

Direct Effects of Pesticides and Other Grassland Management Practices on the North American Monarch Butterfly (*Danaus plexippus plexippus*): A systematic review

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ABSTRACT

This review synthesizes recent scientific literature on the effects of grassland management practices and pesticide applications on monarch butterflies (*Danaus plexippus*), focusing on studies published since the 2020 U.S. Fish and Wildlife Service Species Status Assessment (SSA). While the SSA highlighted habitat conservation efforts to enhance monarch populations, there has been limited empirical research on the direct effects of these management strategies on monarch survival and fitness. Following PRISMA guidelines, we conducted a systematic review to evaluate research published during and after the SSA (2019-2024) on the direct effects of common grassland management practices (mowing, grazing, burning, haying) and pesticides (herbicides, insecticides, fungicides) across monarch life stages. This search identified 29 studies that met our criteria. Of these, only two studies addressed the impacts of grassland management on monarchs, indicating a substantial and important information gap. The other 27 studies reviewed indicate significant risks of pesticide exposure to monarchs, particularly at immature life stages from lethal and sublethal effects. The effects of chronic insecticide exposure, particularly neonicotinoids, are especially critical to consider, as research shows insecticides can result in impairment of growth, development, and reproductive behaviors. Additionally, fungicides pose overlooked risks to monarchs due to their toxicity to non-target organisms. Notably, most studies evaluating pesticide effects were conducted in a laboratory setting and thus it is unclear how these results relate to field-realistic exposure, as well as exposure over multiple life history stages and potential synergistic effects of pesticides. Additional relevant information gaps remain, especially on studies of southern migratory paths critical for monarch population persistence. Data gaps at relevant scales for grassland management combined with growing evidence of pesticide persistence and subsequent threats to insects, highlight how more ecologically applicable metrics for monitoring environmental chemicals could help improve understanding of the effects of pesticides on monarchs and characterize contamination of milkweed and nectar resources.

Keywords

Monarch butterfly, *Danaus plexippus*, pesticides, insecticide, herbicide, fungicide, mowing, grazing, haying, burning, survival, fitness, viability, management

INTRODUCTION

RATIONALE AND BACKGROUND

The iconic migration of the eastern monarch butterfly (*Danaus plexippus plexippus*) across North America has captivated observers and scientists for centuries, yet these phenomenal insects face an uncertain future. Based on over 20 years of observations of the overwintering colonies in Mexico, migratory monarch populations show declining long-term trends and are predicted to fall below the historic minimum, and near the quasi-extinction threshold, at least three times in the next 20 years (Rendón-Salinas et al., 2023; Semmens et al., 2016; Zylstra et al., 2022). The long-term average area occupied by monarchs at the overwintering area in Mexico from 1993-2003 was 8.82 ha (range 4.02-12.8 ha), however, a recent report notes a “new normal” wintering area calculated from 2004-2022 as 3.25 ha (range 1.5-5.0 ha) (Rendón-Salinas et al., 2024). In 2020, the United States Fish and Wildlife Service (USFWS) determined the eastern monarch population was “warranted but precluded” for listing under the Endangered Species Act, acknowledging the population’s precarious status while prioritizing other conservation efforts (U.S. Fish and Wildlife Service, 2020). Listing the monarch would likely have far-reaching implications, as their habitat range essentially covers the entirety of the continental United States. Habitat conservation efforts are considered to play a key role in supporting monarch populations to prevent a need for federal protection and ensure the survival of the migration phenomenon for future generations (K. Oberhauser et al., 2017). The Monarch Conservation Database was established by the USFWS to track habitat conservation efforts implemented by community conservationists, non-governmental organizations, businesses, and government agencies to create, restore, and improve habitat for monarch

butterflies. Habitat improvement has largely focused on planting milkweed (*Asclepias* spp.), the sole host plant for larval monarchs, and other native flowering plants historically found in grassland ecosystems. As of June 2023, the Monarch Conservation Database report estimated just over 381,000,000 milkweed stems added to the landscape through conservation efforts and remaining CRP easements (Midwest Association of Fish and Wildlife Agencies, 2023.) Despite these efforts, monarch overwintering population indices continue to decline (Rendón-Salinas et al., 2023). However, the extent and causes of the monarch population decline are equivocal and highly debated in the literature (Davis & Dyer, 2015; Davis et al., 2024; Fordyce et al., 2020; Inamine et al., 2016; K. Oberhauser et al., 2017; Pleasants et al., 2016; Taylor et al., 2020; Thogmartin et al., 2020). Beyond milkweed loss, threats such as illegal logging in overwintering areas, habitat degradation, insecticide exposure, increased disease rates, and climate change have all been linked to population declines (U.S. Fish and Wildlife Service, 2020). Although these factors are included in the USFWS Species Status Assessment population model, it is still unclear how each independent factor's spatial and temporal nuances directly affect monarchs across life stages, and how that can ultimately influence population growth rates (Farr et al., 2024; Moreno-Sanchez et al., 2019). This uncertainty in monarch population status and response to anthropogenic land use and climate change underscores how a better understanding of the impact of threats on transgenerational monarch populations can help to develop improved conservation strategies for their remaining habitats (Belsky & Joshi, 2018)

Land management practices have historically influenced monarch populations, a trend that continues today. Before the 1950s, there is evidence to suggest monarch and milkweed populations were increasing, likely due to forest clear-cutting and the subsequent increase in

low-intensity agricultural lands (Boyle et al., 2019; Vane-Wright, 1993). It has been suggested that disturbance-tolerant *Asclepias* species, such as *Asclepias syriaca*, were abundant in these newly created landscapes (Vane-Wright, 1993). Research by Li et al. (2023) supports this pattern with reconstructed historical land cover and land use changes showing a decline in forests due to cropland expansion between 1800 and 1950, with slower conversion rates afterward.

Monarch populations are considered to have begun declining after the 1950's as a consequence of land conversion to cropland coinciding with the rise of herbicides and intensified agricultural practices (Boyle et al., 2019; X. Li et al., 2023). The introduction of genetically modified (GM) crops in 1996 further accelerated monarch habitat degradation, the mechanism for which was the presence of *Bacillus thuringiensis* (*Bt*) crystalloproteins, considered toxic to monarch larvae, in GM corn (Malcolm, 2018). However, subsequent research indicated the risks associated with the direct effects of exposure to GM crops on monarchs were outweighed by the fact that agricultural fields still supported substantial milkweed populations (Malcolm, 2018; Oberhauser et al., 2001). In time, concern for monarch populations shifted from the direct effects of *Bt* crystalloproteins to the indirect and direct effects of habitat loss and degradation after the widespread adoption of herbicide-resistant GM crops eradicated milkweed from treated fields (Malcolm, 2018). As GM corn and soybean monocultures became ubiquitous across the Midwest, pesticide use has become central to modern agriculture, raising concerns about ecosystem disruption and widespread insect declines (Douglas et al., 2022; Wagner, 2020). Pesticides (herbicides, fungicides, and insecticides) can have both indirect and direct effects on non-target species and have been implicated in insect declines not only in the United States but

across the globe; a phenomenon now known as the “insect apocalypse” (Gilburn et al., 2015; Goulson, 2019.; Van Deynze et al., 2022).

Many species, including the migratory monarch, rely on the capacity for large-scale movements across grassland landscapes to track available resources and avoid inclement environmental conditions (Augustine et al., 2021). Most grasslands in North America were lost following European settlement in the mid-1800's, primarily through conversion to agriculture (Lauenroth et al., 1999; Samson et al., 2004), a trend that has continued into the 21st century as the demand for biofuels has increased in recent years, driving more grassland conversion to row crop agriculture (Zhang et al., 2021). For remaining grassland ecosystems, common mechanical management techniques, which focus on removal of vegetation biomass, including burning, grazing, mowing, and haying can reduce woody plant encroachment, control invasive species, and promote native plant and animal productivity. However, the complexity of these management practices and the ecosystem response require long-term monitoring for a holistic assessment (Wagle & Gowda, 2018).

Mechanical management practices and pesticide application are commonly employed across the Great Plains. Grazing and fire management may become more prominent features of grassland management with the acknowledgment that natural disturbance regimes at larger spatial scales are necessary to conserve landscape-scale processes such as animal movement and metapopulation dynamics (Augustine et al., 2021). However, the direct effects of grassland management and pesticide application on monarch survival and fitness are likely complex and require comprehensive evaluation across life stages (Orr & Garland, 2017). The USFWS SSA for the monarch butterfly restricted their evaluation to the larval and adult stages. Although

pesticides were included in the assessment and there was evidence to suggest pesticides, particularly insecticides, were present on the landscape in biologically relevant concentrations, information was limited, and no conclusions were reached regarding the effects of insecticides on monarch population viability. Further, the SSA contains no information on the direct effects of grassland disturbance management practices on monarch butterflies. However, since the publication of the SSA in 2020, more than 4,000 records investigating various aspects of monarch butterfly ecology and conservation have been published (Figure 1). Thus, synthesizing recent knowledge can help to improve understanding of the potential long-term impacts of common land management practices across the Great Plains on monarch population viability and informing the development and implementation of effective conservation measures by land managers.

In this review, our objective was to synthesize the most recent empirical literature assessing the direct effects of the most common grassland management practices (mowing, grazing, burning, haying) and pesticide (herbicide, insecticide, fungicide) applications on monarchs across all life stages, identify information gaps and uncertainties, and assess the potential impact on future monarch population viability.

This review focuses on management of the eastern monarch butterfly's breeding habitat, including what we define as mechanical grassland management techniques (grazing, burning, mowing, haying), with the primary purpose of removing vegetation biomass, and pesticide application (herbicides, insecticides, or fungicides). We discuss the implications of the findings in the context of monarch population viability, following the definition put forth by the SSA as "the species' ability to withstand environmental stochasticity (resiliency), catastrophes

(redundancy), and changes in its environment (representation)” (U.S. Fish and Wildlife Service, 2020). We reviewed the literature published from 2019-2024 to include studies published during and after the publication of the 2020 USFWS Species Status Assessment and focus on research that investigates the direct effects of management and pesticides on monarch butterflies at any life stage. We also review the potential for cumulative effects across life history stages and synergistic effects of management practices.

METHODS

PROTOCOL

We conducted a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page, McKenzie, et al., 2021) and developed a protocol to document the process, outlined briefly below. A systematic review is defined as “a review that uses explicit, systematic methods to collate and synthesize findings of studies that address a clearly formulated method.” (Page, McKenzie, et al., 2021). These guidelines were constructed to standardize the framework for systematic reviews to ensure a standard of quality, transparency, and reproducibility. The following sections are a summary of the protocol we developed to conduct the review (Supplemental Material S1).

