UNIVERSIDADE DE SÃO PAULO

CRISTINA AKEMI KITA

Uma meta-análise dos efeitos dos agrotóxicos sobre as abelhas e o serviço de polinização prestado por elas no contexto das práticas agrícolas intensivas

A meta-analysis of the effects of pesticides on bees and their pollination service in the context of intensive agricultural practices

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Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Ecologia de Ecossistemas Terrestres e Aquáticos.

Orientador: Marco Aurelio Ribeiro de Mello

Coorientadora: Laura Carolina Leal de Sousa

SÃO PAULO
2022

FICHA CATALOGRÁFICA

Kita, Cristina Akemi
Uma meta-análise dos efeitos dos agrotóxicos sobre as abelhas e o serviço de polinização prestado por elas no contexto das práticas agrícolas intensivas / Cristina Akemi Kita ; orientador Marco Aurelio Ribeiro de Mello; coorientadora Laura Carolina Leal de Sousa -- São Paulo, 2022.

86 p.

Dissertação (Mestrado) -- Instituto de Biociências da Universidade de São Paulo. Programa de Pós-Graduação em Ecologia.

1. Interações entre espécies. 2. Serviços ecossistêmicos. 3. Análise de dados. 4. Revisão sistemática. I. Ribeiro de Mello , Marco Aurelio, orient. II. Leal de Sousa, Laura Carolina , coorient. III. Título.

BANCA EXAMINADORA:

Prof(a). Dr(a).

Prof(a). Dr(a).

Prof. Dr. Marco Aurelio Ribeiro de Mello

Orientador

AGRADECIMENTOS

Se não fosse pela ajuda que recebei ao longo da minha jornada como cientista, principalmente durante o mestrado, esta dissertação não estaria concluída. Por isso, não poderia deixar de agradecer às pessoas que caminharam junto comigo até aqui.

Agradeço primeiramente aos meus pais, que não apenas investiram nos meus estudos sem medir esforços, mas também me apoiaram e me incentivaram a batalhar pelos meus sonhos. Também agradeço à minha tia Mitie pelo suporte e motivação, que suavizaram a minha jornada.

Agradeço também aos meus professores da graduação pelos ensinamentos, especialmente à Profa. Cinthia Brasileiro por ter me apresentado ao mundo acadêmico durante a iniciação científica na Universidade Federal de São Paulo (UNIFESP) e por ter aberto a porta que me levou ao meu orientador, Prof. Marco Mello. Receber a orientação desse excelente profissional foi crucial para o desenvolvimento desta dissertação e da minha carreira como cientista. Por isso, sou extremamente grata ao Prof. Marco pelos sábios conselhos, valiosos ensinamentos e grandes oportunidades que recebi durante esses anos do mestrado.

Também sou imensamente grata à Profa. Laura Leal por ter se disposto a me coorientar e ter feito isso de forma brilhante, ensinando-me a fazer meta-análise e, assim, tornando possível a execução das minhas ideias. Agradeço também à Profa. Tereza Giannini e à Profa. Isabel Alves pelas valiosíssimas sugestões, conselhos e direcionamento durante as reuniões do Comitê de Acompanhamento. Sem essas pesquisadoras admiráveis e seu vasto conhecimento sobre abelhas, eu não teria desenvolvido e amadurecido esta dissertação como fiz.

Também sou grata por ter tido a sorte de conhecer e trabalhar com os meus colegas Natalya Zapata-Mesa, Sebastián Montoya-Bustamante, Renata Muylaert e Guillermo Florez-Montero. Agradeço muito pela troca de ideias, confiança, ajuda e experiências. Agradeço

também aos colegas do “Grupo de ajuda com metas!” pela troca de informações e ajuda com meta-análise.

Por fim, agradeço ao Programa de Pós-Graduação em Ecologia da Universidade de São Paulo (PPGE) e à Coordenação para o Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo apoio, especialmente durante a pandemia de Covid-19.

SUMÁRIO

RESUMO

O crescimento da população humana previsto para as próximas décadas aumentará a demanda por alimentos. Para atender a essa demanda, é prevista uma intensificação das práticas agrícolas. Dentre essas práticas está a aplicação de agrotóxicos. Contudo, a aplicação de agrotóxicos ameaça as abelhas, que são os principais polinizadores dos cultivos agrícolas que dependem do serviço prestado por animais, portanto ameaçando também a nossa segurança alimentar. Assim, entender a magnitude dessas ameaças tem se tornado cada vez mais urgente. Nesta dissertação, utilizando uma abordagem meta-analítica, estimamos os efeitos da aplicação de agrotóxicos sobre a comunidade de abelhas e o serviço de polinização prestado por elas. Vimos que a sobrevivência de abelhas e a continuidade do serviço de polinização das lavouras são ameaçadas pela aplicação de agrotóxicos. Por fim, concluímos que a preservação de áreas naturais e a adoção de práticas que minimizem o uso de agrotóxicos é essencial para mitigar essa ameaça. Além disso, a partir das dificuldades e limitações encontradas durante a extração de dados primários para a nossa meta-análise, elaboramos um segundo capítulo. Propusemos dez regras simples para reportar informações relacionadas a métodos de coleta de dados, dados brutos, dados processados e resultados de modelos de estudos sobre interações entre espécies, a fim de melhorar sua reprodutibilidade e usabilidade. Por fim, concluímos que produtores de dados primários podem contribuir para o avanço do conhecimento não apenas publicando suas descobertas, mas também compartilhando informações de forma mais eficiente, o que pode beneficiar suas equipes e toda a comunidade.

ABSTRACT

The human population growth predicted for the next decades will increase the demand for food. To meet this demand, agricultural practices are expected to be intensified. Among these practices is the application of pesticides. However, applying pesticides threatens bees, the primary pollinators of animal-dependent crops, thus also threatening our food security. Therefore, understanding the magnitude of these threats is becoming increasingly urgent. In this dissertation, using a meta-analytic approach, we estimated the effects of pesticide application on the bee community and its pollination service. Our results show that the survival of bees and the continuity of crop pollination are threatened by pesticide application. Finally, we conclude that the preservation of natural areas and the adoption of practices that minimize the use of pesticides are essential for mitigating this threat. Furthermore, we prepared a second chapter based on the difficulties encountered during the extraction of primary data for our meta-analysis. We propose ten simple rules for reporting information related to data collection methods, raw data, processed data, and model results from studies on species interactions to improve their reproducibility and usability. Finally, we conclude that primary data producers can help advance knowledge not only by publishing their findings, but also by sharing information more effectively, which benefits their teams and the entire community.

INTRODUÇÃO GERAL

O dilema do crescimento

Nas próximas décadas, a população humana atingirá patamares inéditos. Estimativas indicam que poderemos atingir 8,5 bilhões de pessoas ao redor do mundo em 2030, 9,7 bilhões em 2050 e 10,9 bilhões em 2100 [1]. Acompanhando esse crescimento, a demanda por alimentos também deverá aumentar, levando a uma maior sobrecarga em sua produção [2].

Como a produção de alimentos está diretamente relacionada ao uso da terra, é esperado que haja um uso mais intensivo das áreas agrícolas existentes e uma expansão dessas áreas nas próximas décadas para atender a essa demanda futura por alimentos. Contudo, essa intensificação agrícola resultará em uma perda maciça da biodiversidade global [3]. Isso porque a conversão de áreas de vegetação natural para uso agrícola gera impactos ambientais que podem ser sintetizados em três eixos principais: (1) mudanças em larga escala da cobertura da terra, como a conversão de habitats naturais em paisagens agrícolas principalmente por desmatamento; (2) mudanças na estrutura da paisagem, como a redução da heterogeneidade ambiental devido à especialização das lavouras (monoculturas); e (3) mudanças no manejo da terra, como o uso intensivo de agrotóxicos [4].

Desta forma, o aumento esperado da população humana nos próximos anos está diretamente relacionado à intensificação agrícola que, por sua vez, ameaça a biodiversidade devido à degradação ambiental, gerando uma demanda conflitante entre produzir e conservar.

Ameaças à biodiversidade

Essa demanda conflitante cria ameaças diretas à biodiversidade, por exemplo aos polinizadores [5], cuja sobrevivência está intimamente relacionada à saúde do ambiente. Isso porque a perda e a fragmentação de habitat pelo desmatamento e expansão das áreas agrícolas, a redução da complexidade ambiental por meio da especialização das lavouras, e a contaminação ambiental pelo uso intensivo de pesticidas e fertilizantes colocam em risco a disponibilidade de recursos cruciais para os polinizadores [6]. Dentre esses recursos podemos destacar aqueles necessários para a nidificação, como as cavidades de árvores, e os alimentos, como o pólen e o néctar [7]. Por exemplo, a redução da complexidade ambiental por meio da expansão das monoculturas reduz a disponibilidade desses recursos no espaço e no tempo, resultando em uma redução da riqueza de espécies de polinizadores no ambiente [8].

Para complicar ainda mais esse cenário de reduções da complexidade ambiental, a ameaça aos polinizadores também prejudica muitas plantas, pois aproximadamente 87,5% das espécies de angiospermas no mundo dependem, em algum grau, dos polinizadores para a sua reprodução sexuada [9]. Como algumas dessas espécies de plantas fornecem frutos que são a base da dieta de muitos animais, a ausência da polinização pode prejudicar não só as plantas e os polinizadores, mas a fauna como um todo [10]. Assim, as ameaças aos polinizadores podem gerar um efeito em cascata na comunidade [11], que pode desencadear consequências ao bem-estar humano.

Menos polinizadores, menos comida

A ameaça aos polinizadores causada pela intensificação agrícola pode afetar a própria produção agrícola. Isso porque os polinizadores aumentam a quantidade e a qualidade de frutos e sementes de cerca 75% dos 115 cultivos mais importantes produzidos no mundo [12]. Dentro dessa produção, estão incluídas *commodities* agrícolas de alto valor econômico, como o café, o cacau e a canola [13].

Estima-se ainda que o serviço de polinização aumente o valor de mercado da produção agrícola mundial anualmente. Por exemplo, o valor desse aumento chegou a ser estimado em 235 a 577 bilhões de dólares [13]. Isso porque cultivos que dependem da polinização feita por animais frequentemente possuem preços de venda mais altos do que cultivos que não dependem. Outra estimativa que reforça a contribuição dos polinizadores para a produção agrícola é a de que a ausência desse serviço implicaria em uma perda de 5 a 8% da produção agrícola mundial [14].

Com essa possível perda da produção e do fornecimento de frutos e sementes, não só a economia seria afetada, mas também a nossa segurança alimentar, tanto em termos da disponibilidade quanto da qualidade dos alimentos [3,15]. Portanto, indo além do ponto de vista ecológico, a perda de polinizadores também resultaria em impactos econômicos e sociais devido ao importante papel que eles desempenham na agricultura.

O papel das abelhas

Dentre os polinizadores mais importantes na agricultura podemos destacar os insetos, pois a grande maioria das plantas cultivadas depende da polinização feita por eles, principalmente pelas abelhas, que prestam esse serviço a mais de 90% das principais culturas agrícolas mundiais [12]. Ao redor do mundo, há mais de 20.000 espécies de abelhas descritas [16], sendo a abelha europeia *Apis mellifera* a espécie mais manejada para fins agrícolas [17]. Entretanto, nas últimas décadas, o rápido declínio populacional dessa espécie vem sendo reportado por apicultores ao redor do mundo, especialmente na América do Norte [18].

Paralelamente a esse declínio conhecido como Distúrbio do Colapso das Colônias (CCD, na sigla em inglês), a redução da riqueza e abundância de espécies de abelhas não-manejadas também vem sendo observada em outras partes do mundo [19]. Essa perda também é muito prejudicial para a agricultura, pois a polinização por *Apis mellifera* não substitui a polinização feita por abelhas silvestres, já que essa espécie presta apenas um serviço suplementar [20]. Dependendo do cultivo, a polinização só é bem-sucedida se feita por abelhas silvestres, como no caso da acerola que é polinizada por abelhas coletoras de óleo do gênero *Centris* [21]. Além disso, outros cultivos dependem de interações especializadas, como a polinização por vibração realizada por abelhas dos gêneros *Bombus* e *Xylocopa* [22]. Assim, tanto o declínio de abelhas manejadas quanto o de abelhas silvestres impactam a polinização das culturas agrícolas.

Esse declínio de abelhas é associado a diversas causas, dentre elas, a contaminação por agrotóxicos e a redução da disponibilidade e variedade de recursos alimentares em campos agrícolas [18]. Contraditoriamente, essas causas prejudiciais às abelhas são características das práticas agrícolas intensivas aplicadas em lavouras que dependem do serviço de polinização prestado por elas [23]. Como essas lavouras têm se expandido rapidamente nas últimas cinco décadas, esse declínio pode ser visto como o princípio de um problema que pode vir a se agravar com o aumento esperado da produção agrícola no futuro [24], já que o *déficit* de abelhas e da polinização pode desencadear problemas ecológicos, econômicos e sociais. Dessa forma, investigar a magnitude dos efeitos das ameaças provenientes da intensificação agrícola sobre as abelhas e o serviço de polinização prestado por elas torna-se cada vez mais urgente.

Investigando o problema

Para ajudar a entender melhor esse problema, a presente dissertação tinha como objetivo original utilizar uma abordagem meta-analítica para quantificar a magnitude dos efeitos dos três principais aspectos da intensificação agrícola sobre a comunidade de abelhas e o serviço de polinização que elas prestam. Assim, quantificaríamos perda de habitat, homogeneização da paisagem e uso intensivo de agrotóxicos.

Contudo, após uma revisão sistemática da literatura e a triagem dos artigos seguindo os nossos critérios de inclusão (veja detalhes a seguir), obtivemos um baixo tamanho amostral para as análises investigando o efeito da perda de habitat e da homogeneização da paisagem sobre a comunidade de abelhas e o serviço de polinização prestado por elas. Isso implicaria em estimativas de tamanho de efeito pouco confiáveis para esses dois processos, devido a um baixo poder estatístico. Portanto, optamos por focar na ameaça dos agrotóxicos à comunidade de abelhas e ao serviço de polinização das lavouras prestado por elas, pois nesse caso obtivemos um tamanho amostral bom o suficiente para gerar resultados sólidos.

