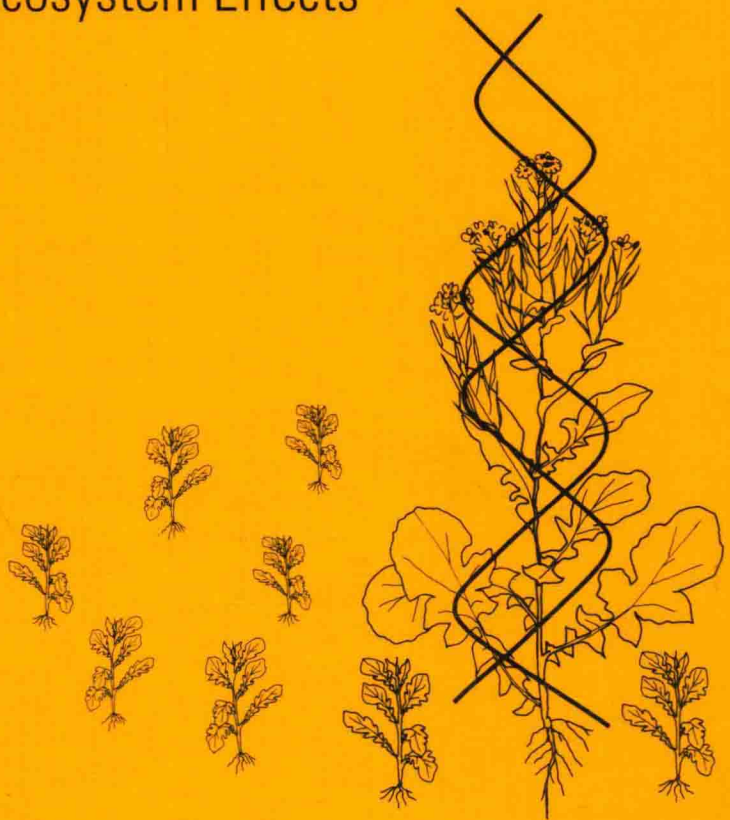


Gösta Kjellsson  
Vibeke Simonsen

# Methods for Risk Assessment of Transgenic Plants

I. Competition,  
Establishment  
and Ecosystem Effects



Birkhäuser

Gösta Kjellsson  
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I. Competition,  
Establishment  
and Ecosystem Effects

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## Preface

The present work was initiated in autumn 1991, with the aim to compile a catalogue of test methods useful for the risk assessment of transgenic plants, hereafter synonymously referred to as genetically modified plants (GMP). The steering group decided that the methods included in the catalogue should be based on recent literature with the main emphasis on competition, establishment, and ecosystem effects. The main user groups for the catalogue are scientists and students working in plant ecology and risk assessment and administrators with responsibility for the legislation of transgenic plants. A broad range of ecological and genetic methods in contemporary use has been included in the catalogue.

It is our hope that the present work will also be useful as a reference for experimental research in general plant population ecology and genetics. Most of the methods covered in the catalogue do indeed originate from these research fields.

We would like to thank the members of the steering group, Hans Erik Svart and Søren Mark Jensen from the National Forest and Nature Agency, and Kaj Juhl Madsen from the Danish Environmental Protection Agency for their support and assistance in initiating this work, and for valuable comments during its preparation.

Several people gave highly valuable technical assistance: Lilian Møx-Jørgensen, Lone Møller, Anna Marie Plejdrup, Karsten Rasmussen and Bodil Thestrup. Caroline Burke, Winnie Meilstrup, and Lone Thøgersen generously helped in correcting the language. Kåthe Møgelvang is acknowledged for the drawing on the front cover.

Special thanks to Dr. Michael Burke for drawing our attention to herbivory as a major factor for seedling mortality, and for letting us add "chemical exclusion experiment" to the list of methods.

Finally, it is a pleasure for us to express our sincere gratitude to the voluntary reviewers of the first draft of the catalogue who gave us many valuable comments. They are: Dr. R. K. M. Hay, Scottish Agricultural Science Agency, Scotland; Dr. H. Gaugitsch, Umweltbundesamt, Austria; Dr. J. Pedersen and Dr. F. Eriksen, National Food Agency of Denmark, Denmark; Dr. L. Val Giddings, Biotechnology Coordination and Technical Assistance, USA; Dr. L. Espeby, Naturvårdsverket, Sweden; Dr. F. Pythoud, Bundesamt für Umwelt, Wald und Landschaft, and Dr. F. Felber, Institut de Botanique, Neuchâtel, Switzerland.