Eligibility Criteria

We searched for empirical studies published in scientific journals, books, preprints, or conferences as well as Master theses and Doctoral dissertations from 2019-2024 and followed a set of predefined eligibility criteria (Table 1). To be included, publications needed to be available

in full text and English but did not have to be open source. We sought primary literature with quantitative analytical and statistical methods (excluding observational, opinion, commentary, reference, or educational material) with a focus on the North American subpopulation of monarch butterflies and the direct effects on monarch populations or viability by a) our focal common grassland management strategies (burning, haying, mowing, or grazing) and/or b) pesticidal application. We define a direct effect as the impact from physical or chemical interactions that affect an organism without being mediated/transmitted by a third-party organism or object (e.g. destruction or harm of a monarch at any life stage due to grassland management techniques, or ingestion/contact with a pesticide).

Table 1. Eligibility criteria for inclusion or exclusion of papers with justification provided

	Inclusion Criteria	Justification
Title/Abstract	Articles in Journals, Books, Preprint, Master's Thesis, Doctoral Dissertations, or Conference from 2019-2024	Provision of comprehensive review
	Full text is available	Easy access and availability for current and future researchers, prevent conclusions made from misleading partial text
	Articles focused on monarchs (any subpopulation of monarch is acceptable)	Most articles are expected to be focused on North American populations and information on other populations can be included as discussion points
	The study indicates an assessment of the effects of management and chemical application on monarch (or other insect pollinator) populations or viability, specifically the aspect of resiliency. Resiliency is measured by survival, reproduction, and growth rates as well as good body condition.	Assess papers testing specific hypotheses related to our research questions

Full Text	Study indicates an assessment of the degree or extent of risk of insecticide exposure to monarchs (or other insect pollinator) at any life stage, including oviposition preference	Assess papers testing specific hypotheses related to our research questions
	Study assesses monarch response (individual or population) to management technique (grazing, mowing, haying, or sometimes generally referred to as “disturbance” or “disturbance regimes”)	Assess papers testing specific hypotheses related to our research questions
	Exclusion Criteria	Justification
Full Text	Papers not available in English	English is an international language. Reports on monarchs in the overwintering area are provided in English. The authors of the review are not bilingual
	No formal data analysis (observational, notes, letters, commentary, opinion, review, reference/educational material (not including meta-analysis))	Prevents reviewers from drawing conclusions from inadequate studies for inference
	Studies should focus on the effects of the management technique or chemical application to monarchs at any life stage	Avoid papers that assess indirect effects of chemicals, this information has been synthesized recently
	Monarch butterflies not mentioned, study organism does not include monarch butterflies	Publication does not address the primary taxon of interest for this literature review

Information Sources

We used Web of Science, Scopus, and Google Scholar to conduct the literature search. No authors were contacted for additional correspondence or resources for the search.

Search strategy

Two search strings were used for each database (Table 2). Search results were restricted by year of publication (2019-2024), language (English only), and were sorted by relevance.

Table 2. Number of results from the selected search string and database combinations.

Search String	Database	Search	Results
	Google Scholar	Web of Science	Scopus
(monarch OR "monarch butterfly" OR "danaus plexippus") AND (habitat* OR grassland* OR landscape*) AND (mow* OR graze* OR burn* OR disturbance OR manage*)	1020*	69	61
monarch* OR "monarch butterfly" OR "danaus plexippus" AND (herbicide* OR pesticide* OR fungicide* OR insecticide*)	2330*	3828*	57

Asterisks indicate search results from which only the first 200 records were reviewed. Unavailable papers were not included. Final number of records reviewed was 714.

Selection process

After all searches were conducted with each string and database combination, we pooled records and removed duplicates (i.e. repeated publications across multiple databases). Search results were sorted by relevance. For three of the search string/database combinations that returned over 1,000 records, we opted to review only the top 200 publications due to the diminishing relevance of articles beyond this threshold (Table 2). Next, we systematically screened these publications by reviewing the title and abstract of each paper to assess relevance to the topic. Non-relevant publications were excluded from the review at this stage, after which we conducted a full-text review of the remaining records and included or excluded those based on the methodology of the reviewed study (Page, Moher, et al., 2021). The final number of records we reserved for in-depth review was 28 (Figure 2).

Data collection process

Authors reviewed each study and extracted relevant data into a spreadsheet (supplemental material S2). Data items included were:

1. Author name(s)
2. Year of publication
3. Article keywords
4. Article scope (study site)
5. Article objectives
6. Additional study organisms
7. Study site location (North America, other)
8. Study type (lab/field)
9. Monarch subpopulation (Eastern, Western, North American, Mexican, NA)
10. Life stage (egg, larval, pupal, adult, NA)
11. Sample size
12. Dependent variable (survival, fitness)
13. Dependent variable metric (survival rate over time, growth, wing size, body condition, etc..)
14. Independent variable (grazing, mowing, haying, burning, pesticide application)
15. Independent variable metric (LD50 dosage units, hectares mowed, burned, etc..)
16. Method exclusion
17. Pesticide type (herbicide, insecticide, fungicide, NA)
18. Pesticide family (neonicotinoid, organophosphate, pyrethroid)
19. Pesticide active ingredient(s)
20. Database
21. Search string
22. Main findings for each life stage if applicable (Positive, Negative, No Effect, Inconclusive)

Study risk of bias assessment

Authors referred to the protocol to prevent bias in study selection (Page et al., 2021). Any uncertainties were discussed among the authors.

Synthesis methods

Independent results were created in our excel sheet for each independent variable (i.e. pesticide, mowing, grazing, burning) represented in the study (Supplemental Table 1). We first grouped the results by mechanical management or pesticide application. For publications assessing the effects of pesticide application on monarchs, we grouped studies by pesticide type, pesticide family, life stage and dependent variable (survival, fitness). Further, we discuss the results of the effects of pesticides in the categories outlined in Van Deynze et al. (2024) (weed control, reactive and prophylactic insect control) because these groupings capture when and how pesticides are used, as well as their mode of action. To clarify, prophylactic insecticides are often systemic insecticides applied to crops preemptively to deter damage from insects, while reactive insecticides are applied after insect damage is noticed for quick action, and are often delivered through aerial, ground, or foliar spraying, or fogging for broad or spot treatments (Van Deynze et al., 2024).

RESULTS

Study Selection

Following the protocol described in the initial phase of this study resulted in a combined 714 records using the two search strings across three databases. Records fitting our search criteria comprised approximately 6% of the overall monarch literature, which was returned using the search string “Monarch butterfly OR *Danaus plexippus*”, published since 2019 (pie chart, Figure 2). Next, we removed 219 duplicate products across databases and screened the remaining 495 titles and abstracts for relevance to our objective. The largest number of records were excluded

in this step (n = 452) leaving 43 records relevant to our objective to screen for full-text availability and further eligibility criteria. All 43 records had full text available, but 15 did not meet our criteria for inclusion or were theses or dissertations of which the relevant chapters had been published and collected by our search methods. This resulted in 29 articles relevant to our objective (flowchart, Figure 2).

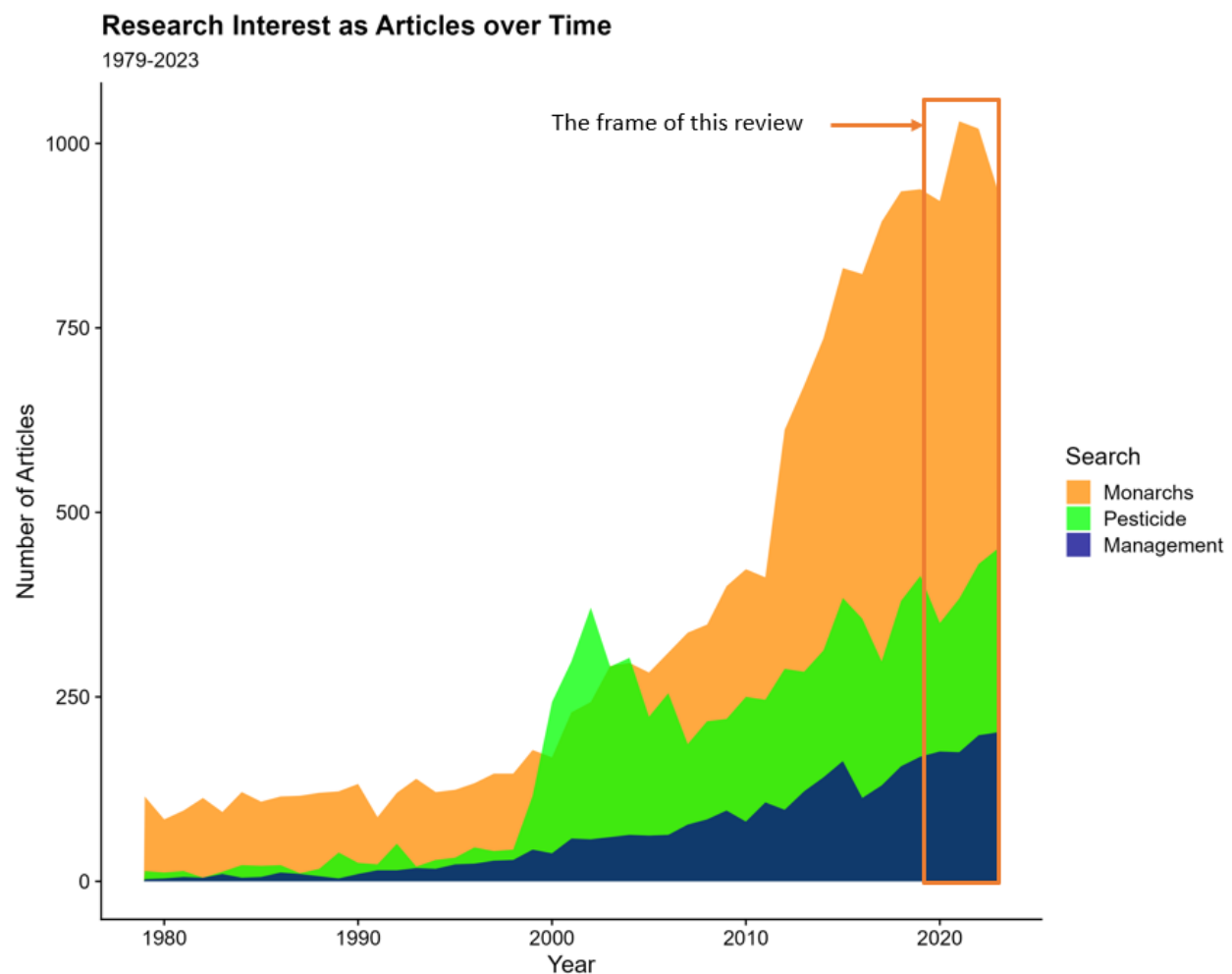


Figure 1. The number of search results from 1979-2023 on monarchs (orange) and using our search strings for pesticide use (green) and mechanical grassland management (blue) as a general indication of research interest in these topics over time. The frame of this review is indicated by the orange rectangle.

