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Review

Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating arthropod-resistant genetically engineered crops ☆

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HIGHLIGHTS

- Non-target risk assessment (RA) of transgenic crops is supported by toxicity studies.
- No clear rationale exists for selecting test species for RA of transgenic crops.
- We propose a rationale based on methods used for pesticides and biocontrol agents.
- Species are selected according to their sensitivity, reliability, and relevance.
- This increases the quality and efficiency of RAs for cultivating transgenic crops.

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ABSTRACT

Arthropods form a major part of the biodiversity in agricultural landscapes. Many species are valued because they provide ecosystem services, including biological control, pollination and decomposition, or because they are of conservation interest. Some arthropods reduce crop yield and quality, and conventional chemical pesticides, biological control agents and genetically engineered (GE) crops are used to control them. A common concern addressed in the ecological risk assessment (ERA) that precedes regulatory approval of these pest control methods is their potential to adversely affect valued non-target arthropods (NTAs). A key concept of ERA is early-tier testing using worst-case exposure conditions in the laboratory and surrogate test species that are most likely to reveal an adverse effect. If no adverse effects are observed in those species at high exposures, confidence of negligible ecological risk from the use of the pest control method is increased. From experience with chemical pesticides and biological control agents, an approach is proposed for selecting test species for early-tier ERA of GE arthropod-resistant crops. Surrogate species should be selected that most closely meet three criteria: (i) *Potential sensitivity*: species should be the most likely to be sensitive to the arthropod-active compound based on the known spectrum of activity of the active ingredient, its mode of action, and the phylogenetic relatedness of the test and target species; (ii) *Relevance*: species should be representative of valued taxa or functional groups that are most likely to be exposed to the arthropod-active compound in the field; and (iii) *Availability and reliability*: suitable life-stages of the test species must be obtainable in sufficient quantity and quality, and validated test protocols must be available that allow consistent detection of adverse effects on ecologically relevant parameters. Our proposed approach ensures that the most suitable species are selected for testing and that the resulting data provide the most rigorous test of the risk hypothesis of no adverse effect in order to increase the quality and efficiency of ERAs for cultivation of GE crops.

☆ This paper summarises the outcome of an expert panel convened by the International Organisation for Biological and Integrated Control of Noxious Animals and Plants/West Palaearctic Regional Section (IOBC/WPRS) (http://www.iobc-wprs.org/).

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Contents

1. Introduction

Arthropods form a major part of the biodiversity in agricultural landscapes. Many arthropod species are pests that reduce crop yield and quality. Current pest control methods include conventional chemical pesticides, biological control, and host plant resistance including genetically engineered (GE) crops that produce arthropod-active compounds. Most arthropod species within agricultural landscapes provide ecosystem services, including control of pest arthropods, pollination, and decomposition (Gurr et al., 2003; Mulder, 2006; Kremen et al., 2007). Some arthropods are protected species because they are of conservation value (ESA, 1973; IUCN, 2010). Consequently, certain arthropods or the ecosystem services they provide are regarded as entities to be protected from pest control measures (EFSA, 2010a; Sanvido et al., 2012).

Ecological risk assessments (ERAs) of regulated pest control methods evaluate, among other things, their potential to adversely affect valued non-target arthropods (NTAs). During problem formulation for an ERA, conceptual models are constructed that describe pathways whereby the stressor, in this case the arthropod-active compound or a biological control agent, could harm an arthropod's abundance or ecological functions provided by arthropods. Subsequently, risk hypotheses are formulated and tested (Raybould, 2006, 2011). A common hypothesis is that the stressor does not reduce the abundance of, or functions provided by, valued NTAs under field conditions. This hypothesis is typically tested following a tiered approach that is conceptually similar for the different regulated pest control methods (Touart and Maciorowski, 1997; Hill and Sendashonga, 2003; van Lenteren et al., 2006; Garcia-Alonso et al., 2006; Romeis et al., 2008).

Not all NTAs present in the receiving environment (the ecological area where the pest control technology will be used) can be tested. Consequently, surrogate species must be identified to represent the entities to be protected. Surrogate species are typically used because specific at risk arthropods and test systems are not available or are difficult to develop and because surrogates can provide high quality animals supported by well validated test protocols. Ideally, surrogate species have equal or greater sensitivity to the pesticidal active ingredient or biological control organism than do the species they represent in the ERA and thus knowledge of the effects on these species provides reliable predictions about effects on many other species (Raybould et al., 2011).

Early-tier testing, using worst-case exposure conditions in the laboratory, for adverse effects of stressors on surrogate test species for valued NTAs provides a conservative test of the risk hypothesis. These early-tier tests have high power to detect adverse effects because (i) the impact of the stressor is isolated, (ii) tests can be conducted with many replicates using validated protocols with surrogate arthropods reared under standardised conditions, and (iii) organisms are exposed to concentrations of the toxin exceeding conservative estimates of field exposures (Raybould et al., 2011; Romeis et al., 2011). If no adverse effects are detected under these conditions, ecologically relevant effects in the field can be excluded with high confidence. Accordingly, early-tier testing identifies uses of products that pose negligible ecological risks, allowing assessors to focus on uses that pose significant risks or uncertainties. More complex and realistic higher-tier assessments under semi-field or field conditions are only necessary when adverse effects indicating potentially unacceptable risk have been detected in early tier testing or when unacceptable uncertainties remain. Recent meta-analysis of non-target effects of GE plants producing insecticidal crystal (Cry) proteins derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) showed that laboratory studies "predicted effects that were on average either more conservative than or consistent with effects measured in the field" (Duan et al., 2010). Thus, ERAs based on results of these NTA ecotoxicological tests provide protection of biological control organisms and other non-pest species in and around fields of GE crops (Romeis et al., 2006; Marvier et al., 2007; Wolfenbarger et al., 2008; Naranjo, 2009; Duan et al., 2010).

Other approaches to ERA have proposed the identification and testing of "keystone" (Chapman, 2002) or "ecologically significant" (Andow and Hilbeck, 2004; Birch et al., 2004) species in the receiving environment. These approaches have numerous problems: keystone or ecologically significant species may not be known, may not be testable, may differ among areas in which the GE crop will be grown, or may simply not occur because an ecological function will depend on species diversity rather than the presence of a particular species (Raybould et al., 2011). Furthermore, even if the ecologically most important species were identified and testable, it does not follow that they necessarily should be tested. It is a common mistake to believe that the best way to test the hypothesis of no harm to valued species A is to test species A. Species B may be preferable because it may be more sensitive or more easily tested and thus more likely to show an adverse effect than species A. If species B shows no effect, no further testing may be necessary. If species B were affected, tests, perhaps including species A, could be conducted to characterise the risk further.

The precise array of surrogate NTA species tested for ERAs of currently commercialised GE crops was and is not specified in regulations, although some broad categories are indicated (e.g., US EPA, 1996; Rose 2007; EFSA 2010b). This is in part not to be prescriptive, but also in part because a defined process is not in place. Instead, it evolved from a combination of needs and constraints such as regulatory requirements to test certain groups of species (e.g., pollinators), the availability of suitable test methods, experience with chemical pesticides, and from reviews of regulatory ERAs of the first GE crops (e.g., from the Scientific Advisory Panels of the United States Environmental Protection Agency). Accordingly, a systematic justification of the efficiency and efficacy of the selection of surrogate species for tests in ERAs of GE crops is needed.

