



BIODIVERSITY SECTOR
ECOLOGICAL IMPLICATIONS OF GMOS

Robust methodologies for ecological risk assessment

Final report: Inductive hazard analysis for GMOs

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Executive summary

Hazard identification is arguably the most important component of any risk assessment. Hazards that are not identified in the early stages of a risk assessment are not carried through the assessment, leading ultimately to underestimates of risk. Hazard identification for all new technologies initially must be inductive. As operating experience grows, and adverse events are recorded, the analysis can also adopt deductive approaches. The most common (deductive) approaches are checklists and unstructured brainstorming. Checklists may be lengthy and well developed or quite short, and are clearly the “status quo” in the majority of risk assessment frameworks for GMOs. This is surprising for such a new technology and worrying—checklists do not ask “what can go wrong” with the system in question, and do not confirm that all components of the system have been questioned. Indeed they tend to mislead the analyst into believing that all aspects of the system have been questioned without confirming this to be true.

The objective of this report is to apply two inductive techniques – Hierarchical Holographic Modelling (HHM) and Fault Tree Analysis—to identify the potential ecological hazards associated with the unconfined release of Herbicide Tolerant (HT) canola, *Brassica napus*. The aim of the analysis is to demonstrate the potential value of inductive hazard identification techniques as applied to GMOs. It does not aim to identify hazards specific to a particular product. The demonstration does not therefore apply to a particular type of HT canola nor identify particular herbicides or release conditions in a particular environment. It does, however, identify the general types of ecological hazards that may be associated with HT canola. In a real analysis of a GMO intended for release, the identification of hazards would be supported by product-specific and geography-specific information that is not presented here.

Hierarchical Holographic Modelling (HHM) captures the complexity of a large system by identifying the components and processes of all sub-systems and analysing how they interact with each other. The technique decomposes the system by looking at it from many different perspectives including, for example, the functions, activities, geo-political boundaries, or structures of the system. The analyst constructs an HHM by first identifying the most appropriate perspectives for the problem in hand. These are used to define the sub-systems which in turn are further decomposed into components, processes, functions or activities, which may or may not overlap with other sub-systems. The analyst(s) identifies hazards by comparing potential interactions between the sub-systems in a qualitative fashion. This is best achieved by a team expert in one or more of the chosen perspectives.

The Hierarchical Holographic Model developed here identified a total of 153 potential hazards, 13 potential benefits and 30 event scenarios that may present a benefit or a hazard depending on the specific environmental and agricultural conditions. It is important to note that the analysis did not actively seek to identify potential benefits—the ratio of hazards to benefits in this study is not in any way indicative of the cost-benefit ratio that might result from the introduction of HT canola to any given area. Approximately 43% of hazards were identified only once. A further 42% of were identified between 2 and four times, whilst 2 hazards (1%) were identified over 15 times. All hazards were grouped into broad categories and scored by degree of concern and confidence. The final hazard score does not represent a formal assessment of risk and uncertainty—it is simply a way to prioritise each of the hazards for further analysis. In particular, some hazards, which are probably quite unlikely, might have received a disproportionately high hazard score because one team member, perhaps unfamiliar with that particular biological process or group of organisms, over-rated the likelihood or the severity of the consequences.

The incidence of HT volunteers (and HT resistant weeds) on the farm has the highest average score of all the hazard categories. HT volunteers on farm occur due to the significant seed loss during harvest and via a variety of natural process (e.g. ants and earthworms) that encourage seed burial and re-emergence. Dispersal of the HT gene beyond the farm (off-site) has the second highest average score of all the hazard categories identified here. There are a large number of ways in which the HT gene might disperse beyond the farm, either as HT canola pollen and seed, or as HT pollen and seed of a weedy relative following gene flow.

Adverse changes to weed spectra were the most frequently identified hazard of HT canola, although this ranked relatively low. Farming practice associated with HT canola, particularly the expected increase in post-emergent herbicide application and subsequent selection of herbicide resistant hybrids or volunteers, may increase the resources available to brassicaceous pests whilst at the same time reducing the resources available to beneficial insects and invertebrates. The largest source of potential ecological hazards arises through potential changes to farming practice that may follow widespread use of HT canola. Most of these hazards are associated in one way or another with either the way in which HT canola is grown—such as closer crop rotations, minimum tillage and post-emergent application of herbicides—or with the farmer’s more attentive behaviour to a high value/high return crop—such as increasing acreage into marginal or remnant land areas and altered spray strategies.

In summary, the top ten hazards reflect an underlying concern that commercialisation of HT canola, without careful management, will increase the incidence of HT volunteers, both on and off-farm, which facilitate “secondary” seed and pollen-mediated dispersal of the trait over large distances. This coupled with the potential development of herbicide tolerance amongst weeds, may necessitate the use of alternative, and potentially more toxic, weed control strategies across large areas of agricultural and non-agricultural land. The impact of HT canola, and associated farming practice, on soil fauna communities and processes, also figure prominently in the HHM analysis. This appears to be an important, but as yet poorly understood, aspect of the new technology.

The main drawback with the HHM analysis is the time required to complete it, and the need to coordinate experts that, as in this case, might be drawn from several different institutions. It is often difficult to maintain continuity and consistency in these groups. Redundancy and duplication within the analysis also tend to reduce its efficiency. It is difficult, however, to determine *a priori* where duplication is likely to occur. *A posteriori* analysis of the hazards and their HHM references may help analysts to design more streamlined approaches to the assessment that require less time without threatening the rigour and completeness of the analysis. This is an area for future research.

Fault-trees are a “top-down” hazard-analysis tool—the analyst specifies a failure event (the “top-event”) and then, using two logical functions OR and AND, identifies all of the events that cause the specified failure. The causative events are laid out in a tree with the branches connected by “gates” comprising either of these logical functions. A fault-tree is therefore a graphical model of all the parallel and sequential combinations of events that lead to the top event.

In this report, a fault tree analysis is applied to a well-documented hazard—gene flow between herbicide tolerant canola and a weedy relative. It lays out the logical chain of events that must occur for the gene conferring herbicide resistance to become stably integrated in a weed population. Fault tree analysis can augment a Hierarchical Holographic Model by defining the necessary event chain behind the potential hazards suggested by the model.

The principal advantage of the fault tree analysis is its structured and rigorous approach to identifying exposure pathways. The analysis here has helped to identify potential rate limiting steps and

speculates on possible hazard scenarios, such as the potential interaction between viral and bacterial pathways, that do not appear to have been addressed in the literature to date.

Another advantage of a fault tree is that it quickly identifies knowledge uncertainty in the system. This analysis highlights how uncertainty increases moving from sexual gene flow pathways to viral, bacterial and then fungal pathways. Particular areas of uncertainty include:

- sexual gene flow: the importance of mass effect in maintaining competitively neutral or inferior hybrids;
- viral: the relative frequency of RNA-RNA, DNA-DNA, RNA-DNA and DNA-RNA recombination;
- bacterial: the mechanisms of homologous recombination in bacteria, the rate at which endophytic bacteria come into contact with host DNA, the possibility of bacterial mediated transformation of plant cells by species other than *A. tumefaciens*, and the possibility of bacteriophage infection of plant cells; and,
- fungal: the potential role of fungal-mediated gene flow.

The fault tree provides an excellent platform to quantify the potential for gene flow under commercial conditions because it breaks down the event chain to individual elements that can be analysed experimentally, and therefore potentially quantified. Indeed quantitative estimates already exist for some of the basic events in the tree. Furthermore, many of the current appeals to the safety of GM products rely on the incredibly rare likelihood of undesired events such as bacterial gene flow. These appeals, however, are undermined by the large number of potential exposure pathways (demonstrated by the complexity of the fault tree) and extremely high exposure (billions of plants in commercial production). Quantitative fault tree analysis provides a means to explore the overall effect of these two opposing themes. Developing quantitative estimates of the frequency for each of the basic initiating events within the tree, and the undeveloped events, within a case-specific analysis, is therefore an important avenue of future research.

Fault tree analysis is not designed to identify all potential hazards. Unexpected interactions (outside the experience or imagination of the analyst) could result in additional unidentified hazards or hazard inducing mechanisms. By virtue of its holistic approach HHM analysis is more likely to identify, or at least suggest, unexpected interactions. Taken together these tools enable the analyst to postulate certain hazards and then investigate in more detail how they might occur. There is no guarantee, however, that these processes together will identify all hazards. There are no such guarantees in any form of hazard analysis or risk assessment (hence the need to continually compare the predictions of a risk assessment with reality). The logical and rigorous structure of HHM analysis and fault tree analysis, however, helps minimise the probability of missing important casual pathways and it performs much better in this regard than unstructured brainstorming techniques.

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Description of terms

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| Allelopathy | Inhibition of one species of plant by chemicals produced by another plant. |
| Bacteriophage | A virus that infects bacteria. The genetic material is always housed in the centre of the phage particle and may be either DNA or RNA—either double- or single-stranded. Some temperate phages (e.g. Lambda phage,) can insert their genome into their host's genome and be replicated within it, others (e.g. P1) replicate within a bacterial plasmid. This non-lethal infective relationship is termed lysogeny. Transduction occurs when a temperate phage from one lysogenic bacterial culture infects a second bacterial culture, taking with it a small amount of closely linked DNA that remains as a stable feature of the recipient cell. Antibiotic resistance can be transferred in this way. |
| Conjugation | Union in which two individuals or filaments fuse together to exchange or donate genetic material. In bacterial conjugation only a portion of the genetic material is transferred from the donor cell. To effect conjugation, the donor cell containing the plasmid must establish a physical contact (<i>pilus</i>) with the recipient cell. |
| Cytoplasm | All cell contents within the plasma membrane but excluding any nuclear region or nuclei. In eukaryotic cells it comprises cytoplasmic matrix (cytosol) in which organelles are suspended, plus crystalline or otherwise insoluble granules of various kinds. |
| DNA | Deoxyribonucleic acid. The nucleic acid forming the genetic material of all cells, some organelles, and many viruses. A major component of chromosomes and the sole component of plasmids. Nuclear DNA is normally found as duplex DNA and this form is the ultimate store of molecular information for all cells, but single stranded DNA viruses occur. |
| Duplex | Of a molecule composed of two chains or strands, usually held together by hydrogen bonds; e.g. double stranded (duplex) DNA. |
| Eco-type | Term generally applied in botanical contexts, referring to a species population exhibiting genetic adaptation to the local environment, whose phenotypic expression withstands translocation of the plant, or its offspring, to a new environment. |
| Endophyte | An organism that lives within a plant. |
| Hazard | The propensity for a substance or activity to cause harm. Hazard is a function of the intrinsic properties of the substance or activity, and the circumstances surrounding its use or implementation. |

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| Integron | Large complex transposons, discovered in the 1980's, that include genes of a site-specific recombination system capable of capturing and mobilising genes contained in mobile elements called gene cassettes. Integrons consist of two conserved regions (<i>intI</i> regions and <i>suII</i> region) flanking a variable region (gene cassette) that can contain multiple genes enabling survival under strong selective pressure (e.g. antibiotic resistance genes). An "empty" integron has been found on plasmid pVS1 in which the conserved regions are immediately adjacent. How genes are recruited into integrons is unknown. |
| Kairomone | A chemical or mixture of chemicals emitted by an organism (e.g. a plant) that induces a response in an individual of another species (e.g. an insect) that is beneficial to the receiving organism. An example of a kairomone is a plant scent that makes the plant more easily identifiable to an insect pest. |
| Lysis | Destruction of cells through damage to or rupture of the plasma membrane. In bacteria may be brought about by infection with bacteriophages. |
| Phytoalexins | Complex antimicrobial organic compounds (often phenol based) produced by plants in response to infection that inhibit further growth of the pathogen. As infected cells die they, and the immediately surrounding cells, produce phytoalexins. |
| Plasmid | A piece of symbiotic DNA, mostly in bacteria but also in yeast, not forming part of the normal chromosomal DNA of the cell and capable of replicating independently of it. Sometimes plasmids house genes encoding enzymes (e.g. conferring drug resistance) of critical value to the host cell or organism. In theory any gene can be transferred by plasmids. Some plasmids, if not all, can be transferred from one cell to another by conjugation, transduction or transformation. |
| Pleiotropy | The ability of allelic substitutions at a gene locus, or cell product, to affect or to be involved in the development of more than one aspect of phenotype. For any given allele some effects may be dominant, other recessive. A gene locus may have pleiotropic effects if it encodes an enzyme or regulatory protein whose product is involved in several biogeochemical pathways. |

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| Recombination | Any process other than point mutation by which an organism produces cells with gene combinations different from any it inherited. In bacterial transformation and transduction (examples of homologous recombination) homologous DNA duplexes first align, the donor duplex separates into its two strands (denaturation) and one strand invades the host duplex, aligning with the host strand having the greatest base-pairing conformity. It is then cut (nicked) by an enzyme, while the host strand without a partner is nicked at two places, donor DNA getting inserted by ligases in its place. |
| Risk | A measure of the likelihood and consequence of an undesired event. The consequences of an undesired event are usually expressed in terms of impact on human, economic or environmental values. |
| Reverse transcription | The copying of an RNA molecule back into its DNA complement. The enzymes that perform this function are called reverse transcriptases. Reverse transcription is used naturally by retroviruses to insert themselves into an organism's genome. |
| RNA | Ribonucleic acid. Nucleic acid class differing from DNA in being usually either single stranded or looped, in containing ribose not deoxyribose, and in that uracil replaces thymine. The three RNA types, messenger (mRNA), transfer (tRNA) and ribosomal (rRNA) are all involved in protein synthesis. In RNA viruses, RNA is sometimes double stranded, serving as genetic code. In some RNA viruses the RNA is transcribed into DNA and in others (retroviruses) it is reverse-transcribed into DNA. |
| Transcription | Production of RNA molecules by an RNA polymerase enzyme, using a DNA strand as a template. |
| Transduction | Process in which usually a bacteriophage picks up DNA from one bacterial cell and carries it to another, where the DNA fragment may become incorporated into the bacterial host's genome. |
| Transformation | Change in certain bacteria (occasionally other cells) which, when grown in the presence of killed cells, culture filtrates or extracts from related strains, take up foreign DNA and acquire characters encoded by it. |
| Transposable element | Genetic elements that are incapable of replication independently of the host cell genome, and inherited only when physically integrated into that genome or that of a plasmid or bacteriophage. |

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| Transposon | A genetic element, varying from 750 base pairs to 40 kilobase pairs in length, having at least the genes necessary for its own transposition (movement from a site in one genome to another site in the same or in a different genome). Simple transposons carry this information alone. Complex transposons carry additional genes such as those encoding antibiotic resistance proteins; genes encoding proteins involved with novel catabolic activities, such as the degradation of toluene and proteins that confer resistance to heavy metals such as mercury. |
| Translation | Ribosomal phase of protein synthesis. |
| Virion | A fully formed virus particle containing its nucleic acid or nucleoprotein core either within a naked coat of protein (<i>capsid</i>) or within a capsid enveloped by one or more host cell membranes acquired during exit from an infected cell. It is important to distinguish between the bio-system called a virus and the individual virus particles (Virions). Whereas a virion possesses inherent structural and biochemical properties that can be studied <i>in vitro</i> , a virus possesses additional functional activities and a variety of biotic interactions than can only be recognised inside its host as a result of the infection process. |
| Virus | One of a group of minute infectious agents (20 – 300 nm long or wide), unable to multiply except inside living cells of the host. Not normally regarded as “living” since none have any enzyme activity away from its host. They are, however, biological systems since each contains molecular information in the form of nucleic acid (but unlike cells never both DNA and RNA), transcribed and replicated within the host cell. |

1 Introduction

1.1 Hazard identification and risk assessment

Hazard identification is arguably the most important component of any risk assessment. Hazards that are not identified in the early stages of a risk assessment are not carried through the assessment, leading ultimately to underestimates of risk. Hazard identification techniques also play two other important roles within a risk assessment. First, they are an effective and appropriate way to involve stakeholders and other interested parties in the risk assessment—indeed the views and opinions of these groups often provide a deeper and richer appreciation of the problem in hand (Stern and Fineberg, 1996). Second, they can help in the design of statistically valid monitoring strategies by highlighting where and when to look for potential adverse events—it is much easier to monitor a situation when you know what to look out for.

Hazard identification for all new technologies, including genetically modified organisms (GMOs) initially must be inductive. As operating experience grows, and adverse events are recorded, the analysis can also adopt deductive approaches. The most common (deductive) approaches are unstructured brainstorming and checklists. Checklists may be lengthy and well developed (OGTR, 2001) or quite short (OECD, 1992), and are clearly the “status quo” in the majority of risk assessment frameworks for GMOs (Hayes, 2002). This is surprising for such a new technology and worrying—checklists do not ask “what can go wrong” with the system in question, and do not confirm that all components and processes of the system have been questioned. Indeed they tend to mislead the analyst into believing that all aspects of the system have been questioned without confirming this to be true.

These concerns are particularly pertinent in complex ecological systems. Ecological hazards may manifest in natural, arable and marginal environments and cut across all levels of biological organisation. Furthermore ecological hazards can arise through subtle, multi-stage events often involving complex interactions and feedback between physical, biological and chemical components and processes. In these circumstances, systematic inductive hazard identification techniques are much more likely to identify potential hazards than deductive techniques that rely solely on the operating experience of the analyst.

1.2 Background and objectives

This report is the third and final report of KRA Project 1: Robust methodologies for ecological risk assessment jointly funded by Environment Australia and CSIRO Biodiversity sector on the ecological implications of GMOs. The first report (Hayes, 2002) defines best practice relative to the scientific principles, hazard identification, risk calculation, social appraisal and monitoring components of an ideal ecological risk assessment. It then evaluates 8 international and national frameworks for GMO risk assessment against each of these components. The second report is a short guide to best practice risk assessment based on this review.

The objective of this report is to apply two inductive techniques—Hierarchical Holographic Modelling (HHM) and Fault Tree Analysis—to identify the potential ecological hazards associated with the unconfined release of Herbicide Tolerant (HT) canola, *Brassica napus*. The aim of the analysis is to demonstrate the potential value of inductive hazard identification techniques as applied to GMOs. It does not aim to identify hazards specific to a particular

product. The demonstration does not therefore apply to a particular type of HT canola nor identify particular herbicides or release conditions in a particular environment. It does, however, identify the general types of ecological hazards that may be associated with HT canola. In a real analysis of a GMO intended for release, the identification of hazards would be supported by product-specific and geographic-specific information that is not presented here.

This is the first time that inductive hazard identification techniques have been applied in earnest to a GMO. Recently, simple fictitious fault-trees have been mooted (National Research Council, 2002) but the advantages and pitfalls of inductive hazard identification techniques applied to complex ecological systems have yet to be fully explored. The example portrayed here is not polished—it represents the first tentative steps into a demanding but important component of best practice ecological risk assessment for GMOs.

2 Hierarchical Holographic Modelling

2.1 Introduction

Large complex systems typically consist of multiple, linked sub-systems, nested within a hierarchy, that interact in a non-linear fashion. One of the cardinal rules of risk analysis is to understand the relationships among the many components and processes of each sub-system and the way they interact with other sub-systems in the hierarchy. The high dimensionality (large number of variables) and complexity (non-linear interactions) of such large systems present daunting hurdles to the risk analyst (Haimes, 1981; 2001).

Hierarchical Holographic Modelling captures the complexity of a large system by identifying the components and processes of all sub-systems and suggests ways in which they might interact with each other based on established/supportive information. The technique decomposes the system by looking at it from many different perspectives including, for example, the functions, activities, geo-political boundaries, or structures of the system. HHM can be used in one of two ways—as a hazard identification tool or as a comprehensive analytical modelling tool. The analyst constructs an HHM by first identifying the most appropriate perspectives for the problem in hand. These are used to define the sub-systems which in turn are further decomposed into components, processes, functions or activities, which may or may not overlap with other sub-systems. The analyst can investigate the quantitative properties of the system if the functions, activities, components or processes of the system can be described by a series of overlapping models, subject to overall system constraints. The analyst(s) can also identify hazards by comparing potential interactions between the sub-systems in a qualitative fashion. This is best achieved by a team, whose members are expert in one or more of the chosen perspectives.

The most difficult part of constructing an HHM is selecting the most appropriate perspectives, system boundaries and level of aggregation or reductionism in the model. Here the focus of study is the analysis of the ecological implications of GM canola. It deliberately avoids socio-political or economic perspectives. We also avoid a specific geographic perspective (such as a particular agricultural region of Australia) because this is simply a demonstration of the techniques rather than an actual hazard analysis. The perspectives chosen for this analysis therefore focus on the anthropogenic, biological, chemical and physical components and processes of the environment that define the following sub-systems: biological hierarchy; biological components; biological processes; physical components; physical processes;

chemical processes and components; anthropogenic components; and anthropogenic processes. Together, these sub-systems provide a complete description of the canola environment. Each sub-system is further decomposed into its constituent parts, as deemed most appropriate by the assessment team. For example, the biological component sub-system lists all organisms that might occur in the GM canola environment by major biological group—e.g. microorganisms, plants, insects, other invertebrates, birds, mammals, reptiles/fish/amphibians and humans. Similarly, a list of biological processes can comprehensively include all known processes such as growth, decay, predation, excretion, etc. (Figure 1).

