

An ecologically-based method for selecting ecological indicators for assessing risks to biological diversity from genetically-engineered plants

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Abstract: The environmental risks associated with genetically-engineered (GE) organisms have been controversial, and so have the models for the assessment of these risks. We propose an ecologically-based environmental risk assessment (ERA) model that follows the 1998 USEPA guidelines, focusing on potential adverse effects to biological diversity. The approach starts by (1) identifying the local environmental values so the ERA addresses specific concerns associated with local biological diversity. The model simplifies the indicator endpoint selection problem by (2) classifying biological diversity into ecological functional groups and selecting those that deliver the identified environmental values. (3) All of the species or ecosystem processes related to the selected functional groups are identified and (4) multi-criteria decision analysis (MCDA) is used to rank the indicator endpoint entities, which may be species or ecological processes. MCDA focuses on those species and processes that are critical for the identified ecological functions and are likely to be highly exposed to the GE organism. The highest ranked indicator entities are selected for the next step. (5) Relevant risk hypotheses are identified. Knowledge about the specific transgene and its possible environmental effects in other countries can be used to assist development of risk hypotheses. (6) The risk hypotheses are ranked using MCDA with criteria related to the severity of the potential risk. The model emphasizes transparent, expert-driven, ecologically-based decision-making and provides formal methods for completing a screening level-ERA that can focus ERA on the most significant concerns. The process requires substantial human input but the human capital is available in most countries and regions of the world.

Key words: genetically engineered organisms; environmental risk assessment; ecosystem services

1 INTRODUCTION

Environmental risk assessment (ERA) models for genetically-engineered (GE) organisms can be informed by conventional eco-toxicological models or

more recent exotic species models (Andow & Hilbeck, 2004), which has contributed to various suggestions about appropriate models for ERA (Andow *et al.*, 2006a; Cowgill & Atkinson, 2003; Dutton *et al.*, 2003;

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Garcia-Alonso *et al.*, 2006; Hilbeck *et al.*, 2008; Romeis *et al.*, 2008). In the first view, the GE organism is merely a species containing a novel or modified chemical, which motivates an eco-toxicological approach to ERA for GE organisms (Romeis *et al.*, 2008). However, GE organisms are not merely organisms with novel chemicals. Gene-gene interactions and genotype-environment interactions are fundamental to the ontogenetic process, which is itself channelized or plastic and molded by evolution, and the ecotoxicology model may be inadequate as a general approach for environmental risk assessment for present and future GE plants (Andow *et al.*, 2006a).

The second perspective views the GE organism like an exotic species and relies on expert judgment to identify potential adverse effects and assess the associated risks qualitatively (Orr *et al.*, 1993). It is doubtful that a GE organism is so different from the source species that it could be justifiably consider to be a novel species. More probably, a comparison with the non-GE parent organism or genotype will illuminate the potential environmental effects associated with the GE organism, as has been commonly done (NRC, 1987).

The aim of this paper is to outline a risk assessment model for GE organisms that is a blend of the strengths of the two models, using advances in ecology to organize the model and prioritize ERA research into affordable and manageable projects. Here we outline the first step of an ecologically-based ERA, considering first today's commercial GE plants. These are primarily maize, soybean, cotton, and canola with a herbicide tolerance (HT) gene and/or an insect resistance (IR) gene. Future GE plants will likely span a broader range of species, traits, environments and activities, including different types of insect resistance genes (Shahidi-Noghabi *et al.*, 2009), stress tolerance (Hu *et al.*, 2006), reduced lignin content (Kawaoka *et al.*, 2006), modified biosynthesis (Degenhardt *et al.*, 2009), pharmaceuticals, forests, landscaping, biological control, altered nutrient content, production of industrial chemicals, biofuels, and

bioremediation (Naranjo & Vicente, 2008). Our framework will be useful for ERA for these future GE plants as well. We address two issues associated with the first step of an ERA: what is the stressor (aka hazardous substance) that could cause environmental effects, and what are useful ecological indicators that are relevant to the problem.

2 STRESSOR IDENTIFICATION

A GE plant is one with an altered genetic composition that has not been generated via sexual or vegetative reproduction, and nearly always requires some *in vitro* manipulation of the genome to alter its genomic composition. This broad definition includes both trait deletions and additions, which can be of genetic material from the same or different species. Variation in the scope of environmental risk regulation for GE organisms has been a contentious issue (Andow & Zwahlen, 2006; NRC, 2000, 2002, 2004), but there remains broad scientific consensus that ERA must consider the introduced trait, the organism that is transformed, the environment into which the GE organism will be released, and interactions among these (CBD, 2012; ESFA, 2010b; NRC, 1987; Snow *et al.*, 2005). Beyond this, there have been relatively few points of consensus, and the risk assessment process has been undergoing continual development and evaluation by groups with differing perspectives and priorities (Andow *et al.*, 2006b; Romeis *et al.*, 2006; Waltz, 2009).