In this paper, we examine selection criteria for surrogate species in early-tier tests to support ERAs. Experience from the assessment of chemical pesticides and biological control agents is applied to the selection of test species for the ERA of GE crops. A clearer rationale for surrogate species selection may help in utilising effects data in ERAs for GE crops in countries other than those for which the data were originally obtained (Romeis et al., 2009a). This will be particularly useful for developing countries with limited resources that are developing GE crops to support food security (Raybould and Quemada, 2010; Huesing et al., 2011). We do not propose a list of organisms that must be tested to support the ERA of GE crops because concerns vary among jurisdictions, and therefore we cannot be prescriptive for all ERAs. What we present in this paper are sound criteria for surrogate arthropod species selection for early tier laboratory tests to assess the ecological risks from cultivating arthropod-resistant GE crops.

2. Non-target risk assessment of chemical pesticides: the European system

Evaluation of risks posed by chemical pesticides to the environment is mandatory prior to placing these products on the market (EC, 2009). Side-effect testing of pesticides on beneficial NTAs (i.e., predators and parasitoids) has a long history in Europe (Hassan and Vogt, 2006). Testing was originally performed to identify pesticides that would be compatible with Integrated Pest Management (IPM) and Integrated Crop Management (ICM) practices to provide pest control programmes with limited adverse effects on beneficial arthropods under field conditions. The International Organisation for Biological and Integrated Control of Noxious Animals and Plants/West Palaearctic Regional Section (IOBC/WPRS) developed tiered ERA by outlining iterative tests, selection of test species, and data interpretation. The approach begins by testing a small set of non-target species in the laboratory using worst-case exposures, and moves to more realistic exposures under semi-field or field conditions only if adverse effects are identified at lower tiers. Standard laboratory, semi-field and field methods were developed for numerous beneficial arthropods, including predatory mites, generalist predators, and parasitoids. Several international testing programmes were established, and the results were published as pesticide lists with the corresponding side-effects on beneficial arthropods (Hassan and Vogt, 2006).

Since 1991, studies of each pesticide on NTAs, along with ERAs that are based on the results of those studies, must be submitted in the European Union for registration of all active ingredients and formulated products (Council of the European Union, 1991, 1996; EC, 2009). NTA data and ERA requirements for European registration were defined in two international multi-stakeholder workshops: ESCORT 1 (European Standard Characteristics of Non-Target Arthropod Regulatory Testing; Barrett et al., 1994) and ESCORT 2 (Candolfi et al., 2001). The proposed procedures were subsequently implemented by the European Union (EC, 2002) and also adopted by the European and Mediterranean Plant Protection Organization (EPPO, 2003).

ESCORT 1 proposed tiered testing based on the work of the IOBC/WPRS and comprised NTA data from four to six species: two sensitive species [*Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) and *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae)], and up to four crop-relevant arthropod species amenable to laboratory testing and representative of ground- and foliage-dwelling predators. If any early-tier laboratory study did not rule out unacceptable risk, higher-tier studies and ERA must be performed on the affected species to characterise the risk further (Barrett et al., 1994). Standardised and validated methods for the NTA tests were published (Candolfi et al., 2000).

Current European NTA data requirements and ERAs for regulatory approval of chemical pesticides (EC, 2002), based on ESCORT 2 (Candolfi et al., 2001), start with the evaluation of mortality of two sensitive indicator (surrogate) test species under worst-case laboratory conditions (Tier 1). Species selection was based on two sensitivity analyses of laboratory studies: one covering 95 pesticides and 12 NTA species in seven Orders and nine Families (Candolfi et al., 1999); and a second covering 75 pesticides and 23 NTA species in eight Orders and 16 Families (Vogt, 2000). Both analyses demonstrated that *T. pyri* and *A. rhopalosiphi* were the most sensitive species, and by testing these two species, predictions of the effects on other NTA species could be made with high confidence. A lethal or sub-lethal effect on any arthropod species could be predicted for 95.8% of the pesticides considered by testing *T. pyri* and *A. rhopalosiphi* under worst-case laboratory conditions (Candolfi et al., 1999). The probability of missing significant adverse effects when testing only these two indicator species is extremely low.

At Tier 1, laboratory worst-case dose–response studies are performed (Grimm et al., 2001) and the in- and off-field ERA is performed using a Hazard Quotient (HQ) approach (Campbell et al., 2000; Candolfi et al., 2001). If the HQ is above a certain threshold value, unacceptable risk cannot be excluded and higher-tier studies and ERAs with the affected and additional species (one or two additional species depending if only in-field or also off-field risk is identified) have to be performed. Proposed species are *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae), and *Aleochara bilineata* Gyllenhal (Coleoptera: Staphilinidae). These species were selected because they are commercially available, amenable to testing in the laboratory, have reliable test protocols, provide sufficient phylogenetic and functional diversity, and are present in agricultural fields and thus potentially exposed (Barrett et al., 1994; Candolfi et al., 2001).

In addition to testing predators and parasitoids, most regulatory jurisdictions (e.g., EC, 2002), require testing of honey bees (*Apis mellifera* Linnaeus; Hymenoptera: Apidae), as well as testing of selected soil organisms, if exposure of the organisms is anticipated. For *A. mellifera*, a sequential testing and risk assessment scheme is applied that starts with a worst-case oral and contact exposure of adult bees under laboratory conditions. If the product will be used in glasshouses, data on bumble bees may also be requested.

For soil organisms, testing and ERA also are based on a tiered approach (EC, 2002), whereby the Tier 1 assessment is based on structural endpoints (e.g., mortality, reproduction) while the higher tier assessment is based on functional endpoints (e.g., degradation of organic material). To perform the Tier 1 ERA, laboratory studies with the surrogate arthropod species *Folsomia candida* Willem (Collembola: Isotomidae) or *Hypoaspis aculeifer* (Canestrini) (Acari: Gamasidae) are performed.

3. Non-target risk assessment of arthropods for biological control

Exotic natural enemies (predators and parasitoids) introduced for the biological control of pests may pose ecological risks to non-target species (Louda et al., 2003; van Lenteren et al., 2006). Consequently, the introduction of exotic natural enemies requires an ERA prior to their release in some countries (Bigler et al., 2005; Hunt et al., 2008). IOBC/WPRS developed a guideline on information requirements to conduct the ERA (Bigler et al., 2005). One key variable is host specificity; that is, whether the introduced species will parasitize or prey on NTAs in the area of introduction in addition to the target species (Van Driesche and Reardon, 2004; Kuhlmann et al., 2006; van Lenteren et al., 2006).

Tiered testing has also emerged as the most efficient assessment system for ERA of biological control agents (van Lenteren et al., 2003, 2006).

Given the limited resources available for biological control projects, extensive NTA testing will often be unrealistic and impractical (Van Driesche and Hoddle, 1997; Messing, 2001). Sands (1997) suggested that testing more than 10 non-target species is infeasible. It is essential, therefore, that species selected for testing are the most predictive for the NTA effects of the biological control agent.