2.2 Hazard identification

The hazard identification proceeds by examining a system matrix (Figure 2). The study team was asked to suggest potential ecological hazards by considering a series of pair-wise interactions between the constituent parts of each sub-system i.e. within each cell of the matrix. The number of pair-wise interactions (pwi) is given by the number of cells (C) in the matrix and the number of interactions (I) within each cell

$$pwi = C \times I \quad . \quad [1]$$

The number of cells in the matrix is determined by the number of sub-systems or perspectives (p) in the HHM i.e. the number of columns in Figure 1

$$C = \left[\frac{p(p-1)}{2} \right] \quad , \quad [2]$$

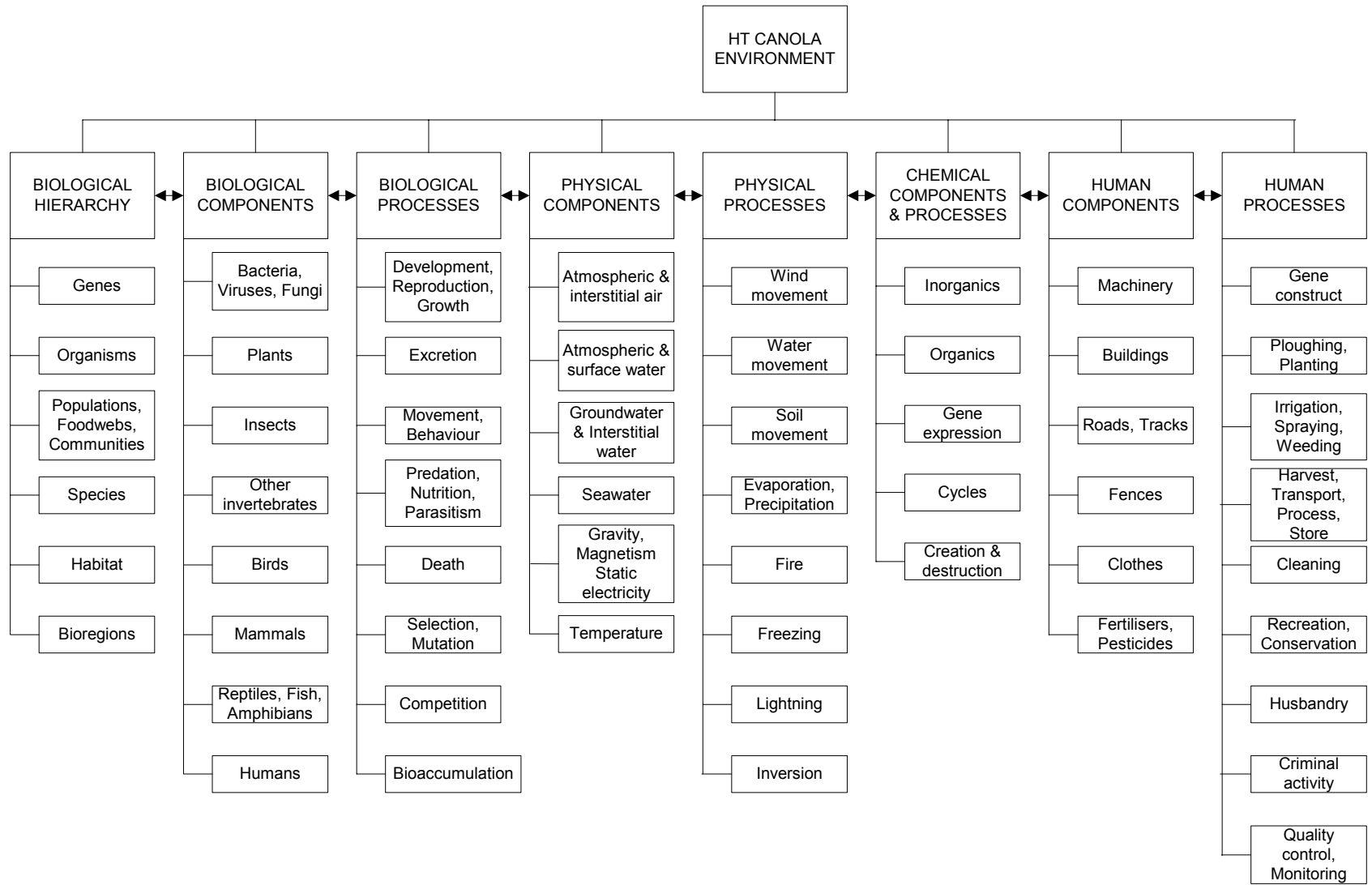
whilst the number of interactions depends on the constituent parts (s) of the two sub-systems i and j compared within each cell of the matrix

$$I = \sum_{i=1}^n \sum_{j=1}^m (s_i \cdot s_j) \quad . \quad [3]$$

In this analysis, the study team was asked to consider 1356 interactions within the HT canola environment for their potential to cause adverse ecological impacts. The team comprised an ecologist, a soil biologist, an agronomist, two entomologists, two weed scientists and a risk analyst. The team met on five occasions (one of which was via video conference), totalling a period of approximately five days, in order to complete the hazard matrix.

Initially, hazards were identified without reference to their likelihood or consequence. Subsequently, each member of the study team was asked to score their degree of concern (high, medium, low) with each hazard and their degree of confidence (from 0.1, 0.2, ... to 1) in the plausibility of the hazard. The scores were aggregated across individuals by taking the sum of concern (high = 3, medium = 2 and low = 1) and multiplying this with the average confidence. The final hazard score does not represent a formal assessment of risk and uncertainty—it is simply a way to prioritise each of the hazards for further analysis. In particular, some hazards that are probably quite unlikely might have received a disproportionately high hazard score because one team member, perhaps unfamiliar with that particular biological process or group of organisms, over-rated the likelihood or the severity of the consequences.

Figure 1 Hierarchical Holographic Model (HHM) used to represent the HT canola environment



During the hazard identification process, some of the pair-wise comparisons did not identify any hazards whilst others elicited several. Furthermore, the same hazard was often identified by more than one pair-wise comparison. Null sets (comparisons that do not identify hazards) are inevitable with such a rigorous and systematic approach—we did not discard any potential interactions prior to the assessment. Duplication (the same hazard identified by more than one comparison) often indicates multiple event chains leading to the same undesired outcome—in this manner the HHM model adds additional value to the hazard identification process by identifying the different circumstances by which an undesired event might be realised.

3 Potential HT canola hazards

The analysis identified a total of 153 potential hazards, 13 potential benefits and 30 event scenarios that may present a benefit or a hazard depending on the specific environmental and agricultural conditions. These events were grouped into 13 broad hazard categories including three that are not (strictly speaking) ecological: these categories being social, criminal and product segregation. Table 1 lists all the potential events identified in the analysis, ranked by hazard score, and the number of times they were identified in the matrix. Approximately 43% of events were identified only once. A further 42% of events were identified between two and four times, whilst two events (1%) were identified over 15 times (Figure 3).

Appendix A lists all events by their identification number and original HHM reference. The HHM reference indicates precisely where in the analysis the event was identified. The reference is a unique three-part identifier. The first two numbers identify a particular cell within the hazard matrix—i.e. the sub-systems being compared. These numbers correspond to the table numbers in Appendix B. The third part identifies a particular pair-wise comparison and corresponds to the number within the appropriate table (see Appendix B). For example 1.1.13 refers to comparison number 13 in Table 1.1 between “Irrigate-Spray-Weed” and “Genes” in the person-made processes/biological hierarchy cell.

It is important to note that the analysis did not actively seek to identify potential benefits—the ratio of hazards to benefits in this study is not in any way indicative of the cost-benefit ratio that might result from the introduction of HT canola to any given area. In many instances the potential events identified in this study are not unique to HT canola and may occur (or even be more significant) in conventional canola crops. This possibility is not addressed here. However, it is interesting to note that the potential costs and benefits of biotechnology may only be assessed properly by applying a much greater level of scrutiny to conventional agricultural practice (National Research Council, 2002).

3.1 *HT volunteers on farm*

The incidence of HT volunteers (and HT resistant weeds) on farm has the highest average score of all the hazard categories. HT volunteers on farm may occur due to the significant seed loss during harvest and via a variety of natural process (e.g. ants and earthworms) that encourage seed burial and re-emergence. HT volunteers may contaminate subsequent crops and necessitate their destruction. Here we assume that some form of segregation may be necessary to prevent contamination of certified seed or non-HT canola. Farmers must also invest time and resources to monitor for volunteers and may use more toxic, destructive or labour-intensive weed control strategies to eliminate HT volunteers on farm following HT canola crops.

Figure 2 The HT canola hazard matrix. Numbers within each cell represent the number of pair wise comparisons between the constituent parts of the sub-systems defined by the HHM. The sum of the cells within the matrix is the total number of potential interactions (1356)

| | | | | | | | | |
|---------------------------------|-----------------|----------------------|-----------------------|----------------------|--------------------|---------------------|---------------------------------|--|
| | Human processes | | | | | | | |
| Biological hierarchy | 54 | Biological hierarchy | | | | | | |
| Biological components | 72 | 48 | Biological components | | | | | |
| Biological processes | 72 | 48 | 64 | Biological processes | | | | |
| Physical processes | 72 | 48 | 64 | 64 | Physical processes | | | |
| Physical components | 54 | 36 | 48 | 48 | 48 | Physical components | | |
| Chemical components & processes | 45 | 30 | 40 | 40 | 40 | 30 | Chemical components & processes | |
| Human components | 54 | 36 | 48 | 48 | 48 | 36 | 30 | |

Figure 3 Duplication size-frequency distribution—the number times individual events were identified in the HHM analysis

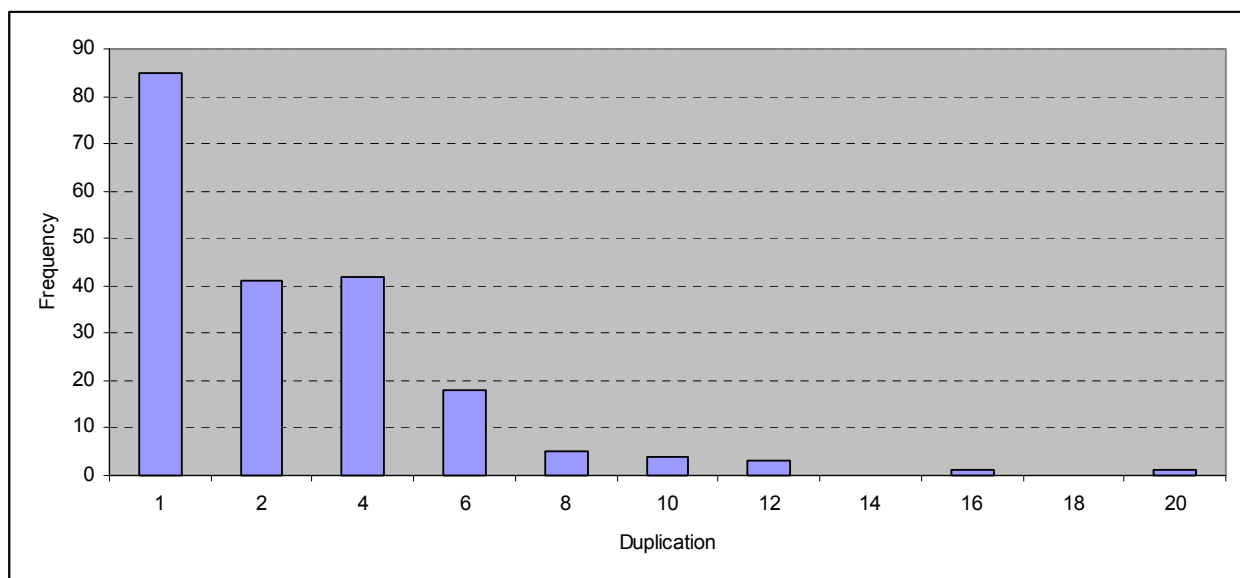


Table 1 Ranked potential hazards (and benefits) associated with HT canola, showing hazards identified by the HHM and those identified (•), implied (-) or not identified (x) in an equivalent checklist approach.

| Hazard category | ID | Potential hazard | +/- | Rank | Checklist |
|-------------------------|-----|---|-----|------|-----------|
| Farming practice | 99 | > acreage of HT canola > weed tolerance development | - | 14.8 | - |
| HT dispersal (off-farm) | 33 | Off-site transport of HT canola pollen by pollinating insects (incl. long distance noctunids) | - | 14.7 | • |
| HT volunteers (on-farm) | 49 | Subsequent crop seed contaminated by HT canola seed | - | 14.3 | • |
| HT dispersal (off-farm) | 28 | Off-site transport of HT canola seed via farm machinery (impossible to clean completely) | - | 13.8 | • |
| Segregation | 57 | HT canola requires segregation from harvest to process to consumer | - | 12.9 | x |
| HT dispersal (off-farm) | 22 | Seed dispersal (& accumulation) along transport routes | - | 12.2 | • |
| HT volunteers (on-farm) | 48 | Significant seed loss during harvest | - | 12.0 | • |
| Segregation | 62 | Different building (process, storage, drum master, waste disposal) required if segregation required | - | 12.0 | x |
| HT volunteers (on-farm) | 50 | Farmer needs alternative (> toxic) weed management strategy to eliminate HT volunteers | - | 11.3 | • |
| Unexpected expression | 7 | Gene expression in the roots modifies exudation | - | 11.2 | • |
| HT volunteers (on-farm) | 53 | Additional monitoring needed for volunteers & other HR weeds | - | 11.2 | • |
| Farming practice | 153 | Construct or associated farming practice facilitates herbicide resistance in weeds | - | 10.9 | - |
| Farming practice | 150 | Organic farming operations inhibited in HT canola regions | - | 10.7 | x |
| Gene flow | 157 | Gene stacking possibility following hybridisation with another GM plant | - | 10.7 | • |
| Farming practice | 151 | Avoidance of HT canola regions by other agricultural stakeholders (e.g. organic farmers, bee keepers). | +/- | 10.2 | x |
| HT dispersal (off-farm) | 29 | Off-site transport of HT canola seed via soil (e.g. equipment, tools, erosion, subsequent crops, soil samples, boots) | - | 10.2 | • |
| Farming practice | 73 | HT canola < crop rotation options | - | 10.1 | x |
| HT dispersal (off-farm) | 43 | GM pollen spreads into conservation areas | - | 10.1 | - |
| HT dispersal (off-farm) | 37 | HT canola in roadsides can only be removed with more toxic herbicides | - | 9.6 | • |
| HT dispersal (off-farm) | 23 | Disposal of cleaning waste, spoilt, low quality or excess HT seed may lead to dispersal | - | 9.5 | x |
| HT dispersal (off-farm) | 30 | Off-site transport of HT canola seed via sale or movement (legal or illegal) of contaminated stock (e.g. mud on hooves) | - | 9.4 | • |
| Segregation | 58 | HT canola requires segregation if unsuitable for animal feed | - | 9.2 | x |
| Unexpected selection | 9 | Plants have chemical defences & various levels of HT that will be selected by > herbicide application | - | 9.2 | x |
| HT dispersal (off-farm) | 32 | Off-site transport of HT canola pollen by wind (incl. fire generated thermals, inversions) | - | 9.0 | • |
| Farming practice | 125 | Altered spray schedules, insecticides & fungicides (because of > valued crop) has acute & chronic impacts on non-target insects | - | 8.8 | x |
| Gene flow | 155 | HT gene flow to weedy relatives (via insect pollinators) | - | 8.7 | • |
| Farming practice | 124 | Altered spray schedules of insecticides & fungicides (because of > valued crop) has acute & chronic impacts on microbes | - | 8.7 | x |
| Farming practice | 76 | > acreage of HT canola cultivation > impact on soil biological functions | - | 8.6 | x |
| HT dispersal (off-farm) | 24 | Off-site transport of HT canola seed via birds (plumage, gut, etc.) | - | 8.3 | - |
| Unexpected invasion | 19 | Aggregation of weedy hybrids or volunteers along fence lines = haven for HT canola & canola pathogens | - | 8.0 | • |
| HT dispersal (off-farm) | 38 | HT canola in national parks can only be removed with more toxic herbicides | - | 7.9 | • |
| Farming practice | 154 | Resistance management may require re-introduction of ploughing which has implications for soil degradation & soil erosion | - | 7.7 | x |
| Segregation | 61 | Different machinery required if segregation required | - | 7.7 | x |
| Farming practice | 77 | > acreage of HT canola cultivation > pressure on remnant vegetation & native plants | - | 7.7 | x |
| HT dispersal (off-farm) | 39 | Additional cultivation to remove HT canola from unwanted areas (e.g. buffer zone) degrades soil structure | - | 7.6 | x |

Table 1 Potential hazards (and benefits) associated with HT canola continued...

| Hazard category | ID | Potential hazard | +/- | Rank | Checklist |
|-------------------------|-----|---|-----|------|-----------|
| Farming practice | 118 | Minimum till associated with HT canola encourages bioaccumulation of pesticides (if they reach soil) | - | 7.5 | x |
| Farming practice | 84 | > acreage of HT canola > herbicide & insecticide application in new areas—effects on insect/invertebrate fauna | - | 7.4 | x |
| Gene flow | 159 | Gene flow augmented in disturbed areas due to > weeds, volunteers, unusual wind conditions, birds, mice movement | - | 7.3 | x |
| Biodiversity | 162 | Decreasing canola variety diversity in Australian acreage < the gene pool > susceptibility to pathogens (e.g. blackleg) | - | 7.3 | x |
| HT volunteers (on-farm) | 51 | Destruction of subsequent crop | - | 7.2 | x |
| Farming practice | 127 | Altered spray schedules—insecticides & herbicides & fungicides > surface water pollution | - | 7.0 | x |
| HT dispersal (off-farm) | 27 | Off-site transport of HT canola seed via clothing | - | 6.8 | • |
| Farming practice | 88 | > acreage of canola directly > out crossing rate | - | 6.7 | x |
| Segregation | 65 | Cannot sell soil used to grow HT canola if segregation required | - | 6.5 | x |
| Farming practice | 130 | > post emergent spraying (finer droplets) > herbicide drift (under temperature inversion conditions) > impact on beneficials | - | 6.5 | x |
| HT dispersal (off-farm) | 36 | Off-site transport of HT canola pollen and plant residues via contaminated machinery & air filters (incl. emergency/utility vehicles) | - | 6.4 | • |
| HT dispersal (off-farm) | 35 | Off-site transport of HT canola pollen via clothing | - | 6.4 | x |
| Farming practice | 106 | Different irrigation regime influences soil temperature | +/- | 6.4 | x |
| HT dispersal (off-farm) | 41 | Additional cultivation/spraying to remove HT canola from unwanted areas (e.g. fence lines) damages native plants & animals | - | 6.3 | x |
| Farming practice | 132 | > post emergent spraying > monitoring for (pesticide/herbicide) residues > exposure of personnel | - | 6.2 | x |
| Farming practice | 83 | > acreage of HT canola > herbicide & insecticide application in new areas—effects on flora (e.g. nectar availability) | - | 6.2 | x |
| Farming practice | 109 | > fertiliser if > yield potential > non-point pollution | - | 6.2 | x |
| HT volunteers (on-farm) | 52 | Seed accumulation at equipment cleaning or storage sites | - | 6.0 | • |
| Plant biochemistry | 199 | HT canola diverts metabolic energy into GM protein, away from other insect/invertebrate pest resistance processes > susceptibility | - | 6.0 | • |
| HT dispersal (off-farm) | 34 | Off-site transport of HT canola pollen & seed by surface water run-off (incl. irrigation) + aggregation | - | 5.9 | • |
| Segregation | 64 | > QC & monitoring required for inorganic/organic residues if segregation required | + | 5.8 | x |
| Farming practice | 100 | > acreage of HT canola alters carbon turn-over & affects soil creation | - | 5.7 | x |
| Farming practice | 85 | > acreage of HT canola > herbicide & insecticide application in new areas—effects on birds, mammals, reptiles, amphibians, fish | - | 5.6 | x |
| Farming practice | 120 | Minimum till associated with HT canola alters surface run-off & drainage | - | 5.6 | x |
| Farming practice | 95 | > acreage of HT canola into non-agricultural areas &/or > herbicide & pesticides < air quality | - | 5.5 | x |
| Unexpected selection | 11 | Weed eco-type strains selected along with herbicide tolerance—loss of plant gene pool | - | 5.5 | x |
| Farming practice | 149 | Reliance on one crop > susceptibility to pests & pathogens | - | 5.5 | x |
| HT dispersal (off-farm) | 31 | Off-site transport of HT canola seed via losses from seed meal processing plants | - | 5.5 | - |
| Farming practice | 112 | Minimum till associated with HT canola leads to > problems with seedling pests (red legged earth mite, wire worms) | - | 5.4 | x |
| Unexpected expression | 4 | Temperature stress affects gene expression (e.g. failure to express)? | - | 5.4 | x |
| Farming practice | 91 | > acreage of HT canola into non-agricultural areas < air quality (pollen, dust) | - | 5.3 | x |
| HT dispersal (off-farm) | 40 | Additional cultivation to remove HT canola from unwanted areas > seed burial | +/- | 5.1 | x |
| HT volunteers (on-farm) | 47 | Bioturbation (earthworms, ants, etc) > seed burial depth | +/- | 5.0 | x |
| HT dispersal (off-farm) | 44 | GM volunteers in recreation/conservation areas < beneficial (e.g. mycorrhiza) & introduce pathogenic soil microbes | - | 5.0 | x |
| Unexpected selection | 10 | Selection of herbicide-tolerant microbes (because of additional application) reduces efficacy of herbicide? | - | 5.0 | x |
| Farming practice | 128 | > post emergent spraying makes HT canola < competitive because of stress placed on the plant by the herbicide. | + | 4.8 | • |

Table 1 Potential hazards (and benefits) associated with HT canola continued...

| Hazard category | ID | Potential hazard | +/- | Rank | Checklist |
|-------------------------|-----|---|-----|------|-----------|
| Toxicity | 163 | HT canola (+ residues), lethal or sub-lethal effects on soil microbe diversity & function (decomposition, OM turn-over, N & S levels) | - | 4.8 | • |
| Plant biochemistry | 194 | Construct affects N levels in HT canola leaves > nutritional value > insects on HT canola > insecticide or < yield | - | 4.7 | x |
| Unexpected expression | 1 | Water stress affects gene expression (may > root exudation) | - | 4.7 | x |
| Unexpected expression | 3 | Eco-physiological stress < expression > conventional herbicide application | - | 4.7 | x |
| Farming practice | 90 | > acreage of HT canola &/or HT > spectrum (susceptibility) > international/state transport of pests (weevils, moths) & pathogens | - | 4.6 | x |
| Unexpected selection | 15 | Irrigation selects for weeds that flower or grow in appropriate period > potential for out crossing with these species & HT canola | - | 4.6 | x |
| Farming practice | 121 | Minimum till associated with HT canola < soil loss | + | 4.6 | x |
| Toxicity | 175 | Accumulation of pesticides in localised areas via predominant wind, water or soil movement | - | 4.5 | x |
| Biodiversity | 161 | Altered weed spectrum alters hosts for insects > brassicaceous weeds > resources to brassica pests < resources to beneficials | - | 4.5 | x |
| Farming practice | 96 | > acreage of HT canola into non-agricultural areas &/or > herbicide & pesticides < surface water quality issues | - | 4.5 | x |
| Unexpected invasion | 16 | HT canola is selected towards less competitive varieties in the laboratory | + | 4.4 | • |
| Farming practice | 107 | > fertiliser application to avoid stress induced failure to express &/or extend canola | - | 4.4 | x |
| Farming practice | 89 | > acreage of HT canola &/or irrigation indirectly > out crossing rate (may change the flowering pattern of canola) | - | 4.3 | x |
| Farming practice | 114 | Minimum till associated with HT canola—effect on dormancy & persistence of volunteer seed | +/- | 4.3 | • |
| Farming practice | 82 | > acreage of HT canola (& irrigation) will change insect fauna leading to possible adverse impacts (e.g. less ants, more pests) | - | 4.3 | x |
| Farming practice | 115 | Minimum till associated with HT canola selects for seedling vigour & thereby > invasive potential of HT canola | - | 4.1 | x |
| Plant biochemistry | 196 | Construct affects inorganic/organic chemical composition of the plant, stubble inputs into the soil & soil bio-geochemical cycles | +/- | 4.1 | • |
| Farming practice | 129 | > post emergent spraying damages HT canola > susceptibility to pests & pathogens | +/- | 4.1 | • |
| Toxicity | 171 | HT canola root exudates or biofumigants have lethal or sub-lethal effects on soil microbes, insects or invertebrates | - | 4.0 | • |
| Farming practice | 123 | Minimum till associated with HT canola leads to increased organic matter, changes timing of mineralisation rates | +/- | 4.0 | x |
| Farming practice | 79 | > acreage of HT canola > invasion along “road margins” associated with new roads or tracks | - | 3.9 | x |
| Unexpected expression | 5 | Nutrient stress affects gene expression | - | 3.9 | x |
| Farming practice | 116 | Minimum till associated with HT canola selects for persistence & thereby > invasion potential of HT canola seed | - | 3.9 | x |
| Farming practice | 104 | > irrigation to avoid stress induced failure to express | - | 3.9 | x |
| Farming practice | 97 | > acreage of HT canola into non-agricultural areas &/or > herbicide & pesticides < ground water quality | - | 3.9 | x |
| HT dispersal (off-farm) | 46 | Monitoring for emergence along transport routes > exposure of personnel to traffic accidents | - | 3.8 | x |
| Segregation | 59 | > cleaning if segregation required >run-off & non-point pollution (surface, ground, coastal) | - | 3.8 | x |
| Unexpected selection | 12 | Pesticide & fertiliser drift & run-off causes selection in adjacent environments | - | 3.7 | x |
| Farming practice | 93 | > acreage of HT canola into non-agricultural areas > water demand > evaporation, surface water run-off & non-point pollution | - | 3.6 | x |
| Farming practice | 131 | > post emergent spraying makes HT canola less desirable as feed due to herbicide residues | - | 3.6 | x |
| Farming practice | 102 | > irrigation > water movement—recharge, drainage—non-point pollution hazards & rising water tables (salinity issue) | - | 3.6 | x |
| Farming practice | 113 | Minimum till associated with HT canola leaves seed on the ground for longer—attracts > insects & birds including nuisance birds | - | 3.6 | x |
| Unexpected expression | 2 | Irrigation reduces water stress causing > gene expression | + | 3.5 | x |
| Farming practice | 119 | Minimum till associated with HT canola < levels of soil-borne pathogens | + | 3.5 | x |
| Farming practice | 110 | Minimum till associated with HT canola favours perennial weeds | - | 3.5 | x |
| Social | 56 | Erosion of clean, green, organic image in areas where HT canola is grown | na | 3.5 | x |

