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RESEARCH ARTICLE

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Meta-analysis on effects of Bt-maize on nontarget invertebrates – Data transportability across continents

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Societal impact statement
Maize varieties producing insecticidal proteins from *Bacillus thuringiensis* (Bt) have become an important component of integrated pest management worldwide. For regulatory approval of such plants, risks to the environment need to be assessed. To make such assessments less expensive and time-consuming, conclusions drawn from data collected in one region could be used in regulatory submissions in other regions. By comparing published data of invertebrates recorded in Bt maize field experiments worldwide, we contribute to the discussion of data transportability across continents. The results are of value to regulatory authorities throughout the world and ultimately of benefit to growers and consumers.

Summary

- For insecticidal crops, adverse effects on non-target invertebrates including beneficial decomposers, predators, and parasitoids are of particular concern. This work focuses on data transportability across continents by comparing non-target invertebrate data from published Bt maize field studies.
- Data derived from a comprehensive global database were summarized for taxonomic composition and subjected to meta-analyses considering taxonomy, Bt maize target insect order, and ecological functional group. Each dataset represents a replicated comparison of an invertebrate population recorded in Bt maize with the respective population in non-Bt (control) maize.
- Taxonomic composition at order or higher taxonomic level was comparable across continents. Meta-analyses revealed that most analyzed invertebrates were equally abundant in Bt and non-Bt maize, while robust adverse effects were only observed on specialized parasitoids of target pests. The conclusions drawn from the North American data were confirmed for Europe and Asia.
- The similarity of species communities at order level as well as outcomes of meta-analyses across continents indicate that conclusions from field studies are generally transportable across geographies. High-quality, well-designed, well-described, and independent studies from multiple locations and years, and open-access data

availability (transparency), increase trust in the conclusions drawn and the usefulness for submissions to multiple regulatory systems.

KEYWORDS
Bacillus thuringiensis corn, environmental risk assessment, genetically modified plants, hedges d, meta-analysis, non-target organisms

1 | INTRODUCTION

Genetically engineered (GE) crops need regulatory approval for environmental release and commercial production under every jurisdiction (SCBD, 2000). Maize varieties producing insecticidal proteins from *B. thuringiensis* (Bt) are among the most widespread GE crops cultivated on several continents (ISAAA, 2019). Environmental risk assessment is part of the regulatory dossier and includes an assessment of adverse effects on non-target organisms (Romeis et al., 2008). For insecticidal crops, adverse effects on beneficial invertebrates, such as decomposers, predators, parasitoids, and pollinators, are of particular concern, as these ecosystem services are generally agreed upon as protection goals (Devos et al., 2015). Non-target risk assessments are performed in a hypothesis-driven, tiered approach (Romeis et al., 2008). Following a problem formulation phase, the non-target testing of insecticidal crops commonly starts with purified proteins that are equivalent to the proteins expressed in the GE plant (lower tier studies). Selected test organisms that represent different taxonomic and ecological functional groups (surrogate species) are exposed to concentrations of the protein exceeding the expression levels *in planta* to create a worst-case exposure situation. In this way, the stressor of concern can be tested without the disturbing influence of other variables, such as environment, plant physiology, or species interactions. If those experiments indicate the sensitivity of certain taxonomic groups, more species of this group or closely related groups are tested and exposure scenarios are refined to be more realistic, e.g., by using GE plant material instead of purified protein (higher tier studies). If unacceptable risks cannot be ruled out with sufficient certainty based on the results of such tests, field experiments are conducted to investigate how non-target invertebrate populations are affected under realistic conditions. Even though this approach suggests that higher tier testing is not required if no hazard is identified at lower tiers, many jurisdictions, e.g., the European Union (EU), call for field experiments in any case. Direct effects of the expression of a novel protein on non-target species are difficult to detect in the field because of the many confounding factors causing high variability. The advantage of field experiments, however, is that ecological consequences of the GE plant can be assessed, such as food-web or other indirect effects. In general, risk assessments should make use of existing information and only request additional data if necessary to reach a confident conclusion of acceptable risk (Romeis et al., 2009). For example, it is widely accepted that the results of non-target tests conducted with surrogate species in lower-tier laboratory testing are applicable across jurisdictions, i.e., not restricted to the location where the test has been performed (Romeis et al., 2009). GE crops undergo regulatory evaluation by transformation event. For insect-resistant crops, the most relevant data for the risk assessment of new transformation events are those previously collected on the same insecticidal protein, if applicable (Romeis et al., 2009). However, expression patterns cannot be assumed to be similar between transformation events and need to be known before conclusions from one event can be transferred to another. Similarly, when data from related proteins are to be considered in risk assessments, mode of action, activity spectrum, and most importantly, expression patterns need to be known and comparable. If this is the case, conclusions from studies might be transported among transformation events within or between crops and geographies, e.g., from Cry1Ab-expressing maize to Cry1Ab-expressing cowpea (Ba et al., 2018), from crops cultivated in the Americas to African or Asian countries (Melnick et al., 2023), or from GE crop-producing countries to importing countries (Nakai et al., 2015). Although the particular arthropod communities differ among crops and continents, the sensitivity or lack of sensitivity of insecticidal proteins is usually determined by the presence or absence of receptors in higher taxonomic levels (such as families or orders).

Confined field trials for regulatory purposes are commonly conducted in agro-ecosystems that are representative of those where the GE crop is intended to be cultivated (Garcia-Alonso et al., 2014). Such field trials are commonly performed to obtain material for compositional analyses, to quantify transgene expression levels, to collect phenotypic and agronomic data over the growing season (Garcia-Alonso et al., 2014), and, as described previously, to evaluate potential ecological risks for valued non-target species. One matter of debate in regulatory science is how suitable (transportable) field data from one location are for the risk assessment in another location, and whether the uncertainty in the risk assessment regarding potential effects is sufficiently high to justify additional field studies when introducing the same event into other geographies. It has been proposed that data from well-designed confined field trials, where the GE and the nearest non-GE lines are compared side-by-side in a randomized block design, are best transported to regions with similar agro-climatic zones and agricultural practices (Garcia-Alonso et al., 2014; Melnick et al., 2023). If data from multiple sites at an adequate spatial scale and from diverse environmental conditions do not indicate risks specific to certain environments, the conclusion of environmental safety might be transported to other jurisdictions, and additional local field testing might not be necessary. In practice, however, the degree to which field-data generated in other regulatory jurisdictions is accepted by local authorities is variable (Nakai et al., 2024). Madrid et al. (2018)

discuss data transportability more specifically for field trials with Bt maize, where non-target arthropods were recorded in five diverse Mexican maize growing regions representing four ecoregions. The most relevant and abundant taxa as well as the outcome of the individual trials were similar across ecoregions, which led the authors to conclude that non-target arthropod data from field trials should be useful for the whole of Mexico, and potentially also for North and South America.

