Monarch Butterfly Ecology, Behavior, and Vulnerabilities in North Central United States Agricultural Landscapes

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*The North American monarch butterfly (*Danaus plexippus*) is a candidate species for listing under the Endangered Species Act. Multiple factors are associated with the decline in the eastern population, including the loss of breeding and foraging habitat and pesticide use. Establishing habitat in agricultural landscapes of the North Central region of the United States is critical to increasing reproduction during the summer. We integrated spatially explicit modeling with empirical movement ecology and pesticide toxicology studies to simulate population outcomes for different habitat establishment scenarios. Because of their mobility, we conclude that breeding monarchs in the North Central states should be resilient to pesticide use and habitat fragmentation. Consequently, we predict that adult monarch recruitment can be enhanced even if new habitat is established near pesticide-treated crop fields. Our research has improved the understanding of monarch population dynamics at the landscape scale by examining the interactions among monarch movement ecology, habitat fragmentation, and pesticide use.*

Keywords: monarch butterfly conservation, spatially explicit agent-based modeling, movement ecology, pesticide toxicology, habitat connectivity

The monarch butterfly (*Danaus plexippus*) is native to North America, with populations west and east of the Rocky Mountains (see box 1). Eastern monarchs, the focus of this article, migrate from the United States and Canada in the fall to high elevation oyamel fir (*Abies religiosa*) forests, predominantly in Michoacán, Mexico (Brower et al. 2012). In late winter and early spring, overwintering adults in Mexico break reproductive diapause and migrate to Texas and Oklahoma, where they lay eggs and then expire. Adults from the new generation migrate in late spring to the North Central and Northeast regions of the United States and southern Canada. During the summer months, there may be two to four nonmigratory breeding generations. Adults that eclose in mid to late August and September enter reproductive diapause and migrate to Mexico (Oberhauser et al. 2017). Stable isotope analyses of monarchs collected in the overwintering forests suggest 38%–56% originate in the North Central region of the United States (Wassenaar and Hobson 1998, Flockhart et al. 2017).

An individual monarch's life cycle consists of four life stages, including the egg (3–4 days), larva (five instars, 9–14 days), chrysalis (pupa, 9–15 days), and adult (Oberhauser 2004). In the wild, breeding adults live 10–14 days (Zalucki and Lammers 2010, Zalucki et al. 2016). Larvae develop only on milkweed (*Asclepias* spp.), but adults feed on nectar from a wide variety of forbs. Breeding adult females are vagile (figure 1), moving extensively among milkweed and blooming wildflowers in the landscape (Zalucki and Lammers 2010). At the landscape scale, the spatial arrangement of habitat with different densities of milkweed and forbs can influence the number of eggs a female lays in its lifetime (i.e., realized fecundity; see Zalucki et al. 2016, Grant et al. 2018). When migrating in the spring, monarch butterflies can fly up to 50 kilometers (km) per day (https://monarchjointventure.org), but when breeding and laying eggs, females fly up to 1.5 km per day, resulting in 10–15 km net lifetime displacement depending on the landscape (Zalucki and Kitching 1984, Zalucki and Lammers 2010, Zalucki et al. 2016). Highly mobile species such as the monarch have open population

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Box 1. The global reach of monarch butterfly populations.

Despite differences in migratory behavior and overwintering sites, the eastern and western migratory North American monarch butterfly population is considered panmictic (Pyle 2015). The studies to date have not revealed significant genetic differentiation between the monarchs collected in overwintering habitats in Mexico and California on the basis of neutral markers from nuclear DNA (Lyons et al. 2012, Pierce et al. 2014, Zhan et al. 2014, Pfeiler et al. 2017, Talla et al. 2020) or mitochondrial DNA (Brower and Jeansonne 2004, Servín-Garcidueñas and Martínez-Romero 2014, Pfeiler et al. 2017). Monarchs that do not migrate reside in southern Mexico, Central America, Florida, the Caribbean, Hawaii, Australia, New Zealand, and Southern Europe. These populations are genetically differentiated from migratory North American populations (Lyons et al. 2012, Pierce et al. 2014, Zhan et al. 2014, Pfeiler et al. 2017). The North American migratory monarchs account for approximately 90% of the worldwide population (USFWS 2020).

structures (Zalucki and Lammers 2010, Zalucki et al. 2016) and a greater capacity to colonize unoccupied or new habitats in fragmented landscapes than do species that have closed or metapopulation structures characterized by limited movement between sites (Wiens 1997, Thomas 2016).

The eastern and western North American monarch populations have experienced population declines of approximately 80% and 99%, respectively, over the past three decades (Brower et al. 2012, Pleasants and Oberhauser 2013, Semmens et al. 2016, Pelton et al. 2019, USFWS 2020). On the basis of these declines and anticipated future declines, the US Fish and Wildlife Service (USFWS) recently categorized the North American monarch as a candidate species for listing under the Endangered Species Act (ESA; USFWS 2020). Multiple factors associated with monarch butterfly decline include weather conditions, loss of overwintering and breeding habitat, loss of floral (nectar) resources during the spring and fall migrations, and pesticide use (Brower et al. 2012, Pleasants and Oberhauser 2013, Flockhart et al. 2015, Inamine et al. 2016, Oberhauser et al. 2017, Boyle et al. 2019, Pelton et al. 2019, USFWS 2020). Conservation of the eastern monarch is currently approached from multiple perspectives based on conditions in the Southern Plains and the North Central states (Flockhart et al. 2015, Oberhauser et al. 2017, USFWS 2020). Up to half of the overwintering monarchs originate in the North Central region of the United States (Wassenaar and Hobson 1998, Flockhart et al. 2017), making conservation actions in this region especially important, including an emphasis on establishment of blooming forbs for adult feeding and milkweed to support larval development (USFWS 2020).

Sustaining the breeding generations of the eastern monarch butterfly will require a significant conservation effort in the North Central region of the United States (Flockhart et al. 2015, Oberhauser et al. 2017, Zaya et al. 2017, USFWS 2020), including establishment of an estimated 1.3–1.6 billion milkweed stems over the next 10–20 years (Pleasants 2017, Thogmartin et al. 2017). This goal can be reached only with substantial habitat establishment in agricultural landscapes, which represent approximately 75% of available land (Thogmartin et al. 2017). Maize and soybean fields account for 75% of this agricultural land cover (USDA-NASS 2020). The areas available for establishment of milkweed and nectar resources include rural roadsides, marginal croplands, portions of existing Conservation Reserve Program (CRP) lands, pastures, grasslands, and grass borders of maize and soybean fields (Thogmartin et al. 2017). Figure 1 illustrates a typical North Central landscape a monarch female faces in finding milkweeds to lay eggs and the issues associated with establishing milkweed for conservation purposes.

The USFWS monarch Species Status Assessment (USFWS 2020) concluded that exposure to insecticides and herbicides in agricultural landscapes are threats to population recovery. To address this concern, the US Department of Agriculture Natural Resource Conservation Service recommends limiting establishment of new monarch habitats to areas beyond 30–38 meters (m; 100–125 feet) of treated crop fields to reduce pesticide exposure to milkweed leaves, wildflower nectar, and monarchs themselves (USDA-NRCS 2016, USDA-NRCS 2018). Although reducing pesticide exposure, implementing this recommendation would substantially reduce the amount of land available for habitat establishment. For example, in Story County, Iowa, a 38-m buffer would reduce noncrop landcover and road right of ways available for habitat establishment by 38% and 84%, respectively (Grant et al. 2021).

To assess the conservation costs and benefits of establishing habitats for flower-visiting insects in close proximity to crop fields, Uhl and Brühl (2019) and Topping et al. (2020) noted the need for spatially explicit landscape-scale assessments. This perspective is consistent with broader calls for research addressing how animal movement within the landscape influences habitat use (Lima and Zollner 1996, Mueller and Fagan 2008, Nathan et al. 2008, Mueller et al. 2011, Turner and Gardner 2015, Wallentin 2017). Our research is a significant step toward addressing the information needs highlighted by these authors.

Research to Support Monarch Conservation

In this Overview, we synthesize findings from a large, multifaceted project designed to determine how habitat fragmentation, the spatial configuration of habitat, habitat quality, and pesticide use in a landscape dominated by maize and soybean production interact to influence the size of the eastern monarch's breeding population in the North Central region of the United States (figure 2). A model-guided approach (Restif et al. 2012) was used to study interactions across biological, spatial, and temporal scales based on the current understanding of monarch biology, behavioral ecology, ecological modeling, environmental toxicology, risk

Figure 1. A breeding female monarch butterfly surveys a midsummer agricultural landscape in the North Central region of the United States. The landscape includes soybean fields, maize fields, and roadside vegetation. To lay its eggs, the female must find milkweed plants, the only plants larvae will eat. The inset shows a larva on roadside milkweed. Female monarchs lay only a few eggs on a cluster of milkweed plants before moving on to find more milkweed. In the summer, the female, the eggs she lays, and the subsequent larvae could be exposed to insecticide spray drift from the crop duster plane depicted in the background. Earlier in the season, herbicide spray drift and runoff of insecticides used as maize and soybean seed treatments could potentially reduce the quality of milkweed and negatively affect larval development and survival, respectively. Establishing new monarch habitat in North Central agricultural landscapes is a key component in federal and state conservation plans. How can we develop and use knowledge about the monarch's life history, movement behavior, and responses to pesticide exposure to support habitat restoration efforts that maximize population growth?

assessment, and pest management. We illustrate how modeling coupled with experimental and observational research provides a powerful approach to evaluate and predict conservation benefits and risks of habitat establishment options in spatially explicit agricultural landscapes.

Using figure 2 as a reader's guide, we first summarize the development of a spatially explicit, agent-based model that simulates female movement and egg laying (in the top right of figure 2). Outputs of this model are linked to a Bayesian life-stage model that estimates background survival probabilities from the egg through the adult stage (see the bottom right of figure 2; see the "Spatially explicit landscape-scale simulation of adult monarch production" section). Using this modeling framework, we had the means to estimate adult monarch recruitment on the basis of different habitat establishment scenarios in spatially explicit landscapes. However, to simulate monarch flight patterns, we relied on expert elicitation (see the acknowledgments section in Grant et al. 2018) for values of several key parameters that underlie monarch flight behavior. To assess the strengths and limitations of these model assumptions, we undertook a series of studies to empirically quantify monarch flight behavior and habitat use (see the top left of figure 2 and the "Monarch movement ecology and habitat use" section). With increased confidence in our model assumptions, combined with the generation of life-stage-specific toxicity data and field-scale exposure estimates for commonly used herbicides (see the center left of figure 2) and insecticides (in the bottom left of figure 2), we could simulate landscape-scale monarch population growth on the basis of different assumptions of habitat establishment rates at different distances from maize and soybean fields with different pesticide use patterns (see the "Evaluating risks of pesticide use to milkweed and monarchs" section). In the conclusion, we summarize key findings.

Spatially explicit landscape-scale simulation of adult monarch production

Models are useful for studying aspects of monarch ecology that cannot be observed or measured directly (Grant and Bradbury 2019). Millions of individual monarchs of multiple generations move through the North American continent annually. Because we cannot know the location and activities of each monarch, we rely on different categories of models to understand system dynamics and predict the system state under different scenarios (see box 2). Significant advances have been made in modeling continental-scale monarch population dynamics, including migration to and from Mexico (e.g., Flockhart et al. 2015, Oberhauser et al. 2017). Valuable insights have also been gained from research at the field scale—from 1 square meter plots of milkweed to a few hectares of prairie—where monarchs are amenable to direct observation (Fisher et al. 2020a, Zalucki and Kitching 1982a, Fisher and Bradbury 2021). But little has been published on midscale (i.e., county, state) population processes that link field and continental scales. Important midscale questions include how nonmigratory, reproductive female monarchs move over North Central landscapes, how they find milkweed for oviposition, how

