2020

Assessing field-scale risks of foliar insecticide applications to monarch butterfly (Danaus plexippus) larvae

Niranjana Krishnan
Iowa State University, nkrish@iastate.edu

Yang Zhang
Beijing Great-Agri Institute of Pesticide Technology

Keith G. Bidne
U.S. Department of Agriculture

Richard L. Hellmich
U.S. Department of Agriculture, rlhellmi@iastate.edu

Joel R. Coats
Iowa State University, jcoats@iastate.edu

See next page for additional authors

Assessing field-scale risks of foliar insecticide applications to monarch butterfly (Danaus plexippus) larvae

Abstract

Establishment and maintenance of milkweed plants (*Asclepias* spp.) in agricultural landscapes of the north central United States are needed to reverse the decline of North America's eastern monarch butterfly (*Danaus plexippus*) population. Because of a lack of toxicity data, it is unclear how insecticide use may reduce monarch productivity when milkweed habitat is placed near maize and soybean fields. To assess the potential effects of foliar insecticides, acute cuticular and dietary toxicity of 5 representative active ingredients were determined: beta-cyfluthrin (pyrethroid), chlorantraniliprole (anthranilic diamide), chlorpyrifos (organophosphate), and imidacloprid and thiamethoxam (neonicotinoids). Cuticular median lethal dose values for first instars ranged from 9.2 × 10−3 to 79 μg/g larvae for beta-cyfluthrin and chlorpyrifos, respectively. Dietary median lethal concentration values for second instars ranged from 8.3 × 10−3 to 8.4 μg/g milkweed leaf for chlorantraniliprole and chlorpyrifos, respectively. To estimate larval mortality rates downwind from treated fields, modeled insecticide exposures to larvae and milkweed leaves were compared to dose–response curves obtained from bioassays with first-, second-, third-, and fifth-instar larvae. For aerial applications to manage soybean aphids, mortality rates at 60 m downwind were highest for beta-cyfluthrin and chlorantraniliprole following cuticular and dietary exposure, respectively, and lowest for thiamethoxam. To estimate landscape-scale risks, field-scale mortality rates must be considered in the context of spatial and temporal patterns of insecticide use.

Keywords

Monarch butterfly, Insecticides, Toxicology, Risk assessment, Conservation

Disciplines

Ecology and Evolutionary Biology | Entomology | Natural Resources and Conservation | Population Biology

Comments

This article is published as Krishnan, Niranjana, Yang Zhang, Keith G. Bidne, Richard L. Hellmich, Joel R. Coats, and Steven P. Bradbury. "Assessing field-scale risks of foliar insecticide applications to monarch butterfly (Danaus plexippus) larvae." *Environmental Toxicology and Chemistry* (2020). doi: 10.1002/etc.4672.

Authors

Niranjana Krishnan, Yang Zhang, Keith G. Bidne, Richard L. Hellmich, Joel R. Coats, and Steven P. Bradbury

Environmental Toxicology and Chemistry—Volume 00, Number 00—pp. 1–19, 2020
Received: 1 November 2019 | Revised: 5 December 2019 | Accepted: 15 January 2020

Hazard/Risk Assessment

OPEN DATA

Assessing Field-Scale Risks of Foliar Insecticide Applications to Monarch Butterfly (*Danaus plexippus*) Larvae

Niranjana Krishnan,[a,*] Yang Zhang,[b] Keith G. Bidne,[c] Richard L. Hellmich,[c] Joel R. Coats,[a] and Steven P. Bradbury[a,d]

[a]Toxicology Program and Department of Entomology, Iowa State University, Ames, Iowa, USA
[b]Beijing Great-Agri Institute of Pesticide Technology, Beijing, China
[c]Corn Insects and Crop Genetics Research Unit, US Department of Agriculture, Ames, Iowa, USA
[d]Department of Natural Resource Ecology and Management, Iowa State University, Ames, Iowa, USA

