Bt Research 2024, Vol.15, No.2, 87-95
http://microbescipublisher.com/index.php/bt

Feature Review Open Access

Ecological Impact of Bt on Non-target Invertebrates

Sibin Wang
Hainan Institute of Tropical Agricultural Resources (HITAR), Tropical Animal Resources Research Center, Sanya, 572025, Hainan, China
Corresponding email: sibin.wang@hitar.org
Bt Research, 2024, Vol.15, No.2 doi: 10.5376/bt.2024.15.0009
Received: 20 Feb., 2024
Accepted: 30 Mar., 2024
Published: 18 Apr., 2024

Preferred citation for this article:
Wang S.B., 2024, Eclgical impact of Bt on non-target invertebrates, Bt Research, 15(2): 87-95 (doi: 10.5376/bt.2024.15.0009)

Abstract The ecological impact of *Bacillus thuringiensis* (Bt) crops on non-target invertebrates has been a subject of extensive research and debate. This study synthesizes findings from multiple studies to provide a comprehensive understanding of the effects of Bt crops on non-target invertebrate populations. Meta-analyses indicate that non-target invertebrates are generally more abundant in Bt crop fields compared to non-transgenic fields managed with insecticides, although certain taxa are less abundant in Bt fields compared to insecticide-free control fields. Laboratory and field studies reveal that Bt crops have varying impacts on non-target Lepidoptera, with some species showing adverse effects under laboratory conditions, while field studies often report minimal impacts. The presence of Bt proteins in soil and their potential effects on soil-dwelling organisms, such as earthworms, have also been investigated, with findings suggesting no deleterious effects on growth and reproduction. Additionally, the coexistence of Bt and conventional crops can influence insect abundance and plant fitness, with Bt crops providing benefits by reducing target insect populations and thereby enhancing the growth of non-transgenic plants. Despite these findings, the long-term ecological risks and the potential for resistance development in target pests remain areas of concern that require ongoing monitoring and research. This study underscores the importance of evidence-based risk assessments and the need for more ecologically realistic studies to fully understand the ecological implications of Bt crop cultivation.
Keywords Bt crops; Non-target invertebrates; Ecological impact; Resistance development; Soil-dwelling organisms

1 Introduction

Bacillus thuringiensis (Bt) is a gram-positive bacterium that produces crystal proteins (Cry proteins) during sporulation, which are toxic to specific insect orders such as Lepidoptera, Coleoptera, and Diptera. These proteins have been harnessed in agriculture through the development of Bt transgenic crops, which express these insecticidal proteins to protect against pest infestations. Bt crops, such as Bt maize and Bt cotton, have been widely adopted globally due to their effectiveness in reducing pest populations and minimizing the need for chemical insecticides (Clark et al., 2005; Marvier et al., 2007; Li et al., 2022).

While Bt crops offer significant benefits in pest management, there is growing concern about their potential impacts on non-target invertebrates. Non-target organisms, which are not the intended recipients of the Bt toxins, may be exposed to these proteins through various environmental pathways, including soil, water, and plant residues. Understanding the ecological consequences of Bt crops on non-target invertebrates is crucial for several reasons. Firstly, non-target invertebrates play essential roles in ecosystem functioning, including pollination, decomposition, and as part of the food web. Secondly, assessing the non-target impacts of Bt crops is vital for ensuring the sustainability and environmental safety of this technology (Clark et al., 2005; Lang and Otto, 2010; Caquet et al., 2011).

This study aims to summarize the findings from field and laboratory studies on the impact of Bt crops on non-target invertebrate populations. It evaluates the methodologies used in these studies to assess the non-target effects of Bt crops. By identifying knowledge gaps and areas needing further research, this review seeks to enhance our understanding of the ecological risks associated with Bt crops. Additionally, the study offers recommendations for future research and risk assessment practices to ensure the safe and sustainable use of Bt technology in agriculture. By achieving these objectives, this review aims to contribute to evidence-based risk analysis and inform regulatory decisions regarding the application of Bt crops in agricultural systems.

2 Overview of Bt Toxins

2.1 Types of Bt toxins

Bacillus thuringiensis (Bt) produces a variety of insecticidal proteins, primarily Cry and Cyt toxins, which are used in genetically modified (GM) crops to target specific insect pests. The most commonly used Bt toxins in agriculture include Cry1Ab, Cry1Ac, Cry2Ab, Cry3Bb1, and Cry3Aa. These toxins are highly specific to certain insect orders, such as Lepidoptera (moths and butterflies) and Coleoptera (beetles) (Federici, 2003; Kostov et al., 2014; Krogh et al., 2020).

