MEETING

ABSTRACTS OF PRESENTATIONS ON SELECTED TOPICS AT

THE XIVTH INTERNATIONAL PLANT PROTECTION CONGRESS (IPPC)

July 25–30, 1999
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A: ISSUES FACING THE REGULATORY COMMUNITY AS REGARDS BIOTECHNOLOGY, GENETICALLY MODIFIED ORGANISMS AND TRANSGENIC CROPS

Biological and Environmental Risks Involved in the Use of Transgenic Crops

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The release of transgenic organisms has evoked an unusual legal process in that laws governing it are prospective on perceived risks rather than retrospective on experienced risks as is the usual case with legislating against problems. Most countries undertaking transgenic releases have adopted a regulatory structure usually comprising controlled releases to address questions of perceived risks followed by uncontrolled commercial releases. There has been an increasing number of commercial releases from approximately 11 million hectares of transgenic crops in 1997 to more than 27 million hectares in 1998. Most of these commercial releases have been in industrialized countries with only a small proportion in developing countries. The controlled releases, together with laboratory experiments, have addressed a range of perceived risks which can be put into three groups: risks to humans and domesticated animals, risks to the environment, and commercial risks. These perceived risks have to be assessed against the baseline of current and projected farming practices with non-transgenic crops. Few, if any, of these perceived risks have been shown to be real risks which are significantly more important than the non-transgenic situation. The situation with plants transgenically protected against virus infection was discussed. In some countries, the discussions on transgenic crop releases have entered the public domain. The debate has raised various ethical issues and reflects the wish of society to be involved in the adoption of new technologies. *[L]*

Risks of the Release of Transgenic Herbicide-Resistant Plants with Respect to Both Humans and Animals, and the Environment

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Cultivation of transgenic crops, produced by recDNA technology, has increased markedly during the last 3 years. Introduction of herbicide-resistant crops bears potential agronomic and environmental advantages, such as replacement of persistent herbicides by environmentally more benign compounds, reduction of herbicide use, and prevention of soil erosion. Potential disadvantages of the cultivation of such crops may be an increase in dependency on chemical weed control methods, increase in the development of resistance in weeds or the formation of multiple-herbicide-tolerant weeds through cross pollination, and a negative impact on biodiversity. Development and release of transgenic herbicide-resistant crops in the environment and market introduction of derived foods and animal feed have been the subject of thorough risk assessment of issues related to human/animal and environmental safety. Various national and international regulatory bodies have issued guidelines and regulatory directives which cover the legal requirements for release of these crops. Assessment of risks for humans and animals involves: (i) characterization of the donor and host organisms; (ii) characterization of the molecular/genetic aspects of the genetic modification; (iii) potential for gene transfer, in particular the relevance of genes coding for antibiotic resistance; (iv) safety of gene products and metabolites; (v) establishment of substantial equivalence, *i.e.*, a systematic analytical comparison of the composition of genetically modified plants and derived food or feed products with that of non-modified control varieties grown under identical conditions; and (vi) determination of the metabolism of applied herbicides on transgenic plants and levels of residues on these plants and in the foods of animals fed transgenic plant material. Evaluation of environmental issues related to cultivation of transgenic herbicide-resistant plants is focused on (i) potential for gene transfer/gene escape between modified crops and other plants; (ii) treatment of volunteers infesting follow-on crops; (iii) safety assessment for non-target organisms, *i.e.*, birds, insects and other invertebrates and soil organisms; and (iv) resistance/tolerance issues like selection pressure for resistant weeds, shifts in weed composition, and the formation of new resistant weeds. Experience with large-scale breeding of transgenic herbicide-tolerant crops has been gained mainly in the USA, whereas in Europe cultivation of such crops is limited to relatively small field-plot experimentation. Long-term effects of large-scale breeding depend on specific agricultural infrastructure and ecological conditions, and can therefore not easily be extrapolated between countries. Further studies are therefore needed on transgenic crop cultivation with respect to overall use of herbicides, development of resistance in weeds, changes in weed composition, and influences on biodiversity. Based on this information, codes of practices and specific monitoring programs may be designed. *[L]*

Transgenic Arthropods for Pest Management Programs: Risks and Realities

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It is now possible to manipulate genetically both pest and beneficial arthropods using recombinant DNA methods. A variety of transposable element and viral vectors can be used to insert DNA into the chromosomes of arthropods and a variety of potentially useful genes have been cloned. The genetic manipulation of both pest and beneficial arthropods opens new opportunities for improving pest management programs but also creates new responsibilities because we will have to evaluate the potential risks of releasing such transgenic organisms into the environment. The deployment of genetically engineered arthropods in pest management programs will require the resolution of several scientific and environmental issues, including quality control. Another fundamental issue is whether we understand how to deploy the genetically manipulated arthropod. Potential risks associated with permanent release into the environment include the possibility of altering the transgenic strain's behavior, ecological range, or host specificity. Risks will vary with the arthropod species, its effect on human health or its role in the environment; risks also will vary depending upon the genes inserted and the method of insertion used. Measuring the potential

risks of horizontal gene transfer is difficult. At present no guidelines are available that would allow transgenic arthropods to be released permanently into the environment and scientific, environmental, and policy issues remain to be resolved before transgenic arthropods can be deployed in practical pest management programs. *[L]*

Assessing the Risks of Releasing Genetically Modified Virus Insecticides

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Insect pathogens are one of the underexploited groups of biological control agents and their genetic modification offers great potential for expanding their use into major agricultural markets and even control of disease vectors. The technical problems posed by the modification of insect viruses are undoubtedly considerable, but this development also poses major challenges in terms of regulating their release and devising a usable framework which addresses meaningful questions of risk assessment. Baculoviruses are at the forefront of insect virus modification. Baculoviruses with enhanced speed of action have already been developed and successfully field-tested, and current research efforts are focusing on a second generation of baculoviruses which can be modified to attack specific pest complexes. However, baculoviruses are not the only insect viruses that have the potential to be altered genetically and work is already underway on other groups of insect viruses. Our knowledge of the biology and ecology of these other groups of viruses is poor, highlighting the need for more fundamental studies in advance of their wide-scale release. The potential risks that could be associated with releasing genetically modified insect viruses were discussed together with how these issues have been addressed in recent risk assessment studies. The questions that should be asked when considering the release of genetically modified bioinsecticides are essentially the same as those that need to be addressed for any biological control agent; however, microbial bioinsecticides have tended to be viewed more as chemicals, in terms of their risk assessment. A possible framework that could be applied to a broad spectrum of pest control agents was discussed. *[L]*

Risk Assessment of *Bt* Toxins Adsorbed or Bound to Clay Minerals

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The release of transgenic plants expressing genes from various subspecies of *Bacillus thuringiensis* that encode toxins active against several insect pests could result in the accumulation of these active proteins in the soil. These toxins may be adsorbed or bound to soil constituents such as clay minerals or to the clay-size fraction of the soil. Therefore, it is important to evaluate the potential risk of the toxins produced by transgenic plants after the non-harvested remainder of the plant biomass containing the toxins is incorporated into the soil. This is particularly important for transgenic plants containing truncated genes that encode active toxins rather than the nontoxic protoxins. Toxins and protoxins from *B. thuringiensis*, free or adsorbed or bound on pure clay minerals, are being tested against different insect pests: cotton leafworm, *Spodoptera littoralis* (*B. thuringiensis* subsp. *kurstaki*); Colorado potato beetle, *Leptinotarsa decemlineata* (*B. thuringiensis* subsp. *tenebrionis*); and the mosquito *Culex pipiens* (*B. thuringiensis* subsp. *israeliensis*). First we tested the insecticidal activity of purified toxins against the pests mentioned above. We found that the purified toxins presented lower insecticidal activities against the cotton leafworm and Colorado potato beetle than the commercial preparations from which the toxins were obtained. Experiments on the activity of toxins adsorbed and bound to clays are now underway. *[L]*

Gene Silencing: Ups and Downs in the Expression of Transgenes

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Impressive progress has been made in applying DNA transformation technology for crop improvement. Several major crop plants have been engineered with genes imparting resistance to viruses, to insect pests, and to certain herbicides. Unexpectedly, transgenes introduced into plants can inactivate expression of additional copies of the transgenes as well as host genes with similar nucleotide sequences. This effect, called gene silencing, is a general phenomenon reported for many plant species transformed with a wide variety of different transgenes. Gene silencing is a form of epigenetic modification which is stable but can undergo spontaneous as well as developmentally regulated reactivation. Although the underlying mechanisms are still unknown, evidence suggests there are two major forms of silencing: transcriptional gene silencing (TGS), which is often meiotically transmissible and associated with DNA methylation; and post-transcriptional gene silencing (PTGS), which is usually not meiotically transmitted and depends on interactions between transcribed sequences, shows pronounced developmental and environmental regulation, and can sometimes spread systemically throughout a plant. Silencing is a crucial factor in obtaining reliable expression of agronomically important transgenes under field expression. It also offers opportunities for 'knocking out' host genes for crop improvement and suggests novel mechanisms for gene regulation and the interaction of viral pathogens with plants. The aim of this workshop was to review recent advances leading to the identification of parameters important for regulating TGS and PTGS and to explore possible applications of silencing. *[L]*

Transcriptional Gene Silencing

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Transgenes and endogenous plant genes can become silenced at the transcriptional level. Transcriptional gene silencing (TGS) of transgenes can vary in intensity leading to a partial or complete inhibition of transcription, and can appear directly after the transfer of the transgene or progressively during the development of the plant or in progeny plants. Therefore, TGS represents a serious problem for the use of transgenic plants in agriculture. TGS is frequently associated with a condensation of the chromatin structure of the transgene, coupled with hypermethylation. Despite the fact that the mechanisms are still not completely elucidated, several features can be distinguished that contribute to TGS, such as the expression level of the transgene, its homology with other sequences present in the genome, its 'foreign' characteristics with regard to the host genome, the presence of vector sequence in the transgene, its secondary structure and its integration site in the genome. We discussed the current models for TGS, and the ways to avoid TGS in order to produce stable expressing transgenic plants. *[L]*

Mechanisms of Post-Transcriptional Gene Silencing

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The introduction of transgenes with sequence homology to endogenous genes frequently results in events of gene silencing. This suppression of endogenous gene and transgene activities has

been linked to hypermethylation of promoter regions that inhibit transcription or to enhanced post-transcriptional RNA degradation. Although extensive studies were initiated almost 10 years ago to understand the sequence-specific, post-transcriptional RNA degradation, the mechanisms remain obscure. However, in most of the recent models for post-transcriptional gene silencing, including those for RNA-mediated virus resistance, a homologous RNA molecule has been suggested to act as the primary trigger or perpetuator of degradation, *e.g.* an inefficiently processed RNA, an aberrant RNA or an unproductive RNA. It has also been suggested that these irregular RNAs result either directly from foreign transgene structures or indirectly from the conversion of the genes or their transcripts into aberrant structures following homologous DNA-DNA, RNA-DNA or RNA-RNA interactions or from antisense RNA production. Double-stranded (ds)RNA intermediates formed by inter- or intramolecular RNA pairing appear to be a common structure in all the post-transcriptional gene silencing systems under investigation. These dsRNAs may be the signaling molecules for the maintenance of the silencing state *via* autocatalytic cycles of RNA degradation, for the assumed intracellular cross-talking between the cytoplasm and the nucleus, and for the observed systemic spreading of gene silencing. *(L)*

Applications of Gene-Silencing Technologies in Plant Biotechnology

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Ever since gene silencing was discovered, considerable research effort has been applied to understanding the mechanisms underlying this phenomenon. In the late 1980s we decided to use this approach to generate genetically modified processing tomatoes in which the cell wall enzyme, polygalacturonase (PG), was inhibited. From several hundred lines which were generated in the laboratory, very few were selected for commercial testing. These tomato lines have now been grown in the glasshouse and released into the environment for the past 9 years. The knowledge obtained from this work and the implications for the application of gene silencing technologies to applied plant biotechnology were discussed. *[L]*

C: INTERNATIONAL TRANSFER OF PLANT PROPAGATION MATERIAL, GERMPLASM AND RELEVANT QUARANTINE POLICIES AND REGULATIONS; INTERNATIONAL RESEARCH COOPERATION IN IPM