Abstract: Establishment and maintenance of milkweed plants (*Asclepias* spp.) in agricultural landscapes of the north central United States are needed to reverse the decline of North America's eastern monarch butterfly (*Danaus plexippus*) population. Because of a lack of toxicity data, it is unclear how insecticide use may reduce monarch productivity when milkweed habitat is placed near maize and soybean fields. To assess the potential effects of foliar insecticides, acute cuticular and dietary toxicity of 5 representative active ingredients were determined: beta-cyfluthrin (pyrethroid), chlorantraniliprole (anthranilic diamide), chlorpyrifos (organophosphate), and imidacloprid and thiamethoxam (neonicotinoids). Cuticular median lethal dose values for first instars ranged from 9.2×10^{-3} to 79 μg/g larvae for beta-cyfluthrin and chlorpyrifos, respectively. Dietary median lethal concentration values for second instars ranged from 8.3×10^{-3} to 8.4 μg/g milkweed leaf for chlorantraniliprole and chlorpyrifos, respectively. To estimate larval mortality rates downwind from treated fields, modeled insecticide exposures to larvae and milkweed leaves were compared to dose–response curves obtained from bioassays with first-, second-, third-, and fifth-instar larvae. For aerial applications to manage soybean aphids, mortality rates at 60 m downwind were highest for beta-cyfluthrin and chlorantraniliprole following cuticular and dietary exposure, respectively, and lowest for thiamethoxam. To estimate landscape-scale risks, field-scale mortality rates must be considered in the context of spatial and temporal patterns of insecticide use. *Environ Toxicol Chem* 2020;00:1–19. © 2020 SETAC

Keywords: Monarch butterfly; Insecticides; Toxicology; Risk assessment; Conservation

INTRODUCTION

In North America, the eastern population of monarch butterflies (*Danaus plexippus*) has declined significantly in the last 2 decades (Brower et al. 2012; Oberhauser et al. 2017). The historically low 2013 overwintering monarch population, combined with the 2-decade trend, prompted a petition to the US Fish and Wildlife Service (2016) to list the monarch as a threatened species under the Endangered Species Act. From 2004 to 2018, the eastern population occupied an average of 3.46 hectares (ha) of overwintering forest canopy (Monarch Watch 2019). This level is well below a long-term average of 6 ha that is needed to support a resilient population and mitigate the potential loss of the North American migration (Semmens et al. 2016). Approximately 40 to 50% of the monarchs overwintering in Mexico originate in the north central United States (Flockhart et al. 2017), and it is vital to improve summer breeding success in this region (Oberhauser et al. 2017). To maintain a resilient monarch population, an estimated 1.3 to 1.6 billion additional milkweed stems need to be added to the north central US landscape (Thogmartin et al. 2017). Milkweed species (*Asclepias* spp.), and primarily common milkweed (*Asclepias syriaca*) in the north central states (Malcolm et al. 1993), are obligate hosts for monarch larvae. The habitat goal for the north central United States can only be met through a significant conservation effort in agricultural landscapes, including rural roadsides, marginal cropland, portions of existing Conservation Reserve Program land, pastures, and grassy areas bordering maize and soybean fields (Thogmartin et al. 2017).

In the north central United States, monarch larvae are present from mid-May to late September (Prysby and Oberhauser 2004; Nail et al. 2015; Pleasants 2015) and could be exposed to insecticides used to manage early- and late-season pests in conventional maize and soybean production, which are the dominant crops in the region (see Figure 1). Soybean aphid

This article includes online-only Supplemental Data.
* Address correspondence to nkrish@iastate.edu
Published online 21 January 2020 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.4672