Higher-tier field studies performed to evaluate potential adverse effects of insecticidal Bt maize on invertebrate communities have been conducted in many countries worldwide. The objective of our work is to contribute to the discussion of data transportability by examining and comparing meta-analyses of Bt maize non-target studies across continents. Previous global meta-analyses have revealed minimal effects of Bt maize on non-target invertebrates, particularly when compared to effects of pyrethroid and other insecticides (Meissle et al., 2022a; Naranjo, 2009). Using a publicly available, high-quality and comprehensive dataset (Meissle et al., 2022b), here we conducted and compared analyses for different continents. We provide analyses for all Bt proteins combined, for Lepidoptera-active, Coleoptera-active, as well as stacked Bt maize, and for individual Bt proteins and transformation events. Using Bt maize as an example, we discuss the results of the analyses in the context of data transportability across continents and provide implications for risk assessment to enhance data transportability for novel transgenic proteins beyond the well-known Bt proteins.

2 | METHODS

2.1 | Database and data selection

The basis for the analyses in this work is the database of non-target invertebrates recorded in field experiments of Bt maize and corresponding non-Bt maize by Meissle et al. (2022b). Data in this database were retrieved by systematic literature searching, and a critical appraisal scheme was applied to each data record to assess external (relevance) and internal validity (risk of bias). The database contains 7,279 records of non-target invertebrate abundance, activity density, predation rate, or parasitism rate extracted from 120 articles up through August 2019. The field experiments were conducted between 1994 and 2017. Each record in the database represents a quantitative comparison of one invertebrate taxon recorded in Bt maize with the same taxon recorded in non-Bt maize, including mean abundance, standard deviation, and sample size (Meissle et al., 2022b). The database frequently contains multiple records of the same population of non-target invertebrates, i.e., when data on individual species as well as data summarized on family level were entered, when life stages were entered both individually and combined, and when different sampling methods recorded the same taxa. To ensure that each population is analyzed only once, analyses were conducted with a subset of the full database, as described by Meissle et al. (2022a). Selected data ensured comparable taxonomic levels, i.e., on family level, or for some taxa on higher levels. Furthermore, only one record per Bt maize line and experiment was selected for each analysis (preferably all life stages combined and the sampling method with the lowest coefficient of variation). For the purpose of this work, an experiment is defined as a field trial at one location in one year, and replicates are defined as different plots of the same maize type at that location. Analyses were conducted with records where neither Bt nor non-Bt maize received insecticide treatments or, in a minority of cases (<5%), where both maize types received the same treatments (such as seed treatments). We used the dataset as provided in Additional File 5 of Meissle et al. (2022a). Only the records with "TRUE" in the column "Analyse1" were selected for all analyses except for the analyses on species level (see below). Records with low external or internal validity ("High Risk" in the column "AnyRed") were excluded. Information on continents was added to the data subset from the original database (Meissle et al., 2022b).

2.2 | Data analysis

Statistical data analyses were conducted in R (Version 4.4.2, R Core Team, 2024). Graphs were built with the package 'ggplot2'. To illustrate where the data were collected, geographical coordinates of the records were plotted on a world-map (package 'maps') using geom_map. A list of countries producing GE crops in general and Bt maize in particular was derived from ISAAA (2019) with additional information from Agbioinvestor (2024).

For a comparison of the number of records of different taxonomic orders (or higher taxa) that were collected in different continents, bar charts were created. Africa was excluded because sufficient taxonomic data were not available (only three records for Hymenoptera and 16 for non-specified orders). Records of taxa with three or less records across all continents were summarized as "others". In some cases, the order was not specified by the authors, e.g., if taxa from several orders were combined in one record (labelled as "not specified"). In addition to plotting data on order level, we also analyzed data on family level for Coleoptera and Hemiptera, the orders with the largest number of records. Furthermore, we investigated species data available from field studies of the different continents. This was done using data on 40 species as selected for "specific analyses" by Meissle et al. (2022a) ("TRUE" in the column "Analyse5" in Additional File 5 of Meissle et al., 2022a).

Meta-analyses were conducted for each continent separately. The following variables (effect modifiers) were considered: different taxonomic entities, target orders of the Bt proteins, ecological functional groups, and influence of private sector contribution (see Figure 2 in Meissle et al., 2022a). For each record, Hedges' d and its variation were calculated (package 'metafor', escalc function), and the effect sizes with 95% confidence intervals and heterogeneity were estimated using random-effects models with restricted maximum-likelihood estimators for heterogeneity (rma function, method = "REML"). Effect sizes were considered significant if their confidence intervals did not include zero. Negative effect size

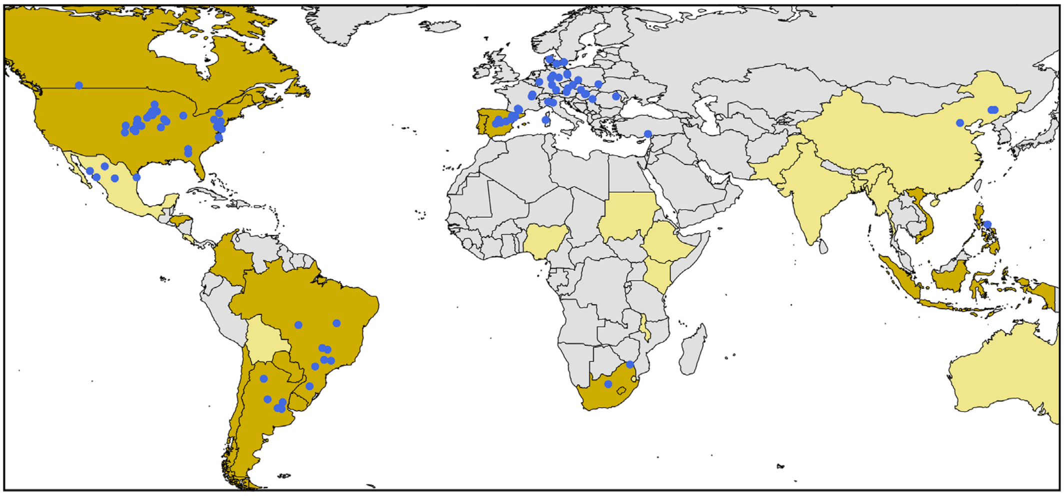

FIGURE 1 Locations where non-target invertebrates were recorded in Bt maize field experiments. Blue circles represent experimental locations. Countries producing Bt maize commercially are filled with golden color. Countries that produce no Bt maize but other genetically engineered (GE) crops are filled with a light yellow color. Countries without current GE crop production are plotted in grey.

estimates were associated with lower abundance, activity density, predation rate, or parasitism rate in Bt maize compared with non-Bt maize. Individual meta-analyses were only conducted when at least five records were available for a given taxon and comparison type and when those records were derived from at least three different articles to ensure an adequate level of data independence (Meissle et al., 2022a). Taxa not fulfilling the requirements for individual analysis, however, were included in analyses on higher taxonomic levels (where the same rules applied).