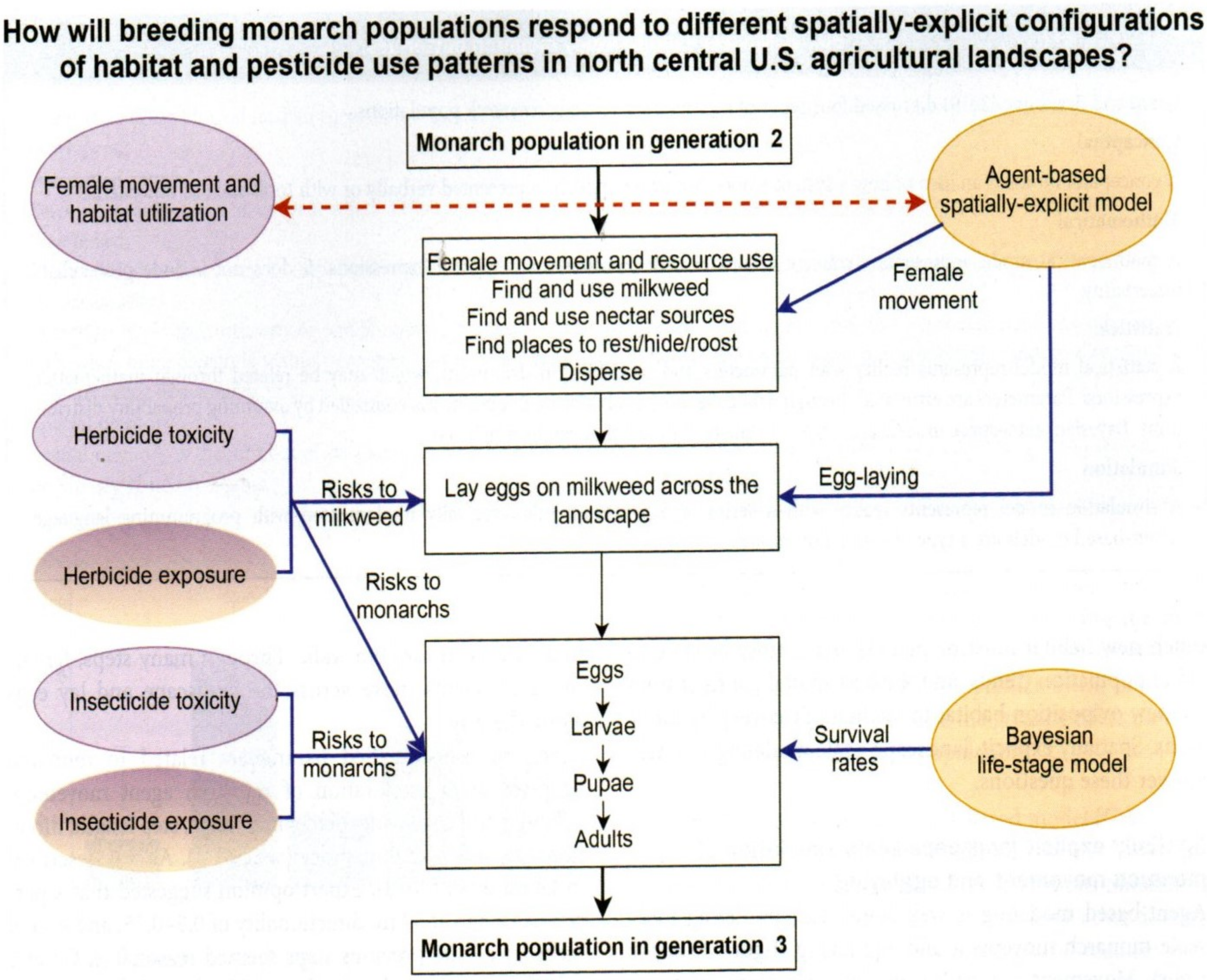


Figure 2. A research scheme that depicts integration of information needed to simulate changes in monarch population size on the basis of spatially explicit habitat restoration options and pesticide use patterns in agroecosystems of the North Central region of the United States. The boxes in the top and bottom of the center column of the figure denote the adult monarch population size in generation 2 and generation 3, respectively. The production of adult monarchs in generation 3 depends on the ability of females in generation 2 to detect and use milkweed in the landscape, the resultant number of eggs laid, and the respective life-stage survival rates from egg through adult (see the sequence of boxes in the center of the figure). Simulation of monarch population size requires empirical data from experimental and observational studies (the purple ovals), as well as information from models (the tan ovals). The design of empirical and modeling research is coordinated to incrementally refine understanding of processes and expand knowledge bases. The red arrow at the top of the figure highlights the importance of linking the development, evaluation, and refinement of an agent-based spatially explicit model (the tan oval at the top right of the figure) that simulates female movement and egg laying (see the "Spatially explicit landscape-scale simulation of monarch movement and egg laying" section in the text) with empirical studies of female movement and habitat use (the purple oval at the top left; see the "Monarch movement ecology and habitat use" section in the text). To estimate background life-stage-specific survival rates from eggs through adults, a Bayesian life-stage model (the tan oval at the bottom right) was developed using data from monarch monitoring studies (see the "Bayesian life-stage model" section in the text). The spatially explicit agent-based model combined with the Bayesian life-stage model provides the means to simulate monarch population sizes of generation 3 (see the "Simulating adult monarch production" section in the text). To address the extent to which pesticide use could influence default assumptions in the agent-based and Bayesian models (see the "Evaluating risks of pesticide use to milkweed and monarchs" section in the text), empirical studies were undertaken to assess the effects of representative herbicides on milkweed quality and resultant female use and larval development effects (the purple toxicity oval and the purple and tan gradient oval, center left). The risks of representative insecticides to monarch survival were estimated on the basis of results from empirical toxicity studies combined with empirical and modeled insecticide exposure data (the purple and purple and tan ovals, respectively, bottom left). By incorporating findings from empirical and modeling studies, population sizes of generation 3 can be simulated under different assumptions of habitat establishment rates and pesticide use patterns (see the "Simulating landscape-scale insecticide effects on monarch populations" section in the text).

Box 2. Models used to study monarch butterflies.

Grant and Bradbury (2019) discussed four types of models used to study monarch populations.

Conceptual

A conceptual model is an idea of how a system works. It may be implicit, represented verbally or with tools such as flowcharts.

Mathematical

A mathematical model represents a conceptual model with equations or analytical expressions. It does not include probabilistic uncertainty.

Statistical

A statistical model represents reality with parameters and probability distributions, which may be related through mathematical expressions. Parameters are estimated through data collection. Probabilistic uncertainty is controlled by assuming probability distributions. Bayesian state–space models are a type of statistical model that can be employed.

Simulation

A simulation model represents reality with a series of algorithmic rules, typically implemented with programming language. Agent-based models are a type of simulation model.

much new habitat must be planted per county or state to affect population trends, and the best spatial configuration for new oviposition habitat to facilitate discovery by monarchs. Spatially explicit, landscape-scale modeling can help answer these questions.

Spatially explicit landscape-scale simulation of monarch movement and egglaying

Agent-based modeling is well suited for simulating mid-scale monarch movement and egg laying (figure 2, upper right). Movement is a critical model component as it dictates how monarchs use the landscape (Nathan et al. 2008, Zalucki et al. 2016). By modeling this mechanism explicitly, we gain important insights into how they use the landscape. In agent-based models, individual agents act independently, allowing emergent behavior to arise that cannot be predicted or modeled through other means. Monarch agents in our original model (Grant et al. 2018) move within a geographic information system (GIS) model of the Story County, Iowa, landscape (148,665 ha) during the months of July and August. Using aerial imagery, the landscape model was developed by dividing the landscape into 17 landcover types. Each distinct area of a landcover type is a GIS polygon. Monarch agents begin at a randomly generated location on the landscape, and therefore, each agent is presented with unique local landscape and movement decisions. In our novel movement algorithm, female monarch agents query the landscape model to discover polygons within perceptual range (see box 3 for a definition of model parameters) and then "choose" which direction to move (see figure 3 in Grant et al. 2018). Agents move one step and decide whether to lay eggs before choosing a direction for another step and so on. Agents have a higher probability of moving toward and laying eggs in landcover polygons with higher milkweed density. If there is only one type of landcover type within perceptual range (e.g., if they were in the middle of a maize field or large grassland), the monarch agent moves in a correlated random walk. Through many steps, female monarch agents move across the landscape and lay eggs along the way.

Several user-specified parameters related to monarch behavior allow exploration of monarch agent movement behavior and egg laying: perceptual range, step length, directionality, and spatial memory (see box 3). As was described in Grant et al. (2018), expert opinion suggested that a perceptual range of 50 m, directionality of 0.5–0.75, and spatial memory of 100 previous steps seemed reasonable. Greater values for perceptual range (e.g., 400 m) resulted in limited movement across the landscape, and the eggs became highly concentrated in a few sites. Lower values of perceptual range (e.g., 50 m) generated more realistic female movement patterns, with eggs distributed more evenly across the landscape, a pattern consistent with empirical observations. Spatial memory and directionality assumptions had more subtle effects on model outputs. Greater spatial memory decreased the number of eggs laid in roadside polygons, because they are relatively small and arranged on the landscape in a way that makes it easy for monarch agents to move to a new polygon they have not visited. Directionality had the greatest effect on eggs laid in grassland or pasture, because low directionality resulted in monarch agents spending more time in this landcover type. The number of monarch eggs laid in each GIS polygon is the primary model output, along with monarch movement paths. The number of eggs in any polygon is an emergent property of all movement and egg-laying decisions of all simulated monarchs. Most eggs were laid in the landcover types of grassland or pasture, roadsides, and nongenetically modified (herbicide intolerant) crops.

Subsequent to the publication of Grant et al. (2018), Blader (2018) provided the means to calibrate model outputs for rural roadsides by surveying geolocated milkweed stems for monarch eggs along two 1.6-km roadsides adjacent to maize and soybean fields in rural Story and Boone Counties

Box 3. Agent-based model of monarch movement.

Grant et al. (2018) introduced an agent-based model of monarch movement and egg laying. Four critical parameters govern monarch movement.

Perceptual range

Perceptual range is the distance at which a monarch agent can perceive a habitat polygon from its location on the landscape.

Step length

Monarch agents move step by step, from one X, Y coordinate to the next. Step length is the distance a monarch agent flies per step.

Directionality

Directionality is the tortuosity or straightness of movement paths when a monarch agent moves in a correlated random walk (occurs only when one polygon is within perceptual range). This parameter varies from 0 (steps occur in a completely random direction) to 1 (steps occur in a straight line).

Spatial memory

Spatial memory is the number of past steps that a monarch agent can "remember" where it was—that is, how long it can remember previously visited polygons.

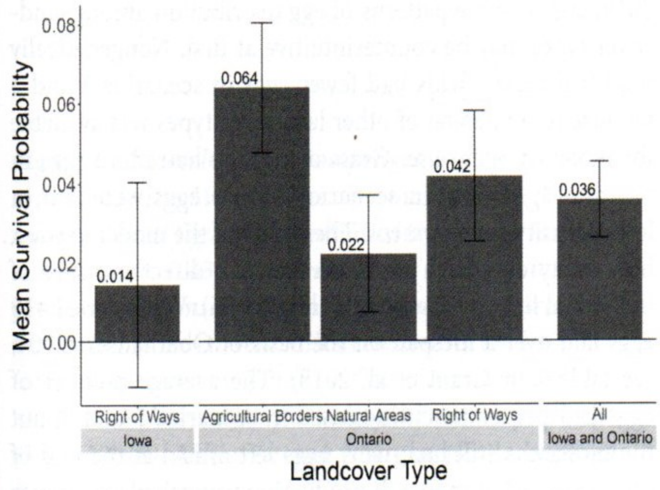


Figure 3. Monarch larval survival probability estimates from a new degree day model obtained by reanalyzing the empirical data reported in Grant et al. (2020). Point estimates are the mean and error bars are the standard deviations. "All" denotes the survival probability based on data pooled across Iowa road right of ways, Ontario agriculture field borders, Ontario natural areas, and Ontario road right of ways.

in Iowa (Grant and Bradbury 2019). Prior to calibration, estimates of egg densities in these locations were two to five times higher than was reported by Blader (2018). Following calibration, simulated egg densities for these roadsides were within a factor of two of the empirical egg density estimates (Grant and Bradbury 2019). If future empirical data sets of milkweed stem and egg density were available for other adjoining landcover classes (e.g., pastures, CRP), a more robust model calibration could be undertaken (Grant and Bradbury 2019).

The model of Grant et al. (2018) is a powerful tool for studying monarch movement and egg laying at the midscale level. The parameter values governing movement provide impetus for empirical studies to refine understanding of monarch movement (see the "Monarch movement ecology and habitat use" section). By examining where eggs are laid across the landscape, we can identify important characteristics of landcover types and polygons receiving the most eggs. We can also create landscape models of hypothetical arrangements of new habitats and predict which conservation management plans will have the greatest effect on nonmigratory breeding monarch populations.

Bayesian life-stage model. The agent-based model (Grant et al. 2018) simulates the number of eggs laid on the landscape. How many of these eggs survive to adulthood? Simulating the number of next-generation monarch adults that develop from the eggs laid in the agent-based model requires estimates of egg and instar survival probabilities (see the lower right of figure 2). Estimating arthropod survival probability has long been a statistical challenge, in part because larvae molt at the end of each instar and, therefore, cannot be marked. However, using monarch development time in relation to ambient temperature, we developed a Bayesian state–space model to estimate survival from egg laying to pupation on the basis of the data collected on Iowa roadsides (Grant et al. 2020). In Grant et al. (2020, 2021), we assumed a pupal survival probability of 0.76 on the basis of Nail et al. (2015). The mean cumulative survival from egg to pupation was estimated as 0.014 (95% CI = 0.004–0.024). The model was tested extensively against field data for bias in detection probability and individual variation in stage duration (Grant et al. 2020). With this new method and framework, we can estimate the number of adults that will be produced from the monarch eggs simulated by the agent-based model.

We have continued to make incremental improvements to this base model. Grant et al. (2020) used degree-day development estimates for Australian monarchs fed *Gomphocarpus fruticosus* (previously known as *Asclepias fruticosa*; Zalucki and Kitching 1982b). These degree-day estimates have been used in several other modeling efforts for North American monarchs (e.g., Cockrell et al. 1993, Feddema et al. 2004, Zalucki and Rochester 2004). However, development of North American monarchs reared on common milkweed (*Asclepias syriaca*) and other native milkweed species occurs at different rates (Erickson 1973, Rawlins and Lederhouse 1981, Oberhauser 2004, Krishnan et al. 2021a, Keith Bidne, United States Department of Agriculture, Corn Insects and Crop Genetics Research Unit, Agriculture Research Service, Ames, IA, USA, personal communication, 2 November 2021). We updated the model of Grant et al. (2020) with a new degree-day submodel based on *A. syriaca* development times and estimated new survival probabilities (figure 3, supplemental figure S1). The details of this new submodel can be found in the "Bayesian life-stage model" section of the supplemental material. Our new cumulative larval survival estimate of 0.036 is used in the following analyses to estimate the number of monarchs produced on the landscape.

Simulating adult monarch production. With the agent-based model providing the number of eggs laid and the Bayesian model providing survival probabilities, we had the ability to simulate the number of adult monarchs that reach the next generation (figure 2; Grant et al. 2020). In Grant et al. (2021), we created three GIS landscape models representing three scenarios of milkweed habitat establishment in Story County, Iowa: Scenario 1 is the current condition, scenario 2 is maximum milkweed establishment, and scenario 3 is moderate milkweed establishment. We used the assumptions in Thogmartin et al. (2017) and commitments in IMCC (2018) as guides to develop these scenarios. Scenario 3 represents a moderate increase in milkweed density in grasslands or pastures, low-intensity development, and roadsides, whereas scenario 2 represents the maximum possible milkweed density in these same areas. We used our agent-based model and survival estimates to simulate the number of adult monarchs produced in each scenario. The estimates of monarch production are best viewed as relative to scenario 1, because the absolute value of monarch production is highly dependent on the starting number of egg-laying adult females, which is an estimate and varies from year to year in the wild. Therefore, we reported results as percent difference relative to the baseline scenario 1 and found that scenario 2 and scenario 3 produced 24.7% and 9.3% more monarchs than scenario 1, respectively (Grant et al. 2021).

Do these results hold at larger spatial scales? For this overview, we extended our spatially explicit simulations for Story County (Grant et al. 2021) to an area in Iowa that is 21 times larger. Monarch movement and egg laying were simulated in the Iowa portion of the Des Moines Lobe (DML; supplemental figure S2), an ecoregion (Griffith et al. 1994), or major land resource area (number 103; USDA-NRCS 2006), present in three North Central states. The DML is excellent cropland, with nearly all the ecoregion devoted to maize and soybean production (supplemental figures S3 and S4). The landscape model of the Iowa DML (3.16 million hectares [ha]) includes 868,617 polygons of 17 landcover types, whereas the Story County landscape model reported by Grant et al. (2021) covered 148,650 ha with 41,326 polygons. We employed the three milkweed establishment scenarios of Grant et al. (2021). We also used the same model parameters as in Grant et al. (2021)—that is, a perceptual range of 50 m, a step length of 30 m, a directionality of 0.5–0.75, and a spatial memory of 100. We ran 200,000 monarch agents in the DML, which is approximately the same monarch density (1 monarch agent per approximately 15 ha) used for Story County (Grant et al. 2018, 2021).

