## ABSTRACT

RICHARD, JENEL. Revisiting the Impacts of Bt-corn on Non-Target Lepidoptera: A Scoping Review (Under the direction of Dr. Jennifer Richmond-Bryant).

This scoping review sought to verify the conclusions of Lang and Otto's (2010) publication assessing the risk of Bt-corn on non-target lepidopteran species. Bt-corn has been an innovation in the agricultural sector in regard to increasing yield while minimizing pesticide application. If the EPA deemed Bt-corn harmful to unintended protected species, it would be subjected to planting restrictions, such as buffer zones, that safeguard habitats. In addition to replicating the literature review, the time period was increased to include contemporary research through December 2023 and search terms were added to include emerging technology. The sixteen laboratory studies used in Lang and Otto's paper were successfully identified during the search process. Three additional published laboratory studies were identified by the search. One identified article was published prior to 2010, and the other two were published between 2010 and 2023. No additional field studies were identified during the expanded scoping review. The results from this scoping review support conclusions of the original paper that pollen from Bt-corn does not pose a significant risk to non-target lepidopteran species.

Revisiting the Impacts of Bt-corn on Non-Target Lepidoptera: A Scoping Review

by
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# DEDICATION

I dedicate this accomplishment to my family. Your support, encouragement, and love have been my constant source of strength throughout my graduate journey. To Tommy, your patience, understanding, and encouragement have been invaluable during this experience. To my children, your smiles and joy have been my motivation. Thank you all for standing by me, for your endless love, and for being my rock. This achievement is as much yours as it is mine.

## BIOGRAPHY

Jenel Richard currently resides in Gibsonville, North Carolina with her family. She earned a Bachelor of Science in Biology with a concentration in Environmental Biology from the University of North Carolina at Greensboro in 2015 and continued her studies with a Graduate Certificate in the Regulatory Sciences in Agriculture from North Carolina State University in 2023. During her undergraduate studies, she conducted literature review on the hormonal regulation disruption caused by Fenoxycarb in freshwater holometabolous insects and invertebrates, assisted research projects related to the effects of triclosan on stream microbial communities, and studied the effects of urban runoff on stream invertebrates.

Ms. Richard is a Regulatory Specialist with Syngenta Seeds, LLC, Research Triangle Park, N.C. She has four years' experience in the agrochemical and agricultural biotechnology industry specializing in the registration and maintenance of pesticide products.

The Environmental Assessment Master's program at North Carolina State University has not only given her a deeper understanding of how to evaluate the risks that technological advancements pose to our environment but also provided a comprehensive insight into the extensive studies and reviews required before products receive registration approval from government agencies. Through detailed coursework and hands-on projects, she has learned about the rigorous scientific research, environmental impact assessments, and regulatory compliance checks that are essential in ensuring new technologies are safe and sustainable before they can be approved for public use.

# ACKNOWLEDGMENTS

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To my professors, your dedication, expertise, and passion for teaching have been truly inspiring. The knowledge and skills I have gained under your guidance have been invaluable, and your commitment to my growth has profoundly impacted both my academic and personal development.

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## TABLE OF CONTENTS

## LIST OF TABLES

## Introduction

*Bacillus thuringiensis* (Bt) is a soil bacterium that produces proteins toxic to certain insect pests (Schnepf et al., 1998; Bravo et al., 2011). These proteins, known as Bt toxins are pesticidal crystal (Cry) proteins, are used in agriculture because they specifically target crop insect pests like caterpillars and beetles without harming humans, animals, or beneficial insects (Tabashnik, 2015).

From an agricultural standpoint, Bt proteins are significant because they have been incorporated into crops such as corn or cotton through genetic engineering. The Bt-crops then produce the toxin themselves, providing a built-in protection against pests, reducing the need for chemical pesticides, and contributing to higher yields (USDA, 2014). In 1996, first Bt-corn was commercially introduced to the U.S. It was developed to address the destruction caused by pests like the *Ostrinia nubilalis* European corn borer (ECB) and *Helicoverpa zea* corn earworm (CEW) (USDA, 2014). However, concerns over the development of resistance in pest populations and potential impacts on non-target organisms (NTOs) were raised within the agricultural community (Tabashnik et al., 2013).

Over time, some insect pests developed resistance to Cry proteins (Tabashnik et al., 2013). This resistance was a major concern as it reduced the effectiveness of Bt-crops. To delay the evolution of resistant pest populations, vegetative insecticidal protein (Vip) was presented as a solution. Additional solutions included the use of refuge (non-Bt-crop) in fields and "stacking" multiple Bt proteins with different modes of action in Bt-crops (Tabashnik et al., 2015). Vip proteins differ from the "stacked" or pyramidal Cry crops by targeting distinctive receptors in the insects' guts (Gupta et al. 2021).