2.2 Mode of action in target organisms

Bt toxins function by binding to specific receptors in the gut cells of target insects, leading to cell lysis and death. When ingested by susceptible insects, the Cry proteins are solubilized and activated in the alkaline environment of the insect midgut. The activated toxins then bind to receptors on the midgut epithelial cells, forming pores that disrupt cell integrity, causing the insect to stop feeding and eventually die (Naranjo, 2009; Schrijver et al., 2015). This mode of action is highly specific, which is why Bt toxins are considered safe for non-target organisms, including humans and other vertebrates (Federici, 2003; Schrijver et al., 2015).

2.3 Use in agricultural practices

Bt crops, such as Bt cotton and Bt maize, have been widely adopted in agriculture to control major insect pests, reducing the need for chemical insecticides. These crops express Bt toxins throughout their tissues, providing continuous protection against pests. The adoption of Bt crops has led to significant reductions in insecticide use, which has environmental and economic benefits (Federici, 2003; Naranjo, 2009; Kostov et al., 2014). For instance, Bt maize and Bt cotton have been shown to reduce insecticide applications by millions of kilograms globally, contributing to more sustainable agricultural practices. Additionally, Bt crops have been found to have minimal adverse effects on non-target invertebrates compared to conventional insecticide treatments (Marvier et al., 2007; Wolfenbarger et al., 2008; Yang et al., 2017).

3 Non-Target Invertebrates: Categories and Roles

3.1 Pollinators

The ecological impact of *Bacillus thuringiensis* (Bt) crops on non-target invertebrates is a critical area of study, given the widespread adoption of these genetically modified organisms. Non-target invertebrates play essential roles in ecosystems, including pollination, soil health, aquatic systems, and biological control. This section studys the impact of Bt crops on various categories of non-target invertebrates. Pollinators are vital for the reproduction of many plants, including crops. The impact of Bt crops on pollinators has been a subject of concern, although studies specifically focusing on pollinators are limited. The general consensus is that Bt crops do not significantly affect pollinator populations. However, indirect effects through changes in plant-pollinator interactions or habitat alterations cannot be entirely ruled out and warrant further investigation.

3.2 Soil invertebrates

Soil invertebrates, such as nematodes, earthworms, and mites, are crucial for nutrient cycling and soil structure. The impact of Bt crops on these organisms has been extensively studied. A systematic study and meta-analysis found no significant overall effect of Bt crops on soil invertebrate populations, although there was considerable variation among different orders of soil invertebrates (Shu et al., 2011; Krogh et al., 2020). Another study indicated that Bt rice did not affect nematode abundance or community composition but did enhance trophic connections within nematode communities, suggesting a complex interaction between Bt crops and soil ecosystems (Liu et al., 2018).

3.3 Aquatic invertebrates

Aquatic invertebrates are essential for maintaining water quality and supporting aquatic food webs. The use of Bt var. *israelensis* (Bti) for mosquito control has raised concerns about its impact on non-target aquatic invertebrates. Long-term studies in French coastal wetlands have shown that Bti applications did not significantly affect the taxonomic structure or abundance of non-target aquatic invertebrate communities (Lagadic et al., 2014; Liu et al., 2018). These findings suggest that, when used according to recommended practices, Bti is environmentally safe for non-target aquatic invertebrates.

3.4 Predators and parasitoids

Predators and parasitoids are important for natural pest control. The impact of Bt crops on these functional guilds has been mixed. Meta-analyses have shown that Bt crops can reduce the abundance of certain parasitoids, particularly those that are specialists on target pests, while having less consistent effects on generalist predators (Dang et al., 2007; Wolfenbarger et al., 2008; Duan et al., 2009). Laboratory studies have generally been conservative, often predicting greater impacts than those observed in the field (Duan et al., 2009). This discrepancy highlights the need for comprehensive field studies to accurately assess the ecological risks of Bt crops.