The agent-based model output was compiled and analyzed as in Grant et al. (2021), except that we also report on the number of eggs laid per monarch agent. The agent-based model output showed that scenarios 2 and 3 increased eggs laid over current conditions by 24.2% and 10.9%, respectively (supplemental figure S5), very similar to the 24.6% and 9.3% for Story County (Grant et al. 2021). Roadsides, low-intensity development, and grassland or pastures were the landcover types with the greatest increase in number of eggs, consistent with findings for Story County (Grant et al. 2018, 2021). Some patterns of egg distribution among landcover types may be counterintuitive at first. Nongenetically modified maize fields had fewer eggs in scenarios 2 and 3 because more habitat of other landcover types was available for monarch agent use. Grass or pasture had more eggs in scenario 3, because in scenario 2, more eggs were laid in low-intensity development. The ability of the model to track how many eggs each monarch lays is a direct measure of individual fitness (supplemental figure S6). We assumed 410 eggs laid over a lifespan on the basis of Oberhauser (2004; see table 2 in Grant et al. 2018). The average number of eggs laid per monarch increased in scenarios 2 and 3, but the monarchs still had many eggs left unlaid at the end of their lives. Adult monarch production was calculated as was reported in Grant et al. (2021), except we used the new 0.036 cumulative larval survival probability (see the "Bayesian life-stage model" section). The results show a pattern (figure 4) similar to that reported by Grant et al. (2021) for Story County, with increases of 24.2% and 10.9% for the DML. As we described in Grant and Bradbury (2019), it is difficult to know how increases of 24.2% or 10.9% in the DML or other intensive agricultural areas would contribute to the overall population size that overwinters in Mexico without a continental scale multigenerational annual model. However, it seems likely that under any circumstances, a 24% boost in nonmigratory populations would be impactful.

As Grant et al. (2018) noted, we would like to predict egg density for individual polygons. With a firm understanding of the factors that increase egg density in model polygons, we could, perhaps, improve egg density for milkweed establishment areas in the wild. We hypothesized that the area, perimeter, shape, and location of individual polygons likely influence monarch egg density in the model. We opted to choose one landcover type to test these hypotheses. A 1.6-km grid of secondary roads demarcates section boundaries in the DML. Cropland occurs within sections in which landscape features such as streams do not discourage agriculture. Smaller-order streams, where they are present, often have grassland or pasture along their length instead

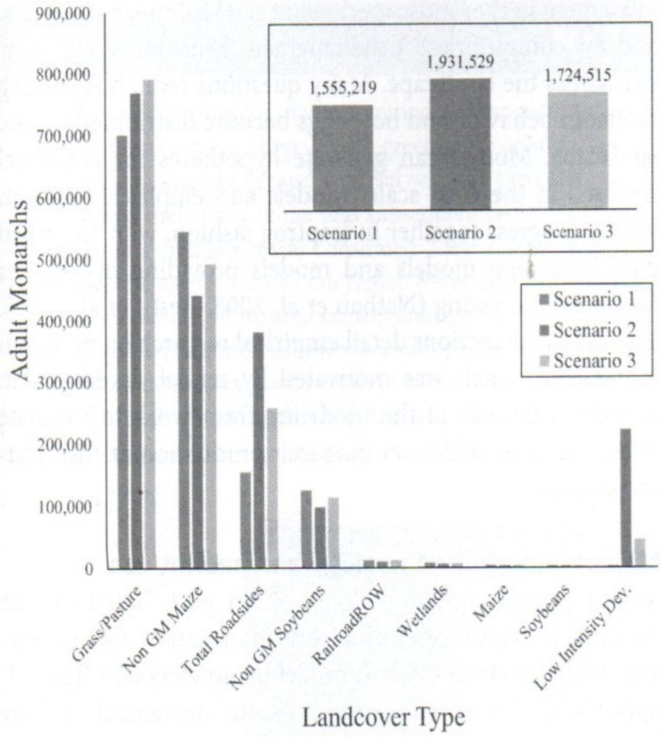


Figure 4. Monarch production per generation by landcover type for the Des Moines Lobe. The number of eggs laid (supplemental figure S3) multiplied by average larval survival rate (0.036) is the number of adult monarchs produced. The inset is the total production per scenario. Scenario 1 is the current milkweed density on the landscape; scenarios 2 and 3 reflect maximum and moderate milkweed habitat establishment, respectively (IMCC 2018). Abbreviations: GM, genetically modified; ROW, right of ways.

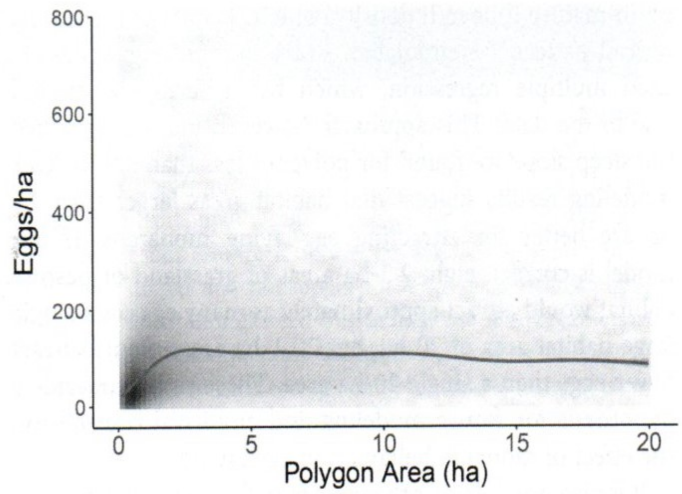


Figure 5. Generalized additive model regression fit to egg density by area for polygons less than 20 hectares (ha; 97% of the points). The 63,375 plotted points are represented using kernel density, where darker shades represents a higher density of points. The estimated maximum egg density is 117.6 eggs per ha at 2.87 ha and decreases to 84.1 at 20 ha. At 0.2 ha, near the smallest polygon size, egg density is 26.5per ha. R2 = .225, *p* < .001. The 95% confidence interval is shown as a gray band, which is narrow and visible only at approximately 20 ha.

of cropland. Larger-order streams and rivers also often have forests along their lengths. These few elements describe most of the landscape features monarchs must navigate. On the basis of the number of eggs laid, the most important landcover type for monarchs is grassland or pasture (figure 4). This landcover type occurs along streams, in isolated areas along roadsides, and between agricultural fields or is otherwise embedded in the landscape. Grassland or pasture polygons also have the most variable shape and distribution across the landscape.

We examined the relationship between egg density (eggs per ha) for each grassland or pasture polygon in the GIS landscape model and the polygon characteristics of area, perimeter length, shape index, and fractal index. The shape index is an improved index of the perimeter to area ratio, independent of scale (Patton 1975, McGarigal and Marks 1995). The shape index equals 1 if the polygon is a perfect square and increases above 1, with no maximum limit, as the polygon shape diverges from a square. The fractal index is a similar measure that quantifies the convolutedness of the polygon shape (Krummel et al. 1987, McGarigal and Marks 1995). A compact round or square shape will have a fractal index near 1. As the shape becomes more complex, the fractal index increases to a maximum of 2. The shape and fractal indices were calculated for each polygon according to McGarigal and Marks (1995).

We used generalized additive model (GAM) regression to examine the relationship between egg density and the main effects of polygon area, perimeter length, shape index, and fractal index. The GAM regression indicated that egg density increases quickly with area to a maximum of 2.9 ha, after which it plateaus and decreases slightly (figure 5). This pattern is likely the result of the complex interplay of several factors. Egg density should simply be a function of the amount of time monarch agents spend in a polygon; therefore, factors that determine the time spent in a polygon are most relevant (Grant et al. 2018). The monarch spatial memory feature in the agent-based model is likely one important factor. With spatial memory, monarch agents have a higher probability of leaving polygons they have already visited. In smaller polygons, monarch agents likely have a higher chance of leaving because they encounter edges more often. In larger polygons, it can take more time before a monarch agent encounters a polygon edge. As the polygon size reaches 2.9 ha, the spatial memory effect becomes less important.

An equivalent behavior in the wild would be when monarchs encounter edges more often in small habitat areas. In these instances, monarchs would be more likely to leave the area because they retain some information about where they have been. But if habitat areas are very large, the butterflies rarely encounter edges and simply wander through the habitat. The empirical research to date does not contradict our model results. Bruce et al. (2021) surveyed patches ranging from 0.9 to 400 ha and found only a weak effect of patch size on immature monarch density, which is consistent with the overall pattern we simulated. Bruce and colleagues (2021) used multiple regression, which fits a relatively straight line to the data. This approach, however, likely overlooked the steep slope we found for polygons less than 2.9 ha. Our modeling results suggest that habitat areas larger than 2.9 ha are better for attracting egg-laying monarchs. If our model is correct, eight 2.5-ha areas of grassland or pasture habitat would attract approximately as many eggs as a single large habitat area of 20 ha, but 20 1-ha areas would attract fewer eggs than a single 20-ha area. These results provide a hypothesis for future modeling and empirical research on the effect of monarch behavior on egg laying.

It is also possible that the pattern in figure 5 is the product of many factors in the agent-based model that are difficult to separate and comprehend. Area alone predicted only 22.5% of the variability in egg density in grassland or pasture landcover polygons, suggesting that additional important factors drive egg density. However, the shape index and fractal index had little effect on egg density (supplemental figures S7 and S8), and perimeter length showed a similar pattern but with a lower R^2 than area did (supplemental figure S9). We expected that elongate polygons (i.e., polygons with larger shape and fractal indices) would be better at intercepting monarchs moving across the landscape, but that does not appear to be the case. Perhaps they do intercept more monarchs, but this may be counterbalanced by increased edge encounters from within an elongate polygon and, therefore, increased opportunities to leave. It is unclear what other factors might explain the remaining 77.5% variability in egg density.

Our finding that simple measures such as habitat size and shape do not precisely predict egg density even in a relatively simple model (compared with the real world) emphasizes the difficulty in understanding monarch landscape use. Recent empirical studies of landscape factors affecting immature monarch density also had poor predictive ability (Bruce et al. 2021). Egg density in any polygon is likely due, in part, to landscape configuration at variable distances from the polygon, which we found difficult to quantify. The interplay between monarch use of roadsides as corridors and discoverability of suitable habitat at some distance from roadsides also is likely important. Future analysis could use the coordinates of each monarch agent after each step. The distribution and density of these coordinates would be useful in visualizing and quantifying monarch movement corridors and movement patterns. Analyses also could be performed on individual lifetime movement paths. We hypothesize that patterns of habitat stepping stones, corridors, and distance to the nearest high-quality habitat, in combination, are important factors, but integrating these features to predict egg density for a particular polygon is a significant challenge.

Our agent-based model and Bayesian survival models provide the tools to simulate monarch reproduction through one generation at the midscale level (figure 2). Our research to date has provided important insights into monarch movement in the landscape, habitat establishment prospects, and the complexities of the emergent behavior of egg density across the landscape. Many questions remain regarding monarch behavior and how eggs become distributed on the landscape. Models can generate hypotheses for empirical research at the field scale. Models and empirical research ideally progress together in leapfrog fashion, with empirical data informing models and models providing hypotheses for empirical testing (Nathan et al. 2008, Restif et al. 2012). The following sections detail empirical research on monarch movement, which was motivated by model development, as well as the use of the modeling framework to simulate effects of insecticides on monarch production at the landscape scale.

Monarch movement ecology and habitat use

Robust population simulations are, in part, based on the quality and relevance of empirical information about monarch biology, which informs model parameters (see figure 2, upper left). Our recent research on the movement ecology of nonmigratory, breeding-season monarchs is meant to address uncertainties associated with the assumptions related to movement components in the agent-based model: perceptual range, step length, and directionality (see box 3). The results of our research also provide the means to interpret model outputs on the basis of observations in the field, which inevitably generate new questions and hypotheses to test.

An organism's movement creates a path traversed over a defined temporal scale, from a few seconds to a lifetime. The mechanistic components that lead to an observed movement path include the internal state of the organism—the motivation to move; its motion capacity—the biomechanical mode and physiological capacity to engage in movement; its navigation capacity—the ability to orient in space (where to move) and time (when to move); and external factors—both abiotic (e.g., wind, temperature) and biotic (e.g., predators, competitors; Nathan et al. 2008). These components may interact to different extents at different times, and they are not all necessarily involved in generating any particular observed movement path. For most lepidopteran species, the movement pattern traversed over an individual's lifetime is largely determined by the adult's movement because of their greater motion capacity than larvae's (e.g., flying versus crawling). Moreover, adult movement paths can have substantial fitness consequences across generations; for example, oviposition site selection by females has a large impact on offspring survival (box 4). Larval and adult habitats differ greatly in spatial scale and composition, and movement paths at one scale can influence fitness at the other.

Broad categories of behaviors are key to producing movement paths of individuals, including migration, station keeping, and ranging (Kennedy 1985, Dingle and Drake 2007, Dingle 2014). Migratory behavior is characterized as persistent, straight line, nonappetitive (undistracted) flight (Kennedy 1985, Dingle 2006, Chapman et al. 2011, 2014, 2015, Reynolds et al. 2017). Monarch butterflies are known

Box 4. Larval movement.

Milkweed establishment that accommodates the natural behavior of monarch larvae at the host plant scale is necessary for effective conservation or restoration. Under greenhouse conditions, we explored the effect of milkweed ramet (stem) density on larval search behavior, milkweed use, and survival without predation, parasitism, or competition (figure 6; Fisher et al. 2020b). Monarch larvae abandoned their natal ramet and subsequent ramets they foraged on prior to the prepupal wandering stage and before all available leaf biomass on a ramet was consumed. Larvae consumed biomass from three or four milkweed ramets that totaled the approximate biomass of a single 10 to 35-cm ramet. Movement ecology suggests that isolated ramets may not support development through pupation, even though an isolated ramet could provide enough biomass. The establishment of milkweed with at least two to four ramets of closely spaced common milkweed would provide sufficient biomass for development and increase the likelihood that larvae moving in random directions would encounter nonnatal ramets to support development. Movement ecology and biomass requirements are critical aspects of monarch larval biology that should be considered in habitat restoration and maintenance plans, monitoring survey designs and protocols, and population modeling.