Além disso, a partir das dificuldades enfrentadas durante a extração de dados dos artigos-fonte e após discussões com colaboradores, pensamos na elaboração do segundo capítulo desta dissertação. A missão desse segundo capítulo é fazer a ponte entre os produtores de dados primários e os pesquisadores que dependem desses dados para a realização de trabalhos de síntese, como revisões sistemáticas, meta-análises e *data papers* [25].

Sentimos essa necessidade, porque muitos dados que poderiam ser incorporados nas nossas análises infelizmente tiveram que ser excluídos. Essas exclusões aconteceram principalmente devido à falta de transparência, acesso e clareza na forma com que os métodos, dados e resultados foram reportados, tornando impossível extrair as informações necessárias para a nossa meta-análise. Essa preocupante situação, recorrente em estudos ecológicos, limita a reprodutibilidade, o reuso de dados, a síntese das informações e o avanço do conhecimento, prejudicando toda a comunidade científica [26].

Desta forma, o segundo capítulo teve como objetivo contribuir para aumentar a usabilidade e reprodutibilidade dos dados primários de estudos sobre interações entre organismos de espécies diferentes. Para isso, visando uma melhor comunicação entre produtores e consumidores de dados, elaboramos um guia contendo dez regras simples para reportar informações sobre interações entre espécies, seguindo o estilo de uma famosa série da revista *PLOS Computational Biology* (*https://collections.plos.org/collection/ten-simple-rules/*). Esse guia, além de melhorar a reprodutibilidade e usabilidade dos métodos, dados e resultados dos estudos primários, também beneficia os produtores de dados, fazendo com que seus estudos sejam mais lidos, citados e usados de forma ampla, expandindo também as oportunidades de colaboração e coautoria. Também explicamos o nosso guia em uma matéria em português publicada no Jornal da USP, voltada para um público bem mais amplo (*https://jornal.usp.br/?p=565105*).

O primeiro capítulo, que tem como título "*A meta-analysis of the effects of pesticides on bees and their pollination service in the context of intensive agricultural practices*", será submetido à revista *Journal of Applied Ecology*. O segundo, "*Ten simple rules for reporting information on species interactions*", está formatado de acordo com as normas da revista *PLOS Computational Biology,* na qual já foi publicado em 2022 (*https://doi.org/10.1371/journal.pcbi.1010362*).

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CAPÍTULO 1

A meta-analysis of the effects of pesticides on bees and their pollination service in the context of intensive agricultural practices

Manuscrito a ser submetido para a revista Journal of Applied Ecology

A meta-analysis of the effects of pesticides on bees and their pollination service in the context of intensive agricultural practices

Cristina A. Kita[1,2*], Laura C. Leal[3], Marco A. R. Mello[1]

1. Department of Ecology, Institute of Biosciences, University of São Paulo, São Paulo, Brazil.

2. Graduate School in Ecology, Institute of Biosciences, University of São Paulo, São Paulo, Brazil.

3. Department of Ecology and Evolutionary Biology, Federal University of São Paulo, Diadema, São Paulo, Brazil.

*c.akemikita@gmail.com (CAK)

INTRODUCTION

In the past decades, a critical problem has worried humankind: managed and wild bee populations are declining all around the world [1]. This decline is associated especially with changes in land use, configuration, and management that reduce the availability and diversity of food and nesting resources crucial to bees [2].

In the opposite direction, pollination-dependent crops have expanded rapidly over the past decades due to growing market demands for food production, suggesting a trend towards monoculture expansion following the global human population growth [3]. However, this expansion associated with a decrease in agricultural diversity reduces food and nesting resources for pollinators, creating a conflicting scenario in which the rapid expansion of pollination-dependent crops under intensive agricultural practices jeopardizes the same bees that these crops depend on [4,5].

The problem depicted in this scenario goes beyond the reduction of food and nesting resources for bees. In intensively agricultural systems, pesticide application is a common practice to protect crops against yield loss [6]. However, pesticides contaminate bees [7]. This contamination can occur directly through pesticide overspray, contact with treated leaves, flowers, seeds, and soil, consumption of contaminated water, guttation fluid at plant leaf tips or margins, pollen, and nectar, as well as indirectly through the collection of contaminated materials [8].

The contamination of bees is worrisome, especially in the face of an increasing demand for food production, because it can cause lethal and sublethal effects capable of impairing the delivery of their pollination service (e.g., [9]). For instance, crop pollination can be affected by a reduction in bee abundance, richness, and diversity within agricultural crops [10] caused by lethal effects. Those effects result in a decreased seed set, yield, marketable quality, and commercial value of those crops [11,12].

Pollination can also be affected even when bees survive contamination (sublethal effects), since contamination can change their behavior and physiology [13]. For instance, changes in the bees' ability to buzz pollinate reduce pollen collection of plants that depend on this specialized system [14]. Sublethal effects can also cause abnormal foraging behaviors such as reduction of forager mobility or motionlessness [15], reduction of olfactory learning and memory [16], and reduction of distance traveled [17], which affect forager navigation capacity (sensu [18]) and, consequently, the pollination service.

The contamination of individual foragers is not only harmful to themselves, but also to their colony due to the long-term effects of hive contamination [19]. Hive contamination occurs through contaminated materials from agricultural crops. Foragers can transport contaminated pollen and nectar that are stored in the hive or used as food to feed all individuals, including larvae [20], contaminating the entire colony. This generalized contamination can affect the fecundity of workers and the production of queens [21,22]. Contamination can also increases adult and larvae mortality [23,24], reducing colony growth, fitness, and long-term viability [25,26].

Those lethal and sublethal effects of pesticides go beyond the economic losses imposed by a decrease in agricultural production through a disruption of the pollination service. They also affect our food supply and consequently threaten our food security. In the near future, the human population will continue to grow, increasing the demand for food production and, consequently, leading to intensive agricultural expansion accompanied by harmful pesticide application [27,28].

Given the consequences to our food production and security that can be triggered by widespread pesticide application in intensive agricultural systems, the objective of our study was to investigate the magnitudes of lethal and sublethal pesticide effects and their consequences for the bee community and its pollination service within crops, using a meta-analytical approach. We aimed at evaluating the hypothesis that pesticide application has strong negative lethal and sublethal effects on bees, leading to consequences for the bee community, which ultimately impair its pollination service within crops. Evidence shows that pesticide application in intensive crops can result in bee population decrease within them, in addition to causing severe cumulative effects on bees, from individuals to the entire colony.

MATERIAL AND METHODS

Literature search

We searched for articles on the databases Web of Science (Core library - *https://www.webofscience.com*) and Scopus (*www.scopus.com*) using all available years up to January 2021. We conducted an advanced search based on title, abstract, and keywords using the following combination of terms: (bee* NOT beetle* OR wild bee OR native bee OR crop pollinator) AND (agrochemical* OR pesticide* OR insecticide* OR fertilizer*) AND (pollination OR transfer of pollen OR crop pollination).

Screening

On the selected databases, we identified 198 articles. Initially, we imported the complete list of articles into R [29]. Using the *litsearchr* package [30], we removed duplicates (N = 44). Following the screening stages proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA: *http://www.prisma-statement.org/*). Then we read the title and abstract of the 154 remaining articles after the deduplication phase. In this stage of the screening process, only the articles that met the following criteria were included in our meta-analysis:

1. The study was related to agricultural pollination by bees;
2. The authors investigated pesticide effects on bees, at least in one of these categories: (i) survival (lethal effect); (ii) bee behavior, physiology, or colony viability (sublethal effect); or (iii) consequences of pesticide application for the bee communities that could impair their pollination service within agricultural crop fields, e.g., effects on bee abundance and richness (consequences of lethal and sublethal effects).

After applying this first inclusion criteria, 65 studies remained in our data set. Finally, we read the methods and results sections of all those studies. In this second stage, only the articles that met the following criteria were included in our meta-analysis:

1. The study was a primary research;
2. If the study was conducted in the field, data were collected in agricultural crops. If the study was conducted in the laboratory, the protocol was based on at least the field doses of pesticides commonly applied in crops;
3. The study reported information that enabled to contrast control and treatment groups (i.e., without pesticide application and with pesticide application).

After those steps, 21 studies remained and were considered relevant for our meta-analysis (Fig.1).

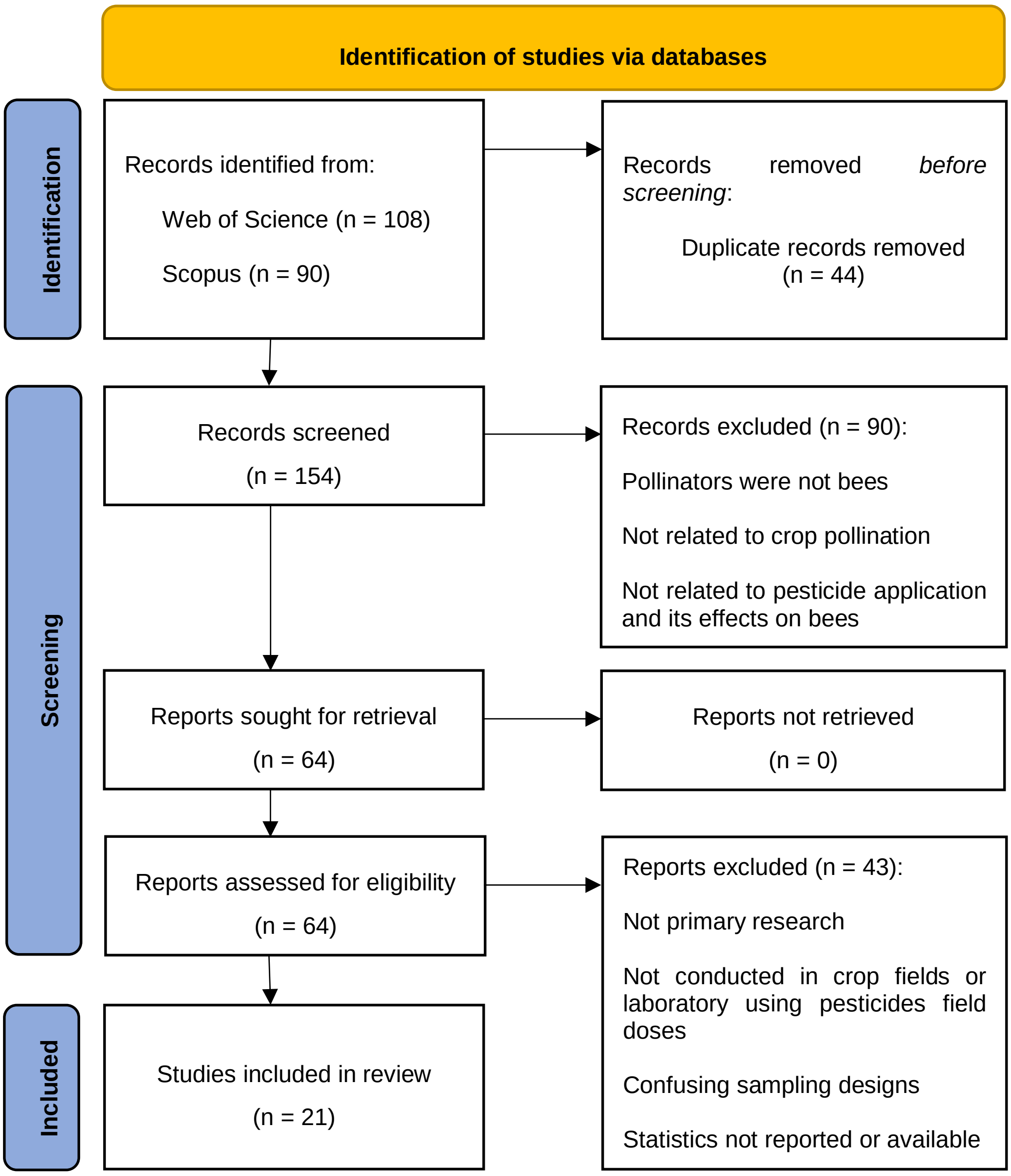

Fig. 1. Screening flow of this meta-analysis based on the PRISMA flow.

It is important to highlight the difficulties found when trying to review, extract, and summarize information from the literature. Most of the studies left out after our screening procedures did actually fit the scope of our meta-analysis. However, they lacked transparency or clarity in the way they reported their methods and results. That is why we urgently need better standardization of information reporting in ecology and conservation, in order to improve different kinds of syntheses, including meta-analyses [31].

Data extraction and effect size calculation

We classified the 21 selected studies into three groups by the kind of investigation conducted. In the first group, composed of six studies, we included the studies in which authors investigated lethal effects of pesticides on bees (hereafter, lethal effects). In the second group, composed of 10 studies (two of which shared with the first group), we included the studies in which the authors investigated sublethal effects of pesticides on bees (hereafter, sublethal effects). In the third group, composed of eight studies (one of which shared with the second group), we included the studies in which the authors investigated the consequences of lethal and sublethal effects of pesticides for the bee communities that could impair their pollination service within crops (hereafter, consequences of pesticide application).

In the first group, to investigate lethal effects, we considered studies in which the authors investigated bee survival probability after pesticide exposure. This variable was measured using the initial number of bees and the number of bees alive after pesticide exposure.

To calculate the effect sizes reported in the studies from this first group, we used the *escalc* function for R from the *metafor* package [32]. In this case, we used *odds ratio* as a metric of effect size. This metric represents the odds of an event happening in one group relative to the odds of the same event happening in the other group based on a contingency table that compares two groups (treatment and control) and the observed counts for two possible outcomes [33], in our case, bees alive or dead. Thus, to calculate *odds ratio* values (effect sizes) and their variances, we needed the number of bees alive and dead after pesticide exposure in the control and treatment groups. Then, we extracted the initial number of bees used in the experiment and the number of bees alive after pesticide exposure in each group. Subtracting the initial number of bees from the number of bees alive at the end of the experiment, we calculated the number of dead bees in the control and treatment groups at the end of the experiment.

