Genetically Modified Pest Protected Plants

and

Organic Agriculture

Brian Baker, Ph.D.
Technical Director
Organic Materials Review Institute

Presentation to the National Academy of Sciences
May 24, 1999

Genetically modified pest protected plants or Genetically Modified Plant Pesticides (GMPPs) have radically changed agriculture in the few years they have been commercially released. The effects that this and other techniques of genetic engineering in agriculture will have on farming are not yet apparent. The organic community is concerned about the impact that this technology will have both on their immediate growing conditions and the long term sustainability of agriculture.

The National Organic Standards Board (NOSB) of the US Department of Agriculture defines genetic engineering as

> made with techniques that alter the molecular or cell biology of an organism by means that are not possible under natural conditions or processes. Genetic engineering includes recombinant DNA, cell fusion, micro- and macro-encapsulation, gene deletion and doubling, introducing a foreign gene, and changing the positions of genes. It does not include breeding, conjugation, fermentation, hybridization, in-vitro fertilization, or tissue culture. (NOSB, 1996)

This has generally been the definition accepted by the organic community in the US. Genetically engineered organisms in general and GMPPs in particular are considered incompatible with organic production for a number of reasons. Among these are:

(1) The recombination of DNA changes the molecular structure of the target organism by biochemical--as opposed to biological--means.

(2) Genetic engineering develops and produces products in a way not possible or practical with traditional techniques used in organic production and processing.

(3) Gene transfers between organisms not capable of exchanging genetic materials by sexual reproduction are intentionally produced to develop a desired trait.

The process of splitting DNA, and deleting, inserting or changing the position of genes involves the use of various compounds. By these means, rDNA techniques take advantage of the means by which organisms repair damaged genetic material. The molecular structure of rDNA is different from the structure of both the donor and recipient DNA in ways that would not be possible through a natural biological process.

Genetic engineering's promoters often state that rDNA techniques differ only slightly from the classical plant breeding of pest or disease resistant plants. However GMPP corn differs in a number of ways from a hybrid variety. Traditional breeding makes use of natural reproductive mechanisms to select desirable traits expressed by a given set of genes from among the many traits present in a population of closely related organisms. While human manipulation is part of the system, the use of natural reproductive mechanisms limits the use of traditional breeding to a single species or to closely related species that can crossbreed. Genetic engineering is clearly distinct from those advances based on the extension of traditional breeding, propagation, and improvement programs. Hybridization, tissue culture, in-vitro fertilization, and transconjugation are all applications of biotechnology that stop short of genetic engineering. While these applications can create problems for genetic diversity and the agroecosystem, such techniques have long been a part of organic farming systems and are well understood.

By contrast, genetic engineering breaches the natural reproductive barriers between or among species. The laboratory procedures involved in rDNA methods isolate genes from one organism and then transfer those genes directly into another organism. This makes it possible to transfer genes from any organism on the planet to virtually any other organism, regardless of their sexual compatibility or the evolutionary distance between them. Genetic engineering can also be used to transfer genes between members of the same species, in order to duplicate or delete that gene or alter its information content. All organisms modified using recombinant DNA techniques are called “genetically engineered” or “transgenic,” regardless of whether recombinant manipulations involve the transfer of genes between highly divergent species or between members of the same species.

GMPPs and Sustainable Agriculture

The prime application of significance at this forum is the introduction of genes from *Bacillus thuringiensis (Bt)*, into several different cultivated plant species. This has enabled those plants to express the same toxins produced by Bt. The organic community has heard a number of arguments both supporting and opposing genetic engineering's compatibility with organic principles. Among the arguments made in favor of allowing GMPPs in organic production include:

- GMPPs are simply an extension of traditional breeding techniques for developing resistant varieties.
- Farmers can reduce pesticide use by planting varieties engineered for pest resistance.
- GMPPs are based on natural toxins already used in organic agriculture.