Current Quarantine Principles and Germplasm

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In order to minimize impact on trade, various treaty obligations require National Plant Protection Organizations (NPPOs) to manage phytosanitary risks in a transparent and non-discriminatory manner. The necessary scientific data to make decisions are considered in pest risk analyses (PRAs). Decision-making processes need to accommodate provision for a range of equivalent risk management options. Improved breeding techniques, including biotechnology, have challenged NPPOs by increasing the rate of germplasm movement. Germplasm as vegetative material (budwood, tubers, etc.) presents a high risk pathway for introduction of vascular pests. The pest risk identified may warrant specific restrictive management options. Testing requirements for latent pests normally include post-entry quarantine. Many PRAs result in import conditions for other forms of germplasm (tissue cultures and seed) being less restrictive, as the risk of vascular pests is reduced. In order to facilitate safe access, the Food and Agriculture Organization (FAO) and the International Plant Genetic Resources Institute (IPGRI) have developed germplasm exchange guidelines at a generic

level for a number of crops. These contain pest information that NPPOs can use to develop phytosanitary measures. With the intention of maintaining pest risk offshore, import conditions, as specified in an import permit, may require phytosanitary certification by the exporting NPPO. Treaties also oblige governments to publish their phytosanitary measures, immediately on their adoption, and transmit their intent to affected parties. NPPOs must provide the rationale for measures on request, so that others can evaluate their technical validity. NPPOs have to recognize that phytosanitary measures for germplasm must be based on assessed risk and equivalent in terms of accepted risk to those for products of the same crop, *e.g.* citrus budwood and fruit with respect to citrus canker (*Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin). *[L]*

International Movement of Plant Germplasm

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Crop improvement efforts require access to a wide range of genetic diversity. Breeding programs therefore often have to introduce germplasm from other countries. It is important that the necessary precautions be taken to avoid the simultaneous introduction of pests and diseases that may cause serious damage in the introducing country. This is particularly true for vegetatively propagated plants, with which the risks involved are considerably greater than with seed propagated plants. Germplasm has specific characteristics that allow and/or require it to be handled in a different way from bulk shipments of commodities. A range of precautions can be taken in order to minimize the risks involved with the introduction of germplasm. These include the choice of the origin of the material, the choice of the type of material, the application of various types of treatments, performing disease detection tests and observation of the material in intermediate or post-entry quarantine. The decision on how to handle the introduction must always be based on risk assessment. For a number of important species, IPGRI, in collaboration with the Food and Agriculture Organization (FAO), has produced technical guidelines for the safe movement of germplasm, in which specific recommendations are made in order to minimize the risks involved with the introduction of germplasm. *[L]*

International Transfer of Plant Propagation Material

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The production and worldwide distribution of plant materials consisting of whole plants, cut flowers, seeds and vegetative propagants have become an integral part of the agricultural trade. In addition to commercial bulk shipments of plants and plant products, there is considerable movement of germplasm to *ex situ* collections, breeding programs and other scientific purposes. One of the concerns regarding movement of plant material is an accidental introduction and distribution of quarantine pests (harmful biotic agents) into areas where they do not occur or are not widely distributed. Vegetatively propagated plant materials infected by pathogens that are often symptomless (*e.g.* viruses) pose special risks. Phytosanitary regulations and policies, established by individual countries and by multi-country organizations in Europe and North America, were designed to prevent entry and spread of pests over natural barriers. These regulations are enforced by quarantine and other authorized inspection agencies. High standards are imposed by many countries for import of plant material and highlight problems involved with safe movement of pest-free plant material, such as post-entry quarantine protocols, rapid virus detection methods, etc. Within the framework of this workshop, several key issues were presented and discussed by a panel of experts. *(L)*

Examples of International Research Cooperation in Integrated Pest Management

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One of the priority areas of German development policy is protection of the environment and natural resources. Increased food production with the aim of food security has to be achieved in an environmentally sustainable manner. Integrated Pest Management is therefore considered a very important component in projects supported within the framework of international agricultural research. Many projects include aspects of IPM, whereas others focus solely on pest control in an environmentally sound way. Examples of successful research projects are the following:

- *Striga* control strategies for cropping systems in Kenya
- Integrated disease management in cereals and legumes in West Asia/North Africa
- Integrated technologies for the management of banana weevils
- Biological control of the larger grain borer
- Integrated control of bacterial diseases of cassava and cowpea

Research collaboration of international agricultural research centers, national agricultural research systems, and bilateral technical cooperation with a high degree of farmer participation, integrates all interested parties and is therefore likely to achieve the goal of sustainable yield increase. *[L]*

D: MINOR CROPS: ISSUES OF RESIDUES AND OFF-LABEL REGISTRATION

Promoting Pesticide Registration for Minor Crops in Israel

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The rationale for the development of export crops in Israel relies on an aggressive introduction and adoption of new crops. This development policy is especially true for the following industries: flowers and ornamentals, herbs and spices, vegetables and subtropical fruit crops. Throughout their adoption process, most of these crops have high technical needs in the area of chemical pest, disease and weed control. Since the adoption process is a gradual one, even the more promising new crops are grown for several years on relatively small areas. However, due to their intensive cropping patterns, most of these crops present high risks in the use of pesticides. The chemical industry is usually reluctant in this case to generate data to support applications for registration and subsequent use. To alleviate some of these difficulties, while maintaining the high safety standards applicable in the country, an off-label approval scheme for use on minor crops will be granted where the following conditions are met: (*a*) crops are grown on an area of less than 500 ha in the country; (*b*) biological efficacy will be extrapolated from major crops and their respective pests, pathogens and weeds; (*c*) application will be supported by evidence with regard to safety for non-edible crops, maximum residue levels and safety for edible ones; (*d*) off-label arrangements will not be granted to aerial applications or to the use of restricted chemicals; (*e*) off-label arrangements will be reviewed by the pesticide registration committee; and (*f*) growers would be held responsible for the application of off-label approved pesticides. *(L)*

The Challenge of Minor Crop Pest Management in the USA: Contributions and Future Directions of the IR-4 Program

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As regulatory requirements for pesticides are intensified under the 1996 Food Quality Protection Act, and many older compounds are lost, the declining availability of pest management tools for minor crops is a serious concern in the USA. The challenge of obtaining clearances for crop protection chemicals on minor crops continues to fall primarily on the Minor Crop Pest Management (IR-4) Program. This USDA-funded program has been active for 35 years and in 1999 has a budget of US$12.4 million. These funds are used to run field trials and residue analyses to establish the magnitude of residue data. Most of these studies are conducted at 25 field research centers located strategically across the US. Residue analyses are conducted at the 16 IR-4 regional and satellite laboratories. The program is coordinated from headquarters at Rutgers University. Project priorities are set in conjunction with registrants, university specialists and growers' groups, and are reviewed with the U.S. Environmental Protection Agency (EPA). Since 1963 the program has obtained almost 5,000 clearances for food uses and a comparable number for ornamental crops. Fifty clearances for biopesticides have been obtained since this program began in 1982. In responding to the minor crop challenge, the future directions for the IR-4 program include: (i) Focusing effort preferentially on reduced risk pesticides; (ii) ensuring earlier availability of new chemistry to minor crop users by working on active ingredients before a major registration has been approved; (iii) expanding the scope of crop groupings to gain maximum benefits from residue studies; (iv) increasing collaboration with other countries in conducting joint residue trials and sharing data; and (v) encouraging the registration of genetically engineered minor crops with pest or pesticide resistance. *(L)*

Minor Crops: Issues of Residues and Off-Label Registration – The USA Perspective

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Minor crops comprise approximately 40% of the value of U.S. crop production. Included in this category are most fruits and vegetables, ornamentals, herbs and spices. Increasing regulatory requirements over the last 30 years have steadily raised the costs of pesticide registrations. This situation has had negative impacts on the availability and variety of pesticides used in pest management for the minor crops. The Food Quality Protection Act (FQPA), which amended the two major statutes governing pesticide registration and use, increased concerns over adequate pest management tools for minor crops. This factor was recognized in FQPA, and part of the legislation addresses this issue. However, in efforts to keep residues within the limitations imposed by FQPA, many currently registered uses appear likely to be dropped. This situation raises questions for the producer about growing and marketing the crop as well as for consumers about the continued availability, quality and price of minor crops. Various options must be considered, ranging from cessation of production of some commodities or seasonal availability only, to finding alternatives to current pest management systems. All options for pest management tools should be examined. These include research on new chemistries, biological control, delivery systems for pest management materials, integrated farming systems, and new crop varieties utilizing biotechnology to incorporate pest resistance. Public as well as private investments will be required to maintain viable minor crop production systems. Growers, the crop protection industry, universities and the government must combine efforts to maintain and enhance minor crop production. *(L)*

E: GENETICALLY MODIFIED MICROBIAL CONTROL AGENTS

Genetic Engineering of Endotoxin Synthesis in *Bacillus thuringiensis* for Improved Efficacy

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Most current products based on *Bacillus thuringiensis* consist of wild-type strains active against lepidopterous, coleopterous, or dipterous insects. While still effective, these products face increasing competition from new chemical insecticides and insecticidal transgenic plants. To remain competitive, bacterial insecticides based on *Bt* are being improved by engineering: (i) strains to produce novel combinations of toxins, (ii) more toxic proteins, and (iii) toxin production to increase toxicity per unit cell weight. More than 80 genes encoding insecticidal toxins have been cloned and sequenced. In addition, genetic elements have been identified that can be manipulated to increase net toxin synthesis per cell. Wild-type and recombinant *Bt* genes have been combined with genes for helper proteins and various genetic elements to achieve increases in endotoxin production ranging from 1.25- to 10-fold. The two helper proteins identified to date are the 20-kDa protein encoded by the *cry11A* operon and the 29-kDa protein encoded by the *cry2A* operon. Genetic elements include strong single and dual promoters, recombinant promoters, mRNA stabilizing sequences such as the 5' STAB-SD sequence of the *cry3A* gene, and various 3' stem-loop structures. Adding the 20-kDa protein gene to wild-type and recombinant *Bt*'s, for example, increases Cry toxin production from 1.25 to 5-fold, depending on the strain. Adding the 29-kDa protein gene to *cry* gene constructs also increases toxin production, by 1.2- to 1.5-fold. Combining the STAB-SD sequence with *cry* genes and driving expression with *cyt1A* promoters increases toxin production from 1.5- to more than 8-fold, with the best results obtained with naturally truncated endotoxins such as Cry2A and Cry3A. Using these elements together with new combinations of Cry proteins provides a variety of tools to increase bacterial insecticide efficacy further. *[L]*

Analysis and Genetic Engineering of Baculovirus Genomes for the Control of Insect Pests

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Baculoviruses are members of a family of rod-shaped viruses with a unique pathology in insects and crustaceans. These viruses are accommodated in two genera, Nucleopolyhedro-virus (NPV) and Granulovirus (GV). The NPVs consist of two morphotypes, depending on whether a single (S) or multiple (M) nucleocapsids are packaged into an envelope. The virions are usually occluded into large proteinaceous crystals or polyhedra. These baculoviruses are successfully used as control agents of insect pests, but lack, *inter alia,* sufficient speed of action. Using genetic engineering strategies some of these baculoviruses, *e.g. Autographa californica* MNPV, have been engineered and provided with genes encoding insecticidal proteins. In order to engineer these baculoviruses successfully, the location, structure and regulation of target baculovirus genes must be known in some detail, in particular their promoters. In addition, potential insecticidal genes and suitable transfer systems should be available to introduce the insecticidal gene into the baculovirus genome. Finally, extensive laboratory and field testing, including ecological impact studies, are required to achieve commercialization. In this contribution the 'state of the art' in the engineering of baculoviruses for improved insecticidal properties was reviewed. In addition, we reported in particular on the engineering of the *Heliothis armigera* SNPV, taking advantage of the information obtained from genomic analysis. This research was supported by grant 29/97 from The Joint Dutch–Israeli Agricultural Research Program 1998-2000. *[L]*

Ecology and Risk Assessment of Genetically Modified Baculoviruses: Field and Laboratory Studies