The data were extracted from texts and figures. To extract data from figures, we used the software WebPlotDigitizer [34]. We also extracted information about bee species assessed, exposure type (i.e., topical or oral), pesticide type applied, hours of exposure and study sites. The group in which bees were exposed to pesticides was considered the treatment and the group in which bees were not exposed to pesticides was considered the control. Thus, negative values of *odds ratio* indicate a reduction in bee survival probability.

As we hypothesized that pesticide application would have a strong negative lethal effect on bees, we predicted that bee survival probability would be lower when bees were exposed to pesticides due to the lower expected number of bees alive in the treatment group (with pesticide application) compared to the control group (without pesticide application). Therefore, we expected a negative mean *odds ratio* value.

In the second group, to investigate sublethal effects, we considered studies in which the authors investigated bee behavior, physiology and colony viability after pesticide exposure. In the studies, the variables describing the pesticide effects on bee behavior were: distance walked by the bees (i.e., locomotion ability), number of foragers entering and exiting the hive (i.e., foraging activity), pollen consumption (i.e., attractiveness for pollen), and proboscis extension (i.e., olfactory learning). The variable related to bee physiology was phenoloxidase activity. The variables related to colony viability were: number of bees in hives (i.e., colony strength), number of offspring produced and nest construction rate (i.e., reproductive fitness) and mean lethal time (i.e., longevity).

To calculate the effect sizes for the second group, we used the *metafor* package for R [32]. For this group, we used Hedges' g [35] as the metric of effect size for all studies in the second group. This metric represents the standardized mean difference between treatments and includes a correction for small sample sizes [33]. Therefore, our effect sizes result from standardized differences between the means of treatments in which pesticides were applied (treatment group) and in which pesticides were not applied or the farm was certificated as organic (control group). Then, we extracted the mean, standard deviation, and sample size of the control and treatment groups of each variable. From studies that did not explicitly report mean values, sample sizes, and variances, we extracted the statistics, such as F, t, and r^2.

The data were extracted from texts and tables of studies in the second group. In some cases, we used the software WebPlotDigitizer [34] to extract data from figures. We also extracted information about bee species assessed, pesticide applied, exposure type (i.e., topical or oral), study sites, sampling method, and type of study (i.e., field or laboratory). If the study was conducted in the field, we also extracted information about farm type (i.e., monoculture or polyculture) and crop type (i.e., plant species).

Using the default parameters in the *escalc* function for R from the *metafor* package, we inputted the mean, standard deviation, and sample size of the control and treatment groups as the default arguments of the *escalc* function to calculate the Hedges' g values (effect sizes) and their sampling variances. From the studies that did not explicitly report mean values, variances, and sample sizes, we used the *compute.es* [36] package for R to calculate Hedges' g values from the statistics reported in the papers. We used the treatment group as a reference (with pesticide application), subtracting from it the mean value of the control group (without pesticide application or organic farms). Thus, negative values of Hedges' g occur when the mean from the control group was higher than that of the treatment group, indicating a negative effect of pesticide application on the bee behavior, physiology and colony viability.

As we hypothesized that pesticide application would have a strong negative sublethal effect on bees, we predicted that the pesticide application would impair bee behavior, physiology and colony. Therefore, we expected a large negative mean Hedges' g value. We interpreted the magnitude of the Hedges’ g effect sizes as small (≥ 0.20), medium (≥ 0.50), and large (≥ 0.80) [37].

In the third group, to investigate the consequences of pesticide application, we considered studies in which the authors investigated the consequences of lethal and sublethal effects of pesticide application for the bee communities that could impair their pollination service within crops. The consequences of pesticide application for bee communities within crops were measured in the original studies using: number of individuals of each bee species (i.e., abundance), number of bee species (i.e., richness), variance of species abundance distribution calculated using Simpson’s Diversity Index (i.e., diversity), and individual bees per flower (i.e., density).

To calculate the effect sizes for the third group, we used the *metafor* package for R [32]. We also used Hedges’ g [35] as the metric of effect size for all studies in the third group. Therefore, as in the second group, our effect sizes result from standardized differences in the means of treatments in which pesticides were applied (treatment group) and in which pesticides were not applied or the farm was certificated as organic (control group). The mean, standard deviation, and sample size of the control and treatment groups were extracted from texts and tables of studies in the third group. In some cases, we also used the software WebPlotDigitizer [34] to extract data from figures. From studies that did not explicitly report mean values, variances, and sample sizes we extracted the statistics. We also extracted information about farm type (i.e., monoculture or polyculture), pesticide applied, exposure type (i.e., topical or oral), crop type (i.e., plant species), sampling method and study sites.

Using the default parameters in the *escalc* function for R from the *metafor* package and the *compute.es* package, we calculated Hedges’ g values. We also used the treatment group as a reference (with pesticide application). Therefore, negative values of Hedges' g occur when the mean from the control group are higher than that of the treatment group, indicating a negative consequence of pesticide application for the bee community that could impair its pollination service within crops.

As we hypothesized that pesticide application would have a strong negative lethal and sublethal effects on bees, we predicted that the consequences of pesticide application for the bee community that could impair its pollination service within crops would also be strong and negative. Therefore, we also expected a large negative mean Hedges' g value.

Statistical analysis

Mean effect sizes

Using a restricted maximum-likelihood estimator (REML), we built meta-analytic mixed-effects models to calculate the mean effect sizes. We used the function *rma.mv* from the *metafor* package for R in all analyses. We considered the *odds ratio* or Hedges' g metrics as the response variables and the inverse of the variance as the weight of effect sizes, depending on the group of studies being analyzed.

To calculate the mean effect size of lethal effects (mean *odds ratio* value), we used all effect sizes calculated with data from the first group of studies. We built a model without moderators using study identity, bee species, exposure type, pesticide applied, and hours of exposure as random factors to control for pseudoreplication associated with more than one data point coming from the same study and to control for different bee species, bee exposure type to pesticides, type of pesticides, and duration of experiments, respectively. This way, we could calculate the mean lethal effect controlling for some of the predictable biases. In order to incorporate phylogenetic uncertainty regarding tree topology or branch lengths, and considering that bee survival rate is correlated with life history, we built a phylogenetic covariance matrix among the bee species using the Interactive Tree of Life online tree generator (iTOL: *https://itol.embl.de/*) database consulted through the *ape* and *rotl* packages for R [38,39]. The information about the study sites were used when discussing the results.

To calculate the mean effect size of sublethal effects (mean Hedges' g value), we used all the effect sizes calculated with data from the second group of studies. We built a model without moderators using study identity, type of study (i.e., field or laboratory), and sampling method as random factors to control for pseudoreplication associated with more than one data point coming from the same study, to control for different experimental conditions and sampling efforts, respectively. This way, we could calculate the mean sublethal effect controlling for biases. The information about bee species assessed, pesticide applied, exposure type, farm type (i.e., monoculture or polyculture), and crop type (i.e., plant species) were not incorporated into the model due to not available data (NAs), but they were considered when discussing the results as well as the information about the study sites.

To calculate the mean effect size of the consequences of pesticide application (mean Hedges' g value), we used all the effect sizes calculated with data from the third group of studies. We built a model without moderators using study identity, sampling method, farm type (i.e., monoculture or polyculture) and crop type (i.e., plant species) as random factors to control for pseudoreplication associated with more than one data point coming from the same study, to control for different sampling efforts, the influence of crop diversity and plant species, respectively. This way, we could calculate mean effect of the consequences of pesticide application for the bee community that could impair its pollination service within crops controlling for biases. The information about pesticide applied and exposure type (i.e., topical or oral) were not incorporated into the model due to not available data (NAs), but they were considered when discussing the results as well as the information about the study sites.

Processed data and R scripts used to calculate the models are available in the online supplement (appendix S1).

Heterogeneity

We estimated the heterogeneity of each model and the heterogeneity of each random variable using the I^2 statistic with a 95% confidence interval (CI). This way, we were able to analyze the overall heterogeneity of each model and the influence of each random variable on overall heterogeneity across studies in each model. I^2 describes the percentage of variation across studies due to data heterogeneity and not by chance [40]. The values of heterogeneity 25%, 50%, and 75% were considered as small, medium, and high, respectively, as suggested by Higgins (2003).

Publication bias

We tested for publication bias in each model using an adapted version of Egger's regression [41] for multilevel models (*rma.mv*), including the random factors. In this test, we ran a linear regression contrasting the model's residuals and the variances of each effect size. A publication bias is pointed out when the intercept of the regression significantly deviates from zero.

RESULTS

Lethal effects

We obtained 40 effect sizes (k = 40) from studies that investigated the lethal effect of pesticide exposure on bee survival. Pooling together all the effect sizes calculated with data from the first group of studies, we found a mean overall effect of -4.702 (± 1.538, SE) which means that the survival probability of bees exposed to pesticides is almost five times lower than that of bees not exposed to pesticides (Z = -3.057, p = 0.002, CI = -7.716 to -1.688; Fig. 2). All effect sizes came from laboratory experiments, mostly conducted in Brazil (Appendix S3. Fig. S6). The three most common bee species used were: *Plebeia emerina*, *Tetragonisca fiebrigi* and *Scaptotrigona xanthotricha*.

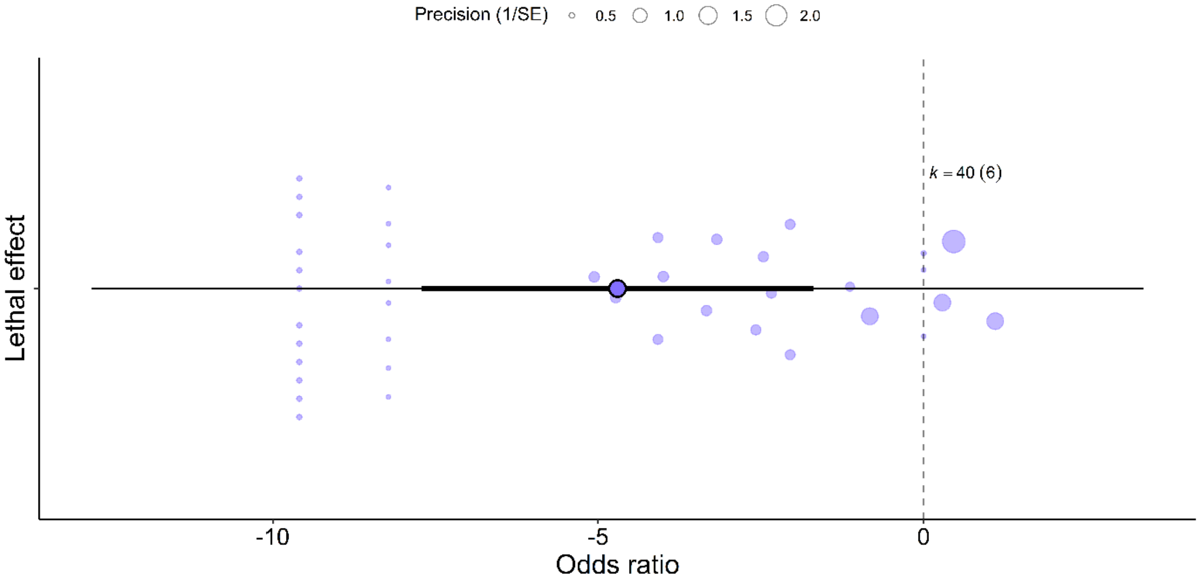

Fig. 2. Mean lethal effect size of pesticide application on bees and the effect sizes calculated using the data extracted from primary studies. The purple dots represent the effect sizes. The central dot in the thick black line represents the mean effect size for lethal effects. The other dots represent weighted individual effect sizes calculated from studies that investigated the effect of pesticide exposure on bee survival. Effect sizes are weighted by their precision (1/standard error, SE). The dashed line represents zero effect. The thick black line represents 95% confidence intervals of the mean effect size (CI), and an effect is considered significant when CI does not overlap 0. The thin black line represents the prediction intervals (PI). Number of effect sizes used to calculate the mean effect size: *k* = 40. Number of studies used to calculate the mean effect size = 6.

Sublethal effects

We obtained 41 effect sizes (k = 41) from studies that investigated sublethal effects. Pooling together all the effect sizes calculated with data from the second group of studies, we found a mean overall effect of -0.885 (±0.290, SE), which represents a large negative mean effect of pesticide application on the bees (Z = -3.050, p = 0.002, CI = -1.454 to -0.316; Fig. 3). Out of 41 effect sizes, 35 came from laboratory experiments, mostly conducted in the United States. From field experiments, four effect sizes came from studies conducted in Mexico and three conducted in the United States (Appendix S3. Fig. S7). The most common bee species used was *Bombus impatiens*.