A stressor is any physical, chemical, or biological entity that can induce an adverse environmental response, either directly or indirectly (US EPA, 1998). GE organisms typically comprise multiple stressors (US EPA, 1998)—the transgene product, the inserted transgene/s, and the GE organism itself are all potential stressors. Any of these may generate secondary stressors, which are components of the ecosystem on which the primary stressor acts, which in turn can induce adverse environmental responses (US EPA, 1998). The transgene product, typically a protein or RNA, can have properties similar to environmental toxins, and in those cases, can be assessed using eco-

toxicological methods. The transgene product may produce environmental effects directly or indirectly. For example, Cry1Ac in *Bt* cotton, may kill heliothine pests (a direct effect) thereby enabling secondary, non-target pests to become major pests (an indirect effect) (Lu *et al.*, 2010; Zeilinger *et al.*, 2011).

In some cases, the effects associated with secondary stressors may be more important for ERA than the original product itself. These may include biosynthetic, regulatory, and degradation products of the original transgene product. For example, β -carotene-producing GM rice is engineered to produce two protein enzymes, phytoene synthase and *crt1* (Ye *et al.*, 2000). These enzymes complete the biosynthetic pathway for the synthesis of β -carotene in rice seeds, making β -carotene a secondary stressor. The quantities of phytoene synthase and *crt1* are small compared to the quantities of β -carotene, so the ERA might focus on a secondary stressor (the enzyme product) rather than the primary stressors (the enzymes).

The transgene itself can also be a stressor, independently of the transgene product. Effects associated with transgene flow depend on the transgene, not its product. Transgene flow can reduce genetic diversity in recipient wild populations and contaminate non-GE commercial seed. The transgene can also have effects, depending on where it is integrated in the recipient genome. If a transgene integrates into a functional locus in the recipient genome, it would likely affect the expression of that locus. While such events can be minimized during the development of a GE organism (Tran *et al.*, 2008), a change in expression of the functional locus would be a secondary stressor.

Finally, the GE organism can also be a stressor, independent of the transgene product and transgene. It can act as a whole organism, a seed or pollen grain via food webs, ecosystem functions, and human-mediated activities. Industrial waste from GE low-lignin trees could possibly have higher decomposition rates (Weedon *et al.*, 2009), in turn releasing greenhouse gases. For some GE traits, such as drought tolerance in plants, the GE organism would probably be the fo-

cus of the risk assessment. GE organisms that are trait-deleted are not expected to produce new gene products, so ERA of these cannot be done using ecotoxicological methods because there is no chemical product produced by the GM organism. Unintended environmental effects could derive from the interruption of plant gene functions by the trait deletion, so an ERA would focus on the whole GE plant as the stressor.

The fact that a GE organism comprises multiple stressors means that an ecotoxicological model will have limited applicability because that approach focuses only on one of the possible stressors, the transgene product. An exotic species model also will have limited application, because it focuses only on one of the possible stressors, the GE organism.

3 IDENTIFYING INDICATORS OF RISK

Environmental indicators are used to characterize the effects that human activities exert on the environment (Heink & Kowarik, 2010). Three major kinds of adverse environmental effects have been identified for GE organisms (NRC, 2002; Snow *et al.*, 2005; Snow & Moran-Palma, 1997): (1) Adverse effects associated with gene flow and its consequences; (2) Adverse effects on biological diversity; and (3) Adverse evolutionary effects in the organisms associated with the GE organism, such as evolution of resistance in the organism to transgene products. In this paper we focus on the adverse effects on biological diversity, including species and ecosystem processes.

Our model follows the structure of the US EPA (1998) model, and is a classic "tiered" ERA (Andow & Zwahlen, 2006), designed to focus the ERA on the most significant risks. This model is not prescriptive and readily adjusts to differing spatial, temporal and organizational scales. By focusing on the most significant risks, it can minimize the average cost of an ERA. The model does not commit a regulatory authority to a specific analytical method for estimating risk, and it encourages methodological innovation so that the final ERA is useful for regulators and decision-makers. This flexibility would enable a country to deter-

mine how best to assess its own concerns. Earlier versions of this ERA model were developed by scientists involved in the "GMO Guidelines" and "GMO ERA" projects that worked on case studies involving GE insecticidal plants in different countries of the developing world (Andow & Hilbeck, 2004; Birch *et al.*, 2004; Hilbeck *et al.*, 2006a, 2008).

Identifying relevant indicators for an ERA can be accomplished via the following six step process (Fig. 1).

(1) Identify the important environmental values that could be compromised by the GE plant in the recipient local/regional environment, and identify possible adverse changes to those values.

(2) Identify the ecological processes and functional groups that are necessary for the continued existence of those environmental values. Possible environmental risks associated with *unselected* ecological processes and functional groups are judged to be below the level of concern (*de minimis* risks) and are not evaluated further.

(3) Identify possible indicator endpoint entities (e. g., list up possible indicator species).

(4) Rank these indicator entities using multi-criteria decision analysis (MCDA) and select ones most closely related to environmental risk. This yields a short list of assessment endpoint entities that are associated with the possible adverse effects, and are the indicators of possible environmental risks. The many possible adverse effects associated with *unselected* endpoint entities simultaneously are judged *de minimis*, insignificant, or acceptable, and are not evaluated further.