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The ongoing development of genetically modified baculoviruses provides the opportunity to extend the use of these more benign methods of pest control and reduce chemical input, particularly in agricultural systems. Baculoviruses with a more rapid speed of kill have already been developed and successfully field-tested, and future targets for genetic modification include the alteration of host range. However, before these novel organisms are released we must investigate whether there are likely to be any negative environmental consequences, particularly on non-target hosts. Over the past 5 years we have carried out a program to assess the risks of releasing genetically modified baculoviruses (GMBVs). As a model system we have used the alfalfa looper, *Autographa californica,* nucleopolyhedrovirus (AcNPV) and a recombinant which expresses an insect selective scorpion toxin (AcNPV-ST3). Using an ecological approach which combines both empiricism and host-pathogen theory, we have attempted to develop a general framework for the risk assessment of GMBVs. As a first step in this process, the relative fitness of the wild type and genetically engineered AcNPV has been compared using detailed laboratory assays and small-scale manipulative field experiments. Simple mathematical models highlight four key parameters which can alter the basic reproductive rate of the virus, thereby influencing whether it is likely to establish in non-target populations. These parameters: speed of kill, productivity, transmission and persistence, have been estimated for these two viruses. The results of these experiments and their implications for risk assessment were discussed, together with the implications of future developments in baculovirus modification. *[L]*

Increased Infectivity of a Baculovirus Recombinant Expressing an Insect-Selective Neurotoxin in *Spodoptera exigua* Larvae

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Baculoviruses are naturally occurring pathogens which can infect a wide range of lepidopteran pest insect species. These viruses are environmentally sound alternatives to chemical insecticides as control agents of insect pests. The agricultural use of baculoviruses has been limited *inter alia* due to their restricted specificity, high production costs and slow speed of action as compared with chemical insecticides. Recombinant baculoviruses expressing insect-specific neurotoxins are promising candidates as improved (*i.e.*, fast-acting) biological control agents of insects. The infectivity and speed of action of an *Autographa californica* baculovirus (AcMNPV) recombinant expressing the insect neurotoxin from *Androctonus australis* Hector (AaIT) were determined in *Spodoptera exigua* larvae and compared with wild-type AcMNPV. AcMNPV-AaIT showed a 25% reduction in median lethal times (*i.e.*, faster acting), but had also strongly reduced LD_{50} values and steeper probit regression lines for second, third and fourth instar *S. exigua* larvae. The steeper regression lines may indicate reduced variability in larval susceptibility for the AcMNPV-AaIT recombinant. The reduced LD_{50} values may also suggest that AcMNPV-AaIT-infected larvae produce altered polyhedra or that the lowered values are the result of an interaction between AaIT and the larval defense system. A lower LD_{50} entails an agronomic advantage (lower dose needed), but may also constitute an additional environmental risk of AcMNPV-AaIT recombinants. This research was supported in part by grant no. 805.18.758 from the Stichting Technische Wetenschappen of the Dutch Organization for Scientific Research. *[P]*

Strategies to Enhance the Insecticidal Potency of Recombinant Baculoviruses Expressing Anti-Insect Toxins

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Engineering baculoviruses with cDNAs encoding anti-insect selective toxins results in enhancement of the speed of kill of their lepidopteran pest targets. However, despite the progress achieved in this direction, further enhancement of the speed of kill of baculoviruses is required to promote their competition with chemical insecticides. Our approach to achieve this goal includes: *(a)* earlier expression of the anti-insect toxin by placing it under the control of various viral promoters; *(b)* the utilization of more potent toxins; and *(c)* synergistic effects among anti-insect selective toxins expressed by one or a combination of baculoviruses. Our data indicate that *Autographa californica* nucleopolyhedro-viruses engineered according to the above principles showed increased speed of kill of *Helicoverpa armigera* and *Heliothis virescens* larvae, thereby paving the way to the implementation of recombinant baculoviruses in pest control. This research was supported by grant IS-2530-95C from BARD, the Binational Agricultural Research and Development Fund. *[P]*

Cloning and Expression of Cell-Wall-Degrading Enzymes from *Trichoderma harzianum*

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Trichoderma harzianum strains are used as biocontrol agents due to their antagonistic activities against a wide range of phytopathogenic fungi and there is a large body of results supporting a key role for cell-wall-degrading enzymes (CWDEs) during *Trichoderma*'s antagonism. Our group has been dealing with the identification, purification and characterization of CWDEs of *T. harzianum* CECT 2413, making a significant contribution to the understanding of the amazingly complex set of lytic enzymes involved in mycoparasitism. More than six different CWDEs have been purified and four genes (cDNA), coding for 42 and 33 kDa endochitinases, a β-1,3-endoglucanase and a β-1,6-endoglucanase, respectively, have been cloned. Transgenic plants expressing the 42 kDa endochitinase showed increased resistance/tolerance against some fungal pathogens. Transgenic *Trichoderma* strains with constitutive high expression of 33 kDa endochitinase have improved mycoparasitic activity compared with the parental strain, in dual culture assays against *Rhizoctonia solani*. The β-1,6-endoglucanase gene (BGN16.2) constitutes the first report of a gene coding for a protein with this enzymatic activity. Other new enzymes with protease and glucanase activity are also under study. These genes can be an important heterologous source of resistance/tolerance to fungal pathogens in plants. They can also be used for improvement of biocontrol abilities of antagonistic microorganisms and their products can be included in effective fungicide formulations with lower health and environmental risks than chemical pesticides. *[L]*

Transformants of *Trichoderma longibrachiatum* Overexpressing the β-1,4-Endoglucanase Gene *egl1* Show Enhanced Biocontrol of *Pythium ultimum* on Cucumber

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Correlation between the production of chitinolytic enzymes and suppression of plant pathogenic fungi containing chitin as the main cell wall constituent has been demonstrated by several authors for many *Trichoderma* species. In spite of the extensive studies of chitinases, little is yet known about the role of cellulolytic enzymes in the biocontrol of plant pathogenic oomycetes, which contain cellulose as the main cell wall component. The purpose of this study was to determine the involvement of the EGL1 β-1,4-endoglucanase (E.C. 3.2.1.4) from *Trichoderma longibrachiatum* in the biological control of damping off of cucumber caused by *Pythium ultimum*. Nine transformants of *T. longibrachiatum* with extra copies of the *egl1* gene were studied for mitotic stability, endoglucanase production, and biocontrol activity against *P. ultimum* on cucumber seedlings. The transformants showed a significantly higher level of expression of the *egl1* gene in comparison with the wild type under both inducing and non-inducing growth conditions. Transformants with the *egl1* gene under the control of a constitutive promoter had the highest enzymatic activity. Both the endoglucanase activity and the transforming sequences were stable under non-selective conditions. When applied to cucumber seeds sown in *P. ultimum*-infected soil, *T. longibrachiatum* transformants with increased inducible or constitutive *egl1* expression were more suppressive than the wild type strain. *[L]*

Molecular Approaches to Increasing Biocontrol Activity of a Soilborne *Enterobacter* Strain by Introducing Heterologous Regulatory Signals

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Enterobacter agglomerans strain IC1270, an antagonist of many phytopathogenic fungi, secretes one endochitinase and two exochitinases. The possibility of further increasing the chitinolytic and antifungal activity of this strain by introducing regulatory genes from *Pseudomonas* was tested. Strain IC1270 was transformed with the *Pseudomonas* genes encoding transcription sigma factors *rpoS* (σ^{38}) and *rpoD* (σ^{70}) or the two-component global regulation system GacA-ApdA, known to mediate changes in gene expression in response to sensor signals. The activity and pattern of chitinolytic enzymes secreted by the parent strain and its derivatives carrying these heterologous regulatory genes were compared in a liquid assay using chromogenic chitin derivatives or by SDS-PAGE using fluorescent chitin derivatives. Several differences from the parent strain were observed in the resultant derivatives, depending on the regulatory gene introduced: an increase in total inducible chitinolytic activity and in the intensity of some bands corresponding to exochitinase production; the appearance of a new endochitinase and of constitutive chitinolytic activity; and an increase in the efficiency of growth suppression of several fungal pathogens, both *in vitro* and under greenhouse conditions. Sequences with partial homology to these regulatory genes of *Pseudomonas* were found in the genomic DNA of strain IC1270. Therefore, the detected differences may be the result of responses to additional sensor signals and/or an imbalance in the sigma-factor ratio. *[P]*

F: CAN BIOTECHNOLOGY ASSIST CROP PROTECTION IN DEVELOPING COUNTRIES?

Importance of Private–Public Sector Cooperation in Advancing Biotechnology for Crop Protection in Developing Countries

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Central to the importance of improved cooperation between the public and private sectors in using biotechnology is the common goal of meeting the needs of humanity for food, feed and fiber in the next millennium. The world's population is anticipated to exceed ten billion in 2050; most of these people will live in the developing countries, and in 'mega-cities'. Approximately 50% more food

has to be produced in the next 20 years on less land, and with less water and less labor. To achieve this requires that science and technology be directed at *(a)* raising yield plateaus, *(b)* narrowing yield gaps of current crop cultivars, and *(c)* maintaining or stabilizing current actual yields in farmer's fields. All three aspects have strong crop protection elements, as well as opportunities for advances from biotechnology. Crop plants genetically engineered for tolerance to herbicides, insect pests and pathogens are now grown on over 28 million ha worldwide, with the USA, Canada and Argentina accounting for 89% of the total area. Biotechnology has therefore moved from the realm of research conducted in the public and private sectors, to the realm of commercialization, mainly by companies in the private sector. This rapid movement would not have occurred without strong collaboration between the two sectors on the regulatory aspects of biosafety and commercialization, especially in North America. Between 1986 and 1998, 45 countries approved >25,000 field trials of transgenic plants of some 60 crop species. Most developing countries now have biosafety guidelines for field tests of transgenic crops. The private sector is concerned about having the freedom to operate with transparent regulations, having a 'leveled' playing field where all entities compete equally; allowing clients to have the freedom to choose; and finally, with continued R&D using partnerships with the public sector, non-governmental organizations and the civilian society. Industry and governments share common objectives relative to agricultural biotechnology. Important issues in cooperation remain, such as in: traits (identification, prioritization); structural genomics; functional genomics (including physiology); biosafety issues; field test protocols; and intellectual property protection. The impact of biotechnology, and its underlying science of molecular genetics, is only just being felt on modern agriculture. Much remains to be discovered through strategic research, and new applications are yet to be found. The private sector is known to outspend the public sector in plant biotechnology R&D by a ratio of approximately 30:1. All this points to the need for greater cooperation between the public and private sectors at a time when the world has to face the critical issues of food sufficiency and environmentally sustainable development. *[L]*

Mapping Genes Governing Resistance to Insect Pests of Rice

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To accelerate the insect resistance breeding programs in rice, molecular markers are quite useful. If resistance genes are tagged with DNA markers, time and money can be saved in moving the genes from one varietal background to another. In India, brown planthopper (BPH), white backed planthopper (WBPH), yellow rice stemborer (YSB) and rice leaffolder (RLF) are the major insect pests limiting rice production. Studies were undertaken to identify the molecular markers linked to the resistance genes of the above pests. Recombinant inbred lines (RILs) were obtained from the *Oryza officinalis* derived lines IR 54745-2-21-12-17-6 (Resistant) and IR 50 (Susceptible) to map the genes for BPH and WBPH. RILs are also being developed with W1263 (R) and Co43 (S) and TNAU LFR831311 (R) and IR36 (S) for YSB and RLF, respectively. In mapping BPH resistance genes, four RAPD primers and two microsatellites were identified as polymorphic between the parents and population. The co-segregating RAPD and microsatellite markers were mapped. The closely linked marker AJ9b was mapped on rice chromosome 3 using the Lemont and Teking population, and cloned, sequenced and converted into STS marker. For WBPH resistance, 64 AFLP primer combinations were used. The primer combination EAGG-MCAA showing co-dominant bands was selected for scoring RILs and parents. A 276 bp DNA fragment that was co-segregated with the resistant phenotype was cloned, sequenced and converted into STS marker. A parental survey with SSR and RAPD markers was done with the YSB and RLF mapping populations which are now in the F_4 and F_3 stages, respectively. *[L]*