- GMPPs can provide organic farmers and processors with more tools to increase the productivity and lower the costs of organic production and processing.
- Organic producers, processors, and handlers will soon lose commonly available inputs as GMPPs displace the original sources of these natural toxins.

These assertions are not well supported by any empirical evidence, and there are a number of reasons that GMPPs appear incompatible with organic principles, such as:

- GMPPs are too new to be able to predict the long-term effects on human health and the environment.
- The amplification of the introduced toxin (high dose) and synergy with the natural toxins and allergens occuring in plants may compromise food safety in ways that cannot be predicted based on the toxicological profile of the individual constituents.
- GMPPs threaten the biodiversity of both the host plant and donor organism.
- Certain products of genetic engineering--combined with a liberal interpretation of the organic standards--would increase, rather than decrease, chemical usage on organic farms. Of particular concern are herbicide tolerant crops.
- GMPP field tests have not gathered adequate data to conduct a valid risk assessment. In particular, protocols reviewed do not evaluate the potential impacts of GMPP crop residue on organisms such as beneficial insects and microorganisms that play a vital role in humus formation and cycling crop plant nutrients.
- GMPPs have the potential to select resistant target pests by the widespread release of naturally occurring toxins in more persistent and toxic forms.
- GMPPs could create new weeds or pests not easily controlled through the horizontal transfer of genetic material.

GMPPs are considered 'synthetic' for the purposes of setting an organic standard. The transfer of genetic material under natural conditions is usually limited to within a species. Transfer between species takes place within nature, and is means by which many cultivated varieties of plants have developed (Goodman, et al., 1987). Transfers between more distant relatives may take place through a variety of means (Bevan, 1984; Wolf, 1996). These transfers are known as *gene flow*. The frequency and significance of the flow of genetic material under natural conditions is the subject of academic debate. These uncontrolled transfers are often unstable or result in mutations that are not reproductively viable. GMOs are, by contrast, not random and must be robust enough to bring the desired traits to fruition.

Other researchers dismiss as invalid or--at best--deficient, any evidence that horizontal gene transfers occur under any natural conditions as invalid, or deficient at best (Prins and Zadoks, 1994), thus supporting the case that such the transfer of genes between species is ‘unnatural,’ and therefore synthetic. If horizontal gene flow is common, this increases the probability--and therefore the risk--of ecological damage that results from GMPPs crossing with wild relatives to create new weeds (Rissler and Mellon, 1996). On the other hand, if gene flow is uncommon, then transgenic organisms are more clearly unnatural, or 'synthetic.' It is possible for GMPPs to transfer genes, and for those transfers to have ecological repercussions. Given that scientists disagree over the probabilities and consequences of such an occurrence, the probability of horizontal gene transfer and the consequence of such an event should not be the main consideration to determine whether or not GMPPs can co-exist with organic farming systems.

Why Conserve Bt?

Bacillus thuringiensis is often called the ideal pesticide. It is narrow-spectrum, with each strain effective against a family. One reason that farmers give for not using Bt more often is its selectivity. However, its selective nature makes it an attractive option for farmers who rely heavily on beneficial organisms as part of their pest management strategy. Bt is a naturally occurring soil organism that is found throughout the world, thus not likely to upset soil microbiology. The toxins produced are essentially non-toxic to humans and other mammals. It is effective because it is highly toxic in low doses to the target species.

Bt is most effective against pests that are both highly susceptible and have feeding habits that cause the foliar applied spores required to be ingested. To be effective, the Bt must both be ingested, and the target pest must metabolize the protein to activate the toxin. An insect that does not metabolize Bt will be immune to it as a pesticide, or *resistant.* An insect may be susceptible to one strain of Bt and resistant to another. This is usually related to the specific toxins produced by the strain of Bt, known as the δ-endotoxin. Four different groups of crystal proteins have been identified based on their amino acid structure. CryI and CryII are active against lepidoptera. CryI crystals are found in commercially available strains *aizawai, berliner* and *kurstaki*. The *kurstaki* strain also contains CryII structures. Over 100 species of lepidopteran pests have been identified as susceptible to the *kurstaki* strain. Strains active against coleoptera, such as *tenebrionis,* contain CryIII protein structures. The commercial strain used to control mosquitoes--*israelensis*--contains the CryIV class of crystals.