(*Aphis glycines*) is a major late-season pest of soybean (Hodgson et al. 2012), and true armyworm (*Mythimna unipuncta*) is an emerging early-season pest in maize fields containing rye cover crops (Dunbar et al. 2016). These pests are managed with pyrethroid, organophosphate, or neonicotinoid foliar applications (Hodgson et al. 2012; Dunbar et al. 2016). The percentage of maize and soybeans treated with foliar or soil-applied formulations in the north central states ranges from 8% in Kansas, Minnesota, and Michigan to 20% in Illinois and from 6% in Michigan to 30% in Minnesota, respectively (US Department of Agriculture 2018). Nationally, at least 79% of maize and 34% of soybeans are planted with neonicotinoid-treated seeds (Douglas and Tooker 2015). Consistent with these use patterns, neonicotinoids have been detected in milkweed growing near maize and soybean fields (Olaya-Arenas and Kaplan 2019). Chlorantraniliprole, an anthranilic diamide, recently entered the market in both foliar and seed treatment formulations (Thrash et al. 2013; Carscallen et al. 2019).

The US Fish and Wildlife Service (2017) has identified insecticide exposure as one of the potential threats to monarch butterfly recovery. In 2016 and 2017, the US Department of Agriculture's National Resources Conservation Service's (2016) *Monarch Butterfly Wildlife Habitat Evaluation Guide* discouraged placement of monarch breeding habitat within 38 m of crop fields treated with herbicides or insecticides. Employing a "no habitat buffer" of this size would significantly reduce the area of land available for establishing breeding habitat and hectares of small habitat patches (e.g., 0.4–2.0 ha) that are crucial for supporting increased monarch egg densities across the landscape (Zalucki et al. 2016; Grant et al. 2018). For example, in Story County, Iowa, USA, a 38-m buffer around conventional maize and soybean fields represents approximately 84% of rural roadside rights-of-way and 38% of grassland, Conservation Reserve Program land, pastures, railroad rights-of-way, riparian corridors, and wetlands.

We are developing a landscape-scale approach (Grant and Bradbury 2019; Uhl and Brühl 2019) to test the hypothesis that conservation benefits of establishing monarch breeding habitat in close proximity to maize and soybean fields will outweigh the risks of increased insecticide exposure. However, the current paucity of insecticide toxicity data precludes the means to assess field-scale and landscape-scale mortality rates. Consequently, we are undertaking a series of acute and chronic toxicity studies that are relevant for foliar and seed treatment insecticide formulations. Here, we report larval acute contact and dietary toxicity of 5 insecticides registered for foliar applications to manage early- and late-season insect pests in maize and soybean fields: beta-cyfluthrin (a pyrethroid), chlorantraniliprole (an anthranilic diamide), chlorpyrifos (an organophosphate), and imidacloprid and thiamethoxam (neonicotinoids). Using data from these toxicity studies and exposure estimates obtained from spray drift modeling, we predict larval mortality rates from the edge of a treated field to 60 m downwind following aerial and ground boom applications.

MATERIALS AND METHODS

Monarch butterfly rearing

Monarch colonies at Iowa State University are maintained by the US Department of Agriculture's Corn Insects and Crop