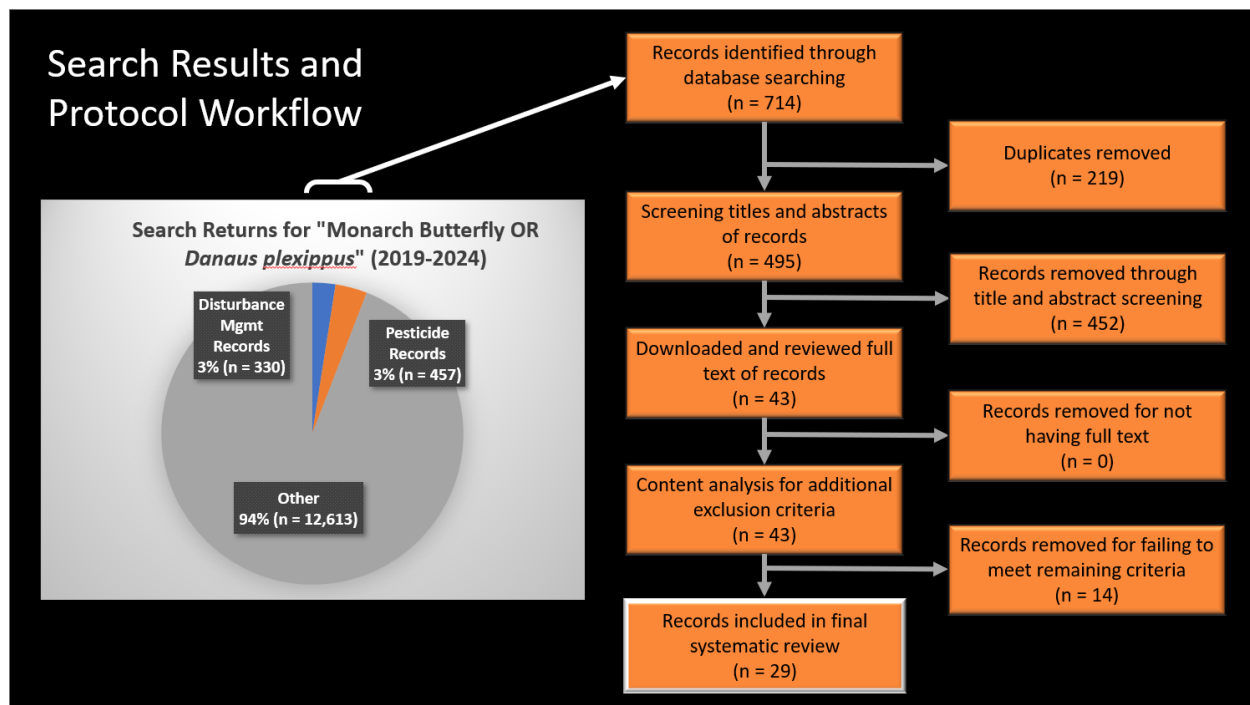


Figure 2. Pie Chart (left) illustrating the distribution of literature between 2019 and 2024 published on monarch butterflies in general compared to the knowledge gap associated with the direct effects of mechanical grassland management and pesticide use on monarchs. Flow Chart (right) of the screening process beginning with pooled records returned from our search strings (n = 714) and ending with the final records (n = 29).

Study Characteristics

Most studies were conducted in the North-Central United States (IA, IL, IN, MN, WI; n = 14), followed by the Southeast (GA, FL; n = 6), Canada (n = 3), Northeast (PA, n=2), and the Pacific Northwest (WA, n=1). Additionally, one study had a broader geographical focus spanning the Midwest, Northeast, and Southeast United States (Table 3). Collectively, all monarch life stages were studied, with the most comprehensive evaluation of the adult stage (Figure 3). Only two studies meeting our criteria assessed the effects of grassland management on monarchs, whereas the remaining 27 studies evaluated the direct effects of pesticide use on monarchs or aimed to quantify monarch pesticide exposure. Twenty-two studies were conducted in the laboratory, six in the field, and one study included both laboratory and field components. Ten of

the studies assessed wild monarchs, nineteen studies used lab-reared monarchs, and one study did not specify whether monarchs were wild or reared. Wild monarchs were either observed or collected in the field and studied at the same life stage in which the specimen was collected. Ten records measured monarch fitness (defined as any endpoint measured regarding the whole-organism performance including the health of the individual (Brownscombe et al., 2017; Orr & Garland, 2017) as the dependent variable, six measured survival and thirteen studies assessed survival and fitness.

Survival and Fitness Traits Tested Across Monarch Life Stages In Response to Pesticide Exposure

Egg  N = 3	Larva  N = 21	Pupa  N = 6	Adult  N = 19
Survival, days to hatch, size, mass	Survival, Weight, Length, Volume, Width, Herbivory, Growth Rate, Metabolic Rate, Signs of Intoxication	Survival, Weight, Development Time, Signs of Intoxication	Survival, Oviposition preference, Number of eggs laid, Emergence time, Mass, Wingspan, Distance flown, Flight (Duration, Speed Velocity), Grip Strength, Longevity, Metabolic Rate, Time to eclosion, Malformation, Successful Emergence, Behavior, Orientation

Figure 3. Traits tested in response to pesticide exposure by the articles reviewed in this search separated by monarch butterfly life stage. N indicates the number of studies that evaluated the designated life stage (Note: some studies monitored multiple life stages).

Table 3. Summary of studies investigating the effects of pesticides on monarch butterfly fitness and survival. Details on pesticide family, active ingredient, life stage evaluated, endpoints measured, study location, type, and origin of monarchs.

Pesticide Type	Pesticide Family	Pesticide Active Ingredient	Life stage	Endpoint Measured	Location	Study Type	Monarch Type	Citation
Insecticide	Neonicotinoid	Clothianidin	Adult	Metabolic rate, flight distance, flight velocity	PA	Lab	Reared	Cibotti 2024
			Adult	Orientation, vector strength, rate of travel, mass, forewing length	Canada	Lab	Reared	Wilcox et al. 2021b
			Larva, Adult	Survival from egg to 5th instar, number of eggs	Canada	Lab	Reared	Knight et al. 2021
			Egg, Larva, Adult	Larval length, width, mass, volume, Adult mass, number of eggs, egg size, egg mass	Canada	Lab	Reared	Wilcox et al. 2021a
			Larva, Pupa, Adult	Mortality rates, larval mass, larval metabolic rate, pupal mass, pupal metabolic rate, adult mass, adult resting metabolic rate, adult flight metabolic rate	PA	Lab, Field	Reared	Cibotti 2023
			Larva, Pupa, Adult	Larval growth, larval survival, pupal survival, adult mass	FL	Lab	Reared	Bargar et al. 2020
		Imidacloprid	Adult	Longevity, oogenesis	WA	Lab	Reared	James 2019
			Adult	Oviposition preference	IA	Lab	Reared	Mullins et al. 2021
			Larva, Adult	survival to adulthood, development time, egg size, egg number, adult body size	MN	Lab	Reared	Kobelia et al. 2020
			Larva, Pupa, Adult	Mortality, signs of intoxication	IA	Lab	Reared	Krishnan et al. 2021a

	Imidacloprid, Clothianidin	Adult	Survival, behavior, reproduction, flight performance, weight	GA	Lab	Wild, Reared	Prouty et al. 2023
	Imidacloprid, Clothianidin, Thiamethoxam	Larva, Adult	Larval growth rate, mass, adult mass, distance flown, flight duration, flight speed	GA, FL	Lab	Reared	Prouty et al. 2021
		Larva, Adult	Dietary exposure	IA	Field	Wild	Hall et al. 2022
Pyrethroid	Deltamethrin	Larva, Adult	Mortality, time to pupation, mass of chrysalis, adult malformation, successful adult emergence, adult mass	FL	Field	Wild	Giordano et al. 2020
	Bifenthrin, Beta-cyfluthrin	Larva	Mortality, weight, behavior	IA	Lab	Reared	Krueger et al. 2021
	Pyrethroid, Diamide, Organophosphate, Neonicotinoid	Larva	Spray drift mortality	IA	Lab	Reared	Grant et al. 2021
		Larva	LD50, weight	IA	Lab	Reared	Krishnan et al. 2020
		Egg, Larva, Pupa, Adult	Egg mortality, days to hatch, Larval mortality, arrested ecdysis, days to instar/pupa, larval/pupal weight, pupal mortality,		Lab	Reared	Krishnan et al. 2021b

		adult eclosion, adult weight, wing span, adult mortality
Bt	Bacillus thuringiensis	Larva 48 hr survival rate WI Lab Not reported Redmond et al 2020 Larva Exposure Midwest, Northeast, Southeast US Lab Wild Nunez-Mur et al. 2021
Herbicide	Chlorinated o-ansic acid Dicamba Nitrophenyl ether Fomesafen Phosphonate, Chlorinated o-ansic acid, Acetic acid Glyphosate, Dicamba, Acetic Acid	Larva, Pupa, Adult Larval weight, leaf area consumed, larval survival, pupa weight, adult wingspan, number of eggs laid IA Field Wild Saghi 2021 Larva, Adult Oviposition preference, instar mortality IA Lab Wild Lizotte-Hall et al. 2019 Egg Mortality of eggs, days until hatch GA Lab Wild Albanese 2019
Fungicide and other	Triazole, Pyrethroid, Neonicotinoid Propiconazole, Bifenthrin, Thiamethoxam Triazine, Triazole, Strobilurin, Neonicotinoid, Chloroacetamide,	Larva Dietary mortality, spray drift mortality GA, FL Lab Reared Greiner 2022

Chloroacetanilide, Pyrethroid, Phenylamide Strobilurin, Triazine, Neonicotinoid, Chloroacetanilide	Clothianidin, Thiamethoxam, Imidacloprid, Deltamethrin, Atrazine, Metolachlor, Acetochlor, 2-4D, Azoxytobin, Pyraclostrobin, Trifloxystrobin, Propiconazole, Metalaxyl, Difenocanazole	Larva	Potential contact and dietary exposure	IN	Field	Wild	Olaya-Arenas et al. 2019
	Clothianidin, Atrazine, S-metolachlor, Azoxytobin, Pyraclostrobin, Trifloxystrobin	Larva, Adult	Oviposition, foraging behavior	GA, IN	Lab	Reared	Olaya-Arenas 2020b
		Larva, Pupa, Adult	Larval weight, larval height, larval survival, herbivory, pupal weight, pupal survival, pupal development time, adult emergence, adult longevity, adult size	IL	Lab	Reared	Olaya-Arenas et al 2020a

We found 23 unique pesticide active ingredients evaluated for potential effects on monarchs across all studies representing 5 broad types of insecticides (Table 3). Among the studies investigating pesticides most evaluated insecticides (24), followed by herbicides (6) and fungicides (4). The 24 studies testing insecticides assessed the effects of neonicotinoids (20), pyrethroids (7), organophosphates (3), diamides (3), and *Bt* (2) on monarch survival and fitness.