Supply of Pest-Free Stock

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Crop production is threatened by a range of pest and disease pressures. The use of healthy planting material is an effective strategy for the prevention of yield losses. Hence, high quality plant propagation material free of pests (harmful biotic factors, including viruses) to ensure a healthy start of the ensuing crop is a prerequisite to high productivity in modern agriculture. In vegetatively propagated plants, viral diseases not only cause yield reduction but also are transmitted through vegetative plant parts to the next generations. This is also true for seedborne pathogens. Thus, disease infection has significant implications in germplasm exchange across national boundaries. The production of pest- and pathogen-free plants is a prerequisite for the international exchange of germplasm, to avoid any risk of introducing diseases and pests into non-affected areas. Meristem-tip culture is commonly applied to eliminate viruses from plants. The fact that some viruses are more difficult to eliminate than others has led to the use of thermotherapy and chemotherapy, along with meristem-tip culture, to increase the efficiency of virus elimination. Tissue culture, done under sterile conditions, also eliminates other pathogens and insects. Tissue culture material is considered to be the most suitable form for international exchange of vegetatively propagated crops. Plants regenerated from meristem-tips, however, are not automatically free of disease infections. Therefore, reliable disease diagnostic protocols are essential in a disease elimination scheme. Development of rapid and sensitive techniques for the identification and detection of pathogens, particularly viruses, has been pursued in order to facilitate germplasm transfer. While enzyme-linked immunosorbent assay (ELISA) was a revolution in the early 1980s, particularly for virus detection, these tests are often not sufficiently sensitive to detect low-titered viruses in infected plants and are complicated by the occurrence of serological diversity among virus isolates. Modern biotechnology provided monoclonal antibodies and polymerase chain reaction (PCR)-based protocols, which are highly sensitive and provide greater confidence in obtaining disease-free materials. Thus, production of pest-free planting materials, using biotechnological approaches, became easier and simpler, and will be highly beneficial to farmers in developing countries. *(L)*

Rapid Propagation of Virus-Tested Potatoes in Kazakhstan

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Potatoes are the second most important crop in Kazakhstan, grown on approximately 425,000 ha. The average yield is only 8 ton/ha, less than 20% of the yields obtained in the USA, Europe and Israel. Seed tubers are at present taken from the same fields grown for market potatoes. In 1992–93, using certified seeds from Holland, yields of approximately 35–40 tons/ha were obtained. Surveys for viruses in potato fields uncovered a high incidence of virus symptoms. After establishing an ELISA laboratory in Almaty and preparing high titered antisera of PVX, PVY, PVS and PVM (potato virus x, y, s and m, respectively), several surveys for viruses and aphids were performed in several regions of Kazakhstan. Local potato varieties were found to be highly infected (15–75%), most infections being due to PVX, followed by PVY and PLRV (potato leafroll virus). Infection rates reaching 15–20% of PVS and PVM were also observed. Two areas with low aphid populations were located. Through meristem cultures and heat therapy, virus-tested plants of the varieties 'Nievsky' and 'Tamasha' were

prepared. These plants were propagated by tissue culturing and minitubers were prepared in Astana. In 1998 approximately 50,000 minitubers were planted in northern Kazakhstan, yielding 28 tons/ha, compared with 14 tons/ha in commercial fields in this region. This scheme of rapid propagation and minitubers shortens considerably the number of generations from the nuclear plants to certified seed and reduces the possibility of exposure to re-infection by viruses. This work was supported by a US AID grant within the CDR/CAR (Cooperative Development & Research/Central Asian Republics) program. *[L]*

'SWOT' Analysis of Transgenic Cotton in India

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India is a booming market for any international cotton seed trader as it stands first in the cotton area in the world. Varied agroclimatic zones in India have made cotton research location-specific. Hence, strength, weaknesses, opportunities and threats (SWOT) analysis of transgenic (*Bt*) cotton presently entering India was undertaken, based on the available and collected cotton statistics as a benchmark. Present cotton situations in all three cotton-growing zones of India were kept as base and the prospects of *Bt* cotton in each zone were estimated. North India is a predominantly *Gossypium hirsutum* area (1.34 mha), grows only varieties and fortunately almost totally irrigated. *Bt* cottons have an opportunity here only if the genotypes are resistant to leaf curl virus, which is jeopardizing cotton cultivation here. For *Bt* varieties unwanted spread is a threat. Central India contains equal areas under hybrids and diploid cottons (1.75 mha), with the hybrid area likely to increase. The *Bt* intrahirsutum hybrids are an open opportunity here but need wider adaptatibility. In South India 60% of the cotton area is under interspecific and intrahirsutum hybrids. Pest outbreaks are a common phenomenon *e.g.* with whiteflies and *Spodoptera*. The best opportunity for transgenic cotton appears to be here. Transgenic cotton needs to be evaluated against *Helicoverpa armigera*, which requires a higher level of resistance than *H. zea* or *H. virescens*. Multiple gene constructs through hybrids have a good chance of success. Continuous cultivation of *Bt* cotton may change the pest scenario, as happened in South India with respect to interspecific hybrids. Location-specific *Bt* cultivars are required in India. The opportunities are equal if weaknesses are overcome by more strength. *[P]*

Genotypic Requirements for the Success of Transgenic Cotton in India

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Insects pests in cotton constitute the main bottlenecks in increasing cotton production in India and involve as much as 60% of the cost of cultivation. Resistance to insect pests in cotton is achieved by mechanisms like non-preference, antibiosis and escape. A decade of research on cotton plant characters involved in imparting tolerance/resistance to insects pests was discussed along with their suitability in utilizing them as raw genetic stocks in the production of successful transgenic cotton genotypes. Studies of genotypes emerged from composite cross *vis-à-vis* the data of cultivars released from the resistant/tolerant material as compiled. The data on these mechanisms of insect pest resistance were pooled for discussion. Jassid and aphid resistance in cotton plants was found to be attainable through plant characters like pubescent thin leaves, petioles and leaf veins *vis-à-vis* anatomical characters like thick coating of epidermis, compact mesophyll cells and dense cortical cells in petioles, stem tips and midribs. The distance between the epidermis and phloem is another factor imparting resistance to sucking pests. Cotton genotypes possessing the following traits were found tolerant to bollworms: glabrousness, light green leaf color, plant pigmentation, long pedicel, cream petal color, smaller bracts, fewer bracteole teeth, thick boll rind, boll hardness, etc. The biochemistry of bollworm tolerance revealed mechanisms such as high-gossypol glands, high tannin, low protein and sugar contents in different plant parts. Studies of multiple pest tolerance (jassid,

aphid and bollworm) indicated involvement of the following characters: glabrousness on the upper surface of the leaf and pubescent lower surface of the leaf, small bracts and fewer bracteole teeth, along with high-gossypol glands. The breeding work which followed has resulted in commercially accepted intrahirsutum and interspecific hybrids along with *Gossypium hirsutum* varieties. Selection of genotypes for production of transgenic cotton has to be based on the characters conferring tolerance/resistance to pests. This will not only help in sustaining the crop's resistance but also avoid primary infection through non-preference. Such genotypic specificity is needed in transgenic programs, where insecticidal genes require a congenial environment for proper results. *(P)*

G: TRANSGENIC CROPS RESISTANT TO HERBICIDES – AGRICULTURAL AND ENVIRONMENTAL IMPLICATIONS

Transgenic Crops Tolerant to Herbicides on the Market

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Herbicide tolerance was one of the first agronomic traits introduced into crops by genetic engineering. There are several reasons for this: biochemical and molecular mechanisms were known and available when genetic engineering of plants was developed in the early 1980s; tolerance to herbicides is easy to score and can be evaluated in the laboratory, greenhouse and field; there is a demand by farmers for better weed control to allow flexibility of treatments, larger return on products, better protection of the crops; and finally, there is an economic incentive for the farmers, and for the seed and agrochemical companies. Ten years later, several crops tolerant to herbicides are on the market in different countries. Tolerance to various herbicides such as bromoxynil, glyphosate, glufosinate, sulfonylureas, imidazolinones, 2,4-D, quizalofop and sethoxydim, have been developed. Two main strategies have been used: detoxification of the herbicide or introduction of a mutated target. Crops tolerant to herbicides are available mainly on the Canadian market, in the USA and Argentina. Europe is behind for various reasons. In certain countries and for certain crops, the tolerant crops represent more than 50% of the market. Stacking with other traits, such as insect resistance, is appearing on the market, mainly for corn and cotton. Herbicide tolerance is also a good marker system either during the regeneration process or to select transformed plant cells. It is also a good breeding tool to select stacked systems. *[L]*

Global Use of Roundup Ready®Crops

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Green plants with natural tolerance, resistance or selectivity to glyphosate have been difficult to find. Roundup Ready®crops are genetically enhanced to resist glyphosate and approved crops have been tested to insure that plant phenology and ontogeny are no different from conventional crops. Broad spectrum efficacy and favorable toxicological and environmental properties of glyphosate have resulted in a wide range of non-crop, before-crop, after-crop and under-crop uses for this herbicide. With the advent of genetically enhanced crops that resist glyphosate, the herbicide can now be used in-crop at application rates providing excellent crop safety and broad spectrum weed control. Global competitiveness of this technology, relative to current in-crop/herbicide systems, depends on quick canopy closure, which can be aided by uniform plant populations and narrow row spacing. In addition, each crop exhibits its own canopy development characteristics which influence number and timing of herbicide applications. Questions are being asked about natural occurrence of weed resistance given possible exclusive use of the Roundup Ready®system. Resistant weed populations can arise only from weeds that survive herbicide application. Given no soil residual herbicide activity,

weeds surviving glyphosate application can be identified 10 to 20 days following application during scouting and corrective action can be taken immediately. The best way to prevent an initial case of weed resistance to glyphosate is to use the recommended application rate for the largest and most difficult weed. *[L]*

Current Use of Transgenic Herbicide-Resistant Soybean and Corn in the USA

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The use of herbicide-resistant crops has increased, primarily with glyphosate-resistant soybeans. In 1996, approximately 404,700 ha of glyphosate-resistant soybeans were planted; 3,642,300 ha in 1997; and 11,331,600 ha in 1998; and most important, use is anticipated to increase markedly in 1999. Currently there are over 300 glyphosate-resistant soybean varieties available. New licensing agreements with Novartis, NuForm, and Cheminova will probably increase glyphosate use. In addition, Zeneca anticipates the registration of sulfosate for use in glyphosate-resistant soybeans. Corn hybrids that are resistant to glyphosate have not been widely adopted. Estimates suggest that between 1,618,800 ha and 2,023,500 ha of glyphosate-resistant corn will be planted in 1999. There has been little adoption of sethoxydim-resistant corn and glufosinate-resistant corn although imidazolinone-resistant corn hybrids have been widely planted. Although no instances of weed resistance to glyphosate have been documented, there are indications that *Amaranthus rudis* populations will increase in response to this technology. Furthermore, isolated populations have demonstrated a variable rate response to glyphosate. *Abutilon theophrasti* populations have also increased in some fields treated with glyphosate. As herbicide-resistant crop technologies are typically based on herbicides without soil residual, multiple in-season applications are often used. This results in a greater risk of off-target movement of herbicides. Pesticide off-target complaints in Iowa increased 54% from 1997 to 1998 and glyphosate off-target complaints accounted for 25% of the total. *(L)*

Transgenic Apple Clonal Rootstocks Resistant to the Herbicide Basta

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One of the most important problems of modern horticulture is production of dwarf fruit plants on clonal rootstocks. Herbicides are widely used in nurseries and orchards. Herbicide-resistant fruit rootstocks represent a new means of conferring selectivity and enchancing fruit crop safety and production. The *bar* gene cloned at the Bioengineering Center RAS has been used in our research for obtaining phosphinotricine (PPT)-resistant apple rootstocks. This gene encodes a phosphinotricine acetyl transferase (PAT) which converts PPT into the non-toxic acetylated form and prevents the accumulation of toxicity in plant tissues. Based on the development transformation protocols for apple, more than 20 transgenic plants of semi-dwarf apple rootstock N545 have been obtained. The *bar* gene integration into plant genomes was confirmed by PCR analysis. One of the transgenic plants successfully proliferated on medium supplemented by 10 mg/l PPT and rooted on 5 mg/l PPT. Greenhouse tests of transgenic plants expressing various levels of PAT for its resistance to the commercial herbicide Basta (glufosinate) are in progress. *[L]*