Losey et al. (1999), ignited a significant ecological concern surrounding Bt-corn cultivation by suggesting potential harm to NTOs, specifically monarch butterflies. Based on their field experiments and laboratory analysis, that butterfly larvae ate less, grew more slowly, and experienced higher rates of mortality when reared on leaves dusted with transgenic corn pollen. Losey et al. (1999) considered their findings along with the fact that corn pollen can be dispersed up to 60 feet by wind and concluded that Bt-corn pollen was harmful to monarch butterflies.

Experiments motivated by the Losey et al. (1999), paper provided insight into the ecological impact of Bt-corn on NTOs. Studies conducted across multiple disciplines led to conclusions that contradicted the findings of Losey et al. regarding the harm to NTOs in both field and laboratory settings. These experiments utilized multiple means of assessment to determine the effects of Bt-corn pollen on NTOs.

Lang and Otto (2010) published a scoping review and meta-analysis that synthesized the multitude of experiments conducted to assess the ecological impact of Bt-corn on NTOs. By gathering and analyzing the findings from these published studies, the authors sought to provide a comprehensive understanding of the extent of the effects, if any, of Bt-corn on non-target lepidopteran species. They concluded that Bt-corn did not have a negative ecological impact on NTOs. However, their review did not assess the Vip protein as it was not commercially available until after their publication.

In this scoping review, I intend to replicate and expand on the literature review conducted by Lang and Otto in 2010 on the effects of transgenic pollen on non-target lepidopteran species. In replicating their literature review, I seek to validate their results and use the results for a baseline comparison to my own scoping review. Additionally, this exercise will allow me to identify limitations in the process. My scoping review will also expand on the literature used in Lang and Otto's (2010) publication by including contemporary articles, additional search terms derived from emerging concepts, and utilizing a secondary database, AGRICOLA, to provide comprehensive coverage.

## Methods and Materials

### Replicating Lang and Otto – Scoping Process

The goal of this process it to capture the state of the literature at the time of Lang and Otto's (2010) paper. The author's review sourced their references primarily through Web of Science. In their publication, the authors included data from an internal database that was inaccessible to me, which likely affected their meta-analysis conclusion and was not applicable to the scoping review component. Search criteria were set to limit language, time period, and peer-reviewed status. Despite the global nature of science literature, the publications were restricted to English only. For the purpose of replicating Lang and Otto's results, the search parameter was restricted to papers published before January 1$^{st}$, 2010. Of the peer-reviewed publications, only those with original data from laboratory or field trials were considered to ensure the quality and reliability of the analyzed studies.

Search terms were designed to capture articles that investigated the direct effects (mortality, feeding behavior, weight, etc.) of exposure to Bt-corn pollen, anthers, and its insecticidal proteins on Lepidopteran species. These terms included, but were not limited to "Lepidopt*," "Butterfly*," "moth*," "non-target*," "Bt," "Bacillus thuringiensis," and "Cry." A complete list of search terms can be found in APPENDIX A.

Studies focused on crops such as rice, cotton, and oilseed rape were excluded from the analysis because they do not rely on wind for pollination, which significantly reduces the risk of pollen drift affecting adjacent ecosystems (Mendelsohn et al., 2003; Yao et al., 2008). While it is possible for larvae to encounter Bt toxins indirectly through the consumption of corn leaves, roots, or decaying organic matter, the central theme of this exercise revolves around the exposure pathways associated with the dispersal of pollen. This review prioritized studies that detailed the impacts of artificial diets infused with the specific insecticidal proteins inherent to Bt-corn on non-target lepidopteran larvae. Therefore, research focused on lepidopteran intentionally targeted for pest control or in which larvae were exclusively fed green corn plant tissue were omitted from consideration. Publications that explored ovipositor behavior or embarked on a broader ecological risk assessment for lepidopterans were also deliberately excluded from the evaluation.

**Replicating Lang and Otto – Data Compilation**

Observations were defined as the quantification of a specific variable during a unique test trial. Observations required actual data or relevant statistics and were categorized into several groups, including mortality, food consumption, body mass, time to pupation, behavioral patterns, pupal mass, length of pupal period, adult sex ratio, body size and mass, and egg and larvae abundance in field studies (Lang and Otto, 2010).

For the observations, such as consumption and body mass, only the value for the final larval stage was considered (Lang and Otto, 2010). Results from studies that segregated data points by sex were combined under the adult stage classification, while measurements for different developmental stages were included. Replication efforts required consideration of 'time to pupation' and 'time to emergence' because the original authors sought to consider their potential correlation. Body mass data did not consider the measurements of lipid content.