Gösta Kjellsson and Vibeke Simonsen  
Silkeborg, April 1994

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# 1. Introduction

## *Origin of the catalogue*

The present catalogue is the result of the cooperation between three Danish environmental authorities: The National Forest and Nature Agency, The Danish Environmental Protection Agency, and The National Environmental Research Institute, Department of Terrestrial Ecology. The aim of the work presented was to compile a catalogue of test methods useful in risk assessment of genetically modified plants (GMPs), with the emphasis on competition, establishment, and ecosystem effects. A GMP may be defined as a plant with genes inserted from another plant or organism through non-conventional gene transfer (transformation). Thus, by this definition, natural sexual processes (i.e., mating and natural recombination) are excluded. Characters which have been altered in cultivated plants by transformation include: herbicide resistance, insect resistance, pathogen resistance, drought and frost tolerance, improved nitrogen fixation, increased protein or starch content, etc. (140).

## *Genetically modified plants (GMP) - definition*

### 1.1. Reasons for initiating the work

## *EC directive*

The basis for the present work is provided by the EC directive of April 23, 1990, on the deliberate release into the environment of genetically modified organisms (90/220/EC). This directive lays down the basic terms for risk assessment of GMPs in the European Communities.

## *Technical and commercial development*

The development of efficient methods to genetically modify various organisms is increasing rapidly, and by March 1994, a total of 210 requests for deliberate release of genetically modified organisms had been received by the authorities in the EC. It may be expected that the number of requests for deliberate release for test purposes will increase rapidly in the coming years, and requests for commercial releases will appear. Several international companies are ready to apply for commercial release of different transformed plants. One of the first GMPs which is to be introduced commercially is a modified tomato. The modification involves a gene that suppresses the expression of a natural gene which produces a cell-wall degrading enzyme in the tomato fruit (415). Suppression of this gene will produce tomatoes with the potential for prolonged storage, which is a commercially desirable characteristic. To our knowledge, no information is available on possible pleiotropic effects of the inserted gene, on possible transmission of the gene to related species, and its risk to natural ecosystems. In the future, we will no doubt experience the results of the gene transfer into very diverse plant groups, from citrus and oranges to plums and pears, deriving from all parts of the world (203, 524).

*Public concern and ecological research*

The use of biotechnology to produce genetically modified plants may meet the needs of society and the public for more plentiful and improved plant food, with an additional reduction in the use of fertilizers and pesticides. On the other hand, there is an increasing awareness about the potential negative impact on the natural flora and fauna. Few actual experiments have been performed with real GMPs in natural habitats. The PROSAMO project in the U.K. has been working with the ecological effects of releasing transgenic plants, such as oilseed rape, *Brassica napus* L., which is modified for resistance against fungus attack (131). This and other studies may provide strongly needed information on the ecological consequences of GMPs in nature.

## 1.2. Main purposes of the work

*Risk assessment of GMPs*

The main purposes of the work are to

- compile a catalogue of methods which may be used in ecological risk assessment of GMPs
- give a review of useful methods for studying competition and establishment of GMPs
- indicate where new test procedures need to be developed for risk assessment of GMPs

*General reference work*

The catalogue focuses on ecological and genetic methods and the main topics are competition, establishment, and ecosystem effects. The genetic aspects concerning hybridization and pollination are not included in the present work, but a second volume covering these aspects is in preparation. **The catalogue should, furthermore, be useful as a general reference of methods for population ecology and population genetics.** Methods covering seed predation are generally not included, as this would have made the amount of literature unmanageable. Methods for vegetation analysis (M64) are covered only in the provision of general suggestions. Some statistical procedures are presented, but in general, standard textbooks on the subject should be consulted.

*Dispersal of inserted genes*

There are two general ways by which the spreading of inserted genes may take place in nature. The first way is the dispersal by seeds or vegetative organs of the GMP itself, and its subsequent survival and establishment in a new habitat. The second way is the transmission of the inserted genes by pollination to non-modified individuals of the same species, or to related species by hybridization. A possible third way, that of genetic transmission in nature between non-related organisms such as plants and micro-organisms, has not been definitely proven so far.

*Focus of the catalogue*

The present work focuses solely on aspects of dispersal and establishment of the modified plant into nature and the competitive interactions with the local flora. Questions concerning genetic transmission by pollination or hybridization is thus not included in the present work (volume in prep.), and information must be sought in standard text books.