Unbiased Decision-Making on the Agricultural and Ecological Risks of Gene Transfer from Biotechnologically Derived Herbicide-Resistant Crops

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One of the risks of biotechnologically derived herbicide-resistant crops (BD-HRC) is transfer of the resistance genes to wild, genetically related species or populations. In evaluating this risk three aspects should be weighed: the risk of the occurrence of the event itself and its possible agricultural and ecological consequences, possible economic consequences, and the (politically inspired) acceptability of a chosen risk level. Since the authors are neither economists nor politicians we deal only with the risk of the occurrence of the event and its consequences for agriculture and the environment. *Genetic risk:* Factors considered are dispersal and weediness of the BD-HRC, mode of propagation (sexual/non-sexual), existence of opportunity for outcrossing (identical and related species within crossing distance), maternal *versus* paternal inheritance, phenology of flowering, homology of chromosome in donor and acceptor species, occurrence of field hybrids, possibility of obtaining fertile hybrids in the laboratory and the status of wild relatives: major or minor weed, or non-weedy species. Evaluation of these factors is done through a decision tree recognizing five potential risk levels. *Agricultural/ecological risk:* Each of the potential risk levels is the basis of an evaluation of agricultural or ecological risk through decision trees. Factors considered are cropping system, major *versus* minor crops, herbicide use, and other weed management practices. The combination of genetic risk with agricultural or ecological environment leads to the indication of one out of five risk levels. *[L]*

So What If There is Introgression to Weeds? Implications and Mitigation

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Transgenic herbicide-resistant crops are being released in areas where there are interbreeding weed species, *e.g.* oilseed rape (*Brassica napus*) where there is *B. campestris*, which shares the B genome with rape; rice where con-specific red rice grows. The Canadian authorities claim other herbicides can kill *B. campestris* if it introgresses resistance from rape, but do not consider export implications. Rice farmers are happy to be able to remove red rice from rice, albeit temporarily. Red rice is weedy in no other crops; without biological pollution of other agroecosystems. In both cases the short-term risk is negligible, but for the long term there are few replacement herbicide resistance genes. Introgression can often be genetically delayed, and its effects can be mitigated transgenically. Crops such as wheat and oilseed rape have multiple genomes, not all of which are homologous to related weeds. Thus, the C genome in rape and the AB genomes of wheat are not homologous to *B. campestris* and *Aegilops* spp. genomes, respectively. Rare homeologous crossing over would be required to transfer the genes to the weeds. Cytogenic techniques can 'weed out' the transformants where the trait is on the 'at risk' weed-homologous chromosomes. The use of tandem constructs, where a few genes that are good for the crop (besides herbicide resistance) but bad for weed, should be linked to the herbicide resistance gene. These mitigate introgression when it does occur. Such potential mitigating genes include dwarfiness, non-shattering, lack of shade recognition, and lack of secondary dormancy. Biennialism (no bolting) is a great trait for carrots and beets, sterility fine for potatoes. As these traits are 'tightly linked', they will remain with herbicide resistance, rendering

introgressed individuals unfit and non-competitive. Thus introgression can both be delayed as well as mitigated, thereby lessening the hazards. *[L]*

Pollen-Mediated Gene Transfer: A Holistic View

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For the first time, transgenic plants will be sown on a large scale during the coming years. Newly implanted genes in the modified crops could escape through cross pollination with related wild strains. This genetic flux varies widely depending on the strain and the region. The risk is low or non-existent in Europe and North America in the case of soybean, maize, wheat, rye, barley, potato, tomato and some types of clover; by contrast, it is moderately high for endive, turnip, oilseed rape, cabbage, radish and chicory; and for carrot, alfalfa, and most species and strains of wild grass which today are subject to intensive cultivation (for lawns, sports fields and golf courses), the risk is very high. In the last group of crops it is in fact highly probable that genes will escape, which does not necessarily mean that this will have a negative effect on the environment. Mass cultivation of transgenic crops with an extremely high gene flow and high dissemination dynamics should be carefully monitored on a long-term basis. Unwelcome outcrossing can be avoided: in many crops traits have been developed which express the transgenes in the chloroplasts of plant cells, which then would make outcrossing impossible. However, we should also bear in mind that new oilseed rape traits have already outcrossed to other rape fields and certainly also to their wild relatives. In a truly holistic view this has to be balanced out with the new outcrossing events. *[L]*

Potential Effects of the Introgression of Virus Resistance Transgenes into Natural Populations of *Brassica oleracea*

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A study was conducted to assess the impacts of turnip mosaic virus (TuMV), turnip yellow mosaic virus (TYMV) and cauliflower mosaic virus (CaMV) on the survival, growth and reproduction of wild cabbage plants. Three groups of 200 wild cabbage seedlings with TuMV, TYMV or water (control) were inoculated. The plants were transplanted into a common garden and their survival, growth, flowering and seed production were measured. In a separate trial, we compared CaMV-inoculated seedlings with controls. After 18 months, mortality was significantly higher in the TYMV-inoculated and TuMV-inoculated groups (51.3% and 34.1%, respectively) compared with the control group (21.7%). TYMV also suppressed flowering (75% of survivors) compared with TuMV (89.2%) and the controls (85.2%). Although only TYMV-inoculated plants had significantly reduced dry weights, both TYMV and TuMV reduced seed production per plant to *ca* 50% of the controls. We have detected no effects from inoculation with CaMV. It is concluded that introgression of transgenes for TYMV or TuMV resistance has the potential to alter the dynamics of wild cabbage populations, whereas CaMV genes are much less likely to have any effect. Risk assessment of transgenic virus resistance in a given species must be virus-specific. *(L)*

I: TRANSGENIC CROPS RESISTANT TO PATHOGENS AND INSECTS

Transgenic Plants Resistant to Bacteria and Fungi

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Apple cultivars were transformed with heterologous genes to produce transgenic lines with resistance to the bacterial disease fire blight (*Erwinia amylovora*), and to the fungal disease apple scab (*Venturia inaequalis*). Genes for the lytic proteins (LP), attacin E, and the cecropin analog SB-37 were transferred to the fire blight-susceptible cv. 'Gala' by *Agrobacterium*-mediated transformation. Field tests of transgenic lines containing each of the LP genes have shown significantly increased resistance to fire blight following inoculation. Transgenic line TG138, expressing attacin E, had only 5% shoot length blighted compared with 56% in non-transgenic Gala controls. LP transgenic Gala lines are now being evaluated for fruit quality. Genes for chitinolytic enzymes from *Trichoderma harzianum* were transferred to the scab-susceptible cv. 'McIntosh' by *Agrobacterium*-mediated transformation. In greenhouse tests, an endochitinase gene (*ech42*), gave a high level of resistance to scab in transgenic McIntosh plants, but caused severe reduction of plant growth. An exochitinase gene, N-acetyl-B-D-glucosaminidase (*nag1*), gave a lower level of scab resistance, but no growth reduction. Lines transgenic for both genes were selected for low *ech42* and high *nag1* expression, and had a high level of scab resistance without significant growth reduction. Synergism between *ech42* and *nag1* for scab resistance was observed in lines containing both genes. Selected lines are now being evaluated in the field for resistance, growth, and fruit quality. *[L]*

Enhanced Resistance to Bacterial and Fungal Pathogens in Transgenic Crops

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Bacterial and fungal diseases pose serious problems in crop production. Particularly, enhanced resistance to bacterial pathogens has not been achieved so far by conventional breeding in many crop species. Also, there is no effective resistance to fungal pathogens in many cases. By means of genetic engineering, new perspectives in resistance breeding are opened up. A prominent example of resistance engineering to bacteria is the expression of phage T4 lysozyme as a novel antimicrobial agent. Transgenic potato plants are under extensive evaluation in the lab, greenhouse, and field. Testing for enhanced resistance to soft rot and black leg caused by *Erwinia carotovora* suggests a high potential for application in resistance breeding. In addition, optimization of the genetic constructs, *e.g.* by inclusion of an anaerobically and *Erwinia*-inducible promoter, is in progress. We might unexpectedly detect enhanced resistance to *Phytophthora infestans* races 1–11, the causal agent of late blight disease in potato. In leaf-disc inoculations, disease symptoms were reduced by 60–70% as compared with non-transgenic controls. Taken together, these investigations led to the discovery of a broad-spectrum microbicidal activity of lysozymes. In conclusion, this approach promises to be a valuable tool in the hands of crop breeders. Some of the experiments were performed at the Federal Center for Breeding Research on Cultivated Plants, Quedlinburg, Germany. *[L]*

Isolation of Disease Resistance Gene Candidates from Wild Emmer Wheat, *Triticum dicoccoides*, Using Degenerate PCR Primers

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Wild emmer wheat, *Triticum dicoccoides,* the progenitor of cultivated wheat, is a promising source for disease resistance genes. Genes cloned from diverse plants for resistance to different pathogens have sequence similarities in domains presumably involved in pathogen recognition and signal transduction pathway responsible for triggering the defense response of the host plant. Our goal is to study the genomic organization and diversity of disease resistance genes in wild emmer

wheat by cloning and mapping disease resistance gene candidates. Polymerase chain reaction (PCR) primers based on the conserved regions of resistance gene were used to amplify genomic DNA of two tetraploids that are near-isogenic lines for the yellow rust resistance gene *Yr15*. Using PCR primers based on conserved nucleotide binding site sequences of cloned disease resistance genes from rice and barley we were able to amplify PCR products in the range of 100–700 bp, with some bands showing polymorphism between the resistant and the susceptible near-isogenic lines. Several PCR products in the range of 400–500 bp of the resistant *T. dicoccoides* line were extracted from the gel and cloned using a TA cloning kit. Sequence analysis showed a high similarity between the cloned PCR products (more than 90%), and a high homology to disease resistance genes from barley (84–95% identity of DNA sequence), rice (79–83% identity of DNA sequence) and cultivated wheat *T. aestivum* (86–89% identity of DNA sequence). The cloned disease resistance gene candidates will be used to target the yellow rust resistance genes *Yr15* and *YrH52* derived from *T. dicoccoides* and mapped previously on the short arm of chromosome 1B of wheat. *[L]*

Comparative Pathobiology/Compatibility Approaches in Generating Transgenic Disease-Resistant Plants

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Two approaches are being used to generate disease-resistant transgenic plants. While studying mechanisms of microbial disease development, an essential virulence determinant was identified. Plants harboring genes whose products interfere with the expression of this factor have been produced; thus the pathogen is not killed but is unable to elaborate disease. This form of resistance may be quite durable. Alternatively, it was determined that when genes relevant to animal disease are inserted into plants, they influence the response to pathogen challenge. Using well developed animal models of apoptosis, we are analyzing known mammalian mediators of programmed cell death (and cell cycle regulation) in the context of plant disease, using both viral (brome mosaic virus, BMV; and tobacco mosaic virus, TMV) and fungal (*Colletotrichum trifolii, Sclerotinia sclerotiorum*) pathogens, with corn, *Arabidopsis* and tobacco as plant hosts. In the past year the appropriate constructs were made and transgenic tobacco plants harboring the genes BCL_2, CED-9, IAP and E1B 19K were generated. Results of these studies as well as a discussion of approaches towards creating durable transgenic forms of plant disease resistance were presented. *(L)*

Transgenic Plants Resistant to Viruses: A Dream Come True?