**Expanding on the Literature**

To expand upon Lang and Otto (2010), additional search terms were employed to capture articles relevant to emerging technology, such as Vip protein implementation in corn crops (Appendix A). In an effort to capture the most up-to-date research and to provide a more exhaustive review of the literature, the same methodology (search terms, criterion, etc.) was replicated for the period from January 1, 2010, through September 30, 2023.

As an additional measure, the same search terms, limiting parameters, and time periods were used to generate results from the AGRICOLA database. A methodical process of cross-referencing was undertaken to identify and remove any duplicates among the discovered articles (Appendix B).

## Results

Twenty relevant publications were captured during the replication efforts. These twenty articles were also captured during Lang and Otto's (2010) scoping review. Of the twenty resulting articles, three assessed both laboratory and field trials. Aligned with the principles set by Lang and Otto, each data set or trial was treated as an independent entity, thereby bringing the comprehensive tally of the results to twenty-three. Sixteen identified publications were laboratory studies, while the remaining seven explored field-based experiments.

Expanding the time period to include studies published between January 01, 2010, and September 30, 2023, resulted in the identification of two additional laboratory studies (Müller et al., 2012; Schuppener et al., 2012). Both tested for adverse effects of Bt-corn on Small Tortoiseshell larvae, with Muller et al. (2012) exploring the effects of Cry1Ab and Schnuppener et al. (2012) exploring the effects of a pyramidal Cry crop.

The implementation of additional search terms led to the discovery of one new source of laboratory-derived data (Lee et al., 2003). This newly identified article, which was published within the timeframe utilized by Lang and Otto (2010), specifically examined the effects of Vip proteins on monarch butterflies.

The seven field-based experiments utilized in Lang and Otto's (2010) publication were identified. No new data were discovered during this scoping review, but all 2010 data were successfully replicated.

## Discussion

### Discrepancy Between Databases

All relevant literature found in AGRICOLA was also found in Web of Science, but not all studies utilized in the scoping review were captured in AGRICOLA (Hanley et al., 2003; Hellmich et al., 2001; Jesse and Obrycki, 2000; Jesse and Obrycki, 2002; Lang and Vojtech, 2006; Losey et al., 1999; Müller et al., 2012; Schuppener et al., 2012; Shirai and Takahashi, 2005; Stanley-Horn et al., 2001; Wraight et al., 2000; and Zangerl et al., 2001). The discrepancy observed between the literature captured in Web of Science and AGRICOLA databases suggests that, while AGRICOLA contains a significant portion of the relevant literature, it may not encompass the entire literature repository required for replicating the scoping review. This discrepancy could arise due to differences in the scope, coverage, or indexing methodologies employed by these databases. Interestingly, some uncaptured literature was located within the AGRICOLA database (Hanley et al., 2003; Hellmich et al., 2001; Jesse and Obrycki, 2000; Jesse and Obrycki, 2002; Stanley-Horn et al., 2001; Wraight et al., 2000; Zangerl et al., 2001), indicating that the search algorithms or query sensitivity may function differently between these databases. This highlights the necessity of consulting multiple databases to obtain a comprehensive collection of data for a thorough analysis.

### Data Analysis

Due to the variations in the volume of experiments and the observed variables across the identified publications, the findings were aggregated and synthesized based on the cumulative count of publications, experiments, and observations. The location of the identified studies remained relatively consistent with that reported by Lang and Otto (2010), with one additional study originating from the U.S. and two from Germany. However, the newly identified publications did not substantially alter the dataset, as nearly half the data were derived from three papers (Jesse and Obrycki, 2000; Hellmich et al., 2001; Anderson et al., 2005).

In this review, studies were conducted on the larvae of 12 lepidopteran species, spanning nine families, as outlined in Table 1. Notably, 25% of the species observed were categorized as secondary pests, indicating their inclination to inflict damage on crops other than corn, or on bee hives. The Papilionidae and Nymphalidea families were represented by two species while the remaining families were represented by a single species. Unsurprisingly, the Monarch butterfly was the predominate subject of interest, featured in nearly two-thirds of the experiments.

In addition to the corn events and Cry proteins evaluated in the 2010 meta-analysis, the newly identified articles explored the effects of a Cry1A.105/Cry2Ab2 stack (Schuppener et al., 2012) and Vip3A (Lee et al., 2003), a vegetative insecticidal protein. The majority of experiments tested the effects of Cry1Ab (78.7%), including a new study (Muller et al., 2012). Test materials included pollen (65.2%), anthers (13.2%), purified toxins (13.2%), and pollen-anther combination (9.4%). The materials tested were either applied to leaf disks, whole leaves, whole plants, or added to an artificial diet and introduced to the test species as a food source. The most common stage for observations was the first Instar of development (61.1%) followed by third instars (19.5%), second instars (16.8%), fourth instars (1.6%) and unknown developmental stage (1.1%).