4 Field Studies and Real-world Impacts

4.1 Long-term field monitoring

Long-term field monitoring is essential to understand the real-world impacts of Bt crops on non-target invertebrates. Studies have shown that Bt crops can influence the abundance and diversity of non-target invertebrates over extended periods. For instance, a meta-analysis of 42 field experiments indicated that non-target invertebrates are generally more abundant in Bt cotton and Bt maize fields compared to non-transgenic fields managed with insecticides, although certain taxa were less abundant in Bt fields compared to insecticide-free control fields (Marvier et al., 2007). Another study conducted over four consecutive years in French coastal and continental wetlands found no immediate or long-term detectable effects of Bt formulations on the taxonomic structure and abundance of non-target aquatic invertebrate communities (Yaqoob et al., 2016; Zhou et al., 2020). These findings suggest that while Bt crops can have some impact on non-target invertebrates, the effects may vary depending on the specific taxa and environmental conditions.

4.2 Comparative studies with non-Bt areas

Comparative studies between Bt and non-Bt areas provide valuable insights into the ecological impacts of Bt crops. The study and meta-analysis comparing soil invertebrates in Bt and conventional crop fields found no significant effect of Cry proteins on soil invertebrates across different orders (Wolfenbarger et al., 2008). Similarly, a meta-analysis on the effects of Bt cotton, maize, and potato on non-target arthropod functional guilds revealed no uniform effects, with insecticide use having a more significant impact than Bt crops themselves (Figure 1) (Dang et al., 2007; Li et al., 2022). In China, a meta-analysis of Bt rice showed that while parasitoid populations decreased slightly, detritivores increased, indicating that Bt rice poses negligible risks to non-target functional guilds in large-scale agroecosystems (Kostov et al., 2014). These comparative studies highlight the importance of considering both Bt and non-Bt areas to isolate the specific effects of Bt crops.

4.3 Observed ecological changes

Field studies have documented various ecological changes associated with Bt crops. For example, a study on the decomposition of Bt maize residue found that Bt maize decomposed similarly to non-Bt maize in large mesh bags, but decomposed faster in small and medium mesh bags during winter, suggesting potential seasonal effects on decomposition processes (Zwahlen et al., 2007). Another study on the effects of Bt maize on non-target Lepidoptera reported adverse effects on caterpillars in both laboratory and field settings, although the overall risk to butterflies and moths remains inconclusive due to limited data and the need for more ecologically realistic experiments (Lang et al., 2016). Additionally, a study of Bt rice's impact on spiders, major predators in rice fields, indicated that Bt protein can transfer and accumulate in spiders, affecting their physiology and spreading to higher trophic levels (Yang et al., 2017). These observed ecological changes underscore the complexity of Bt crop impacts and the need for comprehensive, long-term studies to fully understand their ecological implications.

5 Mechanisms of Non-target Effects

5.1 Direct toxicity

Direct toxicity refers to the immediate harmful effects of Bt crops on non-target invertebrates due to the ingestion or contact with Bt toxins. Laboratory studies have shown that exposure to high doses of Bt Cry proteins can reduce the survival rates of non-target Lepidoptera and other invertebrates (Duan et al., 2009; Marroquin et al., 2020). However, field studies often reveal a more nuanced picture. For instance, while laboratory tri-trophic studies predicted reduced abundances of parasitoids, these effects were not always observed in field conditions, suggesting that direct toxicity might be overestimated in controlled environments. Additionally, some studies have indicated that Bt crops do not significantly affect the survival or abundance of non-target Coleopteran species (Figure 2) (Duan et al., 2009; Naranjo, 2009; Brühl et al., 2020).