Table 1 Potential hazards (and benefits) associated with HT canola continued...

| Hazard category | ID | Potential hazard | +/- | Rank | Checklist |
|-------------------------|-----|--|-----|------|-----------|
| HT dispersal (off-farm) | 25 | Off-site transport of HT canola seed via mammals (fur, gut, etc.) | - | 3.4 | • |
| Farming practice | 98 | > acreage of HT canola into non-agricultural areas &/or > herbicide & irrigation (attracts fauna) > exposure of fauna to chemicals | - | 3.4 | x |
| Farming practice | 144 | Tram line effects associated with spray intensive HT canola changes surface water run-off > soil erosion | - | 3.4 | x |
| Plant physiology | 189 | Construct > pollen allergenicity | - | 3.4 | • |
| Farming practice | 105 | > irrigation > direct impact (pump damage) on fish | - | 3.3 | x |
| Farming practice | 143 | > volume of herbicide > root exudation | - | 3.3 | x |
| Farming practice | 126 | Altered spray schedules, insecticides & fungicides (because > valued crop) > toxic to non-target vertebrates | - | 3.3 | x |
| Farming practice | 138 | > volumes of herbicide & different types of herbicide—effects on microbial diversity & function (exudation ∞ to volume) | - | 3.2 | x |
| Unexpected invasion | 20 | Aggregation of HT canola seed by hoarding insects | - | 3.2 | x |
| Farming practice | 133 | < pasture associated with HT canola < fire hazard | + | 3.2 | x |
| Unexpected expression | 6 | Insect pest density affects gene expression (insects attracted by kairomones to damaged plants) | - | 3.1 | x |
| Farming practice | 117 | Minimum till associated with HT canola prolongs root exudates & therefore > their affect above & beyond conventional canola | - | 3.1 | x |
| Toxicity | 164 | HT canola (+ residues) has lethal or sub-lethal effects on soil microbe diversity & hence soil structure | - | 3.0 | • |
| Farming practice | 78 | > acreage of HT canola > migration barriers and habitat fragmentation associated with new roads or tracks | - | 3.0 | x |
| Farming practice | 135 | < pasture associated with HT canola < surface water pollution by animal waste | + | 3.0 | x |
| Farming practice | 134 | < pasture associated with HT canola < methane | + | 3.0 | x |
| Farming practice | 101 | > acreage of HT canola into new temperature zones changes expected pesticide degradation rates and species sensitivity | - | 3.0 | x |
| Farming practice | 94 | > acreage of HT canola into non-agricultural areas > water demand < groundwater infiltration & recharge | - | 2.9 | x |
| Unexpected expression | 8 | Pleiotropic uncertainty | + | 2.9 | • |
| Plant biochemistry | 200 | Bioaccumulation of HT canola metabolites following > herbicide resistance | - | 2.8 | x |
| Plant biochemistry | 198 | Construct affects symbiotic relationships with fungi, parasitic plants | +/- | 2.8 | - |
| Farming practice | 137 | > volume of herbicide & different types of herbicide—effects on soil bio-geochemical cycles? | +/- | 2.8 | x |
| Segregation | 60 | Different cleaning compounds if segregation required (effects of this?) | +/- | 2.8 | x |
| Farming practice | 81 | > acreage of HT canola cultivation > attraction of vertebrate pests to storage areas | - | 2.8 | x |
| Plant biochemistry | 195 | Construct affects N/carbohydrate ratio—food chain effects for insects that feed on plant (e.g. aphids & their parasites) | +/- | 2.7 | x |
| Farming practice | 145 | Non-point pollution on coastal resources (e.g. coral) | - | 2.7 | x |
| Unexpected selection | 14 | Brassica growth favoured in high salt environments (sea spray, saline soils) | - | 2.7 | x |
| Farming practice | 108 | > fertiliser application needed following HT canola because of adverse affects on VAM fungi | - | 2.6 | x |
| Farming practice | 86 | > acreage of HT canola leads to insufficient time to clean insect pests from silos & harvesting equipment | - | 2.6 | x |
| Plant biochemistry | 193 | Construct affects N levels in HT canola leaves—effects survival of immature insects that have co-evolved with low N | +/- | 2.6 | x |
| Farming practice | 92 | > acreage of HT canola into non-agricultural areas < surface water quality (dust) | - | 2.5 | x |
| Farming practice | 103 | > irrigation > evaporative loss (contingent on increased take up over conventional strains) | - | 2.5 | x |
| Farming practice | 146 | Effects of > traffic volumes (e.g. herbicide tankers) in agricultural areas (accidents, road-kill, nuisance) | - | 2.5 | x |
| Toxicity | 173 | HT canola has allelopathic effects on other plants | - | 2.4 | x |
| Social | 54 | Public do not accept GM technology | na | 2.4 | x |
| HT dispersal (off-farm) | 45 | Attempts to control HT volunteers leads to > traffic & > exposure to other harmful microbes | - | 2.4 | x |

Table 1 Potential hazards (and benefits) associated with HT canola continued...

| Hazard category | ID | Potential hazard | +/- | Rank | Checklist |
|-------------------------|-----|--|-----|------|-----------|
| Farming practice | 80 | > acreage of HT canola cultivation > exposure of new areas to plant pathogens & weeds via soil contaminated machinery | - | 2.3 | x |
| Farming practice | 140 | > volumes of herbicide & different types of herbicide—non-target impacts on fauna | +/- | 2.3 | x |
| Gene flow | 156 | Horizontal gene flow of HT gene to weedy relatives (via bacteria, viruses or fungi) | - | 2.2 | • |
| Toxicity | 169 | HT canola (+ residues) affects parasitoid micro-environment | - | 2.1 | x |
| HT dispersal (off-farm) | 26 | Off-site transport of HT canola seed via reptiles, fish or amphibians | - | 2.1 | - |
| Farming practice | 122 | Minimum till associated with HT canola < evaporation = cooler soils with implications for microbes & chemical reaction rates | +/- | 2.0 | x |
| Criminal | 69 | Black market in HT canola seed | - | 2.0 | x |
| Toxicity | 165 | HT canola (+ residues) has lethal or sub-lethal effects on insects & other invertebrates (incl. predatory soil fauna) | - | 2.0 | • |
| Plant physiology | 185 | Construct affects root phenology & biopore development | +/- | 1.9 | x |
| Farming practice | 87 | > acreage of HT canola > precision farming techniques (< pollution and drift) | + | 1.9 | x |
| Toxicity | 176 | Toxicity of secondary products following GM protein breakdown? | - | 1.9 | • |
| Farming practice | 139 | > volumes of herbicide & different types of herbicide—selection for certain fungi > spore dispersal of certain spp. | - | 1.8 | x |
| Unexpected invasion | 18 | Gene product confers competitive advantage to seed | - | 1.8 | • |
| Toxicity | 174 | HT canola (+ residues) less palatable or acceptable to insects & other invertebrates—behavioural changes? | +/- | 1.8 | x |
| Unexpected invasion | 17 | Different flowering patterns of HT canola in recreation/conservation areas changes seed production, out crossing & spread | - | 1.8 | • |
| Farming practice | 147 | Corporation farming dependant on limited seed source | - | 1.8 | x |
| Unexpected invasion | 21 | Canola transported by water competes with natives and other desirable pasture plants in riparian areas | - | 1.8 | x |
| Criminal | 70 | Vandalism (machinery, buildings, crop) at GM crop sites | - | 1.7 | x |
| Segregation | 67 | Burning of stubble if segregation required—reduces soil pathogens | + | 1.6 | x |
| Toxicity | 166 | HT canola (+ residues) has lethal or sub-lethal effects on birds feeding on seed or plant | - | 1.5 | • |
| Segregation | 63 | Dedicated GM transport routes (e.g. avoid national parks) & > traffic volumes | - | 1.5 | x |
| HT dispersal (off-farm) | 42 | Walkers & flower collectors inadvertently spread seed, plants or pollen | - | 1.4 | x |
| Plant physiology | 179 | Construct affects seed viability, dormancy or longevity? | - | 1.4 | • |
| Toxicity | 167 | HT canola (+ residues) has lethal or sub-lethal effects on reptiles/amphibians feeding on seed or plant | - | 1.3 | - |
| Farming practice | 136 | < pasture associated with HT canola > feed lot production > localised concentration of animal waste | - | 1.3 | x |
| Segregation | 66 | Burning of stubble if segregation required—soil biogeochemical & fauna/flora impacts | - | 1.3 | x |
| Toxicity | 170 | HT canola residues influence soil temperature via different decomposition rate of crop residues | +/- | 1.3 | x |
| Farming practice | 111 | Minimum till associated with HT canola leads to > harvesting ants | - | 1.2 | x |
| Farming practice | 74 | HT canola = closer crop rotations < pasture—soil chemistry (wet/dry cycles) & microbial implications | - | 1.2 | x |
| Plant physiology | 177 | Construct affects pollen production rate & timing > synchronicity of pollination with weedy relatives? | - | 1.2 | • |
| Gene flow | 160 | Surface & ground-water transport of bacteria | +/- | 1.2 | x |
| Farming practice | 142 | > volumes of herbicide & different types of herbicide > bioaccumulation in food chain of herbivores & predators | - | 1.2 | x |
| Toxicity | 172 | Gene product causes an allergic reaction in humans | - | 1.1 | • |
| Plant physiology | 186 | Metabolic cost of protein production < competitive behaviour in presence of tolerant weeds/pests > insecticide use | - | 1.1 | • |
| Unexpected selection | 13 | Sulphur precipitation favouring brassica growth in sulphur limited environments | - | 1.0 | x |
| Toxicity | 168 | HT canola (+ residues) has lethal or sub-lethal effects on mammals feeding on seed or plant | - | 1.0 | • |

Table 1 Potential hazards (and benefits) associated with HT canola continued...

| Hazard category | ID | Potential hazard | +/- | Rank | Checklist |
|--------------------|-----|--|-----|------|-----------|
| Farming practice | 141 | > volumes of herbicide & different types of herbicide > leaf litter & changes composition | +/- | 0.9 | x |
| Plant biochemistry | 197 | Construct affects biofumigant properties of canola | +/- | 0.8 | • |
| Farming practice | 152 | Bee-keeper aggregation in HT canola region > nuisance | - | 0.8 | • |
| Farming practice | 148 | One planting at one time—improved air quality? | + | 0.8 | x |
| Farming practice | 75 | HT canola = closer crop rotations = changes to soil moisture regime | +/- | 0.7 | x |
| Plant physiology | 190 | Construct alters pollen decomposition rate (transport & surface water quality issues) | +/- | 0.7 | • |
| Plant physiology | 182 | Construct affects seed hardness | +/- | 0.7 | • |
| Plant physiology | 181 | Construct affects seed shatter characteristics | +/- | 0.7 | • |
| Plant physiology | 180 | Construct extends seed maturity period (yield lag) | +/- | 0.7 | • |
| Gene flow | 158 | Horizontal gene transfer to gut bacteria via insect predation, HT feed | - | 0.6 | • |
| Criminal | 72 | Manufacturer sued for failed gene expression? | - | 0.6 | x |
| Criminal | 68 | Theft of germplasm | - | 0.6 | x |
| Plant physiology | 178 | Construct affects pollen production—wind & water dispersal implications | - | 0.6 | • |
| Criminal | 71 | Criminal spread of HT seed | - | 0.5 | x |
| Plant physiology | 187 | Construct affects frost tolerance of plant | +/- | 0.5 | • |
| Plant physiology | 184 | Construct affects rate of chromosome mutation | +/- | 0.4 | x |
| Plant physiology | 183 | Construct affects seed dispersal directly (physiological—lodgement) or indirectly (changes insect movement patterns) | +/- | 0.4 | - |
| Plant physiology | 192 | Construct alters salt tolerance | - | 0.4 | x |
| Plant physiology | 188 | Construct alters evapotranspiration rate of plant | +/- | 0.4 | x |
| Plant physiology | 191 | Construct alters pollen static properties | +/- | 0.3 | - |
| Social | 55 | Public de facto acceptance of GM technology | na | 0.1 | x |

3.2 *HT dispersal off-farm*

Dispersal of the HT gene beyond the farm (off-site) has the second highest average score of all the hazard categories identified here. There are a large number of ways in which the HT gene might disperse beyond the farm, either as HT canola pollen and seed, or as HT pollen and seed of a weedy relative following gene flow. In the first instance, the most significant losses of HT canola seed will probably occur along seed transport routes (Crawley and Brown, 1995). Significant losses may also occur around canola meal processing and/or storage plants. Other important anthropogenic vectors of canola seed and pollen include: contaminated machinery especially hired or contract machinery; vehicles; soil movements; stock movements; the clothing and activities of farm personnel; whole plant movements (root ball soil); walkers or flower collectors; theft, legal and illegal seed sales; and, waste disposal of spoilt, low quality or excess HT canola seed, and seed-cleaning residues. Contaminated machinery may carry seed, contaminated soil and even pollen, for example in air filters and inaccessible crevices and ledges.

Biological vectors of canola seed and pollen (in order of likely significance) are insects, birds, mammals, reptiles and amphibians, whilst physical vectors include wind and surface waters. Most pollen is probably dispersed by insects, which operate over short ranges (1 km or less), such as honey bees (Ramsay et al., 1999). However, some potential insect vectors such as noctuid moths are capable of transporting pollen hundreds of kilometres (Gregg, 1993). Birds and mammals may disperse seed via ingestion/excretion or simply through mud on feet or fouling of plumage and fur, or even twig collections. However, the extent to which canola seeds remain viable having passed through an animal's gut is largely unknown and undoubtedly differs among taxa. Anecdotal evidence from Canada suggests that canola seed remained viable and subsequently emerged after being fed to chickens and distributed as chicken manure spread on a field 12 months later (quoted in OGTR, 2002a). The viability of canola pollen after extended periods of time on insect vectors does not appear to have been investigated. Further dispersal of the HT gene, via the same biological vectors, is also possible if the gene is successfully integrated into the genome of a weedy relative via vertical or horizontal gene flow (see below).

The dispersal of the HT gene beyond the farm is viewed as a potential negative side effect of the unconfined release of HT canola. Canola is thought to reduce levels of beneficial soil microbes, such as mycorrhizae (Gavito and Miller, 1998). The extent to which this might impact on off-site plant and soil communities is not known. Conventional canola, however, is only a significant weed in disturbed habitats, and does not persist in undisturbed natural habitats (OGTR, 2002a), and HT canola is not thought to be significantly different in this respect (Salisbury, 2000). Attempts to remove HT volunteers in areas outside of the farm, particularly in recreation or conservation areas, may have physical, biological and chemical knock-on effects such as trampling and introduction of weeds and harmful pathogens by personnel and equipment, and non-target impacts of (the more toxic) herbicides required to eliminate HT varieties.

3.3 *Biodiversity*

Adverse changes to weed spectra were the most frequently identified hazard of HT canola, although this ranked relatively low. Farming practice associated with HT canola, particularly the expected increase in post-emergent herbicide applications of a chemical with a single mode of action and subsequent selection of herbicide resistant hybrids or volunteers, may

increase the resources available to pests of the Brassicaceae whilst at the same time reducing the resources available to beneficial insects and invertebrates. This may cause canola pest numbers to increase, damaging the crop and/or encouraging farmers to apply higher levels of pesticides and insecticides. Similar effects have recently been claimed for Bt cotton in China (Nanjing Institute of Environmental Sciences, 2002), however, there is no evidence for such changes in insect biodiversity with Bt cotton in Australia except for declines in the abundance of specific parasites of major cotton pests, an effect probably limited to within the crop (Fitt and Wilson, 2002).

The study team also noted the possibility of a decline in canola varieties in Australia if a single (or very few) HT canola variety is widely adopted. This may locally reduce the canola gene pool and thereby increase susceptibility of the crop as a whole to pathogens such as blackleg (*Leptosphaeria maculans*).

3.4 Segregation

As noted above, segregation may be required for reasons of seed purity or may be demanded to maintain organic farming certification and/or consumers' choice of GM free products. Maintaining separate production, transport and processing streams may lead to minor ecological impacts such as pollution of surface waters with cleaning products and disturbance/habitat loss through additional building requirements. Segregation may reduce the farmer's ability to move or sell soil or soil related products (e.g. whole plants), transfer machinery between farms or use certain transport routes, and may also require significant quality control resources. Segregation may also necessitate burning of HT canola stubble. This may reduce soil borne pathogens (a potential benefit) and may also have a minor effect on soil biochemistry and the composition of soil fauna and flora.

3.5 Unexpected expression

Unexpected expression of the HT gene—i.e. failure to express, excessive expression, expression in unexpected parts of the plant or unexpected interactions—may occur if the plant is stressed by water, temperature, nutrients or insects attracted to damaged plants by kairomones, or simply as a result of pleiotropic uncertainty. Water and temperature stress, for example, are thought to reduce Cry1Ac gene expression in Bt cotton (Daly and Fitt 2000). Farmers may attempt to compensate for failures of expression by additional irrigation, or application of extra fertiliser or pre-emergent herbicide. These changes in farming practice may have knock-on effects for the environment (see below). Expression of the HT gene in the root and root hairs of canola may modify root exudates, which may have important implications for soil fauna, flora and bio-geochemical cycles. This is known to occur in Bt corn (*Zea mays*) where high concentrations of Bt toxin have been recorded in the rhizosphere soil (Saxena et al., 1999) but is yet to be investigated in HT canola.

3.6 Unexpected selection

Unexpected selection may occur via a series of physical, chemical or biological events. Pesticide and fertiliser drift and/or run-off may select for certain plants or weeds in environments adjacent to canola crops, GM or otherwise. This may be more significant for HT canola, however, if this process selects for weedy relatives capable of hybridising with the GM plant. Similarly irrigation may select for plants that flower and grow at similar times to canola and thereby increase the potential for or rate of out-crossing. Additional post-emergent herbicide sprays associated with HT canola crops may also select for plants that have

naturally high levels of herbicide tolerance. Again this may or may not increase out-crossing rates. The selection of eco-types that are naturally tolerant to herbicide may also result in losses to the plant gene pool, both locally and regionally. Herbicide resistance in plants and microbes is also likely to develop in response to additional herbicide application or a reduction in the types of herbicides used (see farming practice below). The significance of these hazards, however, needs to be weighed against the negative impacts of alternative strategies for managing weeds that are implemented for conventional HT canola. Furthermore, the magnitude and extent of impact of such hazards, if expressed, would be influenced greatly by the extent of use of HT canola. Canola is also thought to be slightly tolerant of frost (OGTR, 2002a) and salt (Steppuhn et al., 2001), although this is not enhanced in HT varieties (Redmann et al., 1994; OGTR, 2002b). Nonetheless canola volunteers may have a slight competitive advantage in saline soils, areas subject to frost or sea-spray.

3.7 *Unexpected invasion*

Unexpected invasion is most likely to occur via weedy hybrids or HT volunteers aggregated along fence-lines and/or roadsides. These areas are known to harbour weeds and volunteers and are a source of propagules for invasion into fields and adjacent habitats (Pessel et al., 2001). These propagules would have a competitive advantage in the presence of the subject herbicide, applied directly or via drift into these adjacent environments. Competitive advantage (or disadvantage), leading to unexpected invasion dynamics, may occur in a variety of other ways. Less competitive varieties of canola may be selected in the laboratory—the so-called laboratory weakling scenario. This would act to reduce invasiveness and therefore is seen as a potential benefit. Alternatively, the gene product may confer a competitive advantage to the HT canola seed, although this is considered to be relatively unlikely. Other possible, but relatively unlikely scenarios include: HT canola volunteers exhibiting different flowering patterns in natural areas compared with arable areas, thereby confounding predictions regarding spread or colonisation based on arable characteristics; and, canola seed or pollen is transported and aggregated beyond the planted field or farm, by hoarding insects or surface water run-off.

3.8 *Farming practice*

The largest source of potential ecological hazards arises through potential changes to farming practice that may follow widespread use of HT canola. Most of these hazards are associated in one way or another with either the way in which HT canola is grown—such as closer crop rotations, minimum tillage and post-emergent application of herbicides—or with the farmer's more attentive behaviour to a high value/high return crop—such as increasing acreage into marginal or remnant land areas and altered spray strategies for a range of insect pests and weeds.

Some of the most significant hazards identified in the analysis presume that high value canola will encourage increased HT canola acreages in existing agricultural land. Without careful management this would increase the rate of out-crossing to weedy relatives, encourage the development of herbicide resistance, and inhibit organic farming operations. Herbicide resistance may develop in on-site weeds via selection and in off-site weeds through transgene transfer. Increased herbicide resistance in weeds, on and off-site, is the most important hazard identified in this section. Techniques to manage herbicide resistance (such as ploughing) may also degrade soil and encourage soil erosion.

An increase in acreage of HT canola into non-agricultural areas may be accompanied by a series of ecological impacts similar to those associated with conventional agriculture, as well as a suite of impacts unique to GM crops. Agriculture (conventional or otherwise) often reduces native habitats and remnant vegetation, causes significant changes to soil biochemistry and reduces air quality. Fields, fences, roads and tracks may present barriers to migration, fragment the natural landscape, facilitate invasion by introducing weeds, pests and pathogens, and disturb natural communities that otherwise provide biotic resistance to the establishment of non-native species and native weeds (Mack, 1996). The extent to which these impacts are associated with HT canola will be difficult to determine because gene technology is only one of many factors (such as market price and demand for canola oil) that determine the acreage under canola.

New agricultural activity may place additional demands on surface and ground water for irrigation purposes or threaten these resources through the run-off of herbicides, pesticides and insecticides. Irrigation may attract fauna to bodies of open water in new agricultural areas and thereby increase their potential exposure to agricultural chemicals. However, HT canola may encourage precision farming techniques that will help to minimise chemical drift and non-point pollution. Increased acreages of HT canola into new temperate zones may lead to a number of subtle changes in the plant's physiology. Farmers may attempt to compensate for the influence of temperature stress on gene expression by additional irrigation or application of fertiliser. Additional fertiliser may also be applied because of the greater yield potential of canola or may be needed in subsequent crops because of the potentially adverse effect of canola on beneficial soil fungi.