**Table 1.** Summary of laboratory-based studies. Listed are the numbers of publications, experiments, and observations per country, species, Bt event/toxin, material tested, larval food, and instars tested. Percentages in parentheses.

| | | Number of Publications | Number of Experiments | Number of Observations |
|---|---|---|---|---|
| Country | | | | |
| | USA | 12 (63.2) | 38 (76.0) | 122 (75.8) |
| | Germany | 5 (26.3) | 10 (20.0) | 33 (20.5) |
| | China | 1 (5.3) | 1 (2.0) | 5 (3.1) |
| | Japan | 1 (5.3) | 1 (2.0) | 1 (0.6) |
| Species | | | | |
| | *Danaus plexippus* (Danaidae) | 8 (38.1) | 33 (66.0) | 115 (71.4) |
| | *Papilio polyxenes* (Papilionidae) | 2 (9.5) | 2 (4.0) | 3 (1.9) |
| | *Papilio machaon* (Papilionidae) | 1 (4.8) | 1 (2.0) | 7 (4.3) |
| | *Piersis rapae* (Pieridae)[1] | 1 (4.8) | 1 (2.0) | 3 (1.9) |
| | *Piersis brassicae* (Pieridae)[1] | 1 (4.8) | 1 (2.0) | 2 (1.2) |
| | *Pseudozizeeria maha* (Lycaenidae) | 1 (4.8) | 1 (2.0) | 1 (0.6) |
| | *Inachis io* (Nymphalidea) | 1 (4.8) | 2 (4.0) | 3 (1.9) |
| | *Euchaetes egle* (Noctuidae) | 1 (4.8) | 1 (2.0) | 2 (1.2) |
| | *Antheraea pernyi* (Saturniidae) | 1 (4.8) | 1 (2.0) | 5 (3.1) |
| | *Plutella xylostella* (Plutellidae)[1] | 1 (4.8) | 1 (2.0) | 2 (1.2) |
| | *Galleria mellonella* (Pyralidae)[1] | 1 (4.8) | 2 (4.0) | 2 (1.2) |
| | *Algais urticae* (Nymphalidea) | 2 (9.5) | 4 (8.0) | 16 (9.9) |
| Event/toxin | | | | |
| | MON810 event (Cry1Ab) | 6 (18.2) | 9 (14.8) | 58 (36.0) |
| | Bt176 event (Cry1Ab) | 9 (27.3) | 17 (27.9) | 34 (21.1) |
| | Bt11 event (Cry1Ab) | 6 (18.2) | 19 (31.1) | 35 (21.7) |
| | MON863 event (Cry3Bb1) | 1 (3.0) | 1 (1.6) | 4 (2.5) |
| | MON89034 x MON88017 (Cry1A.105/Cry2Ab2) | 1 (3.0) | 1 (1.6) | 4 (2.5) |
| | Cry1Ac event | 1 (3.0) | 1 (1.6) | 1 (0.6) |
| | Cry1F | 2 (6.1) | 2 (3.3) | 2 (1.2) |
| | Cry9C | 1 (3.0) | 2 (3.3) | 2 (1.2) |
| | Cry1Ab/Cry2Ab2 event | 1 (3.0) | 1 (1.6) | 4 (2.5) |
| | Cry1AB toxin | 1 (3.0) | 3 (4.9) | 6 (3.7) |
| | Cry1Ac toxin | 1 (3.0) | 1 (1.6) | 2 (1.2) |
| | Cry1F toxin | 1 (3.0) | 1 (1.6) | 2 (1.2) |
| | Cry9C toxin | 1 (3.0) | 1 (1.6) | 2 (1.2) |
| | Vip3A | 1 (3.0) | 1 (1.6) | 1 (0.6) |

| | | | | |
|---|---|---|---|---|
| Material tested | | | | |
| | Purified toxin | 2 (8.7) | 7 (12.5) | 13 (8.1) |
| | Pollen | 16 (69.6) | 37 (66.1) | 100 (62.1) |
| | Anthers | 3 (13.0) | 7 (12.5) | 34 (21.1) |
| | Pollen and anthers | 2 (8.7) | 5 (8.9) | 14 (8.7) |
| Larval food | | | | |
| | Artificial diet | 3 (13.6) | 9 (18.0) | 15 (9.3) |
| | Leaf discs | 13 (59.1) | 28 (56.0) | 97 (60.2) |
| | Whole leaves | 5 (22.7) | 11 (22.0) | 33 (20.5) |
| | Whole plant | 1 (4.5) | 2 (4.0) | 16 (9.9) |
| Instar | | | | |
| | L1 | 13 (43.3) | 32 (53.3) | 116 (61.1) |
| | L2 | 7 (23.3) | 12 (20.0) | 32 (16.8) |
| | L3 | 6 (20.0) | 12 (20.0) | 37 (19.5) |
| | L4 | 3 (10.0) | 2 (3.3) | 3 (1.6) |
| | Unknown | 1 (3.3) | 2 (3.3) | 2 (1.1) |

