and replicase genes will facilitate the creation of new viruses. Defective copies may still provide enough material for effective recombination.
- Genetically engineered satellite RNAs may be able to be replicated by viruses other than their host virus, which may lead to new types of infections and could increase (or decrease) pathogenicity.
- Very little is known about the distribution of plant viruses in nature, and few researchers are currently working in the area of viral ecology. Yet, information in this area is critical to conduct proper and scientifically defensible risk assessments of plants genetically engineered to resist viruses.
- We know little more about the biology of viruses in plants, in particular in multiple infections. Some evidence indicates temporal and spatial separation of different viruses

in multiple infections in nature, so that recombination in non-GE plants is more rare than would be found in a plant continually expressing a viral gene.

- Non-pathogenic viruses that make their way into the host cell, and then can't move, may have movement facilitated by the engineered transgene, which might serve as a movement protein. Even though they are not normally a pathogen of that particular plant, the non-pathogenic virus may be able to cause disease in the presence of the transgene, and/or have the opportunity to recombine with the transgene and in this way acquire pathogenic potential.

Given the possibility that transgenic virus-resistant plants may broaden the host range of some viruses or allow the production of new virus strains through recombination and transcapsidation demands careful further experimental investigation.

General Conclusions and Recommendations

The available scientific information allowed the group to conclude that although no catastrophic impacts have yet been recorded from the massive use of transgenic crops, the known and potential risks are substantial from an ecological point of view. It was generally agreed that because of the widespread use of transgenic crops, and the impossibility of effectively removing them once they are released, even more effects might persist and accumulate and eventually cause serious ecological impacts. For example nobody can really predict the impacts that will result from the Bt toxin that is released into the soil from roots during the growth of thousands of hectares of Bt corn , or the effects to the soil and general ecosystem from pollen during corn tasseling and as a result of the incorporation of tons of plant residues after crop harvesting.

Not enough research has been done to evaluate the environmental and health risks of transgenic crops, an unfortunate trend as most scientists feel that such knowledge was crucial to have before biotechnological innovations were upscaled to actual levels. There is a clear need to further assess the severity, magnitude and scope of risks associated with the massive field deployment of transgenic crops.

Much of the evaluation of risks must move beyond comparing GMC fields and conventionally managed systems to include alternative cropping systems featuring crop diversity and low-external input approaches. This will allow real risk/benefit analysis of transgenic crops in relation to known and effective alternatives.

The potential for ecological risks is to a large extent "event and context-specific". The particular risks which may be identified for the first wave GE offerings do no exhaust the list of potential risks from events yet in the pipeline. By the same token, ecological risks identified in the US or Canada may not be relevant to risks in Malaysia or Mexico - whether due to gene flow issues or to disruption of natural pest controls in more biodiverse environments. Risks in a "normal" weather year may not be predictive of those in a dry year (e.g. RR soybean stem splitting in Georgia), or to those experienced by farmers burdened by sporadic pest outbreaks. In short, identification and quantification of risks seems likely to remain an obligate and ongoing complement to the development and release of each new GE crop.

The repeated use of transgenic crops in an area may result in cumulative effects such as those resulting from the buildup of toxins in soils. For this reason, risk assessment studies not only have to be of an ecological nature in order to capture effects on ecosystem processes, but also of sufficient duration so that probable accumulative effects can be detected. The application of multiple methods will provide the most sensitive and comprehensive assessment of the potential ecological impact of transgenic crops.

Further empirical studies of the ecological impact of commercial-scale cultivation of transgenic plants are clearly needed, particularly with regard to the following questions:

- Which cultivated plants have sexually compatible wild relatives that could become troublesome weeds after inheriting fitness-related transgenes, and to what extent will this conversion to weediness occur?
- Will the propagation of certain transgenic plants result in the evolution of newly resistant plant pests (microbial pathogens, insects, and weeds), and if so, how can the evolution of these resistant biotypes be delayed or avoided?
- What effects will plant-produced pesticides have on the population dynamics of non-target organisms, especially beneficial predators, parasitoids, pollinators, components of soil food webs, and fundamental ecological processes?

Ecologists can provide valuable input in the planning and evaluation of high-risk genetically engineered plants, but does documenting the risks of such crops entails the best use of scarce ecological talent? Or should ecologists devote their time and skills to developing the best environmentally sound approaches to deal with real agricultural limitations, which in many cases are management options not related to biotechnology but rather to agroecology?

Overall the group felt that although biotechnology is an important tool, at this point alternative solutions exist to address the problems that current GMCs are designed to solve. The dramatic positive effects of rotations, multiple cropping, and biological control on crop health, environmental quality and agricultural productivity have been confirmed repeatedly by scientific research. Biotechnology should be considered as one more tool that can be used, provided the ecological risks are investigated and deemed acceptable, in conjunction with a host of other approaches to move agriculture towards sustainability.