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ENVIRONMENTAL EVIDENCE

SYSTEMATIC REVIEW PROTOCOL Open Access

How susceptible are different lepidopteran/coleopteran maize pests to Bt-proteins: a systematic review protocol

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Abstract

Background: Lepidopteran and coleopteran species are the most important pests in maize. They can be controlled using genetically modified crops expressing insecticidal *Bt*-proteins. The long term success of this technology demands a pest resistance management. Important information for a successful management of resistance is the baseline susceptibility of the different targeted pests towards the different *Bt*-proteins. The data on baseline susceptibility should enable risk assessors and managers to assess whether a) *Bt*-maize events represent a high-dose to specific target organisms, b) resistance has developed while monitoring the commercial introduction of *Bt*-maize events and c) potential knowledge gaps can be identified.

Methods: This protocol describes our suggested methods for conducting a systematic review to give an overview of the European target pests in maize and their susceptibility to insecticidal *Bt*-proteins. Both published and unpublished data shall be collated. Different sources of information will be searched in order to maximize the coverage of the search. All identified publications will be stored in a database. Relevant information for the review will be identified in a three step approach based on inclusion criteria. This data set will be an important basis to model and assess the potential for evolution of resistance of different crop-*Bt*-protein-species combinations.

Keywords: Systematic review, *Bacillus thuringiensis*, Insect pests, Corn, Toxicity test, Coleoptera, Lepidoptera, Cry protein

Background

Lepidopteran and coleopteran species are the most important pests in maize. The effectiveness of common control measures is affected by several factors: The hidden life style of some pest species reduces exposure to insecticides, which makes it difficult to control these species effectively. Resistance evolution to conventional insecticides can make them ineffective, the environmental harm associated with the use of insecticides should be reduced or economic considerations lead to higher pest pressure (no tillage, maize on maize cultivation). An alternative approach to common control measures is the use of genetically modified (GM) crops expressing *Bt*-proteins.

However, one concern with growing *Bt*-maize is the potential for evolution of resistance. Resistance evolution against agricultural control measures is a well known problem for more than 100 years [1]. It occurs regularly where pest populations are exposed to a uniform, strong and continuous selection pressure [1-3] and is therefore expected also for insect resistant GM crops expressing *Bt*-proteins. In consequence *Bt*-products might lose their effectiveness against pests both as conventional spray application and as transgenic trait of GM crops. Therefore pest resistance management must accompany the cultivation of *Bt*-crops to delay the evolution of resistance to *B. thuringiensis* products [4].

The regulatory risk assessment does not consider the occurrence of resistance evolution in general, but the efficacy of strategies by applicants delaying the expected resistance evolution. The most common strategy is the "high-dose/refuge" strategy e.g. [1,5]. The common

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approach to forecast the evolution of resistance is the use of models.

The principles of the high-dose/refuge strategy used in these models are that (1) the protein is killing nearly all pest organisms feeding on GM crops (high dose), (2) the frequency of resistance alleles is low, (3) the inheritance of resistance is fully recessive, (4) rare resistant pests surviving on *Bt*-crops mate with abundant susceptible pests from nearby refuges of host plants without *Bt*-proteins [5], and (5) fitness costs are associated with the evolution of resistance [6]. Therefore data on the biology of the target organisms, the characteristics of the modified plant and the GM trait are needed to support the model with all relevant information. In the case that not all requirements for the high dose refuge strategies are fulfilled, a modified strategy or additional measures might be needed.

One of the most important pieces of information to model the potential to evolve resistance of a pest/crop system is the baseline susceptibility of the target organisms to the Cry proteins. This was confirmed by stakeholders (see Appendix 1) who were consulted during a workshop and by a prioritisation process of several proposed review questions.

The goal of the systematic review (SR) will be to collect as much data as possible on baseline susceptibility of different lepidopteran/coleopteran maize pests to *Bt*-proteins to have a broad data basis to model and assess the potential for evolution of resistance of different crop-*Bt*-protein-species combinations. Data on baseline susceptibility for a specific pest population need to be generated before *Bt*-maize will be cultivated; which means before an intervention takes place. These data will be independently collected for different populations of lepidopteran or coleopteran pests. Since study design, the used *Bt*-proteins, biological activity of different batches of *Bt*-proteins, and the age of the test organisms might differ between studies data from different sources are hardly comparable. Therefore the results of the SR will comprise a collection of relevant information, but will not enable a comparison of study results.