[1] Denotes secondary pests

The observations were classified into ten categories (Table 2). Significant effects (P-value < 0.05) were considered adverse. Observations for survival, food consumption, body mass, and developmental time were the most frequently recorded. Survival, food consumption and body mass had most observations recorded as having an adverse effect on the larvae. Observations for larval developmental time had 45.8% adverse effects recorded.

**Table 2.** Summary of laboratory and field-based effects on lepidopteran larvae. Listed are the numbers of observations for variables recorded in the experiments analyzed in the review; -, not studied. Percentages in parentheses.

| Parameter | Laboratory | | Field | |
|---|---|---|---|---|
| | Adverse effect | No effect | Adverse effect | No effect |
| Survival[1-22] | 24 (60.0) | 16 (40.0) | 5 (19.2) | 21 (80.8) |
| Food consumption[1,2,4,7,11,13,14,16,20,21,23] | 15 (75.0) | 5 (25.0) | 0 (0.0) | 1 (100.0) |
| Larval body mass[1,2,4,7,12-14,16-18,20,21-23] | 23 (60.5) | 15 (39.5) | 3 (27.3) | 8 (72.7) |

| | | | | |
|---|---|---|---|---|
| Larval developmental time[2,3,9,14,16,20,21] | 11 (45.8) | 13 (54.2) | 3 (42.9) | 4 (57.1) |
| Larval behavior[2,23] | 1 (20.0) | 4 (80.0) | - | - |
| Pupal mass[1,2,3,9,11,16] | 2 (14.3) | 12 (85.7) | 2 (25.0) | 6 (75.0) |
| Length of the pupal period[1,2,3,11,16] | 5 (50.0) | 5 (50.0) | 2 (40.0) | 3 (60.0) |
| Adult body mass[1,3,11,16] | 1 (16.7) | 5 (83.3) | 1 (14.3) | 6 (85.7) |
| Adult size[11] | 1 (20.0) | 4 (80.0) | 0 (0.0) | 6 (100.0) |
| Adult sex ratio[3] | - | - | 0 | 3 (100.0) |

[1]Anderson et al. (2004) [2]Anderson et al. (2005) [3]Dively et al. (2004) [4]Felke et al. (2002) [5]Gathmann et al. (2006) [6]Hanley et al. (2003) [7]Hellmich et al. (2001) [8]Jesse and Obrycki (2002) [9] Jesse and Obrycki (2000) [10] Jesse and Obrycki (2003) [11]Lang and Vojtech (2006) [12]Li et al. (2005) [13]Losey et al. (1999) [14]Mattila et al. (2005) [15]Shirai and Takahashi (2005) [16]Stanley-Horn et al. (2001) [17]Wraight et al. (2001) [18]Zangerl et al. (2001) [19]Lee et al. (2003) [20]Müller et al. (2012) [21]Schuppener et al. (2012) [22]Felke and Langenbruch (2003) [23]Prasifka et al. (2007)

The effects of the Cry1Ab protein were observed largely via events Bt176, Bt11, and MON810, with Bt176 having the most adverse effects (Table 3). The vegetative insecticide protein, Vip3A only tested one variable while the stacked proteins were observed for four observed responses. There were low observations recorded for adult stage of development with majority of observations recorded as no effect (Table 3).

**Table 3**. Laboratory-based effects of Bt-corn on lepidopteran larvae. Listed are the numbers of observations for variables recorded in the experiments analyzed in the review; -, not studied.