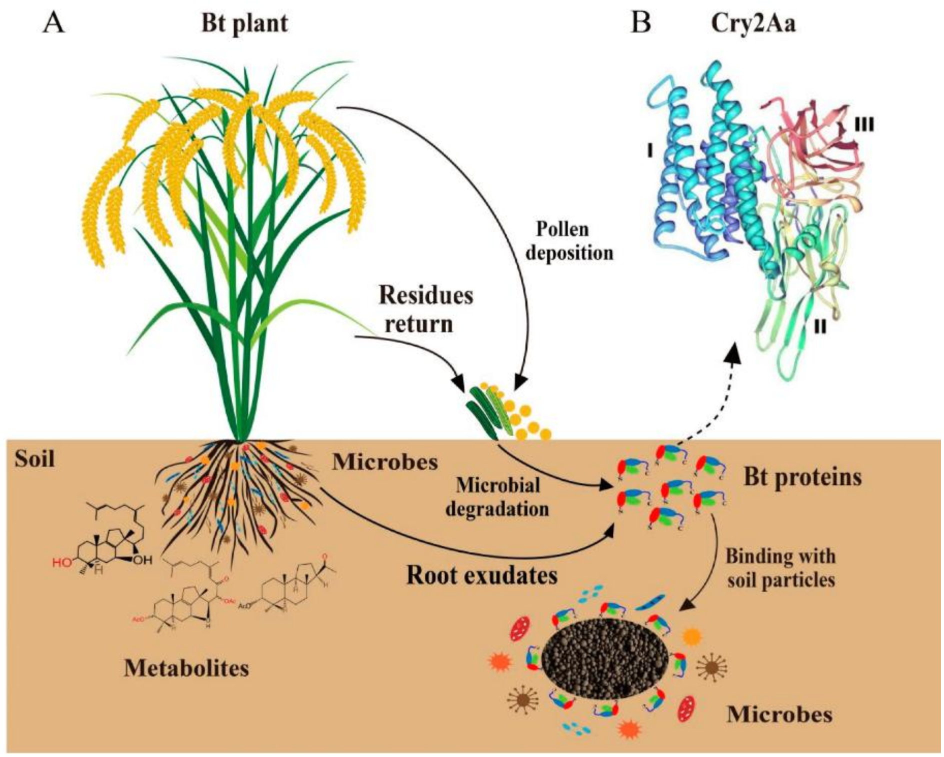

Figure 1 Environmental behaviors of Bt protein (A) and its three-dimensional structures (B). I, II, and III: domains I, II, and III (Adopted from Li et al., 2022)
Image caption: This figure illustrates the interactions between Bt plants (transgenic plants) and the environment, with a particular focus on the fate and ecological processes of Bt proteins (Cry2Aa) (Adopted from Li et al., 2022)

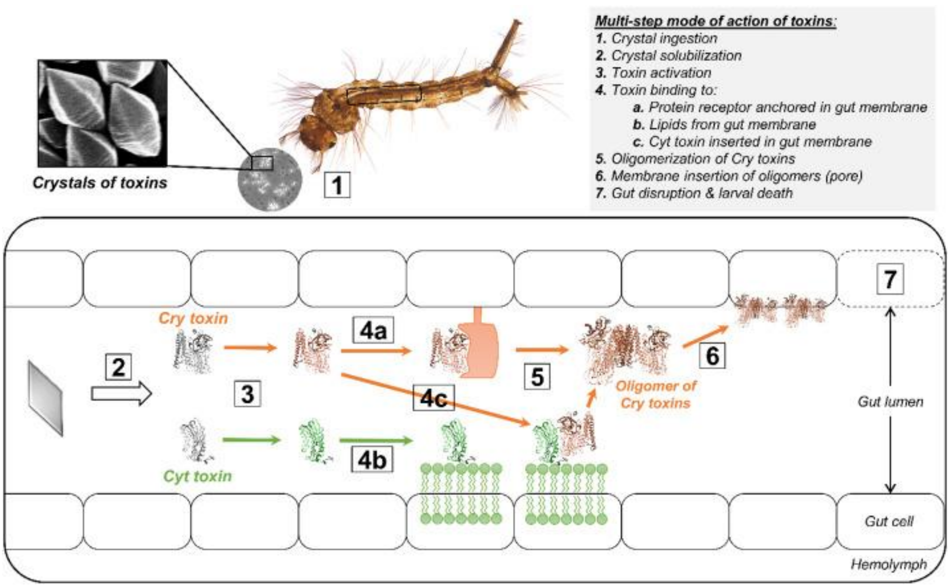

Figure 2 Schematic diagram of the mode of action of *Bacillus thuringiensis* subsp. *israelensis* Cry (orange) and Cyt (green) toxins in larval mosquito gut (Adopted form Brühl et al., 2020)
Image caption: This figure illustrates the multi-step mode of action of Cry and Cyt toxins on the gut cells of mosquito larvae (Adopted from Brühl et al., 2020)

5.2 Sub-lethal effects

Sub-lethal effects are those that do not cause immediate death but can impair the physiological or behavioral functions of non-target organisms. For example, Bt proteins have been shown to accumulate in spiders, leading to unintended physiological effects (Bordalo et al., 2021). These sub-lethal impacts can manifest as reduced feeding rates, impaired reproduction, or altered development, which can have long-term consequences on population dynamics. In aquatic ecosystems, sub-lethal effects of Bt-based insecticides on shredders and chironomids have been observed, leading to reduced leaf decomposition rates and altered ecosystem functioning (Bordalo et al., 2021).