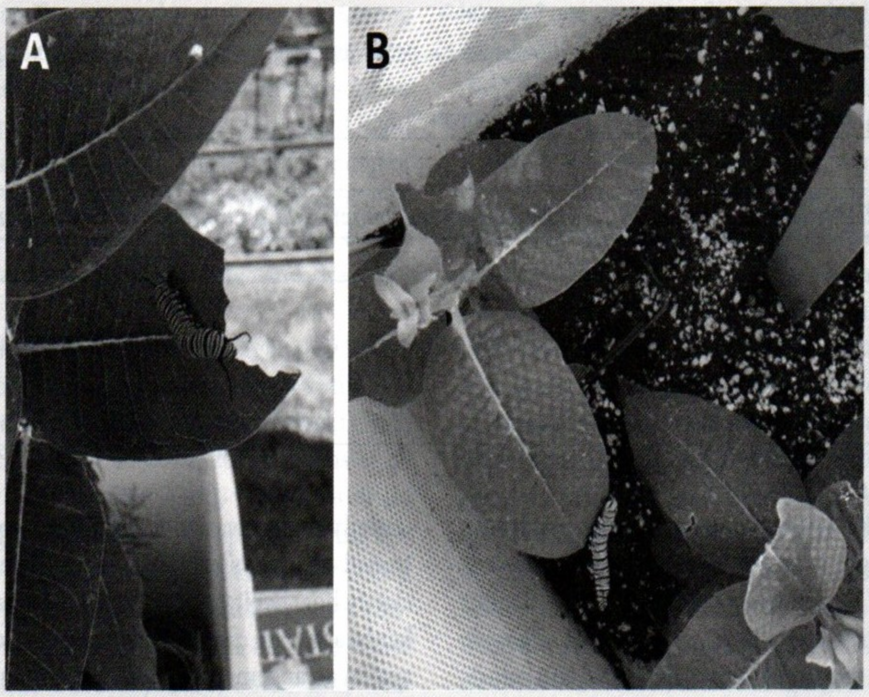


Figure 6. (A) Monarch larva feeding on a milkweed leaf. (B) A fifth instar monarch at the edge of a milkweed leaf searching for new milkweed ramet. Notice the feeding injury on two milkweed ramets in the photo. Only one larva was in the cage with these plants.

The motivations for milkweed ramet abandonment behavior are unclear. The hypotheses include finding young leaves that have higher nitrogen concentrations and are easier to digest (see the "Herbicides" section), finding plants with lower concentrations of herbivore-induced defensive chemicals, predator avoidance, or thermoregulation. Additional studies analyzing the impact of vegetation toughness, age, and induction of defensive compounds with feeding, as well as predators and competition, are underway.

for their annual continental-scale migration; this phenomenon is a collective result of many individuals engaging in migratory behavior. Station-keeping behavior, on the other hand, is characterized by appetitive, foraging, and oviposition flights in which the insect seeks a resource within a habitat or local landscape. Ranging behavior is also appetitive, but it results in movement across multiple habitats at a landscape scale. Ranging occurs in response to a perceived shortage of a resource in an individual's immediate surroundings so that it leaves that habitat in search of better habitat, resulting in a net movement sequence described as emigration, matrix transit, and immigration.

Station-keeping and ranging behaviors dominate the movement ecology of breeding-season, nonmigratory monarch adults. Breeding-season female and male adults have fundamentally different movement ecologies because of differences in movement motivations (table 1) and the components of reproductive success: Females lay as many eggs as possible, and males mate with as many females as possible. A mated, gravid female monarch is frequently motivated to find suitable oviposition substrates. Breeding-season males are often observed patrolling habitat in search of females, whereas females fly less frequently and often rest in vegetative cover, avoiding harassment by courting males (Pliske

Table 1. Motivations and resultant station-keeping movement behaviors of adult monarchs during the summer breeding season				
Motivation	**Station-keeping appetitive behavior**	**Habitat type**	**Adult female**	**Adult male**
Hunger	Food search	Nectar from flowering forbs (including milkweed)	X	X
Reproduction	Mate seeking, aerial courtship	Flowering forbs, milkweed, opportunistic		X
	Copulation, postnuptial flight	Sheltered perch, trees, shrubs		X
	Oviposition	Milkweed	X	
Escape	Courtship avoidance	Erratic flight to high altitude, dense vegetation, drop to ground	X	
	Predator avoidance	Erratic flight to high altitude	X	X
		Dense vegetation, drop to ground, hide under leaves	X	X
Thermoregulation	Basking	Sunny perch	X	X
	Shade	Trees, shrubs, dense vegetation, milkweed leaves	X	X
Overnight inactivity	Roosting	Trees, shrubs	X	X

Table 2. Terms to define habitat classes by resource (milkweed ramets and blooming forb inflorescences) density.		
Habitat class	**Resource density**	**Example**
Zero density	0 resources per square meter (m^2)	Crop field
Low density	Resources present but fewer than 3 resources per m^2	Roadside with scattered resources
Intermediate density	5–26 resources per m^2	Pasture
High density	Greater than 30 resources per m^2	Newly established prairie restoration

1975). However, flight activity is energetically expensive, and nonmigratory flight is fueled by carbohydrates replenished by ingestion of nectar from blooming forbs (Brower et al. 2006, Kral-O'Brien et al. 2020). Hunger may override other motivations for movement until the monarch's caloric requirements are met. Other day-to-day motivations for monarch flight activity may include escape from predators, finding shade in which to shelter from excessive heat, and finding a roosting site for overnight inactivity.

In a series of studies, we observed wild-captured breeding-season females under field conditions in free-flying, radio-telemetry experiments (supplemental figure S10) and in tethered-flight trials (supplemental figure S11). In automated (Fisher et al. 2021, Fisher and Bradbury 2022) and handheld radio-telemetry studies (Fisher et al. 2020a, Fisher and Bradbury 2021, 2022), we documented temporally and spatially fine-grained movement paths of female monarchs within habitat classes of varying resource quality and composition. We aimed to better understand monarch butterfly movement patterns, including natural step lengths (distance between two stopping locations), directionality in association with habitat classes, and the perceptual distance relative to nectar and milkweed resources. We also sought to understand potential external factors and internal states affecting motivations, which can quickly vary within an individual monarch and probably predominate in a hierarchical manner depending on circumstances (table 1). Our results illustrate the difficulties of interpretation when the underlying motivation for flight cannot be definitively controlled or always ascribed. Nevertheless, our findings help clarify how habitats used by female monarchs in fragmented landscapes are functionally connected and how different spatial arrangements of restored habitats may increase adult recruitment.

Movement patterns. Previous studies indicate that adult lepidopteran movement is influenced by habitat classes defined by resource density (table 2), with patterns representative of station-keeping and ranging behaviors (Zalucki and Kitching 1982a, Zalucki 1983, Delattre et al. 2010, Schultz et al. 2012, 2020a, Evans et al. 2020b, Kral-O'Brien and Harmon 2021). Turn angles, the angle between three successive points on a movement path, are often used to describe movement ecology and are a measure of directionality. Directed movements (i.e., those of small turn angles) with large natural steps are expected when traversing a habitat with zero or a low density of resources. Conversely, torturous flight patterns with short flight steps, indicative of foraging and ovipositing behavior, are predicted in intermediate or high-density resource habitats. We found evidence to support these predictions in the case of monarchs, as is presented in the following paragraphs, but discovered that their movement patterns were more complicated than this simplified framework. Movement patterns are likely context specific and result from a variety of interacting factors (Nathan et al. 2008).

Our research demonstrated that, as we anticipated, turn angles differed in high-density and low-density habitats (defined in table 2). Monarchs in resource-devoid habitats, such as sod fields (Fisher and Bradbury 2021) and crop fields (Fisher and Bradbury 2022), traveled in straight lines as evidenced by small turn angles, suggesting they do not waste time and energy searching for resources where they are unlikely to be encountered. Conversely, the flight paths were torturous when the monarchs were in high-density habitat and surrounded by milkweed and nectar resources, reminiscent of the looping, foray flight patterns described by Conradt et al. (2003). However, monarchs within intermediate-density habitats traveled with a high degree of directionality between clusters of resource plants (Fisher and Bradbury 2022). When the monarchs were moving among resource plants, foraging was the dominant behavior, suggesting that the monarchs were efficient at locating resources when they were surrounded by a grass-dominated land cover in an intermediate habitat class. These findings suggest that movement patterns are likely a product of resource density and distribution, because the resources were not evenly distributed within the habitat classes (Fisher and Bradbury 2022).

Although prior work suggested natural flight step length should differ on the basis of habitat class, similar to the way turn angles do (Zalucki and Kitching 1982a, Zalucki 1983, Delattre et al. 2010, Schultz et al. 2012, 2020a, Evans et al. 2020b, Kral-O'Brien and Harmon 2021), we observed no difference in step length, with 90% of all steps measuring less than 50 m (Fisher et al. 2020a, Fisher and Bradbury 2022). Using radio telemetry, we were able to quantify large emigration step lengths (i.e., the remaining 10% of steps; Fisher et al. 2020a, Fisher and Bradbury 2021, 2022) that were previously only theorized (Crone and Schultz 2022). Long-distance emigration occurred periodically via single steps with lengths from 50–1900 m and was initiated from well within the habitat class boundaries (i.e., not because the butterfly intercepted the boundary while foraging). These long-distance emigrations were initiated significantly more often in high or intermediate-density habitats than in resource-devoid or low-density habitats, which suggests these steps followed foraging and ovipositing in an apparently still-suitable habitat class. The internal or external stimuli associated with these large emigration steps are unclear; however, we hypothesize that this behavior increases parental fitness by promoting a wide distribution of an individual's eggs across the landscape, thereby spreading location-related risks to their offspring (see Fisher and Bradbury 2022). Such risks could include density-dependent increases in predation, parasitism, disease, and intra- and interspecific larval competition for milkweed. The reasoning is similar to the migratory escape hypothesis favoring long-range migration of monarchs (Altizer et al. 2011, Bartel et al. 2011, Flockhart et al. 2018, Menz et al. 2019). Density-independent risks could include the catastrophic loss of localized larval habitat to events such as fire, hail, and flooding.

Although not the annual continental-scale migration, the behavior associated with the long emigration flights is reminiscent of migratory behavior in that it is in a straight line, is undistracted, and covers distances greater than during local foraging (Dingle and Drake 2007). There are also hints that these flights are nonappetitive (i.e., not resource directed), a key characteristic of migratory behavior (Kennedy 1985, Dingle 2014). For example, the motivation to initiate the long steps is not obviously a facultative response to inadequate resources in the current habitat, and the flight appears to end spontaneously, regardless of the habitat encountered (see Fisher and Bradbury 2022). Incorporation of nonappetitive emigration flights into breeding monarch movement ecology is of great interest phenomenologically, because nonappetitive flight behavior by individuals is commonly only associated with population-level displacement (Sappington 2018). In addition to universal characteristics of migratory behavior—for example, in a straight line and undistracted—the continental-scale migration of monarchs is also associated with common but not universal characteristics such as high-altitude flight, celestial navigation, and reproductive diapause, none of which are characteristic of the long-step displacements of breeding-season monarchs. The description of two types of nonappetitive flight behavior in a species is rare and emphasizes the nature of migration as a syndrome or suite of traits that can vary within a species depending on the life-history goal at hand (Sappington and Showers 1992, Dingle 2006, 2014). Accounting for short-range nonappetitive flights could be of practical importance as well when modeling monarch movement ecology and habitat connectivity. Future telemetry research designed to track monarchs through the full sequence of habitat emigration, matrix transit, and immigration could be used to test the hypothesis that long-step flights are nonappetitive.

For butterflies, perceptual ability is likely dominated by vision at a fine scale (e.g., within 5 m of a resource; Garlick 2007) and olfaction at longer distances (greater than 30 m; see Rutowski 2003, MacDonald et al. 2019) with individuals flying upwind in response to tactile cues detected by sensilla, in and out of an odor plume, until they reach the source of the attractant chemical signal (Bell 1991, Cardé and Willis 2008). The perceptual ability of butterflies is species dependent, with estimates ranging from at least 1 m for the cabbage white (*Pieris rapae*; Fahrig and Paloheimo 1987) to 100 m for the speckled wood (*Pararge aegeria*; Merckx and Van Dyck 2007). Although perceptual abilities to find milkweed for oviposition or blooming forbs for nectaring likely differ, on the basis of our observations of monarchs flying upwind directly toward and then landing on a resource plant (i.e., guided movement), we estimate the monarchs' perceptual ability can range up to 125 m. This estimate is conservative, because perception at longer distances cannot be excluded (Fisher and Bradbury 2021, 2022). For instance, although not moving upwind, we observed a monarch perform a straight flight of 230 m that terminated on milkweed (Fisher and Bradbury 2021). We also observed two monarchs traverse more than 500 m across crop fields and terminate their movement in habitat with forage resources (Fisher and Bradbury 2022).

Because insects use tactile cues to determine wind direction and fly upwind when performing search behavior (Bell 1991, Cardé and Willis 2008, MacDonald et al. 2019), we expected the external factor of wind direction to influence movement patterns. Although most free-flying butterfly movement studies do not report wind conditions, or it is assumed wind direction does not confound experimental designs (e.g., Merckx and Van Dyck 2007, Schultz et al. 2012, Fernández et al. 2016, MacDonald et al. 2019, 2020a, Evans et al. 2020b), the results from our studies consistently indicated a strong influence of wind direction on orientation. We found that mildly food-deprived monarchs attached to a flight mill consistently oriented upwind with small turn angles, indicating directed flight into the wind whether or not milkweed or nectar sources were available within a grass-dominated field (Mullins 2021). We also observed upwind flight regardless of the distance between tethered monarchs and resources (3–25 m) or wind direction relative to the location of the resources (Mullins 2021). Free-flying monarchs also generally traveled upwind when released in 50–64-ha mosaics of resource-devoid, low-, and intermediate-density habitats (Fisher and Bradbury 2022). However, when released in a 4–32-ha resource-devoid habitat (a sod field) 5–75 m from potted milkweed and nectar resources, 90% of the monarchs (n = 145) oriented downwind and away from plant resources, likely because they were repelled by the turf or did not perceive the potted plants (Fisher and Bradbury 2021). Breeding-season female monarch flight appears to be strongly influenced by wind direction, but whether orientation is upwind or downwind likely depends on the motivation for movement.