| Parameter | MON810 | | Bt176 | | Bt11 | | Vip3A | | MON89034 x MON88017 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Adverse effect | No effect | Adverse effect | No effect | Adverse effect | No effect | Adverse effect | No effect | Adverse effect | No effect |
| Survival[1-18] | 2 | 5 | 12 | 2 | 4 | 2 | 0 | 1 | 1 | 0 |
| Food consumption[1,2,4,6,9,11,12,16,17,19] | 4 | 4 | 4 | 0 | 3 | 0 | - | - | 1 | 0 |
| Larval body mass[1,2,4,6,10-12,14,16-19] | 4 | 6 | 4 | 1 | 6 | 2 | - | - | 1 | 0 |
| Larval developmental time[2,3,8,12,16,17] | 5 | 5 | 2 | 2 | 2 | 3 | - | - | 1 | 0 |
| Larval behavior[2,19] | 1 | 4 | - | - | - | - | - | - | - | - |
| Pupal mass[1,2,3,8,9] | 1 | 5 | 1 | 2 | 0 | 5 | - | - | - | - |
| Length of the pupal period[1,2,3,9] | 3 | 3 | 1 | 0 | 1 | 2 | - | - | - | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Adult body mass[1,3,9] | - | - | 1 | 2 | 0 | 3 | - | - | - | - |
| Adult size[9] | - | - | 1 | 2 | 0 | 2 | - | - | - | - |
| [1]Anderson et al. (2004) [2]Anderson et al. (2005) [3]Dively et al. (2004) [4]Felke et al. (2002) [5]Hanley et al. (2003) [6]Hellmich et al. (2001) [7]Jesse and Obrycki (2002) [8]Jesse and Obrycki (2000) [9]Lang and Vojtech (2006) [10]Li et al. (2005) [11]Losey et al. (1999) [12]Mattila et al. (2005) [13]Shirai and Takahashi (2005) [14]Wraight et al. (2001) [15]Lee et al. (2003) [16]Müller et al. (2012) [17]Schuppener et al. (2012) [18]Felke and Langenbruch (2003) [19]Prasifka et al. (2007) | | | | | | | | | | |

As no new literature sources were identified regarding the effects of Bt-corn in field studies, the data collected in Lang and Otto's (2010) review were replicated for quality assurance and did not differ. Therefore, the summary table was omitted from this paper.

**Discrepancy Between Laboratory Results and Field Results.**

Since no new studies were identified during the scoping review, only replicated results by Lang and Otto (2010) were reported. Seen in Table 2, field-based studies by (Dively et al., 2004; Gathmann et al., 2006; Jesse ad Obrycki, 2003; Li et al., 2005; Stanley-Horn et al., 2001; Wraight et al., 2000; Zangerl et al., 2001) resulted in significantly less adverse effects on lepidopteran larvae compared with laboratory studies (Anderson et al., 2004; Anderson et al., 2005; Dively et al., 2004; Felke et al., 2002; Felke and Langenbruch, 2003; Hanley et al., 2003; Hellmich et al., 2001; Jesse and Obrycki, 2000; Jesse and Obrycki, 2002; Lang and Vojtech, 2006; Li et al., 2005; Losey et al., 1999; Mattila et al., 2005; Prasifka et al., 2007; Shirai and Takahashi, 2005; Wraight et al., 2000; Lee et al., 2003; Müller et al., 2012; Schuppener et al., 2012). This could be attributed to environmental complexities not easily replicated in laboratory settings. Natural ecosystems are more complex and variable than controlled laboratory environments. Biotic and abiotic factors could potentially influence the adverse effect of pollen on the larvae. In the field, the larvae host plants are exposed to wind and rain which may dilute the concentration of pollen available for larvae consumption.

**Lack of Modeling Studies**

Lang and Otto (2010) made the decision to exclude studies utilizing modeling assessments. These models incorporated factors such as wind patterns, topography, climate conditions, and biological characteristics of plants to predict how pollen moves across landscapes. Leclerc et al. (2018), developed a model that accounts for the agricultural landscape and assesses a realistic exposure impact of Bt-corn on non-target organisms.  Modeling assessments also studied the risks concerning the temporal overlap between pollen dispersal and sensitive larval stages as seen in the LepiX model (Fashe et al., 2018).

Lab studies occur under controlled conditions, while field environments had many fluctuating biotic and abiotic variables. Modeling could help estimate how factors like temperature, precipitation, alternate food sources, etc. may influence the effects seen in lab studies. Perry et al. (2010), developed a mathematical model that integrated a relationship between mortality,pollen dose based on laboratory experiments and the relationship between pollen amounts and distance from crops based on field measurements for MON810. The mathematical model of exposure was later used to model the effects of Cry1F (Perry et. al., 2012).  Including modeling could help researchers in better replicating natural settings when utilizing laboratory settings for the reported studies. A combination of lab experiments, field monitoring, and modeling efforts could provide a more holistic understanding of the ecological risks.

## Conclusion

The scoping review successfully replicated the meta-analysis conducted by Lang and Otto (2010), validating their original conclusion that pollen from Bt-corn does not pose a significant risk to non-target lepidopteran species under field conditions. By expanding the search to include more recent literature from 2010-2023 and additional search terms related to emerging biotechnology, a few new laboratory studies were identified. However, these additional studies did not contradict the original findings.

The expanded scoping review identified two new laboratory studies examining effects on the Small Tortoiseshell larvae, as well as one pre-2010 study on Vip proteins that was not included in the original meta-analysis. The three studies found Bt-corn had no significant effects on non-target lepidopteran and were aligned with the conclusion drawn by Lang and Otto (2010). While laboratory studies can provide useful data, their artificial conditions may not fully represent field environments.