No meta-analyses were conducted for Africa (only one available article). For South America, three articles were available, but one of them contained only one record (predation rate, no taxonomic specification), so meta-analyses for South America were only conducted for all Bt proteins and all taxa combined as well as for the ecological function of predation.

More detailed analyses (so-called moderator analyses) were conducted to assess the impact of individual Bt proteins for Lepidoptera-targeted, Coleoptera-targeted, or stacked Bt maize (but not for the analyses where all Bt proteins were combined). Because regulatory approval of GE crops is granted for individual transformation events, we also conducted analyses on event-level whenever sufficient data were available.

For significant effect sizes (95% confidence intervals do not include zero) in all previously described meta-analyses, the robustness of the effect was evaluated by calculating the fail-safe number, which is the number of studies with effect size zero that need to be added to the analysis to turn the outcome non-significant (Rosenberg, 2005). We added the threshold of robustness according to Rosenberg (2005), who suggested that the fail-safe number should be at least five times the number of records in the analysis plus 10. We furthermore repeated each significant analysis multiple times while leaving one record, experiment, or article out at the time to identify if individual records, experiments, or articles influenced the result (Supplemental Material, Table S1).

3 | RESULTS

3.1 | Geographic locations and time periods of collected non-target data

When plotting the locations of non-target field experiments with Bt maize in the database on a world map, it becomes obvious that most data have been collected in North America and Europe (Figure 1). Fewer datasets were available from South America, Asia, and Africa. The main adopters of Bt maize are North and South American countries, where Lepidoptera- and Coleoptera-active maize is cultivated. While 975 records from 31 articles were available for North America, only 91 records from three articles were available for South America. In Europe, only Lepidoptera-active Bt maize is currently produced in Spain and Portugal (transformation event MON810). Nevertheless, many field studies have been conducted with Lepidoptera- and Coleoptera-active Bt maize in a range of European countries (767 records from 65 articles). In Asia, where Bt maize against Lepidoptera pests is cultivated in Vietnam, Indonesia, and the Philippines, altogether 124 records from nine articles on such Bt maize were available. From Africa, only one article from South Africa with 19 records on Lepidoptera-active Bt maize was available.

Early studies were conducted with Bt maize producing a single Lepidoptera-active Bt protein (Cry1Ab) (Figure S1). From the year 2000 onwards, Coleoptera-active Bt maize was field-tested in North America and from 2005 also in Europe. Data on stacked maize producing Lepidoptera- and Coleoptera-active Bt proteins were available for field trials from 2006 onwards.

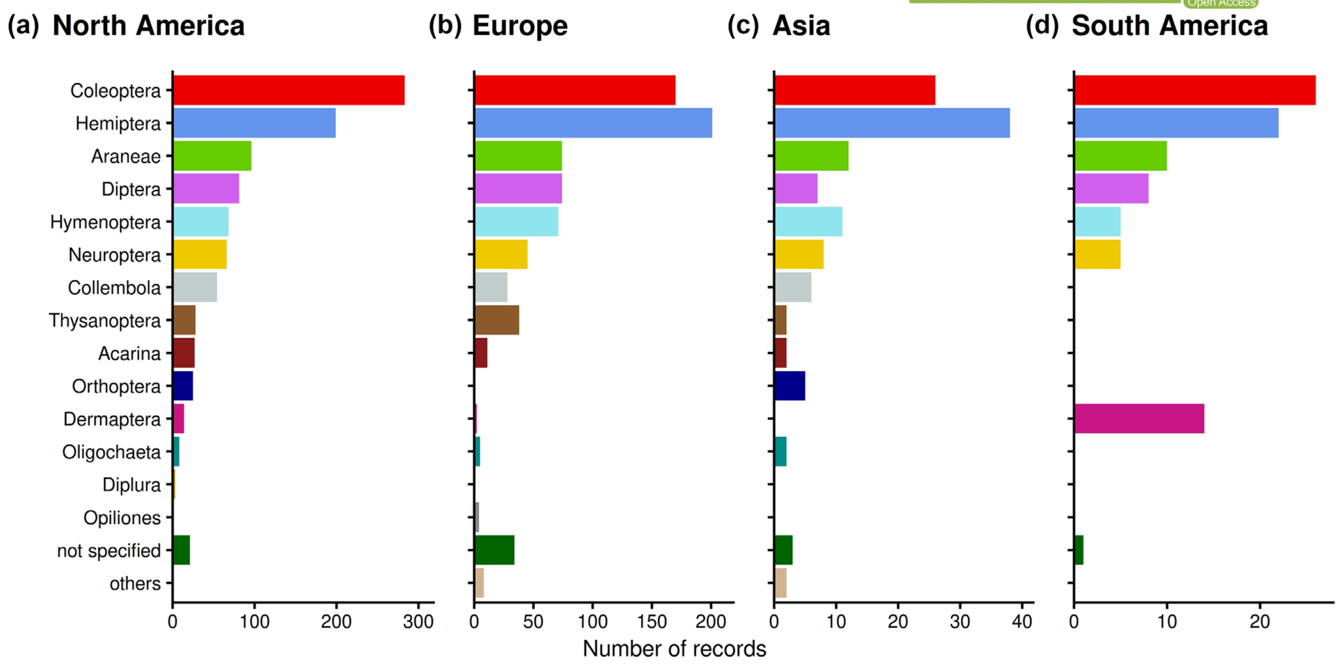

FIGURE 2 Taxonomic composition (order level) of available non-target invertebrate data for a) North America, b) Europe, c) Asia, and d) South America. The x-axis represents the number of records available for meta-analyses. Note the different scales of the x-axes. "Not specified" are records in the database that are not attributed to a specific order. "Others" comprise all orders with < 3 records for each continent.

3.2 | Taxonomic composition of non-target data

The taxonomic composition on the order level is comparable among continents (Figure 2). Coleoptera and Hemiptera are the most recorded orders in all continents, followed by Araneae, Diptera, Hymenoptera, and Neuroptera. Less frequently recorded were Collembola, Thysanoptera, Acarina, Orthoptera, Dermaptera, Oligochaeta, Diplura, and Opiliones. In South America, comparatively many records on Dermaptera were available (15% of all records).