NTA species are tested under replicated laboratory conditions for their susceptibility or acceptability as hosts or prey for natural enemies. They are selected based on knowledge of the ecological host range of the biological control agent in its native range and its biology and physiology (van Driesche and Hoddle, 1997; Messing, 2001; Sands and Van Driesche, 2004; Withers and Browne, 2004; Barratt et al., 2006). Kuhlmann et al. (2006) proposed a method that extends the centrifugal-phylogenetic method used successfully for evaluation of biological control agents for weeds (Wapshere, 1974; Londsdale et al., 2001). Three main criteria are used when compiling the initial non-target species list to be considered for testing: ecological similarities – species that live or feed in the same microhabitat as the target species; phylogenetic affinities – species that are related to the target species; and safeguard considerations – beneficial arthropods or rare and endangered species that belong to the same family or order as the target species.

The initial test list is generally too long for all species to be tested, so species are filtered out according to attributes that affect their likelihood to be attacked by the biological control agent. Filter 1 comprises spatial, temporal and morphological attributes; non-target species most likely at risk have ecological attributes similar to the target pest, including their climate requirements, geographical and temporal distribution, and morphological attributes such as size. Filter 2 is availability: many species from the initial list may not be testable; testing should focus on species readily available for laboratory host specificity testing, and rare and endangered species would have to be replaced by surrogates. Filtering on these criteria typically leads to a list of 10–20 species that may be tested (Kuhlmann et al., 2006). Testing is iterative, and species can be removed or added depending on the outcome of previous tests.

Host-specificity tests under confined conditions in the laboratory frequently overestimate the host range of biological control agents (Sands and Van Driesche, 2000; Toepfer et al., 2009). Artificial testing conditions omit factors that influence the host searching and acceptance behaviour of the biological control organisms, and consequently the physiological, not the ecological host range is assessed (Van Driesche and Hoddle, 1997; Barratt et al., 2006). Bigler et al. (2010) documented the case of the egg parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), which is mass-released in maize to control the European corn borer *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). Under laboratory conditions, eggs from only one of 25 butterfly and moths species were rejected by *T. brassicae*. Subsequent semi-field and field studies demonstrated that all species tested were parasitized insignificantly when eggs were laid or glued on their host plants. Hence, if a species is not attacked by a natural enemy under the artificial, worst-case conditions in the laboratory, the probability that the species is attacked in the field is negligible (Toepfer et al., 2009). Study design considerations have been proposed to maximise the probability of detecting attacks on NTAs, including food deprivation of predators and no-choice experiments (Withers and Browne, 2004).

The ERA can be stopped at an early stage if none of the non-target test species ïs attacked under the worst-case conditions in the laboratory (van Lenteren et al., 2006). Should some of the non-target species be attacked, more complex assays are required to assess the risks (see Fig. 1 in van Lenteren et al., 2006).

4. Non-target risk assessment of GE plants expressing arthropod-active compounds

Similar to ERAs for pesticides and biological control agents, species should be selected for testing effects of GE plant-expressed arthropod-active compounds that represent species, species groups or guilds that are most at risk, i.e., valued species most likely to be exposed to the arthropod-active compound in the field and to be sensitive to the compound.

4.1. Identification of species most likely to be exposed

Three questions are relevant to the exposure assessment for a compound with an oral route of exposure: Which valued arthropod species, species groups and guilds occur in the respective crop in the area of introduction of the GE variety? Which NTAs are most likely to ingest the arthropod-active compound based on their biology and mode of feeding? What is the level and duration of exposure?

Knowledge of the arthropod fauna and functional guilds is available for most crops, and species contributions to particular valued ecosystem functions have been identified (CABI, 2007; Van Driesche et al., 2008; Meissle et al., 2012). There also is information about the concentration of arthropod-active compound in different GE plant tissues throughout crop development. This information forms an important part of any regulatory package submitted to a regulatory authority (Mendelsohn et al., 2003; OECD, 2007). Various arthropod exposure routes have been identified in the crop and in adjacent habitats for above-ground species (Garcia-Alonso et al., 2006; Romeis et al., 2009b). Studies of *Bt*-transgenic crops have revealed that exposure to *Bt* Cry proteins vary widely among different herbivore feeding guilds and species (Raybould et al., 2007; Romeis et al., 2009b). Arthropods such as predators or parasitoids are mainly exposed to the plant-produced toxins when preying on or parasitizing herbivores that have fed on GE crops. There is evidence that the concentration of the arthropod-active compound is usually diluted as it moves up the food chain and does not accumulate (Romeis et al., 2009b; Meissle and Romeis, 2009, 2012).

Soil-inhabiting species may be exposed because toxins expressed in arthropod-resistant GE crops (e.g., *Bt* crops) can enter the soil e.g. *via*: senescent plant material, dispersed pollen, root exudates, dead arthropod material, or faeces (Icoz and Stotzky, 2008). There is no evidence that the *Bt* Cry proteins accumulate in the soil and persistence appears to be limited and largely depending on soil characteristic, crop management and different environmental factors (Head et al., 2002; OECD, 2007; Icoz and Stotzky, 2008; Gruber et al., 2012).

Arthropod species outside the crop might be exposed for a limited period in time to the plant-expressed arthropod-active compounds contained in wind-dispersed pollen or plant litter distributed aerially, e.g., during harvest. The best known example for such off-crop exposure is that of lepidopterous larvae that ingest maize pollen deposited on their host plant (Oberhauser et al., 2001).

GE plant material and bioactive protein also may enter water bodies and, thus, aquatic organisms may be exposed to the protein. The routes through which GE crop biomass or arthropod-active protein may enter an aquatic ecosystem include erosion of soil-bound protein, surface runoff of freely-soluble protein, aerial dispersal of pollen and crop dust, and spreading of plant tissue or

senescent crop residue. Exposure in aquatic habitats may vary, depending on the crop, the region in which it is grown, the purpose for which it is grown (e.g., seed *vs*. fodder maize), crop management, and the spatial distribution between aquatic and terrestrial environments (Carstens et al., 2012).

4.2. Identification of species most likely to be sensitive

Assuming a similar mode of toxicity, NTAs phylogenetically closely related to the target pests are the most likely to be affected by the arthropod-active compound. Prior to the selection of species for early-tier testing, existing knowledge about the compound's activity spectrum should be acquired. Useful information comes primarily from the scientific literature and expert knowledge, particularly from the developers of the GE product. Evidence for the narrow spectrum of activity has been compiled for the most commonly used Cry proteins expressed in today's *Bt* crops; the evidence comprises observations on the effects of Cry proteins on various species (e.g., US EPA, 2001; Mendelsohn et al., 2003; Romeis et al., 2006; OECD, 2007; Naranjo, 2009), and data on the conditions necessary for Cry protein activity, such as gut pH and Cry protein receptors (Bravo et al., 2007). For a novel arthropod-active compound, an indication of its spectrum of activity may come from comparison to other compounds. In many cases the arthropod-active protein is designed to control a specific set of target pests; for example, MIR604 maize produces a modified Cry3A (mCry3A) for control of certain *Diabrotica* species (Coleoptera: Chrysomelidae). While native Cry3A from *B. thuringiensis* subsp. *tenebrionis* has little or no activity against *Diabrotica* species, the modified version contains an introduced cathepsin G-recognition site that allows activation of the protein by gut proteases in the guts of target insects (Walters et al., 2008). Data for native Cry3A, and the design of the modified protein, predicted that activity spectrum of mCry3A is likely to be the same as Cry3A, except for the extended range to *Diabrotica* species (Raybould et al., 2007). A similar approach would apply for the assessment of hybrid toxins, i.e., synthetic proteins comprising portions of at least two other proteins (Naimov et al., 2003; Mehlo et al., 2005).