Notably, no new relevant field studies were found during the expanded search period of 2010-2023. This replication and expansion of Lang and Otto (2010)'s work strengthens the evidence that transgenic Bt crops, which have become widely adopted due to their agricultural benefits, do not detrimentally impact populations of non-target lepidopteran species in the field. However, continued monitoring and research remains prudent as new biotechnologies emerge.

## REFERENCES


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# APPENDICES

**Appendix A**

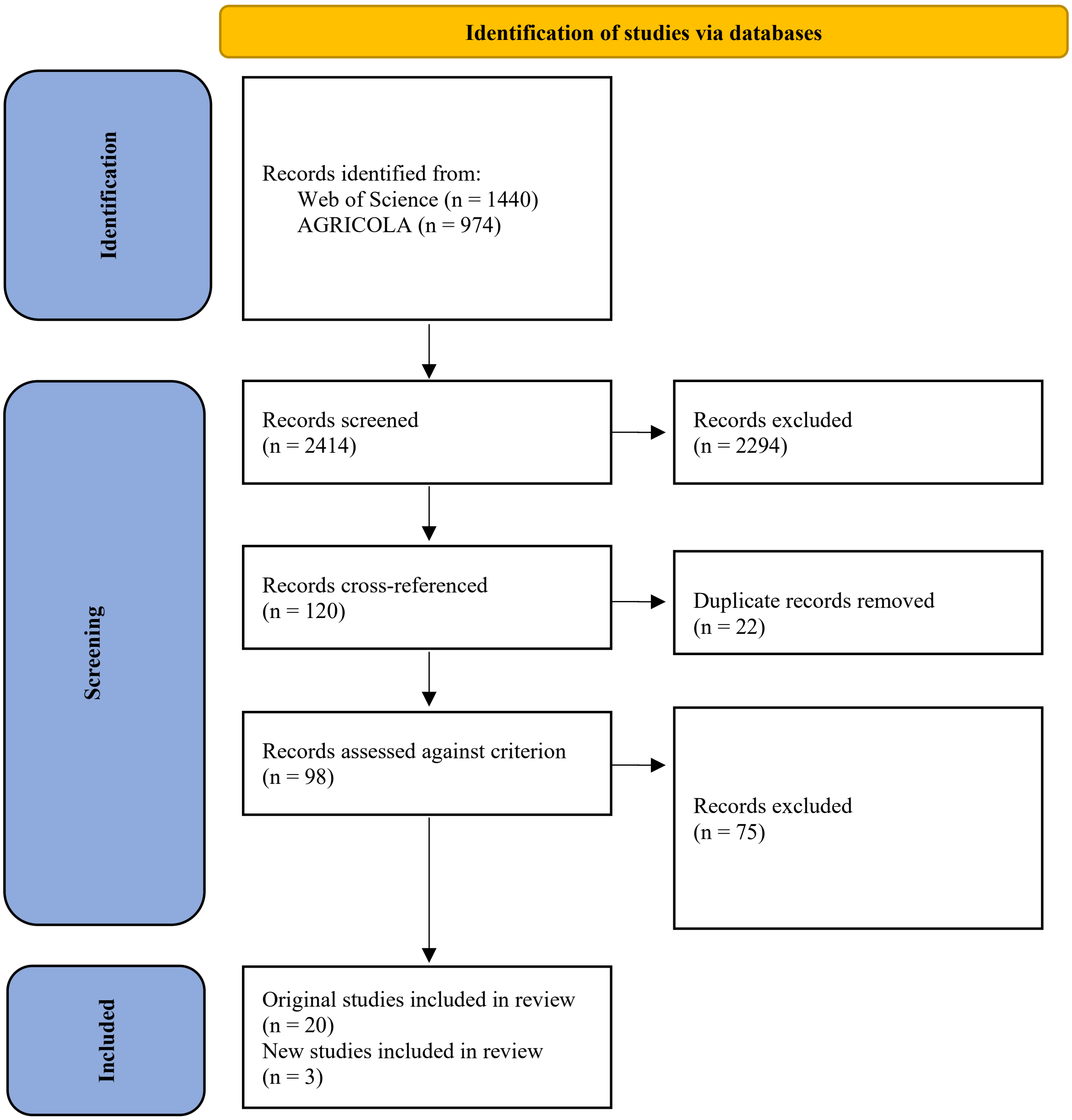
Aggregate results across search terms within individual databases per time period.