Families within the orders of Coleoptera and Hemiptera also were comparable among continents (Figure S2). For example, in the order of Coleoptera, Coccinellidae and Carabidae were reported from four continents, Staphylinidae from North and South America and Europe, and Chrysomelidae from North America, Europe, and Asia. For Hemiptera, Anthocoridae, Cicadellidae, and Aphididae were recorded in four continents, while records of Miridae were available from North America, Europe, and Asia. Some families received more attention on one or two continents, but not on the others. For example, Nitidulidae, Cicindelidae, Melyridae (all Coleoptera), and Geocoridae (Hemiptera) were more frequently reported from the Americas than from Eurasia, while Nabidae, Delphacidae, and Derbidae (Hemiptera) were more frequently reported from Eurasia than for the Americas.

On a species level, the continents show larger differences (Figure S3). Despite some cosmopolitan species, such as *Coccinella septempunctata*, *Harmonia axyridis*, *Pterostichus melanarius*, *Bembidion quadrimaculatum* (Coleoptera), *Chrysoperla carnea* (Neuroptera), or *Rhopalosiphum maidis* (Hemiptera), the remaining 34 species were unique to either North America, Europe, or Asia. No data at the species level were available for South America.

3.3 | Meta-analyses of non-target invertebrates in Bt maize

For Lepidoptera-active Bt maize, sufficient data for detailed meta-analyses were available for North America, Europe, and Asia (Figure 3). Effect sizes without significant deviation from zero were evident for 16 taxa from North America, 20 taxa from Europe, and 11 taxa from Asia. Positive effects (more individuals in Bt maize) were observed for Coccinellidae in North America, Anthocoridae in Europe, and Neuroptera in Asia. Negative effects (fewer individuals in Bt maize) were evident for Diptera, Hymenoptera, and all taxa in North America and for Tachinidae and Diptera in Europe. Fail-safe numbers indicate that the observed significant effects are not robust, except the effect on Hymenoptera in North America (Table S2). Leave-one-out analyses showed that the study by Pilcher (1999) was mainly responsible for this effect.

Moderator analyses on individual Bt-proteins revealed that Bt maize producing Cry1Ab was responsible for most effects (except for effects on all taxa in North America and effects on Neuroptera in Asia) (Figure 3). More specifically, event Bt11 was associated with adverse effects on Hymenoptera in North America, Bt176 with adverse effects on Tachinidae, Diptera, and all taxa in Europe and positive effects on Coccinellidae and Coleoptera in North America, and MON810 with adverse effects on Coccinellidae in Europe (Table S1). In Asia, the moderator analyses showed that Cry1Ac-producing maize (event Bt799) resulted in higher numbers of invertebrates when all taxa were combined (Figure 3, Table S1).

For Coleoptera-active Bt maize, data were available for North America and Europe (Figure 4). For North America, no significant

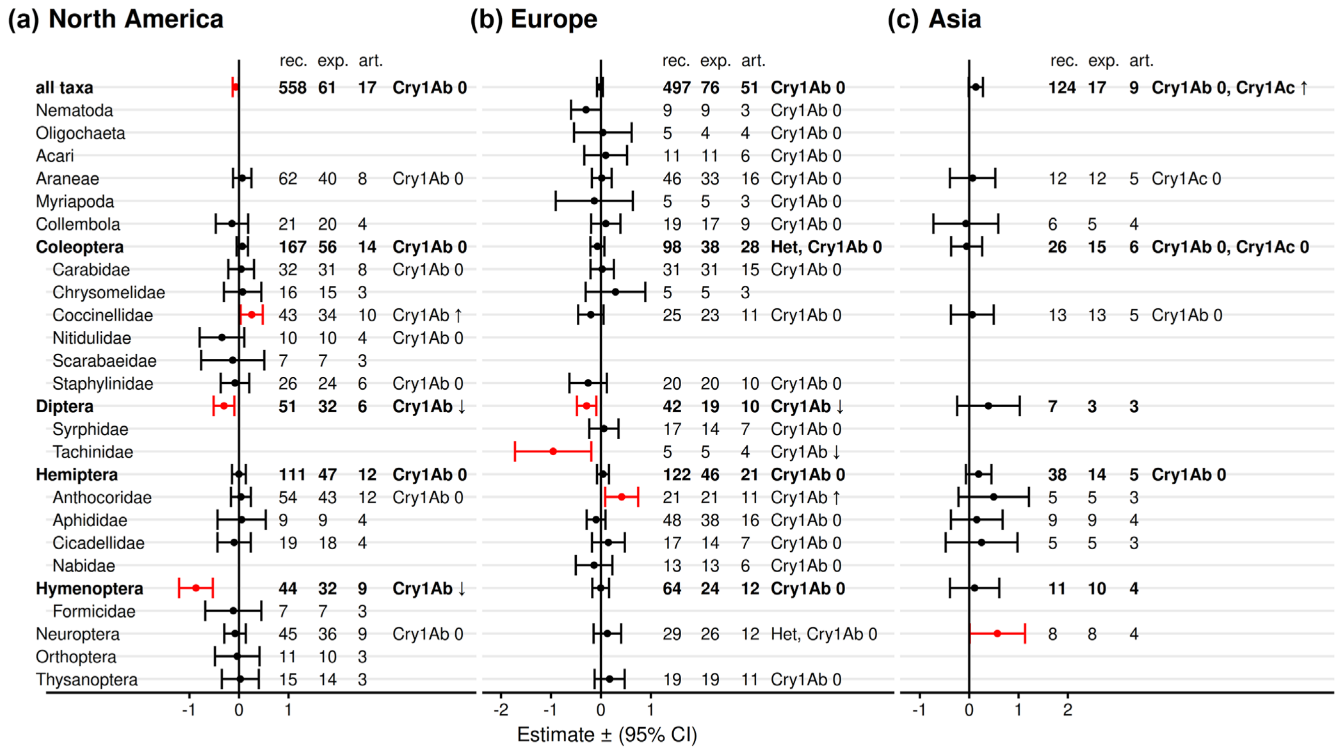

FIGURE 3 Meta-analyses on effects of Lepidoptera-active Bt proteins on non-target invertebrates for a) North America, b) Europe, and c) Asia. Records with any red flag in the critical appraisal were excluded. For each taxon, the effect size estimate and the 95% confidence interval are given (negative effect sizes indicate lower populations in Bt compared with non-Bt maize and vice versa). Significant intervals (red) do not include 0. Fail-safe numbers indicate that the observed significant effects are not robust, except the effect on H↓ymenoptera in North America (Table S2). On the right side is the number of records (rec.), experiments (exp.), and articles (art.) included in each analysis. Het indicates significant (P < 0.05) heterogeneity. Results of moderator analyses for individual Bt proteins are indicated with arrows. ↑: higher values in Bt compared to non-Bt treatment (positive effect size), ↓: lower values (negative effect size), 0: no effect.

effects were observed for 12 taxa. A negative effect was obtained for Staphylinidae and Coleoptera, but fail-safe numbers and leave-one-out analyses indicate that the effect is not robust (Table S2). No effects were observed for all 12 European taxa. Moderator analyses revealed that the effect on Staphylinidae can be attributed to Cry3Bb. No effects were evident when Coleoptera-active transformation events (MON863, MON88017, MON89034 & MON88017) were analyzed individually (Table S1).