The flowering pattern of HT canola may vary from one temperate zone to another, leading to an increase (or decrease) in out-crossing rate above and beyond that directly attributable to the increased acreage alone. Furthermore, the rate at which farm chemicals degrade and the sensitivity of fauna and flora to these chemicals may vary among temperate zones, either augmenting or reducing their potential impacts above or below that predicted from existing canola regions. The movement of canola crops into new regions also may introduce new pests and pathogens into these areas, or simply increase pest numbers. Again this effect may be greater than that directly attributable to the increased acreage if HT canola is more susceptible to these pests and pathogens because, for example, the plant may direct metabolic energy to gene expression and away from natural defence mechanisms. An increase in HT canola acreage might cause a decrease in pasture acreage, in mixed farming regions, and reduce options for crop rotation. Decreased pasture acreages may reduce non-point pollution of surface waters by animal waste (a potential benefit) but encourage more feed lot production and potential point source pollution. Reduced options for rotation of crops may have positive or negative impacts on soil moisture regimes, chemistry and microbial communities.

A variety of ecological effects may occur even if acreages of HT canola do not increase. Farming practice may change in at least two important ways if HT canola replaces conventional canola in existing agricultural regions: increases in minimum tillage and applications of post-emergent herbicide, and changes to spray schedules of insecticide and fungicide.

Minimum tillage tends to favour perennial weeds and may encourage seedling pests such as red-legged earth mites and wireworms. Seeds tend to remain on the surface of the soil for longer and may attract more birds (increasing the potential for seed dispersal and the exposure of birds to farm chemicals). Simultaneously, minimum tillage may select for seedling vigour

and persistence, thereby increasing invasion potential. Minimum tillage may also prolong the effect of root exudates on soil microbes, increasing the impact of exudates above that of conventional canola and beyond that potentially associated with the gene construct. Additionally minimum tillage may encourage bioaccumulation of pesticides in soil, reduce levels of soil erosion (a potential benefit), and retain soil moisture, causing cooler soils, increase organic matter and change mineralisation rates (the effects of which are unclear).

Post emergent spraying may make HT canola less competitive (relative to weedy hybrids), and more susceptible to pests and pathogens, because of the stress it places on the plant. For example, glyphosate is known to inhibit the production of phytoalexins that defend against plant pathogens in a number of crops (Termorshuizen and Lotz, 2002). Post emergent spraying also involves finer sprays than pre-emergent sprays, and may therefore increase levels of herbicide drift although this may be offset by precision farming practices (see above).

Herbicide residues may make HT canola less suitable as a feedstock. If the total volume of herbicide applied to HT canola increases, the herbicide may bioaccumulate more or faster in leaf litter and the food chains of herbivores and their predators (if the herbicides are at all bioaccumulative).

Post-emergent application of herbicide and/or increased application of herbicide lead to a larger biomass of slowly decaying root material than weed control strategies based on pre-emergent spraying. This may select for certain weeds and fungi, and may also have important implications for microbial diversity and function (such as organic matter turn-over and mineralisation). For example, a well-known side effect of glyphosate and glufosinate ammonium is the emergence of “herbicide synergists”—opportunistic root pathogens that accelerate the death of herbicide sensitive roots. These synergists may be more aggressive in HT canola fields because of the increased mass of decaying root material (Termorshuizen and Lotz, 2002). Soil microbial communities, including beneficial microflora, may also be influenced by additional root exudates that may be proportional to the rate at which herbicide is applied to the crop.

The farmer may change schedules of insecticide and fungicide spray because HT canola has a higher value than conventional canola. This may lead to more acute and chronic impacts on leaf litter communities, soil microbes, non-target insects, birds, mammals, reptiles, amphibians etc. and increase levels of surface water pollution. Finally, there are a variety of potential socio-economic impacts associated with the large-scale adoption of HT canola such as dependence on limited seed suppliers, reliance on a single crop variety, and implications for beekeepers. These were not explored in detail in this analysis.

3.9 Gene flow

Gene flow is perhaps the most obvious and widely publicised hazard associated with HT canola. This is discussed in detail in numerous other publications (see for example Rieger et al., 1999; Wilkinson et al., 2000). This analysis notes that gene flow may occur via insects, wind, soil and gut bacteria, viruses or fungi, and by combinations of these vectors (Snyder et al., 1999). Gene flow may be augmented in disturbed areas (e.g. roadsides) because of increased numbers of weedy relatives and volunteers, and the increased activity of seed/pollen transport vectors (birds, mice, trucks etc.). Gene flow to other GM plants may lead to “gene stacking” (Orson, 2002). Gene flow is discussed in more detail in section 4 of this report.

3.10 Toxicity

HT canola or the residues associated with it may have lethal or sub-lethal impacts across all levels of biological hierarchy. Many of these impacts will be similar to those of residues from conventional canola, but some might be different. Root exudates, biofumigants or the herbicide residues associated with the plant may impact on soil microbe diversity and function. This may have adverse implications for decomposition, organic matter turn-over, biogeochemical cycles (nitrogen, phosphorus, sulphur, etc.) and soil structure. Root exudates, biofumigants and/or the genetic construct may also influence soil insect and other organisms. Acute and chronic toxicity of these residues towards canola pests may be beneficial; however, similar impacts on natural predators and parasites may be harmful, disturbing the natural checks and balances of insect and invertebrate communities (Groot and Dicke, 2002). This may encourage higher numbers of pests than would otherwise occur. Potential allelopathy, intoxication of birds, reptiles, mammals, etc, and allergenicity in humans are also identified as potential hazards. HT canola residues may decompose at a different rate than those associated with conventional canola and therefore may influence soil temperature, although the potential effects of this are unclear.

3.11 Plant physiology

The HT construct may have a number of impacts on the plant's physiology including rate and timing of pollen production, rate of pollen decomposition, static and/or allergic properties, seed viability, dormancy, longevity, maturity period (yield lag) and chromosome mutation rate. It may also alter pod shattering characteristics, seed hardness and dispersal characteristics. All of these effects may or may not increase the plant's invasive potential in agricultural and natural environments. Evidence to date, however, suggests that HT canola is not significantly different from conventional canola in these traits (Salisbury, 2000). The construct may also alter root phenology and biopore development, frost and salt tolerance, and the plant's rate of evapotranspiration. Again the likelihood of these changes was considered low, and their effects unknown. The metabolic cost of protein production may make HT canola less competitive in the presence of tolerant weeds or pests. This may reduce its invasion potential but also encourage farmers to apply different types and/or quantities of herbicides and pesticides.

3.12 Plant biochemistry

The HT construct may lead to a series of subtle, but potentially significant, changes in the plant's biochemistry. These changes may be caused by the HT gene or its associated genes, for example the non-specific phosphorylation activity of the marker genes (Harding and Harris, 1997). One of the more significant concerns in this context is the possibility that HT canola diverts metabolic energy into the GM protein and away from other natural pest resistance processes, thereby increasing its susceptibility to pests. Again, however, the agronomic characteristics and pest potential of HT canola are thought to lie within the normal range of values displayed by conventional canola (Salisbury, 2000).

The HT gene also may cause an increase in nitrogen content of the leaves. This may influence the survival of immature insects that have co-evolved with lower levels of nitrogen in conventional canola or increase the nutritional value of the leaves to adult insect pests thereby encouraging pest populations that feed on the plant. Similarly, the construct may have a small effect on the nitrogen/carbohydrate ratio in the plant's cells, with positive or negative implications for the food chains of insects that feed on canola (e.g. aphids and their parasites).

It may alter chemical composition of the plant residues and stubble inputs into the soil. Finally, the construct may affect the biofumigant properties of the plant and/or its symbiotic relationship with beneficial fungi (e.g. vesicular-arbuscular mycorrhizae), parasites or insects, and the chemical composition of the plant residues and stubble inputs into the soil.

4 Fault tree analysis

4.1 Introduction

Fault-trees are a “top-down” hazard-analysis tool—the analyst specifies a failure event (the “top-event”) and then, using two logical functions OR and AND, identifies all of the events that cause the specified failure. The causative events are laid out in a tree with the branches connected by “gates” comprising either of these logical functions. A fault-tree is therefore a graphical model of all the parallel and sequential combinations of events that lead to the top event. The OR gate represents the union of events attached to the gate. An OR gate can have any number of inputs (branches) running into it. The event above the gate is realised if any one or more of the inputs occur. The AND gate represents the intersection of events attached to the gate. An AND gate can also have any number of inputs running into it, but the event above the gate is only realised if all the inputs occur.

Fault-tree analysis is versatile and systematic. It forces the analyst(s) to carefully examine the system, focus on the events that relate to the top event and describe (using logical functions) the event sequences that lead to this event. The logical structure of the event series is one of the principal advantages of fault tree analysis over conventional hazard identification techniques (such as brainstorming) because:

- it helps emphasise that ecological hazards are a function of the properties of the system in question and the circumstances (in this context) of the GMO release;
- it captures the necessary and sufficient conditions necessary for the top event; and
- it provides a qualitatively coherent model of the system that forms an excellent basis for a quantitative model.

4.2 Fault tree applied to gene flow

Figures 4 to 10 show a fault tree for gene flow between HT canola and a weedy relative. The symbols used in the fault tree are explained in Figure 11. Three events must occur for the HT gene to be expressed in a weedy relative: first-generation transgenic weeds (FGTWs) must be created—i.e. the gene must be transferred from the canola genome to the weed genome; the HT gene must be correctly expressed in the weed; and, the HT gene must be incorporated (introgression) into the chromosomes of the weed species—i.e. the FGTWs must be fertile and capable of sexually reproducing (backcrossing) with its weedy parents (Figure 4).

Creation of FGTWs is a necessary but not sufficient condition for the creation of herbicide tolerant weeds. The FGTWs must have the requisite biochemical machinery to transcribe and translate the gene into a functional protein for phenotypic change to occur. A variety of transcription and/or translation errors can prevent phenotypic change (Landis et al., 2000). These are dependant *inter alia* on the specific nucleotide sequence conferring herbicide tolerance, the weedy relative and gene flow mechanism. They are not therefore addressed in detail in this demonstration.

Ultimately the FGTWs may or may not persist in the environment. Persistence is not a requisite of gene flow—transgenic individuals can be maintained through mass effect (see below)—but it influences the likelihood of gene flow by increasing the amount of HT pollen and seed on and off the farm. The FGTWs must survive long enough to flower, however, in order to backcross with its weedy parents. Backcrossing requires the transfer of FGTW pollen to, and successful fertilisation of, the ovary of its weedy parents. Both plants must therefore flower at the same or nearly the same time, and the pollen must be transported between flowers. Pollen transport can occur via wind, water or animals such as pollinating insects. The frequency of successful backcross and F2 production varies depending on the weedy relatives. For example, field experiments with HT canola have demonstrated a low frequency of successful backcrossing between F1 hybrids and *Raphanus raphanistrum* and *Sinapis arvensis* (Table 2).

F1 fertility following sexual reproduction is determined by the compatibility of the genomes that are crossed and whether or not the canola parent is male or female. The Brassicaceae family consists of over 3000 species in 370 genera. Approximately 52 genera and 160 species are present in Australia, of which 8 are important weeds: *Brassica rapa*, *B. juncea*, *B. tournefortii*, *Diploaxis tenuifolia*, *Raphanus raphanistrum*, *Sinapis arvensis*, *Sisymbrium officinale* and *S. orientale*. Field studies have shown that *B. rapa* hybrids produce fertile pollen with either parent (but backcross capability has not been demonstrated yet) whilst *R. raphanistrum* hybrids only produce fertile pollen with male canola parents. *S. arvensis* appears to have limited hybridisation potential (Table 2). Field based information for the other weedy relative of *B. napus* is not currently available.

Creation of FGTWs may occur in one of two ways: sexually or non-sexually. Sexual hybridisation is a common and well-documented gene transfer mechanism. Sexual hybridisation occurs if HT pollen is transferred to, and successfully fertilises, the ovary of a weedy relative. The process can occur the other way round—i.e. pollen of a wild relative fertilises the ovary of an HT plant. Reciprocal crosses can be different in several ways, most notably the rate of successful hybridisation, fertility of the F1 hybrid and the fate of its seed. The rate of successful hybridisation and the fertility of the F1 depend on the degree of sexual compatibility between the donor and recipient plant. Like hybrid fertility, data on compatibility between members of the Brassicaceae family is available in the literature (Table 2), and it can be measured in field and laboratory studies (Scott and Wilkinson, 1998; Wilkinson et al., 2000). It is not therefore developed further in the fault tree.

The seed of a wild relative fertilised by HT pollen will fall wherever the female parent resides—predominately along roads, fence lines, etc. and natural habitats. The seed produced by HT canola fertilised by the pollen of a wild relative must either set before harvest or physically escape harvest (the plant is too low to be harvested, the seed is too small to be collected, the plant is not physically removed by the farmer prior to harvest, etc.) in order to reach the soil (Figure 5). These considerations do not apply to HT canola plants that are already located beyond the farm (by natural and anthropogenic seed dispersal) because seed set is unlikely to be hindered by anthropogenic harvesting activity. The incidence of HT canola plants off-site is therefore an important factor controlling the rate of gene flow.

Figure 4 Fault tree analysis for gene flow between HT canola and a weedy relative

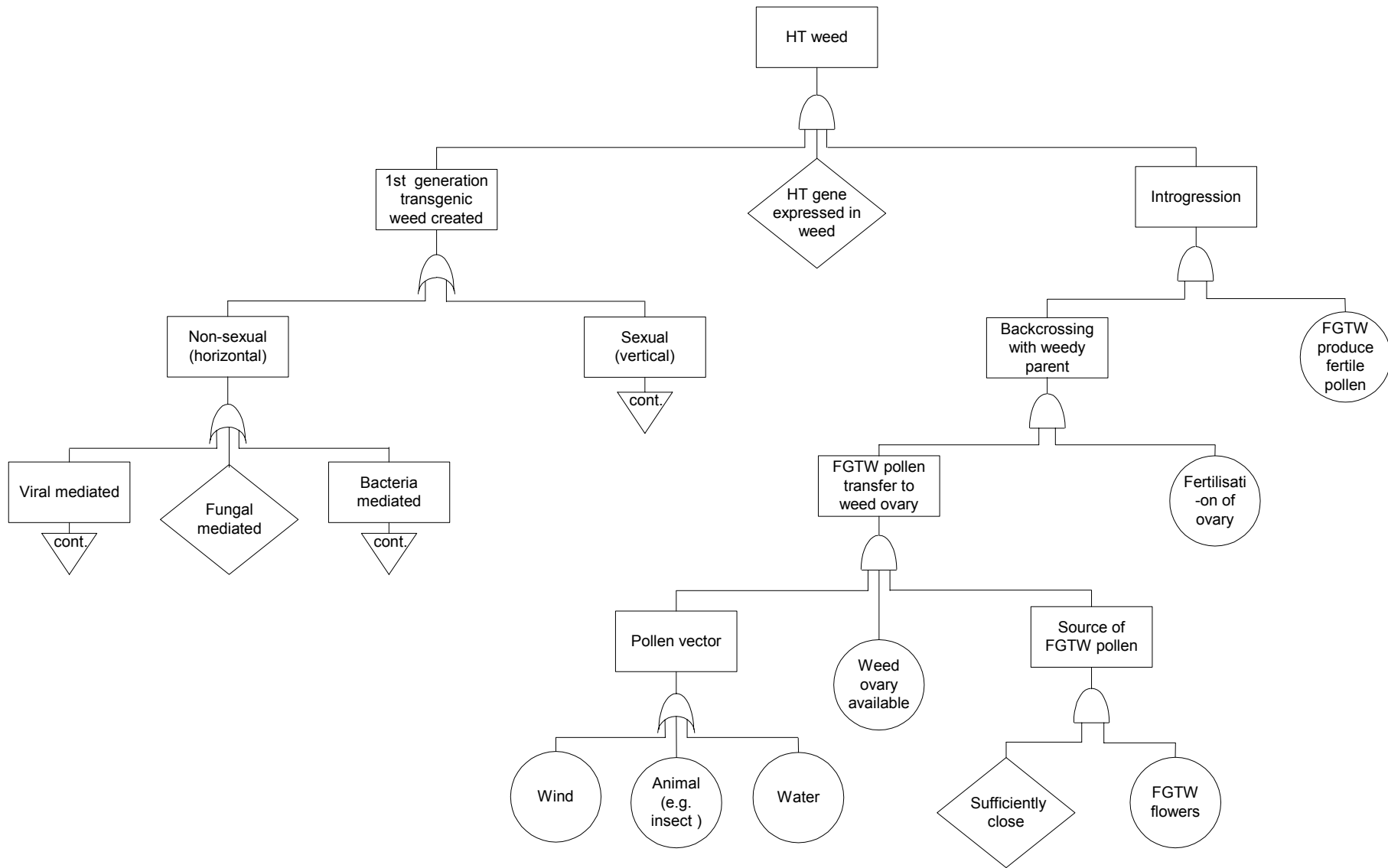


Figure 5 Fault tree analysis for gene flow continued—sexual creation of first generation transgenic weed

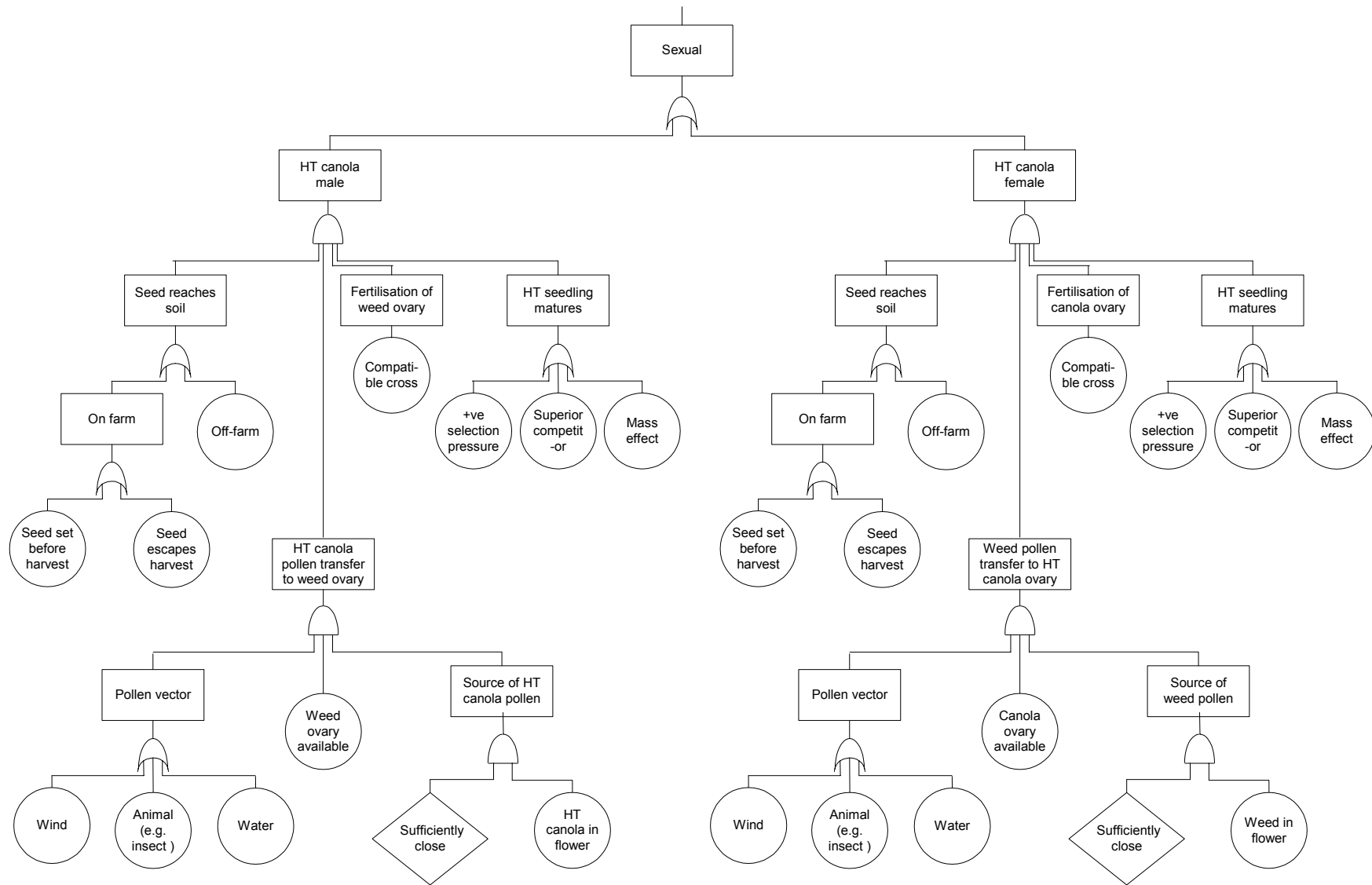


Figure 6 Fault tree analysis for gene flow continued—viral mediated horizontal gene transfer

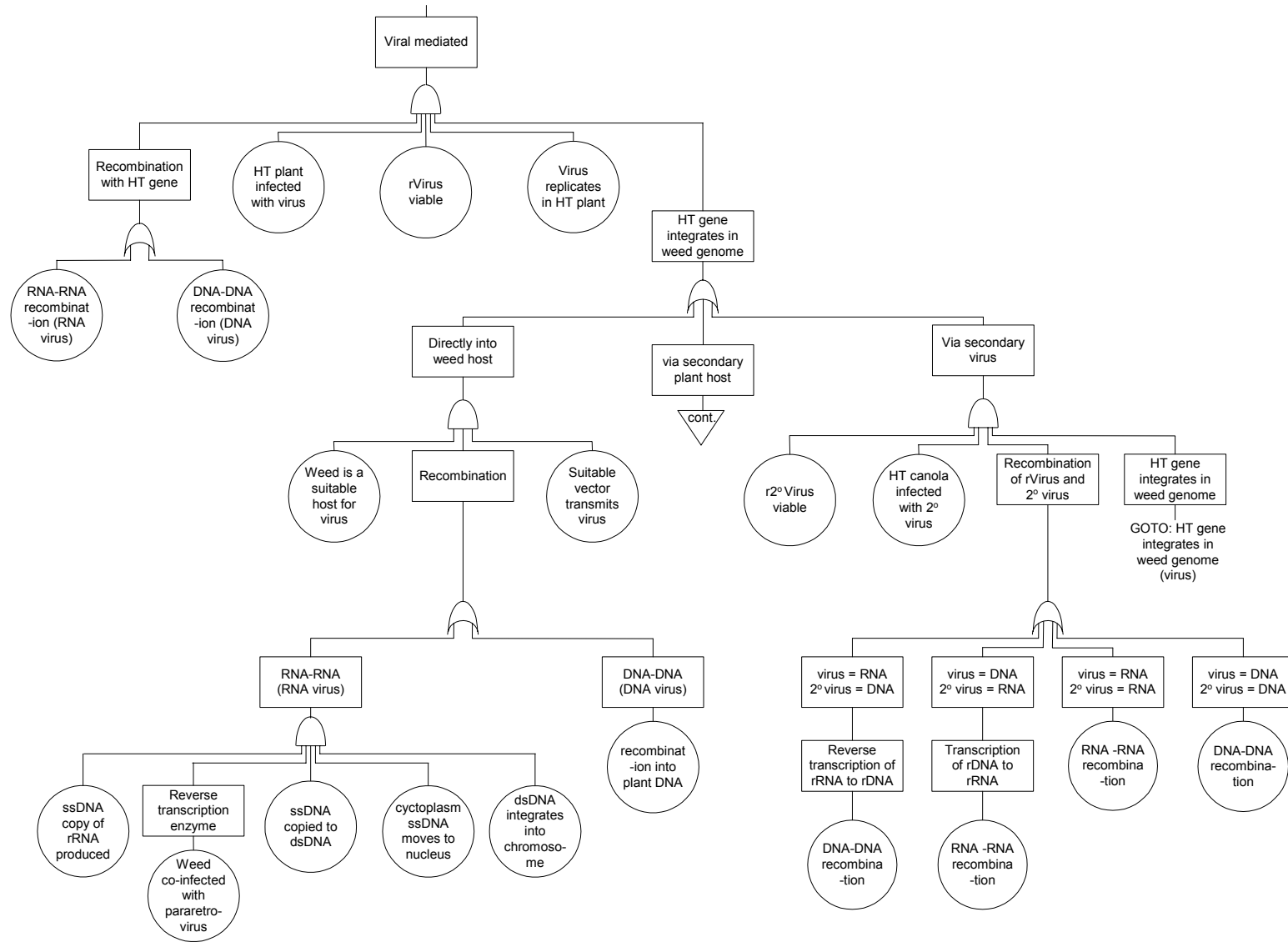


Figure 7 Fault tree analysis for gene flow continued—viral mediated horizontal gene transfer via secondary plant host