### 1.3. Organization of the catalogue

*Literature search*

The catalogue is based on a literature search in the library databases BIOSYS and CAB, and supplemented with references known by the two authors. **The authors decided upon a selective search within the above databases, covering the last 5 years of natural science journals and books.** Before initiating the literature search it was necessary to create a list of relevant key words. This list consists of two parts, one concerning the level of the organism, and the other with processes related to the level (see chapter 2). By **level**, the authors understand **life stage** (e.g., seed, seedling, etc.), **genome** (native and modified), **population**, **ecosystem**, and **test system** (related to how and where the methods are performed). By **process**, the authors understand either the phases (e.g., seed bank, seed germination, etc.) related to the specific level (e.g., life stage - seed), or the processes (e.g., invasion) which they are influenced by. A comprehensive explanation of the words used in this context is given in chapter 2.

*Levels and processes**Contents - chapter 3-6*

Chapter 3 is a list of **processes** with corresponding methods, and chapter 4 is a list of **methods** relevant for each process. A synopsis of processes and recommended methods, and comments on processes which lack relevant methods, will be presented in chapter 5. Chapter 6 contains detailed descriptions of the different methods.

*Contents - chapter 7-8*

Suggestions for general procedures and methodologies are presented in chapter 7. Finally, a list of references is added in chapter 8. The list is compiled from the RISK database, which has been elaborated specifically for this work in the bibliographic system Pro-Cite.





## 2. Levels and corresponding processes

As mentioned in the previous chapter, it was necessary to construct a list of key words for the literature search. For this purpose the authors selected the following main topics which would be of value in the compilation of the method catalogue: **life stages** in the life cycle of a plant, the **genome**, and interactions among plants, **population** and **ecosystem**. The authors then added the relevant process words and initiated the library search. The initial search list contained more process words than the present one, because it included synonyms or similar terms for some processes (e.g., juvenile and seedling). Furthermore, the process words **risk assessment** and **release** under the level **test system** were included in the search profile. The final list of levels and processes is shown below (section 2.1). Process words shown in parentheses are largely treated as synonyms. An explanation of the process words will be given in the glossary (section 2.2).

### 2.1. List of levels and processes

Level		Process
Life stage	Seed	Seed production
		Seed set
		Seed weight (seed size)
		Seed dispersal
		Seed bank
		Seed dormancy
		Seed survival (seed viability)
		Seed germination
	Seedling	Seedling competition
		Seedling survival (persistent seedlings)
		Seedling growth
		Seedling mortality
		Seedling selection
	Adult plant	Plant size
		Plant growth
		Plant competition
Vegetative	Vegetative reproduction (clone)	
	Clonal growth (vegetative growth)	
	Vegetative allocation	

2.1. continued

Level	Process	
Life stage	Flowering	(Sexual reproduction) Reproductive allocation
Genome	Natural	Genotype Genetic markers Polymorphism
		Quantitative characters QTL (quantitative trait locus)
		Chromosomes (polyploidy)
		Proteins
	Modified	DNA RNA
		Genetically modified organism Genetically modified plant
Population		Population dynamics
		Density-dependent mortality Density-dependent competition Density-dependent regulation (density-dependent selection)
		Parent-offspring competition (parent-offspring selection) Sibling competition
		Spatial pattern (spatial distribution) Neighbourhood
		Recruitment Phenology Allelopathy

2.1. continued

Level	Process
Ecosystem	Biodiversity Community structure
	Invasion (colonization) Disturbances
Test system	Vegetation dynamics Ecosystem effects Genotype-environment interactions
	Risk assessment
	Release (pre-release) Deliberate release Small scale release Large scale release Contained trial (confined trial)
	Growth chamber experiment Greenhouse experiment Field experiment (field test) Laboratory experiment Experimental design Data analysis Model

## 2.2. Glossary of terms and abbreviations

Methods are referred to as M-numbers, see section 6.2.

Allelopathy	Chemical effects (usually negative effects) of live or dead plant tissues on neighbouring plants
Antibody	A specific protein which is produced in an individual exposed to a specific antigen. The antibody specifically recognizes the antigen, binds selectively to it, and thus eliminates the antigen from the individual
Antigen	A macromolecule which causes an antibody production
Biodiversity	Number and relative composition of species or ecotypes in a certain habitat
Chromosomes	Small bodies in the cell carrying the genetic information. They are present in nearly all cells of an individual. The chromosomes consist of nucleic acid (DNA) and proteins (histones).
Clonal growth (vegetative reproduction)	Growth and multiplication of asexually reproducing plants
Community structure	Composition and diversity of an assemblage of species in a habitat of well defined physiognomic appearance
Confined trial	Experiment which is performed under very strict control to prevent the transfer of genes from test organisms into the environment
Data analysis	Analysis of data by statistical and other methods so that simple conclusions may be drawn
Deliberate release	Release of an organism (e.g., genetically modified) into managed or natural habitats