The focus of most Bt research conducted over the past ten years has been on recombining genes that express the various Cry toxins into organisms of different genuses, often across different kingdoms. These new techniques have had a number of implications for the toxicity, persistance, and even mode of action of the Bt toxin. Transgenic plants have been engineered to express the toxin directly, rather than depend upon the Bt organism to be applied to the foliage to deliver the toxins. While it is possible to introduce multiple genes into another organism, transgenic products are usually engineered to express a single toxin.

GMPP policy based solely on experience with Bt as the donor organism will not produce valid results for other toxins expressed by other donor organisms. Transgenic Bt is a prototype for other GMPPs (Fishchoff, 1996). Numerous other natural toxins are being screened as potential insecticides (see, for example, Davidson, et al., 1996). The same technique could be extended to a number of other toxins that have greater environmental or human health impacts than the Bt toxin. These toxins could be derived from plants such as pyrethrum, derris and qubé (rotenone), and *Nux vomica* (strychnine); from viruses, bacteria or other microorganisms, or arachnid-derived neurotoxins. Any model used to evaluate GMPPs should take into account the specific host-pest-toxin interaction and the ecological system that is exposed to the release of that organism to provide a valid assessment of the impacts.

GMPPs and the Organic Evaluation Criteria

Transgenic corn, cotton, and potatoes were introduced commercially in 1996. The release of these products has raised a number of issues from the unknown effect of concentrated toxins, increased persistence, and the potential for the Bt gene to escape to wild relatives of the receiving organisms. The very lack of practical experience, knowledge, and predictability of the outcomes of introducing GMPPs into the ecological system is itself a factor in questioning the technology's sustainability. Since the outcomes of these manipulations cannot be predicted as fully as the outcomes of traditional breeding, there is tremendous uncertainty associated with genetically engineered crops both in terms of environmental impacts and food safety. Risk assessment requires data on potential outcomes, their probabilities, and the costs associated with those outcomes. Since the outcomes from adopting the technology can't be predicted *ex ante,* and the probabilities and costs associated with those outcomes are indeterminate, the risks cannot be determined and risk-benefit analysis is not a valid model to make scientific decisions. Add to this the likelihood that some of the potential outcomes are irreversible, another model for technological decision-making is more appropriate.

Organic agriculture has been more conservative in adopting most new technologies than other farming sectors. However, in some cases, organic farmers have been innovators, pioneers and early adopters of biopesticides. NAS may want to consider the evaluation process and criteria used to set the standard for organic farming. Organic farming standards are based on the premise that synthetic materials are prohibited, unless they are explicitly allowed. Before a synthetic material can be allowed, the NOSB is required to review substances against the following criteria (See 7 USC 6518(m)):

(1) the potential of such substances for detrimental chemical interactions with other materials used in organic farming systems;
(2) the toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment;
(3) the probability of environmental contamination during manufacture, use, misuse or disposal of such substance;
(4) the effect of the substance on human health;
(5) the effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock;
(6) the alternatives to using the substance in terms of practices or other available materials; and
(7) its compatibility with a system of sustainable agriculture.

Given that GMPPs are synthetic, then these criteria can be applied to evaluate their compatibility with organic standards.

(1) Detrimental Interactions

The ecological consequences of releasing GMPPs into the environment are not obvious, and numerous effects of genetically engineered products cannot be predicted with accuracy prior to their commercial release. Bt toxins expressed by other host organisms may have different interactions with the environment than the non-GMO bacteria. The interaction of different strains can have a synergistic effect (Wu and Chang, 1985). These strains vary widely in their host range. Given the complexity of the biochemistry of different plants, the substances naturally exuded by plants may cause detrimental interactions with the Bt toxins.