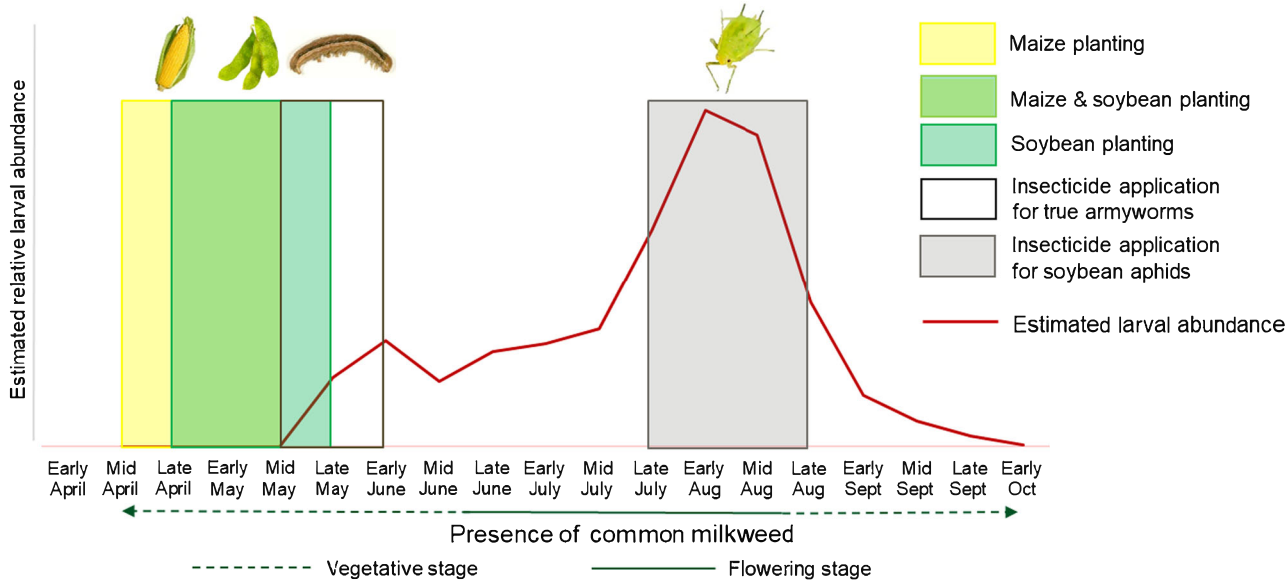

FIGURE 1: Conceptual model depicting maize and soybean planting dates, periods of economically significant true armyworm and soybean aphid populations, monarch larval abundance, and common milkweed phenology in Iowa. Monarch larval abundance (red line) for the north central United States was estimated for the years 1997 to 2014 (Prysby and Oberhauser 2004; Nail et al. 2015; Pleasants 2015). A supplementary file (*Monarch abundance calculations*) contains data used to derive these estimates. Approximate dates for maize and soybean planting (yellow and green bars, respectively) were obtained from Iowa State University extension reports (Pedersen 2007; Elmore 2012). Approximate insecticide application dates for managing true armyworm (white bar) and soybean aphid (light gray bar) populations exceeding economic thresholds in Iowa were based on Dunbar et al. (2016) and Hodgson et al. (2012), respectively. Presence and stage of common milkweed (solid and dotted green line) from April to September in the north central United States were obtained from Kaul et al. (1991) and Journey North (2016).

Genetics Research Unit in Ames, Iowa. Every spring and summer from 2014 through 2017, monarch butterfly eggs were collected from common milkweed plants in rural roadsides and Iowa State University farms in Boone and Story Counties, Iowa, to establish 2014, 2015, 2016, and 2017 colonies. Adult male and female monarchs, obtained from the respective colonies, were housed in aluminum frame cages (~60 × 60 × 60 cm) with brass screens (14 × 18 mesh). Stems of tropical milkweed (*Asclepias curassavica*) with leaves, and occasionally flowers, were placed in the cages to facilitate egg laying. After 3 to 4 h, the stems were removed and kept for 3 d in an I-35VL incubator (Percival Scientific) maintained at 21.1 °C, 65% relative humidity, and a 16:8-h light:dark cycle. On day 4, eggs were moved to another incubator maintained at 26.6 °C (65% relative humidity and 16:8-h light:dark cycle) to induce hatching. Newly hatched larvae (0–12 h old) were individually plated onto Petri plates (60 × 15 mm) with a thin layer of 2% agar:water and a freshly picked and surface-sterilized (washed in 10% bleach:water solution, followed by 3 water rinses) milkweed leaf. The larvae were reared in the 26.6 °C incubator and fed additional tropical milkweed leaves ad libitum, except from June through September when larvae were raised on freshly picked and surface-sterilized common milkweed leaves collected from nonagricultural sites in Story and Boone Counties, Iowa. On day 11, individual larvae were transferred to 8-oz Comet plastic tumblers (Waddington North America) inverted over an open Petri plate (100 × 15 mm) fitted with a 90-mm disk of Whatman No. 1 filter paper (GE Healthcare). When the larvae initiated pupation (typically days 15–17), they were held at room temperature. After eclosion (typically days 29–32), butterflies were screened for *Ophryocystis elektroscirrha*, using the method described by Altizer et al. (2000); infected individuals were sacrificed. Adult monarchs were provided Gatorade Glacier Cherry Frost Thirst containing sugar and dextrose (The Gatorade Company) as a nutritional source. Toxicity bioassay studies were undertaken with the 2014 and 2015 colonies in 2017, 2018, and the first half of 2019. The cumulative survival from egg stage through pupation when bioassays were undertaken ranged from approximately 75 to 80%.