(5) Identify plausible risk hypotheses based on known ecological interactions starting from the GE organism and ending with the selected endpoint entities. Risk hypotheses comprise possible exposure pathways and causal mechanisms leading to an adverse environmental effect. They can consist of an indirect sequence of exposure-effects pathways.

(6) Rank the risk hypotheses using MCDA and select ones most closely related to environmental risk. The risk hypotheses that are selected will clearly specify the attributes of the endpoint entities that should be

assessed. The many possible risk hypotheses that are *unselected* are considered *de minimis*, insignificant, or acceptable, and are not evaluated further. It will usually be necessary to evaluate the selected risk hypotheses using some combination of laboratory, greenhouse, and/or field experiments (Andow *et al.*, 2008).

The model guides attention onto the most important potential adverse environmental effects using the most important environmental indicator entities guided by the most important risk hypotheses. It concentrates on human values, considers principles of ecological structure, and specific ecological knowledge associated with the GE plant, and therefore should be possible to generalize to other GE organisms. The model is a screening-level ERA (SL-ERA, US EPA, 1998). SL-ERAs are structured to provide a high level of confidence in determining *de minimis* risks, which are eliminated from further consideration. Our model does this for environmental values, indicator endpoint entities, and risk hypotheses. SL-ERAs are not designed nor intended to provide definitive estimates of actual risk. Rather, their purpose is to assess the need, and if required, the level of effort necessary, to conduct a definitive ERA.

3.1 Identify environmental values and possible adverse environmental effects

Damage, harm, or adverse effects can occur to things that people value. Thus the model starts by identifying the important environmental values in the intended receiving environment (Malone *et al.*, 2010; Nelson & Banker, 2007). An ERA, logically, should identify the environmental values which a society does not want to damage. These include both instrumental values (e. g., ecosystem services) (Lövei, 2001; MEA, 2005) and intrinsic or existence values (e. g. biodiversity *per se*) (Bowman, 2002). Ecosystem provisioning services should not be damaged. Provisioning services include agricultural, forestry, fisheries, apicultural, and sericultural production (MEA, 2005). Ecosystem services that support these provisioning services, such as biological control and pollination, should also not be damaged. Cultural values are frequently

Screening-Level ERA

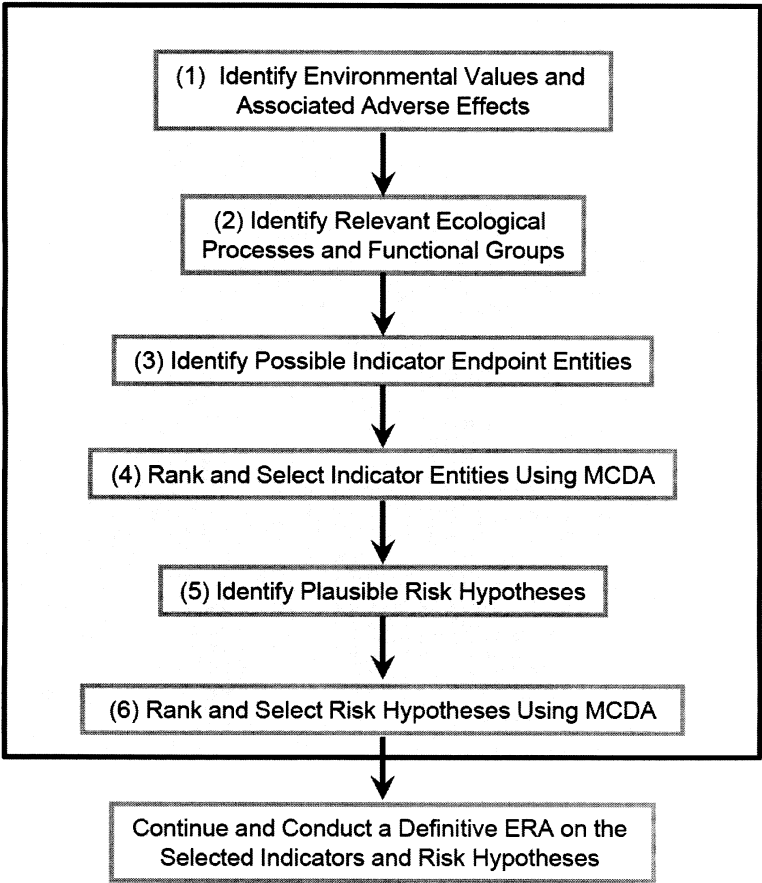


Fig. 1 Steps in an ecologically-based screening-level (SL) ERA for biological diversity
A SL-ERA identifies issues that need to be assessed in a definitive ERA.

overlooked. Some plants and animals have an important cultural role in society. For example, bald eagles *Haliaeetus leucocephalus* L. and monarch butterflies *Danaus plexippus* L. are cultural icons in the US. The Bhodi tree *Ficus religiosa* L. is an important cultural icon in South and Southeast Asia. Killing or weakening these species is often unacceptable in those societies. Direct valuation of intrinsic and existence values is notoriously difficult, so we suggest it be done indirectly through the supporting ecosystem services. For example, human health is intrinsically valued, and rather than attempt to value a human life directly, we can identify the instrumental ecosystem services that are necessary to maintain or improve human health and value these services. One such service is those ecological factors that regulate the abundance, distribution or virulence of a human disease vector. Thus, an ERA

might focus on incidence of a human disease, which society would want to avoid, rather than the more nebulous concept of human health.