Density-dependent competition	Competition in a species for abiotic resources (e.g., light, water, nutrients) affected by the density of plants
Density-dependent mortality	Mortality in a plant population caused by density-dependent competition
Density-dependent regulation (density-dependent selection)	Regulation of survival, growth, and reproduction of individuals due to density-dependent effects in the population
DFA	Discriminant Function Analysis, see M9
Disturbances	Man-made or naturally inflicted changes to the vegetation cover or to the physical properties of a habitat
DMP	Dry Matter Production, see M12
DNA	DeoxyriboNucleic Acid, a molecule containing the genetic information of an organism
Ecosystem effects	Effects on the composition of an ecosystem resulting from abiotic or biotic pressures
Ecosystem	Relatively self-contained ecological system defined by the abiotic factors and the organisms living in the area and the interactions between them
Experimental design	Practical arrangements used in experimental work
Field experiment (field test)	Experiment performed in the field for general purposes with exposure to environmental variations
Gene	The functional unit of inheritance
Genetic marker	Recognizable trait, which is inherited by an individual
Genetically modified	An organism with genes inserted from another organism through non-conventional gene transfer (transformation)

Genetically modified plant	A plant with genes inserted from another organism through non-conventional gene transfer (transformation)
Genome, modified	The total genetic constitution of an organism, modified compared to the natural genome
Genome, natural	The total native genetic constitution of an organism
Genotype	The genetic constitution of an organism. Often used for a single gene
Genotype-environment interactions	Interactions between the genotypes in a population and environmental factors in the habitat
GMP	Genetically modified plant
Greenhouse experiment	Experiment performed in the greenhouse for general purposes with variable control
Growth chamber experiment	Experiment performed in the growth chamber for general purposes with strict control
HI	Harvest Index, see M26
Invasion (colonization)	Dispersal and establishment of a new species in a certain area or habitat
Isozymes	Proteins with the same catalytic ability but with different electric charge
Laboratory experiment	Experiment performed with laboratory facilities for very specific purposes
LAD	Leaf Area Duration, see M36
LAI	Leaf Area Index, see M35
LAR	Leaf Area Ratio, see M35
Large scale release	Release of plants and other genetically modified organisms in extensive areas mainly for agricultural test production

Life stage: adult plant	The period in a plant's life where reproduction is possible
Life stage: flowering	The period in a plant's life where sexual reproduction is possible
Life stage: seed	The period in a plant's life where dispersal and dormancy in the soil take place
Life stage: seedling	The period in a plant's life from the germinating seed to the adult plant
Life stage: vegetative	The period in a plant's life where vegetative reproduction is possible
Model	Mathematical, statistical, and other models predicting (e.g., by use of computer simulation) an outcome of a certain biological event, which may be validated in the field
NAR	Net Assimilation Rate, see M48
Neighbourhood	Characterization of the area available to an individual plant, of the distance to its neighbours, and of the composition of neighbouring species
NPP	Net Primary Production, see M49
Parent-offspring competition (parent-offspring selection)	Competition between parents and offspring (e.g., seedlings) for abiotic resources
PCA	Principal Component Analysis, see M54
PCR	Polymerase Chain Reaction, see M53
Phenology	Study of periodically occurring biological events in relation to seasonal changes of abiotic factors (e.g., climate conditions)
Plant competition	Competition among adult plants for abiotic factors resulting in differential growth of individual plants
Plant growth	Accumulation of plant biomass, increased plant size, and increased number of organs in relation to the life stage



Plant size	Measured as height, biomass or dry matter
Polymorphism	A trait which may occur in different forms either in a population or in an individual at different life stages
Polyploidy	An integer multiplication of the chromosome number
Population	A group of individuals of a species, which either have been or are able to exchange genes between individuals. Often living in a certain area and type of environment
Population dynamics	Demographic processes influencing the survival, reproduction, and growth-rate of a population
Primer	A short chain of DNA or RNA which initiates the amplification of a certain area of the DNA molecule
Probe	A chain of DNA or RNA complementary in base sequence to a nucleic acid from a given source, and used to selectively isolate the nucleic acid from a mixture of other nucleic acids
Protein	A molecule with ampholytic character consisting of amino acids. The molecule may have catalytic ability and, in this case, the protein is an enzyme
QTL	Quantitative Trait Locus, a locus which is either involved in the determination of a quantitative character, or closely linked to a locus involved in the determination of the quantitative character
Quantitative character	A morphological trait which is determined by many genes and often influenced by the environment
RAPD	Random Amplified Polymorphic DNA, see M56
RCC	Relative Crowding Coefficient, see M57