In summary, we learned that monarchs initiate long emigration flights up to 1900 m, their perceptual range can be quite large (approximately 125 m, but most commonly at least 50 m), and their flight orientation is strongly influenced by external factors, including wind direction and resource density. These findings help shed light on aspects of monarch biology that have been elusive, such as their ability to locate and lay eggs on isolated stems of common milkweed (Zalucki and Kitching 1982b) and their displacement of up to 1.5 km per day and 10–15 km net over their breeding-season lifetime, depending on the landscape (Zalucki and Kitching 1984, Zalucki and Lammers 2010, Zalucki et al. 2016). On the basis of this information, establishing habitat patches approximately 50 m apart along an axis of the predominant wind direction should facilitate a functionally connected landscape for breeding monarch butterflies. However, because monarchs occasionally take large emigration steps greater than 1000 m, meeting the ideal of such close placement of habitat patches is likely not necessary.

Empirical evidence informs options for agent-based model refinement and generates new hypotheses. Evaluation of the agent-based model assumptions (i.e., behavioral attributes of monarch agents) and outputs (egg densities in multiple habitats across adjacent land cover classes in spatially explicit landscapes) can identify new areas of research. As we noted previously (see the "Spatially explicit landscape scale simulation of monarch movement and egg laying" section), Grant and Bradbury (2019) used empirical egg density data collected by Blader (2018) to calibrate model estimates for rural roadsides. Grant et al. (2018) noted that Kasten et al. (2016) reported increasing monarch egg density with increasing milkweed density on roadsides, consistent with model predictions. In the "Simulating adult monarch production" section, we noted that model simulations showed a weak association of monarch egg density with habitat size, which is consistent with trends reported by Bruce et al. (2021) in their monitoring study. Fully evaluating simulations for Story County, Iowa, or the DML will require empirical egg and milkweed density data collected in adjoining land cover classes at spatial scales similar to that addressed in the model (Grant et al. 2018). To date, those data sets are not available, but future large-scale monitoring programs based on statistically rigorous sampling schemes could provide such data (Grant et al. 2018). In addition, Grant et al. (2018), citing Wallentin (2017) and Batty and Torrens (2005), noted that comparing model simulations with empirical data is not the only means to evaluate uncertainty. For example, an evaluation of model input assumptions can help identify and reduce sources of uncertainty. In this regard, Grant et al. (2018) identified specific areas of empirical study that would improve understanding of monarch movement behavior.

The results of our empirical studies (Fisher et al. 2020a, Fisher and Bradbury 2021, 2022, Mullins 2021, 2022) indicate the input assumptions used in the agent-based model (Grant et al. 2018, Grant and Bradbury 2019, 2021) are reasonable; however, the newly acquired information provides options for model refinements and raises new questions about monarch movement that could be the subject of future research. New versions of the model could be developed to simulate egg distribution at finer spatial scales (e.g., within land cover polygons), explore how floral resources affect monarch movement and identify future spatial patterns of monarch habitat that could maximize egg laying. Although model improvements would likely provide new insights into how monarch movement can influence egg distribution on the landscape, the costs and benefits of developing more complex algorithms should be assessed (Grimm et al. 2005).

Grant et al. (2018) concluded that a perceptual range of 50 m was a reasonable model assumption based on uncertainty analyses and expert opinion. Empirical data (Fisher and Bradbury 2021, 2022) confirm that 50 m is a reasonable average value for modeling but also reveal that perceptual range varies depending on habitat. In resource-devoid habitats, the perceptual range can be greater than 50 m (Fisher and Bradbury 2021), and in habitats with a high density of resources, monarchs appear to be responding to stimuli at shorter distances (Fisher and Bradbury

2022). Greater model realism could be gained by implementing a variable perceptual range, in which monarch agents query the landscape at successively larger distances to choose the direction for the next flight step. Although this refinement may not substantially change simulations of egg distribution at the landscape scale, it could provide the means to better simulate egg distributions within individual polygons and help formulate hypotheses for future research on monarch movement ecology at finer spatial scales. Calculating the area of habitat within the perceptual range is, however, the most computationally intensive aspect of the current movement algorithm. If adding this realism to the model would be useful for conservation planning, the costs of increased calculations could likely be managed.

As was noted above, the assumed perceptual range in the model plays an important role in determining the direction a monarch agent flies when two or more polygons are detected. Our empirical studies (Mullins 2021, Fisher and Bradbury 2022) also demonstrated that monarchs often orient in response to wind direction, typically by flying upwind. Wind direction, incorporated by randomly sampling daily wind roses, could be used as a factor affecting movement direction. Movement could be weighted with higher probabilities toward upwind directions, except in areas of low resource density (Fisher and Bradbury 2021), where movement could be weighted downwind.

Our model (Grant et al. 2018) currently employs a constant 30-m step length. Empirical studies suggest that although the assumption of 30-m steps is generally reasonable given that most steps are less than 50 m, female monarchs periodically make flights much greater than 50 m (Fisher et al. 2020a, Fisher and Bradbury 2021, 2022). These long-distance steps are probably responsible for distributing an individual's eggs widely across the landscape and may be important to include in the model. Although movement paths in nature are continuous, they are typically modeled with discrete steps of constant length or time (Turchin 1998). Our empirical observations (Fisher et al. 2020a, Fisher and Bradbury 2021, 2022) suggest that there may be a need to model monarch movement paths using natural step lengths, which are the distances between discrete landing locations. Employing natural step lengths would likely improve the realism of simulated movement paths at finer spatial scales; however, the extent to which increased model complexity would lead to different conclusions about egg distribution at landscape scales is unclear. A significant challenge to implementing natural step lengths in the model is determining how often females lay eggs at the termination of a step. The current lack of empirical egg density data collected at the landscape scale precludes the means to calibrate the model. Regardless of whether the full distribution of natural step lengths is employed in a future movement algorithm, it seems appropriate to include periodic long-distance steps in the current algorithm.

Spatial memory was incorporated into our original model (Grant et al. 2018) as a mechanism to replicate natural behavior. Without spatial memory, monarch agents often stayed in the same polygon indefinitely, which is inconsistent with an observed predilection to emigrate from habitat, as was discussed previously. As with many behavioral phenomena, it is difficult to determine exactly why and how the decision to leave an area is made by monarchs. Regardless of the underlying decision-making process, the spatial memory mechanism in the model replicates observed behavior. Memory is rarely included in insect models, but more evidence is accumulating that monarchs can remember colors and odors, perhaps as an adaptation for navigating the landscape and migration (Blackiston et al. 2011, Rodrigues and Weiss 2012, Gegear 2021). Furthermore, empirical studies addressing the role of memory in monarch movement could inform future model refinements.

The agent-based modeling framework we have developed (Grant et al. 2018, 2021, Grant and Bradbury 2019; see the “Simulating adult monarch production” section) simulates midscale egg distributions for one nonmigratory monarch generation in North Central landscapes. With our empirical evidence summarized in the present article, we can provide more realistic parameters such as natural step lengths of more than 50 m, occasional large steps (greater than 1000 m), and flights oriented upwind. Future research should aim to understand if modified parameters affect the overall egg distribution across the current spatially explicit modeled landscape. Subsequently, the influence of habitat establishment in various spatial configurations on egg distribution across the landscape can be tested to identify areas that will produce the largest impact (i.e., an increase in eggs). For example, our hypothesis that habitats established within 50 m of other habitat areas will facilitate functional connectivity can be tested with future modeling efforts.

Evaluating risks of pesticide use to milkweed and monarchs

To determine whether the potential benefit of establishing habitat near crop fields outweighs the risks of pesticide exposure, it is first important to ascertain if milkweed or monarchs are likely to be exposed to agricultural pesticides. The potential for exposure can be evaluated by comparing the spatial and temporal overlap of pesticide applications (and postapplication fate and transport) and the presence of monarch or milkweed (figure 7). Because exposures to herbicides and insecticides are possible, estimating potential risks requires quantifying the range of expected pesticide exposure concentrations and comparing these values with concentration–response curves obtained from laboratory or field-based toxicity studies (see figure 2, center left and bottom left). These comparisons provide the means to characterize pesticide risks at the field scale. Because monarchs are a vagile species, risk estimates should ultimately be estimated at the landscape scale (figure 8), which can be facilitated with the previously described agent-based movement and egg laying model and the Bayesian life-stage model.

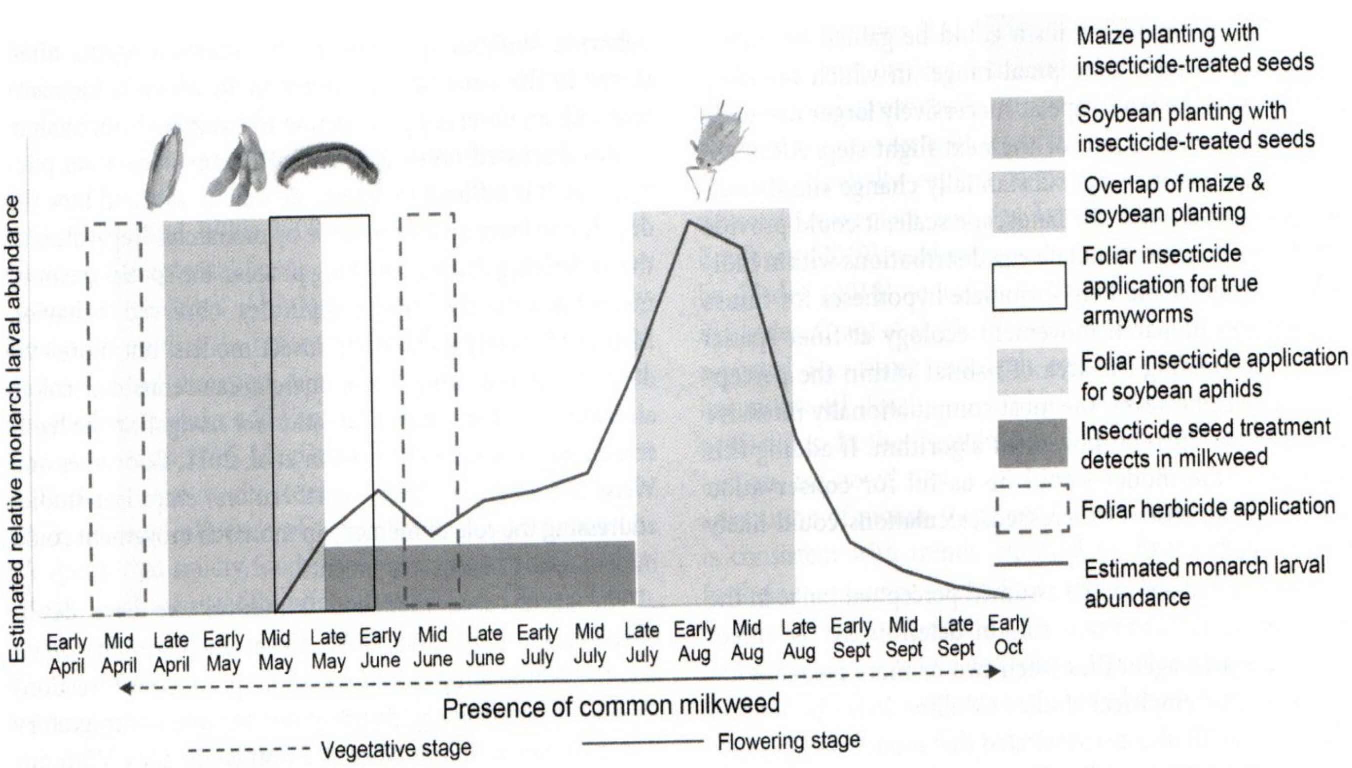


Figure 7. Conceptual model depicting a temporal overlap of monarch larval presence in North Central states with maize and soybean planting dates in Iowa, common milkweed phenology, detectable neonicotinoid insecticides in milkweed downslope of crop fields planted with treated seeds (Hall et al. 2021), foliar insecticide applications to manage economically significant true armyworm and soybean aphid populations, and foliar herbicide applications to no-till fields planted with herbicide-tolerant soybeans. The figure has been adapted from Krishnan et al. (2020).

Herbicides. Approximately 92% of maize acres and 95% of soybean acres in the North Central states are currently treated with herbicides (USDA-NASS 2020). Hartzler (2010) reported that common milkweed in Iowa crop fields declined by approximately 90% between 1999 and 2009, when the use of glyphosate, which effectively suppresses common milkweed (Cramer and Burnside 1981), increased with the introduction of hybrids genetically engineered to be glyphosate tolerant (Brookes 2014, Kniss 2017). Suppression of milkweed in maize and soybean fields is considered a factor in the decline of the fall migratory population of monarch butterflies in the North Central region of the United States (Pleasants and Oberhauser 2013, Pleasants 2015).

As is depicted in figure 7, foliar herbicide applications in Iowa typically occur in mid-April, prior to the arrival of monarchs, and again in mid-June. Herbicide spray drift downwind of treated crop fields could kill milkweed or, more likely, adversely affect the growth or quality of milkweed and indirectly affect monarch survival, growth, or development. Larval mortality or reduced growth and development could also be affected by direct exposure to spray drift or consumption of herbicide residues on spray-drift exposed milkweed (figure 2, left center).

To help assess the potential risks of foliar, postemergent herbicides, we examined the effects of fomesafen (Lizotte-Hall and Hartzler 2019) and dicamba (Saghi 2021) on common milkweed and on monarch use and development. Fomesafen's herbicidal mode of action is based on the inhibition of protoporphyrinogen oxidase, which causes localized damage to exposed foliage (HRAC 2021). Fomesafen and related compounds were first registered for use on soybean in the 1980s. Dicamba is a systemic auxin mimic (HRAC 2021) that has been used in maize and other grass crops since the 1960s. Dicamba was recently approved for use on soybean genetically engineered for dicamba tolerance (Mortensen et al. 2012).