Not all of these values will always be relevant when assessing potential risks of different GE organisms and receiving environments. For GE insecticidal plants, the most relevant kinds of adverse effects and associated environmental values include (Andow *et al.*, 2008): (a) adverse effects on crop or animal production; (b) reduced soil health, soil quality, or water quality; (c) reduced value of economic activities not directly related to the production of the GE plant, such as honey or silk production; (d) reduced cultural value (spiritual, aesthetic, etc.) (CEC, 2004; Losey *et al.*, 1999); (e) increased conservation concern (loss of rare species); (f) impaired ecosystem services or reduced sustainability (e. g. loss of

species involved in biological control or pollination); and (g) increased human disease via environmental change (WHO, 2000). All of these should be considered for every GE insecticidal plant during this first step, but only a subset of these are likely to be relevant in any particular case.

For example, a Vietnam case study (Andow *et al.*, 2008) considered the introduction of *Bt* cotton and concluded that potential adverse effects include reduced productivity of cotton and nearby crops, impaired soil health, adverse effects on honey production by bees, and effects on pollinators and butterflies. The most important of these values was associated with cotton production, because the livelihoods of many poor small-scale farmers depend on it. Soil health is vital for long-term agricultural productivity. Honey is a major export commodity, so a GE plant should not interfere with this market. The instrumental value of pollinators and conservation value of butterflies was recognized. Such initial choices greatly simplify the scope of the subsequent ERA.

3.2 Identify relevant ecological processes and functional groups

The second step of the model connects ecological processes and functional groups to the possible adverse environmental effects associated with the identified environmental values. A functional (or guild) group is a group of species that carries out a similar ecological role in a similar manner (Root, 1967). The different taxa in the functional group contribute to maintain an ecological process or ecosystem service. Most agricultural fields in temperate regions contain many hundreds or a few thousands (Mészáros *et al.*, 1984) of species, and even more species are found in tropical, small-scale farming systems. It is clearly impractical to assess all of these species, nor are all of equal importance. By organizing and grouping biological diversity into ecological functions, the model simplifies the assessment of diverse taxonomic groups and species, which is particularly helpful in megadiverse countries. Other risk assessment models (Malone *et*

al., 2010; Romeis *et al.*, 2008) do not use this functional approach.

Because the potential adverse effects will differ for each GE plant, the functions and functional groups of concern will also differ. A broad perspective at this initial stage is essential, in order not to overlook critical functional groups, and, thereby, erroneously omit them from the ERA. In the process, knowledge gaps are identified early in the ERA process, so these can be timely addressed.

The choice of functional groups to be included in the ERA for each receiving environment is guided by the values and possible adverse environmental effects identified in step 1. At this step the kind of trait in the GE plant becomes important because the trait may cause changes in management practices, which can have adverse environmental effects. For example, a herbicide-tolerant GE plant is likely to change the herbicide treatment regime, which will alter the weed community, which in turn may affect other species associated with those weeds (Haughton *et al.*, 2003). Thus, in this case, weeds would be an important functional group to consider. Insect resistant GE plants may reduce or shift insecticide use, releasing previously controlled pests and reducing crop yields (Lu *et al.*, 2010; Zeilinger *et al.*, 2011). In that situation, insect herbivores (both pest and non-pest species) would be an important group to consider.

The relevant functional groups will also depend on the biology of the plant being transformed. For example, if the plant (such as canola) requires insect pollinators to ensure or enhance yield, assessing the impact on the pollinator group would be important for evaluating the effects on crop production. For wind pollinated plants (e.g., maize), bees that feed on pollen could be evaluated in relation to potential adverse effects on honey production, other bee products and (possibly) the pollination of nearby crops. Information about how the plant interacts with other organisms can be used to identify additional, potentially affected functional groups.

**Table 1 Association of ecological functional groups with specific adverse effects
for consideration in ERA for *Bt* cotton in Vietnam**

Functional Groups	Potential Adverse Effects						
	Crop ↓	Soil health ↓	Non-crop economic value ↓	Cultural value ↓	Conservation concern ↑	Environmental quality ↓	Human & animal disease ↑
Pest herbivores							
Predators/Parasitoids							
Pollinators of crop							
Soil decomposers							
Endangered species							
Plant disease							

Dark boxes indicate the chosen associations, and cross-hatched boxes are ones that were considered (Andow *et al.*,2008). Arrows indicate the direction of change in the effect that is considered adverse.