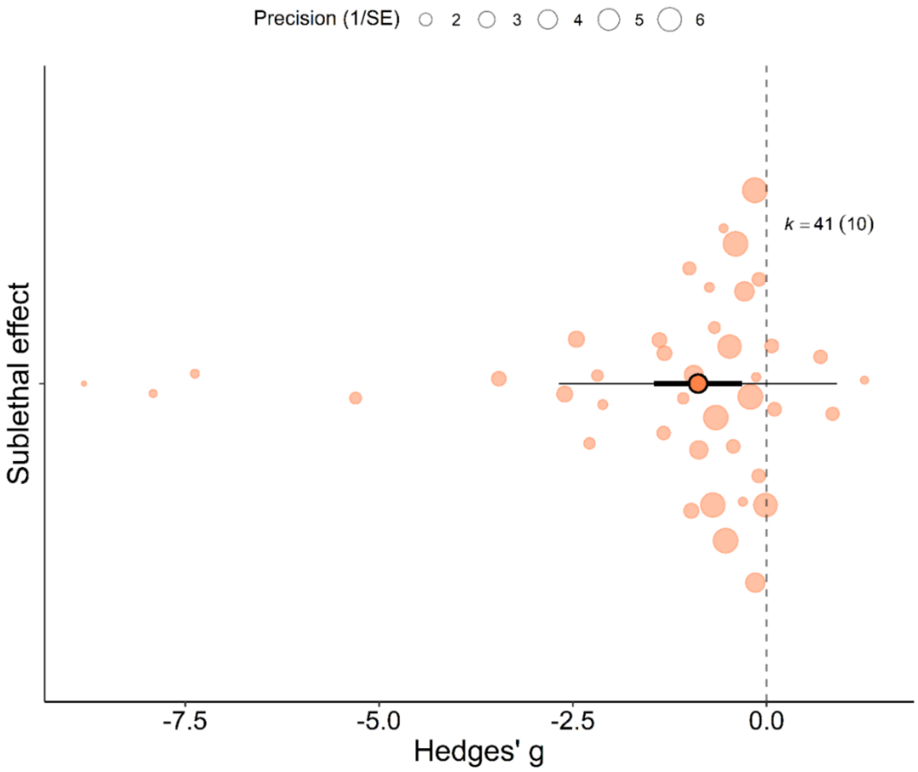

Fig. 3. Mean sublethal effect size of pesticide application on bees and the effect sizes calculated using the data extracted from primary studies. The orange dots represent the effect sizes. The central dot in the thick black line represents the mean effect size for sublethal effects. The other dots represent weighted individual effect sizes calculated from studies that investigated sublethal effects. Effect sizes are weighted by their precision (1/standard error, SE). The dashed line represents zero effect. The thick black line represents 95% confidence intervals of the mean effect size (CI), and an effect is considered significant when CI does not overlap 0. The thin black line represents the prediction intervals (PI). Number of effect sizes used to calculate the mean effect size: *k* = 41. Number of studies used to calculate the mean effect size = 10.

Consequences of pesticide application

We obtained 32 effect sizes (k = 32) from studies that investigated the consequences of lethal and sublethal effects for the bee communities that could impair their pollination service within crops. Pooling together all the effect sizes calculated with data from the third group, we found a mean overall effect of -0.341 (±0.192, SE), but it did not differ from zero (i.e., overlapped with zero). It means that the consequences of pesticide application for the bee communities that could impair their pollination service within crops are probably very small or null (Z = -1.772, p = 0.076, CI = -0.718 to 0.036; Fig. 4). All effect sizes came from field experiments, mostly conducted in the United States (Appendix S3. Fig. S8). It was not possible to identify the most common bee species, because not all study reported the bee species or the number of species collected.

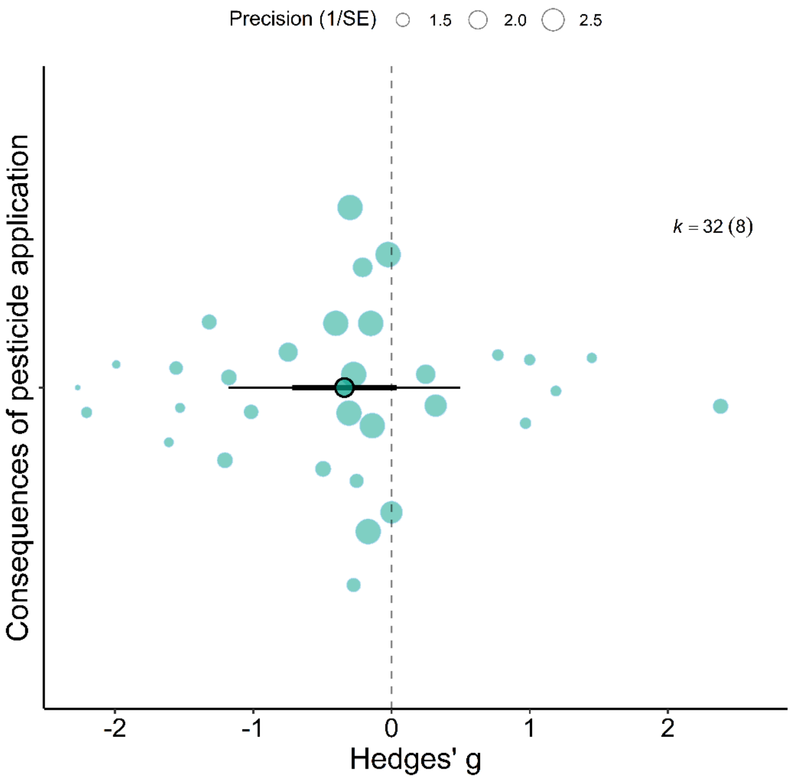

Fig. 4. Mean effect sizes of the consequences of lethal and sublethal effects for the bee communities that could impair their pollination service within agricultural crop fields. The green dots represent the effect sizes for the consequences of lethal and sublethal effects. The central dot in the thick black lines represents the mean effect size. The other dots represent weighted individual effect sizes calculated for lethal and sublethal effects from

studies that investigated lethal and sublethal effects. Effect sizes are weighted by their precision (1/standard error, SE). The dashed line represents zero effect. The thick black lines represent 95% confidence intervals (CI), and an effect is considered significant when CI does not overlap 0. The thin black lines represent the prediction intervals (PI). Number of effect sizes used to calculate the mean lethal effect size: k = 32. Number of studies used to calculate the mean effect size = 8.

Heterogeneity

In the model related to lethal effects, we observed a high level of heterogeneity (I^2 = 90.55 %; CI = 88.63 to 92.47; Table 1a), explained mainly by the type of pesticide applied. In the model related to sublethal effects, we observed a medium level of heterogeneity (I^2 = 69.38%; CI = 67.46 to 71.30; Table 1b), explained mainly by the study identity. In the model related to the consequences of pesticide application for the bee communities that could impair their pollination service, we observed a small level of heterogeneity (I^2 = 22.19%; CI = 20.27 to 22.58; Table 1c), explained mainly by the plant species.

Table 1: Heterogeneity estimates obtained from the models. I^2 = percentage of total variation across studies due to heterogeneity rather than chance. σ2 = heterogeneity given in the summary of the models.

a) Lethal effects

	I^2 (95% CI)	σ2 (95% CI)
Study ID	24.69 (22.77, 26.62)	3.99 (0.29, 30.47)
Bee species	$3.11e^{-07}$(-1.92, 1.92)	0.00 (0.00, >10.00)
Pesticide applied	65.85 (63.93, 67.77)	10.64 (3.68, 38.27)
Hours of exposure	$9.36e^{-11}$ (-1.92, 1.92)	0.00 (0.00, 1.85)
Exposure type	$8.06e^{-12}$ (-1.92, 1.92)	0.00 (0.00, >10.00)
Total	90.55 (88.63, 92.47)	-

b) Sublethal effects

	I^2 (95% CI)	σ2 (95% CI)
Study ID	69.38 (67.46, 71.30)	0.75 (0.32, 2.33)
Study type (laboratory or field)	$7.17e^{-07}$ (-1.92, 1.92)	0.00 (0.00, >10.00)
Sampling method	$9.58e^{-08}$ (-1.92, 1.92)	0.00 (0.00, 4.47)
Total	69.38 (67.46, 71.30)	-

c) Consequences of pesticide application

	I^2 (95% CI)	σ2 (95% CI)
Study ID	2.69 (0.76, 4.61)	0.02 (0.00, 0.79)
Sampling method	$3.46e^{-07}$(-1,92, 1,92)	0.00 (0.00, 0.91)
Farm type (monoculture or	$3.65e^{-10}$ (-1.92, 1.92)	0.00 (0.00, 5.58)
Plant species	19.51 (17.59, 21.43)	0.13 (0.00, 1.05)
Total	22.19 (20.27, 22.58)	-

Publication bias

We found evidence of publication bias in our dataset of lethal effects and sublethal effects, but not in our dataset of the consequences of pesticide application model (Table 2). Visual analysis are available in the online supplement (Appendix S2. Fig. S1-S3).

Table 2: Summary of the Egger's test for each meta-analytical model. CI = Confidence interval.

a) Lethal effects

Moderator	**Estimate**	**t-value**	**P value**	**Upper CI**	**Lower CI**
None (overall pesticide effect)	10.33	5.80	$1.11e^{-06}$	13.95	6.72

b) Sublethal effects

Moderator	**Estimate**	**t-value**	**P value**	**Upper CI**	**Lower CI**
None (overall pesticide effect)	1.61	2.13	0.04	3.14	0.08

c) Consequences of lethal and sublethal effects

Moderator	**Estimate**	**t-value**	**P value**	**Upper CI**	**Lower CI**
None (overall pesticide effect)	0.59	0.90	0.38	1.95	-0.76

DISCUSSION

In the present study, we have made worrisome findings. We focused on understanding how agricultural intensification in response to the increasing human demand for food might affect crop pollination by bees, which in turn might threaten our own food security. As a study model of a key impact of agricultural intensification on bees, we chose pesticide application. First, we have found out that pesticides might reduce bee survival by a factor of five. Second, another major impact might be easily overlooked. In addition to killing bees, pesticides have severe sublethal effects, which alter not only the behavior and physiology of forager bees, but also their colony viability. The consequences of these effects for the bee community and its pollination service within crops might not be evident in the short-term, but they can be serious in the near future. Here, we discuss the implications of our findings and suggest ways to overcome the problem.

We began to assess the problem of pesticide application by running separate models, so we could better understand the potential impacts of pesticides on different types of bee responses. The first model, as predicted, showed that pesticide application reduces bee survival by a factor of five. In addition, the second model showed that even when bees survive contamination, pesticide application has a large negative effect on their behavior, physiology, and long-term colony viability, also as predicted. However, the third model did not show a negative consequence of these effects for the bee communities within crops, contrary to what we predicted. In a nutshell, it means that pesticide application might not have impaired the crop pollination by bees yet.

Let us expand on this idea. Pesticide application is harmful to bees and, thus, affects crop pollination. This statement holds, as pesticide exposure reduces bee survival and leads to sublethal effects that hinder the pollination service delivered by bees. Still, we need to consider three issues. First, pesticide effects depend on environmental context. Second, farm management can influence the magnitude of pesticide effects. Third, pesticide effects also depend on bee species sensitivity. With this in mind, we interpret our results from environmental, farm management, and biological perspectives.

From an environmental perspective, bees are influenced by several factors. These factors can affect bee abundance, richness, diversity, and density within crops, as well as bee pollination service, especially because they can interact with pesticide effects [42]. This interaction can enhance the negative effects of pesticides or buffer them. For instance, a landscape with poor nutritional resources can increase the decline of bees within a monoculture due to the additive effect of nutritional stress combined with pesticide contamination [43]. On the other hand, a high percentage of natural vegetation surrounding a monoculture can buffer this decline, as natural vegetation provides food and refuge from pesticides [44]. In addition, natural areas function as sources of bees to crops [45,46], especially to monocultures that represent low-quality habitats to bees [47]. Moreover, crop distance from natural areas also influence the visitation rate of bees to crops [48], as well as the quality of their pollination service (e.g.,[49]). Apart from natural vegetation, weather conditions can also influence pesticide effects on bees because some pesticides can have higher or lower toxicity depending on field temperatures (e.g.,[50]). Therefore, under field conditions, the effect of pesticide application on bee communities and their pollination service depends on environmental context.

This dependence can explain the neutral effect of the consequences of pesticide application found and its contrast with the negative lethal and sublethal effects. 100% of lethal and 85% of sublethal effect sizes came from laboratory experiments while 100% of the effect sizes of the consequences of pesticide application came from field experiments. In addition, as 84% of our data about the consequences of pesticide application came from monocultures that were surrounded by natural or semi-natural areas, there might be a strong influence of these areas on the abundance, richness, diversity, and density of bees within crops, an influence that is absent under laboratory conditions. Thus, it is possible that the neutral mean effect of the consequence of pesticide application is an evidence that environmental factors, especially natural vegetation areas, can buffer the negative effects observed in controlled laboratory experiments than an indication that pesticides have no pervasive effects on bee communities. This evidence reiterates the paramount importance of conserving natural areas surrounding agricultural areas.

From a farm management perspective, in intensive farms, bees are exposed to a cocktail of pesticides, combined in different mixtures, application methods, and crop types, so that pesticide effects can show different magnitudes [51,52]. In a cocktail, pesticides can interact with each other resulting in a synergistic effect [53], which increases bee mortality and changes bee behavior compared to the isolated effect [54,55] of a single pesticide. In our sublethal dataset, a study exemplifies this synergistic effect. The study showed that the effect of the glyphosate herbicide when combined with 2,4-D herbicide resulted in an effect size three times more negative than each of the pesticides alone (Appendix S1).

In addition to increasing pesticide toxicity, a bad combination can even turn a non-harmful pesticide into a harmful one when applied together with other pesticides [56]. Moreover, pesticides can also interact with other stressors, such as the pathogenic fungus *Nosema apis* (Microsporidia), amplifying the negative effects of pesticides [57]. Therefore, despite 100% of our lethal and 90% of our sublethal effects sizes having resulted from single pesticide effects under laboratory conditions, they give us a clue about the magnitude of these effects under field conditions. In other words, their negative effects can be worse in intensive farms, especially in intensive farms isolated from natural areas.

From a biological perspective, pesticide effects also depend on the sensitivity of each bee species (e.g., [58]), which varies according to life history traits. Traits such as body weight, sociality (i.e., solitary or social), flight season, voltinism (i.e., number of generations in a year), floral specialization (i.e., polylectic or olygolectic), nesting location, and sex vary among bee species. Thus, a species' vulnerability depends on the combination of those traits [59]. For instance, lighter species, such as leafcutting bees (e.g., *Megachile rotundata*; average weight = 26 mg), are more sensitive to pesticides than heavier species, such as bumblebees (e.g., *Bombus rufocinctus*; average weight = 140 mg) due to their higher surface area to volume ratio, which increases their contact absorption area [60,61]. In addition to those traits, evolutionary adaptations may also explain differences in sensitivity between bee species. For instance, honeybees are less sensitive to synthetic alkaloids of insecticides than bumblebees due to their ancestral adaptation to feed on tropical nectars, in which natural alkaloids are prevalent [62]. Therefore, the magnitude of the pesticide effect is not only influenced by the pesticide itself, but also by the bee species assemblage of different localities, landscapes, or biomes from all around the world.

