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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.