For GE cotton in Vietnam, several functional groups were considered in relation to the potential adverse effect on crop production (Table 1) (Andow *et al.*,2008). The functional groups adversely affecting production were invertebrate pests, pathogens, and weeds of cotton. Functional groups that can improve cotton production include pollinators and plant growth promoting organisms, such as mycorrhizal fungi, endophytic bacteria, free-living nitrogen fixing bacteria, and beneficial rhizosphere bacteria. Other ecologically significant functional groups can affect the population dynamics of the above groups, and include organisms providing biological control, such as predators, parasitoids and pathogens. Because of the high value accorded to cotton production in Vietnam, four functional groups were selected: non-target herbivore pests (including insect disease vectors), predators, parasitoids, and pollinators. The most important plant disease (cotton blue disease), caused by a virus (Correa

et al.,2005) is an insect-vectorred disease, and this vector (cotton aphid) was included in the non-target herbivore pest group (Nguyen *et al.*,2008).

Using a functional classification of biological diversity in this way significantly reduces the complexity of an ERA, because it results in the selection of few functional groups that address the potentially important adverse effects associated with a GE plant. For example, for a herbicide tolerant soybean (Table 2), the functional groups would probably include pest herbivores, soil decomposers, endangered plant species, weeds, and useful plants. These selected functional groups contain only a small fraction of the total biological diversity associated with the GE plant and although the species are likely to vary from region to region, this focuses the ERA on the appropriate group of species, while at the same time, eliminating from consideration many species associated with the unselected functional groups.

Table 2 Association of ecological functional groups with specific adverse effects for consideration in ERA for an herbicide tolerant soybean

Functional Groups	Potential Adverse Effects						
	Crop ↓	Soil health ↑	Non-crop economic value ↓	Cultural value ↓	Conservation concern ↑	Environmental quality ↓	Human & animal disease ↑
Pest herbivores							
Predators/Parasitoids							
Pollinators of crop							
Soil decomposers							
Endangered species							
Weeds							
Useful plants							

Dark boxes indicate possible associations that would be reasonable to select. Arrows indicate the direction of change in the effect that is considered adverse.

3.3 Identify potential indicator endpoint entities

By limiting the number of functional groups, we also limit the number of species and ecosystem processes that should be considered. All species and ecosystem processes that are associated with the ecological function should be identified and listed; about 30 ~ 100 species are likely to be listed in any functional group. Some species may be listed in more than one functional group, because many species have multiple functions in an ecosystem.

In some cases, species cannot be easily identified and the effect is better characterised by considering changes in the intensity or status of an ecosystem function. For example, soil health could possibly be measured by following changes in the density of selected soil-dwelling species, but it might more usefully be measured via soil respiration or decomposition of organic matter. In such cases, it may be more efficacious to

concentrate the ERA on the ecosystem process, rather than on the species comprising that function or process.

3.4 Rank and select indicator endpoint entities

The list of species in each functional group can be ranked using multi-criteria decision analysis (MCDA), which is a tool that synthesizes expert judgments transparently (Dodgson *et al.*, 2009; Fenton & Neil, 2001; Kiker *et al.*, 2005). From these ranks, several species will be selected to serve as potential indicator endpoint entities in the ERA.

The criteria are designed to prioritize locally important and geographically widespread species/processes that have the closest association with the GE plant and the most significant role in the ecological function under consideration, for examples see (Andow *et al.*, 2008; Hilbeck *et al.*, 2006). Each criterion receives a qualitative score from 1 (high) to 3 (low). Criteria

related to association (see below) and functional significance are each averaged, and these averages are combined as unweighted additive criteria to give a composite rank, which is used to select possible indicator endpoint entities. A key advantage of MCDA is that it makes the expert judgments transparent.

The association criteria for species include:

- geographic distribution (scored as wide, regional, restricted),
- prevalence in the habitat of the recipient organism (frequency of occurrence in suitable habitat; proportion of suitable habitat patches that are occupied; always, often, or rarely),
- abundance (average population density in occupied habitat; common/abundant, medium or low),
- phenological (temporal) overlap between the recipient organism and the potential endpoint species (throughout the growing period, over most of the growing period, or restricted to one stage or a short time only), and
- habitat specialization (generalist, occurring in many habitats, restricted, or specialist).

The relevance of the above criteria will vary for different functional groups and should be individually evaluated (Hilbeck *et al.*, 2008).

Functional significance is evaluated independently of association. For example, the functional significance criteria for predaceous natural enemies that were considered for *Bt* cotton in Vietnam are (Pham *et al.*, 2008):

- biological control of pests associated with the GE plant,
- food for other natural enemies,
- biological control in nearby crops, and
- biological control in natural areas.

Predators are significant in cotton agricultural ecosystems because of their potential role in the biological control of plant pests. Disruption of the biological control function can lead to flare-ups of secondary pests, resulting in loss of agricultural production or shifts in the species composition in adjacent vegetation (Pham *et al.*, 2008). In addition, some species of

predators may be important intra-guild prey for other species of predators.