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The development and practical use of transgenic plants that are resistant to plant viruses has come of age since the first report in 1986 that transgenic tobacco expressing the coat protein gene of tobacco mosaic virus (TMV) was protected against TMV. It has been shown conclusively that selected transgenic plants expressing viral transgenes, such as coat protein, replicase, movement proteins, and even gene fragments show resistance to the viruses from which the transgene is derived. This approach of pathogen-derived resistance is applicable to nearly all of the RNA viruses. Much progress has been made towards understanding the underlying mechanism(s) of resistance. More recent revelations show that resistance need not be protein-mediated and, indeed, the majority of resistance seems to be RNA-mediated. Furthermore, much progress has been made in showing that transgenic plants of horticulturally valuable crops provide good protection against viruses under greenhouse and limited field conditions. However, only a handful of virus-resistant transgenic plants have been deregulated for widescale field experiments and even fewer have been commercialized. Will this very useful approach remain in the academic arena? The possible reasons were discussed

for the rapid development of the technology and of information towards understanding the underlying resistance mechanisms, along with the disappointingly slow implementation of these transgenic plants for practical virus control. *[L]*

Transgenic Wheat Plants Resistant to Barley Yellow Dwarf Virus Obtained by Pollen Tube Pathway-Mediated Transformation

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Barley yellow dwarf virus (BYDV) is of economic importance in most cereal-producing countries and is probably the most economically important virus of cereals. To date no unaffected forms of resistance have been described in common wheat. GPV is a Chinese serotype isolate of BYDV that has no reaction with antiserum of PAV, MAV, SGV, RPV or RMV. Based on the BYDV-GPV coat protein (CP) sequence, the cDNA of CP was produced by RT-PCR. The full length 606nt cDNA of CP was inserted into expression plasmids pJ3x35SN and pEmu-mcs-N. The recombinant plasmids were named pPPI1 and pPPI5, verified using an a-^{32}P-dATP labeled CP probe and a r-^{32}P-ATP labeled GPV RNA probe, and sequenced to confirm the presence of the unmodified GPV CP gene cDNA in the plasmids. Pollen tube pathway transformation was used in the research. The NPT II kanamycin resistance gene was used as a selectable marker to screen transgenic seedlings. Molecular analysis such as polymerase chain reaction (PCR), Southern and Western blots, and ELISA were used to detect CP gene in transgenic wheat plants. Molecular analysis of the CP gene in transgenic plants confirmed the stable integration of the CP gene into the wheat genome and inheritance of the gene to the T1, T2, T3 and T4 generations. An additional T4 generation was confirmed using back-crossing to the original common wheat variety Beijing837. The CP gene segregated as a dominant Mendelian trait in T1 selfed plants. Upon inoculation with GPV in greenhouse tests, transgenic plants that expressed the CP gene exhibited a significant delay of symptom development and reduced virus accumulation compared with control plants. In field trials, our results showed a higher level of resistance to virus infection in T2, T3 and T4 plants. An efficient pollen tube pathway-mediated transformation protocol was developed for the generation of transgenic wheat plants that express coat protein of GPV. *[P]*

Genetic Engineering of Plants for Resistance to Insects

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The economic as well as environmental costs of insect control in agriculture are very high. In recent years, transgenic plants expressing insect resistance genes were evaluated for use in agriculture. A number of genes have been identified that have been shown to confer resistance to attack by insects. We presented a brief review of the origins of genes useful for generating insect-resistant plants. The endotoxins from *Bacillus thuringiensis (Bt)* are perfect candidates for transformation of plants as they are single, unmodified proteins coded by single genes. As a result, the *Bt*-transformed crops were among the first genetically engineered crops to be commercialized and several *Bt*-transformed crops are now in use. Also, many plants contain proteins that can inhibit animal proteolytic enzymes. Overexpression of genes for these protease inhibitors in plants was therefore very promising and some were shown to confer resistance to insect attack. However, limited success in plant protection was obtained in field tests. Genes that code for insect hormones, insect pheromones, or proteins that disrupt feeding or prevent the insect from recognizing the crop as a food source will be described. The use of insect-specific toxins is more often found in the transformation of baculoviruses than in crops, but if these toxins are effective *per os* they can be used in transgenic crops too. An approach to achieve such an effect was discussed. *[L]*

Seasonal Changes in the Efficacy of Transgenic *Bt* Cotton

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Genetically engineered cotton plants expressing insecticidal proteins from *Bacillus thuringiensis* (*Bt*) were developed using constitutive promoters so that efficacy could be maintained throughout plant development. The initial pest management and resistance management strategies devised for *Bt* cotton were built around this model. In Australia, in both experimental trials and commercial fields, cotton engineered to express the *Bt* endotoxin, CryIAc, has shown significant declines in efficacy for the control of *Helicoverpa* spp. from mid-summer, so that crops require alternative treatments when the plant starts to set bolls. By late season, larvae can develop and pupate on the *Bt* cotton. Furthermore, some plants have variable efficacy in early season, even before squaring has commenced. There are many possible causes for the decline. At this stage we have evidence for a decline in the amount of *Bt* protein present and also for possible interference by plant factors in the availability of the *Bt* protein to the insect. Stress factors also play a role. Changing efficacy during plant growth has consequences for pest management and may exacerbate selection for *Bt* resistance and thereby compromise resistance management strategies designed to minimize the risk of resistance. These consequences were discussed. *[P]*

Field Results Obtained with Transgenic Sweet Potato Plants Expressing the *cry3A* Gene from *Bacillus thuringiensis* var. *tenebrionis*

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Some clones of sweet potato (*Ipomoea batatas* L.) transgenic plants belonging to the 'Jewel' cultivar were obtained. These plants carried the *cry3A* gene from *Bacillus thuringiensis* var. *tenebrionis*. Molecular tests and biological activity experiments against the sweet potato weevil (*Cylas formicarius*) were performed. Transgenic plants were obtained using an efficient transformation and regeneration protocol developed in the lab. An increase in the transformation frequency, compared with that previously reported, was obtained. Several biological activity experiments were performed to evaluate the insecticidal capacity of the transgenic plants. The parameter percent of infestation was used to compare the tolerance levels of the different clones tested. All the experiments were performed using random block designs with three or four replicates and fulfilled the conditions required for this kind of work with genetically modified organisms. Even when the expression levels of the Cry3A toxin were very low, there was an inverse correlation between the toxin detection and the level of insect damages. The best resulting clone was also checked by a genomic Southern blot and one single gene copy seems to be present. Work to improve the expression levels of the Cry3A toxin is in progress. *[P]*

Synthetic *cry3A*-like Gene for High Levels of Expression in Transgenic Plants

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The obtainment of high expression levels of foreign proteins in plants, especially of bacterial origin, has included the modification of the nucleotide sequence of the bacterial genes. One of the approaches has been focused on improving the plant codon usage, and to avoid the presence of certain signals that can interfere with the desired expression of the foreign gene. We described the obtainment

of five DNA fragments, sized from 200 to 400 base pairs, that were designed to have a plant-like *cry3A* gene from *Bacillus thuringiensis* var. *tenebrionis*. The five fragments were synthesized having a ten nucleotides overhang to allow the ligation with the following sequential fragment. Ligation reactions combined with polymerase chain reaction, restriction and cloning were used to get the completed gene. The PCR from the first to the fifth fragment was used to check the proper size of the inserts. The clones were checked by restriction analysis. The ones that showed the expected behavior were checked by sequencing and expression in *Escherichia coli*. Work with plant expression vectors is in progress. *[P]*

Transgenic Crops Resistant to Parasitic Weeds

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Orobanche (Orobanchaceae) is a non-photosynthesizing root holoparasitic angiosperm. Its spread in the Mediterranean area during the last decades has represented an economic threat to crop growth unsolved by agronomic technology and herbicide use. Producing transgenic crops resistant to the parasitic weed might solve the economic threat and reduce the use of herbicides. Our basic approach was to increase cell wall rigidity in the host root in the hope of avoiding *Orobanche* establishment. This was done by introducing into the host additional copies of the gene encoding caffeoyl-CoA-methyl transferase, an enzyme involved in cell wall lignification and ester-ferulation of other cell wall components. *Nicotiana tabacum* was used as a model plant. Transformation was done in the laboratory of A. Zilberstein by R. Marely. We have obtained some transformed lines with increasing lignification and resistance to *Orobanche*. *[P]*

J: APPLICATION OF GENOMICS FOR PLANT PROTECTION

Exploitation of Genetics in Providing Durable Control of Turnip Mosaic Virus (TuMV)

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Turnip mosaic potyvirus (TuMV) is a very important pathogen affecting brassicas worldwide. Extreme forms of resistance to TuMV have been identified in the A genome of *Brassica rapa* and *B. napus*, but not in the C genome of *B. oleracea*. This contribution describes the establishment of a gene-for-gene relationship between TuMV and the *Brassica* A genome; the characterization and mapping of plant resistance genes and the viral determinants of virulence/ avirulence; the movement of resistance genes from the A to the C genome; and the development of strategies to provide durable resistance to TuMV. The interactions between brassicas and TuMV were determined by mechanical inoculation of plants. Plant lines that were homozygous for resistance to TuMV were challenged with TuMV isolates originating from different regions of the world to characterize the specificities of the different resistances. Genes were mapped using bulk segregant analysis and RFLP (restriction fragment length polymorphism) markers. Virulence/avirulence determinants of TuMV for different resistance genes are being identified using a full-length infectious clone of TuMV and naturally occurring mutants. Marker-assisted transfer of resistance genes from the A to the C genome is being attempted by inter-specific hybridization and subsequent back-crossing. The interactions among four brassica differentials and >140 isolates of TuMV revealed 12 different patterns of interaction (=pathotypes) with most of the isolates belonging to pathotypes 1, 3 or 4. New sources of resistance

to TuMV with new specificities have been identified in *B. rapa*. The first resistance gene to a virus (*TuRB01*) has been mapped and a putative viral determinant of virulence/avirulence for this gene has been proposed. Interspecific hybrids between resistant *B. rapa* and susceptible *B. oleracea* plants have been produced with functional resistance to TuMV. *(L)*

Application of Genomic Tools for the Exploitation of Wild Wheat Germplasm: Molecular Tagging of Stripe Rust Resistance Genes Derived from Wild Emmer Wheat

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Traditional approaches for exploitation of disease resistance genes from wild germplasm are sometimes slower than the appearance of new pathogenic races in the fields. Fortunately, the advanced genomic technology available today can help to increase the efficiency of utilization of wild resources for crop improvement. Stripe rust, caused by *Puccinia striiformis* f.sp. *tritici*, is one of the most severe diseases of wheat. Wild emmer wheat, *Triticum dicoccoides*, was found to be a valuable source for novel stripe rust resistance (*Yr*) genes. Previously, it was estimated that more than ten novel *Yr* genes are present in a collection of wild wheat accessions from Israel. However, it is not clear yet which *T. dicoccoides* accessions carry identical, allelic or different resistance genes. This question can be unambiguously resolved by using molecular markers and genetic linkage maps to identify the chromosome location of each gene. Based on a microsatellite survey of 19 wild wheat accessions, two accessions conferring a single dominant gene each, designated as *Yr15* and *YrH52*, were selected for mapping studies. The *Yr15* gene was mapped on chromosome 1BS and several markers linked to the gene were identified. Among 79 segregating microsatellite loci, nine were found to be linked to *YrH52* and a genetic map of chromosome 1BS carrying *YrH52* was constructed. Using the marker *Nor1*, common to the two maps, it was estimated that the distance between *YrH52* and *Yr15* is approximately 10 cM. Further studies are under way to clone disease resistance gene homologs as tools to target *Yr15* and *YrH52*, and to test whether they reside within a cluster of resistance genes. *[L]*

Marker-Assisted Breeding for Disease Resistance Genes in Tomato and Pepper

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Tagging disease resistance genes by molecular markers has the potential to accelerate greatly breeding programs aimed to introgress these genes into elite cultivars. The advantages of marker-assisted resistance breeding are: (i) rapid accumulation of multiple resistance genes in a single parent by simultaneous genotyping for different resistance genes; (ii) avoidance of quarantine; (iii) screening at an early stage of the plant; and (iv) avoidance of progeny tests. In tomato, genes conferring resistance to different types of pathogens were mapped. These include: viruses (tobacco mosaic virus, TMV; tomato spotted wilt virus, TSWV; tomato yellow leaf curl virus, TYLCV), fungi (*Leveillula, Alternaria, Fusarium, Cladosporium*), bacteria (*Xanthomonas, Pseudomonas*) and nematodes. In some cases the resistance genes were cloned and their sequences can be used themselves as markers, *e.g. Pto, I2, Cf-9* and *Mi*, conferring resistance against *Pseudomonas, Fusarium, Cladosporium* and nematodes, respectively. In pepper, monogenic resistances to viruses such as potato virus Y, PVY,

TMV and TSWV have been mapped. In addition, QTL (quantitative trait locus) for quantitatively inherited resistances such as for PVY, *Phytophthora* and cucumber mosaic virus, CMV, have been identified. The implications of the results for marker-assisted breeding and pyramiding of resistance genes were discussed. *[L]*

Construction of an Ultra-High-Density Map of the Potato and Its Application for Cloning of Disease Resistance Genes