For stacked maize producing Coleoptera- and Lepidoptera-active Bt proteins, only few data are available that matched the criteria of at least five records from three articles, mainly from Europe (Figure 5). In North America, only three studies investigated invertebrates in stacked Bt maize. While no effect was observed on Coleoptera, a negative effect on all taxa became evident. For Europe, no effect was observed for 12 taxa, but there was a positive effect on Neuroptera. Fail-safe numbers and leave-one-out analyses showed that the significant effects in North America and Europe were not robust (Table S2). Event-specific analyses for stacked Bt maize expressing Lepidoptera- and Coleoptera-active Bt proteins were not possible.

When all Bt proteins were combined, meta-analyses of effects on individual taxa demonstrated no significant effects on 21 taxa from North America, 24 taxa from Europe, and 11 taxa from Asia (Figure S4). In North America, adverse effects were revealed for Syrphidae (and Diptera), Braconidae (and Hymenoptera), and all taxa combined, while positive effects were obtained for Coccinellidae. In Europe, adverse effects were obtained for Tachinidae (and Diptera) and positive effects for Anthocoridae. The outcome of this analysis is thus similar to the analysis on Lepidoptera-active proteins (with the additional analyses of Syrphidae and Braconidae that were not possible when Lepidoptera-active maize was analyzed alone). The addition of the Coleoptera-active proteins and the stacks did not change the result. In Asia, only data for Lepidoptera-active maize were available, so the analysis of all Bt proteins combined is identical with the analysis of Lepidoptera-active maize. In South America, a positive effect was observed when all taxa were combined for meta-analysis (Table S3). The effect, however, was not robust (Table S2).

On ecological functional group level, no significant effects were observed for Europe and Asia (Figure 6). For North America, no effects were observed on any functional group for Coleoptera-active Bt maize and for stacks. In contrast, adverse effects were evident for parasitoids in Lepidoptera-active maize and parasitoids and herbivores when all Bt proteins were combined. Fail-safe numbers suggest that the significant effect on parasitoids is robust for Lepidoptera-active

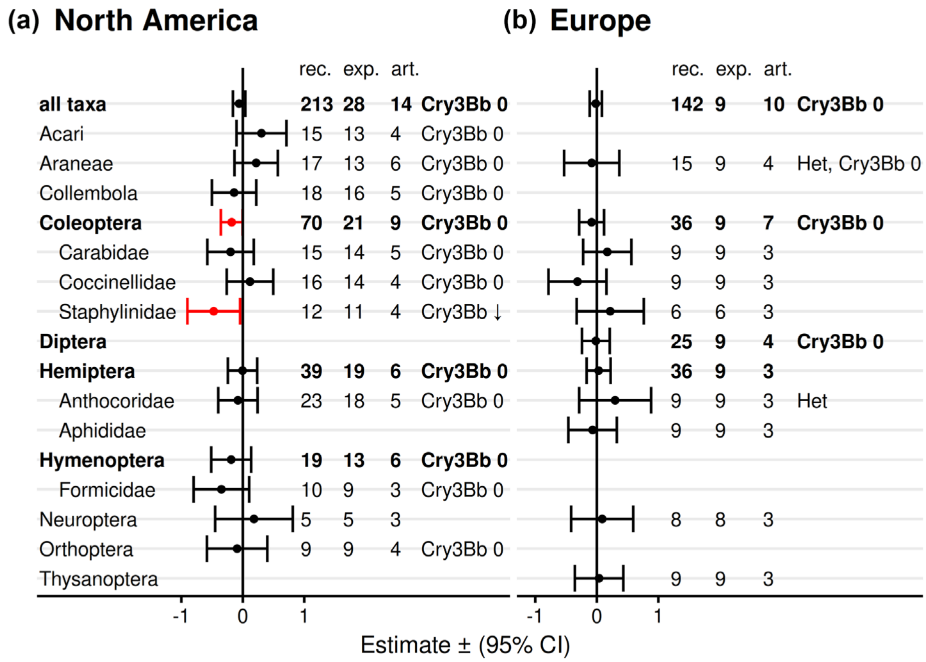

FIGURE 4 Meta-analyses on effects of Coleoptera-active Bt proteins on non-target invertebrates for a) North America and b) Europe. Records with any red flag in the critical appraisal were excluded. For each taxon, the effect size estimate and the 95% confidence interval are given (negative effect sizes indicate lower populations in Bt compared with non-Bt maize and vice versa). Significant intervals (red) do not include 0. Fail-safe numbers indicate that the significant effects are not robust (Table S2). On the right side is the number of records (rec.), experiments (exp.), and articles (art.) included in each analysis. Het indicates significant ($P < 0.05$) heterogeneity. Results of moderator analyses for individual Bt proteins are indicated with arrows. ↓: lower values in Bt compared to non-Bt treatment (negative effect size), 0: no effect.

