Modern Biotechnology in Integrated Plant Production
IOBC-WPRS Bulletin Vol. 163, 2023
pp. 69-75

Bt maize and non-target animals – a systematic review

Michael Meissle[1], Steven E. Naranjo[2], Jörg Romeis[1]
[1]Agroscope, Research Division Agroecology and Environment, Zürich, Switzerland; [2]USDA-ARS, Arid-Land Agricultural Research Center, Maricopa, Arizona, USA
e-mail: michael.meissle@agroscope.admin.ch

Abstract: Systematic reviews follow predefined standards to ensure unbiased, comprehensive, transparent, repeatable, and robust evidence synthesis including statistical meta-analyses. For various stakeholders, such systematic reviews help to address uncertainties regarding environmental impacts of genetically modified (GM) plants. The current work, following the stringent standards of the Collaboration for Environmental Evidence (CEE), addressed the question: "Does the growing of Bt maize change abundance or ecological function of non-target animals compared to the growing of non-GM maize?". Literature was searched and screened systematically until August 2019. More than 7200 records on invertebrate abundance, activity density, or predation/parasitism rates were stored in a custom-made database. A critical appraisal scheme for field studies on non-targets in GM crops was developed to capture issues associated with internal and external validity of all primary data. Our meta-analyses on different taxonomic levels revealed few and often non-robust significant effect sizes when comparing Bt maize (GM to produce insecticidal Cry proteins from *Bacillus thuringiensis*) and untreated non-Bt maize, largely corroborating earlier meta-analyses. When untreated Bt maize was compared with conventional maize sprayed with insecticides, predator populations in particular were lower after the application of broad-spectrum pyrethroids. Bt maize thus represents a highly selective pest control technology with relatively few negative consequences for non-target invertebrates, especially when compared with the use of broad-spectrum insecticides for managing Bt-targeted pests. One shortcoming of the present and all prior reviews, however, is the limited availability of data. To facilitate future meta-analyses, we strongly encourage researchers to publish full datasets along with scientific articles.

Key words: Bt corn, critical appraisal, environmental risk assessment, genetic engineering, meta-analyses, non-target organisms

Introduction

Genetically modified (GM) maize producing insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) has been grown for more than 25 years in more than a dozen countries and 5 continents (Romeis et al., 2019). Because of the insecticidal mode of action of Bt maize, potential adverse effects on non-target organisms are a major area of concern that is addressed in risk assessments. In particular, beneficial arthropods contributing to decomposition, pollination, and biological control have been the focus of research. Over the years, hundreds of studies on environmental effects of Bt maize have been conducted and published by public sector scientists and product developers. Reviews, and in particular meta-analyses, can help stakeholders to get a complete overview of research results and to address uncertainties regarding environmental impacts of the technology (Kohl et al., 2015). Although data on non-target effects of Bt maize have been subjected to meta-analyses before, many field studies from

Europe and other parts of the world only became available in the last decade, and those data are often not covered in previous publications. Furthermore, systematic reviews on Bt crops and non-targets following standardized protocols are still lacking. Therefore, a systematic review according to the guidelines of the Collaboration for Environmental Evidence (CEE) has been conducted to answer the question: "Does the growing of Bt maize change abundance or ecological function of non-target animals compared to the growing of non-GM maize?" In this article, we summarize the history of previous meta-analyses of non-target species in Bt crops and present general incentives and challenges of systematic reviews. Then, we introduce activities concerning the current review (Meissle et al., 2022a) including stakeholder involvement, review methodology, review findings, and communication of results.

Previous meta-analyses of Bt crops and non-targets

The first meta-analysis on field data of non-target invertebrates in Bt maize and Bt cotton was published by Marvier et al. (2007) (Table 1).

Table 1. Publication history of meta-analyses on non-target organisms in Bt crops.

Reference	**Laboratory/ field studies**	**Bt crops/ Bt proteins**	**Focus**
Marvier et al. (2007)	field (42 publications)	maize, cotton	higher level taxa
Wolfenbarger et al. (2008)	field (47 publications)	maize, cotton, potato	ecological functional guilds
Duan et al. (2008)	laboratory (25 publications)	Bt proteins	honey bees
Naranjo (2009)	laboratory (135), 63 field (63 publications)	13 Bt proteins, maize, cotton, potato, rice, eggplant	ecological functional guilds; life history traits
Duan et al. (2010)	laboratory (74), field (52 publications)	maize, cmotton, potato, rice, eggplant	compare laboratory with field studies
Comas et al. (2014)	field (13 independent field trials)	maize	Spain
Dang et al. (2017)	laboratory (40), field (27 publications)	rice	China
Pellegrino et al (2018)	field (32 publications)	maize	agronomic, environmental and toxicological traits
Krogh et al. (2020)	field (22 publications)	maize, cotton, canola, potato, rice	soil invertebrates
Meissle et al. (2022a)	field (120 publications)	maize	systematic review