The indicator species and ecosystem processes that receive the highest composite rank can be selected and taken to the next step (Fig. 1). Expert judgment, regulatory imperatives, and societal preferences can be used to decide how many of these indicator endpoint entities are retained, but more should be retained than absolutely necessary to minimize type 2 error as is appropriate for SL-ERA. There will be considerable uncertainty in the MCDA due to knowledge gaps. The robustness of the rankings can be evaluated in two ways. High-ranked indicators with many uncertain criteria should be re-examined to determine if they can be excluded from further consideration. This can be done by setting the uncertain criteria to 3 and recalculating the rank. If the indicators retains a high rank, then its rank is robust to uncertainty. If it does not retain a high rank, then it is not robust to uncertainty, and therefore may not be a useful indicator. Similarly, low-ranked, uncertain indicators should be re-examined to determine if they should be included. This can be done by setting the uncertain criteria to 1 and recalculating the rank. If the indicators retain a low rank, then its rank is robust to uncertainty (for more detail see Andow *et al.*, 2008).

If similar taxa are highly ranked, only some should be selected, because they often perform their ecological function in very similar ways, and may be redundant indicators. Instead, we suggest selecting indicators representing a broader range of taxa, because this will allow assessment of a broader range of ecological mechanisms by which a GE plant could cause adverse effects in the environment.

For example, in developing the ERA for *Bt* cotton in Vietnam (Pham *et al.*, 2008), 13 of 67 species of predators in cotton were considered to be widespread and have significant biological control function. Of these 13 species, none had high uncertainty. All species with high uncertainty had low ranks. Robustness analysis indicated that all of these species retained a low rank, so the list of 13 species of predators was robust

Table 3 Highly ranked species of arthropod predators developed in the ERA for genetically-engineered insecticidal cotton in Vietnam (from Pham *et al.*,2008)

Rank	Species or species group	Order: Family
1	<i>Menochilus sexmaculatus</i> (F.)	Coleoptera: Coccinellidae
1	<i>Micraspis discolor</i> (F.)	Coleoptera: Coccinellidae
3	<i>Micraspis vincta</i> (Gorh.)	Coleoptera: Coccinellidae
4	<i>Harmonia octomaculata</i> (F.)	Coleoptera: Coccinellidae
5	<i>Andrallus spinidens</i> F.	Heteroptera: Pentatomidae
5	<i>Eocanthhecona furcellata</i> Wolf.	Heteroptera: Pentatomidae
7	<i>Oxyopes</i> sp.	Araneae: Oxyopidae
7	<i>Lycosa</i> sp.	Araneae: Lycosidae
9	<i>Paederus fuscipes</i> Curtis	Coleoptera: Staphylinidae
9	<i>Paederus tamulus</i> Erich.	Coleoptera: Staphylinidae
11	<i>Ischiondon scutellaris</i> F.	Diptera: Syrphidae
12	<i>Chlaenius xanthopleurus</i> Chaud.	Coleoptera: Carabidae
13	<i>Episyrphus balteatus</i> (Deg.)	Diptera: Syrphidae

Prioritized species are highlighted in grey.

to uncertainty in the ranking process. Of the 13 prioritized species, four were aphidophagous coccinellids (Table 3), two were pentatomids, two were staphylinids and two were syrphids. Given the above taxonomic considerations, only five of these 13 species were retained for the next step (Table 3). These five would be "worst-case indicators" of the reactions of the remaining 62 species.

In the Vietnam *Bt* cotton case, 14 species belonging to four functional groups were selected that were associated with potential adverse effects on cotton production, including the 5 predators just described (Andow *et al.*,2008). By following this model, the vast majority of the >600 identified species (> 98%) that would likely occur in a Vietnamese cotton field were eliminated from further consideration.

3.5 Identify risk hypotheses

Considering all the indicator endpoint entities (species or processes) selected in step 4, plausible risk hypotheses are constructed by coupling exposure of the entity to a stressor with a hypothetical causal scenario that could result in a potentially adverse environmental effect. Risk hypotheses can be constructed readily from known or suspected exposure and adverse

effects pathways. Qualitative event-tree and fault-tree analysis can be helpful in this process.

Construction of risk hypotheses can start with known exposure pathways. Because GE plants comprise multiple stressors, many possible exposure pathways can be identified (Andow & Hilbeck, 2004). Exposure can occur via the food chain, directly to the endpoint species via feeding on GE plant material, or indirectly through tritrophic or multitrophic interactions, through decomposition processes (when decomposers feed on dead plant parts), or by consuming dispersing parts of a GE plant (e.g. , pollen finding its way into aquatic habitats (Rosi-Marshall *et al.*, 2007). Starting with possible exposure pathways, several risk hypotheses can be developed through qualitative event-tree analysis (Hayes *et al.*,2004) by connecting exposure to possible adverse effects via a chain of potential causes. An example of a risk hypothesis developed using a simple event-tree analysis is shown in Fig. 2. When data are available, quantitative event-tree analysis can be performed, where estimated probabilities are associated with each sequential causal event (Hayes *et al.*, 2004 ; NRC,2002).