That said, we must consider that, in our dataset, there are more studies from North America than other continents. Out of 21 studies, 11 came from North America, especially from the United States (nine), six from Latin America, two from Europe, one from Asia and one from Africa (Appendix S3. Fig. S4 and S5). Therefore, our results are likely biased towards North American crops and bee assemblages. Nevertheless, our study is not the first to point to a regional bias related to pesticide effects on bees. In another systematic review of neonicotinoids and their impacts on bees, the authors also identified not only a bias towards North America and Europe but also towards studies on *Apis mellifera* [63]. Apart from social bees, a regional bias was also detected in a recent systematic review of pesticide effects on solitary bees, in which the majority of the studies also came from the United States [64]. Anyway, despite this bias, our meta-analysis reinforces a knowledge gap in other continents and brings new insights into the problem.

For instance, despite the limited number of bee species in our lethal and sublethal datasets (11 species: *Apis cerana*, *Apis mellifera*, *Bombus impatiens*, *Melipona quadrifasciata*, *Melipona scutellaris*, *Osmia lignaria*, *Plebeia emerina*, *Partamona helleri*, *Scaptotrigona mexicana*, *Scaptotrigona xanthotricha* and *Tetragonisca fiebrigi*) and the presence of outliers (Fig. 2 and 3, respectively), our results are consistent. The results corroborates previous studies suggesting negative lethal and sublethal effects on bees, leading to potential damage to crop pollination services [65]. In addition, our sensibility tests show that, after removing the outliers that could influence our results, the estimated mean lethal and sublethal effects are still negative and different from zero (Appendix S4. Fig. S9 and S10), indicating that our mean negative effect sizes are robust. With this in mind and observing that the majority of the effect sizes in our dataset of the consequences of pesticide application were negative and the P-value ($p = 0.08$) was nearly significant (i.e., $p < 0.1$) for a dataset with great intrinsic variation, the mean effect size of the consequences of pesticide application for the bee communities tends to be negative.

The negative consequences of pesticide application might be evident in the near future. If we consider the large mean effect size of sublethal effects of our meta-analysis and the results of other studies showing severe sublethal effects on bees after their contamination, the long-term consequence for bees and their pollination service might be enormous. In a meta-analysis that evaluated the effect of field-realistic doses of imidacloprid on honeybees, the author found that, under laboratory conditions, the field-realistic dietary doses of the insecticide in nectar are unlikely to cause death directly, but it rather causes sublethal effects [66]. Sublethal effects were also observed in wild bees, causing a decrease in colony growth, flight abilities, and adult longevity not only under laboratory conditions, but also in the field [67]. Therefore, we need to be aware not only of short-term consequences, such as a reduction in bee abundance, richness, and diversity immediately after pesticide application, but also of long-term consequences to bee population persistence and their capability to delivery crucial pollination services in the future.

In the coming decades, following the actual path of intensive agriculture expansion, environmental buffers, such as natural vegetation surrounding crops, tend to be reduced due to monoculture expansion. This habitat loss increases the likelihood of pest outbreaks, since natural areas are also sources of natural enemies that spread to crops [68]. With pests spreading to crops, pesticide application tends to increase. This increase negatively affects the pests, but also their natural enemies and bees. Without refuge from pesticides, lack of food, and lack of nesting resources in homogeneous landscapes, bee richness and abundance will decrease quickly [46]. Without natural enemies and bees, crop quality and productivity will also decrease. Thus, it is important to reduce further land conversion and the reliance on pesticides for crop production.

Depending on management practices, crop types, and growing conditions, some organic systems can nearly match the productivity of conventional ones [69]. A promising alternative to intensive agriculture is the practice of ecological intensification. This practice involves actively managing farmlands to increase the intensity of the ecological processes that support production, such as biotic pest regulation, nutrient cycling, and crop pollination to enhance agricultural productivity [70]. Examples of these practices involve restoring or maintaining semi-natural ecosystems to increase the diversity and availability of food and nesting resources for bees. Conservation policy and management that focus on reducing distances between foraging resources to create vegetation patches and planting crop varieties that are attractive to bees are possible solutions to maintain the diversity of bees. In addition, adopting integrated pest management (IPM) protocols to reduce pesticide application and minimize bee contamination is another possible solution [71]. In cases in which the adoption of IPM protocols are not feasible, additional studies investigating the synergistic effect of pesticides on bees will be necessary to better understand what combination could be avoid to minimize their negative effects. After all, our food security strongly depends on bees.

CONCLUSION

Our results shed light on the magnitude of pesticide effects on bees under a scenario of intensified agricultural practices. In this context, growing pesticide application threatens the survival of bees and, most likely, the continuity of their crop pollination service. Therefore, it can also affect our own food security. This problem can be magnified in the coming decades by monoculture expansion associated with an increase in the use of pesticides. To counterbalance those synergistic threats, the reduction of pesticide application, preservation of natural habitats, and adoption of practices that improve bee diversity and survival are crucial in the near future.

ACKNOWLEDGMENTS

We are sincerely grateful to the authors of all studies included in our meta-analysis, whose effort made this synthesis possible. Tereza C. Giannini and Isabel A. Santos, members of the thesis committee of CAK, made invaluable suggestions to study design and result interpretation. CAK thanks the Coordination for the Improvement of Higher Education Personnel (CAPES, 88887.470293/2019-00) and the Graduate School in Ecology of the University of São Paulo (PPGE-IB-USP) for the M.Sc. scholarship. MARM was funded by the Alexander von Humboldt Foundation (AvH, 3.4-8151/15037 and 3.2-BRA/1134644), National Council for Scientific and Technological Development (CNPq, 304498/2019-0), São Paulo Research Foundation (FAPESP, 2018/20695-7), and Dean of Research of the University of São Paulo (PRP-USP, 18.1.660.41.7). We also thank the Stack Overflow community (*https://stackoverflow.com/*), where we solved most of our coding dilemmas.

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CAPÍTULO 2

Ten simple rules for reporting information on species interactions

Artigo publicado em 2022 na revista PLOS Computational Biology: https://doi.org/10.1371/journal.pcbi.1010362

Ten simple rules for reporting information on species interactions

Cristina A. Kita[1,2*], Guillermo Florez-Montero[3], Sebastián Montoya-Bustamante[1,2], Renata L. Muylaert[1,4], Natalya Zapata-Mesa[1,2], Marco A. R. Mello[1]

1. Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

2. Programa de Pós-Graduação em Ecologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

3. Universidade Federal do ABC, Centro de Ciências Humanas e Naturais, Santo André, Brazil.

4. Molecular Epidemiology and Public Health Laboratory, School of Veterinary Science, Massey University, Palmerston North, New Zealand.

*c.akemikita@gmail.com (CAK)

All authors contributed to conceiving this project, writing the manuscript, and designing the figure. CAK coordinated the project, and MARM supervised the team. In addition, MARM acquired funding for the project. Middle authors are listed in alphabetical order of surnames.

INTRODUCTION

“*It is like the fire of a torch: if hundreds or thousands of people would come each with a torch to ignite by that flame, each torch they have ignited from the original one could be used to cook meals and keep a dark house bright, and yet the original torch would stay as bright as it used to be.*”

(Shakyamuni Buddha, “The Sutra of Forty-two Chapters”)

There is growing appreciation of information sharing in science, because it allows reproducibility and boosts usability, thus benefiting the community and helping to advance knowledge [1]. Nevertheless, to be helpful, information sharing needs to be efficient and that depends not only on consistently reporting raw data, but also methods, processed data, and model results. Whenever there are inconsistencies, issues arise. First, issues in reproducibility that arise due to the lack of methodological details reduce science trust. These details are crucial for assessing a study’s reproducibility and reliability [2,3]. Second, issues in the reuse of a study’s raw and processed data that arise due to an incomplete report of them limit their reuse for making synthesis (*sensu* [4]). An incomplete reporting of model results also hinders synthesis work, which slows down the development of a field.

Aiming to solve those issues and improve the reproducibility and usability of primary scientific research, general guidelines have been proposed in the light of the open science culture [5]. Examples of such guidelines are the FAIR Guiding Principles (FAIR) [6] and the Transparency and Openness Promotion (TOP) [7]. Another outstanding example is the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) [8].

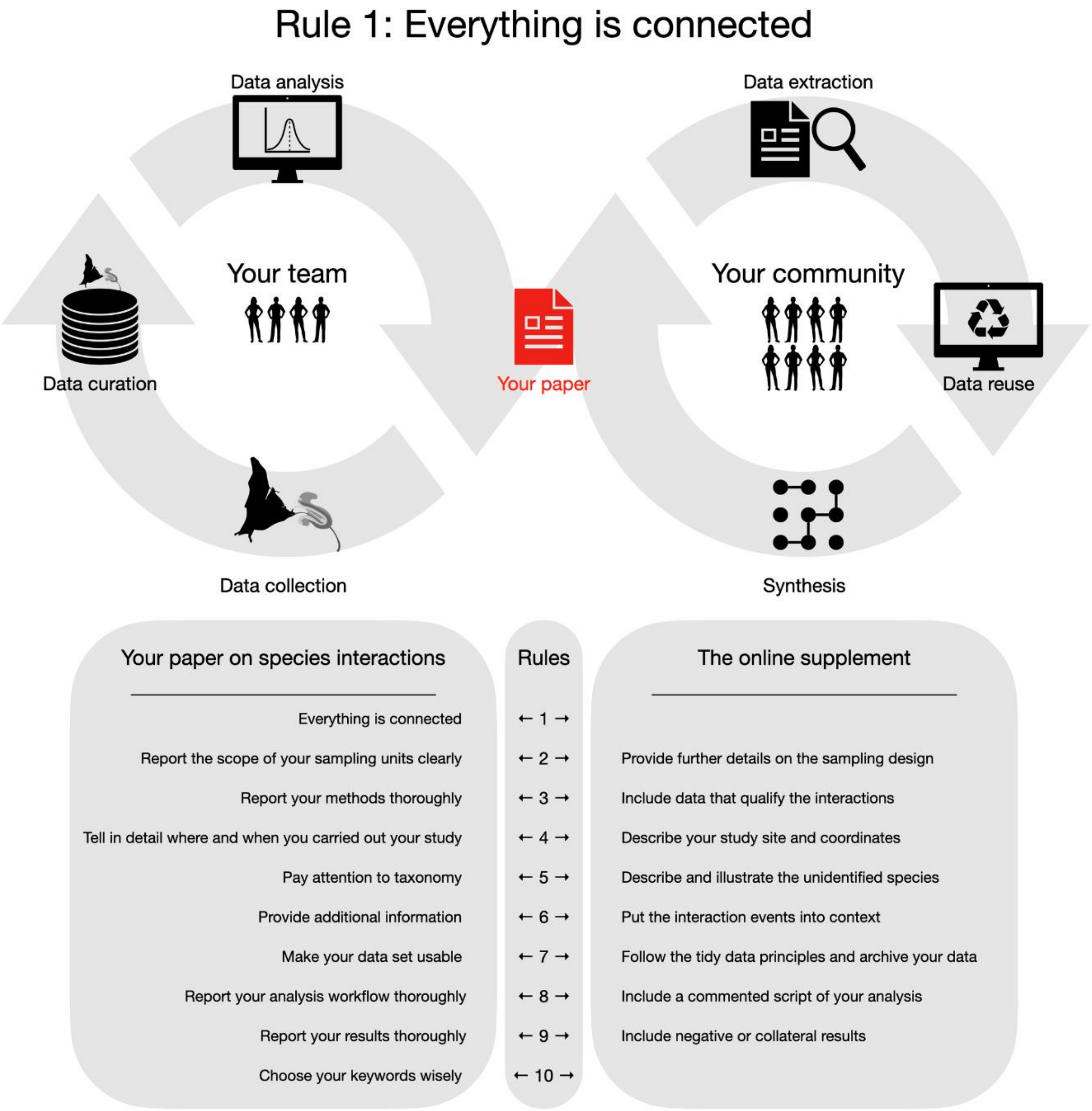
Extensions of those guidelines have also been elaborated to address issues faced in specialized fields. For example, in ecology, our field, there is a new extension known as the Preferred Reporting Items for Systematic Reviews and Meta-analyses in Ecology and Evolutionary Biology (PRISMA-EcoEvo) [9]. Ecologists also use other specialized guidelines, such as the Tools for Transparency in Ecology and Evolution (TTEE), designed to help journals adopt TOP [10].

Those existent guidelines and extensions are crucial as many ecologists rely on primary data for synthesis. However, despite those new roadmaps and tools, issues in reproducibility and usability are still common in ecological studies. We notice them all the time, as our research group specializes in synthesis. Our main topic of interest is ecological interactions between organisms of different species (a.k.a. species interactions), such as pollination and zoonosis. We have struggled to extract information from primary sources when compiling primary interaction data, conducting meta-analysis, re-analyzing processed data, and interpreting model results. Our syntheses strongly depend on you, who collect data on species interactions in the field or lab. Most importantly, we agree that sharing your data collected with so much effort without receiving proper rewards is not fair [11]. Anyway, despite asymmetric rewards and conflicts of interest, both data producers and users can greatly benefit from an open research culture, as we discuss here.

Aiming to tie those loose ends and improve the communication between data producers and users, and by harnessing the framework created by the previously mentioned guidelines, we propose ten simple rules for reporting information related to data collection methods, raw data, processed data, and model results from studies on species interactions. Our objective is to go beyond merely pointing out problems, as we also suggest practical solutions to solve them. Although some of our rules apply to researchers who use primary information for secondary studies, they are addressed primarily to you and all colleagues who produce primary information on species interactions. Because our rules can significantly improve the reproducibility and usability of methods, data, and results, by following them you can improve the citations of your primary studies [12] as well as broaden your collaboration and co-authorship horizons. In other words, if you follow our rules, your hard work can benefit the entire scientific community, including your own research group.