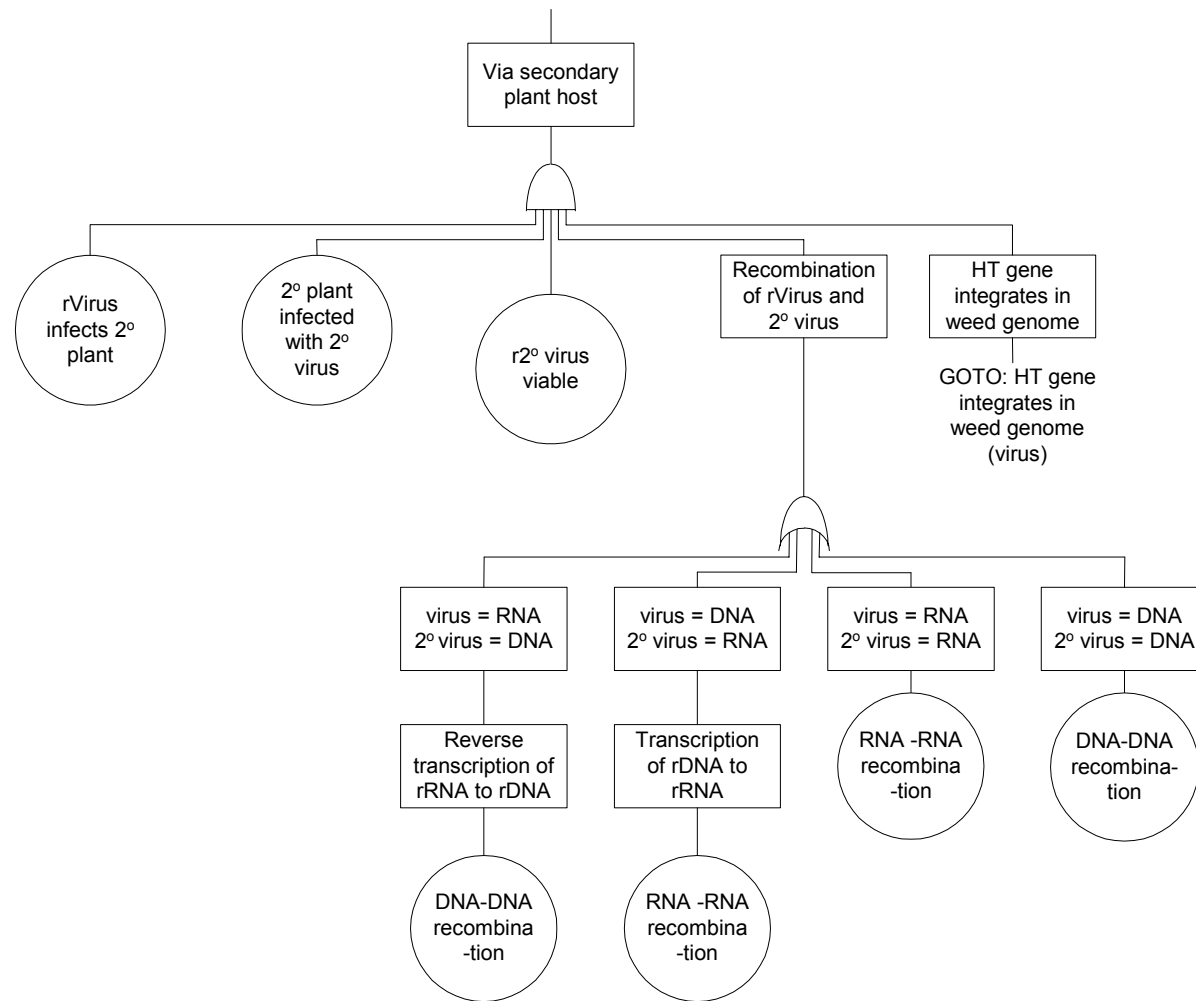


Figure 8 Fault tree analysis for gene flow continued—bacterial mediated horizontal gene transfer

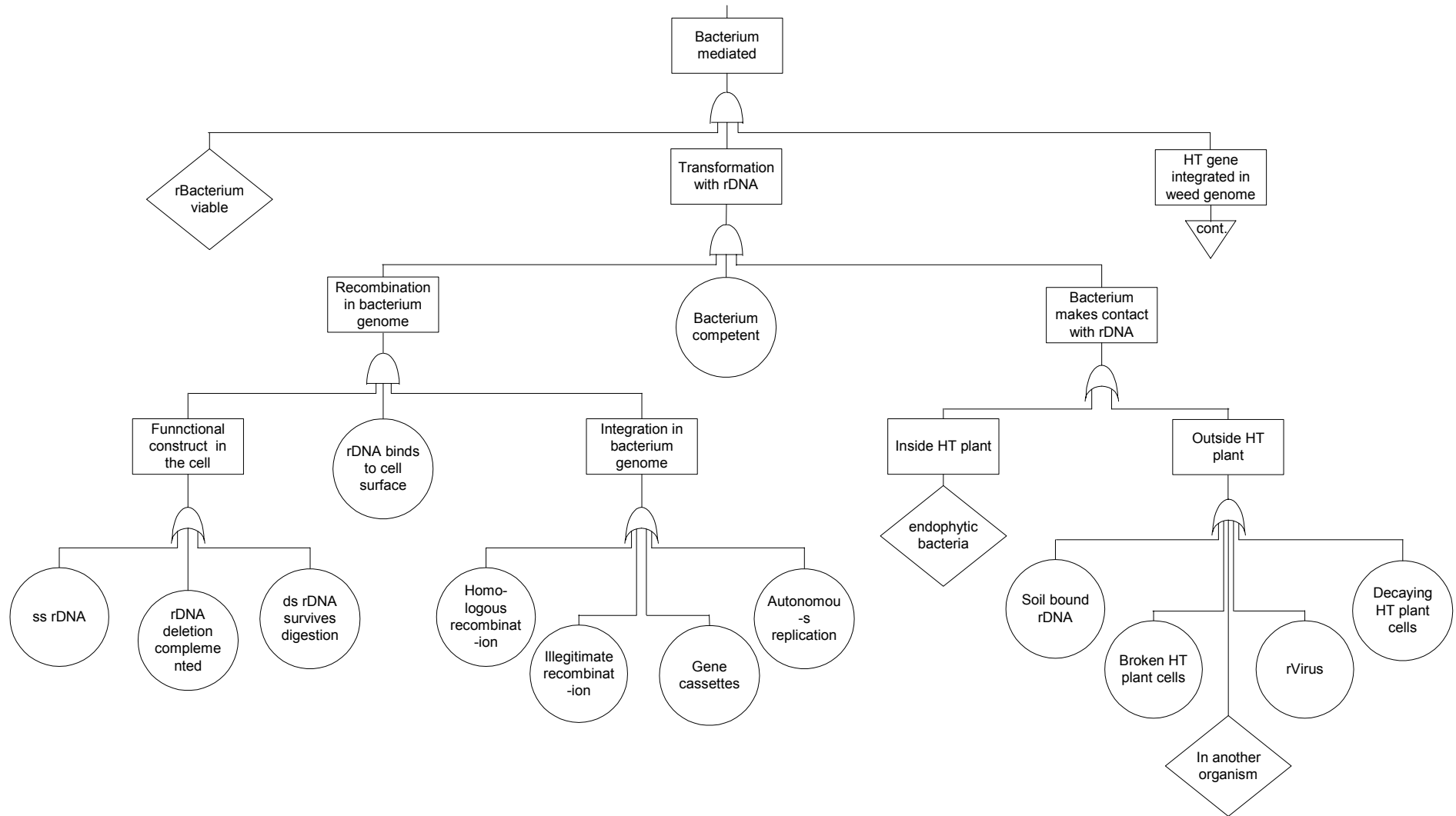


Figure 9 Fault tree analysis for gene flow continued—bacterial mediated gene integration into weed genome

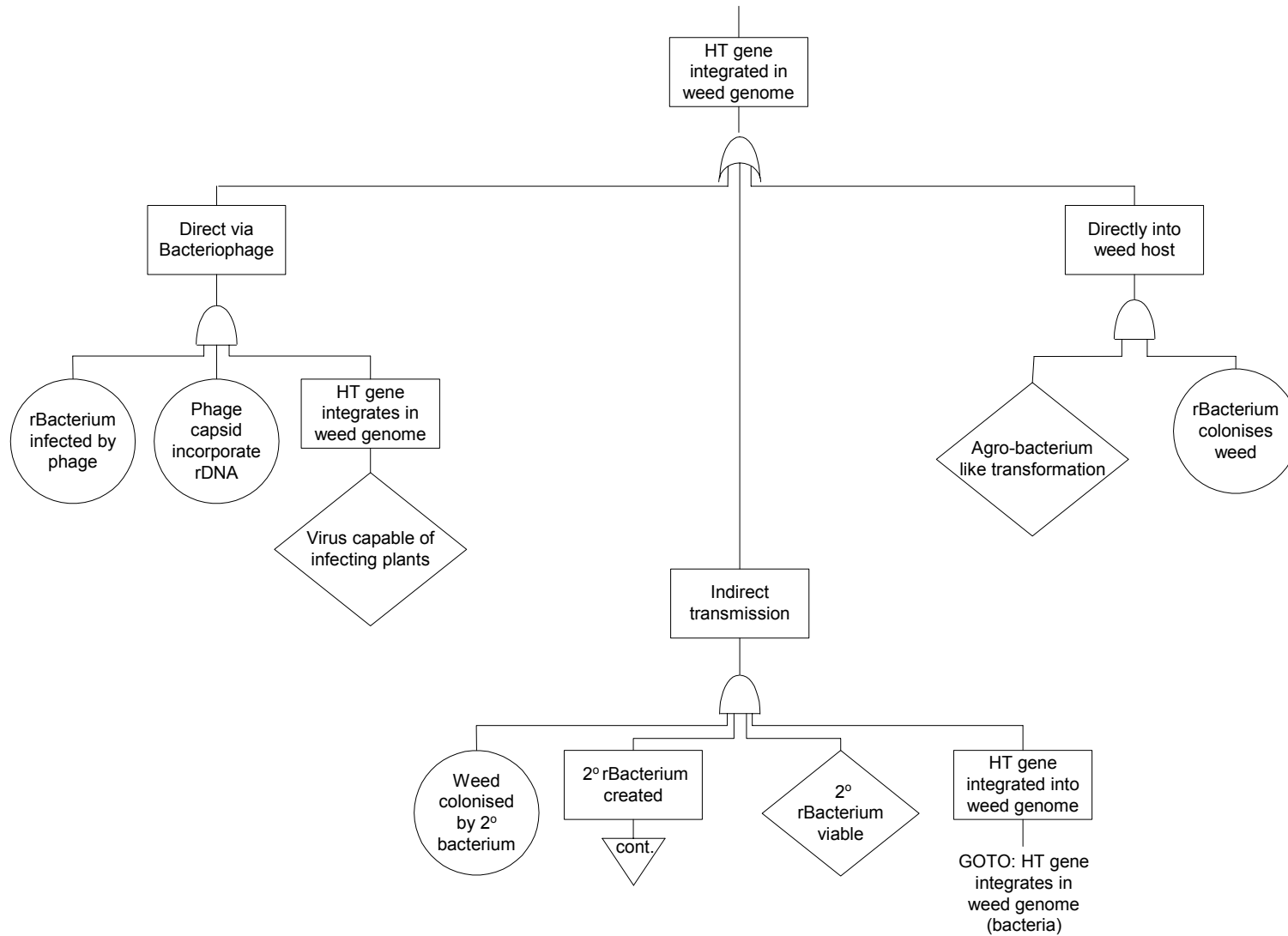


Figure 10 Fault tree analysis for gene flow continued—2° rBacterium created

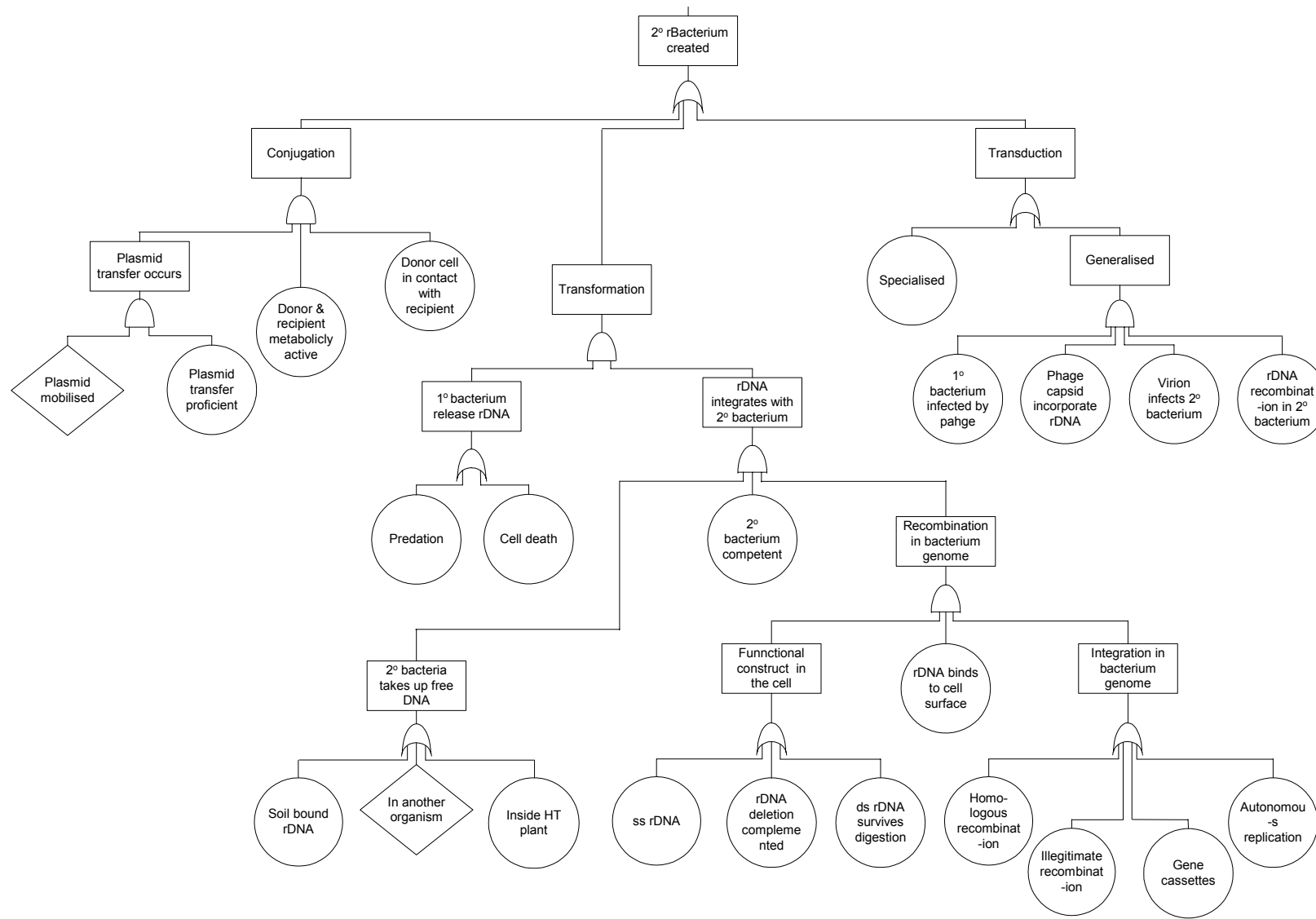


Figure 11 Event symbols used in the fault tree

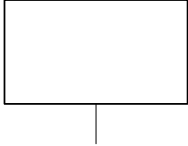
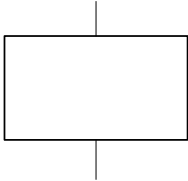
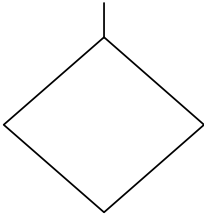
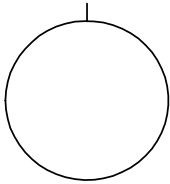
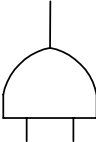
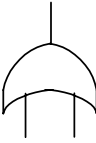
| SYMBOL | MEANING OF SYMBOL |
|---|---|
|  | <p>TOP EVENT the primary undesired event of interest</p> |
|  | <p>INTERMEDIATE EVENT caused by more primary level events described below</p> |
|  | <p>UNDEVELOPED EVENT not developed any further because either it is not useful or data are unavailable</p> |
|  | <p>BASIC INITIATING EVENT does not need to be developed further</p> |
|  | <p>AND GATE logic gate where output occurs only if all inputs occur</p> |
|  | <p>OR GATE logic gate where output occurs if any of the inputs occurs</p> |
| <p>GOTO:</p> | <p>Indicates a loop in the fault tree - text identifies where tree re-commences</p> |

Table 2 Field studies of hybridisation between *Brassica napus* and several weedy species

| Parent | Sample size | F1 hybrid plants | Pollen fertility | F2 or backcross |
|------------------------------|----------------------------|-------------------|-------------------------------|---------------------|
| <i>Brassica napus</i> L. | | | | |
| M/F | 1:1 mixture | 9 – 23% | 21-86% | - |
| F | 1 per 25m ² | 56% | 21-86% | - |
| F | 1 per 25m ² | 93% | 21-86% | - |
| | Unknown | 60% | 21-86% | - |
| <i>Raphanus raphanistrum</i> | | | | |
| M | 1:1 mixture | 1.4 – 88.6 | Sterile – 30% | -/Yes |
| M | 1:1 mixture | 9.63 ^A | 0 – 30% | -/0.05 ^A |
| M | 1:1 mixture | 23.7 ^A | 0 – 30% | - |
| M | 1:1 mixture | 2.8 ^A | 0 – 30% | -/1.7 ^A |
| M | 1:625 mixture ^E | 2 ^B | Low | - |
| F | 1:1 mixture | 806 ^C | - | Yes/yes |
| M | 70184 | 3734 ^D | 65.4% sterile, 34.6%, 1 – 30% | Yes/? |
| <i>Sinapis arvensis</i> | | | | |
| M | 1:1 mixture | 0.18 ^A | 0 – 30% | -/0.12 ^A |
| F | ~2.9 million | 0 | - | - |
| M | 483 | 6 | - | - |

A = seeds per 100 flowers; B = per 956 seeds; C = per 988 seeds; D = per m²; E = 1:625 wild radish: *B. napus*

(Source: Rieger et al., 1999)

Hybrid seed, which reaches the soil, must subsequently germinate and mature. Crawley et al. (1993) describe this process in terms of the hybrid's finite rate of increase (λ):

$$\lambda = (1 - d_1 - g) + g(1 - d_2)F \quad [1]$$

where d_1 is the proportion of seeds that die in one full year, g is the proportion of seeds germinating in the first growing season (e.g. the first spring), d_2 is the proportion of seeds that die between growing seasons (e.g. over winter) and F is the mean fecundity—the mean number of seeds produced per seed that germinates. The first term in the right hand side of equation [1] refers to the carry over of seed from one year to the next. The second term calculates the number of seeds produced by those plants that germinate and grow in competition with the surrounding vegetation.

Hybrid seedlings can grow and mature in the presence of surrounding vegetation in one of three ways: a) they are competitively superior; b) they are competitively neutral or inferior but persist through a mass effect; or, c) they experience positive selection pressure. HT hybrids will experience positive selection pressure in the presence of the herbicide against which they are tolerant. They may therefore persist in fields where the herbicide is applied or, as noted in the HHM analysis, in adjacent environments that are regularly affected by herbicide drift or in any other environments where the herbicide is applied.

Mass effect refers to the circumstances by which species exist in a location from which they would ordinarily be excluded by competition, maintained by a constant delivery of propagules from a source population (Thompson et al., in prep). In this context the propagules are HT hybrid plants and seeds. The importance of mass effect in maintaining populations of GMOs that are competitively neutral or inferior is thought to be determined by how competitively inferior the GMO is compared to its wild relatives, the rate at which propagules are delivered to a location, the carrying capacity of that location and the magnitude of environmental variation experienced by the plants or animals in the location. Each of these factors is site- and hybrid- specific and can only be determined by careful field studies. This event is not developed further in the fault tree because this is a demonstration that does not refer to specific sites or hybrids.

The HT hybrid may also persist because it is competitively superior to the plants around it. The HHM analysis suggests, for example, that increased use of post-emergent herbicide sprays and minimum tillage may select for seedling vigour in weedy relatives or HT volunteers. If gene flow between these plants produces hybrids, the seedling vigour of these hybrids may be sufficient to offset the metabolic costs that may or may not be associated with the HT gene and its expression. These plants may therefore persist in adjacent environments, without mass effects, via a complex pathway of farming practice, selection, hybridisation and invasion.

Transfer of pollen to the ovary of a weedy relative or HT canola requires a source of pollen and a transport vector when the ovary is available—i.e. both plants must flower at or near the same time. Pollen transport usually occurs via wind or insects, although it may also be transferred by water (e.g. washed out of the atmosphere by rainfall) and other animals (e.g. fur) or anthropogenic vectors (e.g. contaminated machinery). Pollen is usually viable for only a few hours or days hence the distance over which pollination may occur depends on the flowering characteristics of donor and recipient plants, wind speed, direction and turbulence, and the flight patterns, range and velocity of, for example, pollinating insects. Again these

factors are site-, plant- and vector-specific, are well described in the literature (Giddings et al., 1997a; 1997b; Lavigne et al., 1998), and are not therefore developed further within the fault tree. It is important to note, however, the various ways (identified in the HHM analysis) in which these factors may vary. These factors may confound gene flow predictions based on a limited set of field observations. Other potential vectors are probably less significant in terms of pollen dispersal to available ovaries but may play a more prominent role in propagule dispersal (e.g. seeds) and hence mass effects.

The importance of non-sexual hybridisation, sometimes called “horizontal gene flow”, is widely disputed—some scientists maintain that horizontal gene flow plays a very minor role, if any, in the evolution of plants and animals, while others argue that it plays a significant role (Rissler and Melon, 2000). Horizontal gene flow may be mediated via fungal, bacterial or viral infections of any HT plants—either the original HT canola or HT weedy relatives—allowing potential feedback loops.