5.3 Indirect ecological interactions

Indirect ecological interactions occur when Bt crops affect non-target organisms through changes in the ecosystem, such as altered prey availability or habitat conditions. For instance, the reduction in target pest populations can lead to a decrease in prey for predators and parasitoids, indirectly affecting their abundance and diversity (Belousova et al., 2021; Bordalo et al., 2021). In some cases, Bt crops have been associated with increased abundance of certain non-target taxa compared to fields treated with conventional insecticides, suggesting that the indirect effects of Bt crops might be less detrimental than those of chemical pest control methods (Marvier et al., 2007; Naranjo, 2009). However, the complexity of these interactions necessitates further research to fully understand the cascading effects within ecosystems (Brunk et al., 2019; Belousova et al., 2021). The mechanisms of non-target effects of Bt crops on invertebrates are multifaceted, involving direct toxicity, sub-lethal impacts, and indirect ecological interactions (Liang et al., 2018; Brühl et al., 2020). While laboratory studies provide valuable insights, field studies are crucial for a comprehensive understanding of these effects in real-world conditions.

6 Mitigation and Management Strategies

6.1 Development of bt varieties with reduced non-target effects

The development of Bt crops with minimized impacts on non-target invertebrates is crucial for sustainable agricultural practices. Research has shown that while Bt crops generally have fewer adverse effects on non-target organisms compared to conventional insecticides, there are still some non-target taxa that are affected (Marvier et al., 2007; Wolfenbarger et al., 2008; Naranjo, 2009). To mitigate these effects, it is essential to focus on the strategies, engineering Bt crops to express Cry proteins only in specific tissues or developmental stages can reduce exposure to non-target organisms. For instance, targeting the expression to parts of the plant that are less likely to interact with non-target species can help minimize unintended impacts (Naranjo, 2014; Romeis et al., 2019). Incorporating multiple Bt genes that target different pest species can reduce the overall amount of each toxin produced, potentially lowering the risk to non-target organisms (Duan et al., 2009; Navasero et al., 2016). Developing new Bt proteins that are highly specific to target pests while being harmless to non-target species can further reduce ecological risks. Studies have shown that different Cry proteins have varying effects on non-target organisms, suggesting that careful selection and design of these proteins are vital (Naranjo, 2014; Krogh et al., 2020).

6.2 Use of buffer zones and refuges

Buffer zones and refuges are critical components in the management of Bt crops to protect non-target invertebrates and delay resistance development in target pests. These strategies involve the planting non-Bt crops adjacent to Bt fields can provide a habitat for non-target organisms and help maintain their populations. This approach also supports the survival of susceptible pest individuals, which can mate with resistant ones, thereby slowing the development of resistance (Naranjo, 2009; Navasero et al., 2016). Buffer zones of non-Bt vegetation around Bt fields can act as a barrier, reducing the movement of Bt toxins into surrounding ecosystems. These zones can also serve as habitats for beneficial non-target species, promoting biodiversity and ecological balance (Wolfenbarger et al., 2008; Naranjo, 2014). Rotating Bt and non-Bt crops spatially and temporally can reduce the continuous exposure of non-target organisms to Bt toxins, allowing their populations to recover and maintain ecological functions (Marvier et al., 2007; Duan et al., 2009).

6.3 Integrated pest management (IPM) approaches

Integrating Bt crops into broader IPM strategies can enhance their effectiveness and sustainability while minimizing non-target impacts. Key components of IPM includethe utilizing natural predators and parasitoids to control pest populations can complement the use of Bt crops. Studies have shown that Bt crops can support conservation biological control by reducing the need for chemical insecticides, which are more harmful to non-target species (Duan et al., 2009; Romeis et al., 2019). Regular monitoring of pest and non-target organism populations can help in making informed decisions about pest management interventions. Implementing action thresholds ensures that control measures are only applied when necessary, reducing unnecessary exposure to Bt toxins (Naranjo, 2014; Romeis et al., 2019). Employing cultural practices such as crop rotation, intercropping, and maintaining habitat diversity can enhance the resilience of agroecosystems and reduce reliance on Bt crops alone for pest control. By adopting these mitigation and management strategies, the ecological impact of Bt crops on non-target invertebrates can be minimized, promoting a more sustainable and environmentally friendly approach to pest management.