Rule 1: Everything is connected

A good study begins with an exciting problem begging to be solved. From the problem come your questions and expectations, and from them follow the methods used to describe new phenomena or contrast expectations against reality. Adequate methods lead to reliable results, allowing robust interpretations and paving the way for discoveries. Nevertheless, all this fine-tuning might not be helpful for yourself and your community if every step taken along the way is not clearly explained. You cannot bake a tasty cake without a nice recipe. Likewise, the reader cannot assess your study's reliability and originality if its methods, data, and results are not thoroughly reported. Therefore, if you follow all rules proposed here, the reader will be able to use your ideas (the main goal of any scientific study), reuse your data, and make synthesis with your results. In addition, you can use our rules as guidelines to design your study from scratch, as many people do in the case of systematic reviews and meta-analyses carried out in the light of PRISMA, because all these rules and guidelines are connected to one another (Fig 1).



Your paper on species interactions	Rules	The online supplement
Everything is connected	← 1 →	
Report the scope of your sampling units clearly	← 2 →	Provide further details on the sampling design
Report your methods thoroughly	← 3 →	Include data that qualify the interactions
Tell in detail where and when you carried out your study	← 4 →	Describe your study site and coordinates
Pay attention to taxonomy	← 5 →	Describe and illustrate the unidentified species
Provide additional information	← 6 →	Put the interaction events into context
Make your data set usable	← 7 →	Follow the tidy data principles and archive your data
Report your analysis workflow thoroughly	← 8 →	Include a commented script of your analysis
Report your results thoroughly	← 9 →	Include negative or collateral results
Choose your keywords wisely	← 10 →	

Fig 1. Our ten simple rules are connected to one another. Follow them and use our roadmap when designing and publishing your study on species interactions. This additional work might boost your study's impact and usability, thus helping your community and your team. Efficient communication goes a long way.

Rule 2: Report the scope of your sampling units clearly

We are sure you have also faced this problem when checking a data sheet. Several studies lack clarity about the scope of the sampling units, especially in terms of the ecological level of organization assessed (i.e., individual, population, community, ecosystem, or biome). When samples are taken from wild organisms, some methods capture or collect individuals, while others only allow direct or indirect observation. Those differences also need to be addressed in the statistical analysis. In addition, be clear about recording interactions between individuals or groups of individuals. For instance, if samples were taken from individuals that were subsequently released without being marked or collected, they should be quantified as “number of captures” rather than “number of individuals”. If samples were taken from direct or indirect observation, they should be quantified as “number of visits” rather than “number of individuals”. These simple changes can improve statistical analysis and communication with your peers.

Rule 3: Report your methods thoroughly

Not all primary studies allow us to reproduce their data collection unequivocally. Naive assumptions must be made when we lack information about study design, which leads to biases [13]. Therefore, provide all details needed to calculate the sampling effort following the standards accepted in your field. Provide rich information about capturing and recording devices (manufacturer, model, size, and material), hours sampled per field session, number of sampling sessions per time unit (e.g., day, week, month, season, or year), and number and size of sampling plots within sampling sites. If multiple sites have been sampled, detailed information should be reported for each one.

In addition to being clear about the sampling sites, informing the size of the team of data collectors, as well as how the team was divided across sites and treatments, can help the readers to understand sampling effort and potential biases. Understanding those key points can prevent serious biases as, for instance, the number of nodes and links in an ecological network tends to increase with sampling effort. This trend can induce a potential bias towards a core of highly-connected species, underestimating the presence of lowly-connected species, which results in flawed assessments of network topology [14,15]. Diagrams are particularly welcome to explain your sampling design. It is also essential to state the limitations of your methods, for instance, in terms of the taxonomic groups included or excluded.

Finally, report interaction attributes that allow telling apart positive and negative interaction events (e.g., the reproductive organs of a flower were touched or not by the visitor?) and explicitly state if your data set has only positive, negative or both types of interactions. This way, you can avoid misinterpretations and clarify potential limitations of your data set. Moreover, remember to explain what the interaction weights mean in your study (e.g., frequency of encounter or number of resources harvested?). Due to the difficulty in comparing interaction strength (measured as, for instance, the sum of dependencies of a species [16]) between different interaction types, we also recommend reporting raw data on species abundance and number of interaction events.

Rule 4: Tell in detail where and when you carried out your study

Unfortunately, critical information about your study area might go unnoticed, despite being essential to make your paper reproducible and usable. Therefore, describing the study area in detail is particularly important when analyzing spatial and temporal patterns of species interactions. Whenever possible, take a professional GPS to the field so that you can georeference your sampling sites, plots, transects, and trails. Afterwards, it is worth reporting all coordinates in decimal degrees using the proper datum, preferably the World Geodetic System 1984 (datum WGS 84). If you do not have a professional GPS, download a geotracking app to your smartphone, as modern models contain GPS receivers with good accuracy in open areas. You can also use indirect approaches, such as identifying site landmarks on Google Maps or Google Earth and extracting their coordinates. Explain the georeferencing in detail as each technique implicates different accuracy levels.

When reporting the seasons sampled, consider universal seasons, such as summer/winter and spring/fall, and local seasons that affect the interactions studied, such as rainy/dry. You can also report microclimatic information, such as temperature, moisture, and rainfall. Next, illustrate the environment, describe its elevation, types of vegetation, average tree height, water sources, and any other information that helps assess the context of the interactions. Finally, explain the land-use regimes of the studied landscape. If possible, include a map in the supplement.

Rule 5: Pay attention to taxonomy

Identifying organisms to the species is no easy task. In addition, many studies do not name species correctly, with mistakes varying from misspelling to outdated names. Those two problems combined create severe limitations for interpreting and reusing interaction data. Scientific names are not a mere formality, but they are key to unlocking a trove of biological knowledge acquired over generations. Access to this knowledge is crucial to correctly interpret the conditions and resources required by the species involved in the interactions. Furthermore, this knowledge is crucial to tell apart closely related types of interactions, such as seed dispersal and seed destruction, based on their potential outcome [17].

Therefore, whenever possible, provide additional information about unidentified species, such as photos, sketches, DNA/RNA sequences, and ultrasound calls, in the supplement so that other scientists can at least tell different morphospecies apart. Thus, when dealing with unidentified species, rather than grouping them all together per higher taxon, it is better to number them individually for each study site. Remember that morphospecies 1 found at site A is not necessarily the same as morphospecies 1 found at site B, especially if the sites are far away from one another.

Most importantly, name the species correctly, following international taxonomic standards [18,19], including correct spelling and up-to-date names recognized for the taxon. There are many publicly available taxonomic databases that can help you, some of them focused on a single taxonomic group such as Mammal Diversity Database (https://www.mammaldiversity.org/) and Plants of the World Online (http://powo.science.kew.org/), and others with broad taxonomic scope such as Catalogue of Life (https://www.catalogueoflife.org/) and Encyclopedia of Life (https://eol.org/). There are also some awesome tools for taxonomic harmonization [20].

Some very helpful packages for R [21] are also available. For instance, packages that allow users to download phylogenic and taxonomic data directly in R such as *rotl* [22], packages for parsing, plotting, and manipulating large taxonomic datasets such as *metacoder* [23], and even brand new packages for checking taxonomic spelling such as *taxspell* (https://github.com/sckott/taxspell). Reporting the taxonomy reference used is also a good practice that improves communication with your peers. Remember that taxonomy changes over time, so names are vital in connecting knowledge from studies separated by decades or centuries.

Finally, when publishing the data, do not only present species codes but also write full scientific names in a data frame used as a species reference in the supplement. Double check that no species code is left without its corresponding full name. And always invite as a coauthor a specialized taxonomist, who can check the names in your database and connect the dots in the literature. There is no substitute for expert knowledge and experience.

Rule 6: Provide additional information

You should always record and report additional information not considered in the original data collection plan. An excellent way to do that is field notes, which shed light on potential sources of bias, such as rain, cloudy days, fires, floods, hurricanes, earthquakes, volcano eruptions, or other outstanding events. This kind of information puts the data into context and helps other researchers interpret outliers and formulate new questions. OK, we know that the current publication ethos pushes us towards being extremely concise in our articles, but we can make unlimited use of online supplements to tell richer stories. For example, if a bat captured on a given night had an abnormal amount of ectoparasitic flies for its species, that is undoubtedly worth mentioning. Reporting additional information was common practice in the time of classical naturalists and has always helped people think outside the box. Just remember Alexander von Humboldt and his marvelous field notes [24].

Rule 7: Make your data set usable

Sharing is caring, so mind the data you share. You should prepare your primary data to be readily used in reanalysis, new analysis, and synthesis [25]. This care may open many new research avenues and boost interest in your work. Unfortunately, many studies report data as tables embedded in the text, usually in PDF format, which seriously hinders manual and automatized data extraction. In addition, typing data from PDFs also increases the chances for errors. Instead, raw data should be shared in data sheets in plain text formats, such as TXT or CSV, which any software running on any operating system can process.

Likewise, you should follow a tidy data format to organize your sheets as it creates human- and machine-readable, easily manipulated data. The principles of tidy data provide a standard way to organize data values within a dataset and are pretty simple [26]: (1) Each variable forms a column, (2) each observation forms a row, and (3) each type of observational unit forms a table. For instance, if you captured bat species A, carrying seeds of plant species X at 19:00 and plant species Y at 20:00, in the mist nets α and β, respectively, the columns of your data table could be named “bat species”, “plant species”, “hour”, and “mist net ID”. Then, the first row would read “A; X; 19; α” and the second, “A; Y; 20; β”. If some information is missing, you can fill a cell with “not available” (i.e., NA).

Once you have a tidy data set, you can use tidy tools for data analysis, in which the output of one tool can be used as the input of another. This allows you to combine multiple tools to solve a complex problem in a reliable and reproducible way. In addition, remember to create a metadata file that explains the content of each table and column, as well as the codes used to summarize information. Many good guides help you accomplish this task [27,28]. Yes, we know that this is much information to include in a manuscript. Therefore, move data and metadata to the supplement or, even better, to open databases and repositories (adequately cited in the manuscript using stable URLs). See more tips for data archiving in the next rules.

Rule 8: Report your analysis workflow thoroughly

Have you ever had trouble understanding and reproducing the analysis workflow of a particular study or even of a study you carried out years ago? Nowadays, large amounts of data in ecology are analyzed by coding, using languages such as R, Python, MATLAB, C++, or Julia. Sadly, code is not shared in most studies [29]. Therefore, the best solution for this problem is to provide a script file with the code used in your analysis, complemented by the processed data that you directly used in the analysis. For instance, instead of only reporting network metrics (e.g., nestedness, connectance, and modularity), report also the language (e.g., R or Python), the package (e.g., *bipartite* or *igraph*), and the function (e.g., *computeModules or cluster_louvain*) you used. You can even go beyond sharing code by writing tutorials in Markdown, LaTeX, and other languages, which guide the reader when reproducing your analysis. If you did not analyze your data by coding, provide a step-by-step textual description (i.e., pseudocode) that describes how you got your results. This enables full reproducibility.

After preparing the supplement carefully, we strongly recommend that you deposit your code and data in an online public repository. This is already a common practice with genetic data put on GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and with animal tracking data that go to MoveBank (https://www.movebank.org/). In our field, you can deposit species interaction data on GloBI (https://www.globalbioticinteractions.org/data), Mangal (https://mangal.io/), Lifewebs (http://www.lifewebs.net/), or Web of Life (https://www.web-of-life.es), among other repositories. If you work with vertebrate-virus associations, you can deposit your data on the VIRION database (https://www.viralemergence.org/data). There are also more general open repositories such as Zenodo (https://zenodo.org) and Dryad (https://datadryad.org), which allow creating stable URLs and citable DOIs for GitHub repositories, help you choose licenses, and provide long-term archiving [25].

Archiving is a practice that ecology and evolutionary biology journals and funding agencies have been encouraging or requiring [30]. By doing this, you contribute to making your data accessible and reusable in a transparent way. Moreover, you can broaden your co-authorship horizons. For instance, the Lifewebs repository (http://www.lifewebs.net/contribute.html) offers to all data contributors authorship in resulting publications in which their datasets were used. In other words, by archiving your data in an online public repository, you can increase not only the citation of your primary studies but also the number of your publications.

Do not worry about making your data publicly available, as when archiving code and data you can inhibit unwanted manners of use by choosing an adequate license. There are six license options on Creative Commons (a.k.a. CC licenses; https://creativecommons.org/), ranging from most to least permissive. The most permissive is CC BY, which allows users to distribute, remix, adapt, and build upon the material in any medium or format, so long as attribution is given to the creator. This license also allows commercial use. The least permissive is CC BY-NC-ND, which allows users to copy and distribute the material in any medium or format in unadapted form only, for noncommercial purposes only, and only so long as attribution is given to the creator. There is also an option called CC0 (a.k.a. CC Zero) that is a public domain dedication tool that allows users to distribute, remix, adapt, and build upon the material in any medium or format, with no conditions. Depending on the repository used, you can choose any of the standard licenses included in the tools.

In addition to repositories and licenses, there are some guidelines available on blogs and other publications that you can follow to improve your script and make it more useful (e.g., [29,31]). This way, readers and users will find all the answers they need, making your analysis workflow easily reproducible. Besides reproducibility, providing your code as a script has other benefits, such as making your results checkable and reliable, which may improve your study's impact. This practice may also be pedagogical for young scientists, which contributes to the open science culture.

Rule 9: Report your results thoroughly

Do not spare any details when reporting your model results after all this work to make your study reproducible and reusable. Many ecological studies report only P-values or cherry-pick the results that support the working hypothesis [32]. Missing results, negative or positive, hamper data extraction and significantly affect reliability and usability. For instance, uncertainty concerning a subset of results can bias a meta-analysis, as excluding studies with missing information worsens the publication bias [33]. Biases of different kinds can lead to worrisome practical consequences, such as not detecting the harmful effect of a pesticide on crop pollination.