Objective of the review

One key assumption for the high-dose/refuge strategy is that the amount of *Bt*-protein expressed in *Bt*-maize is killing nearly all pest organisms feeding on GM crops (high dose). Therefore data on the dose–response relationship is needed to estimate the baseline susceptibility of the target organism. Furthermore changes in the baseline susceptibility of the target organisms are measured in insect resistance monitoring programs indicating resistance evolution [7]. The data on baseline susceptibility should enable risk assessors and managers to assess whether *Bt*-maize events represent a high-dose to a specific target organisms, resistance has developed after commercial introduction of *Bt*-maize events (monitoring) and to identify potential knowledge gaps. Based on the above remarks, the following review question was formulated:

How susceptible are different lepidopteran/coleopteran maize pests to Bt-proteins?

The research question has the following components:

P = Population:

The populations considered are all lepidopteran or coleopteran pest species in maize, which are intended to be controlled by *Bt*-maize in Europe. According to the current applications *Ostrinia nubilalis*, *Sesamia nonagroides*, *S. cretica*, and *Diabrotica virgifera virgifera* are defined as target species. Additionally lepidopteran or coleopteran pest species should be considered, which might be of economic relevance in maize cultivation. The selection of considered species is based on an extended review initiated by EFSA [8].

O = Outcome:

Baseline susceptibility data of lepidopteran and coleopteran pest species on different Cry proteins.

Methods

This review is part of the EU funded project GRACE to collate available evidence on evolution of resistance to *Bt*-crops relevant in Europe. The review team and the stakeholder group for this review are formed by scientist from the project and representatives from NGOs, authorities, industry and science.

Search strategy

The aim of the search is to obtain unbiased and comprehensive information relevant to the review question. Both published and unpublished data should be collated. Different sources of information will be searched in order to maximize the coverage of the search. All searches will be conducted in English.

Search terms

Combinations of the following search terms will be applied to the selected databases.

*denotes a wild card for zero or more characters. The search terms are related to population and outcome. The search will include title, abstract and keywords. As all databases and websites vary in the way they handle complex search strings and the use of Boolean operators the exact search strings used will be listed in the appendix of the review.

Population: (lepidopter* OR butterfl* OR coleopter* OR beetl*).

Outcome: (toxi* OR cry* OR vip3* OR bacillus thuringiensis* OR bt) AND (suscept* OR resist*).

Several search strings have been tested in a preliminary search with Thomson Reuters (formerly ISI) Web of

Science. The results are listed in Table 1. Additionally it was tested whether 15 known relevant publications identified by personal expertise of the review team (reference publications, see Appendix 2) were found with the used search strings.

Because it returned the highest number of hits and found all reference publications (15 of 15) we decided to choose the third search string presented in Table 1 for our search. The "NOT" operator was excluded, as in this case publications dealing with both, maize and e.g. cotton were excluded as well.

Data bases

The following data bases will be considered:

- Thomson Reuters (formerly ISI) Web of Science, New York, USA, http://apps.webofknowledge.com
- Scopus by Elsevier, Amsterdam, The Netherlands, http://www.scopus.com/
- CAB abstracts, CABI, Wallingford, United Kingdom, http://www.cabdirect.org/
- JSTORE by Ithaka, USA, http://www.jstor.org/

Search engines

Additionally the search will be supplemented by the use of internet meta search engines. For this Google Scholar (http://scholar.google.com) will be used. The search terms will be simplified and restricted to pdf or word files. The first 50 hits will be examined from this search.

Specialist sources

Additional grey literature or other useful data will be provided by the stakeholder group. Therefore known experts in the field of resistance management will bee contacted and asked for unpublished data such as Diploma thesis, Phd thesis, technical reports or similar information. The scientists contacted and the information they delivered will be recorded and listed in the report. Furthermore data collected in monitoring reports of the authorisation holders will be considered. Additionally applications for placing on the market will be screened for baseline susceptibility data. However these data can only be considered upon approval by the consent holder.

Study inclusion criteria

All identified publications will be stored in a database using the software Reference Manager Professional Version 12. Relevant information for the review will be identified in a three step approach based on inclusion criteria described below. In the first step publications will be excluded if their titles are considered irrelevant based on the inclusion criteria. In the second step publications will be excluded, when their abstracts are considered to be irrelevant. In both steps, a conservative approach will be used meaning that if there is any doubt about the relevance of the information it will be retained. In the third step the included publications will be viewed at full text.

The first and second step will be conducted independently by two reviewers. To avoid different appraisement of reviewers a kappa test will be undertaken to guarantee consistency in the interpretation of the selection process. Therefore 10% of the papers (up to a maximum for 300 in the first step and 100 for the second step) will be checked and the level of agreement evaluated. If the kappa rating is below 0.6 discrepancies in the decision making need to be discussed and adjusted to assess the remaining articles.