| Database | Parameters | Pre-2010 | Search Terms | Results | Saved to Folder | Peer-Reviewed | Journal Article |
|---|---|---|---|---|---|---|---|
| AGRICOLA | TX All Text Fields | no | “Bacillus thuringiensis” and “monarch butterfly” | 6 | 2 | yes | yes |
| Web of Science | All Fields | no | “Bacillus thuringiensis” and “monarch butterfly” | 10 | 3 | yes | yes |
| AGRICOLA | TX All Text Fields | yes | “Bacillus thuringiensis” and “monarch butterfly” | 12 | 6 | yes | yes |
| Web of Science | All Fields | yes | “Bacillus thuringiensis” and “monarch butterfly” | 23 | 19 | yes | yes |
| AGRICOLA | TX All Text Fields | no | “Lepidopt*” and “non-target*” | 221 | 3 | yes | yes |
| Web of Science | All Fields | no | “Lepidopt*” and “non-target*” | 568 | 20 | yes | yes |
| AGRICOLA | TX All Text Fields | yes | “Lepidopt*” and “non-target*” | 47 | 3 | yes | yes |
| Web of Science | All Fields | yes | “Lepidopt*” and “non-target*” | 196 | 7 | yes | yes |
| AGRICOLA | TX All Text Fields | no | “Cry*” and “Lepidopt*” and “non-target*” | 52 | 11 | yes | yes |
| Web of Science | All Fields | no | “Cry*” and “Lepidopt*” and “non-target*” | 143 | 17 | yes | yes |
| AGRICOLA | TX All Text Fields | yes | “Cry*” and “Lepidopt*” and “non-target*” | 16 | 3 | yes | yes |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Web of Science | All Fields | yes | “Cry*” and “Lepidopt*” and “non-target*” | 40 | 4 | yes | yes |
| AGRICOLA | TX All Text Fields | no | “Bt*” and “Butterfl*” or “Bt*” and “Moth*” | 270 | 6 | yes | yes |
| Web of Science | All Fields | no | “Bt*” and “Butterfl*” | 110 | 12 | yes | yes |
| AGRICOLA | TX All Text Fields | yes | “Bt*” and “Butterfl*” or “Bt*” and “Moth*” | 262 | 10 | yes | yes |
| Web of Science | All Fields | yes | “Bt*” and “Butterfl*” | 59 | 24 | yes | yes |
| AGRICOLA | TX All Text Fields | no | “Bt*” and “pollen” and “non-target*” | 23 | 2 | yes | yes |
| Web of Science | All Fields | no | “Bt*” and “pollen” and “non-target*” | 76 | 16 | yes | yes |
| AGRICOLA | TX All Text Fields | yes | “Bt*” and “pollen” and “non-target*” | 8 | 2 | yes | yes |
| Web of Science | All Fields | yes | “Bt*” and “pollen” and “non-target*” | 38 | 6 | yes | yes |
| AGRICOLA | TX All Text Fields | no | “danaus plexippus” and “bacillus thuringiensis” | 6 | 3 | yes | yes |
| Web of Science | All Fields | no | “danaus plexippus” and “bacillus thuringiensis” | 7 | 2 | yes | yes |
| AGRICOLA | TX All Text Fields | yes | “danaus plexippus” and “bacillus thuringiensis” | 13 | 10 | yes | yes |
| Web of Science | All Fields | yes | “danaus plexippus” and “bacillus thuringiensis” | 14 | 12 | yes | yes |
| AGRICOLA | TX All Text Fields | no | “butterfly*” and “vip*” | 1 | 1 | yes | yes |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Web of Science | All Fields | no | “butterfly*” and “vip*” | 3 | 0 | yes | yes |
| AGRICOLA | TX All Text Fields | yes | “butterfly*” and “vip*” | 0 | 0 | yes | yes |
| Web of Science | All Fields | yes | “butterfly*” and “vip*” | 4 | 2 | yes | yes |
| AGRICOLA | TX All Text Fields | no | “transgenic” and “Pollen” and “Lepidopt*” | 23 | 8 | yes | yes |
| Web of Science | All Fields | no | “transgenic” and “Pollen” and “Lepidopt*” | 72 | 13 | yes | yes |
| AGRICOLA | TX All Text Fields | yes | “transgenic” and “Pollen” and “Lepidopt*” | 14 | 6 | yes | yes |
| Web of Science | All Fields | yes | “transgenic” and “Pollen” and “Lepidopt*” | 77 | 21 | yes | yes |

# Appendix B


Identification of studies via databases
Identification
Records identified from:
Web of Science (n = 1440)
AGRICOLA (n = 974)
Screening
Records screened
(n = 2414)
Records excluded
(n = 2294)
Records cross-referenced
(n = 120)
Duplicate records removed
(n = 22)
Records assessed against criterion
(n = 98)
Records excluded
(n = 75)
Included
Original studies included in review
(n = 20)
New studies included in review
(n = 3)