In addition, fraud is more easily prevented by transparency. When reporting results from tests or models, go beyond significance values by including relevant descriptive statistics, scores of the calculated statistics, sample sizes, degrees of freedom, effect sizes, and statistical powers (e.g., [34]). If a result does not belong to the core of the story being told, but is important to help understand its context, move it to the supplement. When reporting results in figures, use transparency to indicate data overlap and incorporate measures of variability (such as variance, standard deviation, or standard error) in the figure or its caption [29].

Rule 10: Choose your keywords wisely

This last rule might sound trivial but pay close attention to it. All your hard work is lost if people do not find your paper, so choose your keywords wisely. Keywords not only help people interested in the same scientific problem find your paper, but they are also crucial for people who carry out systematic reviews, meta-analyses, and all kinds of synthesis. Unfortunately, several studies on species interactions are overlooked in advanced searches due to poor keyword choice.

Therefore, first, we strongly recommend that you include at least one of the following general keywords, even if they are already contained in the title: "ecological associations", "interspecific interactions", or "species interactions." Second, add keywords related to the expected outcome and intimacy of the interactions, such as “amensalism", "antagonism", "commensalism", "mutualism", or "symbiosis". Third, include some keywords specific to the studied interaction type, such as "blood parasitism", "cleaning symbiosis", "ectoparasitism", "endoparasitism", "extrafloral nectaries", "folivory", "florivory", "frugivory", "infection", "nectarivory", "nectar robbery", "oil collection", "pollination", "pollinivory", "seed dispersal", "trophobiosis", "zoonosis", or whatever fits your study best.

By choosing your keywords wisely, your work will gain visibility and will more likely be found, read, cited, and used in synthesis.

FINAL REMARKS

The reproducibility crisis in global science is also worrisome in the small world of species interactions. Transparency and clarity are crucial to solving this crisis. Furthermore, scientists who collect primary interaction data in the field or lab can significantly benefit from improving the reproducibility and usability of their studies. Like a torch, whose brightness is not diminished by igniting other torches, the reuse of primary data and the synthesis of primary results broaden the scope of your primary studies by multiplying their potential uses, boosting their citations, and creating new opportunities for collaboration and co-authorship. To achieve this promising scenario of mutual benefits, improving communication between data producers and users is of paramount importance. The ten simple rules suggested here can help us reach this goal.

ACKNOLEDGMENTS

We are immensely grateful to all colleagues who collect primary interaction data in the field or lab. We have also spent many days and nights with our feet wet, studying bats, rodents, marsupials, birds, bees, ants, wasps, and many other organisms in their habitats or in captivity experiments. Therefore, we know that synthesis would be impossible without your naturalistic effort. Ecology needs people working both on the empirical and theoretical sides of the Force and communicating effectively with one another.

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CONCLUSÃO GERAL

Através dessa dissertação, contribuímos para um melhor entendimento do problema da aplicação de agrotóxicos e seus efeitos sobre a comunidade de abelhas e o serviço de polinização de lavouras, no contexto das práticas agrícolas intensivas. Por meio da síntese de dados brutos, dados processados e resultados, estimamos os efeitos dos agrotóxicos na sobrevivência de abelhas e na continuidade da polinização das lavouras. Além disso, identificamos lacunas de conhecimento e apontamos vieses, abrindo portas para novas pesquisas sobre esse problema.

Assim, nosso trabalho reforça a importância do compartilhamento de informações para o avanço do conhecimento científico e, ao mesmo tempo, exemplifica como a falta de transparência, detalhamento e disponibilidade de informações pode limitar a compreensão e a resolução de problemas ecológicos, econômicos e sociais a partir de trabalhos de síntese. Isso porque a falta de detalhamento metodológico e o relato incompleto de resultados em muitos estudos primários impossibilitaram o reuso de dados que poderiam enriquecer a nossa meta-análise. Essa situação limitou não só a nossa capacidade de compreender o problema da aplicação dos agrotóxicos mais a fundo, mas também a nossa capacidade de sugerir ações de conservação mais específicas para a preservação das abelhas e a polinização das lavouras, fundamental para a nossa segurança alimentar.

Através dessa experiência observamos na prática um exemplo da importância do compartilhamento de dados primários e o impacto que eles podem ter em trabalhos de síntese envolvendo interações entre espécies. Desta forma, podemos concluir que uma comunicação eficiente entre produtores de dados primários e pesquisadores que dependem desses dados para a realização de trabalhos de síntese é a chave para abrir novas portas do conhecimento e beneficiar não só a comunidade científica, mas também a sociedade como um todo.

ANEXOS

Suplemento do capítulo 1

Appendix S1. Processed data and scripts.

Available on: *https://github.com/CKita/Bees*

Appendix S2. Visual analysis of publication bias.

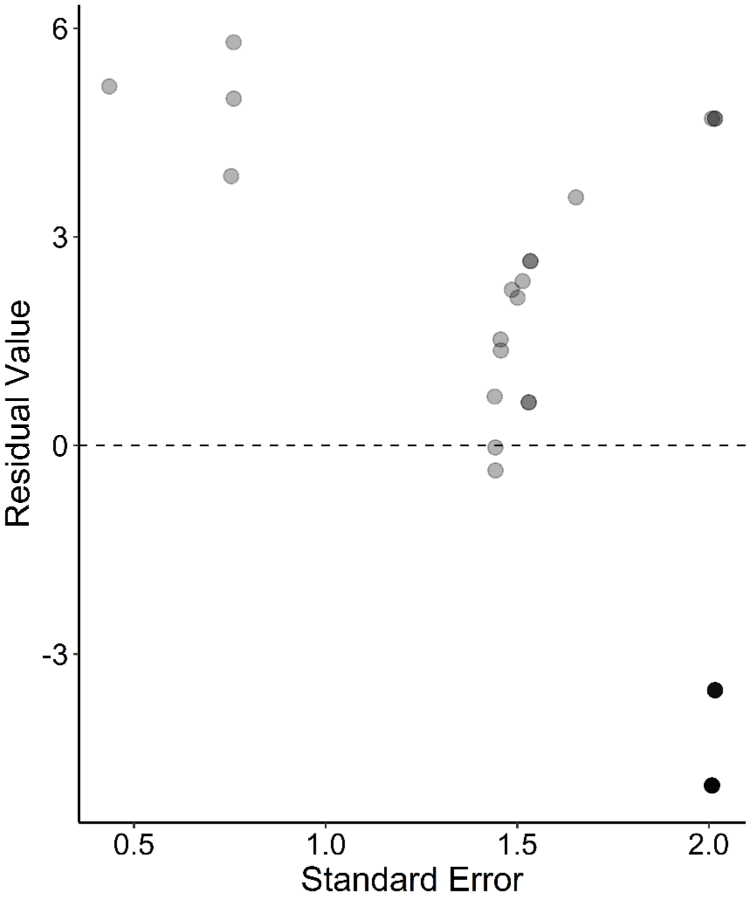

Fig.S1. Visual analysis of publication bias of lethal effects indicating bias. Plot of standard error of the variances plotted against the corresponding standard residuals of original effect sizes.

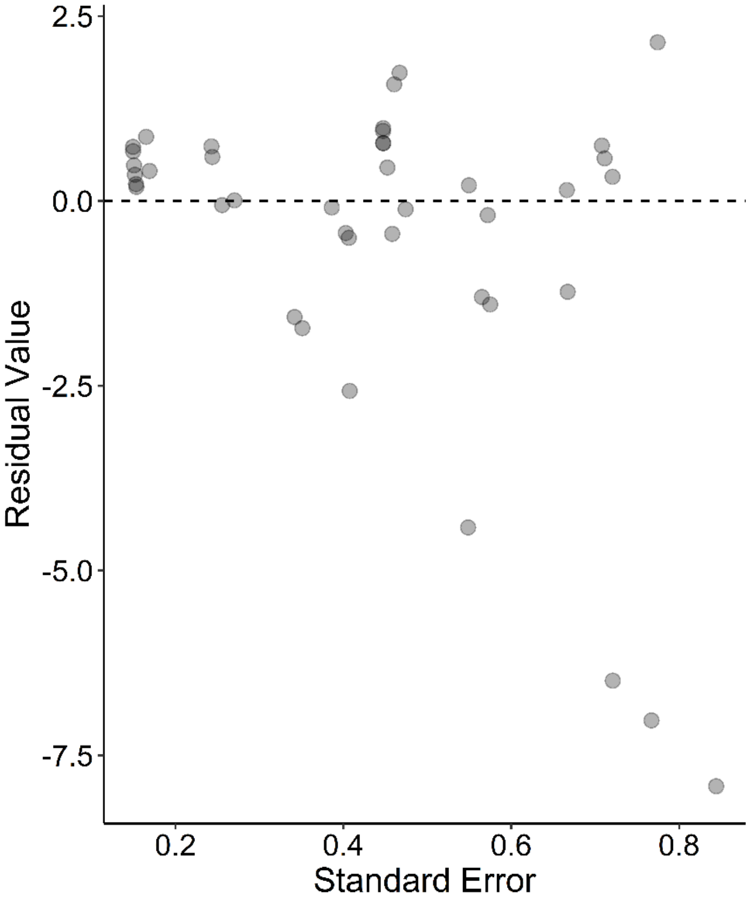

Fig.S2. Visual analysis of publication bias of sublethal effects indicating bias. Plot of standard error of the variances plotted against the corresponding standard residuals of original effect sizes.

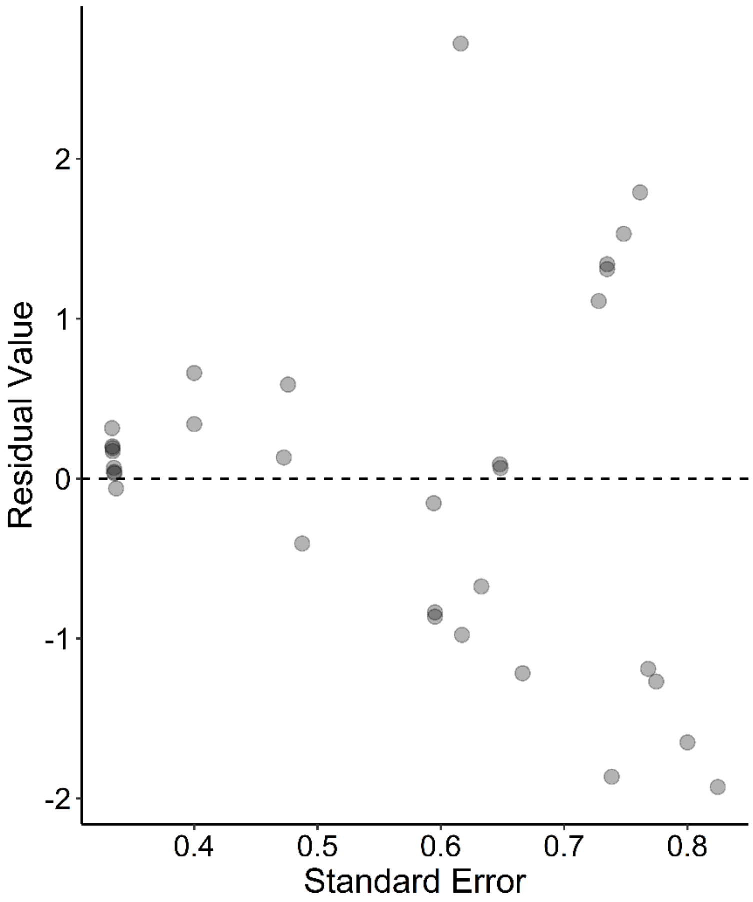

Fig.S3. Visual analysis of publication bias of the consequences of lethal and sublethal effects for the bee communities that could impair their pollination service indicating no bias. Plot of standard error of the variances plotted against the corresponding standard residuals of original effect sizes.

Appendix S3. Study sites.

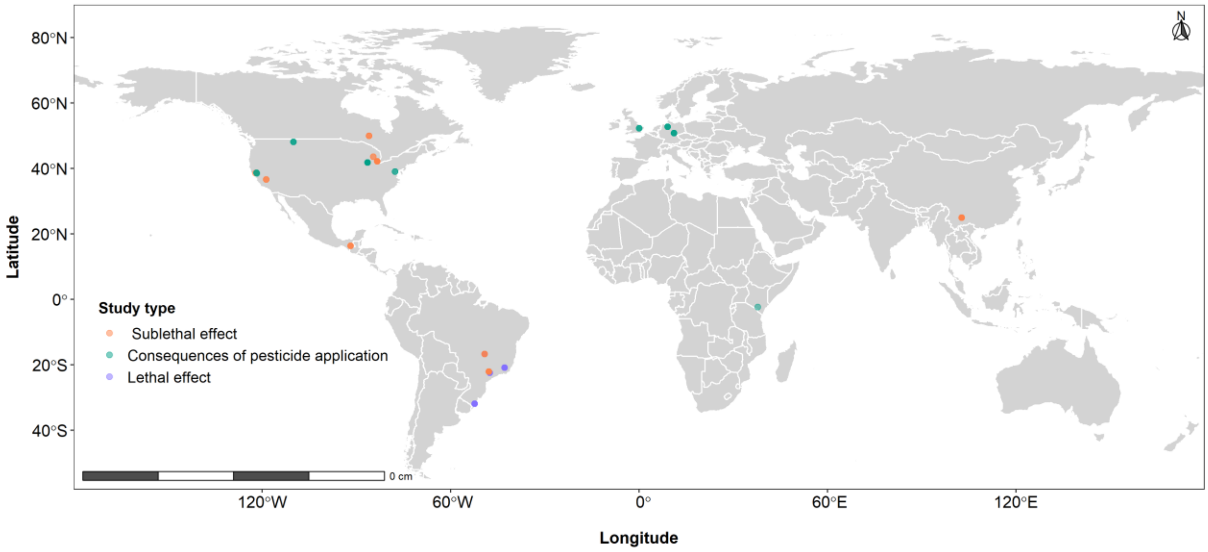

Fig. S4. Geographic distribution of effect sizes by study type.