7 Regulatory and Policy Considerations

7.1 International regulatory frameworks

The international regulatory frameworks for Bt crops are designed to ensure the safety and efficacy of these genetically modified organisms (GMOs) before they are approved for commercial use. Regulatory bodies such as the European Food Safety Authority (EFSA), the United States Environmental Protection Agency (EPA), and the International Service for the Acquisition of Agri-biotech Applications (ISAAA) play crucial roles in the risk assessment and approval processes. These frameworks typically involve rigorous evaluations of the potential environmental and health impacts of Bt crops, including their effects on non-target invertebrates. For instance, the biosafety assessment protocols often include laboratory and field studies to evaluate the ecological risks associated with Bt crops (Marvier et al., 2007; Duan et al., 2009; Kostov et al., 2014).

7.2 Risk assessment rrotocols

Risk assessment protocols for Bt crops involve a tiered approach that starts with laboratory studies and progresses to field trials. Laboratory studies often expose non-target organisms to high doses of Bt proteins to detect any harmful effects, which are then compared with field study results to validate the findings. This approach helps in understanding the potential risks and ensuring that laboratory results are consistent with field observations (Duan et al., 2009; Dang et al., 2017). Meta-analyses and systematic studies are also employed to synthesize data from multiple studies, providing a comprehensive understanding of the ecological impacts of Bt crops on non-target invertebrates ((Marvier et al., 2007; Krogh et al., 2020; Belousova et al., 2021). These protocols are essential for identifying any unintended effects on beneficial insects, soil organisms, and other non-target species, thereby informing regulatory decisions and ensuring environmental safety.

7.3 Public perception and acceptance

Public perception and acceptance of Bt crops are influenced by various factors, including the perceived benefits and risks, media coverage, and the transparency of regulatory processes. The ongoing debate about the ecological and environmental risks of Bt crops, particularly their impact on non-target invertebrates, has led to mixed public opinions. While some studies have shown no significant harmful effects on non-target organisms, others have raised concerns about potential long-term impacts (Lagadic et al., 2016; Yang et al., 2017; Belousova et al., 2021). Effective communication of scientific findings and regulatory decisions is crucial for gaining public trust and acceptance. Engaging stakeholders, including farmers, consumers, and environmental groups, in the decision-making process can also help address concerns and improve the acceptance of Bt crops (Lagadic et al., 2016).

8 Concluding Remarks

The ecological impact of *Bacillus thuringiensis* (Bt) crops on non-target invertebrates has been extensively studied, with mixed results. Meta-analyses and systematic studies indicate that non-target invertebrates are generally more abundant in Bt crop fields compared to non-transgenic fields managed with insecticides, but less abundant compared to insecticide-free control fields. Soil invertebrates, including nematodes, mites, and earthworms, show no significant changes in population abundance and biomass due to Bt crops, although there is considerable variation among different orders. Laboratory studies often predict reduced field abundance of non-target organisms, but these effects are not always realized in field conditions, suggesting that laboratory studies may overestimate ecological risks. Additionally, Bt formulations used for mosquito control, such as Bti, have shown no long-term adverse effects on non-target aquatic invertebrates in various wetland environments.

Continued research and monitoring are crucial to fully understand the long-term ecological impacts of Bt crops and formulations. The complexity of ecological interactions and the potential for indirect effects on non-target organisms necessitate ongoing studies. For instance, Bt strains can affect indigenous microorganisms and establish complex relationships with local plants, which may have cascading effects on the ecosystem. Moreover, the potential for Bt crops to alter food webs and nutrient cycles in soil ecosystems highlights the need for comprehensive, multi-level assessments. Long-term monitoring is also essential to detect any delayed or cumulative effects that may not be apparent in short-term studies.