Inclusion criteria

Relevant subject(s)/population(s):

Pest species of maize with economic relevance or regionally economic relevance in Europe will be considered. The selection of species is based on EFSA data base listing arthropods species in crop fields [8]. Different names for the same species will be considered (in brackets) to minimize information loss by the existence of synonymies [9].

Lepidoptera: *Ostrinia* (*Pyralis*, *Pyrausta*) *nubilalis*, *Sesamia nonagrioides*, *Agrotis segetum*, *Helicoverpa* (*Heliothis*) *armigera*, *Agrotis ipsilon* (*ypsilon*), *Autographa gamma*,

Table 1 Scoping results with different search strings (performed on 13.01.2014)

Nr	Search string	Web of science	Reference publications[a)]
1	TS = ((Bt Maize OR Bt corn OR transgenic maize OR transgenic corn) NOT (cotton OR rice OR soy*) AND (lepidopter* OR butterfl* OR coleopter* OR beetl*) AND (Cry* OR Bt protein* OR Bt toxin OR Bacillus thuringiensis toxin OR vip3*) AND Europe AND (baseline susceptibility OR LC50 OR toxicity OR resistan* OR bioassay))	19	3
2	TS = ((Bt Maize OR Bt corn OR transgenic maize OR transgenic corn) NOT (cotton OR rice OR soya) AND (lepidopter* OR butterfl* OR coleopter* OR beetl*) AND (Cry* OR Bt protein* OR Bt toxin OR Bacillus thuringiensis toxin OR vip3*) AND (baseline susceptibility OR LC50 OR toxicity OR resistan* OR bioassay))	438	4
3	TS = (lepidopter* OR butterfl* OR coleopter* OR beetl*) AND TS = (toxi* OR cry* OR vip3* OR Bacillus thuringiensis* OR bt) AND TS = (suscept* OR resist*)	3887	15

[a)]Reference publications found (out of 15). Reference publications are listed in Appendix 2.
The asterisk (*) denotes a wildcard for zero or more characters.

Mythimna (*Pseudaletia*) *unipuncta*, *Acronicta rumicis*, *Mamestra brassicae*, *Sesamia cretica*, *Xylena vetusta.*

Coleoptera: *Diabrotica virgifera virgifera*

Relevant outcomes:

Toxicity tests with *Bt*-maize expressed *Bt*-proteins, test endpoints LC_{50}, EC $_{50}$, MIC_{50}.

Bt-maize expressing lepidopteran or/and coleopteran specific Cry proteins.

Relevant types of study design:

Laboratory studies with field collected test organisms; this means populations should not have been reared in the laboratory for more than 3 generations.

Study quality assessment

The quality of all included publications is assessed by the two reviewers. The studies are categorized, based on the quality of a specific toxicity study considering following criteria:

- full description of the source of the toxin,
- the bioactivity of the toxin has to be verified, experimental design comprises positive control
- the concentration and integrity must be checked with an ELISA test or Western Blot
- the number of tested larvae should be at least 10 per concentration
- a minimum of two replications should be performed
- the number of toxin concentrations should be not less than five
- the dose response curves should be characterized by $LC_{50}/EC_{50}/MIC_{50}$ and/or $LC_{90}/EC_{90}/MIC_{90}$ with CIs of the different values

The studies will be categorized individually for each criterion. The results will be annexed within an Excel sheet or table.

Data extraction strategy

Next to the authors of the studies, the date and the type of publication are recorded. Definite variables are the tested insect species, the country or region where the insect pests were collected, the description of the Cry protein used. The toxicity tests are classified after their methods (e.g. surface application) and endpoints (LC_{50}, EC_{50}). The number of tested individuals and the statistical reliability is recorded.

A preliminary data extraction and coding sheet is annexed to the protocol. The final format depends on the publications included. In the process two reviewers are involved. Reviewer one is extracting the data. The results will be checked by reviewer two.

Data analysis

Data will be synthesised in two ways. Firstly extracted data will be presented in a table (compare Table 2) or graphs. This will provide an overview of the reviewed studies. Secondly the data will be summarized in a narrative report.

Dealing with missing data

Studies in which relevant data were recorded but not reported in the publication will be identified and the authors will be contacted and asked if they are able to provide the missing datasets.

If the relevant data cannot be obtained or calculated from the given data, the dataset will be marked as incomplete data set.