The potential role of fungi in gene flow is unclear. The first stages of horizontal gene flow between genetically modified plants and plant-associated fungi have been reported. Fungi are known to be transformable. For example, DNA uptake from the host plant has been claimed for *Plasmodiophora brassicae*, and *Aspergillus niger* is reported to have taken up the hygromycin gene from a genetically modified plant (quoted in Nielsen et al., 1998). To date, however, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated. This event therefore remains undeveloped in the fault tree (Figure 4).

Viral mediated gene flow of endogenous genes from one plant to another has never been found, although such a transfer (and that of a transgene) might be possible if a sequence of theoretically possible steps occurs. The first theoretical step requires a virus to replicate within the cells of HT canola and recombination to occur between the viral genome and the HT gene to produce a viable recombinant virus (rVirus in Figure 6).

Recombination with an RNA virus (RNA-RNA recombination) would be most likely to occur via a process called “template switching” wherein the viral RNA-dependent RNA polymerase (RDRP) stops copying the virus template strand, switches to another site on the same or different strand of RNA (e.g. mRNA from HT canola containing the HT gene sequence), and then continues copying (Tepfer, 1993; Nagy and Simon, 1997).

To date there appears to be only one documented example of recombination between the mRNA of a transgenic plant and the replicating RNA of a virus (Greene and Allison, 1994). In this experiment, the pressure and selection for recombination was extremely high. An essential gene of the virus was disabled and the transgene mRNA provided the template to repair it. The rarity of reported recombination events between viral RNA and host mRNA probably reflects the infrequency or the failure of the product viability and the lack of selection for such a product over the wild type virus. Between viruses, intermolecular RNA-RNA recombination is also a rare event but (again) under strong selective pressure has been demonstrated in four groups of plant viruses—alfalfa mosaic virus, bromoviruses, carmoviruses and tombusviruses, and in cauliflower mosaic virus. RNA-RNA recombination can occur between dissimilar RNA molecules, possibly mediated by sites of similar RNA structure (Falk and Bruening, 1994).

DNA-DNA recombination might occur if the HT canola was infected with a DNA virus. There are, however, only three groups of plant viruses with DNA genomes: the

Caulimoviridae, the *Geminiviridae* and the nanoviruses (Hull, 2001). The relative frequencies of DNA-DNA recombination does not appear to have been documented possibly because of the low prevalence of DNA viruses, hence the expected frequency of this event is as yet unclear.

There are three ways that the HT gene may become integrated into the genome of a weedy relative via a recombinant DNA or RNA virus (i.e. one whose genome incorporates the HT gene). Direct integration may be possible if the weed is a suitable host of the recombinant virus, a suitable vector exists that transmits the recombinant virus to the weed, and recombination or reverse transcription followed by recombination takes place within the weed.

First, recombination between the genome of the recombinant DNA virus and the plant's genomic DNA could occur directly. The path for an HT gene in a recombinant RNA virus to enter the plant's genome would require a sequence of steps. Firstly, the recombinant RNA virus would require co-infection of the weed with a pararetrovirus (of which there are only a few known—most notably the cauliflower mosaic virus). Secondly, the reverse transcription enzyme encoded by the pararetrovirus would have to switch from its own RNA template onto the RNA of the recombinant RNA virus to produce a single-stranded (ss) DNA copy of it. This presumably occurs at a similar frequency to template switching of RDRP and may well be a rate-limiting step in this process. Thirdly, the DNA would have to move from the plant's cytoplasm to the nucleus. Fourthly the ssDNA would have to be copied into dsDNA, and then fifthly recombine into the plant's DNA genome. A recent report has shown that chloroplast (cp) DNA can integrate into the plant's genome at a frequency of 1 in 16,000 (Huang et al., 2003). In this experiment the cpDNA included a constitutive plant-viral promoter, without which the frequency of successful gene transfer and expression might be orders of magnitude lower. Steps 3 to 5 in the discussion above are analogous to this experiment with the exception of copying ssDNA to dsDNA.

Second, indirect integration may occur via a secondary infecting virus. Multiple infections of a plant by pathogenic and/or non-pathogenic viruses, one of which is recombinant, could lead to the creation of a recombinant second order virus in either of the two ways discussed above (RNA-RNA, DNA-DNA), or additionally by DNA to RNA recombination (transcription of the DNA to RNA followed by RNA-RNA recombination) or RNA to DNA recombination (via a reverse transcription of the RNA and subsequent DNA-DNA recombination)—giving a total of four possible ways. Again, however, the 2^o recombinant virus must be viable in order for the HT gene to be integrated into a weedy relative. This pathway could theoretically allow gene flow between HT canola and an unrelated weed species if the weed was a suitable host for the secondary recombinant virus but not the first. Clearly, however, this is even less likely than direct integration into the weed genome due to the additional necessary steps. Since gene integration via the secondary recombinant virus can proceed directly or indirectly it is possible to conceive third, fourth, etc order infection sequences each increasingly less likely than the previous one.

Third, indirect integration via a secondary plant is also theoretically possible but more unlikely still than any of the pathways described so far due to the additional necessary steps (Figure 7). Indirect integration via a secondary plant requires that a recombinant virus created in any of the ways described above infects a secondary plant wherein a secondary recombinant virus is created as described above. Integration into the weed genome then

proceeds via this secondary recombinant virus. Again third, fourth and fifth order infections are conceivable but increasingly unlikely.

There are three necessary, but not sufficient, events for bacteria-mediated gene flow: transformation that creates a viable recombinant bacterium (rBacterium), followed by incorporation of the HT gene into the genome of the weedy relative (Figure 8). In this context it is important to note that competitively neutral or slightly inferior recombinant bacteria (and viruses) can be maintained by mass effect in much the same way as recombinant plants. Hence, persistence is not a necessary condition for horizontal gene flow although it is an important determinant of likelihood.

It is important to note that considerable uncertainty surrounds the mechanism and frequency of bacteria-mediated horizontal gene flow. This is due in part to the fact that science has described at species level less than 1% of the bacteria present in the natural environment (Hugenholtz and Pace, 1996). The possibility of horizontal gene flow from plants to bacteria is usually approached from within the framework of the three mechanisms that are known to transfer DNA between bacteria—transformation, transduction and conjugation. To date, however, mechanisms that support conjugative gene transfer from plants to bacteria are not known. Similarly viruses that function in both plants and bacteria, and thereby capable of facilitating transductive gene transfer are also unknown. Therefore, transformation is the only theoretically feasible mechanism for gene transfer between plants and bacteria that has been experimentally demonstrated (Nielsen et al., 1998).

Transformation is probably the most common mechanism of gene transfer in nature and is known to occur in a wide variety of bacterial species (Table 3). Transformation requires contact between a competent bacterium and the recombinant DNA, followed by integration within the bacterial genome. To be competent bacteria must be metabolically active. The proportion of competent bacteria within any population is site- and species- specific. Laboratory experiments with *Bacillus subtilis* suggest 10 – 25% of competency under optimal conditions (Dubnau, 1991). In natural environments bacteria persist under extremes of nutrient availability and other environmental stresses (pH, soil moisture, etc.). Competency rates are therefore likely to be lower and more variable than optimal laboratory conditions.

DNA uptake in competent bacteria is thought to take place through a protein channel that spans the bacterial envelope. This usually occurs after the DNA is bound to the bacterial cell surface through a variety of well-described physical and biological mechanisms (Timms-Wilson et al., 1999). The probability of DNA binding depends on the form of the free DNA (e.g. single stranded, double stranded), the number of binding sites on the bacterial cell and species of recipient bacteria. Some bacteria are only able to bind DNA from closely related organisms (e.g. *Haemoglyphis influenzae*) whilst other species appear capable of binding to any free DNA (e.g. *Bacillus subtilis* and *Acinetobacter calcoaceticus*).

Upon entering the cell, DNA will be exposed to bacterial-encoded restriction enzymes that attack and digest dsDNA, but not ssDNA, which would escape digestion. Restriction enzymes that recognise specific 4 base pair (bp) sequences will leave DNA fragments of approximately 250 bp, whilst larger fragments will be left after digestion by enzymes that recognise longer sequences. Both types of fragments might be able to complement genes with deletions, and larger fragments might even contain functional constructs. Currently, functional constructs are usually greater than 300 bp that mitigates against this possibility.

To be expressed, heterologous rDNA taken up by competent bacteria must integrate into the bacterial chromosome. Integration can be achieved in one of four ways: homologous integration; non-homologous integration via a gene cassette; illegitimate recombination; or autonomous replication based on the presence of the *oriV* gene (Figure 8).

Recombination enzymes are highly selective for sequence identity during the initial stages of denaturation and strand invasion, which requires a minimum length of homology. The rate of homologous integration is logarithmically dependant on the degree of homology between the captured rDNA and the genome of the recipient bacterium, and linearly dependant on the length of sequence homology (the so-called log-linear relationship). The frequency of homologous integration between plant rDNA and bacteria is therefore determined by sequence homology and the length of sequence homology and may therefore be expected to be low. Short regions of homology, however, can mediate recombination with adjacent non-homologous sequences, and bacteria with mutations in some of their *mut* genes (that are involved in the mismatch DNA repair) appear to have less stringent homology requirements (Nielsen et al., 1998). It is also important to note that the widespread use of bacterial genes (promoters and terminators) in gene constructs will contribute to sequence homology and will therefore increase the probability of successful integration.

Gene cassettes are a new class of recently described mobile genetic elements. They are usually found adjacent to integrons, which mediate the expression of the gene cassette and their transposition. Each gene cassette contains a recombination site downstream of the gene, known as the 59-bp element, which mediates transposition by site-specific recombination. Gene cassettes are only usually associated with antibiotic resistance genes. However, some selectable markers in genetically modified plants are identified gene cassettes. These markers may circumvent the usual requirements for homologous recombination because chromosomal integration of the gene cassette can be encoded by the integron (Nielsen et al., 1998).

As noted above considerable uncertainty surrounds the mechanisms of homologous recombination even in the best-studied bacteria such as *Escherichia coli*. Unknown (illegitimate) recombination events have been recorded in various organisms. Indeed the construction of the genetically modified plants is currently based on unknown (illegitimate) recombination events with insertion of genes at random sites within the plant genome. The barriers to heterologous DNA recombination are clearly flexible and may depend on environmental conditions (Nielsen et al., 1998).

Heterologous rDNA can also be integrated in bacteria if it contains replication functions and a bacterial *oriV* gene that enables autonomous replication. Genetic modification techniques (such as electroporation and particle guns) that insert whole plasmids with intact replication functions may enable integration in this manner (Nielsen et al., 1998).

Table 3 Bacterial species capable of natural transformation

| Naturally competent, transformable bacteria | |
|---|---|
| PHOTOLITHOTROPHIC | METHANOTROPIC |
| <i>Agmenellum quadrulicatum</i> | <i>Methylobacterium organophilum</i> |
| <i>Anacystis nidulans</i> | ARCHAEBACTERIA |
| <i>Chlorobium limicola</i> | <i>Methylobacterium thermoautotrophicum</i> |
| <i>Nostoc muscorum</i> | <i>Methanococcus voltae</i> |
| <i>Synechocystis</i> sp. 6803 or sp. OL50 | CLINICAL ISOLATES OF PATHOGENIC SPECIES |
| CHEMOLITHOTROPHS | <i>Campylobacter jejuni</i> |
| <i>Thiobacillus thioparus</i> | <i>Campylobacter coli</i> |
| <i>Thiobacillus</i> sp. strain Y | <i>Haemogyophilus influenzae</i> |
| HETEROTROPHIC | <i>Haemogyophilus parainfluenzae</i> |
| <i>Acromobacter</i> spp. | <i>Helicobacter pylori</i> |
| <i>Acinetobacter calcoaceticus</i> | <i>Moraxella</i> spp. |
| <i>Azotobacter vinelandii</i> | <i>Neissera gonorrhoea</i> |
| <i>Bacillus subtilis</i> | <i>Neissera meningitidis</i> |
| <i>Bacillus licheniformis</i> | <i>Staphylococcus aureus</i> |
| <i>Deinococcus radiodurans</i> | <i>Streptococcus pneumoniae</i> |
| <i>Lactobacillus lactis</i> | <i>Streptococcus sanguis</i> |
| <i>Haemogyophilus</i> sp. | <i>Streptococcus mutans</i> |
| <i>Halobacterium</i> sp. | |
| <i>Methanobacterium thermoautotrophicum</i> | |
| <i>Methanococcus voltae</i> | |
| <i>Methanobacterium organophilum</i> | |
| <i>Micrococcus radiodurans</i> | |
| <i>Mycobacterium smegmatis</i> | |
| <i>Pseudomonas stutzeri</i> (and related species) | |
| <i>Streptomyces</i> spp. | |
| <i>Thermoactinomyces vulgaris</i> | |
| <i>Thermus thermophilus</i> | |
| <i>Thermus flavus</i> | |
| <i>Thermus caldophilus</i> | |
| <i>Thermus aquaticus</i> | |
| <i>Vibrio</i> sp. strain D19 and WJT-IC | |
| <i>Vibrio parahaemolyticus</i> | |
| <i>Neisseria</i> sp. | |
| <i>Streptococcus</i> sp. | |

(Source: Timms-Wilson et al., 1999).

The recombinant DNA can be sourced from either within the canola (via endophytic bacteria) or outside of the canola plant, bound to decaying cells, broken cells, soil, within the protein envelope of a recombinant virus or in the gut of another organism (e.g. earthworm). Again, the rate at which endophytic bacteria come into contact with host DNA is unknown at this stage. Transformation outside the canola plant requires access to free rDNA at the time and place in which competent bacteria develop. Release of DNA from plants, animals and microorganisms occurs as a result of cell death or lysis. DNA is known to be released from decaying plant material, and can occur in considerable quantities in soil and water, where it may persist for substantial periods of time (Table 4). Since viruses can persist in soil over time, their DNA protected by the protein envelope (Marsch and Wellington, 1994), recombinant viruses (e.g. bacteriophages) represent another potential external source of rDNA for bacteria that degrade virus envelopes.

DNA is also known to form complexes with minerals such as clay, quartz and felspar. These complexes are resistant to DNase activity and therefore help DNA to persist in the soil (Timms-Wilson, 1991). The availability of plant DNA to bacteria in soil has not been experimentally demonstrated. However, the fate of DNA released by a recombinant plant is not thought to differ substantially from bacterial chromosomal DNA (Nielsen et al., 1998). It is also possible that bacteria may encounter rDNA in the stomachs of other herbivorous or soil ingesting organisms. In this instance, however, the HT gene would have to survive degradation by food processing acids and nucleases. The likelihood of this is unknown but thought to be very low (OGTR, 2002b).

In summary, the extent to which DNA containing the HT gene is likely to be successfully integrated into the genome of a competent bacterium is therefore very dependent on the particular HT sequence and bacterium involved. On the whole, however, successful transformation of bacteria with recombinant DNA sourced from GM plants is very rare. Experiments performed under ideal conditions (no indigenous competing populations, favourable biotic and abiotic conditions) with *Acinetobacter* sp. suggest transfer rates in the region of 1.5×10^{-10} to 1×10^{-13} recombinant bacteria per inoculated plant. Transformation rates in the field are expected to be lower than this (Timms-Wilson, 1991).

If a stable recombinant bacterium were created, it is possible to conceive of three ways in which the HT gene might be incorporated into the genome of a weedy relative: directly from the bacterium, indirectly via a secondary recombinant bacterium, or indirectly via a bacteriophage-like virus (Figure 9).

Direct integration might proceed in a manner similar to DNA transfer via infection with *Agrobacterium tumefaciens*. The genes and proteins that mediate DNA transfer from *A. tumefaciens* to plant cells have been shown to be similar to the genes and proteins that mediate conjugation between bacteria, suggesting some evolutionary link (Zupan and Zambryski, 1995). It is important to note, however, that *A. tumefaciens* is the only known naturally occurring organism capable of genetically transforming a plant cell, although other strains of *Agrobacterium*, notably *A. rhizogenes*, have been shown to be capable of transforming plants in the laboratory. Hence, this route is speculative, except for the very specific (and therefore very unlikely) case where the Ti-plasmid of *A. tumefaciens* itself is transformed with the HT gene. However, since less than 10% of culturable bacteria have been identified and characterised it is possible that there are other soil bacteria capable of plant transformation.

Table 4 Estimated half-life of DNA in various environments

| Location | Half-life (hours) |
|----------------------------------|---------------------------|
| AQUATIC ENVIRONMENTS | |
| Waste water | 0.017 – 0.17 ^a |
| Waste water | 0.23 ^b |
| Oligotrophic freshwater | 4.2 ^c |
| Eutrophic freshwater | 5.5 ^b |
| Estuarine seawater | 3.4 – 5.2 ^c |
| Estuarine seawater | 5.5 ^b |
| Oligotrophic seawater | 12.8 ^c |
| Phosphorous limited seawater | 4.5 ^c |
| Not phosphorous limited seawater | 45.0 – 83.0 ^c |
| Marine sediments | 235 ^d |
| Marine sediments | 140 ^c |
| TERRESTRIAL ENVIRONMENTS | |
| Loamy sand soil | 9.1 ^e |
| Silty clay soil | 15.1 ^e |
| Clay soil | 28.2 ^e |

^aConversion of super coil into relaxed-circular or linear plasmid DNA

^bLoss of hybridisation signals of plasmid DNA in southern transfer or dot blots

^cLoss of acid precipitated material (colourmetric DNA determination or ³²P labelled plasmid DNA

^dIn dead cells degradation measured as in footnote c

^eLoss of transformation activity of plasmid DNA

(Source: Timms-Wilson et al., 1999)

Many bacteria associated with plants are thought to harbour lysogenic bacteriophages. Gene integration into the weed genome might therefore occur via recombinant bacteriophage infection. Recombinant bacteriophages could be produced if the recombinant bacteria were infected with the bacteriophage whose capsid subsequently incorporated the recombinant DNA (see transduction below). Integration into the weed genome, however, would require that the bacteriophage is also capable of infecting the plant cell (and then progress as per the viral gene integration pathways discussed above). Although viruses that function in different species are known (Chiura, 1997), viruses that function in both plants and bacteria have not yet been identified (Nielsen et al., 1998), hence this route is purely speculative at this time.

Indirect bacterial transmission of the HT gene to a weedy relative requires the creation of a persistent secondary recombinant bacterium that is capable of transforming the weed. As noted above, however, the only naturally occurring bacterium capable of transforming plant cells is *A. tumefaciens*. Secondary recombinant bacteria can be produced via transformation, transduction or conjugation (Figure 10). The transformation process in this instance is the same as that discussed above except that the source of the recombinant DNA is a recombinant bacterium in this case (not HT canola).

Two types of transduction are recognised: specialised and generalised. Generalised transduction occurs when chromosomal or plasmid DNA is accidentally packed into the capsid of a reproducing bacteriophage. The geometry and size of the capsid restricts the

amount of the DNA that can be packaged. The probability that the phage will incorporate recombinant DNA is therefore determined *inter alia* by the size of the recombinant gene. Furthermore most virus bacteriophages attach to specific cell surface receptors and thus the virion will only be able to infect a narrow range of bacterial species, limiting the potential for gene flow.

The recombinant DNA introduced into the secondary bacterium must recombine with the host genome in order to be stably integrated. This requires that the host DNA contain regions of nucleotide sequence homologous to the transducing phage rDNA, which align after release from the virus capsid during the normal process of virus replication. There are a variety of cellular restrictions that may prevent successful integration into the host genome. Successful transduction is therefore a rare event, with expected frequencies (for non-recombinant DNA) in the range of 1×10^{-6} to 1×10^{-9} per capsid generation (Timms-Wilson et al, 1999).

Only DNA associated with the *cos* site can be packed into the capsid of bacteriophage during specialised transduction. Hence only recombinant DNA within close proximity to this site will be incorporated into transducing bacteriophages. The frequency of specialised transduction is therefore expected to be lower than generalised transduction. The possibility and frequency of specialised transduction is likely to be case specific and is not therefore developed further in this demonstration.

Conjugation requires direct contact between a metabolically active donor cell containing a plasmid or transposable element, and a metabolically active recipient cell. Some plasmids are self-transmissible, meaning that they contain all the genes that encode the transfer process. Other plasmids are non-conjugative but can be mobilised by transmissible plasmids. Plasmid transfer is also range limited. Some broad host range plasmids can transfer to many phylogenetically distant species of bacteria, whereas others have very narrow specificity. The frequency of conjugation in soil and plant environs is sensitive to the availability of nutrients, temperature, moisture and pH. Plasmid transfer is thought to be very rare in oligotrophic bulk soil but much more common in the rhizosphere, with expected frequency in the field ranging from 1×10^{-4} to 1×10^{-6} per donor cell on the root (Timms-Wilson et al., 1999).

5 Discussion, conclusions and future directions

This report demonstrates the application of two rigorous hazard analysis techniques to the potential hazards associated with HT canola. The importance of this demonstration lies in both the hazards that have been identified and the event chains that may or may not lead to these hazards. This approach emphasises that hazard is a function of the intrinsic properties of a substance or activity (release of HT canola) and the circumstances surrounding this activity. The HHM analysis helps identify potentially important circumstances by exposing the diversity of mechanism (e.g. different dispersal vectors) and means (standard farming practice, ecological response, farmer response). The fault tree analysis builds on this process by helping the analyst to lay out the logical sequence of events that make up potentially hazardous event chains.

The HHM analysis identifies a broad suite of ecological hazards. Many of these hazards are relatively obvious and would have been identified with a checklist or via unstructured brainstorming. Some of the hazards, however, are much more subtle and involve quite complex event chains, and for these reasons would probably not have been suggested without a structured, rigorous hazard identification procedure. This claim can be substantiated by

comparing the hazards identified in this document with those identified by an actual risk assessment for field trials of HT canola using a checklist based approach (OGTR, 2002b). Table 1 compares the hazards identified in the risk assessment, and those implied but not specifically identified, with the hazards identified in the HHM analysis. Overall the checklist based approach identifies (or implies) 31% of the hazards identified by the HHM analysis. It is important to note that many of the hazards that were not identified in this checklist (e.g. those associated with segregation and supply chain management, changes to agricultural practice) may be addressed in future risk assessments for commercial release of canola. The checklist, however, failed to identify a number of hazards that might be associated with field trials such as: dispersal via disposal of spoilt or low quality seed; the effects of herbicide drift; the effect of temperature, moisture or insect-induced stress on gene expression; the effect of gene expression and post emergent sprays on root exudates and the nutritional quality of canola leaves (and its influence on herbivore behaviour); weed spectrum changes, etc. These hazards are purely speculative but they are plausible and may warrant further investigation or targeted monitoring.

The HHM analysis also provides a suitable platform to rank hazards. This can be achieved via a formal hazard ranking procedure (such as the Analytical Hierarchy Process) or by simply asking the team to score each hazard based on their professional experience and judgement. In this example the study team members were asked to rank the hazards in terms of their degree of concern and confidence in the specified events. It is important to note that this ranking does not represent a formal risk assessment—it is simply a means to prioritise each hazard for subsequent analysis. The top ten hazards reflect an underlying concern that, without careful management, commercialisation of HT canola will increase the incidence of HT volunteers, both on and off-farm, which facilitate “secondary” seed and pollen-mediated dispersal of the trait over large distances. This coupled with the development of widespread herbicide tolerance amongst weeds, may necessitate the use of alternative, and potentially more toxic, weed control strategies across large areas of agricultural and non-agricultural land. The impact of HT canola, and associated farming practice, on soil fauna communities and processes, also figure prominently in the HHM analysis. This appears to be an important, but as yet poorly understood, aspect of the new technology.