The authors conducted systematic literature searches, defined inclusion criteria and rules for selection of datasets for analyses. Furthermore, authors were contacted for missing information and the database was provided freely. Follow-up analyses of the same dataset exploring further, more detailed analyses on functional guilds became available from Wolfenbarger et al. (2008). Duan et al. (2008) focused on laboratory data on honeybees. Naranjo (2009) updated the database and repeated analyses on functional guilds in Bt maize, cotton, and potato. Data from 13 (partly unpublished) independent Bt maize field studies in Spain were meta-analyzed by Comas et al. (2014), a meta-analysis focusing on Bt rice in China was published by Dang et al. (2017), and an analysis of soil invertebrates in different Bt crops became available by Krogh et al. (2020). Pellegrino et al. (2018) analyzed a broad range of agronomic and environmental data on Bt maize, while the number of included datasets for non-targets was rather small. Finally, the systematic review described here was published by Meissle et al. (2022a).

Incentives and challenges of systematic reviews

With the number of published primary research papers increasing exponentially, reviews and meta-analyses become more and more important for informing science as well as policy and management decisions including the environmental sector. However, review objectiveness and comprehensiveness are crucial to prevent misinformation and misjudgments by decision makers (O'Leary et al., 2016).

The Collaboration for Environmental Evidence (CEE) is a global open community of stakeholders to promote and deliver evidence synthesis to environmental policy and practice (https://environmentalevidence.org). CEE provides guidelines for review protocols, systematic reviews and evidence maps (CEE 2022), provides a set of tools for evidence synthesis, and runs a journal for publication of products conducted according to the guidelines and other papers relevant to systematic evidence synthesis (https://environmentalevidencejournal.biomedcentral.com).

Woodcock et al. (2014) defined criteria that should be fulfilled for high quality systematic reviews:

1. Publication of a review protocol that explains the background, a clearly defined review question, the literature search strategy, inclusion criteria, quality assessment, and the data extraction and data analyses approach. The protocol is published and open for comments before the evidence synthesis is conducted.
2. Searching for studies is done in a comprehensive range of sources including peer-reviewed (multiple databases) and grey literature (relevant webpages). Clearly defined search strings ensure systematic searches and detailed documentation ensures transparency and repeatability.
3. Literature screening following clearly defined and documented inclusion criteria determines studies relevant to the review question.
4. Transparent critical appraisal of the methodology of each study following predefined criteria allows objective accounting for variation in study quality for analysis and interpretation of results.
5. Data extraction is documented, repeatable, and consistent.
6. Data are synthesized in a quantitative way (meta-analyses, "study of studies"). Heterogeneity in the effect is investigated statistically and possible publication bias is considered.

While the highest possible standard should always be the aim for any scientific work, conducting a systematic review according to CEE guidelines is time consuming and requires a team of people, leading to substantial costs. Such systematic reviews are projects on their own and need to be commissioned with an adequate budget and time frame, depending on the complexity of the research question, the number of available studies, and the desired depth of analysis. The advantage of a review team is that tedious work can be shared and quality control steps by other team members can be installed.

Stakeholder involvement

Active stakeholder involvement is a key element of many systematic reviews in environmental management (Haddaway et al., 2017), including the one presented here. Stakeholder involvement may lead to a broader acceptance of conducted reviews and ensures their thoroughness and their relevance from a societal perspective. In the EU-funded project GRACE (**G**MO **R**isk **A**ssessment and **C**ommunication of **E**vidence), a workshop with stakeholders representing academic institutions, competent authorities, civil society organizations, industry, and professional organizations was conducted to discuss the importance and relevance of potential reviews for the areas of health, socio-economy, and environment (Spök et al., 2018). All stakeholders were invited to prioritize potential review questions and later on to comment on the draft review protocols for the questions that were ultimately selected. Towards the end of the funded project period, a second stakeholder workshop on the preliminary results of the evidence synthesis activities of GRACE was held and comments provided by the stakeholders helped to finalize the project.

Review methodology

The review protocol for the present systematic review on non-targets in Bt maize outlines the methodology (Meissle et al., 2014). Following this protocol, literature published until August 2019 was searched systematically in 12 bibliographic databases, 17 specialized webpages, and reference sections of 78 review articles. Defined eligibility criteria were applied to screen titles, abstracts, and full texts of the retrieved references (Meissle et al., 2022a; b).