Another important source of information are tests conducted by the developer of a GE crop during product development. Before engineering a crop to express an arthropod-active compound, the compound usually undergoes a number of screening tests to determine its activity against a range of organisms belonging to different taxonomic orders to identify potential target species and indications of significant human health and environmental risks (US EPA, 2001; OECD, 2007; Rose, 2007). The information from these tests helps identify the best tests of the NTA risk hypotheses.

While we have focused on GE plants containing *Bt* proteins, the approach also works for GE plants that produce other arthropod-active compounds, including those based on RNA interference (RNAi). For RNAi plants, knowledge of the specific genome sequence that is targeted adds important information to support species selection. Bioinformatics (i.e., sequence homology) suggests that the phylogenetic relatedness of the NTA to the target pests is of importance (Baum et al., 2007; Whyard et al., 2009).

4.3. Species availability and amenability to testing

Availability and amenability to early-tier testing is vital for species selection. For pesticide testing, the IOBC/WPRS, EPPO and Beneficial Arthropod Regulatory Testing Group (BART) recommended using laboratory-reared arthropods (Barrett et al., 1994; Candolfi et al., 2000, 2001). Standardised, genetically similar test arthropods from laboratory colonies provide consistency between experiments and testing laboratories, which promotes data reproducibility and comparability. Although some phenotypic divergence from wild populations may arise during laboratory breeding, use of laboratory populations is deemed preferable to the unknown and variable physiological state of field-collected specimens. The availability of standardised test arthropods puts limits on species selection. When there is no viable alternative and field-collected NTAs must be used, specimens should be standardised as much as possible, and information on the site and method of collection, as well as details on the handling and maintenance before use in the experiments, should be provided (Rose, 2007; Romeis et al., 2011).

Confidence in extrapolating conclusions of no adverse effects from an early-tier study of a surrogate species to other species depends on the quality of the study and its ability to detect effects. Consequently, studies must fulfil several design criteria to ensure reproducibility and interpretability of the data (Romeis et al., 2011). Good design increases the likelihood that studies will be accepted across regulatory jurisdictions, helping to avoid redundant testing, and requires that the species and life-stages selected are amenable to testing using standardised, validated protocols. The requirement for standardised validated test protocols means that for current ERAs of GE crops similar sets of arthropods are tested in laboratory toxicity studies for GE crop ERAs. Testing those species also has the advantage that data on other compounds are available for comparison. In Table 1 we provide a list of species that are available and amenable for laboratory studies and have been tested in the past to support the ERA of GE crops.

5. Conclusions and implications

Despite the complexity of ecological systems, ERAs for GE crops do not have to be complex; they may follow the simple models used successfully for conventional chemical pesticides and biological control agents.

Construction of conceptual models of how cultivation of the GE crop could pose harm to valued NTAs, and the identification of testable risk hypotheses is an important first step in the ERA (Raybould, 2011). The focus on relevant hypotheses minimises the collection of data that may not be useful for decision-making and diverts resources from evaluation of more serious risks (Craig et al., 2008; Raybould, 2011). The relevance of a non-target study for ERA must, therefore, be judged by its power to refute risk hypotheses of no harm, or less conservatively, risk hypotheses that harm will not occur above a certain frequency or magnitude (Raybould, 2011).

A commonly tested hypothesis for arthropod-resistant GE crops is that the arthropod-active compound does not cause adverse effects to valued NTAs under field exposures. Laboratory studies using controlled worst-case exposure conditions provide rigorous tests of this hypothesis and thereby contribute important data for the ERA of GE crops. Confidence in the conclusions drawn from surrogate NTA studies is increased if the species tested are those most likely to be sensitive, which may be inferred from existing knowledge of the spectrum of activity and the mode of action (MoA). If such surrogate species are not adversely affected under these conditions, valued NTAs are unlikely to be adversely affected and the risk hypothesis may be regarded as rigorously corroborated.

For early-tier laboratory studies to support the ERA of arthropod-resistant GE crops, surrogate species should be selected that most closely meet three criteria:

- *Sensitivity*: species should be the most likely to be sensitive to the arthropod-active compound based on the known spectrum of activity of the active ingredient, its MoA, and the phylogenetic

Table 1
Arthropod surrogates that have been used in regulatory risk assessment studies for insecticidal GE crops (based on US EPA, 2001; OECD, 2007; Biopesticides Registration Action Documents (BRADs), and company information).

Functional group	Species	Order: Family	Common name	Life stage treated	Food and feeding mode	Test substance
Pollinators	*A. mellifera* Linnaeus	Hymenoptera: Apidae	Honey bee	Larva	Feed pollen grains and hypopharyngeal gland extract produced by nursing bees	GE pollen, purified protein
	A. mellifera Linnaeus	Hymenoptera: Apidae	Honey bee	Adult	Feed pollen grains and nectar; flower-visiting	GE pollen, purified protein
Parasitoids	*Brachymeria intermedia* Nees	Hymenoptera: Chalcididae	None	Adult	Larvae live parasitic, adults feed on nectar and honeydew; plant-dwelling	Purified protein
	Ichneumon promissorius Erichson	Hymenoptera: Ichneumonidae	Banded caterpillar parasite wasp	Adult female	Larvae live parasitic, adults feed on nectar and honeydew; plant-dwelling	Purified protein
	Nasonia vitripennis (Walker)	Hymenoptera: Pteromalidae	Jewel wasp	Adult	Larvae live parasitic, adults feed on nectar and honeydew; plant-dwelling	Purified protein
	Pediobius foveolatus (Crawford)	Hymenoptera: Eulophidae	Bean beetle parasitoid	Adult	Larvae live parasitic, adults feed on nectar and honeydew; plant-dwelling	Purified protein
Predators	*Coleomegilla maculata* De Geer	Coleoptera: Coccinellidae	Pink-spotted lady beetle	Larva, adult	Larvae and adults are predators on arthropod herbivores (mainly aphids) and feed on pollen; plant-dwelling	GE pollen, purified protein
	C. septempunctata Linnaeus	Coleoptera: Coccinellidae	Seven-spotted lady beetle	Larva, adult	Larvae and adults are predators on arthropod herbivores (mainly aphids) and feed on pollen; plant-dwelling	GE pollen, purified protein
	Hippodamia convergens Guérin-Méneville	Coleoptera: Coccinellidae	Convergent lady beetle	Adult	Larvae and adults are predators on arthropod herbivores (mainly aphids) and feed on pollen; plant-dwelling	Purified protein
	Poecilus cupreus (Linnaeus)	Coleoptera: Carabidae	Ground beetle	Larva	Larvae and adults are predators on arthropods and also feed on plant material (pollen, seeds); soil-dwelling	GE pollen, purified protein
	A. bilineata Gyllenhal	Coleoptera: Staphylinidae	Rove beetle	Adult	Larvae are parasitoids of fly pupae, adults are predators; soil-dwelling	Purified protein
	C. carnea (Stephens)	Neuroptera: Chrysopidae	Green lacewing	Larva, adult	Larvae feed on arthropods (mainly aphids) (piercing-sucking). Adults feed on pollen and nectar. Both stages are plant-dwelling.	Purified protein
	Orius insidiosus (Say)	Hemiptera: Anthocoridae	Minute pirate bug	Nymph, adult	Nymphs and adults feed on soft-bodied arthropods, pollen and other plant tissue (piercing-sucking); plant-dwelling	GE pollen, purified protein
	O. laevigatus (Fieber)	Hemiptera: Anthocoridae	Minute pirate bug	Nymph, adult	Nymphs and adults feed on soft-bodied arthropods, pollen and other plant tissue (piercing-sucking); plant-dwelling	GE pollen, purified protein
Decomposers	*F. candida* Willem	Collembola: Isotomidae	Springtail	Adult, immature	Feed on decaying plant material and fungus hyphae; soil-dwelling	GE leaf tissue
	Xenylla grisea Axelson	Collembola: Hypogastruridae	Springtail	Adult, immature	Feed on decaying plant material and fungus hyphae; soil-dwelling	GE leaf tissue
Aquatic organisms	*Daphnia magna* Straus	(Crustacea:) Diplostraca: Daphniidae	Waterflea	Adult, immature	Suspension feeder (filter feeder); ingest algae and pollen	GE pollen and leaf tissue, purified protein
	Chironomus dilutus Shobanov, Kiknadze & Butler	Diptera: Chironomidae	Midge	Larva	Feed on detritus	GE plant extract