Table 4. Breakdown of pesticide types, active ingredients, and insecticide families represented in the studies found in this review.

Pesticide Type	N	Active Ingredients/ Strains	Insecticide Family	N
Insecticide	24/27	Clothianidin, Imidacloprid, Thiamethoxam, Deltamethrin, Bifenthrin, Beta-cyfluthrin, Chlorantraniliprole, Chlorpyrifos, <i>Bacillus thuringiensis</i>	Neonicotinoid	20/24
Herbicide	6/27	Glyphosate, Dicamba, Acetic Acid, Fomesafen, Atrazine, S-metolachlor, Acetochlor, (2,4D)	Pyrethroid	7/24
Fungicide	4/27	Azoxystrobin, Pyraclostrobin, Trifloxystrobin, Propiconazole, Difenoconazole, Metalaxyl	Organophosphate	3/24
			Diamide	3/24
			<i>Bacillus thuringiensis</i>	2/24

Synthesis of Monarch Literature

Direct Effects of Mechanical Management Techniques

We found no studies that assessed the *direct* effects of common, non-chemical grassland management strategies on monarchs. As with studies published before 2019, results reported from this literature primarily evaluated management effects on mid-season milkweed stem regeneration for monarch larvae and oviposition (e.g. Alcock et al., 2016; Fischer et al., 2015; Smith & Cherry, 2014). However, the limited research evaluating indirect effects of grassland management on monarchs found monarchs preferentially oviposit on mowed milkweed depending on the time of year in relation to peak monarch abundance (Knight et al., 2019). For example, at ~43 N latitude, Knight et al. (2019) found summer mowing to be most effective when it occurred no more than once around mid-July, ideally leaving a few patches of mature

milkweed behind for asynchronous larval development. Additionally, mowing in specific months has been shown to increase monarch larval survival rates (Haan & Landis, 2020). Haan and Landis (2020) tested the response of monarchs to different mowing regimes and found that milkweed stems regenerating post-mowing had greater instar survival rates (July: 41.5% $\pm$ 4.8, August: 43.8% $\pm$ 6.2) compared to instars on unmowed milkweed (July: 16.7% $\pm$ 3.1, August: 18.9% $\pm$ 3.8).

Direct Effects of Pesticides

The response of monarchs to pesticide exposure can be highly variable and depends on monarch life stage, pesticide family, application method, and location and proximity of the monarch relative to the pesticide application area. However, of the 27 studies that tested either the direct effect of pesticide exposure on monarchs or assessed the degree or extent of exposure risk, 18 indicated pesticides had a negative effect on at least one, if not multiple, monarch fitness metrics at a specific life stage (James, 2019; Knight et al., 2021; Krishnan et al., 2020; Krishnan, Zhang, et al., 2021; Redmond et al., 2020). Several studies found no effect ($n = 3$) (Hall et al., 2022; Lizotte-Hall & Hartzler, 2019; Mullins et al., 2021) or had inconclusive results ($n = 3$) (Nunez-Mir et al., 2021; Saghi, 2021; Wilcox et al., 2021). Interestingly, one study found a positive effect on flight speed and distance of adults (Cibotti et al., 2024). Notably, all studies were done in the context of agricultural pesticide application.

Immature monarchs (i.e. eggs, larvae, pupae) include the life stages most susceptible to lethal effects from acute and chronic pesticide exposure both topically and via ingestion of plant tissue (Krishnan et al., 2021b). Early instar larvae (2nd and 3rd instars) are more susceptible to

mortality via acute exposure, whereas late instar (5th instar) larval mortality is more likely from chronic exposure causing sublethal effects or arrested pupal ecdysis (Krishnan et al., 2020; Krishnan et al., 2021a; Krishnan et al., 2021b). When adult monarchs are exposed topically to pesticides, especially reactive pesticides (organophosphates, pyrethroids, and diamides), within proximity of the application site, the effects can be highly lethal (Krishnan et al., 2021b). The risks associated with lethal effects from acute dietary exposure are challenging to assess in adult monarchs because there is limited information on the concentrations of pesticides found in nectar (Hall et al., 2022; Krishnan et al., 2021b). However, sublethal effects from chronic uptake of low pesticide concentrations (based on field-realistic concentrations) can negatively affect overall individual fitness in subsequent life stages (James, 2019). Two studies reported pesticide ingestion at the larval stage had sublethal effects on adult monarchs such as reduced body size and wing length (Kobiela & Snell-Rood, 2020; Olaya-Arenas, Hauri, et al., 2020). Further, there is evidence to suggest monarchs are likely chronically exposed to sublethal levels of a mixture of pesticides throughout their lifecycle (Olaya-Arenas & Kaplan, 2019).

Monarchs may have behavioral and genetic adaptive capabilities that provide some level of resistance to insecticides (Kobiela & Snell-Rood, 2020; Mullins et al., 2021; Olaya-Arenas, Scharf, et al., 2020), but more research is needed to clarify and support these findings. Two studies tested monarch oviposition preference to pesticide-treated milkweed, with one study reporting deterrence when the milkweed was treated with a mixture of 6 pesticides (Olaya-Arenas, Scharf, et al., 2020). However, the other study, which focused solely on milkweed treated with imidacloprid, found no difference in the number of eggs deposited between the control and test milkweed (Mullins et al., 2021). Collectively, the results of our synthesis indicate that while

monarchs are at a relatively low risk of acute lethal pesticide exposure, chronic, low-level exposure at the larval and adult stages increases the risk of sublethal effects.

Herbicides

We identified four studies that evaluated the direct effects of herbicides on monarchs (Albanese, 2019; Lizotte-Hall & Hartzler, 2019; Olaya-Arenas, Hauri, et al., 2020; Saghi, 2021) and two that assessed the extent of pesticide exposure risk (Olaya-Arenas, Scharf, et al., 2020; Olaya-Arenas & Kaplan, 2019). Collectively, these studies investigated impacts on monarch egg hatch rates, larval and pupal development, larval feeding behavior, larval survival, adult wingspan, and oviposition preferences for treated versus untreated milkweed plants.

Herbicide exposure poses complex, albeit indirect, risks to monarchs, primarily through damage or contamination of their host plants. Dicamba, increasingly used in herbicide-resistant cropping systems, caused significant injury to milkweed, including leaf malformation and cupping shortly after application (Saghi, 2021). This damage reduced monarch oviposition by up to 35% compared to untreated plants in one year of observation, though no effect was observed in the subsequent year. Notably, larvae fed dicamba-treated milkweed (27 days post-application) exhibited no significant effects on development metrics such as weight, pupal size, or adult forewing length (Saghi, 2021). Other herbicides, such as fomesafen, caused chlorosis and necrosis on milkweed leaves, with injury persisting for several weeks. However, no adverse effects on oviposition were observed in either field or greenhouse experiments (Lizotte-Hall & Hartzler, 2019)

Risks extend beyond indirect effects of herbicide-induced plant injury. Atrazine and S-metolachlor, also common agricultural herbicides, were detected on milkweed leaves at field-relevant concentrations, highlighting the potential for pesticide drift into non-target habitats (Olaya-Arenas & Kaplan, 2019). These herbicides negatively impacted larval feeding behavior and adult oviposition, particularly at higher concentrations. For instance, feeding aversion was observed in 1st instar larvae exposed to mean (9.7 ng/g, 1.36 ng/g) and maximum (329.5 ng/g, 15.6 ng/g) concentrations of both atrazine and s-metolachlor, respectively (Olaya-Arenas, Scharf, et al., 2020). Additionally, oviposition on milkweed treated with a pesticide cocktail (including atrazine and S-metolachlor) was reduced by 30% compared to controls (Olaya-Arenas, Scharf, et al., 2020). Direct herbicide contact also impacts monarch survival and fitness. For example, a study found that direct exposure to glyphosate (2%) and a combination of 2,4-D (7.59%) with dicamba (0.854%) significantly reduced larval hatch rates from 65% in control groups to just 6% in treated groups (Albanese, 2019).

In summary, these studies underscore the complexity of herbicide impacts on monarch butterflies. While some herbicides, such as dicamba and fomesafen, primarily harm milkweed with variable effects on monarch behavior and development, others, like atrazine and S-metolachlor, can directly influence monarch feeding and oviposition. The variation in impacts across herbicide types, concentrations, and monarch life stages highlights the need for careful management of herbicide use near monarch habitats (Lizotte-Hall & Hartzler, 2019; Olaya-Arenas, Scharf, et al., 2020).

Prophylactic Insecticides

Most of the studies assessing insecticides (n = 24) investigated effects of prophylactic insecticides (Neonicotinoids and *Bt* strains) on monarch survival and various fitness metrics including (but not limited to) development, behavior, mass, length, herbivory, emergence times, longevity and oviposition (James, 2019; Mullins et al., 2021; Olaya-Arenas, Hauri, et al., 2020; Prouty et al., 2023; Wilcox et al., 2020, 2021). Authors employed a variety of methods among these studies, making comparisons across studies challenging. However, several broad trends emerged. Overall, acute toxicity of prophylactic insecticides to monarchs appeared to be low, especially in comparison with reactive insecticides (organophosphates, pyrethroids, diamides) (Krishnan et al., 2021b; Krueger et al., 2021). Conversely, sublethal effects were noted in some form in almost all studies, although the need for more research to understand the potential population-level impacts of exposure to these chemicals was noted in numerous studies (Olaya-Arenas, Hauri, et al., 2020; James 2019, Bargar et al., 2020, Wilcox et al., 2021, Krueger et al., 2021, Kobiela et al., 2020, Prouty et al., 2023).