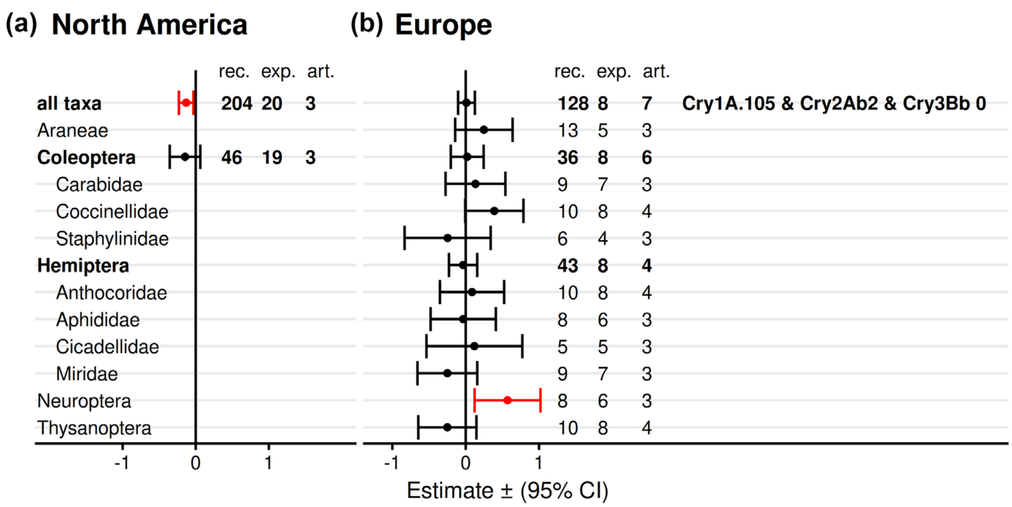

FIGURE 5 Meta-analyses on effects of stacked Coleoptera & Lepidoptera-active Bt proteins on non-target invertebrates for a) North America, b) Europe. Records with any red flag in the critical appraisal were excluded. For each taxon, the effect size estimate and the 95% confidence interval are given (negative effect sizes indicate lower populations in Bt compared with non-Bt maize and vice versa). Significant intervals (red) do not include 0. Fail-safe numbers indicate that the significant effects are not robust (Table S2). On the right side is the number of records (rec.), experiments (exp.), and articles (art.) included in each analysis. Results of moderator analyses for individual Bt protein stacks are indicated; 0: no effect.

proteins and for all proteins combined, while the effect on herbivores is not robust (Table S2). In South America, no effect on predators was evident when all Bt proteins were combined (Table S3).

Moderator analyses with individual Bt proteins indicate that effects on parasitoids in North America are attributed to Cry1Ab (Figure 6). Event-specific analyses revealed that effects on parasitoids

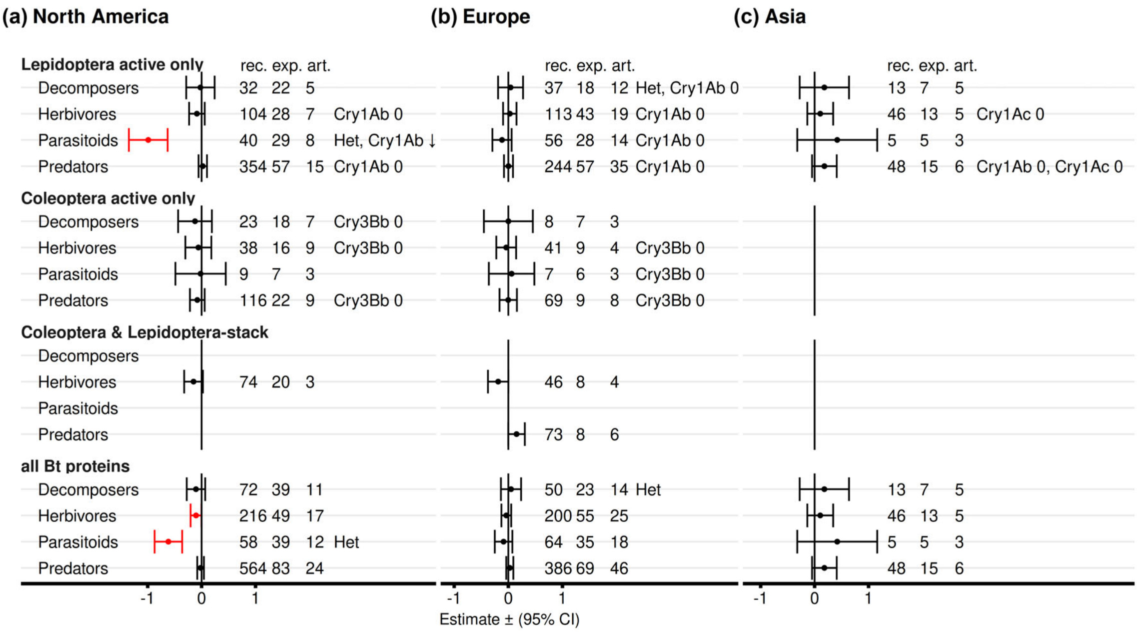

FIGURE 6 Meta-analyses on functional group level for a) North America, b) Europe, and c) Asia. Either records of Lepidoptera-active, Coleoptera-active, stacked Coleoptera- and Lepidoptera-active, or all Bt proteins were included. Records with any red flag in the critical appraisal were excluded. For each functional group, the effect size estimate and the 95% confidence interval are given (negative effect sizes indicate lower populations in Bt compared with non-Bt maize and vice versa). Significant intervals (red) do not include 0. Fail-safe numbers indicate that the significant effect on parasitoids is robust, while the effect on herbivores is not robust (Table S2). On the right side is the number of records (rec.), experiments (exp.), and articles (art.) included in each analysis. Het indicates significant heterogeneity. Results of moderator analyses for individual Bt proteins are indicated with arrows. ↓: lower values in Bt compared with non-Bt treatment (negative effect size), 0: no effect (no moderator analyses conducted for all Bt proteins combined).

are associated with Bt11 in North America and Bt176 in Europe, while no event-specific effects on other functional groups were evident (Table S1).

3.4 | Meta-analyses of private and public sector data

In North America, 68% of the analyzed records (all Bt proteins combined) derived from studies where the private sector contributed by sponsoring or by coauthoring the article. In Europe, only 13% of the records were from studies with private sector involvement, and in Asia, all available data derived from purely public studies. Meta-analyses revealed a negative effect on invertebrates in North American Bt maize in studies with private involvement for Lepidoptera-active Bt proteins and for all Bt proteins, but not for public studies and not for private or public studies with Coleoptera-active maize (Figure S5). Fail-safe numbers indicate that the effect is not robust (Table S2). Moderator analyses showed that this effect can be attributed to Cry1Ab (Figure S5). In Europe and Asia, no effects were evident except for a positive effect of Cry1Ac, event Bt799, in Asia. No other event-specific Bt maize effects were observed (Table S1).