relatedness of the test and target species. Sensitivity means an adverse effect on an ecologically relevant parameter such as survival or growth.

- *Relevance*: species should be representative of valued taxa or functional groups that are most likely to be exposed to the arthropod-active compound in the field.
- *Availability and reliability*: suitable life-stages of the test species must be obtainable in sufficient quantity and quality, and validated test protocols must be available that allow consistent detection of adverse effects on ecologically relevant parameters.

These selection criteria should ensure that the most suitable species are tested and the resulting data provide the most rigorous test of the risk hypothesis of no effect. While the reliability criteria are independent from the particular GE crop that is assessed, the species most sensitive to the stressor of concern and the species that are representative of the ones most likely to be exposed vary among GE crops. Thus test species for early-tier testing need to be selected case-by-case. Ideally, risk assessors from the GE crop developer together with representatives of the regulatory authority agree on a set of suitable test species at an early stage of the risk assessment. The proposed selection criteria should merely guide the identification of the test species.

For example, in the case of a GE plant that produces a Cry3 protein for the control of coleopteran pests, the risk is expected to be higher for beetle species than for species belonging to other taxa. Consequently, more beetle representatives should be tested than for example when assessing a GE crop controlling lepidopterous pests (Raybould, 2006). For a GE crop expressing less specific arthropod-active proteins, such as lectins or a protease inhibitors (Malone et al., 2008), initial tests may cover a broader phylogenetic range of surrogate species for which study material and test protocols are available. Furthermore, if pest species fulfil the criteria for being useful surrogates, they can be included in the testing

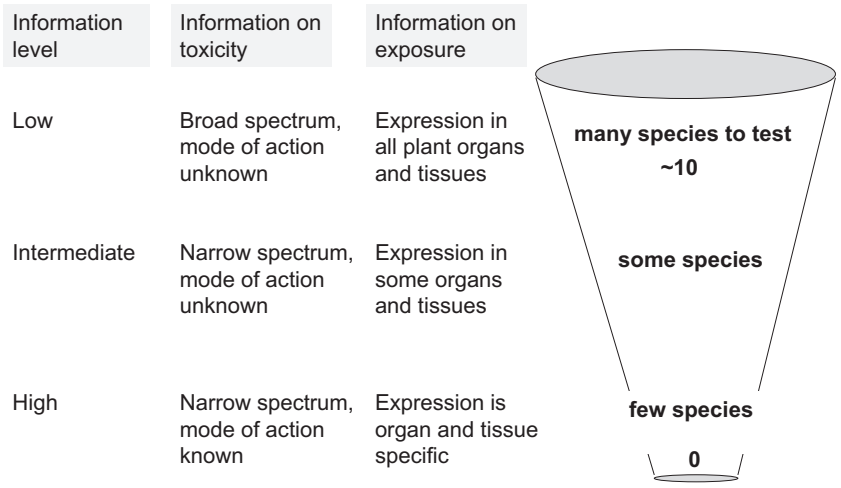

Fig. 1. Information that influences the scope of surrogate species testing for an insecticidal trait early in the ecological risk assessment.

programme. For example, if there were concern that cultivation of a GE crop producing a protease inhibitor might harm certain endangered Lepidoptera, tests of the effects of the inhibitor on lepidopterous pests related to the endangered species would provide useful information. Depending on the results of these initial tests, additional species may need to be tested or higher-tier studies need to be conducted.

Our proposed approach ensures that early-tier laboratory studies provide generic data on the spectrum of activity of the studied arthropod-active compounds that are valuable for ERAs considering the same or similar compounds elsewhere. Selecting the appropriate species for early-tier ecotoxicological tests thus increases data transportability and reduces the need to supplement existing data with results of additional studies that add little to the risk assessment (Romeis et al., 2009a; Raybould and Quemada, 2010). In some respects, NTA testing of Cry proteins is confirmatory. Regulatory tests using non-pest species have not revealed activity that was unexpected based on knowledge in the scientific literature and data collected from pest screens during product development. The lack of unexpected adverse effects in NTA effects tests corroborates the hypothesis that phylogenetic similarity is a good predictor of activity of Cry proteins. As NTA effects data accumulate, it may be possible to reduce the number of species tested for Cry proteins in a manner similar to that for synthetic pesticides. For arthropod-active compounds that are less well-studied, our approach gives confidence that the selected surrogates adequately represent the organisms and functions that require protection. This together with the level of information on the intensity and duration of exposure will affect the number of species that finally have to be tested (Fig. 1).

Our approach of species selection for GE crop ERA provides an effective means of allocating limited regulatory resources: effort is concentrated on riskier products. We recognise, however, that regulatory authorities may require testing of additional species for various reasons. One reason might be for risk communication. Certain species might be tested because they receive a high level of attention from the public and certain interest groups. For example, honey bee studies are often required despite minimal exposure owing to negligible concentrations of active ingredients in pollen.

Our approach should help to restrict the collection of data to those species that are relevant and avoid superfluous data that detract the attention of risk assessors from more serious risks. It will also help risk assessors to evaluate which existing ecotoxicological studies are useful for testing their specific risk hypotheses so that ERAs can make best use of available data. Consequently, it should raise the quality of the risk assessment and thus the environmental safety of the GE products released.

References

Andow, D.A., Hilbeck, A., 2004. Science-based risk assessment for nontarget effects of transgenic crops. BioScience 54, 637–649.

Barratt, B.I.P., Moeed, A., Malone, L.A., 2006. Biosafety assessment protocols for new organisms in New Zealand: can they apply internationally to emerging technologies? Environ. Impact Assess. Rev. 26, 339–358.