The effects of prophylactic insecticide exposure on monarchs depends on life stage, active ingredient, and exposure route. Larvae are more susceptible to dietary toxicity than adults, with ingested neonicotinoids specifically associated with reduced larval survival and development (Krishnan et al., 2020; Krishnan et al., 2021b; Prouty et al., 2023). Also, milkweed sprayed with a *Bacillus thuringiensis* ssp. *galleriae* (*Btg*) insecticide resulted in 97-100% mortality of early instar monarchs (Redmond et al., 2020). Acute and chronic dietary, and acute topical, toxicity studies demonstrated two potential modes of action for neonicotinoid-related monarch mortality. The first mode is mortality caused by arrested ecdysis (shedding of the cuticle). The second is

mortality before pupal ecdysis due to central nervous system neurotoxicity (resulting in cessation of feeding, paralysis, and bleeding) caused by much greater pesticide concentrations (Krishnan et al., 2021a; Krishnan, Zhang, et al., 2021). Overall, first larval instar monarchs experienced higher mortality rates associated with neonicotinoids than did fifth instars, but late fifth instar larvae were significantly more susceptible to mortality via arrested ecdysis (Krishnan et al., 2021a; Krishnan, Zhet al., 2021). Interestingly, when larvae mortality was caused by failed ecdysis, no symptoms were observed before death, leading the authors to hypothesize that exposure to neonicotinoids disrupts neuroendocrine signaling during pupal ecdysis (Krishnan et al., 2021). Notably, failed ecdysis occurred at field-relevant neonicotinoid concentrations (0.76 ug/g dietary exposure to 9.1 ug/g topical exposure imidacloprid caused >90% mortality in 5th instars via arrested ecdysis). Further, exposure to field-relevant doses of clothianidin (25 ng/g soil) from bioaccumulation of the chemical in the tissue of milkweed grown in clothianidin treated soil reduced monarch caterpillar length, width, and volume due to reduced herbivory (Wilcox et al., 2020). Imidacloprid concentrations that caused <70% mortality in larvae through dietary exposure also resulted in surviving larvae being developmentally delayed and significantly lighter in weight, specifically in 3rd instars (Krishnan et al., 2020).

Adult monarch butterflies exhibit greater resistance to neonicotinoids compared to immature monarch stages (Krishnan et al., 2021a; Wilcox et al., 2020, 2021). No acute, adverse effects on adult monarch survival or reproductive output (oogenesis) were observed after ingestion and topical exposure to field-relevant concentrations of neonicotinoids (James, 2019). However, monarchs still exhibit sublethal effects during the adult stage after exposure at the larval or adult stages (Krishnan et al., 2021a; Wilcox et al., 2020, 2021).

Sublethal effects of neonicotinoids on monarchs vary depending on the endpoints assessed and the mode of exposure. For example, two studies found that larvae are exposed to sublethal concentrations of clothianidin when feeding on milkweed tissues that have bioaccumulated the chemical via uptake from soil with field-relevant concentrations. (Bargar et al., 2020, Wilcox et al., 2020). Further, several sublethal effects resulted from dietary exposure to neonicotinoids at either the larval stage or adult stage, including reductions in longevity, body mass, flight metabolic rate, and the number of times males chose to mate (James, 2019; Prouty et al., 2023; Wilcox et al., 2021). However, no effects of neonicotinoids were detected for adult flight orientation, morphology, or rate of travel (James, 2019; Prouty et al., 2023; Wilcox et al., 2021). Interestingly, low-level exposure to clothianidin increased flight velocity and travel distance of adult monarch butterflies (Cibotti et al., 2024). These results contradict a prior study that reported lower flight metabolic rates in adults that fed on clothianidin-treated milkweed at the larval stage (Cibotti, 2023).

Neonicotinoid exposure can also alter adult reproductive behavior and elicit sex-specific responses, but results are widely variable. Egg counts in one study demonstrated no preference by females between milkweed stems treated with varying concentrations of imidacloprid and those sprayed with a control solvent. However, another study found females preferred pesticide-treated milkweed over non-contaminated milkweed stems (Knight et al., 2021; Mullins et al., 2021). Furthermore, other studies found a mixture of pesticides at a high concentration acted as a deterrent for monarch oviposition, with females responding negatively to increasing concentrations of imidacloprid, but not clothianidin (Olaya-Arenas, Scharf, et al., 2020; Prouty et al., 2023).

Studies assessing the effects of fungicides on adult monarchs also report a range of results and likely warrant further investigation. Results of one study showed a larva reared on fungicide-treated leaves exhibited a decrease in adult wing length by 12.5% but also reported increased longevity in the same individuals (Olaya-Arenas, Hauri, et al., 2020). The effects of fungicides on monarch larvae are also complex. Fungicide exposure alone can reduce larval survival, but in some cases, when mixed with other pesticides, the detrimental effects of fungicides are reduced (Greiner, 2022). Greiner (2022) found when the fungicide propiconazole was mixed independently with an organophosphate (chlorpyrifos) and a neonicotinoid (thiamethoxam), larval survival was higher in both cases compared to being treated with propiconazole alone (Greiner, 2022). Interestingly, the same lethal effect was not observed with the combined application of propiconazole and bifenthrin (a pyrethroid) (Greiner, 2022).

Reactive Insecticides

The direct effects of reactive insecticides (pyrethroids, organophosphates, and diamides) on monarchs were assessed by seven studies, which primarily evaluated survival at various life stages. Reactive insecticides were found to be the most lethal compounds when compared to other pesticides. Krishnan *et al.* (2021b) performed chronic and acute dietary toxicity studies and acute topical bioassays to establish the toxicity of various reactive pesticides to monarchs at different life stages and identified mechanisms of action (i.e. cause of mortality/signs of poisoning) for some pesticides. Acute LC50 values indicated that insecticide active ingredients beta-cyfluthrin (pyrethroid) and chlorantraniliprole (diamide) were most toxic to all monarch life stages (Krishnan, Zhang, et al., 2021). For example, topical application of imidacloprid (0.85 ug/cm²), chlorantraniliprole (0.64 ug/cm²), and beta-cyfluthrin (0.01 ug/cm²) to adult monarchs

resulted in 26%, 58%, and 100% mortality, respectively, after adjusting for control mortality rates (Krishnan et al., 2021b). Chlorantraniliprole is 50 to 500 times more toxic to monarch larvae than neonicotinoids and 3000 times more toxic than chlorpyrifos, and exposure to chlorpyrifos or chlorantraniliprole results in 10 to 20% fifth instar mortality due to arrested pupal ecdysis (Krishnan et al., 2021b). Another study investigating the impact of chronic ingestion of pyrethroids on monarchs found an inverse relationship between mortality and distance from the point of application of deltamethrin (i.e. deltamethrin concentrations), though even ultra-low volume treatments of deltamethrin had significant impacts on monarch mortality (Giordano et al., 2020). Larva reared within 25m of a roadside that experienced truck fogger pesticide (active ingredient deltamethrin) application were three times less likely to survive than those further away, and those that did survive experienced significantly longer development time (Giordano et al., 2020). Further, dietary exposure to pyrethroid insecticides (bifenthrin and beta-cyfluthrin) was found to affect the growth and development of fifth-instar larvae, reducing their 24-hour and overall diet consumption and ultimately leading to a reduction in body weight (Krueger et al., 2021). Greiner (2022) used AgDRIFT (an atmospheric model used to predict deposition patterns of pesticides (Teske et al., 2002)) Tier 1 aerial and ground spray drift assessment to estimate concentrations of propiconazole, thiamethoxam, chlorpyrifos and bifenthrin at 0, 5, and 10m from field edges and then conducted pesticide exposure experiments on monarch larvae at those concentrations. Larvae were exposed to an artificial diet containing pesticides at simulated concentrations and to the pesticides combined with the fungicide propiconazole. The study reported 0% survival rate from chlorpyrifos and

bifenthrin exposure at 0m and 5m but higher survival rates at 10m (21.9% and 6.7% respectively) (Greiner, 2022).

The reviewed studies indicate that reactive insecticides, such as pyrethroids, organophosphates, and diamides, are the most lethal to monarch butterflies across various life stages. Acute toxicity studies identified beta-cyfluthrin and chlorantraniliprole as particularly toxic, with chlorantraniliprole being vastly more toxic than neonicotinoids and chlorpyrifos. Mortality due to arrested pupal ecdysis was a common outcome. Proximity to spray points (concentration) significantly impacts monarch survival, as demonstrated by higher mortality and delayed development in larvae near treated areas. Additionally, dietary exposure to pyrethroids reduces caterpillar growth and weight. These findings emphasize the severe risks posed by reactive insecticides.

Exposure Estimates

Exposure estimates of insecticides and fungicides and survival rates were assessed through modeling exercises in five studies: three used toxicity data obtained from bioassays and dietary toxicity studies; one tested the toxicity of two pyrethroids independently; and one assessed interactions between fungicides and a suite of pesticides (Grant et al., 2021; Greiner, 2022; Krishnan et al., 2020; Krueger et al., 2021). All studies used AgDRIFT to predict deposition patterns of pesticides at a range of distances away from the field edge according to different application methods (aerial spray, high boom, low boom). One study assessed potential interactions between the fungicide propiconazole with a representative pyrethroid, organophosphate, and neonicotinoid (Greiner, 2022). Another investigated the potential exposure and 72-h toxicity of two pyrethroids on monarch larvae (Krueger et al., 2021). To build

a comprehensive understanding of monarch exposure and mortality risks, three pivotal studies were conducted, each contributing unique insights- from lab-based toxicity benchmarks (Krishnan et al., 2021b) to field-scale exposure estimates (Krishnan et al., 2020) – cumulating in a county level simulation model representing two common pesticide management regimes (Grant et al., 2021). The first management regime consisted of low ground-boom and high ground-boom application of beta-cyfluthrin, chlorantraniliprole, chlorpyrifos, and imidacloprid to prevent true armyworms (*Mythimna unipuncta*). The second management regime, employed for soybean aphid (*Aphis glycines*) control consisted of aerial and high ground-boom applications of beta-cyfluthrin, chlorantraniliprole, chlorpyrifos, imidacloprid, and thiamethoxam (Grant et al., 2021). Krishnan et al. (2021b) assessed field-scale risk via cuticular dietary exposure of 1st, 3rd, and 5th monarch instars to insecticides applied for soybean aphid and true armyworm control at 0, 15, 30, and 60m from the field edge. Grant et al. (2021) expanded upon Krishnan et al.'s (2021b) work by combining an agent-based model estimating monarch egg laying in various land-use types with pesticide drift deposition modeling and toxicity data to estimate monarch exposure and mortality at the county level. Further, Grant et al. (2021) estimated the resulting monarch population (representing one non-migratory breeding generation) under different scenarios of pesticide application frequency and monarch habitat availability.