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Genes of agricultural importance are often introgressed from a wild species into a domesticated crop plant. The isolation of these genes relies on the identification of the introgression segments which harbor the gene of interest. Current cloning strategies focus on local marker saturation, *e.g.* by bulked segregant analysis, whereby tightly linked markers are used for screening genomic libraries, construction of a contig map and ultimately to 'walk' to the favorite gene. This map-based approach requires an enormous workload and each new cloning project has to start with an extensive marker screening. To speed up map-based cloning in potato, we designed a strategy by which an ultra-high-density (UHD) marker map can be applied directly to 'land' on the gene of interest. The potato genome will be saturated with 16,000 AFLP markers; an average of 16 markers per cM and approximately one mapped marker every 50 kilobases. With the aimed marker density, a coverage of at least one mapped marker per potato-BAC clone should be achieved. Potato BAC libraries contain, *inter alia*, a number of disease resistance genes in addition to other genes of agricultural importance. The AFLP markers mapped will be categorized and their use as a marker catalog in concert with the BAC libraries provides a one-step approach to identify additional markers linked to the gene of interest. As such it can be considered as being an alternative to a genome sequencing project. Because of the high degree of homology among the Solanaceae with regard to linkage order and sequence divergence, the derived AFLP marker catalog may also be informative for other solanaceous species like tomato and pepper. The lecture focused on the UHD mapping strategy. Current mapping approaches which were applied for global mapping of the *Phytophthora infestans* resistance gene *R2* and fine mapping (and isolation) of the nematode resistance gene *Gpa2* were compared with the UHD strategy and the differences were discussed. The project is supported by grant no. FAIR5-CT97-3565 from the European Commission. *[L]*

Application of Large Sequencing Technology in Plant Pathology

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The availability of ultra-high-throughput DNA sequence technology will revolutionize plant pathology research. *A priori* knowledge of the entire genomic sequence of pathogenic micro-organisms will allow us to study in a novel and highly focused way, *e.g.* strategies that these pathogens employ to circumvent the host plant's defense. On the plant level, these DNA sequence techniques will enable rapid isolation of genes, such as resistance genes, which are involved in

plant–pathogen interactions. Coupled to DNA-chip technology, ultra-nigh-throughput sequencing will allow the elucidation of complete pathways which are involved in plant–pathogen interactions, in both the host plant and the pathogen. Knowledge of these pathways will enable the development of entirely novel and environment-friendly strategies to protect crop plants in modern agriculture. In CPRO-DLO's sequence facility GREENOMICS, genomic sequencing projects are conducted on plant species and plant pathogens. One of these projects concerned the cloning of the nematode resistance gene *Gpa*2 from potato. This gene, which confers resistance to the potato cyst nematode *Globodera pallida,* was previously mapped to the same 6 cM genetic interval on chromosome 12 of potato as the virus resistance gene *Rx*. A physical map of the *Rx/Gpa*2 locus was built by screening two separate BAC libraries with *Rx/Gpa*2 linked markers. From this map, it was deduced that the *Gpa*2 gene should be located on one of four overlapping BAC clones. The entire 200 kb DNA region contained within these BAC clones was sequenced and the subsequent analysis of the obtained sequence identified four candidate resistance gene homologs (RGH1-4) within the *Rx/Gpa*2 interval. These four RGHs were selected for complementation analysis, subcloned as genomic fragments of 6-11 kb into a plant transformation vector, and transferred to a susceptible potato genotype. Roots of primary transformants harboring RGH2 showed the same incompatible interaction with *G. pallida* population D383 as the resistant control plants, proving that RGH2 was the sought *Gpa*2 nematode resistance gene. *(L)*

K: FOOD SAFETY AND DIETARY INTAKE OF PESTICIDE RESIDUES

Update on How the US EPA is Progressing in Regulating Dietary Residues under the Food Quality Protection Act

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In the United States, registrants and users of pesticides are faced with a series of new and difficult regulatory challenges. The U.S. Environmental Protection Agency (EPA) is implementing a new law, the Food Quality Protection Act, which requires that tolerances or MRLs (maximum residue limits) be reassessed and that combined exposure from diet, drinking water, and residential use be taken into account in deciding whether existing tolerances are acceptable. In evaluating tolerances, EPA must consider in addition the cumulative effects of exposure to compounds with common toxic mechanisms. Also, EPA is focusing on acute toxicity much more than before. Finally, the new law requires use of an additional 10x safety factor to protect infants and children unless EPA determines it is not needed. Previously, approvals were often given when fairly crude, worst-case estimates of exposure/risk showed that the products satisfied the criteria. However, under the new criteria the old approaches do not always give acceptable answers. EPA is taking comments on its implementation policies and issuing new data requirements. Registrants are conducting acute dietary NOEL (no observed effect level) studies with animals, and some products are being tested in single-dose studies in human volunteers. To refine exposure assessments, probabilistic and calendar-based analyses of dietary and aggregate exposure are being developed, often using data from food and water residue monitoring programs conducted by government or industry. *(L)*

The Use of 'Best Science' in Making Tolerance Reassessment Decisions

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The Environmental Protection Agency (EPA) is responsible for protecting human health and the environment from unreasonable adverse effects that may result from pesticide use. EPA faces many challenges presented through the laws which provide the Agency with the authority to regulate

pesticides and their use in the United States, including most recently the Food Quality Protection Act (FQPA) of 1996. The new law requires that tolerances be 'safe', defined as "a reasonable certainty that no harm will result from aggregate exposure" to pesticides, including all dietary and their non-occupational exposures. Within ten years, following a specific time schedule, EPA must reassess all existing tolerances to ensure that they meet this new safety standard. Many difficult science issues attend EPA's efforts to implement FQPA. Concepts such as aggregate exposure, cumulative effects, and the 10× safety factor, which the Agency was to begin implementing immediately upon enactment of the new law, required the development of new methods, policies, and procedures. Meanwhile, the tenet that EPA's decisions should be based on sound science has been a guiding principle, set forth by Vice President Gore. As a result of working closely with the Tolerance Reassessment Advisory Committee, a group created to ensure smooth implementation of FQPA, EPA announced last autumn a framework for addressing nine science policy issues, and published a schedule for releasing draft documents on each of the following science policy areas: applying the FQPA ten-fold safety factor; using Monte Carlo analyses for dietary exposure assessment; interpreting 'no residues detected' for exposure assessments; estimating dietary exposure; factoring in drinking water exposure; assessing residential exposure; aggregating exposures from all non-occupational sources; conducting cumulative risk assessment for organophosphate or other pesticides with a common mechanism of toxicity; selecting appropriate toxicity endpoints for risk assessments of organophosphate pesticides. EPA has been applying the science policies, as appropriate, in making regulatory decisions for both new tolerances and tolerance reassessments, at the same time as the policies are being refined through peer review and public comment. As these policies are further refined, some of the Agency's decisions may need to be revised. EPA expects to have virtually all of its science policies finalized before organophosphate risk management decisions are completed. *[L]*

Human Health and Risk Assessment: The Adequacy of Scientific Information

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Potential risks from exposures to pesticide residues in food are coming under rigorous scrutiny by regulatory agencies in many countries. JMPR (Joint Meeting on Pesticide Residues) is debating new methodologies to use for assessing acute risks. The universal interest of pesticide regulators in these issues was underscored by a conference in the United Kingdom on variability and acute dietary risk. The passage of the Food Quality Protection Act (FQPA) in the USA in 1996 imposed significant new risk assessment requirements upon organizations submitting pesticide dossiers. The FQPA requires the assessment of aggregate risk (risks from all use patterns of a pesticide), cumulative risk (combined risk from all compounds having the same mechanism of toxicity), and potential additional safety factors when assessing the risks to children. In addition, most compounds now are assessed with respect to acute dietary risk. Today's more stringent safety standards require sophisticated exposure models to demonstrate adequate levels of safety from the use of pesticides. Often, such models are based upon statistical methods, such as Monte Carlo simulation, that require relatively much more extensive data. However, most pesticide registrants conduct studies according to regulatory guidelines. Consequently, the number of trials required for some crops is quite small. Furthermore, most residue analyses are conducted on composite samples, whereas today's concern is with pesticide residues on single servings of fruits and vegetables. The adequacy of the data used in the complex models must be evaluated. Extrapolating from existing data using statistical techniques, using data from surrogate crops, or collecting additional data are all approaches that enhance the data available for sophisticated exposure analyses that may be required to comply with current safety standards. Strategies for addressing these concerns – including new approaches to using existing data – were presented. *(L)*

The Role of Resistance Management in Product Regulation – An Industry View

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Resistance management is important for today's crop protection industry. Development of a new crop protection product normally takes from 7 to 10 years and represents an investment of between 70 million and 100 million US dollars. Crop protection companies therefore cannot afford to jeopardize their investment by failing to evaluate resistance risk and by not implementing resistance management strategies. To protect such an investment, it is important to understand this risk throughout a product's discovery, development and commercial use. With this knowledge there is a commercial incentive to ensure that products are used in ways which do not accelerate or increase the risk of resistance developing. However, in order to be effective in reducing resistance risk, crop protection companies must coordinate their activities. For this reason, the Global Crop Protection Federation (GCPF) coordinates its members' resistance management activities through its insecticide, fungicide and herbicide resistance action committees, respectively IRAC, FRAC and HRAC. Manufacturers cannot solve this complex problem on their own, because it is product use that determines the risk of resistance development. As such, industry recognizes the essential role that can be played by extension services and governmental organizations in the struggle against resistance. While industry believes self-regulation is the most practical way of managing resistance, it is ready and willing to work with regulators and organizations such as the European and Mediterranean Plant Protection Organization (EPPO) to develop resistance risk evaluation guidelines. However, in order to be effective, any such guidelines must be practical to implement. IRAC, HRAC and FRAC are therefore grateful to have the opportunity to work with EPPO on its draft resistance risk evaluation guideline. *[L]*

Resistance Risk in Relation to the Registration of Plant Protection Products

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Loss of performance of a plant protection product because of the development of practical resistance in the target pest can be costly to the grower, the crop protection company and the environment. Registration authorities and crop protection companies recognize that the risk of resistance can be reduced by suitable management strategies, and that it is in their interests to protect the efficacy of products. In Europe, EU Directive 91/414 requires that applicants for product registration provide information on the occurrence and development of resistance and, if a risk is perceived, propose a management strategy. The European and Mediterranean Plant Protection Organization (EPPO), through its Working Party on Plant Protection Products, has many years of experience in developing international standards for procedures in support of registration (particularly efficacy evaluation guidelines). It has recently developed a new international standard on 'Resistance risk analysis'. This provides detailed guidance on the assessment of resistance risk, with the aim of answering the question "Is the risk acceptable, without any restrictions on use?". The standard sets out the type of information to be provided to the registration authority to justify a positive answer to this question. If the applicant concludes that the risk must be modified before it can be considered acceptable, then risk management options must be proposed. The standard provides guidance on resistance risk management and finally advises the Registration Authority on how to consider the information provided by the applicant in making its registration decision. The standard is backed by detailed appendices on resistance to different types of plant protection products (fungicides, herbicides, insecticides/acaricides). *[L]*

Goals and Accomplishments of the Herbicide Resistance Action Committee

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The Herbicide Resistance Action Committee (HRAC), begun in 1989, is an industry-based group whose members include AgrEvo, American Cyanamid, BASF, Bayer, Novartis, DowElanco, DuPont, FMC, Monsanto, Rhône-Poulenc, Tomen Agro Inc., and Zeneca. The mission of HRAC is to facilitate the effective management of herbicide resistance by fostering understanding, cooperation, and communication among industry, government and farmers. Over the last ten years HRAC has actively pursued its mission by commissioning and publishing monographs on various aspects of herbicide resistance; supporting research on resistance management and education of farmers; developing guidelines on resistance management; producing a classification of herbicides based on their mode of action; and supporting the establishment of a database on the occurrence of herbicide-resistant weeds throughout the world; the latter is currently available on the World Wide Web. HRAC has also supported symposia and meetings dealing with herbicide resistance management. In addition, HRAC has helped establish herbicide resistance working groups in Europe, North America, South America, Australia and India. In the future HRAC will continue to foster a responsible attitude to herbicide use that minimizes the development of resistance through a pro-active approach and to extend the use-life of our many efficacious herbicides. *[L]*