In greenhouse experiments, common milkweed survived fomesafen applications up to 280 grams (g) per ha, which is twice the application rate for soybeans (Lizotte-Hall and Hartzler 2019). Although they were temporarily damaged, the milkweed plants showed signs of recovery within 2 weeks of application. In the field, Lizotte-Hall and Hartzler (2019) applied fomesafen to common milkweed stems at the labeled application rate of 140 g per ha. The leaves displayed chlorosis and necrotic lesions 5 days after application. At 2 weeks after application, the plants displayed moderate damage, and the leaves emerging from their apical meristems appeared normal. At 4 weeks, the plants displayed minor injury, and by 10 weeks, the biomass of the treated and the control plants were similar. There was no difference in oviposition by wild monarchs on fomesafen-treated versus -untreated plants; however, monarch larvae appeared to prefer new growth on treated plants. Survival of larvae on treated plants was not affected by fomesafen. These results suggest that monarchs will use common milkweed damaged by fomesafan and perhaps other herbicides in this class, provided sufficient new growth is available when monarchs are actively laying eggs (Lizotte-Hall and Hartzler 2019).

Steps to assess risk of pesticide exposures to monarch butterfly populations at the field and landscape scale

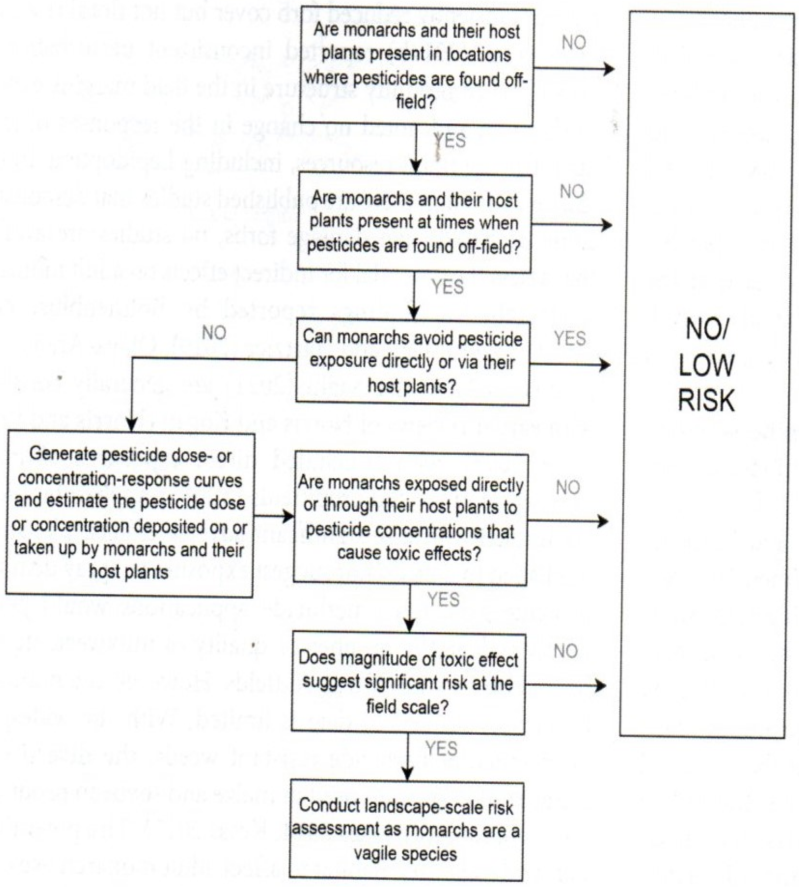


Figure 8. Steps taken to assess the risk of pesticide exposure to monarch butterfly populations at field and landscape scales. Risk is a function of toxicity and exposure. No or* de minimis *risk to individual monarchs is expected if (a) there is no spatiotemporal overlap between pesticide use or residues after application and milkweed or monarch presence, (b) monarchs can avoid pesticide exposure, or (c) the pesticide exposure concentration is lower than the concentration that causes toxic effects. A significant risk to monarch populations cannot be precluded if the exposure concentration exceeds the concentrations that causes adverse effects to individual monarchs. Because monarchs are a vagile species, risk estimates to monarch populations should be characterized at the landscape scale.

With the increase in glyphosate-resistant weeds, crops genetically engineered to be dicamba-tolerant were developed (Behrens et al. 2007). Starting in 2017, expanded use of dicamba was accompanied by an increase in reports of off-target injury to susceptible soybeans and other nontarget plants (Jones et al. 2019). Symptoms associated with dicamba spray drift exposure to nonengineered soybeans include twisting or epinasty of stems and cupping of leaves (Egan et al. 2014a).

Saghi (2021) hypothesized that dicamba spray drift would reduce the quality of downwind common milkweed and, in turn, adversely affect monarch oviposition and larval development. The current dicamba label requires a 73-m in-crop field spray drift buffer (USEPA 2020). Using EPA's AgDRIFT model (USEPA 2011a), estimated exposure at the crop field edge would be approximately 1%–2% of the application rate.

Saghi (2021) treated common milkweed in the field with a dicamba exposure level of 5 g of acid equivalents (ae) per ha, which is approximately 1% of the application rate for maize and dicamba-resistant soybean (Egan et al. 2014b). The milkweed displayed low levels of injury in no more than three leaf nodes, with symptoms typical of synthetic auxin herbicides (e.g., elongation of affected leaves with distorted venation). New leaves emerging within 2 weeks after exposure were malformed, whereas leaves present at application and those emerging after 2 weeks did not exhibit symptoms. The plant height and the date of flowering were unaffected. Dicamba injury to common milkweed reduced monarch oviposition in 2019 (dicamba applied 2 days before first oviposition) but not in 2020 (application 15 days before first oviposition). No relationship was found between visual injury ratings and the number of eggs laid on treated and untreated plants, suggesting that the reduction in egg laying on dicamba-treated plants in 2019 was due to factors such as leaf nutritional quality or quantity of cardenolides (Saghi 2021).

In 4-day and full-life-cycle feeding studies, Saghi (2021) provided larvae leaves harvested 27 days after dicamba field applications as well as leaves treated in the laboratory at an application rate equivalent to 5 g ae per ha. Compared with untreated controls, dicamba-treated leaves did not affect monarch larval weight, pupal weight, adult forewing length, or sex ratio. There were also no differences in protein or fiber content of treated and untreated leaves. Regardless of treatment, larvae that consumed leaves near the apical bud had greater weight gain than larvae that fed on older leaves, likely because of the higher nutritional quality of young leaves (Zalucki and Kitching 1982c, Agrawal 2017). Overall, the results from Saghi (2021) suggest common milkweed plants exposed to dicamba spray drift equivalent to 1% of the application rate would likely produce abnormal foliage when monarchs are ovipositing in May; however, the plants would likely resume normal growth by peak monarch egg laying from late June through mid-August.

Although Saghi (2021) reported no effects of dicamba on monarch oviposition and larval survival and development, the extent to which dicamba concentrations higher than 5 g ae per ha (1% of the application rate) would cause greater impacts on monarch development is unknown. Bohnenblust et al. (2013), however, reported no dietary effects of dicamba on corn earworm (*Helicoverpa zea*) and painted lady (*Vanessa cardui*) larvae fed soybean leaves treated at rates up to 561 g ae per ha (ca. 100% of the application rate). These authors also reported no adverse effects on corn earworm larval survival, growth, or days to pupation when reared on soybean plants treated at concentrations up to 56.1 g ae per ha. Likewise, no adverse effects were reported for painted lady larvae reared on large plum thistle (*Carduus nutans*; basal rosette diameter 17.8–20.32 cm) treated at rates up to 56.1 g ae per ha. On smaller thistles (12.7–15.2 cm diameter) treated at the same application rates, there was no effect on larval survival; however, there were significant declines in larval and pupal weight with increasing application rates.

Prior research indicates atrazine (a photosynthesis system II inhibitor; HRAC 2021) and alachlor (an inhibitor of very long-chain fatty acid synthesis; HRAC 2021) do not significantly affect common milkweed (Cramer and Burnside 1981) at field application rates. To assess the potential direct effects of atrazine and S-metolachlor (a herbicide in the same class as alachlor; HRAC 2021) on monarch larval survival and development, Olaya-Arenas et al. (2020a) undertook full-life-cycle toxicity studies. Larvae were provided common milkweed leaves treated at concentrations that matched the mean and maximum detectable field concentrations of the herbicides, as was reported by Olaya-Arenas and Kaplan (2019). At nominal concentrations of 8.59 and 239 nanograms (ng) of atrazine per g of milkweed leaf, there were no statistically significant effects on larval survival, the amount of leaf tissue consumed (marginal effect at 8.59 ng per g), development time, pupal weight, adult longevity, or adult size (Olaya-Arenas et al. 2020a). At nominal concentrations of 1.23 and 15.3 ng of S-metolachlor per g of milkweed leaf, no adverse effects were reported (Olaya-Arenas et al. 2020a). In a companion study (Olaya-Arenas et al. 2020b), first instars preferred to consume control leaves over leaves treated with S-metolachlor (1.23 and 15.3 ng per g of leaf) and atrazine (239 ng per g of leaf). Second instars, however, showed no preference for untreated milkweed leaves (Olaya-Arenas et al. 2020b).

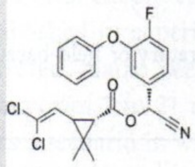
Although transient adverse effects of fomesafen (Lizotte-Hall and Hartzler 2019) and dicamba (Saghi 2021) on common milkweed did not have any effect on monarch fitness, it is possible that herbicides could damage other blooming forbs that serve as nectar sources for flower-visiting insects, including adult monarchs (Mortensen et al. 2012, Egan et al. 2014b, Jones et al. 2019). For example, Ramos et al. (2021) reported increasing reductions in flower size, plant height, and stem diameter in *Brassica rapa* treated with dicamba at 0%, 0.5%, 1%, and 10% of the application rate (560 ng ae per ha). At 25 days after exposure, plants treated at the 0.5% and 1% application rate fully or nearly recovered. Bohnenblust et al. (2016) reported that 1% (5 g ae per ha) of the dicamba field application rate reduced alfalfa (*Medicago sativa* L.) and common boneset (*Eupatorium perfoliatum* L.) blooms and decreased pollinator visits. In a multiyear field study, Egan et al. (2014b) monitored plant and arthropod communities at field edges and in a fallow field treated with dicamba application rates up to 56.1 g ae per ha and reported an application of 5.6 g ae per ha transiently reduced forb cover but not floral resources. Egan et al. (2014b) reported inconsistent perturbations of arthropod community structure in the field margins exposed to dicamba and noted no change in the responses of insect taxa that use floral resources, including Lepidoptera. In conclusion, although there are published studies that demonstrate some herbicides can damage forbs, no studies are available that assess the potential for indirect effects on adult monarchs.

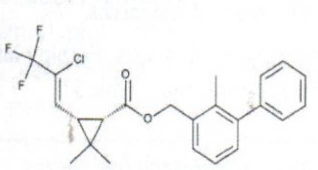
Overall, the findings reported by Bohnenblust et al. (2013), Lizotte-Hall and Hartzler (2019), Olaya-Arenas et al. (2020a, 2020b), and Saghi (2021) are generally consistent with earlier reviews of Norris and Kogan (Norris and Kogan 2000, 2005), who concluded direct exposures of insects downwind of treated crop fields to foliar-applied herbicides are unlikely to cause significant adverse effects. The studies published to date do not suggest exposure to spray drift from postemergent, foliar herbicide applications would permanently reduce the number or quality of milkweed stems in habitat downwind of treated fields. However, the number of herbicides studied to date is limited. With the widespread occurrence of herbicide-resistant weeds, the diversity and number of herbicides used in maize and soybean production are increasing (Brookes 2014, Kniss 2017). The potential for herbicide-exposed habitat to affect adult monarch use or larval survival and development will likely vary with herbicide mode of action, potency, timing of application, and the rate at which milkweed and other nontarget plants recover from herbicide injury. Clearly, additional laboratory and field toxicity studies that report full dose–response relationships for commonly used herbicides are needed to assess the potential impact of spray drift exposure to milkweed downwind of treated maize and soybean fields.

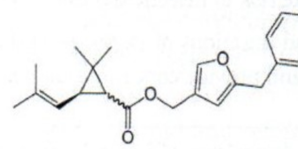
Insecticides. Four insecticide modes of action are commonly used to manage pests in maize and soybean fields (see box 5 for representative compounds): pyrethroids (soil and foliar formulations), organophosphates (soil and foliar), neonicotinoids (foliar and seed treatment), and diamides (foliar and seed treatment). Nearly 100% of maize and 50% of soybean acres in the United States employ neonicotinoid-treated seeds (Tooker et al. 2017). Although diamide insecticides are also registered as seed treatment insecticides, data on their use rates are not publicly available. In addition, 5%–20% of maize and 2%–29% of soybean acres across the North Central states are treated with foliar and soil-applied insecticides (USDA-NASS 2018, 2020). Three neonicotinoids (imidacloprid, thiamethoxam, clothianidin) and a pyrethroid (deltamethrin) have been detected in milkweed leaf tissue collected near maize and soybean fields in Indiana and Iowa (Olaya-Arenas and Kaplan 2019, Hall et al. 2021). Spatial and temporal (figure 7) overlap in use of foliar and seed treatment insecticides and presence of monarchs was documented by Krishnan et al. (2020) and Hall et al. (2021). Spray

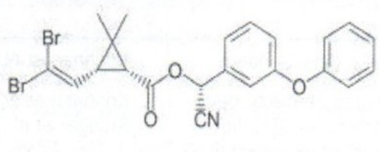
Box 5. Classes of insecticide.

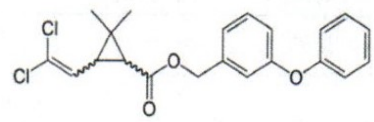
Pyrethroids


Beta-cyfluthrin

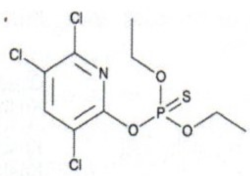

Bifenthrin


Resmethrin

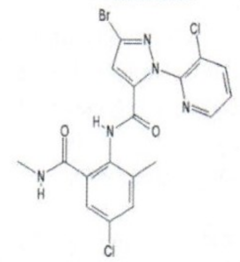

Deltamethrin


Permethrin

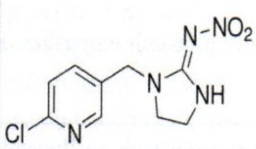
Organophosphates

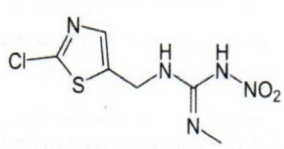

Chlorpyrifos

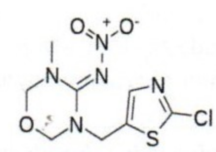
Diamides


Chlorantraniliprole

Neonicotinoids


Imidacloprid


Clothianidin


Thiamethoxam

All four insecticide classes cause an excessive influx of ions in the neuromuscular system that leads to paralysis and death of the insect. Pyrethroids activate voltage-gated sodium channels, neonicotinoids activate nicotinic acetylcholine receptors, diamides activate ryanodine receptors, and organophosphates inhibit acetylcholinesterase resulting in overstimulation of acetylcholine signaling.

drift from foliar insecticides can result in topical exposure of downwind monarchs (eggs, larvae, pupae, and adults) and milkweed and nectar plants that host monarchs. Larvae also can have dietary insecticide exposure from consuming residues on the surface of milkweed leaves. Seed treatment insecticides in runoff can be absorbed by downslope milkweed and wildflower plants; larvae and adults can subsequently be exposed by consuming leaves and nectar, respectively (see figure 2, left, lower oval).