The HHM analysis did not distinguish between hazards that are specific to the GM technology and those associated with agriculture more generally. As a result some of the hazards noted here may be more important in conventional systems. In these circumstances GM technology may offer substantial benefits over current practice. As noted above, however, conventional agricultural practice has not, to date, been subject to the same level of “before the event” scrutiny as biotechnology. Without this information it is difficult to accurately gauge the potential costs or benefits of the new technology.

The main drawback with the HHM analysis is the time required to complete it, and the need to co-ordinate experts that, as in this case, might be drawn from several different institutions. It is often difficult to maintain continuity and consistency in these groups. Redundancy and duplication within the analysis also tends to reduce its efficiency. It is difficult, however, to determine *a priori* where duplication is likely to occur. *A posteriori* analysis of the hazards and their HHM references may help analysts to design more streamlined approaches to the assessment that require less man-hours without threatening the rigour and completeness of the analysis. This is an area of future research.

The principal advantage of the fault tree analysis is its structured and rigorous approach to identifying exposure pathways and the necessary event sequence behind hazards. The analysis here has helped to identify potential rate limiting steps and speculates on possible hazard scenarios, such as the potential interaction between viral and bacterial pathways, that do not appear to have been addressed in the literature to date.

Another advantage of a fault tree is that it quickly identifies knowledge uncertainty in the system. This analysis highlights how uncertainty increases moving from sexual gene flow pathways to viral, bacterial and then fungal pathways. Particular areas of uncertainty include:

- sexual gene flow: the importance of mass effect in maintaining competitively neutral or inferior hybrids;
- viral: the relative frequency of RNA-RNA, DNA-DNA, RNA-DNA and DNA-RNA recombination;
- bacterial: the mechanisms of homologous recombination in bacteria, the rate at which endophytic bacteria come into contact with host DNA, the possibility of bacterial mediated transformation of plant cells by species other than *A. tumefaciens*, and the possibility of bacteriophage infection of plant cells; and,
- fungal: the potential role of fungal-mediated gene flow.

The fault tree provides an excellent platform to quantify the potential for gene flow under commercial conditions because it breaks down the event chain to individual elements that can be analysed experimentally, and therefore potentially quantified. Indeed quantitative estimates already exist for some of the basic events in the tree. Furthermore, many of the current appeals to the safety of GM products rely on the incredibly rare likelihood of undesired events such as bacterial gene flow. These appeals, however, are undermined by the large number of potential exposure pathways (demonstrated by the complexity of the fault tree) and extremely high exposure (billions of plants in commercial production). Quantitative fault tree analysis provides a means to explore the overall effect of these two opposing themes. Developing quantitative estimates of the frequency for each of the basic initiating events within the tree, and the undeveloped events, within a case-specific analysis, is therefore an important avenue of future research.

The fault tree also allows the analyst to identify rate-limiting steps and investigate the knock-on effects of changes to expected conditions. For example, gene flow and persistence through the mass effect, is probably proportional to the bacterial and viral inoculum and therefore to the availability of decaying biomass. The HHM analysis notes that the widespread use of post-emergent herbicide associated with the commercialisation of HT canola is likely to increase the biomass of decaying weeds in agricultural systems, providing the type of system change that could confound risk estimates based on current laboratory and field studies. The effects of this change could be investigated through a case-specific fault tree analysis developed along the lines illustrated here.

Fault tree analysis is not designed to identify all potential hazards. Unexpected interactions (outside the experience or imagination of the analyst) could result in additional unidentified hazards or hazard inducing mechanisms. By virtue of its holistic approach HHM analysis is more likely to identify, or at least suggest, unexpected interactions. Taken together these tools enable the analyst to postulate certain hazards and then investigate in more detail how they

might occur. There is no guarantee, however, that these processes together will identify all hazards. There are, however, no such guarantees in any form of hazard analysis or risk assessment (hence the need to continually compare the predictions of a risk assessment with reality). The logical and rigorous structure of HHM analysis and fault tree analysis, however, helps minimise the probability of missing important casual pathways and it performs much better in this regard than brainstorming techniques. In this context, the fault tree portrayed here should not be regarded as “finished”—indeed the tree should be developed and progressed whenever relevant information becomes available.

HHM analysis and fault-tree analysis are not “objective” processes—their heuristic potential and usefulness depends on the expertise of the analyst(s). Both of these techniques help the analyst deconstruct complex systems into their contributing parts, so long as he or she is sufficiently familiar with the system in question. This is another potential weakness of these techniques—they require substantial expert knowledge, and are ultimately limited by knowledge of the people involved in the analysis. Both techniques are therefore most useful when they are conducted by a team of experts who are able to pool their collective expertise.

Deductive hazard identification techniques may be quicker and easier than HHM or fault tree analysis but otherwise suffer from same types of problems discussed here and, importantly, provide less confidence that all possible hazards have been addressed. Demonstrably thorough hazard identification is an important pre-cursor to both the risk assessment and public confidence in risk-based environmental management of novel technologies such as GMOs. Extra investment in the early stages of an ecological risk assessment will therefore provide substantial benefits throughout the remainder of the process.

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Appendix A

| HazCat | ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|-------------------------|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|----|----|----|----|----|----|----|----|----|
| Unexpected expression | 1 | 1.3.18 | 4.1.10 | 5.2.8 | | | | | | | | | | | | | | | | | |
| | 2 | 1.1.13 | | | | | | | | | | | | | | | | | | | |
| | 3 | 3.4.8 | | | | | | | | | | | | | | | | | | | |
| | 4 | 1.4.6 | 4.2.42 | 5.2.28 | 6.1.28 | | | | | | | | | | | | | | | | |
| | 5 | 4.3.6 | 4.3.8 | 6.1.3 | 7.1.18 | | | | | | | | | | | | | | | | |
| | 6 | 3.4.13 | 5.2.2 | | | | | | | | | | | | | | | | | | |
| | 7 | 1.3.2 | | | | | | | | | | | | | | | | | | | |
| | 8 | 4.3.3 | | | | | | | | | | | | | | | | | | | |
| Unexpected selection | 9 | 1.1.13 | | | | | | | | | | | | | | | | | | | |
| | 10 | 1.2.17 | | | | | | | | | | | | | | | | | | | |
| | 11 | 2.1.26 | | | | | | | | | | | | | | | | | | | |
| | 12 | 4.1.41 | 4.1.42 | 4.3.26 | 4.4.36 | 5.2.1 | 5.2.6 | 5.2.11 | 5.3.12 | | | | | | | | | | | | |
| | 13 | 5.2.16 | | | | | | | | | | | | | | | | | | | |
| | 14 | 6.1.12 | 6.1.16 | | | | | | | | | | | | | | | | | | |
| | 15 | 1.3.22 | | | | | | | | | | | | | | | | | | | |
| Unexpected invasion | 16 | 1.2.23 | 2.2.7 | | | | | | | | | | | | | | | | | | |
| | 17 | 1.3.41 | | | | | | | | | | | | | | | | | | | |
| | 18 | 2.1.10 | | | | | | | | | | | | | | | | | | | |
| | 19 | 3.5.4 | 4.2.43 | 4.4.46 | 5.3.4 | | | | | | | | | | | | | | | | |
| | 20 | 4.2.26 | | | | | | | | | | | | | | | | | | | |
| | 21 | 4.1.50 | | | | | | | | | | | | | | | | | | | |
| HT dispersal (off-farm) | 22 | 1.1.23 | 1.2.32 | 1.3.25 | 1.3.32 | 3.5.9 | 4.4.45 | | | | | | | | | | | | | | |
| | 23 | 1.3.25 | 1.3.35 | 2.3.10 | | | | | | | | | | | | | | | | | |
| | 24 | 1.2.45 | 1.2.37 | 1.2.29 | 1.3.26 | 2.1.13 | 2.3.27 | 3.1.35 | 3.2.34 | 4.1.17 | | | | | | | | | | | |
| | 25 | 1.2.46 | 1.2.38 | 1.2.30 | 1.3.26 | 2.1.14 | 2.3.27 | 3.1.42 | 3.1.43 | 3.2.42 | 4.1.17 | | | | | | | | | | |
| | 26 | 3.1.51 | 3.1.52 | 3.2.50 | 4.1.17 | | | | | | | | | | | | | | | | |
| | 27 | 1.7.23 | 3.2.59 | 4.4.17 | | | | | | | | | | | | | | | | | |
| | 28 | 1.2.26 | 1.2.34 | 7.1.1 | | | | | | | | | | | | | | | | | |
| | 29 | 1.2.26 | 2.3.3 | 2.3.11 | 3.2.3 | 3.2.11 | 3.2.43 | 3.2.51 | 3.2.59 | 5.3.17 | | | | | | | | | | | |
| | 30 | 1.2.56 | 1.7.46 | | | | | | | | | | | | | | | | | | |
| | 31 | 3.1.59 | | | | | | | | | | | | | | | | | | | |
| | 32 | 2.3.1 | 2.3.5 | 2.3.8 | 3.2.9 | 4.1.1 | | | | | | | | | | | | | | | |

| HazCat | ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|----------------------------|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|----|----|----|----|----|----|----|
| | 33 | 2.3.1 | 3.1.19 | 3.2.17 | | | | | | | | | | | | | | | | | |
| | 34 | 2.3.2 | 2.3.10 | 3.2.58 | 4.2.44 | 4.2.47 | | | | | | | | | | | | | | | |
| | 35 | 3.2.57 | | | | | | | | | | | | | | | | | | | |
| | 36 | 2.3.5 | 3.5.7 | | | | | | | | | | | | | | | | | | |
| | 37 | 1.1.35 | | | | | | | | | | | | | | | | | | | |
| | 38 | 1.1.35 | 1.2.43 | 1.6.26 | 1.6.27 | | | | | | | | | | | | | | | | |
| | 39 | 1.1.11 | 1.1.17 | | | | | | | | | | | | | | | | | | |
| | 40 | 1.2.10 | | | | | | | | | | | | | | | | | | | |
| | 41 | 1.2.18 | 1.2.43 | 1.2.44 | 3.5.10 | 3.5.16 | 3.5.22 | 3.5.28 | 3.5.34 | 3.5.18 | 3.5.24 | 3.5.30 | 3.5.36 | | | | | | | | |
| | 42 | 1.2.48 | | | | | | | | | | | | | | | | | | | |
| | 43 | 1.4.41 | 1.4.42 | | | | | | | | | | | | | | | | | | |
| | 44 | 1.2.41 | | | | | | | | | | | | | | | | | | | |
| | 45 | 1.2.41 | | | | | | | | | | | | | | | | | | | |
| | 46 | 1.7.51 | | | | | | | | | | | | | | | | | | | |
| HT volunteers (on-farm) | 47 | 1.2.10 | | | | | | | | | | | | | | | | | | | |
| | 48 | 1.2.26 | 1.2.32 | | | | | | | | | | | | | | | | | | |
| | 49 | 1.2.26 | | | | | | | | | | | | | | | | | | | |
| | 50 | 4.3.30 | | | | | | | | | | | | | | | | | | | |
| | 51 | 1.2.66 | | | | | | | | | | | | | | | | | | | |
| | 52 | 1.3.35 | 1.3.40 | 5.3.19 | | | | | | | | | | | | | | | | | |
| | 53 | 1.3.65 | 1.6.43 | | | | | | | | | | | | | | | | | | |
| Social | 54 | 1.1.31 | 1.2.72 | 2.1.8 | | | | | | | | | | | | | | | | | |
| | 55 | 1.2.02 | | | | | | | | | | | | | | | | | | | |
| | 56 | 1.2.42 | 2.1.8 | | | | | | | | | | | | | | | | | | |
| Segregation | 57 | 1.1.49 | | | | | | | | | | | | | | | | | | | |
| | 58 | 1.2.53 | | | | | | | | | | | | | | | | | | | |
| | 59 | 1.4.33 | 1.5.26 | 1.5.27 | 1.5.28 | 1.7.25 | 1.7.26 | 5.3.7 | | | | | | | | | | | | | |
| | 60 | 1.6.21 | 1.6.22 | 1.6.24 | | | | | | | | | | | | | | | | | |
| | 61 | 1.7.19 | 7.1.7 | | | | | | | | | | | | | | | | | | |
| | 62 | 1.7.20 | 3.5.8 | 4.4.43 | | | | | | | | | | | | | | | | | |
| | 63 | 1.7.21 | | | | | | | | | | | | | | | | | | | |
| | 64 | 1.6.41 | 1.6.42 | | | | | | | | | | | | | | | | | | |
| | 65 | 1.4.59 | | | | | | | | | | | | | | | | | | | |

| HazCat | ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|------------------|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|----|----|----|
| Criminal | 66 | 1.6.10 | 4.1.21 | 4.1.29 | 5.2.21 | 5.2.24 | 5.2.25 | | | | | | | | | | | | | | |
| | 67 | 3.4.5 | | | | | | | | | | | | | | | | | | | |
| | 68 | 1.1.43 | | | | | | | | | | | | | | | | | | | |
| | 69 | 1.2.58 | | | | | | | | | | | | | | | | | | | |
| | 70 | 1.2.58 | 1.4.61 | 1.7.43 | 1.7.44 | | | | | | | | | | | | | | | | |
| | 71 | 1.3.59 | | | | | | | | | | | | | | | | | | | |
| | 72 | 1.6.38 | | | | | | | | | | | | | | | | | | | |
| Farming practice | 73 | 1.2.10 | | | | | | | | | | | | | | | | | | | |
| | 74 | 1.2.49 | 1.6.32 | 1.6.34 | 5.2.9 | | | | | | | | | | | | | | | | |
| | 75 | 4.1.4 | | | | | | | | | | | | | | | | | | | |
| | 76 | 2.1.17 | 2.1.33 | 2.1.41 | | | | | | | | | | | | | | | | | |
| | 77 | 1.2.48 | 2.1.34 | 2.1.35 | 2.1.36 | 2.1.37 | 2.1.38 | 2.1.42 | 2.1.43 | 2.1.44 | 2.1.45 | 2.1.46 | 2.2.13 | 2.2.21 | 2.2.37 | 2.2.45 | 3.1.17 | | | | |
| | 78 | 3.5.33 | 3.5.39 | | | | | | | | | | | | | | | | | | |
| | 79 | 4.4.15 | | | | | | | | | | | | | | | | | | | |
| | 80 | 3.5.1 | 3.5.3 | 4.4.13 | | | | | | | | | | | | | | | | | |
| | 81 | 4.4.14 | | | | | | | | | | | | | | | | | | | |
| | 82 | 1.2.19 | 1.2.20 | 2.2.20 | 3.1.17 | 3.3.18 | 3.3.24 | | | | | | | | | | | | | | |
| | 83 | 3.5.12 | 4.3.21 | | | | | | | | | | | | | | | | | | |
| | 84 | 2.2.35 | 2.2.43 | 3.4.11 | 3.4.16 | 4.3.11 | 4.3.16 | | | | | | | | | | | | | | |
| | 85 | 3.4.21 | 3.4.26 | 3.4.31 | 3.5.42 | | | | | | | | | | | | | | | | |
| | 86 | 1.2.35 | 1.2.36 | | | | | | | | | | | | | | | | | | |
| | 87 | 4.4.19 | | | | | | | | | | | | | | | | | | | |
| | 88 | 2.2.17 | 2.2.33 | 2.2.41 | | | | | | | | | | | | | | | | | |
| | 89 | 1.3.17 | | | | | | | | | | | | | | | | | | | |
| | 90 | 1.2.25 | 1.2.27 | 1.2.28 | 3.5.13 | 3.5.19 | | | | | | | | | | | | | | | |
| | 91 | 1.5.7 | 2.4.25 | 2.4.31 | 5.1.13 | 5.3.24 | 5.3.48 | | | | | | | | | | | | | | |
| | 92 | 5.1.14 | | | | | | | | | | | | | | | | | | | |
| | 93 | 1.5.8 | 2.4.28 | 2.4.34 | 4.1.1 | 4.1.2 | | | | | | | | | | | | | | | |
| | 94 | 1.5.9 | 4.1.1 | 4.1.2 | | | | | | | | | | | | | | | | | |
| | 95 | 1.5.13 | 1.5.19 | 2.2.13 | 5.1.1 | 4.1.36 | 6.2.6 | | | | | | | | | | | | | | |
| | 96 | 1.5.14 | 2.4.26 | 2.4.32 | 5.1.2 | 6.2.12 | | | | | | | | | | | | | | | |
| | 97 | 1.5.15 | 2.4.27 | 2.4.33 | 5.1.9 | 6.2.18 | | | | | | | | | | | | | | | |
| | 98 | 3.3.26 | 3.3.32 | 3.3.38 | 4.1.18 | 4.1.26 | | | | | | | | | | | | | | | |
| | 99 | 2.2.38 | 2.2.46 | | | | | | | | | | | | | | | | | | |

| HazCat | ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|--------|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|----|----|----|----|----|----|----|----|
| | 100 | 4.3.5 | | | | | | | | | | | | | | | | | | | |
| | 101 | 6.2.36 | | | | | | | | | | | | | | | | | | | |
| | 102 | 1.4.18 | 1.5.9 | 4.1.60 | | | | | | | | | | | | | | | | | |
| | 103 | 1.4.20 | | | | | | | | | | | | | | | | | | | |
| | 104 | 1.6.13 | | | | | | | | | | | | | | | | | | | |
| | 105 | 3.5.37 | | | | | | | | | | | | | | | | | | | |
| | 106 | 1.5.18 | | | | | | | | | | | | | | | | | | | |
| | 107 | 1.6.8 | 1.6.11 | | | | | | | | | | | | | | | | | | |
| | 108 | 3.5.6 | | | | | | | | | | | | | | | | | | | |
| | 109 | 4.3.1 | 4.3.2 | | | | | | | | | | | | | | | | | | |
| | 110 | 1.2.10 | | | | | | | | | | | | | | | | | | | |
| | 111 | 1.2.11 | 1.3.11 | | | | | | | | | | | | | | | | | | |
| | 112 | 1.2.11 | 1.2.12 | 1.3.11 | 1.3.12 | | | | | | | | | | | | | | | | |
| | 113 | 1.2.13 | 3.1.19 | | | | | | | | | | | | | | | | | | |
| | 114 | 1.3.9 | 1.3.16 | | | | | | | | | | | | | | | | | | |
| | 115 | 1.3.15 | | | | | | | | | | | | | | | | | | | |
| | 116 | 1.3.14 | | | | | | | | | | | | | | | | | | | |
| | 117 | 1.3.10 | | | | | | | | | | | | | | | | | | | |
| | 118 | 1.3.16 | | | | | | | | | | | | | | | | | | | |
| | 119 | 1.2.9 | | | | | | | | | | | | | | | | | | | |
| | 120 | 1.4.10 | 1.5.8 | 1.5.9 | | | | | | | | | | | | | | | | | |
| | 121 | 1.4.11 | 1.5.7 | 1.5.13 | | | | | | | | | | | | | | | | | |
| | 122 | 1.4.12 | 1.4.14 | 1.5.12 | 1.5.15 | 2.4.18 | 2.4.30 | 2.4.36 | 3.3.6 | | | | | | | | | | | | |
| | 123 | 1.6.9 | | | | | | | | | | | | | | | | | | | |
| | 124 | 1.3.21 | 4.4.30 | | | | | | | | | | | | | | | | | | |
| | 125 | 1.2.19 | 1.2.20 | 1.3.19 | 1.3.20 | 3.1.21 | 3.4.14 | 3.4.19 | 4.3.36 | 4.3.37 | 4.4.24 | 4.4.30 | | | | | | | | | |
| | 126 | 1.2.21 | 1.2.22 | 1.2.23 | 1.2.24 | 3.5.48 | 4.4.30 | | | | | | | | | | | | | | |
| | 127 | 3.3.8 | | | | | | | | | | | | | | | | | | | |
| | 128 | 1.2.23 | 2.2.9 | | | | | | | | | | | | | | | | | | |
| | 129 | 2.2.9 | 3.1.4 | | | | | | | | | | | | | | | | | | |
| | 130 | 1.4.24 | 4.1.8 | 4.1.24 | 4.1.32 | 5.1.43 | 5.3.1 | 5.3.6 | | | | | | | | | | | | | |
| | 131 | 1.6.31 | 1.7.42 | 2.1.6 | 2.1.7 | 2.1.14 | 2.1.15 | | | | | | | | | | | | | | |
| | 132 | 1.7.54 | | | | | | | | | | | | | | | | | | | |
| | 133 | 1.4.13 | | | | | | | | | | | | | | | | | | | |

| HazCat | ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|--------------|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | 134 | 1.5.37 | | | | | | | | | | | | | | | | | | | |
| | 135 | 1.5.38 | | | | | | | | | | | | | | | | | | | |
| | 136 | 1.5.38 | 1.6.32 | | | | | | | | | | | | | | | | | | |
| | 137 | 1.6.14 | 4.4.12 | | | | | | | | | | | | | | | | | | |
| | 138 | 1.2.17 | 1.6.11 | 1.6.12 | 2.1.9 | | | | | | | | | | | | | | | | |
| | 139 | 3.3.1 | | | | | | | | | | | | | | | | | | | |
| | 140 | 3.1.5 | 4.4.6 | 4.4.24 | 4.4.30 | | | | | | | | | | | | | | | | |
| | 141 | 3.1.13 | 3.1.16 | | | | | | | | | | | | | | | | | | |
| | 142 | 3.1.24 | 4.4.48 | | | | | | | | | | | | | | | | | | |
| | 143 | 2.2.10 | | | | | | | | | | | | | | | | | | | |
| | 144 | 5.3.9 | 5.3.15 | | | | | | | | | | | | | | | | | | |
| | 145 | 1.5.10 | 1.5.16 | 2.4.38 | | | | | | | | | | | | | | | | | |
| | 146 | 1.7.24 | 3.5.45 | 4.4.3 | | | | | | | | | | | | | | | | | |
| | 147 | 2.1.8 | | | | | | | | | | | | | | | | | | | |
| | 148 | 2.4.13 | | | | | | | | | | | | | | | | | | | |
| | 149 | 3.1.1 | | | | | | | | | | | | | | | | | | | |
| | 150 | 2.5.11 | 2.5.12 | | | | | | | | | | | | | | | | | | |
| | 151 | 3.1.19 | | | | | | | | | | | | | | | | | | | |
| | 152 | 3.5.14 | 4.4.26 | | | | | | | | | | | | | | | | | | |
| Gene flow | 153 | 1.2.1 | 1.2.2 | 2.1.2 | 2.1.19 | 3.1.15 | 4.2.5 | | | | | | | | | | | | | | |
| | 154 | 3.4.3 | | | | | | | | | | | | | | | | | | | |
| | 155 | 1.3.8 | 2.2.8 | | | | | | | | | | | | | | | | | | |
| | 156 | 1.3.54 | 3.1.22 | | | | | | | | | | | | | | | | | | |
| | 157 | 3.2.13 | 3.2.21 | 3.2.27 | 3.2.45 | 4.4.33 | 7.1.2 | 7.1.3 | 7.1.4 | | | | | | | | | | | | |
| | 158 | 3.3.2 | | | | | | | | | | | | | | | | | | | |
| Biodiversity | 159 | 1.2.19 | 1.1.17 | 1.2.20 | 1.3.17 | 1.3.19 | 1.3.20 | 2.1.18 | 2.1.19 | 2.1.20 | 2.1.21 | 2.1.22 | 2.1.23 | 2.1.27 | 2.1.28 | 2.1.29 | 2.1.30 | 2.2.12 | 3.1.17 | 1.2.13 | 3.1.23 |
| | 160 | 1.3.6 | | | | | | | | | | | | | | | | | | | |
| Toxicity | 161 | 1.2.1 | 1.3.1 | 1.3.3 | 1.3.5 | 1.6.5 | 2.1.1 | 2.1.9 | 2.2.5 | 2.5.5 | 3.4.1 | 3.4.2 | 3.4.4 | | | | | | | | |
| | 162 | 3.2.2 | 3.2.3 | 3.3.20 | | | | | | | | | | | | | | | | | |
| | 163 | 1.3.1 | 1.3.5 | 2.2.5 | 3.1.17 | 4.3.18 | 4.3.19 | | | | | | | | | | | | | | |
| | 164 | 1.2.5 | 1.3.5 | 2.1.13 | 2.2.5 | | | | | | | | | | | | | | | | |
| | 165 | 1.2.7 | 1.3.5 | 2.2.5 | | | | | | | | | | | | | | | | | |
| | 166 | 1.2.6 | 1.3.5 | 2.2.5 | | | | | | | | | | | | | | | | | |
| | 167 | 3.1.22 | | | | | | | | | | | | | | | | | | | |