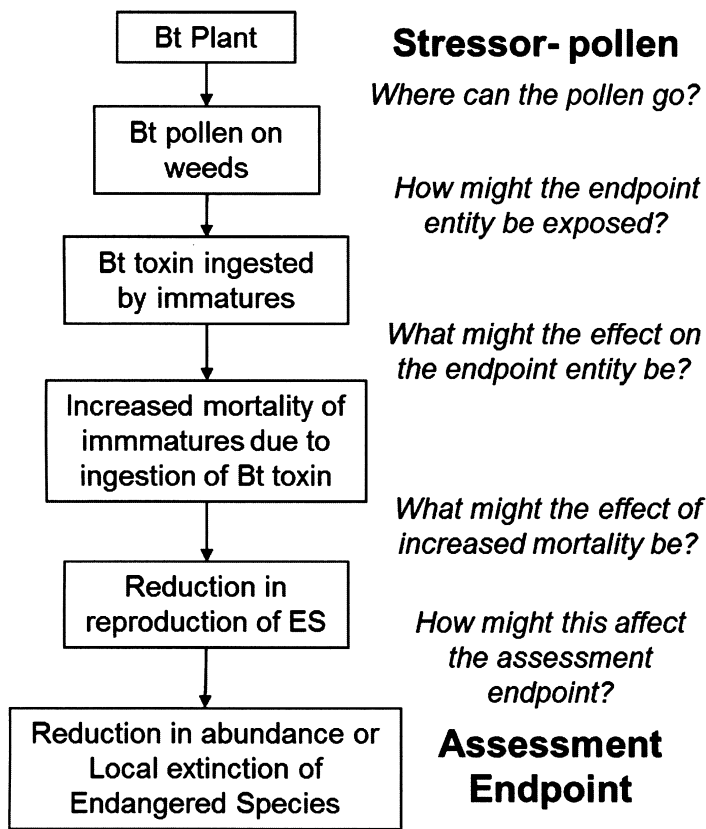


Fig. 2 Simple risk hypothesis from qualitative event-tree analysis

The stressor is a genetically-engineered *Bt* plant. Assessment endpoint is the population size of an endangered species.

An adverse effect pathway is a possible causal chain of events that starts with an exposed indicator endpoint entity and ends with an adverse effect. For species which help to deliver key ecosystem services (Lövei,2001), an adverse effect pathway could begin with a change in a population parameter or a behavior of the indicator species, and end with crop production loss. For an ecological process (e.g. plant residue decomposition), it could begin with a change in the timing, rate or magnitude of the process, and end with a reduction in soil quality. Qualitative fault-tree analysis is a useful technique for systematically identifying multiple risk hypotheses (Hayes,2002; NRC,2002).

Some risk hypotheses for *Bt* cotton in Vietnam that were generated by qualitative fault-tree analysis are shown on Fig. 3. Fault-tree analysis starts with a top event and seeks potential causes until a link to the stressor is found. Starting with a top event of more damage to cotton, three preceding events were connected with an "or gate": higher densities of the pest

insect *Amrasca devastans* Dist., more disease, and a higher density of weeds. An "or gate" indicates that any of the preceding events could cause the subsequent event. More *A. devastans* was connected to four preceding events with an "or gate": increased survival, increased reproduction, decreased emigration, and increased colonization of *A. devastans*. Two of these events were developed further until they could be connected to a stressor from *Bt* cotton. For example, increased survival could be a result of reduced predation by the spider *Oxyopes* (Fig. 3).

Pathways that result from indirect effects of GE organisms are also important. A GE plant may cause changes in management that could cause an adverse effect. Although USA regulation excludes such possibilities from ERA, this is not the case in other countries (e.g. EU Directive 2001/18). Some changes in management practices are deliberately intended, but whether intended or unintended, changes in management could have large environmental consequences.