Barrett, K.L., Grandy, N., Harrison, E.G., Hassan, S., Oomen, P. (Eds.), 1994. Guidance Document on Regulatory Testing Procedures for Pesticides with Non-target Arthropods. ESCORT workshop (European Standard Characteristics of Non-Target Arthropod Regulatory Testing). Society of Environmental Toxicology and Chemistry (SETAC) Europe, Brussels.

Baum, J.A., Bogaert, T., Clinton, W., Heck, G.R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., Pleau, M., Vaughn, T., Roberts, J., 2007. Control of coleopteran insect pests through RNA interference. Nat. Biotechnol. 25, 1322–1326.

Bigler, F., Babendreier, D., van Lenteren, J.C., 2010. Risk assessment and non-target effects of egg parasitoids in biological control. In: Consoli, F.L. (Ed.), Egg Parasitoids in Agroecosystems with Emphasis on Trichogramma. Springer Science + Business Media B.V., pp. 413–442.

Bigler, F., Bale, J.S., Cock, M.J.W., Dreyer, H., Greatrex, R., Kuhlmann, U., Loomans, A.J.M., van Lenteren, J.C., 2005. Guidelines on information requirements for import and release of invertebrate biological control agents in European countries. Perspect. Agr., Vet. Sci., Nutr. Nat. Resour. 1, 001. http://dx.doi.org/10.1079/PAVNNR20051001.

Birch, A.N.E., Wheatley, R.E., Hilbeck, A., Arpaia, S., Lovei, G.L., Songa, J., Sétamou, M., Sithanantham, S., Ngi-Song, A., Anyango, B., Muyekho, F., Ochieno, D., Lelmen, E., Kalama, P., Degaga, E., Soares Melo, I., Pitelli, R., Sujii, E.R., Capalbo, D., Fontes, E.M.G., Wan, F., Ogwang, J., Smith, J., Van Son, N., Quang Tan, T., 2004. Biodiversity and non-target impacts: a case study of *Bt* maize in Kenya. In: Hilbeck, A., Andow, D.A. (Eds.), Environmental Risk Assessment of Transgenic Organisms: A Case Study of *Bt* Maize in Kenya. CAB International, Wallingford, pp. 117–185.

Bravo, A., Gill, S.S., Soberón, M., 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon 49, 423–435.

CABI, 2007. Crop Protection Compendium. 2007 edition. CAB International, Wallingford. <www.cabi.org/cpc/>.

Campbell, P.J., Brown, K.C., Harrison, E.G., Bakker, F., Barrett, K.L., Candolfi, M.P., Canez, V., Dinter, A., Lewis, G., Mead-Briggs, M., Miles, M., Neumann, P., Romijn, K., Schmuck, R., Shires, S., Ufer, A., Waltersdorfer, A., 2000. A hazard quotient approach for assessing the risk to non-target arthropods from plant protection products under 91/414/EEC: hazard quotient trigger value proposal and validation. J. Pest Sci. 73, 117–124.

Candolfi, M.P., Bakker, F., Cañez, V., Miles, M., Neumann, C., Pilling, E., Primiani, M., Romijn, K., Schmuck, R., Storck-Weyhermüller, S., Ufer, A., Waltersdorfer, A., 1999. Sensitivity of non-target arthropods to plant protection products: could *Typhlodromus pyri* and Aphidius spp. be used as indicator species? Chemosphere 39, 1357–1370.

Candolfi, M.P., Blümel, S., Foster, R., Bakker, F.M., Grimm, C., Hassan, S.A., Heimbach, U., Mead-Briggs, M.A., Reber, B., Schmuck, R., Vogt, H. (Eds.), 2000. Guidelines to Evaluate Side-effects of Plant Protection Products to Non-target Arthropods. International Organization for Biological and Integrated Control of Noxious Animals and Weeds, West Palearctic Regional Section (IOBC/WPRS), Gent.

Candolfi, M.P., Barrett, K.L., Campbell, P., Forster, R., Grandy, N., Huet, M.-C., Lewis, G., Oomen, P.A., Schmuck, R., Vogt, H. (Eds.), 2001. Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-target Arthropods. ESCORT 2 workshop (European standard characteristics of non-target arthropods regulatory testing), a joint BART, EPPO/CoE, OECD and IOBC Workshop organized in conjunction with SETAC-Europe and EC. SETAC Press.

Carstens, K., Anderson, J., Bachman, P., De Shrijver, A., Dively, G., Federici, B., Hamer, M., Gielkens, M., Jensen, P., Lamp, W., Rauschen, S., Ridley, G., Romeis, J., Waggoner, A., 2012. Genetically modified crops and aquatic ecosystems: Considerations for environmental risk assessment and non-target organism testing. Transgenic Res. 21, 813–842.

Chapman, P.M., 2002. Integrating toxicology and ecology: putting the "eco" into ecotoxicology. Mar. Pollut. Bull. 44, 7–15.

Council of the European Union, 1991. Council Directive 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC). Off. J. Eur. Commun. L230, 1–230.

Council of the European Union, 1996. Commission Directive 96/12/EC of the 8 March 1996 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market. Off. J. Eur. Commun. L65, 20–37.

Craig, W., Tepfer, M., Degrassi, G., Ripandelli, D., 2008. An overview of general features of risk assessments of genetically modified crops. Euphytica 164, 853–880.

Duan, J.J., Lundgren, J.G., Naranjo, S., Marvier, M., 2010. Extrapolating non-target risk of *Bt* crops from laboratory to field. Biol. Lett. 6, 74–77.

Endangered Species Act (ESA), 1973. US Fish and Wildlife Service. <http://www.fws.gov/endangered/laws-policies/index.html>.

European and Mediterranean Plant Protection Organization (EPPO), 2003. Environmental risk assessment scheme for plant protection products. Chapter 9: Non-target terrestrial arthropods. Bull. OEPP/EPPO Bull. 33, 131–139.

European Commission (EC), 2002. Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC. SANCO/10329/2002 rev 2 final. <http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkdoc09_en.pdf>.

European Commission (EC), 2009. Regulation (EC) No. 1107/2009 of the European Parliament and of the Council of 21 October 2009, concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Off. J. Eur. Union L309, 1–50.

European Food Safety Authority (EFSA), 2010a. EFSA Panel on Plant Protection Products and their Residues (PPR); Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). EFSA J. 8(10), 1821.

European Food Safety Authority (EFSA), 2010b. Guidance on the environmental risk assessment of genetically modified plants. EFSA Panel on Genetically Modified Organisms (GMO). EFSA J. 8(11), 1879.

Garcia-Alonso, M., Jacobs, E., Raybould, A., Nickson, T.E., Sowig, P., Willekens, H., van der Kouwe, P., Layton, R., Amijee, F., Fuentes, A.M., Tencalla, F., 2006. A tiered system for assessing the risk of genetically modified plants to non-target organisms. Environ. Biosafety Res. 5, 57–65.

Grimm, C., Schmidli, H., Bakker, F., Brown, K., Campbell, P., Candolfi, M.P., Chapmann, P., Harrison, E.G., Mead-Briggs, M., Schmuck, R., Ufer, A., 2001. Use of standard toxicity tests with *Typhlodromus pyri* and *Aphidius rhopalosiphi* to establish a dose-response relationship. J. Pest Sci. 74, 72–84.