(2) Toxicity and Mode of Action

There are a number of ways in which genetic engineering can increase the toxicity and enhance the modes of action for many familiar and novel agricultural products. Transgenic plants engineered for resistance to insects and diseases do so by the production of toxins. Many transgenic plants are commodity crops grown as animal feed. Genetically engineered feed may also present health risks to animals through increased levels of toxins, altered nutrient composition, and nutrient availability (see FDA policy 22988). For other products, the toxicity studies have not been performed. GMPPs and their products may result in allergens that cannot be predicted (see FDA policy 22987).

(3) Environmental Contamination

One measure of the adverse effects of genetic engineering on the global environment is *genetic pollution* (Fox, 1997). Hybridization between genetically modified crops and wild relatives threatens the loss of biodiversity and the natural germplasm on which breeders depend for new traits. Recombination also increases the risk of the generation of new viral strains (Greene and Allison, 1994).

When a GMPP is introduced into the environment, it may upset the farm's natural balance. New organisms released into the environment may mutate or interbreed with weedy species, and become pests, pathogens or weeds themselves. Many applications of biotechnology--hybridization and tissue culture, as well as genetic engineering--threaten the diversity that maintains the stability of agricultural systems.

Genetically engineered organisms may displace plants or animals from their niches, thus making them endangered species or even driving them to extinction (Rissler and Mellon, 1993). The escape of a weedy GMPP is a function of the following factors: (a) the ability of the organism to survive; (b) the organism’s propensity to reproduce; (c) the introduced species interaction with other organisms; and (d) the nature of that interaction.

(4) Human Health Concerns
GMPPs may directly create human health and environmental problems. Any genetically engineered product has a dimension of risk that cannot be predicted before its use. Because genes can affect more than one trait (pleiotropism), it is impossible to predict *ex ante* the effects of genetic manipulation (Tiedje, et al., 1989). Models developed for the evaluation of risks of synthetic chemicals do not necessarily provide a meaningful evaluation of the risk of genetic engineering (Goldburg and Greenlee, 1993). For example, insect and disease resistant crops may produce more potent natural toxicants at greater levels than those currently found in plants. New models need to be developed and tested to assess toxicity, allergenicity and antigenicity of expressed products. Also, the stability and form of the Bt toxin may change over several generations that cannot be predicted without long-term research.

(5) Effects on the Agroecosystem
Methodologies to evaluate the environmental impact of the release of live genetically engineered organisms are in their infancy (Smalla and van Elsas, 1996). The effects of such organisms on the ecosystem have been studied only a short time (Gillett et al., 1985; Seidler, 1992). Release of GMPPs into the soil environment could result in "(i) displacement of existing species, (ii) major changes in community structure and function; (iii) perturbation of ecological balance; (iv) accumulation of toxic metabolites; or (v) increased microbial activity due to nutrient input" (Smit, 1992 paraphrased in Smalla and van Elsas, 1996).

The most serious concern is the risk that such products could accelerate the failure of Bt through resistance (Mellon and Rissler, 1998). A more toxic and persistent form of Bt that coincides with the release of transgenic plants theoretically could increase selection pressure for Bt resistant strains of insects. Laboratory resistance to Bt was first reported in the Indian meal moth (*Plodia interpuntella)* (McCaughey, 1985). The diamondback moth was the first species to show resistance in field conditions (Tabashnik, *et al.*, 1990).

Resistance is not inherent to transgenic Bt. Prolonged or constant exposure of the target population to the toxins possibly provides for much greater pressure to select for resistance than with standard foliar Bt (Gould, 1988). Continuous exposure to the *Bt* toxin created the selection pressure for resistant variants of pests (Tabashnik, et al, 1997). Extensive and intensive exposure of pests to *Bt* through transgenic plants may cause widespread pest resistance (Tabashnik, Finson, and Johnson, 1991).