Australian Regulatory Policy, Risk Assessment and Risk Management of Genetically Modified Plant Imports

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Traits conferred on plants by genetic modification may pose phytosanitary risks to Australia's agricultural and natural environments. Importation of all plants and plant products is regulated under the Australian Government Quarantine Act, which is administered by the Australian Quarantine and Inspection Service (AQIS). Proposed importations are subject to controls to manage the risk of introduction, establishment and spread of pests and diseases that may endanger the health or life of humans, animals or plants. AQIS conducts import risk analyses (IRAs) on all genetically modified plants (GMPs) and their products to ensure that they meet our national phytosanitary standards. Risk assessment and management of GMPs is a scientifically based and transparent process which also meets our international phytosanitary obligations under SPS/WTO and the standards developed under the International Plant Protection Convention (IPPC). Assessment of risk elements that may be posed by a GMP are done as a component part of the AQIS IRA process. The policy has been developed in consultation with other government agencies and regulatory authorities, industry groups, primary producer organizations, gene technology R&D organizations, and consumer groups. Establishment of a Gene Technology Office has been mandated by the Government to oversee and coordinate a national framework for the regulation of all aspects of gene technology. *[L]*

The National Committee for Transgenic Plants in Israel: Goals and Activities

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The National Committee for Transgenic Plants (NCTP) was established in 1991, with a mandate to regulate research and breeding programs which utilize transgenic plants and techniques. The 13

members of the committee represent the academic community, the public and the government (the Ministries of Agriculture, the Environment, Sciences, Health, Trade and Industry, and the Prime Minister's Office). The tasks of the NCTP are to (*a*) formulate guidelines for experimentation with transgenic plants (including organisms interacting with plants); (*b*) publish protocols and application forms for genetic engineering experiments with plants; and (*c*) advise government and research institutions on good practices concerning importation and experimentation with transgenic plants. A panel of experts in the fields of molecular biology, environment, and biology (genetics, botany and entomology) reviews applications for experiments with transgenic plants. Applications for field experiments are reviewed in plenary sessions. Teams of NCTP representatives and PPIS inspectors inspect field and glasshouse experiments. Experiments are carried out in Israel on yield improvement (parthenocarpy and shelf life), and on resistance to diseases, pests, herbicides and various stress agents (drought, salinity, etc.). The crops being experimented with are: tobacco, potato, tomato, lemon, zucchini, strawberry, wheat, alfalfa, grapevine, *Arabidopsis thaliana,* petunia, ginseng, poplar, carnation, pine and cotton, in addition to microorganisms (mostly for biological control). The first transgenic plant, a carnation with extended vase-life, was approved for sale. The NCTP is at present preparing the transition from protocols to legislation. *(L)*

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Monitoring Adult Population Fluctuations of *Rhagoletis cerasi* Using Yellow Sticky Traps in Bursa, Turkey

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The European cherry fruit fly, *Rhagoletis cerasi* (L.), is the most important pest of cultivated cherries in northwestern Turkey. The study reported herein was carried out to determine the adult population fluctuations of *R. cerasi* that occurred in family and commercial cherry orchards in Bursa in 1997 and 1998. Adult population fluctuations were monitored by weekly examination of catches of adults in Rebell®-type yellow sticky traps placed in 13 orchards including early-, middle- and late-ripening cherry varieties at seven different localities between Bursa plain and Uludag, where altitudes varied from 150 to 1200 m above sea level. The first *R. cerasi* adults were caught from May to early June, depending on locality, climatic conditions, altitude and other factors. Females emerged slightly earlier than males and predominated in the first catches; at the peak of emergence the sexes were almost equal, and at the end of adult activity the proportion of males had increased. Catches increased in abundance 2–3 weeks after emergence, and the total flight period lasted from May to late July. The total duration of flight ranged from 29 to 57 days. Some 27.5 to 369 and 18 to 507 adults/trap were caught in 1997 and 1998, respectively. The number of females caught per trap was usually greater than that of males, except at Sogukpinar. *(L)*

Integrated Management of *Lobesia botrana* in Northern Greece

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Protection of vineyards against *Lobesia botrana* in Greece is based almost exclusively on insecticides. Because of these tactics, serious problems arise regarding efficient control of insects, development of resistance to insecticides and environmental pollution. During the period of 1996–98 an integrated method of control was used in vineyards in northern Greece. It was based on the

right timing of applications in relation to the mode of action of the insecticide used. In addition, an attempt was made to control *L. botrana* using only *Bacillus thuringiensis (Bt)* toxins. Best results were obtained when two or three sprays were applied along with insect growth regulators and *Bt* toxins, and three or four sprays when only *Bt* toxins were used. *(L)*

Gummosis Disease of Citrus and Its Control

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Gummosis disease is one of the most serious diseases attacking citrus trees in Egypt and other countries throughout the citrus-cultivation areas of the world. It was found that many citrus species and varieties are susceptible to the disease. The percentages of disease incidence ranged between 33.9% and 47.3% at the tested locations in Egypt. Both *Phytophthora citrophthora* and *P. parasitica* are causal agents of gummosis disease under Egyptian conditions. The effect of different irrigation systems and the height of the grafting union were found to be factors associated with disease occurrence. Therefore, it is very important to prevent contact between the irrigation water and the trunks of the trees. Control of gummosis disease through a biocontrol agent was studied. Data showed that, under greenhouse conditions the biocontrol agent *Trichoderma harzianum*, when employed in granule form, completely destroyed the pathogen and the symptoms of the disease disappeared. *(L)*

Control of *Fusarium oxysporum* f.sp. *melonis* by Soil Solarization in Tunnel-Grown Muskmelon

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Fusarium oxysporum f.sp. *melonis* (FOM), the causal agent of Fusarium wilt of muskmelon, causes serious damage to crops grown in unheated tunnels in southern Italy. The possibility of controlling FOM by soil solarization (SoSo) was examined in trials carried out for 46 days during July–August 1998, in tunnels covered by ethylene vinyl-acetate film (EVA). There were six treatments: soil amended with organic matter (1 kg m^{-2}) and covered with one of four different plastic mulches [black polyethylene film, transparent polyethylene film, EVA, or co-extruded EVA-black polyethylene film (COE)]; nonamended plots covered with EVA or COE; in addition to unamended uncovered control. The efficacy of SoSo in controlling FOM propagules placed in the soil at depths of 15 and 30 cm was tested. Solarization increased soil temperature by 6–8°C. Chlamydospore mortality in the nonsolarized plots, in the closed tunnels, was 96.5% and 97.2% at depths of 30 and 15 cm, respectively. Nonamended SoSo treatment resulted in complete kill of FOM propagules at the two tested depths under COE and EVA. It is concluded that SoSo in closed tunnels can control chlamydospores of FOM effectively under the summer conditions of southern Italy. In an additional study in the open field, short solarization (for 12 days) reduced survival of FOM propagules by 50–66%. Combining that treatment with manure, or extending solarization to 27 days, improved the effectiveness of control. *(L)*

Implementation of Soil Solarization and Other Methods for Controlling Soilborne Pathogens in Gaza

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Soilborne pathogens cause heavy losses to the major vegetable and flower crops in the Gaza region, both in the open field and the greenhouse. Methyl bromide is used intensively for their control. Alternative methods were examined with emphasis on reduction of pesticide use. Solarization was found effective in controlling many pathogens and weeds and in increasing the yield in tomatoes, eggplants, strawberries and potatoes, *inter alia.* Combining solarization with methyl bromide at reduced dosage, or with compost or ethylene dibromide, further improved the results. Used plastic was also effective, thus reducing the cost considerably. Space solarization, namely, closing the greenhouse and heating it to 60–65°C, is an effective tool for sanitation purposes. Extension tools to introduce the method to the farmers are needed. *(L)*

Herbicide Resistance in *Echinochloa* spp.

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The aims of this study were to investigate the taxonomy of several populations of the genus *Echinochloa* in Spain, and to determine their resistance mechanisms to the herbicides atrazine, propanil and quinclorac. Botanical determination and PCR-RAPD were used to classify the biotypes. Herbicide resistance was determined under controlled conditions, and the resistance mechanism was studied with fluorescence assays, Hill reaction experiments and techniques for radioactivity studies, GC and HPLC. The genus *Echinochloa* is represented in Spain by five species, but the DNA analysis showed only three groups: *E. colona, E. crus-galli* + *E. hispidula* and *E. oryzoides* + *E. oryzicola.* The group of *E. crus-galli* + *E. hispidula* showed tolerance to quinclorac, being four-fold more tolerant than *E. oryzoides* + *E. oryzicola.* ^{14}C-quinclorac metabolism was shown only by the susceptible biotype. Based on photosystem II studies, an *E. crus-galli* biotype was 80-fold more resistant to atrazine than the susceptible one. Hill reaction assays and fluorescence measurements indicated mutation in the D1 protein. Propanil resistance was studied in American biotypes. Results showed that resistance can be attributed to propanil detoxification by the enzyme aryl acylamilase. Propanil inhibited photosynthesis in both the resistant and susceptible biotypes, but photosynthesis recovered only in resistant biotypes. Metabolism of ^{14}-propanil confirmed the detoxification of herbicide. The study of *Echinochloa* spp. has shown the importance of the correct taxonomy of this genus, due to the tolerance to quinclorac exhibited by some species. The continuous use of propanil and atrazine has led to the appearance of resistant biotypes. Alternative herbicides providing good control of *Echinochloa* should be used to avoid the appearance of resistant populations. *(L)*

New Approach to Herbicide Formulation Chemistry: Organo-Clay Formulations of Chloroacetanilide Herbicides with Reduced Leaching in Soil

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The herbicides alachlor and metolachlor were classified as leachers with high potential to reach the groundwater. We synthesized new controlled release formulations of alachlor and metolachlor with reduced leaching and improved efficacy in soils. The new formulations were synthesized by adsorbing the herbicides to montmorillonite, the surfaces of which were modified from hydrophilic to hydrophobic, by preadsorption of a suitable organic cation, such as benzyltrimethylammonium (BTMA), up to 0.5 and 0.8 mmol/g clay. Alachlor and metolachlor were analyzed by gas

chromatography, and interactions of the herbicides with the organo-clay complexes were studied by Fourier-Transform Infrared spectroscopy (FTIR). An organo-clay complex of 0.5 mmol BTMA/g clay gave optimal adsorption of alachlor and metolachlor. BTMA-clay yielded larger adsorbed amounts of alachlor or metolachlor and larger shifts of the infrared peaks of alachlor than did a benzyltriethylammonium (BTEA)-clay complex. Slow release of the herbicide from the organo-clay complexes to the soil solution reduced its mobility and leaching, and hence maintained the threshold concentration needed for weed control in the top soil, as measured by bioassays. Laboratory and field experiments showed improved weed control with optimal herbicide formulations synthesized by pre-adsorbing BTMA to the clay at a load of 0.5 mmol/g clay, whereas formulations without the organic cations were much less effective. *(L)*

Prevention of Leaching of the Herbicide Norflurazon into Soils by Using Clay Formulations

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The objective of this study was the design of organo-clay formulations of the hydrophobic herbicide norflurazon which would reduce its leaching. The clay surface of montmorillonite was modified from hydrophilic to hydrophobic by preadsorbing the clay mineral surface with organic cations. In addition, a pillared clay (PC) was used without any further treatment. Norflurazon exhibited very high affinity for adsorption on PC. Its very low affinity of adsorption on montmorillonite was enhanced by preadsorbing certain organic cations on it. When the clay was preadsorbed with the organic cation thioflavin-T (TFT), the affinity of adsorption of norflurazon increased by one order of magnitude in comparison with that obtained when using a smaller aromatic organic cation, benzyltrimethylammonium. Slower release of the herbicide, as shown by soil column experiments, paralleled high affinity of its adsorption on the clay or organo-clay. The formulations prepared on the basis of montmorillonite-TFT and PC in aqueous medium were most effective in reducing leaching. Organo-clay formulations with a higher active ingredient were prepared from acetone. These formulations also reduced leaching. It is concluded that formulations from PC and montmorillonite-TFT present herbicidal activity restricted to the top soil layer, whereas their efficiency for weed control is comparable to that of the commercial formulation. *(L)*