| HazCat | ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|----------------------|-----|--------|--------|--------|--------|-------|-------|-------|-------|-------|-------|----|----|----|----|----|----|----|----|----|----|
| | 168 | 3.3.12 | | | | | | | | | | | | | | | | | | | |
| | 169 | 2.2.2 | 3.1.10 | 4.1.11 | 4.2.37 | 4.3.4 | 4.3.9 | | | | | | | | | | | | | | |
| | 170 | 1.2.8 | 2.1.16 | | | | | | | | | | | | | | | | | | |
| | 171 | 2.1.2 | | | | | | | | | | | | | | | | | | | |
| | 172 | 2.1.11 | 2.1.12 | 2.2.11 | 3.1.19 | | | | | | | | | | | | | | | | |
| | 173 | 4.1.57 | 4.1.58 | 4.1.59 | | | | | | | | | | | | | | | | | |
| | 174 | 7.1.30 | | | | | | | | | | | | | | | | | | | |
| Herbicide resistance | 175 | 1.3.1 | 2.2.6 | 3.4.8 | | | | | | | | | | | | | | | | | |
| | 176 | 1.3.13 | | | | | | | | | | | | | | | | | | | |
| | 177 | 3.1.16 | | | | | | | | | | | | | | | | | | | |
| Plant physiology | 178 | 1.3.3 | 1.5.1 | 2.2.1 | | | | | | | | | | | | | | | | | |
| | 179 | 2.4.1 | 2.4.2 | | | | | | | | | | | | | | | | | | |
| | 180 | 1.3.5 | 3.1.9 | 4.2.30 | 4.2.48 | | | | | | | | | | | | | | | | |
| | 181 | | | | | | | | | | | | | | | | | | | | |
| | 182 | 2.3.12 | | | | | | | | | | | | | | | | | | | |
| | 183 | 2.3.14 | | | | | | | | | | | | | | | | | | | |
| | 184 | 2.3.17 | 2.3.25 | | | | | | | | | | | | | | | | | | |
| | 185 | 1.3.6 | 3.1.14 | 4.3.28 | | | | | | | | | | | | | | | | | |
| | 186 | 3.2.10 | | | | | | | | | | | | | | | | | | | |
| | 187 | 1.3.7 | 2.2.7 | | | | | | | | | | | | | | | | | | |
| | 188 | 2.3.30 | | | | | | | | | | | | | | | | | | | |
| | 189 | 1.4.4 | | | | | | | | | | | | | | | | | | | |
| | 190 | 1.5.1 | 5.3.2 | | | | | | | | | | | | | | | | | | |
| | 191 | 1.5.2 | 1.5.6 | 2.2.1 | 2.2.3 | | | | | | | | | | | | | | | | |
| | 192 | 1.5.5 | 2.4.5 | | | | | | | | | | | | | | | | | | |
| | 193 | 4.2.4 | | | | | | | | | | | | | | | | | | | |
| Plant biochemistry | 194 | 1.2.3 | 1.2.4 | | | | | | | | | | | | | | | | | | |
| | 195 | 1.3.4 | | | | | | | | | | | | | | | | | | | |
| | 196 | 3.1.20 | | | | | | | | | | | | | | | | | | | |
| | 197 | 1.6.1 | 1.6.2 | 1.6.3 | 1.6.4 | 2.5.1 | 2.5.2 | 2.5.3 | 2.5.4 | 3.4.7 | 3.4.9 | | | | | | | | | | |
| | 198 | 2.1.25 | 4.3.7 | 6.1.2 | | | | | | | | | | | | | | | | | |
| | 199 | 3.1.4 | 3.1.12 | | | | | | | | | | | | | | | | | | |
| | 200 | 2.1.3 | 2.1.4 | 3.1.1 | 3.4.13 | | | | | | | | | | | | | | | | |

Appendix B

Table 1.1 Man-made processes v biological hierarchy

| | Gene construct | Plough-Plant | Irrigate-Spray-Weed | Harvest-Transport-Process-Store | Cleaning | Recreation-Conservation | Husbandry | Criminal | Quality control – Monitoring |
|------------------------|----------------|--------------|---------------------|---------------------------------|----------|-------------------------|-----------|----------|------------------------------|
| Genes | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 | 49 |
| Organism | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 | 50 |
| Population-Community | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 | 51 |
| Species | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 | 52 |
| Ecosystem habitat | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 | 53 |
| Bioregion-Bioprovinces | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 | 54 |

Table 1.2 Man-made processes v biological components

| | Gene construct | Plough-Plant | Irrigate-Spray-Weed | Harvest-Transport-Process-Store | Cleaning | Recreation-Conservation | Husbandry | Criminal | Quality control – Monitoring |
|--------------------------|----------------|--------------|---------------------|---------------------------------|----------|-------------------------|-----------|----------|------------------------------|
| Bacteria-Viruses-Fungi | 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 | 65 |
| Plants | 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 |
| Insects | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 |
| Other invertebrates | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 |
| Birds | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 |
| Mammals | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 |
| Reptiles-Fish-Amphibians | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 |
| Man | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 |

Table 1.3 Man-made processes v biological processes

| | Gene construct | Plough-Plant | Irrigate-Spray-Weed | Harvest-Transport-Process-Store | Cleaning | Recreation-Conservation | Husbandry | Criminal | Quality control – Monitoring |
|---------------------------------|----------------|--------------|---------------------|---------------------------------|----------|-------------------------|-----------|----------|------------------------------|
| Development-Reproduction-Growth | 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 | 65 |
| Excretion | 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 |
| Movement-Behaviour | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 |
| Predation-Nutrition-Parasitism | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 |
| Death | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 |
| Selection-Mutation | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 |
| Competition | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 |
| Bioaccumulation | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 |

Table 1.4 Man-made processes v physical processes

| | Gene construct | Plough-Plant | Irrigate-Spray-Weed | Harvest-Transport-Process-Store | Cleaning | Recreation-Conservation | Husbandry | Criminal | Quality control – Monitoring |
|---------------------------|----------------|--------------|---------------------|---------------------------------|----------|-------------------------|-----------|----------|------------------------------|
| Wind movement | 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 | 65 |
| Water movement | 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 |
| Soil movement | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 |
| Evaporation-Precipitation | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 |
| Fire | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 |
| Freezing | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 |
| Lightning | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 |
| Inversion | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 |

Table 1.5 Man-made processes v physical components

| | Gene construct | Plough-Plant | Irrigate-Spray-Weed | Harvest-Transport-Process-Store | Cleaning | Recreation-Conservation | Husbandry | Criminal | Quality control – Monitoring |
|--|----------------|--------------|---------------------|---------------------------------|----------|-------------------------|-----------|----------|------------------------------|
| Atmospheric Air – Interstitial air | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 | 49 |
| Surface water – Atmospheric water | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 | 50 |
| Groundwater – Interstitial water | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 | 51 |
| Seawater | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 | 52 |
| Gravity – Magnetism – Static electricity | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 | 53 |
| Temperature | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 | 54 |

Table 1.6 Man-made processes v chemical components and processes

| | Gene construct | Plough-Plant | Irrigate-Spray-Weed | Harvest-Transport-Process-Store | Cleaning | Recreation-Conservation | Husbandry | Criminal | Quality control – Monitoring |
|----------------------|----------------|--------------|---------------------|---------------------------------|----------|-------------------------|-----------|----------|------------------------------|
| Inorganics | 1 | 6 | 11 | 16 | 21 | 26 | 31 | 36 | 41 |
| Organics | 2 | 7 | 12 | 17 | 22 | 27 | 32 | 37 | 42 |
| Gene expression | 3 | 8 | 13 | 18 | 23 | 28 | 33 | 38 | 43 |
| Cycles | 4 | 9 | 14 | 19 | 24 | 29 | 34 | 39 | 44 |
| Creation-Destruction | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 |

Table 1.7 Man-made processes v man-made components

| | Gene construct | Plough-Plant | Irrigate-Spray-Weed | Harvest-Transport-Process-Store | Cleaning | Recreation-Conservation | Husbandry | Criminal | Quality control – Monitoring |
|--------------------------|----------------|--------------|---------------------|---------------------------------|----------|-------------------------|-----------|----------|------------------------------|
| Machinery | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 | 49 |
| Building | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 | 50 |
| Road – Tracks | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 | 51 |
| Fences | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 | 52 |
| Clothes | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 | 53 |
| Pesticides – Fertilisers | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 | 54 |

Table 2.1 Biological hierarchy v biological components

| | Genes | Organism | Population - Community | Species | Ecosystem - Habitat | Bioregions – Bioprovinces |
|------------------------------|-------|----------|---------------------------|---------|------------------------|------------------------------|
| Bacteria- Viruses-Fungi | 1 | 9 | 17 | 25 | 33 | 41 |
| Plants | 2 | 10 | 18 | 26 | 34 | 42 |
| Insects | 3 | 11 | 19 | 27 | 35 | 43 |
| Other invertebrates | 4 | 12 | 20 | 28 | 36 | 44 |
| Birds | 5 | 13 | 21 | 29 | 37 | 45 |
| Mammals | 6 | 14 | 22 | 30 | 38 | 46 |
| Reptiles-Fish- Amphibians | 7 | 15 | 23 | 31 | 39 | 47 |
| Man | 8 | 16 | 24 | 32 | 40 | 48 |

Table 2.2 Biological hierarchy v biological processes

| | Genes | Organism | Population – Community | Species | Ecosystem - Habitat | Bioregions – Bioprovinces |
|---|-------|----------|------------------------|---------|---------------------|---------------------------|
| Development- Reproduction- Growth | 1 | 9 | 17 | 25 | 33 | 41 |
| Excretion | 2 | 10 | 18 | 26 | 34 | 42 |
| Movement- Behaviour | 3 | 11 | 19 | 27 | 35 | 43 |
| Predation- Nutrition- Parasitism | 4 | 12 | 20 | 28 | 36 | 44 |
| Death | 5 | 13 | 21 | 29 | 37 | 45 |
| Selection- Mutation | 6 | 14 | 22 | 30 | 38 | 46 |
| Competition | 7 | 15 | 23 | 31 | 39 | 47 |
| Bioaccumulation | 8 | 16 | 24 | 32 | 40 | 48 |

Table 2.3 Biological hierarchy v physical processes

| | Genes | Organism | Population – Community | Species | Ecosystem - Habitat | Bioregions – Bioprovinces |
|-----------------------------|-------|----------|------------------------|---------|---------------------|---------------------------|
| Wind movement | 1 | 9 | 17 | 25 | 33 | 41 |
| Water movement | 2 | 10 | 18 | 26 | 34 | 42 |
| Soil movement | 3 | 11 | 19 | 27 | 35 | 43 |
| Evaporation - Precipitation | 4 | 12 | 20 | 28 | 36 | 44 |
| Fire | 5 | 13 | 21 | 29 | 37 | 45 |
| Freezing | 6 | 14 | 22 | 30 | 38 | 46 |
| Lightning | 7 | 15 | 23 | 31 | 39 | 47 |
| Inversion | 8 | 16 | 24 | 32 | 40 | 48 |

Table 2.4 Biological hierarchy v physical components

| | Genes | Organism | Population - Community | Species | Ecosystem - Habitat | Bioregions – Bioprovinces |
|---|-------|----------|---------------------------|---------|------------------------|------------------------------|
| Atmospheric Air - Interstitial air | 1 | 7 | 13 | 19 | 25 | 31 |
| Surface water - Atmospheric water | 2 | 8 | 14 | 20 | 26 | 32 |
| Groundwater - Interstitial water | 3 | 9 | 15 | 21 | 27 | 33 |
| Seawater | 4 | 10 | 16 | 22 | 28 | 34 |
| Gravity - Magnetism – Static electricity | 5 | 11 | 17 | 23 | 29 | 35 |
| Temperature | 6 | 12 | 18 | 24 | 30 | 36 |

Table 2.5 Biological hierarchy v chemical components and processes

| | Genes | Organism | Population - Community | Species | Ecosystem - Habitat | Bioregions – Bioprovinces |
|---------------------------|-------|----------|---------------------------|---------|------------------------|------------------------------|
| Inorganics | 1 | 6 | 11 | 16 | 21 | 26 |
| Organics | 2 | 7 | 12 | 17 | 22 | 27 |
| Gene expression | 3 | 8 | 13 | 18 | 23 | 28 |
| Cycles | 4 | 9 | 14 | 19 | 24 | 29 |
| Creation - destruction | 5 | 10 | 15 | 20 | 25 | 30 |

Table 2.6 Biological hierarchy v man-made components

| | Genes | Organism | Population - Community | Species | Ecosystem - Habitat | Bioregions – Bioprovinces |
|-----------------------------|-------|----------|---------------------------|---------|------------------------|------------------------------|
| Machinery | 1 | 7 | 13 | 19 | 25 | 31 |
| Building | 2 | 8 | 14 | 20 | 26 | 32 |
| Roads - Tracks | 3 | 9 | 15 | 21 | 27 | 33 |
| Fences | 4 | 10 | 16 | 22 | 28 | 34 |
| Clothes | 5 | 11 | 17 | 23 | 29 | 35 |
| Pesticides - Fertilisers | 6 | 12 | 18 | 24 | 30 | 36 |

Table 3.1 Biological components v biological processes

| | Bacteria- Viruses-Fungi | Plants | Insects | Other invertebrates | Birds | Mammals (incl. farm animals) | Reptiles-Fish- Amphibians | Man |
|---|----------------------------|--------|---------|------------------------|-------|------------------------------------|------------------------------|-----|
| Development - Reproduction - Growth | 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 |
| Excretion | 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 |
| Movement - Behaviour | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 |
| Predation - Nutrition - Parasitism | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 |
| Death | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 |
| Selection - Mutation | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 |
| Competition | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 |
| Bioaccumulati on | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 |

Table 3.2 Biological components v physical processes

| | Bacteria- Viruses-Fungi | Plants | Insects | Other invertebrates | Birds (incl. farm animals) | Mammals (incl. farm animals) | Reptiles-Fish- Amphibians | Man |
|--------------------------------|----------------------------|--------|---------|------------------------|-------------------------------|------------------------------------|------------------------------|-----|
| Wind movement | 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 |
| Water movement | 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 |
| Soil movement | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 |
| Evaporation - Precipitation | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 |
| Fire | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 |
| Freezing | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 |
| Lightning | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 |
| Inversion | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 |

Table 3.3 Biological components v physical components

| | Bacteria- Viruses-Fungi | Plants | Insects | Other invertebrates | Birds (incl. farm animals) | Mammals (incl. farm animals) | Reptiles-Fish- Amphibians | Man |
|---|----------------------------|--------|---------|------------------------|-------------------------------|------------------------------------|------------------------------|-----|
| Atmospheric Air - Interstitial air | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 |
| Surface water - Atmospheric water | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 |
| Groundwater - Interstitial water | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 |
| Seawater | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 |
| Gravity - Magnetism – Static electricity | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 |
| Temperature | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |

Table 3.4 Biological components v chemical components and processes

| | Bacteria- Viruses-Fungi | Plants | Insects | Other invertebrates | Birds (incl. farm animals) | Mammals (incl. farm animals) | Reptiles-Fish- Amphibians | Man |
|--------------------------|----------------------------|--------|---------|------------------------|-------------------------------|------------------------------------|------------------------------|-----|
| Inorganics | 1 | 6 | 11 | 16 | 21 | 26 | 31 | 36 |
| Organics | 2 | 7 | 12 | 17 | 22 | 27 | 32 | 37 |
| Gene expression | 3 | 8 | 13 | 18 | 23 | 28 | 33 | 38 |
| Cycles | 4 | 9 | 14 | 19 | 24 | 29 | 34 | 39 |
| Creation- Destruction | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 |

Table 3.5 Biological components v man-made components

| | Bacteria- Viruses-Fungi | Plants | Insects | Other invertebrates | Birds (incl. farm animals) | Mammals (incl. farm animals) | Reptiles-Fish- Amphibians | Man |
|-----------------------------|----------------------------|--------|---------|------------------------|-------------------------------|------------------------------------|------------------------------|-----|
| Machinery | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 |
| Building | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 |
| Roads - Tracks | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 |
| Fences | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 |
| Clothes | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 |
| Pesticides - Fertilisers | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |

Table 4.1 Biological processes v physical processes

| | Development- Reproduction- Growth | Excretion | Movement- Behaviour | Predation- Nutrition- Parasitism | Death | Selection- Mutation | Competition | Bio- accumulation |
|--------------------------------|---|-----------|------------------------|--|-------|------------------------|-------------|----------------------|
| Wind movement | 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 |
| Water movement | 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 |
| Soil movement | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 |
| Evaporation - Precipitation | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 |
| Fire | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 |
| Freezing | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 |
| Lightning | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 |
| Inversion | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 |

Table 4.2 Biological processes v physical components

| | Development- Reproduction- Growth | Excretion | Movement- Behaviour | Predation- Nutrition- Parasitism | Death | Selection- Mutation | Competition | Bio- accumulation |
|---|---|-----------|------------------------|--|-------|------------------------|-------------|----------------------|
| Atmospheric Air – Interstitial air | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 |
| Surface water - Atmospheric water | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 |
| Groundwater - Interstitial water | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 |
| Seawater | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 |
| Gravity - Magnetism – Static electricity | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 |
| Temperature | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |

Table 4.3 Biological processes v physical chemical components and processes

| | Development- Reproduction- Growth | Excretion | Movement- Behaviour | Predation- Nutrition- Parasitism | Death | Selection- Mutation | Competition | Bio- accumulation |
|--------------------------|---|-----------|------------------------|--|-------|------------------------|-------------|----------------------|
| Inorganics | 1 | 6 | 11 | 16 | 21 | 26 | 31 | 36 |
| Organics | 2 | 7 | 12 | 17 | 22 | 27 | 32 | 37 |
| Gene expression | 3 | 8 | 13 | 18 | 23 | 28 | 33 | 38 |
| Cycles | 4 | 9 | 14 | 19 | 24 | 29 | 34 | 39 |
| Creation- Destruction | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 |

Table 4.4 Biological processes v man-made components

| | Development- Reproduction- Growth | Excretion | Movement- Behaviour | Predation- Nutrition- Parasitism | Death | Selection- Mutation | Competition | Bio- accumulation |
|-----------------------------|---|-----------|------------------------|--|-------|------------------------|-------------|----------------------|
| Machinery | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 |
| Building | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 |
| Roads - Tracks | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 |
| Fences | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 |
| Clothes | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 |
| Pesticides - Fertilisers | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |

Table 5.1 Physical processes v physical components

| | Wind movement | Water movement | Soil movement | Evaporation-Precipitation | Fire | Freezing | Lightning | Inversion |
|--|---------------|----------------|---------------|---------------------------|------|----------|-----------|-----------|
| Atmospheric Air - Interstitial air | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 |
| Surface water - Atmospheric water | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 |
| Groundwater - Interstitial water | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 |
| Seawater | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 |
| Gravity - Magnetism – Static electricity | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 |
| Temperature | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |

Table 5.2 Physical processes v chemical processes and components

| | Wind movement | Water movement | Soil movement | Evaporation-Precipitation | Fire | Freezing | Lightning | Inversion |
|----------------------|---------------|----------------|---------------|---------------------------|------|----------|-----------|-----------|
| Inorganics | 1 | 6 | 11 | 16 | 21 | 26 | 31 | 36 |
| Organics | 2 | 7 | 12 | 17 | 22 | 27 | 32 | 37 |
| Gene expression | 3 | 8 | 13 | 18 | 23 | 28 | 33 | 38 |
| Cycles | 4 | 9 | 14 | 19 | 24 | 29 | 34 | 39 |
| Creation-Destruction | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 |

Table 5.3 Physical processes v man-made components

| | Wind movement | Water movement | Soil movement | Evaporation-Precipitation | Fire | Freezing | Lightning | Inversion |
|--------------------------|---------------|----------------|---------------|---------------------------|------|----------|-----------|-----------|
| Machinery | 1 | 7 | 13 | 19 | 25 | 31 | 37 | 43 |
| Building | 2 | 8 | 14 | 20 | 26 | 32 | 38 | 44 |
| Roads - Tracks | 3 | 9 | 15 | 21 | 27 | 33 | 39 | 45 |
| Fences | 4 | 10 | 16 | 22 | 28 | 34 | 40 | 46 |
| Clothes | 5 | 11 | 17 | 23 | 29 | 35 | 41 | 47 |
| Pesticides - Fertilisers | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |

Table 6.1 Physical components v chemical processes and components

| | Atmospheric Air - Interstitial air | Surface water - Atmospheric water | Groundwater - Interstitial water | Seawater | Gravity - Magnetism - Static electricity | Temperature |
|--------------------------|--|---|--|----------|---|-------------|
| Inorganics | 1 | 6 | 11 | 16 | 21 | 26 |
| Organics | 2 | 7 | 12 | 17 | 22 | 27 |
| Gene expression | 3 | 8 | 13 | 18 | 23 | 28 |
| Cycles | 4 | 9 | 14 | 19 | 24 | 29 |
| Creation- Destruction | 5 | 10 | 15 | 20 | 25 | 30 |

Table 6.2 Physical components v man-made components

| | Atmospheric Air - Interstitial air | Surface water - Atmospheric water | Groundwater - Interstitial water | Seawater | Gravity - Magnetism - Static electricity | Temperature |
|-----------------------------|--|---|--|----------|---|-------------|
| Machinery | 1 | 7 | 13 | 19 | 25 | 31 |
| Building | 2 | 8 | 14 | 20 | 26 | 32 |
| Roads - Tracks | 3 | 9 | 15 | 21 | 27 | 33 |
| Fences | 4 | 10 | 16 | 22 | 28 | 34 |
| Clothes | 5 | 11 | 17 | 23 | 29 | 35 |
| Pesticides - Fertilisers | 6 | 12 | 18 | 24 | 30 | 36 |

Table 7.1 Chemical processes and components v man-made components

| | Inorganics | Organics | Gene expression | Cycles | Creation-Destruction |
|--------------------------|------------|----------|-----------------|--------|----------------------|
| Machinery | 1 | 7 | 13 | 19 | 25 |
| Building | 2 | 8 | 14 | 20 | 26 |
| Roads - Tracks | 3 | 9 | 15 | 21 | 27 |
| Fences | 4 | 10 | 16 | 22 | 28 |
| Clothes | 5 | 11 | 17 | 23 | 29 |
| Pesticides - Fertilisers | 6 | 12 | 18 | 24 | 30 |