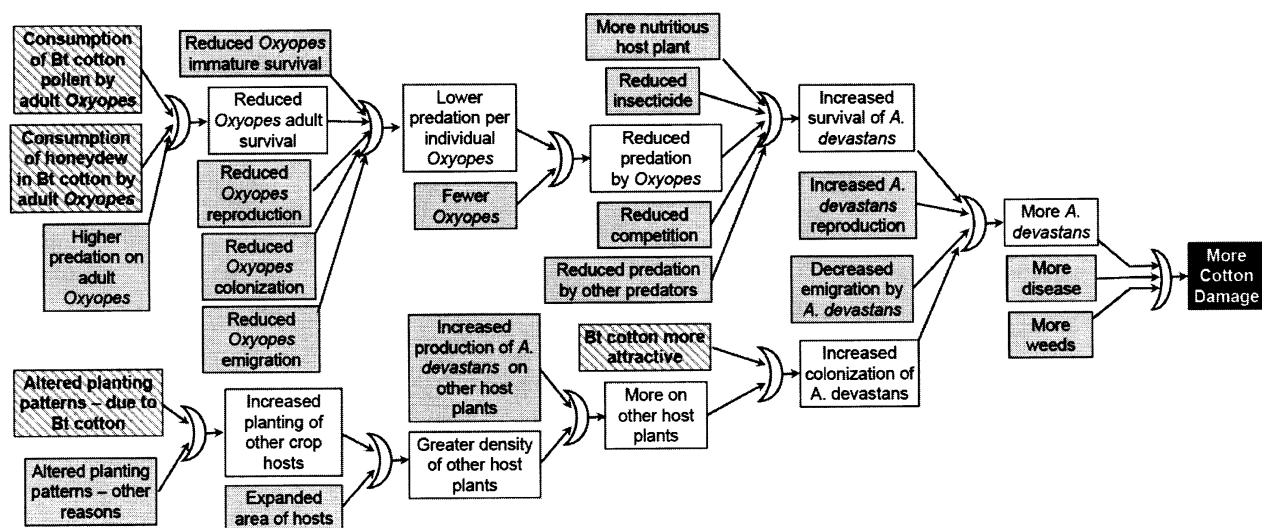


Fig. 3 Risk hypothesis from qualitative fault-tree analysis

The top event is in black box on the right. The stressor is connected via the gray striped boxes. Solid gray boxes indicate undeveloped events.

Boxes are connected with gates, which are all "OR" gates. Only two branches of a full fault-tree analysis are shown because of space limitations. *Oxyopes* is a common predatory spider and *Amrasca devastans* is a leafhopper pest.

The adoption of a GE plant may change crop area, crop rotation or intercropping practices, the seasonal timing of crop production, and previously developed irrigation, tillage, pesticide use, and/or integrated pest management practices (Cerdeira *et al.*, 2007). Such changes in agricultural practices could have a range of environmental effects, including at the landscape scale.

3.6 Rank and select risk hypotheses

Numerous risk hypotheses will be generated for each indicator endpoint entity in Step 4, and they can be ranked using MCDA, using four criteria. First, the entire risk hypothesis can be ranked by (a) its overall likelihood of occurring. The exposure part of the hypothesis can be ranked in terms of its (b) temporal and spatial scale. If exposure is restricted in time, or if exposure is likely to occur only in a limited geographical area, the risk hypothesis may be ranked lower. The potential adverse effect part of the hypothesis can be ranked according to whether it is (c) readily reversible or irreversible (which will also determine whether risk management measures could mitigate the consequences), and whether the (d) affected people consider the magnitude of the consequences to be major and/or unacceptable, or minor and/or acceptable (OGTR, 2005).

Each criterion is ranked 1 (high) to 3 (low), and a combined score is produced by multiplication, because a risk hypothesis that has a high overall rank should be highly ranked for all four criteria. Uncertainty analysis can be incorporated into this MCDA by simulating values for the uncertain criteria. The goal is to identify *de minimis* risks and eliminate them from further consideration, while focusing attention on the potentially larger concerns.

For GE *Bt* cotton in Vietnam, 47 possible risk hypotheses were developed but after ranking, only 15 (32%) were selected for further scrutiny (Andow *et al.*, 2008). Nine of these risk hypotheses concerned potential effects on pollinators (Lê *et al.*, 2008a). Of these, the highest ranked hypothesis was that *Bt* cotton could cause a reduction in *Apis cerana* population density and/or colony quality resulting in reduced production of honey and other bee products. Of the 15 risk hypotheses that were prioritized for additional consideration in Vietnam, (including the aforementioned pollinator hypothesis), only one – the possible effect of GE cotton on transmission of blue disease by cotton aphids – received high ranks for all of the four criteria. Blue disease has recently become the greatest threat to cotton production in Vietnam (Le *et al.*, 2008b). It is an insect-vectored virus disease and can

cause serious yield loss (Correa *et al.*, 2005). If GE cotton induces higher levels of blue disease, small-scale farmers (average farm size, 0.7 ha) could suffer severe losses, a possibility that the country would prefer to avoid. This risk hypothesis can be quantitatively evaluated through a few relatively simple experiments (Nguyen *et al.*, 2008).

Up to this point, the ERA model has not required any laboratory, greenhouse or field experiments. The Vietnam case (Andow *et al.*, 2008) demonstrates that the necessary human capital to conduct the ERA was not excessive, even for a developing country like Vietnam. The model identifies indicator endpoints and risk hypotheses associated with the greatest adverse consequences, thereby allowing targeted and definitive experiments to evaluate the risk quantitatively. These experiments will vary with the risk hypothesis, the GE plant, and the needs of the country, and consequently, are difficult to prescribe and should be developed on a case-by-case basis. An additional advantage of this model is that the identified and selected risk hypotheses should apply for other GE plants of the same crop with similar GE traits that may introduced later into the country. Specifically, the results of the Vietnam case should apply to other GE insecticidal cottons that Vietnam may consider in the future. This allows the costs of the ERA to be spread over multiple ERAs.

4 CONCLUSIONS

The ecologically-based ERA model described here supports hypothesis-driven risk assessment, complies with the provisions in the Cartagena Protocol on Biosafety, the International Plant Protection Convention, and EU Directive 2001/18, and parts have been proposed for use in the EU (EFSA, 2010a, b). The model is based on modern ecological science and takes into account the specifics of the transgene, the GE organism and the intended receiving environment. It provides a well-defined and flexible pathway for requesting and evaluating data around which international systems can be harmonized. Although we have illustrated its use primarily with examples involving GE

plants currently in cultivation, we believe the model is sufficiently general and flexible to screen the risks of new kinds of GE plants and other organisms.

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