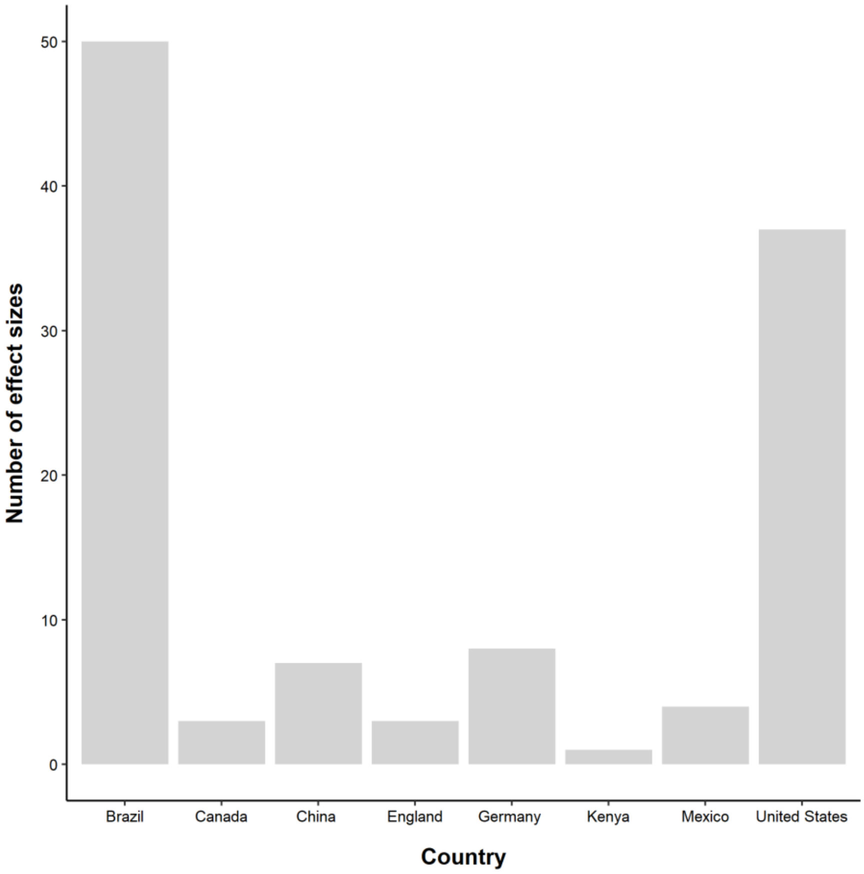

Fig. S5. Number of effect sizes per country including all effect sizes used for this meta-analysis.

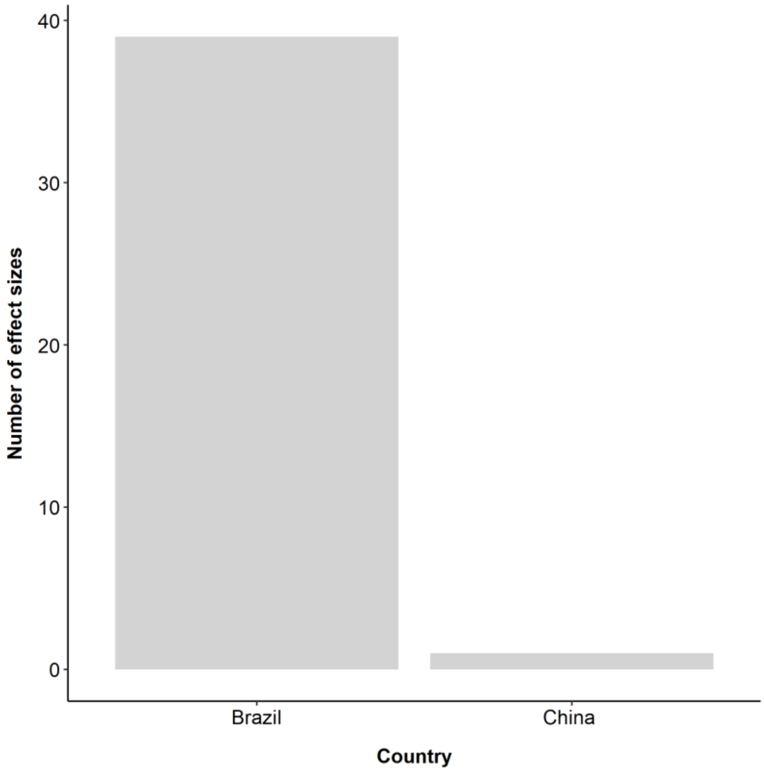

Fig. S6. Number of effect sizes per country including all effect sizes of lethal effects.

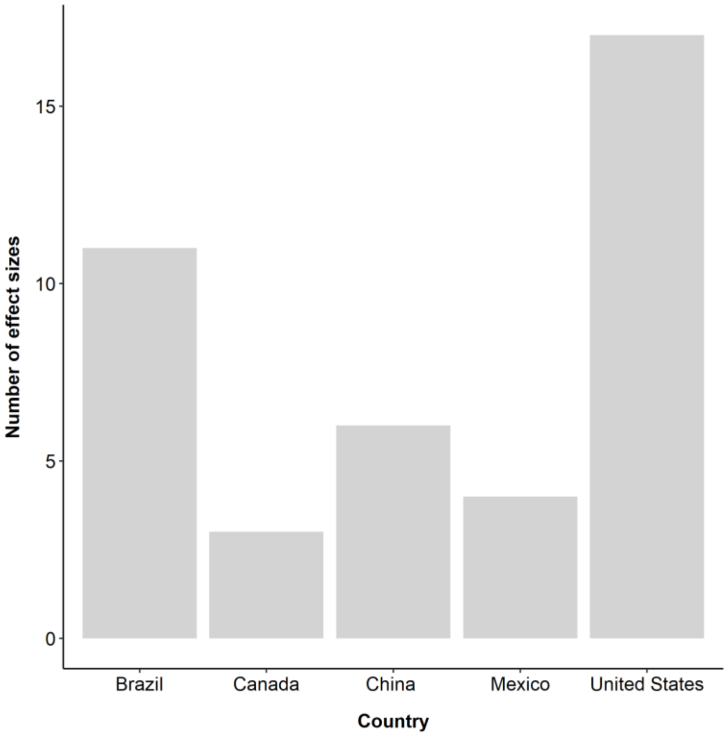

Fig. S7. Number of effect sizes per country including all effect sizes of sublethal effects.

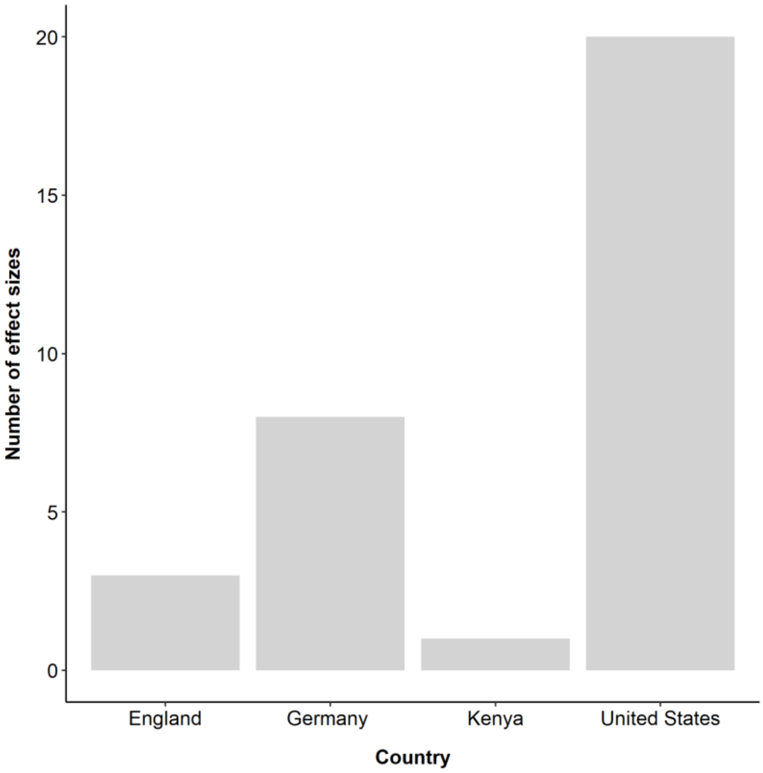

Fig. S8. Number of effect sizes per country including all effect sizes of the consequences of lethal and sublethal effects.

Appendix S4. Sensibility tests.

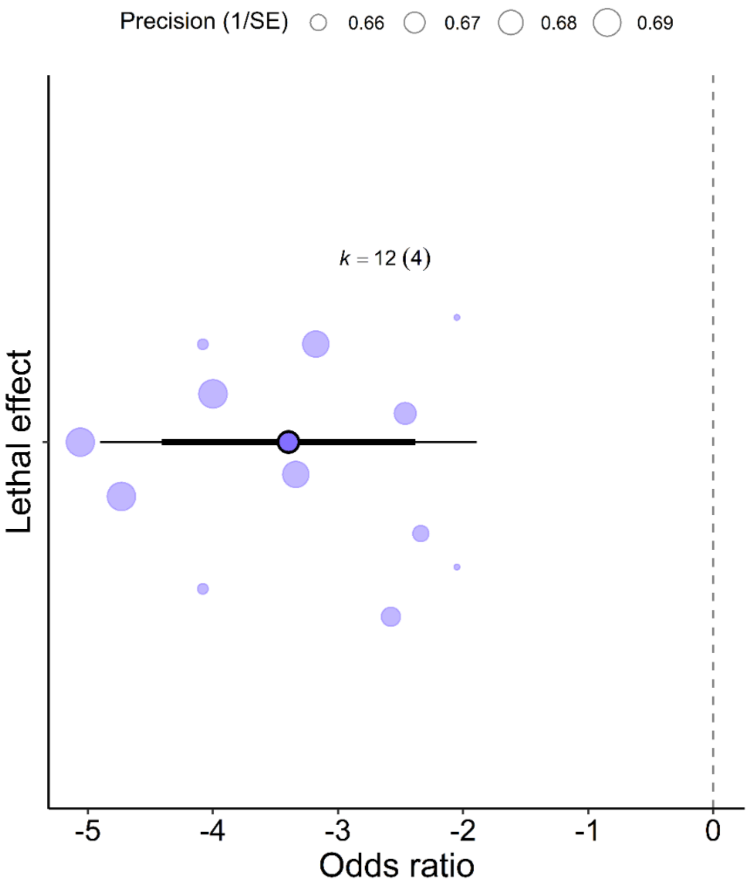

Fig. S9. Mean lethal effect size of pesticide application on bees and the effect sizes calculated using the data extracted from primary studies without outliers. The purple dots represent the effect sizes. The central dot in the thick black line represents the mean effect size for bee survival. The other dots represent weighted individual effect sizes calculated from studies that investigated the effect of pesticide exposure on bee survival. Effect sizes are weighted by their precision (1/standard error, SE). The dashed line represents zero effect. The thick black line represents 95% confidence intervals of the mean effect size (CI), and an effect is considered significant when CI does not overlap 0. The thin black line represents the prediction intervals (PI). Number of effect sizes used to calculate the mean effect size: *k* = 12. Number of studies used to calculate the mean effect size = 4.

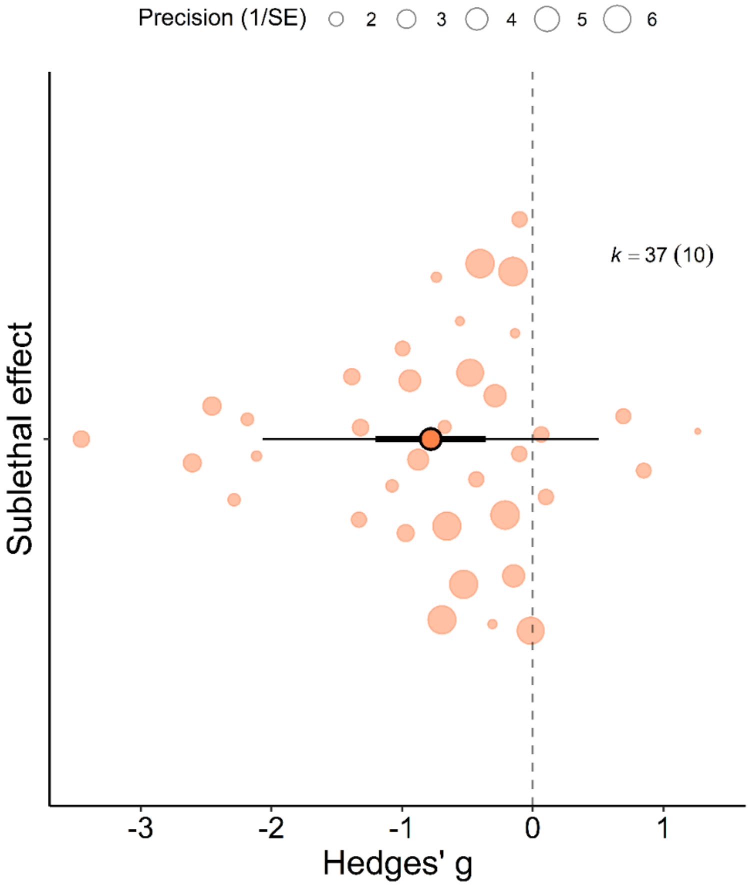

Fig. S10. Mean sublethal effect size of pesticide application on bees and the effect sizes calculated using the data extracted from primary studies without outliers. The orange dots represent the effect sizes. The central dot in the thick black line represents the mean effect size for sublethal effects. The other dots represent weighted individual effect sizes calculated from studies that investigated sublethal effects. Effect sizes are weighted by their precision (1/standard error, SE). The dashed line represents zero effect. The thick black line represents 95% confidence intervals (CI), and an effect is considered significant when CI does not overlap 0. The thin black line represents the prediction intervals (PI). Number of effect sizes used to calculate the mean effect size: *k* = 37. Number of studies used to calculate the mean effect size = 10.