Quantitative synthesis

As outlined above data provided by the evaluated articles will differ in the study design (details see Background) and in the use of different pest populations. These differences make it impossible to directly compare the susceptibility data. Therefore only a narrative discussion of the findings will be provided. The outcome of the study quality assessment will be discussed.

Assessment of heterogeneity:

We will discuss heterogeneity across studies. The influence of variation caused by study design, using different toxin sources, different populations (countries/regions/continents) and different statistical approaches will be explored.

Investigation of heterogeneity:

The influence of different parameters causing heterogeneity will be investigated. For parameters causing high heterogeneity separated analysis of subgroups may be conducted.

Sensitivity analysis:

Sensitivity analyses will be conducted to explore the

- Influence of individual studies
- Influence of funding sources for the overall results
- Influence of study quality
- Influence of data on species, genus, family, or order level

Appendix 1: List of stakeholders consulted during the stakeholder consultation process

The GRACE stakeholder consultation process was divided in three steps: (i) a workshop on evidence synthesis and question formulation; (ii) a written consultation on prioritization of research questions and (iii) a review of the draft protocols. Therefore about 700 stakeholders were invited to participate from different interest groups representing academia (A), competent authorities (CA), industry (I), civil society organisations and non-governmental organisations (CSO/NGO) or other not specified groups (others). Not all stakeholders commented on each of the GRACE research questions, but at least 16 stakeholders

Table 2 List of possible variables extracted for the systematic review on the baseline susceptibility of insect pests to *Bt*-proteins

Variable name	Definition	Type	Closed terms
Article_id	Unique identification number assigned to each publication	Integer	No
Author	Author(s) of the listed publication.	Text	No
Publication_year	Year of publication of study	Integer	No
Citation	Citation, e.g. journal name, volume and page numbers	Text	No
Title	Title of the publication	Text	No
Was_peer_reviewed	Indicates whether study was published in a peer reviewed journal.	String	Yes
Country	Country where insects were collected	String	Yes
Expmt_num	Number of experiment within a study (e.g. different species, population, Bt-toxins, years, etc.)	String	No
Bt_protein	Bt-protein used for the toxicity tests	String	Yes
Target_order	Target taxoNomic order	String	Yes
Target_family	Target taxoNomic family	String	Yes
Target_genus	Target taxoNomic genus	String	Yes
Target_species	Target taxoNomic species	String	Yes
Stage	Stage of the test organism	String	Yes
Test_method	Used test methodology such as surface test, incorporation test, test with discriminate dose or plant material	String	Yes
No_test_organisms	Number of test organisms	Real	No
No_doses	Number of Bt protein doses used	Real	No
Control_No_test_organism	Number of test organism in the control	Real	No
Dose_No_test_organism	Number of test organism in the treatment	Real	No
Model_used	Statistical model used by author to characterize the dose- response relationship.	String	Yes
Endpoint_50	LC_{50}/ EC_{50}/MIC_{50}	String	Yes
Variability_endpoint_50	Variability (CI, SD, SE) of LC_{50}/ EC_{50}/MIC_{50}	String	Yes
Endpoint_90	LC_{90}/EC_{90}/MIC_{90}	String	Yes
Variability_endpoint_90	Variability (CI, SD, SE) of LC_{90}/EC_{90}/MIC_{90}	String	Yes
Slope	Slope ± SE of the dose response curve	String	Yes
X_2	$\chi 2$ goodness of fit test	string	Yes
df	Degrees of freedom of the model	String	Yes

Given is the variable name in the database, the definition of the variable, the type, and whether the variable content is restricted to closed (predefined) terms.

commented on the research questions on resistance evolution.

Stakeholders are not listed by name on the grounds of data protection, but by organisations they represented:

- Scientific Institute of Public Health Brussels, Belgium (others)
- University of Nebraska, USA (A)
- Testbiotech. Germany (CSO/NGO)
- Eurogroup for Animal/ Akademie für Tierschutz (CSO/NGO)
- Federal Agency for Nature Conservation, Germany (CA)
- Unidad de Apoyo D.G. de Producciones y Mercados Agrarios Ministerio de Agricultura, Alimentación y Medio Ambiente, Spain (CA)
- University of Slovenia (A)
- EuropaBio (I)
- 8 anonymous stakeholder (3 CA, 3 A, 1 CSO/NGO, 1 Other)

Appendix 2: Reference publications to assess the quality of the search strings

Avilla C, Vargas-Osuna E, González-Cabrera J, Ferré J, González-Zamora J: **Toxicity of several delta-endotoxins of *Bacillus thuringiensis* against *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Spain**. *J. Invert. Pathol.* 2005, **90**:51–54.