Crops have co-evolved with insects to exude various compounds to deter, repel, or inhibit feeding by pests. Insects overcome these natural defenses in host plants by selection pressure that favors those insects that are able to detoxify those compounds. The ability of these insects to detoxify compounds that plants express increases their ability to damage crops or virulence. This same genetic mechanism also serves as a vehicle for those insect populations to evolve with insecticide resistance as a common trait (Simms, 1987). Data obtained from crops engineered to express the

Bt toxin indicated that resistance can emerge in as few as seven generations (Gould, et al, 1997). This may take as little as two years in some climates.

The maintenance of non-transgenic refugia has been proposed as one resistance strategy (May, 1993). This involves farmers not planting GMPPs for a portion of the acreage in a given crop. It is not possible to predict how large a refuge area is needed before the organism is released. The only way to determine the optimal size of refugia is under actual field conditions (Roush, 1996). By not cultivating GMPPs, organic farmers increase the acreage available as refugia for susceptible gene pool for target pests.

Organic farmers may lose Bt as an effective crop protection tool as a result of accelerated resistance from the release of transgenic Bt (Mellon and Rissler, 1998). Experience with a series of chemicals suggests that if one chemical is used as the primary or only means to controlling a target insect, selection pressure for resistance to that pesticide will be increased (Georghiu and Mellon, 1983). This is consistent with modeled systems that showed GMPP plants could accelerate Bt resistance in target pests (Ferro, 1993).

As molecular ecology and evolutionary population dynamics advance, the risk assessments conducted to evaluate the release of these organisms are not based on sound scientific principles. Most risk assessments are based on small, confined, protected populations that are isolated from both competitors and wild relatives. These experiments are not valid models of the actual field conditions in which crops are grown. It is not scientific to call GMOs 'safe' because 'nothing happened' under these conditions (Regal, 1994). Addressing the impact of genetically engineered Bt on the agroecosystem will require large-scale experiments that examine aspects of both molecular biology and adult insect behavior that have not been of concern to researchers in the past (Roush, 1996).

(6) Alternatives

None-engineered Bt is obviously a viable alternative, one that may be lost as a direct result of the introduction of GMPPs. Bt can be conserved through well-timed applications based on scouting. In addition, there are a number of biological and cultural methods for every one of the target pests of GMPPs. Numerous cultural and biological practices offer options besides GMPPs, Bt, and synthetic pesticides. Among these are rotation of non-host crops, intercropping of non-host crops, habitat management to maintain beneficial organism populations, augmentive releases of beneficial organisms, classical breeding of pest resistant varieties, crop nutrient and water management, timing of planting and harvest, and mating disruption.

Many non-recombinant methods show great promise in improving the efficacy of Bt. Discovery and selection of new strains for novel proteins and field stability through heat and UV resistance show promising developments. Research on adjuvants is one area that has shown great progress, albeit one with little published data. Bt manufacturers have successfully applied transconjugation techniques resulting in a strain with greater efficacy and a broader number of target species.

Sexual reproduction of two Bt strains can produce offspring with a complementary set of genes to express the toxins produced by both parent strains.

(7) Compatibility with Sustainable Agriculture
There are a number of reasons to be skeptical of any claims that the technology is compatible with any system of sustainable agriculture. Time is the only test that will determine the long-term sustainability of GMPPs. The NAS would do well to address these long-term questions about the ecological impact of the technology. To do so requires scientists to develop a methodology based on a whole systems approach to agriculture. Proper evaluation of the technology needs to recognize the uncertainty of outcomes and irreversibility of the consequences.

- Researchers need to design experiments to assess the long-term effects of the use of GMPPs and their products on human health and the agroecosystem.

- Data to review the effects of such products on human health and the environment needs to be over a time of several growing seasons.

- Models to evaluate the data need to take into account the uncertainty and irreversibility in a way that risk-benefit analysis fails to do.