4 | DISCUSSION

4.1 | Bt maize non-target data derive mainly from North America and Europe

Most data on non-target invertebrates were available from North America, where commercialized Bt maize has been developed and grown on large areas for nearly three decades. Early on, Lepidoptera-active maize targeting the European corn borer (*Ostrinia nubilalis*) has been studied, followed by Coleoptera-active maize targeting corn rootworms (*Diabrotica* spp.) and stacked maize carrying both traits. Field testing in North America decreased after 2002. In the USA, stacked Bt maize lines derived from conventional breeding of single-gene lines are not necessarily considered novel traits and subject to simplified regulatory approval, where no additional non-target data from field testing may be needed (De Schrijver et al., 2007). In Europe, many data from Bt maize field studies are available, even though Bt maize is currently only grown in small areas of Spain and Portugal. Field trials have been conducted when Cry1Ab-producing Bt maize became available in 1996. In the early 2000s, many European countries explored potential benefits of Bt maize in local field trials. This includes trials with Coleoptera-resistant Bt maize and stacks that were initiated shortly after the Western corn rootworm (*Diabrotica virgifera virgifera*) invaded the continent. In Europe, each stack is considered a novel trait that also needs to undergo a full risk assessment including field trials (De Schrijver et al., 2007). Experimentation in Europe, however, ultimately ceased when it became clear that the political climate was against GE crops and when the seed companies subsequently retracted their applications for regulatory approval (last data from 2015). Thus, other than the Lepidoptera-targeting transformation event MON810, no new events of Bt maize have received regulatory approval for cultivation in the EU (García et al., 2023). In Asia, the first studies with Lepidoptera-active Bt maize were conducted in 2001, and last available data in our database derived from field experiments in 2017. Coleoptera-targeted Bt maize was not studied in Asia because maize production is not threatened by corn root worm beetles. Only a few Asian countries grow Bt maize in the field (Vietnam, Philippines, Indonesia). Most records, however, derived from China, which conducted several trials with Bt maize, but have yet to approve it for commercial cultivation. Few datasets on Lepidoptera-active, Coleoptera-active, and stacked Bt maize have been retrieved for South America, even though Bt maize is cultivated on large areas and in many countries on the continent. Although non-target data have been regularly recorded in field trials in South American countries, such as Brazil and Argentina, it is evident that a large part of those data have only been used for supporting regulatory submissions and have not been published in the scientific literature. In Africa, Lepidoptera-active Bt maize has been cultivated in South Africa since 1996. However, only one dataset on non-target ecological functional groups is available as of 2019, and none at individual taxonomic levels. For our analyses, we worked with publicly available data, and it remains unclear if more data that meet the quality standards for meta-analysis have been collected, but not published at all, or not in an accessible format.

4.2 | High-level taxonomic composition is similar across continents

The taxonomic composition on order (or higher) level is very similar across continents, and there are no major invertebrate groups that are unique to one specific continent. However, there appear to be differences in local importance of some taxa. For example, Dermaptera are commonly reported from maize fields in South America but have rarely been collected in other continents. Locally important taxa thus need to be considered (but not necessarily tested) when assessing effects of GE crops on non-target species. Similarity across continents decreases when lower taxa, such as families, are compared. For example, some beetle and Hemiptera families were reported more frequently from North America and others more from Europe. Even more pronounced are differences at the species level, as would be expected. Except for some global species, each continent harbors a specific set of species.

When data of Bt maize non-target studies are compared, one question is if the studied taxa are representative of the actual communities in the field. Reported taxa, no matter on which taxonomic level, that have been recorded multiple times in sufficient numbers for a comparison between Bt and non-Bt maize, are likely to have significant importance in a certain region. However, the absence of data does not necessarily indicate the absence of the taxon in the region, it may just not have been investigated in that region. This limitation is specifically important 1) when only a few studies are available from a region, such as South America, Asia, or Africa; 2) for taxa with medium or low abundance; 3) for taxa that are difficult to collect and identify, like mites or other soil-inhabiting species. For example, as mentioned previously, Dermaptera have been reported more often in South American studies than in studies from other continents. If they are truly more important in South American maize fields, or if they are simply understudied or collected less frequently due to sampling methodology in the other continents, remains unclear.

Databases on arthropod species inhabiting different crops including maize, available for Europe (Riedel et al., 2016) and China (Yang, 2018), contain the same main taxa as the Bt maize database of this work (Table S4). One exception is pest Lepidoptera, which are the targets of Lepidoptera-active Bt maize and thus not considered in non-target studies. Even though data on non-target arthropods in African Bt maize are scarce, faunistic studies in South Africa (Truter, 2011) and Kenya (Birch et al., 2004) confirmed that the most important arthropod orders of Africa are covered in our Bt maize database (Table S4).

From a risk assessment perspective, the similarity of arthropod composition at higher taxonomic levels, i.e., the relative prevalence of non-target families and orders across continents, offers a foundation for data transportability. For lower-tier laboratory testing, used to generate data required by many regulatory agencies, surrogate species of different taxonomic orders and ecological functional groups are selected. For the risk assessment of a novel crop in a specific region, it needs to be determined if data from the laboratory testing with purified insecticidal proteins, data from non-target testing of previous transformation events with similar traits, and data from available field trials in other geographies are sufficient for a conclusion of safety, or if uncertainty remains that would require additional field studies in the new region. If there is no evidence from laboratory testing or from field trials in some countries or continents that the mode of action of the novel trait may impair taxa specific to a certain region, the conclusion of "no unacceptable risk for non-target species" should also be informative for the risk assessment of another country or continent. In contrast, if the risk assessment of an insecticidal protein revealed activity in multiple taxonomic orders or if there is a yet undetermined pattern of toxicity, additional (primarily laboratory) tests with locally important orders, such as, for example, Dermaptera for risk assessments in South American regions, may be considered.

4.3 | Meta-analyses do not reveal robust, unexpected effects

Meta-analyses on maize producing Lepidoptera-active Bt proteins revealed adverse effects on Hymenoptera in North America and on Tachinidae (and Diptera) in Europe. In both cases, the effects can be attributed to parasitoids of the target pest, the European corn borer (Meissle et al., 2022a). The effect on Hymenoptera parasitoids in North America is the only robust effect obtained in all meta-analyses (Table S2). No effects, however, were observed on Hymenoptera in Europe. When compared to North America, where many Hymenoptera records represented *Macrocentrus cingulus*, a specialist parasitoid of European corn borer from the family of Braconidae, the available records of Hymenoptera in Europe comprised ten different families, mainly aphid parasitoids without a link to the target pests of Bt maize, and only one record of Braconidae (Figure S6). For Tachinidae, the situation was vice versa. While *Lydella thompsoni* was collected from corn borers in Europe, the records from North America derived from non-identified species, which might have no association with the target pest. Other non-robust positive or negative effects remain largely unexplained (for a detailed discussion, see Meissle et al., 2022a). Depending on the level of data aggregation (target order, Bt protein, or event), such significant effects emerge or disappear. Overall, we believe that non-robust effects should not be overinterpreted, as they are likely to be random or due to artifacts in experimental design of highly influential studies. For example, some individual studies contribute multiple records to the analyses as they include different Bt maize lines, locations, and years (e.g., Madrid et al., 2018; Pilcher, 1999). From a risk assessment perspective, adverse effects of natural enemies of the target pest are expected and generally accepted, while positive effects on natural enemies pose no concern. Ideally, multiple well-designed, high-quality, and independent studies from different regions or continents over several years should be available to increase trust in the data and therefore also the usefulness of the study outcome for multiple regulatory systems.