Milkweed production

Tropical milkweed used to support the colonies and bioassay studies were grown from seed (Johnny's Selected Seeds) in Iowa State University greenhouses at 10 to 41 °C with a 16:8-h light:dark cycle. Seeds were planted in 128-cell plug trays with potting soil (F1-P potting mix; Sun Gro Horticulture) mixed with a fertilizer (Osmocote Pro 19-5-8 + Minors; Hummert International; 500 g/79 L of soil). After approximately 6 wk, 1 or 2 plants were transplanted to 8.9-cm square pots or 3.8-L pots, respectively. Plants were watered twice a day, which included one watering with liquid fertilizer (Peters Professional Peat Lite Special 20-10-20; ICL Specialty Fertilizers; 100 mg/L nitrogen). To manage oleander aphids (*Aphis nerii*) and western flower thrips (*Frankliniella occidentalis*), we released parasitic wasps (*Aphidius colemani*), predatory mites (*Neoseiulus californicus* and *Phytoseiulus persimilis*), and rove beetles (*Dalotia coriaria*) on a regular basis.

Insecticides

Toxicity studies were conducted with the following analytical-grade insecticides (International Union of Pure and Applied Chemistry name; Chemical Abstracts Service number; percentage purity): beta-cyfluthrin ([(*R*)-cyano-(4-fluoro-3-phenoxyphenyl)methyl] (1*S*)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate; 1820573-27-0; 99.3%), chlorantraniliprole (5-bromo-*N*-[4-chloro-2-methyl-6-(methylcarbamoyl)phenyl]-2-(3-chloropyridin-2-yl)pyrazole-3-carboxamide; 500008-45-7; 97.3%), chlorpyrifos (diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-λ^5-phosphane; 2921-88-2; 99.3%), imidacloprid (*N*-[1-[(6-chloropyridin-3-yl)methyl]-4,5-dihydroimidazol-2-yl]nitramide; 138261-41-3; 100%), and thiamethoxam (*N*-[3-[(2-chloro-1,3-thiazol-5-yl)methyl]-5-methyl-1,3,5-oxadiazinan-4-ylidene]nitramide; 153719-23-4; 99.3%). Chlorantraniliprole was provided by DuPont Pioneer (now Corteva Agriscience). The remaining compounds were purchased from Sigma-Aldrich. To prepare insecticide stock solutions for cuticular and dietary bioassays, certified American Chemical Society reagent-grade acetone and Silwet L-77 were purchased from Fisher Scientific.

Toxicity studies

Foliar insecticide applications can result in spray drift landing directly on the larvae (cuticular exposure) and/or on the milkweed (dietary exposure). Toxicity studies were undertaken to mimic these 2 routes of exposure. Cuticular toxicity studies were undertaken using first-, third-, and fifth-instar larvae. Dietary toxicity studies were undertaken with second-, third-, and fifth-instar larvae. First instars were not used in the dietary studies because of their sensitivity to the handling required to execute these bioassays. Individual larvae were held in Petri plates (first to fourth instars) or plastic tumblers (fourth and fifth instars), as described previously (see *Monarch butterfly rearing*), and maintained at 26.6 °C, 65% relative humidity, on a 16:8-h light:dark cycle. For both bioassays, at least 5 insecticide concentrations and an appropriate control carrier were used. Eleven larvae were used in each concentration, and studies were repeated 3 or 4 times. Half of the control larvae were weighed prior to treatment; average weights at the time of treatment for cuticular and dietary studies were calculated (Supplemental Data, Table S1). All bioassays were performed with tropical milkweed. A subset of bioassays was repeated using common milkweed to determine if milkweed species influenced larval sensitivity. Mortality, growth, reduced feeding, signs of intoxication (e.g., spasms, paralysis, loss of hemolymph), arrested ecdysis, and malformed or discolored pupae were recorded every 24 h. Observations were made up to 96 h for first, second, and third instars; fifth instars were observed to pupation. At the end of 96 h or pupation, weights and