We reviewed 15 papers published between 2006 and 2021 (see the supplemental material), in which monarch butterfly responses to chemical insecticides were reported. Toxicity and exposure estimates were derived to assess life stage risks from topical and dietary exposures to spray drift from foliar insecticides and runoff from seed treatment insecticides (box 6).

To date, the researchers in four studies have investigated the extent to which monarch oviposition behavior is influenced by presence of insecticides on milkweed. Our group found that egg-laying females did not discriminate between milkweed exposed to a control solvent and milkweed that contained foliar or systemically applied imidacloprid (Mullins et al. 2021). The imidacloprid concentrations applied in the study were above those detected in the field. We also found no difference in the total number of eggs laid on treated versus untreated plants (Mullins et al. 2021). Olaya-Arenas et al. (2020b) investigated oviposition behavior for a mixture of six pesticides (including clothianidin) applied to common milkweed leaves. Similar numbers of eggs were laid on control plants and those containing a mixture of the pesticides at their mean detectable field concentrations; however, fewer eggs were laid on milkweed

Box 6. Approaches to quantify insecticide toxicity and exposure to assess field-scale risks.

Risk is a function of effects and exposure. Below, we summarize the toxicity and exposure methods employed and reported in the primary papers to estimate risk of insecticides to different life stages of monarchs.

Step 1. Identify routes and durations of exposure to different monarch stages. Employ laboratory or field-based studies that mimic these routes of exposure and provide concentration–response relationships (table 3).

Table 3. Routes and durations of insecticide exposure to monarch life stages.

Monarch stage	Insecticide formulation	Route of exposure	Duration of exposure	Endpoints assessed	References
Egg	Foliar	Topical[a]	Acute	Egg mortality, days to hatch	Krishnan et al. (2021b)
Larva	Foliar	Topical[a]	Acute	Larval or pupal mortality, days to development, weight, diet consumption, adult eclosion	Krishnan et al. (2020), Krueger et al. (2021)
	Foliar and seed treatment	Dietary[b]	Acute and chronic	Larval or pupal mortality, days to development, herbivory, larval size and volume, weight, adult eclosion, wingspan, sex, longevity, fecundity, egg size and mass	Oberhauser et al. (2006, 2009), Krischik et al. (2015), Bargar et al. (2020), Giordano et al. (2020), Krishnan et al. (2020, 2021b), Olaya-Arenas et al. (2020a), Wilcox et al. (2021a), Knight et al. (2021)
Pupa	Foliar	Topical[a]	Acute	Pupal mortality, days to adult, adult health, weight, sex	Krishnan et al. (2021b)
Adult	Foliar	Topical[a]	Acute	Adult mortality	Oberhauser et al. (2009), Krishnan et al. (2021b)
	Foliar and seed treatment	Dietary[b]	Acute and chronic	Adult mortality, fecundity, egg hatch	Krischik et al. (2015), Krishnan et al. (2021b)

[a]Application of insecticide solution on cuticle.
[b]Provision of insecticide-treated milkweed leaves.

Step 2. Determine insecticide exposure concentrations on different matrices, including monarch butterflies, milkweed leaves, and wildflower nectar, either through residue analyses[a] or modeling (table 4).

Table 4. Methods employed to estimate insecticide exposure concentrations in monarch, milkweed and wildflower matrices for foliar and seed treatment formulations.

Matrix	Insecticide formulation	Methods employed	References
Monarch egg, larva, pupa, adult	Foliar	AgDRIFT (USEPA 2011a), a spray drift model, used to estimate pesticide mass per square centimeter at increasing distances downwind of a treatment site. Predicted concentrations were multiplied by the surface area of monarch life stage to estimate monarch dose	Krishnan et al. (2020, 2021b), Krueger et al. (2021)
Milkweed leaf	Foliar	Exposure concentrations estimated from AgDRIFT were multiplied by the surface area of milkweed leaves to estimate leaf concentrations	Krishnan et al. (2020, 2021b)
	Seed treatment	Milkweed leaves sampled from downslope milkweed plants in maize and soybean fields planted with neonicotinoid-treated seeds in Iowa	Hall et al. (2021)
	Foliar and seed treatment	Milkweed leaves sampled at different distances from crop fields in Indiana	Olaya-Arenas et al. (2019)
Wildflower nectar	Seed treatment	Wildflower nectar sampled from edge of neonicotinoid seed-treated rape fields in United Kingdom	Botias et al. (2015)

[a]To quantify spray drift exposure from foliar insecticides, sampling should be done immediately after application, downwind of treated field. To quantify exposure to insecticides used as seed treatments, sampling should be done downslope of treated field.

Step 3. Risk of an adverse effect is estimated by comparing the toxicity data from step 1 with the exposure data from step 2. Risk estimates should be based on exposure and effect data sets with consistent routes and lengths of exposure to different monarch stages.

that contained the maximum field concentrations of all the pesticides in the mixture. The extent to which clothianidin contributed to the oviposition response of the pesticide cocktail, if any, is unknown. Knight et al. (2021) found that milkweed plants established in experimental plots planted with clothianidin-treated maize seeds had clothianidin concentrations ranging from 7 to 21 ng per g. The experimental design mimics a scenario in which milkweed plants are established within seed-treated maize fields (note that monarch conservation plans do not propose reestablishment of milkweed within crop fields; see Thogmartin et al. 2017). Knight et al. (2021) found that female monarchs laid 0.09 eggs per plant per week on milkweed that contained clothianidin and 0.07 eggs per plant per week on control plants. Although this preference for milkweed containing clothianidin was statistically significant, Knight et al. (2021) noted their findings are insufficient to conclude that clothianidin-treated milkweed acts as an ecological trap. Finally, Oberhauser et al. (2006) found that female monarchs did not discriminate against milkweed exposed to permethrin (a pyrethroid) used to manage adult mosquitos (concentrations on milkweed were not reported). Olaya-Arenas et al. (2020b) also found that first-instar monarchs preferred feeding on control milkweed leaf discs rather than on 15 ng per g clothianidin-treated discs (mean detectable field concentration). However, Olaya-Arenas et al. (2020b) reported no preference of first or second instars between control and clothianidin-treated leaf discs treated at the maximum field concentration of 57 ng per g. Together, the data from these studies are inadequate to draw conclusions about whether monarch adults can differentiate between untreated milkweed and milkweed treated with field-relevant concentrations of insecticides. As was suggested by Mullins et al. (2021), electrophysiological studies to determine neurological response to the active ingredients of major insecticides, such as those conducted by Baur et al. (1998) toward plant extracts, would help address the question of detectability. For those compounds detectable by monarchs, follow-up studies to assess female behavioral response to treated milkweed will allow better prediction of how insecticide presence may or may not affect monarch egg distribution in an agricultural landscape.

In our laboratory, we assessed the topical and dietary toxicity of six insecticides on different monarch life stages (egg; first, second, third, or fifth instar larva; pupa; adult; Krishnan et al. 2020, 2021b). We found that chlorantraniliprole (a diamide) and beta-cyfluthrin (a pyrethroid) were the most toxic, followed by clothianidin and imidacloprid (neonicotinoids). Thiamethoxam (a neonicotinoid) and chlorpyrifos (an organophosphate) were generally the least toxic. Except for beta-cyfluthrin, lethal larval doses were similar in both topical and dietary studies, suggesting route of exposure does not noticeably influence toxicity (Krishnan et al. 2021b). We also reported that monarch larvae and eggs are more sensitive to insecticides than adults and pupae; beta-cyfluthrin and chlorantraniliprole were also among the most toxic insecticides to eggs, pupae, and adults (Krishnan et al. 2021b). Interestingly, relatively low doses of neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) prevented final-instar monarchs from completing pupation, possibly by interfering with the function of crustacean cardioactive peptide neurons that regulate pupal ecdysis (Krishnan et al. 2021c). Interlaboratory variation of toxicity and field-exposure data for pyrethroid and neonicotinoid insecticides are summarized in the following paragraphs.

Multiple groups have assessed the toxicity or risk of pyrethroid insecticides on monarch larvae and adults. Krueger et al. (2021) reported a fourfold greater LD_{50} for beta-cyfluthrin in fifth-instar larvae than did Krishnan et al. (2020). Up to fivefold differences in acute toxicity across monarch colonies have been observed for other insecticides (see Krishnan et al. 2021b). Krueger et al. (2021) also found bifenthrin to be two to three times less toxic than beta-cyfluthrin. Both topical and dietary exposures to pyrethroids used in aerial agricultural pest and mosquito management are predicted to cause high rates of larval mortality up to 38 m downwind of treated areas and smaller larval or pupal weights, and extended development times in survivors (Oberhauser et al. 2006, 2009, Giordano et al. 2020, Krishnan et al. 2020, Krueger et al. 2021). Adult females exposed to tropical milkweed freshly sprayed with a formulated permethrin product had lower survival rates than control females (Oberhauser et al. 2006). Topical exposure to aerial and ultralow volume applications of beta-cyfluthrin and resmethrin, respectively, also are predicted to cause high mortality in monarch adults downwind of application sites (Oberhauser et al. 2009, Krishnan et al. 2021b).

Across three research groups, monarch colonies, and milkweed species, chronic exposure to clothianidin concentrations of 47–205 ng per g leaf caused 30%–50% larval mortality (Bargar et al. 2020, Olaya-Arenas et al. 2020a, Krishnan et al. 2021b). Consistent with observations in Bargar et al. (2020), Krishnan et al. (2020, 2021b), and Prouty et al. (2021) found that pupal ecdysis was disrupted in larvae exposed to clothianidin treatments. Knight et al. (2021) found that larvae chronically exposed to leaf concentrations of 7–21 ng per g clothianidin had 3% mortality, whereas Olaya-Arenas et al. (2020a) found chronic exposure to 15 ng per g did not affect larval survival. Chronic exposure to concentrations of 177–1154 ng per g reduced growth in 50% of larvae (Bargar et al. 2020), whereas 5–57 ng per g had no effect on developmental days, adult eclosion, weight, wingspan, longevity, and sex ratio (Olaya-Arenas et al. 2020a, Krishnan et al. 2021b). We found that a tenfold lower concentration (0.5 ng per g of clothianidin) reduced adult weight ($p = 0.044$; Krishnan et al. 2021b). Chronic exposure of larvae to at least 1.2 ng per g of leaf clothianidin did not affect larval survival and had no deleterious effects on reproduction or navigation of subsequent adults (Wilcox et al. 2020, 2021). On the basis of toxicity data reported by Olaya-Arenas et al. (2020a) and Krishnan et al. (2021b), the mean concentrations of clothianidin found in milkweed within or near crop fields in Indiana (0.71 ng per g, Olaya-Arenas and Kaplan 2019) and Iowa (0.41 ng per g, Hall et al. 2021) are unlikely to cause larval mortality (Krishnan et al. 2021b). However, the maximum observed concentrations in milkweed collected in Indiana (56.5 ng per g, Olaya-Arenas and Kaplan 2019) and Iowa (6.6 ng per g, Hall et al. 2021) could cause anywhere between 0% and 30% mortality (Krishnan et al. 2021b), on the basis of toxicity data reported by Olaya-Arenas et al. (2020a) and Krishnan et al. (2021b). On the basis of toxicity data reported by Bargar et al. (2020), Olaya-Arenas et al. (2020a), and Krishnan et al. (2021b), the mean and maximum milkweed field concentrations reported by Olaya-Arenas and Kaplan (2019) and Hall et al. (2021) are unlikely to cause significant sublethal effects in larvae (Krishnan et al. 2021b). In addition, no mortality was observed when adults were fed 140 ng clothianidin per g artificial nectar (Krishnan et al. 2021b); the maximum reported concentration of clothianidin in wildflower nectar is 0.5 ng per g (Botías et al. 2016).

Monarch toxicity studies with imidacloprid also suggest low risk of use in agricultural settings. We obtained a 2-day dietary LC_{90} of 19,000 ng per g (milkweed leaf concentration) for second instars (Krishnan et al. 2020), whereas Krischik et al. (2015) obtained a 7-day dietary LC_{100} for early instars of 10,400 ng per g (milkweed flower concentration; leaf tissue was not analyzed). The mean and maximum imidacloprid concentrations found in milkweed in Indiana and Iowa were 0.01–0.3 ng per g and 2.8–3.7 ng per g, respectively (Olaya-Arenas and Kaplan 2019, Hall et al. 2021). Neither of these concentration ranges is expected to cause any mortality or sublethal effects (developmental days, adult eclosion, weight, wingspan, and sex ratio; Krishnan et al. 2021b). So far, only two monarch adult dietary studies with imidacloprid have been conducted with an appropriate control and experimental design (see Krishnan et al. 2021b for details). Krischik et al. (2015) found a 29-day exposure to 15 and 30 ng per mL imidacloprid did not result in mortality, reduced fecundity, or reduced egg hatch. We found adult monarchs that consumed 0.05 ml of a 250 ng per mL (or 250 ng per g) imidacloprid solution had no mortality over a 4-day postexposure observation period (Krishnan et al. 2021b). The highest imidacloprid concentration reported in wildflower nectar is 0.17 ng per g (Botías et al. 2016).

Like those of the other neonicotinoids, the mean (1.6–1.9 ng per g) and maximum (13–150 ng per g) common milkweed leaf concentrations of thiamethoxam observed in Indiana (Olaya-Arenas and Kaplan 2019) and Iowa (Hall et al. 2021) are also unlikely to cause larval mortality or sublethal effects (Krishnan et al. 2021b). Adult dietary exposure to 330 ng per g thiamethoxam also did not cause any mortality (Krishnan et al. 2021b); the maximum thiamethoxam concentration reported in wildflower nectar is 1.8 ng per g (Botías et al. 2016). Future field studies that quantify residues of chlorantraniliprole seed treatment in milkweed are needed to estimate their risks to monarch larvae. Likewise, adult dietary toxicity and nectar residue data for chlorantraniliprole are needed to assess full life cycle risks.