Data on invertebrate abundance, activity density, or predation/parasitism rates was stored in a custom-made database (Meissle et al., 2022b). Eligible data that did not qualify for the database were presented in tables (Meissle et al., 2022a). A critical appraisal scheme for field studies on non-targets in GM crops was developed to estimate the risk of bias (internal validity) and the suitability of each dataset to answer the review question (external validity). Meta-analyses on different taxonomic levels, functional groups, and types of Bt maize were conducted. Untreated Bt maize was either compared to untreated non-Bt maize, or to insecticide-treated non-Bt maize. The influence of contributions by private sector product developers on reported effects was investigated (Meissle et al., 2022a).

Results

The database on non-target effects of Bt maize field trials contains 7279 records from 233 experiments and 120 articles (Meissle et al., 2022b). Records on individual species and life stages as well as aggregated data for all life stages or higher taxonomic units were stored. Each record represents a comparison of invertebrates in Bt vs. non-Bt maize (non-Bt either untreated or insecticide treated). All records contain means, SDs, N, and many additional variables.

Meta-analyses on different taxonomic levels revealed only few and often non-robust significant effect sizes when both Bt maize and non-Bt maize were untreated (Meissle et al., 2022a). When untreated Bt maize was compared with pyrethroid-treated non-Bt maize, more effect sizes were significant. In particular populations of predators were reduced after pyrethroid treatment, while few data were available for other insecticides. No evidence for publication bias and for influence of vested interests was found.

The critical appraisal revealed that Bt protein expression was often not addressed in the publication of the field studies, which was particularly critical when experimental lines were used. In addition, the consistent availability of data in a form necessary for meta-analyses was a problem. Some studies could not be analyzed because appropriate data could not be obtained from the publication or directly from the authors. This includes the private sector, where getting access to data post publication proved complicated. In the future, however, we hope that availability of data improves through open access initiatives of public institutions in many countries and data availability requirements of journals, as well as initiatives in the private sector (see Morr and Wang, 2023, this issue).

Communication

The project on non-targets in Bt maize comprised three publications, i.e., the review protocol (Meissle et al., 2014), the database (Meissle et al., 2022b), and the systematic review itself (Meissle et al., 2022a). The scientific publications were accompanied by a press release by USDA-ARS (USA) as well as a policy brief by Agroscope (Switzerland) in English, French and German (Meissle et al., 2022c). In addition, a plain language summary is available on the CEE website (Meissle et al., 2022d).

Conclusions

Systematic reviews are helpful for science and decision making if conducted in an objective (unbiased), comprehensive, and transparent way. Following standardized methodology is crucial, although time and resource intensive.

The current systematic review on non-target animals in Bt maize largely confirmed previously published reviews and meta-analyses. The effects of Bt maize on the community of non-target invertebrates were small and mostly neutral. Bt maize showed benefits when compared with the effects of broad-spectrum pyrethroid insecticide treatments. Publication bias and consequences of conflicts of interest were not evident. For future publications of field studies, research data should be made open access.

Acknowledgements

The project has been funded within the GRACE consortium, EU-FP7 project, Grant Agreement KBBE-2011-6-311957.

References

CEE 2022. Guidelines and standards for evidence synthesis in environmental management. Version 5.1. Collaboration for Environmental Evidence (eds: Pullin, A. S., Frampton, G. K., Livoreil, B. and Petrokofsky, G.). URL https://environmentalevidence.org/information-for-authors. Accessed 18. Jan. 2023.

Comas, C., Lumbierres, B., Pons, X., and Albajes, R. 2014. No effects of *Bacillus thuringiensis* maize on nontarget organisms in the field in southern Europe: a meta-analysis of 26 arthropod taxa. Transgenic Res. 23: 135-143. https://doi.org/10.1007/s11248-013-9737-0

Dang, C., Lu, Z., Wang, L., Chang, X., Wang, F., Yao, H., Peng, Y., Stanley, D. and Ye, G. 2017. Does Bt rice pose risks to non-target arthropods? Results of a meta-analysis in China. Plant Biotechnol. J. 15: 1047-1053. https://doi.org/10.1111/pbi.12698

Duan, J. J., Marvier, M., Huesing, J. E., Dively, G., and Huang, Z. Y. 2008. A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). PLoS ONE 3: e1415. https://doi.org/10.1371/journal.pone.0001415

Duan, J. J., Lundgren, J. G., Naranjo, S. and Marvier, M. 2010. Extrapolating non-target risk of Bt crops from laboratory to field. Biol. Lett. 6: 74-77. https://doi.org/10.1098/rsbl.2009.0612

Haddaway, N. R., Kohl, C., Rebelo da Silva, N., Schiemann, J., Spök, A., Stewart, R., Sweet, J. B. and Wilhelm, R. 2017. A framework for stakeholder engagement during systematic reviews and maps in environmental management. Environ. Evid. 6: 11. https://doi.org/10.1186/s13750-017-0089-8