developmental stage of the surviving larvae or pupae were noted. Only data obtained from individual bioassays that had <30% control mortality were analyzed (94 of 116 initiated bioassays; mean control mortality 10%; range 0–27%).

Cuticular toxicity studies. Insecticide stock solutions were prepared in acetone. One μL of an insecticide–acetone solution was placed on the dorsal prothorax using a 50-μL Hamilton syringe. Control larvae were treated with acetone alone. Insecticide stock solution concentrations and subsequent serial dilutions were based on the results of range-finding assays. The measured concentrations of stock solutions were within 75 to 125% of their nominal concentrations. The nominal (measured) stock solution concentrations for beta-cyfluthrin, chlorantraniliprole, imidacloprid, thiamethoxam, and chlorpyrifos were 1 (0.803), 1 (0.810), 10 (9.94), 40 (30.2), and 60 (68.7) μg/μL, respectively. Measured stock solution concentrations were determined by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS or gas chromatography-electron capture detector [GC-ECD]; see *Analysis of insecticide stock solutions*). Nominal concentrations were used to derive dose–response curves (see Supplemental Data, Table S2, and *Statistical analysis*).

Dietary toxicity studies. Larvae were reared on insecticide-treated tropical milkweed leaves for 48 h (second and third instars) or 24 h (fifth instars). Second and third instars surviving the exposure period were then fed untreated leaves ad libitum for an additional 48 h. Surviving fifth instars were fed untreated leaves to pupation. See Supplemental Data, Table S3, for concentrations of insecticide stock solutions and serial dilutions used in the bioassays. Individual second, third, and fifth instars were provided 0.075 to 0.125, 0.350 to 0.450, and 1.8 to 2.2 g of leaf tissue, respectively. Five, 20, or 100 μL of an insecticide suspension made in 0.1% Silwet:water were pipetted on the top surfaces of the leaves (control leaves were treated with 0.1% Silwet:water). The insecticide leaf concentrations used to derive concentration–response curves can be found in Supplemental Data, Table S4. Treated leaves were dried for 5 min and then provided to the larvae. Leaves were photographed prior to treatment, and their surface areas were calculated using ImageJ software (National Institutes of Health) and task-specific code written in Python using the OpenCV computer vision library (Tripathy 2019).

Analysis of insecticide stock solutions

The insecticide acetone solutions and 0.1% Silwet:water suspensions for the neonicotinoids and chlorantraniliprole were analyzed using UHPLC-MS/MS with a Vanquish Flex UHPLC system, including a binary pump, autosampler, and column heater compartment, and a TSQ Altis triple quadrupole MS equipped with a heated electrospray source (Thermo Fisher Scientific). The methods used were as described by Hall et al. (2018), except that UHPLC-MS/MS analyses of chlorpyrifos 0.1% Silwet:water suspensions employed a Hypersil GOLD Aq column (dimensions 100 × 2.1 mm, particle size 1.9 μm; Thermo Fisher Scientific). The binary mobile phases were water:methanol (98:2, v/v) containing 0.1% formic acid and 5 mM ammonium formate (A) and methanol:water (98:2, v/v) containing 0.1% formic acid and 5 mM ammonium formate (B). Acetone solutions and 0.1% Silwet:water suspensions were diluted with acetonitrile prior to injection. The injection volume was 2 μL for the neonicotinoids and chlorantraniliprole and 1 μL for chlorpyrifos. Acetone solutions and 0.1% Silwet:water suspensions of beta-cyfluthrin were analyzed by GC-ECD. Depending on the nominal concentration of a spike solution, a 10- or 100-μL aliquot was concentrated to dryness and then brought up to an appropriate volume with ethyl acetate. Concentrations of beta-cyfluthrin were determined using an Agilent 7890B GC equipped with an Ni^{63} micro-ECD and a Restek Rtx®-5MS w/Integra-Guard® (30 m × 0.25 mm i.d. × 0.25 μm) column. Helium was used as a carrier gas at a flow rate of 1 mL/min, the makeup gas was 5% methane, and the remainder was argon at 60 mL/min. The initial column temperature was 100 °C and held for 1 min. The temperature was then raised to 250 °C at a rate of 25 °C/min, held for 1 min, and then raised to a final temperature of 300 °C (10 °C/min), which was held for 10 min. Both the inlet and detector temperatures were 250 °C. Beta-cyfluthrin's retention time was 14.4 min. Measured concentrations of 0.1% Silwet:water insecticide suspensions are provided in Supplemental Data, Table S3.