The current monarch toxicity database spans four modes of action and 10 compounds. Most studies address the larval life stage; the data are limited for egg, pupa, and adult life stages. Currently, there is incomplete or no published toxicity data available for uses of newer insecticides (e.g., diamides, sulfoximines, and pyropene). To facilitate use of new toxicity data in risk analyses, USEPA (2011b) has published suggested study designs (see the supplemental material).

Attributes to consider when evaluating a terrestrial insect toxicology study. Field studies suggest monarch larvae are likely exposed to mixtures of insecticides, herbicides, and fungicides (e.g., see Olaya-Arenas and Kaplan 2019, Hall et al. 2021). To model the potential toxicity of pesticide mixtures, full dose–response curves for the mixture components are needed (National Research Council 2013). For pesticide mixtures with compounds at concentrations that do not elicit toxicity, no mixture toxicity would be expected if the chemicals act through different mechanisms of action and none of the compounds act as a synergist (National Research Council 2013). If components of a mixture act through a similar mechanism of action, a concentration response model can be employed (National Research Council 2013; see an example for estimating monarch larval toxicity to a mixture of neonicotinoid insecticides in Hall et al. 2021). If mixture components act through different mechanisms of action, response-addition models can be employed (National Research Council 2013). In this regard, foliar and seed treatment fungicides are used in North Central region of the United States (Serrano 2017, Penney et al. 2021), typically in combination with pyrethroid or neonicotinoid insecticides. Although robust dose–response curves are available for these insecticide classes, currently, such data are not available for fungicides. Olaya-Arenas et al. (2020a) were the first to report fungicide toxicity data. They assessed larval toxicity to azoxystrobin, pyraclostrobin, and trifloxystrobin using the mean and maximum detectable milkweed leaf concentrations reported in an Indiana field survey. At mean concentrations, none of the compounds reduced larval survival, herbivory, development time, or pupal weight. At maximum concentrations, significant and marginally significant effects on larval survival were seen with azoxystrobin ($p = 0.03$) and trifloxystrobin ($p = 0.07$). Inconsistent effects on adult wing size and adult longevity were reported.

In summary, a comparison of neonicotinoid toxicity data (Krischik et al. 2015, Bargar et al. 2020, Olaya-Arenas et al. 2020a, Krishnan et al. 2021b) with neonicotinoid concentrations detected in milkweed leaves and wildflower nectar collected from plants in close proximity to crops (Botias et al. 2016, Olaya-Arenas and Kaplan 2019, Hall et al. 2021) indicate these seed treatments pose little to no risk to monarch larvae and adults (Krishnan et al. 2021b). However, foliar-applied insecticides, particularly aerial applications of pyrethroid (beta-cyfluthrin and bifenthrin), diamide (chlorantraniliprole), and organophosphate (chlorpyrifos) insecticides, are expected to cause significant downwind larval mortality via topical and dietary exposure; lower mortality is expected from ground boom applications (Krishnan et al. 2020, Krueger et al. 2021). Aerial applications of beta-cyfluthrin, chlorantraniliprole, imidacloprid, and clothianidin are predicted to also cause high mortality in eggs, whereas pupae are unlikely to be affected (Krishnan et al. 2021b). Adults are at risk from topical exposure to aerial applications of beta-cyfluthrin and chlorpyrifos (Krishnan et al. 2021b). Given the significant field-scale risks associated with foliar insecticide use, a landscape-scale risk characterization that incorporates the vagile behavior of female monarchs is needed (figure 8).

Simulating landscape-scale insecticide effects on monarch populations. Our ability to simulate egg placement in the landscape (Grant et al. 2018, Grant and Bradbury 2019), the survival of monarch eggs and larvae under ambient conditions (Grant et al. 2020), and the instar survival rates on the basis of empirical insecticide exposure and toxicology data (Krishnan et al. 2020) gave us the tools to conduct landscape-scale risk analyses focused on larval topical and dietary exposures to foliar insecticide applications (figures 2 and 6). Our simulations of monarch production reflected different assumptions of insecticide use and habitat establishment over a 10-year period in Story County, Iowa (63,394 ha or 43% maize; 46,164 ha or 31% soybean; Grant et al. 2021). We examined the potential impact of spring insecticide use to manage true armyworm (*Mythimna unipuncta*) in crop fields (see figure 7) and determined that, because of the low number of acres treated, there was minimal impact on adult monarch production (Grant et al. 2021). Below, we summarize our spatially explicit risk analyses for aerial insecticide applications to manage soybean aphid (*Aphis glycines*), a pest of soybean (Grant et al. 2021).

For the soybean aphid, we assumed a single midsummer insecticide application in 1, 3, and 5 years out of 10 to reflect historical variation in populations exceeding economic thresholds on the basis of integrated pest management recommendations. We also modeled insecticide applications in all 10 years to reflect a prophylactic pest management strategy. Only one monarch generation was exposed per year, according to historical patterns of insecticide use. We developed a demographic model that combined larval mortality from insecticide exposure (eggs and pupae are unlikely to be exposed to foliar insecticide drift; see Krishnan et al. 2021b) and natural causes in overall estimates of survival. We employed four milkweed establishment scenarios representing potential conservation actions in the county, consistent with the Iowa Monarch Conservation Strategy (IMCC 2018). Scenarios 1, 2, and 3 were described earlier (see the "Simulating adult monarch production" section) and represent current milkweed density and maximum and moderate habitat establishment throughout the landscape, respectively. We created a new scenario, scenario 4, which represented moderate habitat establishment outside a 38-m buffer around soybean fields, reflecting guidance in the NRCS-USFWS ESA section 7 conference report (USDA-NRCS 2016) to minimize monarch insecticide exposure. For each of these scenarios, we estimated spray-drift-related larval mortality due to contact and dietary exposure within the 38-m buffer to six different insecticides (beta-cyfluthrin, chlorantraniliprole, chlorpyrifos, imidacloprid, clothianidin, thiamethoxam), on the basis of data published in Krishnan et al. (2020). We also included lower and upper bound assumptions of 0% and 100% mortality in the spray drift zone. The drift zone was modeled on northwest sides of soybean fields because the prevailing winds are from the south and east at that time of application. The result was monarch production over 10 years in Story County, under a variety of milkweed establishment and pesticide drift scenarios.

Assuming no insecticide spray drift, 10-year monarch production increased over the current condition (scenario 1) by 24.7% and 9.3%, with maximum (scenario 2) and moderate (scenario 3) habitat establishment, respectively. Three landcover classes accounted for the total increase in production: roadsides, grassland or pasture, and low-intensity development. Assuming moderate habitat establishment only outside the 38-m buffer (scenario 4), which excludes approximately 80% of rural roadsides and 40% of noncrop landcover from new habitat establishment, monarch production increased by 3.5%. We then simulated monarch production following insecticide exposure. In scenario 3, monarch production increased 8.2%–9.3%, depending on the insecticide and how often it was applied (one, three, or five times) over the 10-year period. With maximum habitat establishment, monarch production increased 22.2%–24.7%. Chlorantraniliprole caused the greatest reduction in production, followed closely by chlorpyrifos and beta-cyfluthrin. Thiamethoxam had the smallest effect on production. To address uncertainty in estimated mortality rates, we also assumed an insecticide could cause 100% larval mortality (Grant et al. 2021). Using this upper bound assumption, no adults are produced downwind of a treated field. Under this assumption, and with insecticide applications occurring one, three, and five times over a 10-year period, Grant et al. (2021) reported scenario 3 produced 4.57%, 2.51%, and 0.45% more adults than did scenario 4. Under the same assumption that an insecticide could cause 100% mortality but with applications every year for 10 years, scenario 3 produced 4.70% fewer adults over the 10-year period than did scenario 4 (Grant et al. 2021).

Our simulations reported in Grant et al. (2021) indicate that it is unlikely that habitat establishment within 38 m of agricultural fields in a landscape such as Story County, Iowa, will result in a net decrease in monarch population due to insecticide spray drift. If new monarch habitat is placed near agricultural fields, the increased monarch

Box 7. Research highlights.

Modeling

(see: **Spatially explicit landscape-scale simulation of adult monarch production**)

- An agent-based model was developed that simulates spatially explicit female monarch movement and egg-laying at an ecoregion scale.
- In conjunction with a novel Bayesian model that estimates life-stage survival probabilities, next-generation adult population sizes can be estimated based on different assumptions of future habitat establishment and pesticide use patterns.
- Simulations of ecoregion population responses based on different habitat establishment and pesticide-use scenarios indicate population increases of 10–25% per generation of breeding monarchs can be expected based on proposed habitat establishment rates in monarch conservation plans.
- Shape of landcover polygons is unimportant to simulated egg density, but increasing size up to 2.87 ha increases egg density.
- The highest priority future improvement to the agent-based model is the inclusion of periodic > 50-m flight steps, which would significantly increase realism. With a more extensive effort, the movement algorithm could be based on natural step lengths sampled from empirical distributions, with flight direction biased by prevailing wind direction. Collection of additional milkweed and egg density data across contiguous landcover categories would help reduce uncertainty in landscape attributes and improve the means to evaluate model outputs.

Monarch Movement and Habitat Utilization

(see: **Monarch movement ecology and habitat utilization**)

- Radio telemetry can be employed to study monarch movement.
- Female monarchs are vagile – they have been observed traversing > 500 m of crop fields to resource habitat.
- Wind direction is important in determining orientation during foraging flights.
- Within contiguous habitat classes, flight steps are typically < 50 m, and flight patterns are a function of resource density.
- Monarch perceptual range is estimated to be at least 125 m, which is considerably greater than estimates for other species.
- Monarchs readily cross habitat edges with steps up to 1,900 m. Motivations for these long steps are undetermined. Future studies tracking monarchs emigrating from a resource habitat, traversing the matrix, and immigrating to a new resource habitat could help determine if these long flights are appetitive (ranging) or non-appetitive (where displacement *per se* is the goal).

Risks of Pesticide Use

(see: **Evaluating risks of pesticide use to milkweed and monarchs**)

- Due to a limited number of studies, the extent to which herbicides used in maize and soybean production will adversely affect milkweed or monarchs is uncertain. Currently available data indicate fomesafen and dicamba are unlikely to cause more than transient perturbations in milkweed quality downwind of crop fields. These perturbations are unlikely to affect female use and larval development.
- Use of neonicotinoid-treated maize and soybean seeds pose little to no risk to monarch larvae and adults utilizing milkweed or blooming forbs downslope of crop fields.
- Foliar applications of insecticides, in particular those in the diamide and pyrethroid classes, are expected to kill a large percentage of larvae and eggs downwind of treated crop fields. Although adults and larvae may be extirpated from habitat downwind of treated fields because of insecticide spray drift, these areas will likely be recolonized by other females in the landscape and will not become population sinks. Consequently, establishment of a 38-m "no new habitat" buffer zone around crops for managing insecticide risks would likely be detrimental to monarch population growth in the North Central states.

production from areas around untreated fields and from the upwind side of treated fields outweigh reduced adult production from annual insecticide drift events. In other words, establishing new milkweed habitat within 38 m of agricultural fields will not create monarch population sinks. Other vagile butterfly species that move at spatial scales greater than the scale of potential insecticide spray-drift impacts would likely show similar results (Grant et al. 2021).

Our simulations to date do not include the potential impact of herbicide spray drift on milkweed and potential indirect effects on monarch larvae. So far, studies with fomesafen and dicamba demonstrate temporary effects on milkweed growth and biomass but no effect on monarch egg laying nor survival or growth of immature monarchs (Lizotte-Hall and Hartzler 2019, Saghi 2021). Although these studies do not provide a basis to develop model simulations that include herbicide effects on milkweed viability, if future research demonstrates herbicide exposure adversely affects milkweed stem survival or quality downwind of treated crop fields, these effects on stem density can be included in the agent-based model.

Conclusions

To sustain the eastern North American monarch population, 1.3–1.6 billion new milkweed stems with a diversity of forbs need to be established in the North Central region of the United States (Pleasants 2017, Thogmartin et al. 2017). This goal can be reached only with substantial use of land in agricultural landscapes. Using a model-guided approach, we designed and implemented a research project to determine how the amount and configuration of habitat and pesticide use patterns interact to influence monarch butterfly populations in their summer breeding range. The research effort included development of a spatially explicit, agent-based model of female monarch movement and egg laying, which was linked to a Bayesian life-stage survival model. The development and evaluation of these models were informed by results of laboratory and field-based research on monarch life history, movement ecology, and pesticide toxicology (box 7).

Because of their open population structure, we conclude that breeding monarchs should be resilient to habitat fragmentation and insecticide use in North Central agricultural landscapes. However, adult recruitment can be enhanced to the extent that placement of new habitat parcels containing milkweed and forb resources reduces the distance between existing habitats in the landscape, thus reducing monarch search time and probably increasing lifetime fecundity. On the basis of our spatially explicit, landscape-scale modeling and related empirical studies, we estimate a pattern of habitat tracts containing milkweed and forbs placed at 50 to 100 m intervals would be optimal, even when established next to crop fields treated with insecticides. Simulated population increases are maximized when integrated pest management practices and spray drift mitigation measures are implemented. Simulations indicate that the shape of newly established habitat areas is unimportant, although increasing size up to approximately 2.5 ha may increase egg density.

These landscape-scale findings are unlikely to translate directly to other North Central butterflies of conservation concern. These species are less mobile than the monarch, and their populations are likely less resilient to habitat fragmentation and more vulnerable to extirpation of subpopulations because of pesticide exposure (see Supplemental Information: Butterfly Spcies of Conservation Concern in the North Central USA).

A population increase of 10%–25% per generation of breeding monarchs is our best estimate of what is possible under current land-use patterns and implementation of monarch conservation plans in the North Central states. The question is, how much does this increase contribute to an increase in the overwintering population in Mexico? Over four generations, a 10%–25% increase per generation suggests an overall increase of 46%–144% for the summer reproductive season. Is this enough to reverse the eastern North American population decline? We cannot currently answer that question with our models and empirical research results. Linking our findings with continental-scale models (Flockhart et al. 2015, Oberhauser et al. 2017) would advance understanding of how conservation actions at midrange scales will affect the eastern North American population.

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Supplemental material

Supplemental data are available at *BIOSCI* online.

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