Future studies should focus on several key areas to enhance our understanding of the ecological impact of Bt crops on non-target invertebrates conduct long-term field studies to monitor the cumulative and delayed effects of Bt crops on non-target invertebrate populations and community dynamics. Utilize ecological network analysis to understand the biotic interactions and trophic connections within soil and aquatic ecosystems affected by Bt crops. Incorporate multi-trophic level assessments in laboratory and field studies to better predict the ecological risks associated with Bt crops. Expand research to include diverse geographical locations and habitat types to account for environmental variability and its influence on non-target invertebrate responses. Investigate sub-lethal and indirect effects of Bt crops on non-target organisms, including potential changes in behavior, reproduction, and ecosystem services. By addressing these areas, future research can provide a more comprehensive understanding of the ecological impacts of Bt crops and contribute to the development of sustainable agricultural practices.

Acknowledgments

I would like to thank my supervisor, Professor Jim, for his guidance and support throughout the entire research process.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Belousova M., Malovichko Y., Shikov A., Nizhnikov A., and Antonets K., 2021, Dissecting the environmental consequences of *Bacillus thuringiensis* Application for Natural Ecosystems, Toxins, 13(5): 355.
https://doi.org/10.3390/toxins13050355

Bordalo M., Machado A., Campos D., Coelho S., Rodrigues A., Lopes I., and Pestana J., 2021, Responses of benthic macroinvertebrate communities to a Bti-based insecticide in artificial microcosm streams, Environmental Pollution, 282: 117030.
https://doi.org/10.1016/j.envpol.2021.117030

Brühl C., Després L., Frör O., Patil C., Poulin B., Tetreau G., and Allgeier S., 2020, Environmental and socioeconomic effects of mosquito control in Europe using the biocide *Bacillus thuringiensis* subsp. *israelensis* (Bti), The Science of the Total Environment, 724: 137800.
https://doi.org/10.1016/j.scitotenv.2020.137800

Brunk I., Sobczyk T., and Roth M., 2019, Pest control in German forests: general patterns of biodiversity and possible impacts of Btk, diflubenzuron and lambda-Cyhalothrin on non-target arthropods, birds and bats-a literature review, Journal of Forest and Landscape Research, 4(1): 1-26.
https://doi.org/10.13141/jflr.v4i1.1005

Caquet T., Roucaute M., Goff P., and Lagadic L., 2011, Effects of repeated field applications of two formulations of *Bacillus thuringiensis* var. *israelensis* on non-target saltmarsh invertebrates in Atlantic coastal wetlands, Ecotoxicology and environmental safety, 74(5): 1122-1130.
https://doi.org/10.1016/j.ecoenv.2011.04.028

Clark B., Phillips T., and Coats J., 2005, Environmental fate and effects of *Bacillus thuringiensis* (Bt) proteins from transgenic crops: a review, Journal of Agricultural and Food Chemistry, 53(12): 4643-4653.
https://doi.org/10.1021/jf040442k

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

Duan J., Lundgren J., Naranjo S., and Marvier M., 2009, Extrapolating non-target risk of Bt crops from laboratory to field, Biology Letters, 6: 74-77.
https://doi.org/10.1098/rsbl.2009.0612

Federici B., 2003, Effects of Bt on non-target organisms, Journal of New Seeds, 5: 11-30.
https://doi.org/10.1300/J153v05n01_02

Kostov K., Damgaard C., Hendriksen N., Sweet J., and Krogh P., 2014, Are population abundances and biomasses of soil invertebrates changed by Bt crops compared with conventional crops? A systematic review protocol, Environmental Evidence, 3: 1-9.
https://doi.org/10.1186/2047-2382-3-10

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

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

Lagadic L., Roucaute M., and Caquet T., 2014, Bti sprays do not adversely affect non-target aquatic invertebrates in French Atlantic coastal wetlands, Journal of Applied Ecology, 51: 102-113.
https://doi.org/10.1111/1365-2664.12165

Lagadic L., Schäfer R., Roucaute M., Szöcs E., Chouin S., Maupeou J., Duchet C., Franquet E., Hunsec B., Bertrand C., Fayolle S., Frances B., Rozier Y., Foussadier R., Santoni J., and Lagneau C., 2016, No association between the use of Bti for mosquito control and the dynamics of non-target aquatic invertebrates in French coastal and continental wetlands, The Science of the Total Environment, 553: 486-494.
https://doi.org/10.1016/j.scitotenv.2016.02.096

Lang A., and Otto M., 2010, A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (Bt) maize on non-target Lepidoptera, Entomologia Experimentalis et Applicata, 135(2): 121-134.
https://doi.org/10.1111/j.1570-7458.2010.00981.x