Over all records, adverse effects of Bt maize were observed in studies with private-sector involvement from North America, but not in studies by public institutions on any continent. While non-target studies in North America were mainly conducted or sponsored by the plant developers, studies in Europe and Asia were mainly conducted by public institutions without sponsorship of the private sector. As discussed by Meissle et al. (2022a), the fact that public studies revealed less effects than studies with private-sector involvement indicates that the private sector is willing to publish potentially unfavorable data (showing adverse effects).

4.4 | Implications for data transportability

The transfer of conclusions from non-target field testing across borders may reduce the need for country-by-country field trials, particularly if the countries cover the same agro-climatic zones and if similar agronomic practices are applied (Garcia-Alonso et al., 2014). Agroclimatic information shows that maize is grown worldwide in many climatic zones. Similar climatic zones may indicate better data transportability than different zones, as suggested in a case study on insect-resistant maize (Melnick et al., 2023). Madrid et al. (2018) emphasize, however, that conclusions of non-target field trials might also be relevant across climatic zones in the absence of a plausible hypothesis for an interaction between the GE trait and the environment that would increase adverse environmental impact. When comparing non-target data from different ecoregions in Mexico, they observed no region-specific difference in the outcome of the field trials. Similarly, non-target data spanning a much larger geographic area from Argentina, Brazil, and the USA did not reveal region-specific Bt maize effects (Ahmad et al., 2016). If risk assessment data (including field data) can be used for regulatory submissions in multiple regions, it will not only save time and costs for applicants and regulatory authorities but also contribute to a reduced need for animal testing (including invertebrates), which is on the agenda for chemical legislation in the EU (EC, 2024).

Our meta-analyses conducted on different taxa from multiple continents can be used to assess how well the results from North America, where GE crops are usually developed and first studied in the field, can predict the outcome of analyses in Europe and Asia. Altogether, North America and Europe share analyses on 24 common taxa (family and higher level) for the different protein targets (Table S5). Of those 24 analyses, 19 from North America predicted the outcome of the analyses in Europe correctly (79%). The incorrect predictions, however, did not include a neutral or positive effect in North America being associated with a negative effect in Europe. For Asia, only 11 taxa common with North America were analyzed, and seven from North America predicted the outcome in Asia correctly (64%). Once again, none of the analyses predicted a positive or neutral outcome in North America associated with a negative outcome in Asia (Table S5). This exercise shows that no adverse effects were missed for Europe or Asia when using North American data to predict the non-target effects of Bt maize.

In addition to data transportability between continents, the relationship between data generated in the laboratory and data generated in the field is also informative. Duan et al. (2010) demonstrated in a set of meta-analyses that results from field studies can (largely) be predicted from results of laboratory studies and that laboratory studies are generally more conservative in detecting effects.

The current meta-analyses differ from regulatory risk assessment, which needs to be kept in mind when it comes to data transportability. First, the strength of our meta-analyses is that they cover many different studies accumulated over several decades and conducted for various purposes with a range of different methods. In contrast, regulatory agencies will have only a few data available for novel GE crops with new traits. Second, we pooled data and categorized them by target order of the applied Bt proteins and, if sufficient data were available, also by the individual Bt proteins. The hypothesis behind this pooling is that ecological effects on invertebrate communities should be comparable for different events with the same target pests. While this approach seemed reasonable for the meta-analyses of field studies, regulatory risk assessments are targeted at specific transformation events. Therefore, we also conducted analyses for individual events in cases where sufficient data were available. Third, we did not include data on off-field invertebrate communities in our study, because such data is scarce and no comparison among continents would be possible

(Meissle et al., 2022a). Risk assessments, however, typically also consider impacts on non-crop habitats.

Our results of Bt maize non-target effects may be directly applicable for countries planning to commercialize Bt maize (late adopters). It may also contribute to the regulatory evaluation of other crops producing the same Bt proteins (Ba et al., 2018). Some more general conclusions of our study also may be applicable to risk assessments for novel crops with new modes of action (including genome-edited crops), where multiple datasets are not available: 1) we demonstrated that the taxonomic composition on order (and partly family) level is similar across continents. Thus, conclusions from studies on representatives of those families and orders (surrogates), either from the laboratory or the field, are relevant across geographies; 2) the example of Bt maize shows that the absence of unexpected robust non-target effects of Bt maize field experiments in one geography was generally confirmed in other continents, even though the taxonomic composition on species level varied among geographies. We believe that conclusions of field experiments in one geography are relevant for other geographies also when considering novel GE crops, unless specific risk hypothesis indicate environment-specific interactions of the GE crop with particular non-target species. Overall, this may help mid- to late-adopting countries to incorporate results of previous experiments from other geographies in their risk assessments and reduce the need for local field trials.

4.5 | Conclusions

Most data on invertebrate non-target effects of Bt maize derive from North America and Europe, data for Asia are limited, and few data were retrieved for South America despite large areas of Bt maize cultivation in this region. The taxonomic composition of non-target invertebrates (mainly arthropods) in Bt maize at order or higher taxonomic level is similar across continents, which facilitates data transportability. Locally important orders, such as Dermaptera in South America, should be studied if the risk assessment of an insecticidal protein revealed activity in multiple taxonomic orders. Meta-analyses of non-target field studies from North America show few non-target effects. While effects on specialized parasitoids of target pests or species otherwise related to maize-feeding Lepidoptera are explainable, other effects are non-explainable, but also variable and non-robust. This conclusion has been confirmed in Europe and Asia and corroborates the outcome from laboratory toxicity studies. We thus argue that the conclusion of no unacceptable risk of current Bt maize for non-target invertebrates is transportable among different geographies. High-quality, well-designed, and well-described studies including multiple locations and years, open-access data availability (transparency), and independence of multiple studies will increase trust in the data and thus their usefulness for multiple regulatory bodies. Using Bt maize as an example, we demonstrate that 1) taxonomic composition on invertebrate order level is similar across geographies and 2) the outcome of field studies in one geography was generally confirmed in other geographies. These findings are of value to regulatory authorities throughout the world, also for novel GE crops with different modes of action. Reducing the need for local field trials has the potential to make regulatory processes more efficient, which may ultimately benefit the environment, growers, and consumers.

AUTHOR CONTRIBUTIONS

Conceptualization: MM, SEN, JR. *Data curation*: MM. *Formal analysis*: MM. *Investigation*: MM. *Methodology*: MM, SEN, JR. *Visualization*: MM. *Writing – original draft*: MM. *Writing – review and editing*: MM, SEN, JR.

CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

No research data have been generated. All results of analyses are provided in the Supplemental Material.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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