These modeling exercises broadly indicate that pyrethroids and diamides, and specifically the active ingredients beta-cyfluthrin (pyrethroid) and chlorantraniliprole (diamide), are the most likely to negatively affect monarchs at all life stages (Grant et al., 2021). Additionally, aerial application was shown to be the most lethal application method for most pesticides, and

monarch mortality was projected to be significantly reduced with low boom application (Krishnan et al., 2021b). Both aerial and boom applications of beta-cyfluthrin and chlorantraniliprole are expected to kill nearly all exposed monarch eggs, pupae, or emergent adults up to 60m downwind of application (Krishnan et al., 2021b). A similar pattern was observed with aerial imidacloprid application, which resulted in an estimated 80% egg mortality within 60m downwind, with boom application reducing mortality to approximately 50% (Krishnan et al., 2021b). An agent-based model that estimated monarch populations under various habitat availability and pesticide application scenarios indicated that insecticide spray drift could diminish monarch populations (with the potential to nearly eliminate all monarchs within 38m in a generation) but was not expected to hinder landscape-level population growth in a year (Grant et al., 2021).

Another study conducted a modeling exercise to estimate the risk of the summer breeding monarch population overlapping with the timing of largescale *Bacillus thuringiensis ssp. kurstaki* (*Btk*) insecticide application in the Midwest and Northeastern United States (Nunez-Mir et al., 2021). Their results indicate a marginal temporal overlap between aerial insecticide spray application and monarch larvae presence. The study noted wide variation in monarch arrival date and a lack of information on the toxicity of *Btk* insecticides to monarchs at different life stages. Further, the authors warned that changes in plant and animal phenology driven by climate change may shift the timing of monarch arrival and insecticide application and thus practitioners may want to monitor those changes.

Two other studies attempted to quantify potential pesticide exposure to monarchs in agroecosystems by conducting field-based surveys of pesticide concentrations in milkweed

leaves, forbs, and/or soil either within or adjacent to agricultural lands (Hall et al., 2022; Olaya-Arenas & Kaplan, 2019). The first study detected 4 insecticides, 4 herbicides, and 3 fungicides at a range of concentrations over two years, and found no relationship between landscape composition and the likelihood of detecting pesticide residue (Olaya-Arenas & Kaplan, 2019). Notably, the pesticides thiamethoxam and deltamethrin were detected in > 75% of the samples in the second year of the study. The second study specifically assessed neonicotinoid presence in reconstructed prairies within, or adjacent to, maize or soybean fields and detected at least one neonicotinoid in 100%, 80%, and 80% of soil, blooming forb, and milkweed leaf samples respectively (Hall et al., 2022). Mean pesticide concentrations were relatively low, (i.e. generally well below the chronic LC₅₀) and occurrence did not differ significantly between seasons but did vary across years. The authors concluded monarch larvae were likely to experience chronic dietary exposure to a variety of pesticides in some years, but it was unclear what the effects were (Hall et al., 2022; Olaya-Arenas & Kaplan, 2019).

In summary, modeling exercises on pesticide exposure and survival rates of monarch butterflies reveal significant insights into the impacts of various foliar application methods for a representative selection of compounds.

Genetic and Host-Plant Mediated Resistance to Toxicity of Insecticides

Two studies noted indications of genetic factors that affect the resistance of individual monarchs to insecticide toxicity, specifically neonicotinoids (Kobiela & Snell-Rood, 2020; Krishnan et al., 2021b). Another found host plant properties may mediate the toxic effects of

clothianidin (Prouty et al., 2021). Lineage and sex were the main predictors of larval monarch survival given dietary exposure to imidacloprid, suggesting genetics and sex contribute to susceptibility (Kobiela & Snell-Rood, 2020). Adult monarchs, especially females, fed low levels of imidacloprid as larvae and had reduced body size, but this effect varied across maternal families. While conducting toxicity studies Krishnan et al. (2021b) also noted female monarchs were two and three times as susceptible to topical exposure of imidacloprid and chlorantraniliprole, respectively, as compared to male monarchs. Prouty et al. (2021) found evidence that larvae reared on milkweed host plants with higher levels of cardenolides were less susceptible to the toxic effects of clothianidin, though the mechanism behind this effect is unclear. The authors reared monarch larvae on three species of milkweed (*Asclepias syriaca*, *Asclepias incarnata*, *Asclepias curassavica*) treated with low and high dosages of clothianidin and imidacloprid. Negative effects on monarch development, survival, and grip strength were noted in the high-dose experiment with clothianidin (intended application dose: 51-70 ng/g, residue detected dose 4-70 ng/g). However, these effects were more prominent in larvae reared on *A. incarnata* compared to those reared on *A. curassavica*. The authors suggest the mechanism may be physiological and relate to the monarch's cardenolide tolerance, or be related to the chemical and physical properties of the milkweed itself (Prouty et al., 2021).

DISCUSSION

The 2020 Species Status Assessment for the monarch butterfly reported on five studies testing the effects of neonicotinoids on monarchs (Bargar et al., 2020; Pecenka and Lundgren 2015; Krischik et al., 2015; James 2019; Krishnan et al., 2020), two studies on organophosphates and

pyrethroids (Oberhauser et al., 2006; Oberhauser et al., 2009), and did not report studies evaluating direct effects of the some of the most widely used herbicides in the United States (glyphosate, atrazine, metolachlor, and 2,4 D), particularly in the Midwest (U.S. Department of Agriculture National Agricultural Statistics Service [USA NASS], 2021; 2023.). Our review summarizes an additional 20 studies on neonicotinoids, seven on pyrethroids, three on organophosphates, three studies on diamides, two on *Bt*, and a single study on each of the herbicides mentioned above. Additionally, although the SSA highlights increasing milkweed and diverse nectar resources as best management practices for monarch conservation, it lacks information on the direct effects of grassland management strategies on monarch populations. This overall information gap was highlighted in this review, which identified only two empirical studies examining how grassland management impacts monarch fitness or survival. Both studies found that the effects of grassland management practices were indirect, mediated through changes in host plants or nectar availability (Haan & Landis, 2020; Knight et al., 2019).

Grassland Management: A Double-Edged Sword

Historically, agricultural land management practices since the 1950's have contributed to monarch habitat loss and degradation (Boyle et al., 2019; Van Deynze et al., 2024; James, 2024; Malcolm, 2018; Pleasants & Oberhauser, 2013; Stenoien et al., 2018). Recent advancements in ecological knowledge provide opportunities to shift this paradigm and promote pollinator-friendly management practices that benefit both monarchs and broader grassland ecosystems. However, the lack of sufficient habitat and complex interactions of management practices present unique challenges.

Only 1% of the relevant search results for research that met our search criteria, specifically published between 2019 and 2024 and assessing monarchs and common grassland management strategies, evaluated the impacts of grassland management strategies (mowing, haying, grazing, or burning) on monarch fitness or survival. To determine whether this scarcity of research represented a recent or longer-term trend, we replicated our search methods to review papers published between 2010 and 2018. Similar to publications found in our original search, the most relevant publications (3% of search results) technically met the eligibility criteria for inclusion in our review, but reported effects of specific mechanical management strategies on floral and host plant resource availability and composition and were not examples of direct effects of grassland management on monarchs (Alcock et al., 2016; Baum & Sharber, 2012; Fischer et al., 2015).

Among the earlier published papers that met our search criteria, two assessed the effects of mowing (Alcock et al., 2016; Fischer et al., 2015) and one evaluated the impacts of fire (Baum & Sharber, 2012) on pre-migrant monarch oviposition. These publications reached conclusions similar to those published after 2019: namely that mechanical management strategies stimulating new growth of the host plant, *Asclepias* spp., can be beneficial for monarch reproduction (Haan & Landis, 2020; Knight et al., 2019). Increased monarch oviposition on newly regenerated host plants indicates younger milkweed plants are likely more attractive to ovipositing monarch females than mature milkweed. Additionally, all the papers highlighted the importance of the timing of management strategies, as milkweed regeneration must align with monarch migratory and reproductive phenology for it to be beneficial.

Most published research assessing the impacts of grazing and burning on butterfly communities has primarily been conducted in Europe, where grassland butterfly declines are well documented and the evaluation of conservation efforts is further along (Warren et al., 2021). Low to moderate grazing typically supports abundant butterfly populations, with rotational grazing showing potential benefits, although further research would be needed to confirm these effects (Warren et al., 2021). Variations in grazing and other management strategies can maintain heterogeneous landscapes and support diverse butterfly communities with a range of life history strategies and resource requirements (Bussan, 2022; Fumy & Fartmann, 2023; Schwarz & Fartmann, 2021). There is a positive relationship between livestock density and the presence of butterfly grassland habitat generalists, which indicates grazing pressure can affect not only butterfly abundance but also community composition (Kasiske et al., 2023). However, extensive grazing pressure has the potential to negatively affect monarchs, as livestock have been shown to graze on milkweed, resulting in lower stem counts in grazed areas compared to ungrazed areas (Dickson et al., 2023).

Fire - whether through prescribed burns or wildfires - has received attention for its potential to enhance population growth rates and increase population density in disturbance-dependent and prairie-specialist butterflies (Moranz et al., 2012; Warchola et al., 2018). However, research on fire effects remains limited, particularly due to lack of frequent fire severity data and other critical fire characteristics in studies (Mason et al., 2021). Growing season-prescribed burns have been shown to stimulate milkweed growth and attract greater numbers of ovipositing, pre-migrant monarchs, compared with unburned areas (Baum & Sharber, 2012).

One approach that combines the benefits of both fire and grazing is patch-burn grazing, which theoretically promotes a mosaic of successional vegetation stages across the landscape and benefits diverse butterfly species while supporting other grassland taxa and broader community dynamics (Geest et al., 2023). Some species such as the monarch may benefit from fire in grassland ecosystems every 1-2 years, whereas other species need longer intervals between burns (Geest et al., 2023). However, the resulting efficacy of patch-burn grazing for specific species is unclear (Moranz et al., 2012) and often depends on the interaction between fire frequency and severity of fire with grazing pressure (Dickson et al., 2023). The inherent variation in this combination depending on the ecosystem for which it is prescribed makes it challenging to replicate and observe subsequent patterns in species response (Larkin & Stanley, 2021; K. Oberhauser et al., 2017).