Conclusions and Recommendations

GMPP and organic acreage are both growing at rapid rates, therefore the collision of the two are inevitable. Because GMPPs and their products have become ubiquitous in such a short period of time, some applications of genetic engineering will inevitably spill over into organic farming systems through a variety of means.

Organic agriculture offers a potential reservoir of genetic material from classically bred organisms. Organic farms would become a refuge, a safety valve that allows experimentation in genetic engineering to take place with the safety net that some of the germplasm will be saved that is grown out free from genetic modification. This requires not only that GMPPs are not cultivated or used on such preserves; it also requires that measures be taken to monitor for and prevent genetic pollution. The effectiveness of this strategy is diminished as genetic drift becomes more commonplace. Organic agriculture could also serve as a resource to conserve non-GMO strains of Bt.

Organic farmers are forced to co-exist with GMPPs. The application of genetic engineering has become so ubiquitous that it is not possible to guarantee that many food products are GMO-free. This has quickly become analogous to the situation with pesticides, where drift, atmospheric deposition, and background contamination makes pesticide residues unavoidable. While the economic damage that this will cause organic farmers may not be the NAS's concern, the potential for such drift to reduce biodiversity, accelerate pesticide resistance, undermine efforts to manage that resistance, and disrupt populations of non-target species should not be underestimated.

References

Bevan, M. 1984. Binary *Agrobacterium* vectors for Plant Transformation. *Nucleic Acids Research* 12: 8711-8721.

Davidson, E.W., R.B.R. Patron, L.A. Lacey, R. Frutos, A. Vey, and D.L. Hendrix. 1996. Activity of Natural Toxins Against the Silverleaf Whitefly, *Bemisia argentifolii,* using a Novel Feeding Bioassay System. *Entomologia, Experimentalis et Applicata* 79: 25-32.

Ferro, D. 1993. Potential for Insect Resistance to *Bacillus thuringiensis:* Colorado Potato Beetle (Coleoptera: Chrysomelidae)--A Model System. *Amer. Entomol.* 39: 38-44.

Fischoff, D.A. 1996. Insect-resistant Crop Plants, in G.J. Persley (ed.) *Biotechnology and Integrated Pest Management*: 214-227. Oxon, UK: CAB International.

Fox, M. 1997. Biotechnology, Monocultures and Genetic Pollution. Washington: Humane Society of the United States.

Georghiu, G.P. and R. B. Mellon. 1983. Pesticide Resistance in Time and Space, in Georghiu and Saito, *Pest Resistance to Pesticides*: 1-46. New York: Plenum Press.

Gillett, J.W., A.R. Stern, S.A. Levin, M.A. Harwell, and D.A. Andow. 1985. *Potential Impacts of Environmental Release of Biotechnology Products: Assessment, Regulation and Research Needs.* Ithaca: Cornell University Ecosystems Research Center.

Goldburg, R. and Greenlee, W.F. Technical Risk Assessments and Regulations, in *Agricultural Biotechnology: A Public Conversation About Risk*: 21-23. (Ithaca: National Agricultural Biotechnology Council, 1993).

Goodman, R.M., H. Hauptli, A. Crossway, and V.C. Knauf. 1987. Gene Transfer in Crop Improvement. *Science* 236: 48-54.

Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferre, F.J.Silva and W.J. Moar. 1992. Broad Spectrum Resistance to *Bacillus thuringiesis* toxins in *Heliothis virescens. Proc. Nat. Acad. Sci.* 89: 7986-7990.

Gould, F. 1988. Evolutionary Biology and Genetically Engineered Crops. *BioScience* 38: 26-33.

Gould, F., A. Anderson, A. Jones, D. Sumerford, D. G. Heckel, J. Lopez, S. Micinski, R. Leonard, and M. Laster. 1997. Initial Frequency of Alleles for Resistance to *Bacillus thuringiensis* Toxins in Field Populations of Heliothis virescens, *Proc. Nat. Acad. Sci. USA*. 94: 3519-3523.