Gruber, H., Paul, V., Meyer, H.H.D., Müller, M., 2012. Determination of insecticidal Cry1Ab protein in soil collected in the final growing seasons of a nine-year field trial of *Bt*-maize MON810. Transgenic Res. 21, 77–88.

Gurr, G.M., Wratten, S.D., Luna, J.M., 2003. Multi-function agricultural biodiversity: pest management and other benefits. Basic Appl. Ecol. 4, 107–116.

Hassan, S.A., Vogt, H., 2006. Working group pesticides and beneficial organisms (1974-present). In: Boller, E.F., van Lenteren, J.C., Delucchi, V. (Eds.), International Organisation for Biological Control of Noxious Animals and Plants (IOBC): History of the First 50 Years (1956–2006). IOBC. pp. 221–228.

Head, G., Surber, J.B., Watson, J.A., Martin, J.W., Duan, J.J., 2002. No detection of Cry1Ac protein in soil after multiple years of transgenic *Bt* cotton (Bollgard) use. Environ. Entomol. 31, 30–36.

Hill, R.A., Sendashonga, C., 2003. General principles for risk assessment of living modified organisms: lessons from chemical risk assessment. Environ. Biosafety Res. 2, 81–88.

Huesing, J.E., Romeis, J., Ellstrand, N.C., Raybould, A., Hellmich, R.L., Wolt, J.D., Ehlers, J.D., Dabiré-Binso, L.C., Fatokun, C.A., Hokanson, K.E., Ishiyaku, M.F., Margam, V.M., Obokoh, N., Mignouna, J.D., Nang'ayo, F., Ouedraogo, J.T., Pasquet, R.S., Pittendrigh, B.R., Schaal, B.A., Stein, J., Tamò, M., Murdock, L.L., 2011. Regulatory considerations surrounding the deployment of *Bt*-expressing cowpea in Africa. GM Crops 2, 211–224.

Hunt, E.J., Kuhlmann, U., Sheppard, A., Qin, T.-K., Barratt, B.I.P., Harrison, L., Mason, P.G., Parker, D., Flanders, R.V., Goolsby, J., 2008. Review of invertebrate biological control agent regulation in Australia, New Zealand, Canada and the USA: recommendations for a harmonized European system. J. Appl. Entomol. 132, 89–123.

Icoz, I., Stotzky, G., 2008. Fate and effects of insect-resistant *Bt* crops in soil ecosystems. Soil Biol. Biochem. 40, 559–586.

International Union for Conservation of Nature and Natural Resources (IUCN), 2010. The IUCN Red List of Threatened Species. Cambridge, United Kingdom. <http://www.iucnredlist.org>.

Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., Potts, S.G., Roulston, T., Steffan-Dewenter, I., Vázquez, D.P., Winfree, R., Adams, L., Crone, E.E., Greenleaf, S.S., Keitt, T.H., Klein, A.-M., Regetz, J., Ricketts, T.H., 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. Ecol. Lett. 10, 299–314.

Kuhlmann, U., Schaffner, U., Mason, P.G., 2006. Selection of non-target species for host specificity testing. In: Bigler, F., Babendreier, D., Kuhlmann, U. (Eds.), Environmental Impact of Invertebrates for Biological Control of Arthropods. Methods and Risk Assessment, CAB International, Wallingford, pp. 15–37.

Londsdale, W.M., Briese, D.T., Cullen, J.M., 2001. Risk analysis and weed biological control. In: Wajnberg, E., Scott, J.K., Quimby, P.C. (Eds.), Evaluating Indirect Ecological Effects of Biological Control. CABI Publishing, Wallingford, pp. 185–210.

Louda, S.M., Pemberton, R.W., Johnson, M.T., Follett, P.A., 2003. Nontarget effects: the Achilles' heel of biological control? Annu. Rev. Entomol. 48, 365–396.

Malone, L.A., Gatehouse, A.M.R., Barratt, B.I.P., 2008. Beyond *Bt*: alternative strategies for insect-resistant genetically modified crops. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), Integration of Insect-Resistant Genetically Modified Crops within IPM Programs. Springer Science + Business Media B.V., pp. 357–417.

Marvier, M., McCreedy, C., Regetz, J., Kareiva, P., 2007. A meta-analysis of effects of *Bt* cotton and maize on nontarget invertebrates. Science 316, 1475–1477.

Mehlo, L., Gahakwa, D., Nghia, P.T., Loc, N.T., Capell, T., Gatehouse, J.A., Gatehouse, A.M.R., Christou, P., 2005. An alternative strategy for sustainable pest resistance in genetically enhanced crops. Proc. Natl. Acad. Sci. USA 102, 7812–7816.

Meissle, M., Romeis, J., 2009. The web-building spider Theridion impressum (Araneae: Theridiidae) is not adversely affected by *Bt* maize resistant to corn rootworms. Plant Biotech. J. 7, 645–656.

Meissle, M., Romeis, J., 2012. No accumulation of Bt protein in *Phylloneta impressa* (Araneae: Theridiidae) and other arthropods in Bt maize. Environ. Entomol. 41, 1037–1042.

Meissle, M., Álvarez-Alfageme, F., Malone, L.A., Romeis, J., 2012. Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU. Supporting Publications 2012:EN-334. European Food Safety Authority (EFSA), Parma, Italy, 170pp. <http://www.efsa.europa.eu/en/supporting/pub/334e.htm>.

Mendelsohn, M., Kough, J., Vaituzis, Z., Matthews, K., 2003. Are *Bt* crops safe? Nat. Biotechnol. 21, 1003–1009.

Messing, R.H., 2001. Centrifugal phylogeny as a basis for non-target host testing in biological control: is it relevant for parasitoids? Phytoparasitica 29, 187–190.

Mulder, C., 2006. Driving forces from soil invertebrates to ecosystem functioning: the allometric perspective. Naturwissenschaften 93, 467–479.

Naimov, S., Dukiandjiev, S., de Maagd, R.A., 2003. A hybrid *Bacillus thuringiensis* delta-endotoxin gives resistance against a coleopteran and a lepidopteran pest in transgenic potato. Plant Biotechnol. J. 1, 51–57.

Naranjo, S.E., 2009. Impacts of *Bt* crops on non-target organisms and insecticide use patterns.. Perspect. Agr., Vet. Sci., Nutr. Nat. Resour. 4, 011. http://dx.doi.org/10.1079/PAVSNNR20094011.

Oberhauser, K.S., Prysby, M., Mattila, H.R., Stanley-Horn, D.E., Sears, M.K., Dively, G.P., Olson, E., Pleasants, J.M., Lam, W.-K.F., Hellmich, R.L., 2001. Temporal and spatial overlap between monarch larvae and corn pollen. Proc. Natl. Acad. Sci. USA 98, 11913–11918.

Organisation for Economic Co-operation and Development (OECD), 2007. Consensus Document on Safety Information on Transgenic Plants Expressing *Bacillus thuringiensis*-derived Insect Control Protein. Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 42. Organisation for Economic Co-operation and Development, Paris. <http://www.olis.oecd.org/olis/2007doc.nsf/LinkTo/NT00002DF6/$FILE/JT03230592.PDF>.