In conclusion, low-intensity grassland management techniques replicating natural processes, such as resetting successional dynamics, likely contribute to creating heterogeneous landscapes that support biodiversity (Kasiske et al., 2023, 2024). Asynchronous, patchwork management using diverse techniques holds the greatest potential to support both generalist species such as monarchs and grassland specialists (e.g. *Cercyonis pegala*, *Speyeria idalia*), enriching habitat diversity and ecological function (Leone et al., 2022; Moranz et al., 2012). Designing these landscapes to have high habitat connectivity supports the life history of mobile species like the monarch and creates a network suitable to support the metapopulation dynamics of various species during natural cycles of resource variation driven by climate or other natural factors (Augustine et al., 2021). However, these findings all represent indirect effects on butterfly communities, and all conclusions regarding the direct effects of fire (mortality by incineration)

and grazing (consumption/ trampling of eggs or larvae on milkweed by cattle) remain speculative.

Pesticides: A Growing and Ubiquitous Threat to Monarchs

Rapidly increasing pesticide use in the United States poses a major threat to monarch butterflies, with sublethal exposure affecting most monarch life stages and potentially contributing to their population declines. Pesticides have been consistently detected in surface water, groundwater, and soils across the United States, constituting a persistent threat to ecosystem integrity (Rani et al., 2021; Tudi et al., 2021). For example, several pesticides were detected in more than 90% of water and fish samples collected from streams in the US (Rose et al., 2018). There are over 800 active chemical components of pesticides belonging to more than 100 classes of chemicals that are available in a broad range of commercially assessable products (Nasiri et al., 2020). Recent studies have begun to quantify the widespread exposure of monarchs and other non-target insects to pesticides in agricultural areas through consumption of milkweed plant tissues and nectar contaminated at sublethal levels (James, 2024; Malcolm, 2018; Van Deynze et al., 2022; Wagner, 2020). Pesticides have been detected on milkweed leaves, within leaf tissues, and even in the nectar of milkweed flowers and other nectar resources (Hall et al., 2022; James, 2024; Naujokaitis-Lewis et al., 2024; Olaya-Arenas & Kaplan, 2019). Further, contamination from pesticides is not contained to agricultural areas. Wild pollinators foraging in conservation areas near agricultural land are also exposed to a range of pesticides at harmful concentrations (Halsch et al., 2020; Main et al., 2020). Proximity of remaining pollinator habitat to agricultural areas likely exacerbates the issue. For example, one study estimated that over 80% of monarch habitat in Story County, Iowa, was located within 38

meters of an agricultural field, bringing to question potential exposure from pesticide drift and volatilization (Grant et al., 2021).

A study on worldwide pesticide use reported that over a billion pounds of pesticides were used in the United States in 2021, with herbicides accounting for 73%, followed by insecticides (15%), and fungicides (7%) (Ritchie et al., 2022). Glyphosate, one of the most commonly used herbicides, is well-known for causing milkweed loss, but new research suggests glyphosate can directly and indirectly affect monarch survival by reducing hatch success, altering larval feeding behavior and deterring oviposition (Albanese, 2019; Olaya-Arenas et al., 2020). The reviewed study highlights the potential for significant negative effects of herbicides, particularly glyphosate and 2,4-D, on monarch butterfly eggs and larval development (Albanese, 2019). Atrazine and S-metolachlor also have detrimental effects, especially at higher concentrations, negatively impacting larval feeding behavior and adult oviposition (Olaya-Arenas et al., 2020). Even intermediate herbicide levels associated with pesticide drift commonly encountered in the field can deter monarch reproduction and development, as evidenced by altered larval feeding behavior and changes in adult oviposition preferences (Olaya-Arenas et al., 2020). Uncertainty surrounding the immediate and long-term effects of herbicide exposure, both direct and indirect, on monarch populations emphasizes the importance of careful management and herbicide application near monarch habitats. These research findings also demonstrate the complexity of herbicide impacts on monarchs and how ongoing research can help to better understand these effects and approaches for mitigating them.

The current degree of pesticide use in the United States poses a complex and substantial threat to monarchs, with exposure across all life stages likely contributing to their decline (Naujokaitis-

Lewis et al., 2024; Olaya-Arenas & Kaplan, 2019). The lack of readily available data on pesticide use further complicates efforts to fully understand and mitigate these risks (Douglas et al., 2022). Contrary to some expectations that pesticide use would decline if economic development occurred with a concurrent societal recognition of the ecological cost of industrial technologies, studies show a positive correlation between the two, suggesting that current agricultural and economic strategies will likely lead to continued growth in agrochemical demand (Hedlund et al., 2020). Addressing the pesticide threat to monarchs will likely require a multi-pronged approach, including increased transparency in pesticide use reporting, a better understanding of sublethal effects, and the development of more sustainable agricultural practices that reduce pesticide reliance.

Insecticides: Navigating Acute and Chronic Toxicity

Current research indicates insecticides pose a substantial and pervasive risk to monarchs. Both acute and chronic exposure have been shown to result in substantial mortality rates and a range of sublethal effects, which could have negative demographic consequences for monarchs across their multiple life stages. Acute exposure to reactive insecticides, such as organophosphates, pyrethroids, and diamides, results in the highest mortality rates across monarch life stages when compared to other insecticides (Giordano et al., 2020; Grant et al., 2021; Greiner, 2022; Krishnan et al., 2021b; Krueger et al., 2021). This finding is unsurprising given the purpose of reactive insecticides (for quick lethal effects on pests, typically lepidopterans) and the common practice of spraying that faces many challenges associated with drift (Bish et al., 2021).

However, one study suggested that given monarch metapopulation dynamics and the immense scale of monarch mobility, isolated instances of pesticide application may not have lasting

metapopulation-level effects, even though the effect of acute exposure to reactive insecticides may be deadly to exposed individuals (Grant et al., 2021). Greater threats arise within areas with persistent annual insecticide use, as continual monarch losses in these areas may result in population sinks (Grant et al., 2021).

While acute insecticide exposure risk may be relatively low, chronic exposure, particularly to neonicotinoids, is likely high and can lead to long-term sublethal effects and prepupal mortality. Documented sublethal effects include impaired growth, delayed development, and altered reproductive behavior, all of which undermine monarch survival and reproduction (Bargar, 2024; Bargar et al., 2020; Cibotti, 2023; Cibotti et al., 2024; James, 2019; Olaya-Arenas, Scharf, et al., 2020; Wilcox et al., 2021). Studies have shown that neonicotinoids accumulate in milkweed plants, leading to sublethal impacts on larvae reared on contaminated foliage (Bargar et al., 2020; Naujokaitis-Lewis et al., 2024). Larvae exhibit prepupal mortality after chronic exposure to low doses of clothianidin at concentrations between 5 to 47 ng/g leaf, which is within the range of concentrations associated with pesticide drift documented in the field (Bargar, 2024; Hall et al., 2022; Halsch et al., 2020; Knight et al., 2021; Olaya-Arenas & Kaplan, 2019). Larvae that ingested milkweed contaminated with neonicotinoids also exhibited reduced growth and delayed development, resulting in smaller adults with lower body mass (Bargar, 2024; Bargar et al., 2020; Wilcox et al., 2021). The impacts of neonicotinoids extend further to adult monarchs, reducing longevity and flight metabolic rate, and altering reproductive behavior (Cibotti, 2023; Cibotti et al., 2024; James, 2019. Olaya-Arenas, Sharf et al., 2020).

Limited research has documented how exposure to neonicotinoids and other insecticides affects key monarch behaviors, such as mate choice, resource selection, and migration, as well

as the potential transgenerational impacts of pesticides (Braak et al., 2018). Several studies investigated the effects of insecticide exposure on adult behavior, including oviposition preference, foraging behavior, and male mating behavior (Mullins et al., 2021; Olaya-Arenas, Scharf, et al., 2020; Prouty et al., 2023). Negative effects were not noted at lower concentrations, but researchers observed reduced flight speed, survival, reaction time, male mating success, and female oviposition at higher concentrations (Prouty et al., 2023). Monarch responses to insecticides are further complicated by genetic variability and sex. For instance, different maternal lines exhibit varying levels of resistance to neonicotinoids (Krishnan et al., 2021b). Further, female monarchs are particularly vulnerable to certain pesticides, including neonicotinoids and diamides (Krishnan et al., 2021b). The long-term effects of these selective pressures on monarch traits, such as morphology and behavior, remain unknown.

Preventative pest management is used widely in modern agriculture, often through the application of prophylactic insecticides that are integrated directly into crops (Van Deynze et al., 2024). This is typically achieved in two ways: by coating seeds with insecticides or by genetically modifying crops to produce their own insecticidal proteins. Although these methods are designed to protect crops from insect pests, the persistent and systemic nature of these insecticides also increases the risk of environmental contamination and unintended exposure to non-target organisms. A growing body of research indicates that these compounds pose significant risks to non-target ecosystems and species, especially invertebrates (Alsafran et al., 2022; Bonmatin et al., 2015). In addition to the immediate lethal effects resulting from the widespread use of these insecticides, the sublethal effects of chronic exposure could potentially undermine monarch populations (Braak et al., 2018). We find that this evidence of

sublethal effects across multiple monarch life stages could contribute to declining fitness, which raises concerns about the species' long-term viability, especially considering the multitude of environmental stressors they face.

Fungicides: Unmasking the Hidden Impacts

Fungicides are often overlooked in discussions of pesticide impacts, despite their critical role in agriculture and plant protection (Zubrod et al., 2019). Although only 4 studies considered for this review examined the effects of fungicides on monarchs, the collective findings suggest notable risks to monarch population viability, suggesting increased attention to this class of chemicals. Historically, research on pesticide impacts on monarchs has been disproportionately focused on insecticides (62%) and herbicides (24%), with fungicides representing only 13% of studies between 1991 and 2013 (Zubrod et al., 2019). This knowledge gap is important, as fungicides are central to agricultural crop protection strategies and are likely to remain so (Beckerman et al., 2023). Fungicides can be highly toxic to non-target organisms because they target basic biological processes that are not exclusive to fungi (i.e. energy and cell production) (Zubrod et al., 2019). Additionally, the long-term effects of fungicide exposure on insect fitness are poorly understood (Greiner, 2022; Margus et al., 2023; Olaya-Arenas, Hauri, et al., 2020). This lack of research, especially compared to the number of studies on insecticides and herbicides on non-target organisms, suggests that the impacts of fungicides on monarch populations may not be fully understood and could therefore be potentially underestimated (Zubrod et al., 2019).