Greene, A.E. and R.F. Allison. 1994. Recombination between Viral RNA and Transgenic Plant Transcripts. *Science,* 263: 1423–1425.

May, R. M. 1993. Resisting Resistance. *Nature* 361: 593-594.

McCaughey, W.H. 1985. Insect Resistance to the Biological Insecticide *Bacillus thuringiensis. Science* 229: 193-195.

Mellon, M. and J. Rissler. 1998. *Now or Never: Serious New Plans to Save a Natural Pest Control.* Cambridge, MA: Union of Concerned Scientists.

National Organic Standards Board. 1996. Biotechnology Policy. Adopted at Indianapolis, IN. Washington: USDA/AMS/NOP.

Prins, T.W. and J.C. Zadoks. 1994. Horizontal Gene Transfer in Plants, a Biohazard? Outcome of a Literature Review. *Euphytica* 76: 133-138.

Regal, P.J. 1994. Scientific Principles for Ecologically Based Risk Assessment of Transgenic Organisms. *Molecular Ecology* 3: 5-13.

Rissler, J. and Mellon, M. 1993. *Perils Amidst the Promise: Ecological Risks of Transgenic Crops in a Global Market*. Cambridge, MA: Union of Concerned Scientists.

Roush, R.T. 1996. Can We Slow Adaptation by Pests to Insect Transgenic Crops? in G.J. Persley (ed.) *Biotechnology and Integrated Pest Management*: 150-163*.* Oxon, UK: CAB International.

Seidler, R.J. 1992. Evaluation of Methods for Detecting Ecological Effects from Genetically Engineered Microorganisms and Microbial Pest Control Agents in Terrestial Systems. *Biotech. Adv.* 10: 149-178.

Simms, E. 1987. Ecological Genetics and Evolution in Insect Pests: Implications for Lower Input Agriculture. *Amer. J. Alt. Agr.* 2: 153-159.

Smalla, K. and J.D. van Elsas. 1996. Monitoring Genetically Modified Organisms and their Recombinant DNA in Soil Environments, in J. Tomiuk, K. Wöhrmann and A. Sentker, *Transgenic Organisms: Biological and Social Implications.* Basel: Birkhauser Verlag Basel.

Smit, E., J.D. van Elsas, and J.A. van Veen. 1992. Risks Associated with the Application of Genetically Modified Microorganisms in Terrestial Environments. *FEMS Microbiol. Rev.* 88: 263-278.

Tabashnik, B.E., N.L. Cushing, N. Finson and M.W. Johnson. 1990. Field Development of Resistance to *Bacillus thuringiensis* in Diamondback Moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* 83: 1671-1676.

Tabashnik, B.E., N. Finson and M.W. Johnson. 1991. Managing Resistance to *Bacillus thuringiensis:* Lessons from the Diamondback Moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* 84: 49-55.

Tabashnik, B.E., Y-B. Liu, T. Malvar, D.G. Heckel, L. Masson, V. Ballester, F. Granero, J.L. Ménsua, and J. Ferré. 1997. Global Variation in the Genetic and Biochemical Basis of Diamondback moth Resistance to *Bacillus thuringiensis*, *Proc. Nat. Acad. Sci. USA*. 94: 12780-12785.

Tiedje, J.M., R.K. Collwell, Y.L. Grossman, R.E. Hodson, R.E. Lenski, R.N. Mack and P.J. Regal. 1989. The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations. *Ecologist* 70: 298-315.

Wolf, K. 1996. Gene Transfer Between Organelles and the Nucleus in Lower Eukaryotes, in E.R. Schmidt and T. Hankeln (eds.) *Transgenic Organisms and Biosafety.* Berlin and New York: Springer.

Wu, D and F.N. Chang. Synergism in Mosquitocial Activity of 26 and 65 kDa Proteins from *Bacillus thuringiensis* subsp. *Israeliensis* Crystal. 1985. *FEBS Letters in Biochemistry.* 190: 232-236.