Raybould, A., 2006. Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environ. Biosafety Res. 5, 119–125.

Raybould, A., 2011. The bucket and the searchlight: formulating and testing risk hypotheses about the weediness and invasiveness potential of transgenic crops. Environ. Biosafety Res. 9, 123–133.

Raybould, A., Quemada, H., 2010. *Bt* crops and food security in developing countries: realised benefits, sustainable use and lowering barriers to adoption. Food Sec. 2, 247–259.

Raybould, A., Stacey, D., Vlachos, D., Graser, G., Li, X., Joseph, R., 2007. Non-target organisms risk assessment of MIR604 maize expressing mCry3A for control of corn rootworms. J. Appl. Entomol. 131, 391–399.

Raybould, A., Caron-Lormier, G., Bohan, D., 2011. Derivation and interpretation of hazard quotients to assess ecological risks from the cultivation of insect-resistant transgenic crops. J. Agric. Food Chem. 59, 5877–5885.

Romeis, J., Meissle, M., Bigler, F., 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat. Biotechnol. 24, 63–71.

Romeis, J., Bartsch, D., Bigler, F., Candolfi, M.P., Gielkens, M.M.C., Hartley, S.E., Hellmich, R.L., Huesing, J.E., Jepson, P.C., Layton, R., Quemada, H., Raybould, A., Rose, R.I., Schiemann, J., Sears, M.K., Shelton, A.M., Sweet, J., Vaituzis, Z., Wolt, J.D., 2008. Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nat. Biotechnol. 26, 203–208.

Romeis, J., Lawo, N.C., Raybould, A., 2009a. Making effective use of existing data for case-by-case risk assessments of genetically engineered crops. J. Appl. Entomol. 133, 571–583.

Romeis, J., Meissle, M., Raybould, A., Hellmich, R.L., 2009b. Impact of insect-resistant transgenic crops on above-ground non-target arthropods. In: Ferry, N., Gatehouse, A.M.R. (Eds.), Environmental Impact of Genetically Modified Crops. CAB International, Wallingford, pp. 165–198.

Romeis, J., Hellmich, R.L., Candolfi, M.P., Carstens, K., De Schrijver, A., Gatehouse, A.M.R., Herman, R.A., Huesing, J.E., McLean, M.A., Raybould, A., Shelton, A.M., Waggoner, A., 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Res. 20, 1–22.

Rose, R.I. (Ed.), 2007. White Paper on Tier-based Testing for the Effects of Proteinaceous Insecticidal Plant-incorporated Protectants on Non-target Invertebrates for Regulatory Risk Assessment. USDA-APHIS and US Environmental Protection Agency, Washington DC. <http://www.epa.gov/pesticides/biopesticides/pips/non-target-arthropods.pdf>.

Sands, D.P.A., 1997. The 'safety' of biological control agents: assessing their impact on beneficial and other non-target hosts. Mem. Museum Victoria 56, 611–615.

Sands, D.P.A., Van Driesche, R.G., 2000. Evaluating the host range of agents for biological control of arthropods: rationale, methodology and interpretation. In: Van Driesche, R.G., Heard, T.A., McClay, A.S., Reardon, R. (Eds.), Proceedings: Host Specificity Testing of Exotic Arthropod Biological Control Agents: The Biological Basis for Improvement in Safety. Forest Health Technology Enterprise Team 1999–01, USDA Forest Service, Morgantown, West Virginia. pp. 69–83.

Sands, D.P.A., Van Driesche, R.G., 2004. Using the scientific literature to estimate the host range of a biological control agent. In: Van Driesche, R.G., Reardon, R. (Eds.), Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A Guide to Best Practice. Forest Health Technology Enterprise Team 2004–03, USDA Forest Service, Morgantown, West Virginia. pp. 15–23.

Sanvido, O., Romeis, J., Gathmann, A., Gielkens, M., Raybould, A., Bigler, F., 2012. Evaluating environmental risks of genetically modified crops - ecological harm criteria for regulatory decision-making. Environ. Sci. Policy 15, 82–91.

Toepfer, S., Zhang, F., Kuhlmann, U., 2009. Assessing host specificity of a classical biological control agent against western corn rootworm with a recently developed testing protocol. Biol. Control 51, 26–33.

Touart, L.W., Maciorowski, A.F., 1997. Information needs for pesticide registration in the United States. Ecol. Appl. 7, 1086–1093.

US EPA, 1996. Microbial pesticide test guidelines: OPPTS 885.4000 Background for nontarget organism testing of microbial pest control agents. EPA712-C-96-336, February 1996. United States Environmental Protection Agency, Washington DC.

US EPA, 2001. Biopesticide registration action document. *Bacillus thuringiensis* (*Bt*) plant-incorporated protectants. United States Environmental Protection Agency (USEPA), Washington DC. <http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm>.

Van Driesche, R.G., Hoddle, M., 1997. Should arthropod parasitoids and predators be subject to host range testing when used as biological control agents? Agr. Hum. Val. 14, 211–226.

Van Driesche, R.G., Reardon, R. (Eds.), 2004. Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A Guide to Best Practice. Forest Health Technology Enterprise Team 2004–03, USDA Forest Service, Morgantown, West Virginia.

Van Driesche, R., Hoddle, M., Center, T., 2008. Control of Pests and Weeds by Natural Enemies. Blackwell Publishing, Malden, USA.

Van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S., Loomans, A.J.M., Menzler-Hokkanen, I., van Rijn, P.C.J., Thomas, M.B., Tommasini, M.G., Zeng, Q.-Q., 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl 48, 3–38.

Van Lenteren, J.C., Bale, J., Bigler, F., Hokkanen, H.M.T., Loomans, A.J.M., 2006. Assessing the risks of releasing exotic biological control agents of arthropod pets. Annu. Rev. Entomol. 51, 609–634.

Vogt, H., 2000. Sensitivity of non-target arthropods species to plant protection products according to laboratory results of the IOBC WG "Pesticides and beneficial organisms". IOBC/WPRS Bull. 23 (9), 3–15.

Walters, F.S., Stacy, C.M., Lee, M.K., Palekar, N., Chen, J.S., 2008. An engineered chymotrypsin/cathepsin G site in domain I renders *Bacillus thuringiensis* Cry3A active against western corn rootworm larvae. Appl. Environ. Microbiol. 74, 367–374.

Wapshere, A.J., 1974. A strategy for evaluating the safety of organisms for biological weed control. Ann. Appl. Biol. 77, 201–211.

Whyard, S., Singh, A.D., Wong, S., 2009. Ingested double-stranded RNAs can act as species-specific insecticides. Insect Biochem. Molec. Biol. 39, 824–832.

Withers, T.M., Browne, L.B., 2004. Behavioral and physiological processes affecting outcomes of host range testing. In: Van Driesche, R.G., Reardon, R. (Eds.) Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A Guide to Best Practice. Forest Health Technology Enterprise Team 2004–03, USDA Forest Service, Morgantown, West Virginia. pp. 40–55.

Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J., Watrud, L.S., 2008. *Bt* crops effects on functional guilds of non-target arthropods: a meta-analysis. PLoS ONE 3 (5), e2118.