Research indicates that fungicides can affect monarch morphology and longevity. In one study, exposure to azoxystrobin and trifloxystrobin (two common fungicidal active ingredients known for their broad-spectrum activity against fungal pathogens (Feng et al., 2020) during the larval stage reduced adult monarch wing length by 12.5%, while increasing life span in the same individuals (Olaya-Arenas, Hauri, et al., 2020). This illustrates the complex effects of fungicides on monarch physiology.

The varied impacts of fungicides on monarch behavior, morphology, and lifespan indicate that even sublethal exposures could have profound population-level consequences. When combined with other pesticides and environmental stressors, these effects may intensify the challenges facing monarchs. For example, body size and forewing length are important characteristics in migratory monarchs that improve flight efficiency while soaring long distances (Y. Li et al., 2016) and enable monarchs to store an ample supply of lipids to survive overwintering (Brower et al., 2006). Therefore, a 12% reduction in wing length and smaller body size combined with the added stressor of limited nectar resources makes for less efficient flight which has higher energy demands while the insect has less lipid storage capacity. Lipid storage is less essential during the migration, but only under the assumption that energy demands from soaring are reduced (Brower et al., 2006). A recent study using citizen science data to track roost size of migrating monarchs found that roost sizes have declined as much as 80% along the migration path during a time that was independent of climate and landscape factors (Davis et al., 2024). Given the precarious state of monarch populations, understanding the multifaceted effects of fungicides is can help to inform conservation strategies and sustainable agricultural practices. To better understand the impact of fungicides on monarchs, future research could focus on the long-term

and interactive effects of fungicides, explore alternative pest management strategies, and assess how environmental factors influence monarch exposure. Addressing these information gaps can help to mitigate further monarch population declines.

Bridging the Gap Between Research and Conservation

Successful conservation of migratory monarchs likely requires more than habitat restoration – comprehensive and effective conservation planning that addresses persistent, pervasive stressors such as pesticide exposure and climate change will be important for slowing monarch population declines. The lack of studies addressing the direct impacts of common grassland management practices (burning, mowing, haying, grazing) on monarchs currently hinders optimal habitat management and population recovery efforts. Monarch populations, like most insect populations, experience unpredictable boom-bust cycles, which affects our ability to discern between natural fluctuations and environmentally driven declines and underscores research gaps in understanding of role environmental factors such as pesticide exposure and climate change have on monarch populations (Thogmartin et al., 2020).

A partnership between state fish and wildlife agencies, local partners, and landowners, called The Midwest Association of Wildlife Agencies, created the 2018 Mid-America Monarch Conservation Strategy aimed to avoid listing the monarch butterfly through the USFWS Endangered Species Act through habitat conservation. After the 2020 listing decision was “warranted but precluded”, the strategy shifted to aiding population recovery (Midwest Association of Wildlife Agencies, 2023). The 2023 update continues to focus on adding 1.3 billion milkweed stems and associated flowering nectar resources to the North Core

conservation unit (Midwest U.S.), especially on lands in UDSA cost-share programs (CRP, WRP, ACEP). Despite progress, only 14% of the milkweed stem goal has been met as of June 2023 (Midwest Association of Wildlife Agencies, 2023). Moreover, there is no evidence that these efforts have led to a sustained increase in monarch populations (Rendón-Salinas et al., 2024; Thogmartin et al., 2020). Furthermore, the USFWS population model indicates that even under best-case scenarios of habitat restoration, population growth may not overcome declining trends (U.S. Fish and Wildlife Service, 2020). Although the Mid-America Conservation Strategy acknowledges the pervasive presence of pesticides on the landscape and continued exposure to insecticides as a primary driver affecting the health of monarchs, there is little guidance about pesticides and best management practices. Simply putting milkweed on the ground is likely insufficient based on the evidence synthesized for this review.

Globally, land conversion, climate change, and agricultural pesticides are likely the leading causes of insect declines, and monarch declines in the U.S. are no exception (Van Deynze et al., 2024; Rani et al., 2021; Wagner, 2020). A recent review of studies examining the nature of monarch declines concluded the expected primary drivers have shifted from overwintering and breeding habitat loss driven by deforestation and glyphosate use (pre-2005) to climate change and pesticide (particularly insecticide) exposure today (2005 – 2022) (Shirey & Ries, 2023). This shift in factors influencing monarch populations is evident in research showing that climate change likely alters monarch ecology (i.e. migration timing, overwintering timing and locations, core breeding habitat), and the growing body of evidence suggesting monarch fitness and survival are likely being impacted via pesticide exposure as demonstrated through this review and others (Belsky & Joshi, 2018; Crossley et al., 2022; Culbertson et al., 2022; James, 2024;

Malcolm, 2018; Naujokaitis-Lewis et al., 2024; Steele et al., 2023). Habitat restoration will likely be ineffective in conserving monarch populations on a broad scale unless combined with conservation strategies and broader policies designed to address widespread environmental threats such as chronic pesticide exposure and climate change. Efforts to ban or restrict pesticide usage globally have demonstrated the potential for safer agricultural practices that can help to recover insect biodiversity and ecosystem services, further highlighting the general and pervasive threat of pesticide use to pollinators (Quandahor et al., 2024). Without this holistic conservation approach, habitat improvements at small scales will likely be insufficient for the recovery of monarch populations. Aside from the knowledge gaps identified in this review, recent studies highlight additional insights that could help to improve monarch conservation efforts. For instance, to clarify the assumption that the number of milkweed stems and monarch populations are directly and positively correlated, targeted research could be conducted to improve our collective understanding of monarch biology, ecology, and environmental pressures (Yang, 2023). Another area of research is a more temporally explicit examination of seasonal changes in monarch-milkweed interactions (Yang, 2023). Specifically, factors such as the complex spatial distribution of milkweed, density-dependent competition, and search time limitations are areas that could benefit from further investigation (Crone & Schultz, 2022). There are also limitations in monarch abundance data across their range, although advanced statistical methods are addressing some of these gaps (Davis et al., 2024; Farr et al., 2024). Addressing seasonal data gaps, particularly in the spring, could improve population estimates at broader spatial and temporal scales (Farr et al., 2024). Further, this information could improve understanding of how monarch populations will continue to respond to novel climate

conditions (Zylstra et al., 2022). Community science programs, including monitoring and marking individual monarchs, have been integral in assessing monarch population dynamics and will likely continue to be a valuable resource for addressing these information gaps (Kasiske et al., 2024; Wali et al., 2017).

While this review includes information on the growing body of research investigating the effects of pesticides on monarchs in the Midwestern United States, as well as some representation in the Northeast and Southeast, there is an apparent lack of research on this topic in the Southern United States where migrants traverse before they overwinter and return to as a final stop to lay eggs for the next summer breeding generation. This data gap is particularly important as recent evidence points to spring monarch abundance as a strong predictor of overwintering counts (Zylstra et al., 2021). Data gaps in pesticide use at management-relevant scales (e.g., landowner scale), combined with growing evidence of environmental persistence of pesticides and subsequent threat to insects, indicate the need for more ecologically relevant metrics for monitoring environmental chemicals to better characterize contamination of milkweed and nectar resources (Bargar, 2024; Douglas et al., 2022; James, 2024; Rani et al., 2021; Yang, 2023).

It is important to note that almost all the studies in this review were conducted in a laboratory, with monarchs reared under controlled conditions. Three collective assumptions of these studies are that monarchs encountering pesticides are not already nutritionally stressed, are not infected with the parasite *Ophryocystis elektroscirrha* (OE), and are exposed to pesticides at consistent rates and concentrations. Thus, these lab studies could underestimate the resiliency of wild monarchs or overestimate the consistency of exposure to pesticides at certain concentrations. Additionally, the toxicity bioassays done in the research we reviewed did not

assess the combined risk of topical and dietary exposure for larvae and adults, which is a likely scenario for monarchs in the wild. Addressing these gaps could not only enhance population models but, when combined with mechanistic experimentation, may help identify causal links between monarch declines and their drivers (Grace, 2024; Pocius et al., 2022; Shirey & Ries, 2023; Yang, 2023).

Limitations of the Review

The objective of this review was to synthesize empirical research published after the initial development of the monarch SSA in 2020. Therefore, any publications with relevant information published before 2019 and not included in the SSA are not systematically represented here. This review was conducted only using a single search string for each type of management investigated instead of a combination of search strings. Additionally, given the sheer magnitude of records returned by some search string and database combinations, we decided to create a threshold of papers to be reviewed, so some relevant records may have been missed in this step. However, because the papers were sorted by relevancy, we expect to have addressed the research most relevant to our objective.

Conclusions

The preponderance of research synthesized for this review indicates monarch butterflies experience sublethal effects from chronic pesticide exposure, but assessing the full impacts of these effects proves to be a major challenge. However, if pesticide use continues at current levels or greater, it could present a persistent, widespread threat to monarch populations that is unlikely to be mitigated by any amount of habitat management or by the creation of new

habitat. Considering growing conservation needs, a holistic approach is increasingly recognized as essential for addressing the complex drivers of monarch population declines. Rather than focusing solely on species-specific interventions, conservation could prioritize addressing broader factors like land use conversion, climate change, and pesticide exposure (Yang, 2023). These upstream drivers shape ecosystems and are considerations when developing conservation strategies to enhance ecological resilience and recovery (Nunez-Mir et al., 2021; Rajski & Papalambros, 2021). By focusing on ecosystem-scale processes—such as species interactions, human impacts, and functional diversity—conservation strategies can better support the long-term recovery of monarchs and other migratory and resident grassland-dependent species. This shift emphasizes the importance of understanding natural resilience mechanisms over direct manipulation of species populations, which can lead to uncertain outcomes (Wali et al., 2017). Integrating principles from landscape ecology and social-ecological systems offers opportunities to improve biodiversity and land use management by transitioning from static to dynamic land use frameworks (Killion et al., 2018). Although this approach requires ongoing monitoring, it has the potential to enhance human-wildlife coexistence and strengthen both ecological and social resilience in a changing world.

OTHER INFORMATION

Registration and Protocol

The protocol for this review was not registered online but is available as supplemental material for this review.

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Competing Interests

The authors declare no competing interests.

Availability of Data, Code, and Other Materials

The comprehensive data file containing information on all studies collected, metadata, and other data extracted from the studies considered for this review is available as supplemental material for this review. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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