Crespo AL, Rodrigo-Simón A, Siqueira HA, Pereira EJ, Ferré J, Siegfried BD: **Cross-resistance and mechanism of resistance to Cry1Ab toxin from *Bacillus thuringiensis***

in a field-derived strain of European corn borer, *Ostrinia nubilalis*. *J. Invert. Pathol.*2011, **107**:185–192.

Farinós GP, Andreadis SS, De la Poza M, Mironidis GK, Ortego F, Savopoulou-Soultani M, Castanera P: **Comparative assessment of the field-susceptibility of *Sesamia nonagrioides* to the Cry1Ab toxin in areas with different adoption rates of Bt maize and in *Bt*-free areas**. *Crop Protect*. 2011, **30**: 902–906.

González-Cabrera J, Farinós GP, Caccia S, Diaz-Mendoza M, Castanera P, Leonardi MG, Giordana B, Ferré J: **Toxicity and mode of action of *Bacillus thuringiensis* cry proteins in the Mediterranean corn borer, *Sesamia nonagrioides* (Lefebvre)**. *App. Environl Microbiol.* 2006, **72**:2594–2600.

Hernández-Rodriguez CS, Hernández-Martinez P, Van Rie J, Escriche B, Ferré J: **Shared midgut binding sites for Cry1A. 105, Cry1Aa, Cry1Ab, Cry1Ac and Cry1Fa proteins from *Bacillus thuringiensis* in two important corn pests, *Ostrinia nubilalis* and *Spodoptera frugiperda***. *PloS one* 2013, **8**:e68164.

Huang F, Leonard BR, Gable RH: **Comparative susceptibility of European corn borer, southwestern corn borer, and sugarcane borer (Lepidoptera: Crambidae) to Cry1Ab protein in a commercial *Bacillus thuringiensis* corn hybrid**. *J. Econ. Entomol.* 2006, **99**:194–202.

Khasdan V, Sapojnik M, Zaritsky A, Horowitz AR, Boussiba S, Rippa M, Manasherob R, Ben-Dov E: **Larvicidal activities against agricultural pests of transgenic *Escherichia coli* expressing combinations of four genes from *Bacillus thuringiensis***. *Arch. Microbiol*. 2007, **188**:643–653.

Li H, Bouwer G: **Toxicity of *Bacillus thuringiensis* Cry proteins to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in South Africa**. *J. Invert. Pathol*. 2012, **109**:110–116.

Li H, Oppert B, Higgins RA, Huang F, Buschman LL, Zhu K yan: **Susceptibility of Dipel-resistant and-susceptible *Ostrinia nubilalis* (Lepidoptera: Crambidae) to individual *Bacillus thuringiensis* protoxins**. *J. Econ. Entomol.* 2005, **98**:1333–1340.

Mahon R, Olsen K, Garsia K, Young S: **Resistance to *Bacillus thuringiensis* toxin Cry2Ab in a strain of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia**. *J. Econ. Entomol*. 2007, **100**:894–902.

Nowatzki T, Lefko S, Binning R, Thompson S, Spencer T, Siegfried B: **Validation of a novel resistance monitoring technique for corn rootworm (Coleoptera: Chrysomelidae) and event DAS-59122-7 maize**. *J. Appl. Entomol*. 2008, **132**:177–188.

Pereira EJG, Lang BA, Storer NP, Siegfried BD: **Selection for Cry1F resistance in the European corn borer and cross-resistance to other Cry toxins**. *Entomol. Experiment. Appl*. 2008, **126**:115–121.

Rios-Diez J, Saldamando-Benjumea C: **Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from central Colombia to two insecticides, methomyl and lambda-cyhalothrin: a study of the genetic basis of resistance**. *J. Econ. Entomol*. 2011, **104**:1698–1705.

Saeglitz C, Bartsch D, Eber S, Gathmann A, Priesnitz K, Schuphan I: **Monitoring the Cry1Ab susceptibility of European corn borer in Germany**. *J. Econ. Entomol*. 2006, **99**:1768–1773.

Siegfried BD, Vaughn TT, Spencer T: **Baseline susceptibility of western corn rootworm (Coleoptera: Crysomelidae) to Cry3Bb1 *Bacillus thuringiensis* toxin**. *J. Econ. Entomol*. 2005, **98**:1320–1324.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
AG designed the review questions, wrote the review protocol and will conduct the literature search with subsequent data extraction and synthesis. KUP was involved in writing the review protocol and will conduct the literature search as well as the data extraction. Both authors read and approved the final manuscript.

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