Li Y., Wang C., Ge L., Hu C., Wu G., Sun Y., Song L., Wu X., Pan A., Xu Q., Shi J., Liang J., and Li P., 2022, Environmental behaviors of *Bacillus thuringiensis* (Bt) insecticidal proteins and their effects on microbial ecology, Plants, 11(9): 1212
https://doi.org/10.3390/plants11091212

Liang Y., Liu F., Li J., Cheng Z., Chen H., Wang X., Xiao N., and Liu Y., 2018, Coexistence of *Bacillus thuringiensis* (Bt)-transgenic and conventional rice affects insect abundance and plant fitness in fields, Pest Management Science, 74(7): 1646-1653.
https://doi.org/10.1002/ps.4856

Liu T., Chen X., Qi L., Chen F., Liu M., and Whalen J., 2018, Root and detritus of transgenic Bt crop did not change nematode abundance and community composition but enhanced trophic connections, The Science of the Total Environment, 644: 822-829.
https://doi.org/10.1016/j.scitotenv.2018.07.025

Marroquin L., Elyassnia D., Griffitts J., Feitelson J., and Aroian R., 2000, *Bacillus thuringiensis* (Bt) toxin susceptibility and isolation of resistance mutants in the nematode Caenorhabditis elegans, Genetics, 155(4): 1693-1699.
https://doi.org/10.1093/genetics/155.4.1693

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

Naranjo S., 2009, Impacts of Bt crops on non-target invertebrates and insecticide use patterns, Cab Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 4: 1-11.
https://doi.org/10.1079/PAVSNNR20094011

Naranjo S., 2014, Effects of GM crops on non-target organisms, Plant Biotechnology, 11: 129-142.
https://doi.org/10.1007/978-3-319-06892-3_11

Navasero M., Candano R., Hautea D., Hautea R., Shotkoski F., and Shelton A., 2016, Assessing potential impact of Bt eggplants on non-target arthropods in the philippines, PLoS ONE, 11(10): e0165190.
https://doi.org/10.1371/journal.pone.0165190

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

Russell T., Kay B., and Skilleter G., 2009, Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna, Aquatic Biology, 6: 77-90.
https://doi.org/10.3354/ab00156

Schrijver A., Clercq P., Maagd R., and Frankenhuyzen K., 2015, Relevance of Bt toxin interaction studies for environmental risk assessment of genetically modified crops, Plant Biotechnology Journal, 13(9): 1221-1223.
https://doi.org/10.1111/pbi.12406

Shu,Y., Ma H., Du Y., Li Z., Feng Y., and Wang J., 2011, The presence of *Bacillus thuringiensis* (Bt) protein in earthworms Eisenia fetida has no deleterious effects on their growth and reproduction, Chemosphere, 85(10): 1648-1656.
https://doi.org/10.1016/j.chemosphere.2011.08.032

Wolfenbarger L., Naranjo S., Lundgren J., Bitzer R., and Watrud L., 2008, Bt crop effects on functional guilds of non-target arthropods: a meta-analysis, PLoS

ONE, 3(5): e2118.
https://doi.org/10.1371/journal.pone.0002118

Yang H., Peng Y., Tian J., Wang J., Hu J., Song Q., and Wang Z., 2017, Review: biosafety assessment of Bt rice and other Bt crops using spiders as example for non-target arthropods in China, Plant Cell Reports, 36: 505-517.
https://doi.org/10.1007/s00299-017-2108-1

Yaqoob A., Shahid A., Samiullah T., Rao A., Khan M., Tahir S., Mirza S., and Husnain T., 2016, Risk assessment of Bt crops on the non-target plant-associated insects and soil organisms, Journal of the Science of Food and Agriculture, 96(8): 2613-2619.
https://doi.org/10.1002/jsfa.7661

Zhou Y., Wu Z.Q., Zhang J., Wan Y.S., Jin W.J., Li Y.Z., and Fang X.J., 2020, *Bacillus thuringiensis* novel toxin Epp is toxic to mosquitoes and prodenia litura larvae, Brazilian Journal of Microbiology, 51: 437-445.
https://doi.org/10.1007/s42770-019-00194-z

Zwahlen C., Hilbeck A., and Nentwig W., 2007, Field decomposition of transgenic Bt maize residue and the impact on non-target soil invertebrates, Plant and Soil, 300: 245-257.
https://doi.org/10.1007/s11104-007-9410-6