Kohl, C., Frampton, G., Sweet, J., Spök, A., Haddaway, N. R., Wilhelm, R., Unger, S. and Schiemann, J. 2015. Can systematic reviews inform GMO risk assessment and risk management? Front. Bioeng. Biotechnol. 3: 113. https://doi.org/10.3389/fbioe.2015.00113

Krogh, P. H., Kostov, K. and Damgaard, C. F. 2020. The effect of Bt crops on soil invertebrates: a systematic review and quantitative meta-analysis. Transgenic Res. 29: 487-498. https://doi.org/10.1007/s11248-020-00213-y

Marvier, M., McCreedy, C., Regetz, J. and Kareiva, P. 2007. A meta-analysis of effects of *Bt* cotton and maize on nontarget invertebrates. Science 316: 1475-1477. https://doi.org/10.1126/science.1139208

Meissle, M., Naranjo, S. E., Kohl, C., Riedel, J. and Romeis, J. 2014. Does the growing of Bt maize change abundance or ecological function of non-target animals compared to the growing of non-GM maize? A systematic review protocol. Environ. Evid. 3: 7. https://doi.org/10.1186/2047-2382-3-7

Meissle, M., Naranjo, S. E. and Romeis, J. 2022a. Does the growing of Bt maize change abundance or ecological function of non-target animals compared to the growing of non-GM maize? A systematic review. Environ. Evid. 11: 21. https://doi.org/10.1186/s13750-022-00272-0

Meissle, M., Naranjo, S. E. and Romeis, J. 2022b. Database of non-target invertebrates recorded in field experiments of genetically engineered Bt maize and corresponding non-Bt maize. BMC Res. Notes 15: 199. https://doi.org/10.1186/s13104-022-06021-3

Meissle, M., Naranjo, S. E. and Romeis, J. 2022c. Bt maize has minimal impact on non-target organisms. Swiss Agricultural Research [WWW document], published online 1 July 2022. URL https://www.agrarforschungschweiz.ch/en/2022/07/bt-maize-has-minimal-impact-on-non-target-organisms/. Accessed 18 Jan. 2023.

Meissle, M., Naranjo, S. E. and Romeis, J. 2022d. Genetically modified insect-resistant maize has little impact on studied non-target invertebrates. Collaboration for Environmental Evidence, Plain Language Summary, June 2022, Systematic Review CEE 14-020 [WWW document]. URL https://environmentalevidence.org/wp-content/uploads/2022/12/CEE-PLS_Meissle-et-al.-2021_Genetically-modified-invertebrates_FINAL.pdf. Accessed 18 Jan. 2023.

Morr, C. and Wang, M. 2023. Bayer's commitments to transparency: opening up our science to build public trust. IOBC-WPRS Bull. 163: 76-78.

Naranjo, S. E. 2009. Impact of Bt crops on non-target invertebrates and insecticide use patterns. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 4: 11. https://doi.org/10.1079/PAVSNNR20094011

O'Leary, B. C., Kvist, K., Bayliss, H. R., Derroire, G., Healey, J. R., Hughes, K., Kleinschroth, F., Sciberras, M., Woodcock, P. and Pullin, A. 2016. The reliability of evidence review methodology in environmental science and conservation. Environ. Sci. Policy 64: 75-82. https://doi.org/10.1016/j.envsci.2016.06.012

Pellegrino, E., Bedini, S., Nuti, M. and Ercoli, L. 2018. Impact of genetically engineered maize on agronomic, environmental and toxicological traits: a meta-analysis of 21 years of field data. Sci. Rep. 8: 3113. https://doi.org/10.1038/s41598-018-21284-2

Romeis, J., Naranjo, S. E., Meissle, M. and Shelton, A. M. 2019. Genetically engineered crops help support conservation biological control. Biol. Control 130: 136-154. https://doi.org/10.1016/j.biocontrol.2018.10.001

Spök, A., Racovita, M., Karner, S., Adduci, G., Smets, G., Rüdelsheim, P., Kohl, C., Wilhelm, R. and Schiemann, J. 2018. Inclusive development and prioritization of review questions in a highly controversial field of regulatory science. Environ. Evid. 7: 1. https://doi.org/10.1186/s13750-017-0113-z

Wolfenbarger, L. L., Naranjo, S. E., Lundgren, J. G., Bitzer, R. J. and Watrud, L. S. 2008. Bt crop effects on functional guilds of non-target arthropods: A meta-analysis. PLoS ONE 3: e2118. https://doi.org/10.1371/journal.pone.0002118

Woodcock, P., Pullin, A. S. and Kaiser, M. J. 2014. Evaluating and improving the reliability of evidence syntheses in conservation and environmental science: A methodology. Biol. Conserv. 176: 54-62. https://doi.org/10.1016/j.biocon.2014.04.020