Estimated insecticide exposure and field-scale mortality

Estimated insecticide concentrations deposited on larval and milkweed surfaces following foliar applications were obtained using the Tier I Aerial and Ground models within AgDRIFT, Ver 2.1.1 (US Environmental Protection Agency 2003) for the following representative formulated products (active ingredients; US Environmental Protection Agency registration number): Baythroid® XL (beta-cyfluthrin; 264-840), Admire Pro® (imidacloprid; 264-827), Swagger® (imidacloprid and bifenthrin; 34704-1045), Lorsban® (chlorpyrifos; 62719-220), Beseige® (chlorantraniliprole and lambda-cyhalothrin; 100-1402), and Endigo® (thiamethoxam and lambda-cyhalothrin; 100-1276). Assuming a wind speed of 10 mph (maximum wind speed allowed per label language), concentrations of active ingredients deposited at 0, 15, 30, and 60 m from the edge of the application area were determined using maximum application rates to manage soybean aphids and true armyworms. Aerial and high-ground boom application scenarios were used for soybean aphid applications. For true armyworm, an early-season pest, low- and high-ground boom scenarios were modeled. Consistent with label instructions, a medium to coarse droplet size was selected for aerial applications, and a fine to medium/coarse droplet size was selected for ground applications. Fiftieth percentile model estimates, which exclude outlier and high wind speed effects, were used for ground applications (Supplemental Data, Table S5).

To estimate larval mortality from cuticular exposure following a spray event, the initial average deposition (μg of insecticide deposited/cm^2 of area) obtained from AgDRIFT was compared to cuticular bioassay dose–response curves, with dose expressed as μg of insecticide/cm^2 larva. Larval surface area was estimated using the cylindrical surface area formula $2\pi rh + 2\pi r^2$. The radius and height represent the thickness and length of the larvae, respectively.

To estimate larval mortality from dietary exposure to milkweed leaves, the predicted initial average insecticide deposition (μg of insecticide deposited/cm^2 of area) was compared to dietary bioassay concentration–response curves, with concentration expressed as μg of insecticide/cm^2 leaf. Average leaf surface areas (and weights) provided to larvae are presented in Supplemental Data, Table S13.

Statistical analyses

All statistical analyses were done in RStudio 1.1.383 (Ver 3.5.2). The drc package (Ver 3.0.1, a nonlinear least squares model) was used to generate dose– (or concentration–) response curves if the data met the assumption of normality. If the data did not meet this assumption, a maximum likelihood estimate model was used (Dixon et al. 2020). Abbott's formula was used to account for control mortality, and analysis of variance was used to analyze final larval weights and percentage adult eclosion in treatment groups that had <70% larval or pupal mortality; when treatment effects were significant, post hoc tests with Dunnett's comparisons were employed.

RESULTS

Cuticular bioassays

Acute cuticular doses that kill 10, 50, and 90% of a treated population (LD10, LD50, and LD90, respectively) for first-, third-, and fifth-instar larvae are provided in Table 1. Based on a comparison of LD50 values and 95% confidence intervals (CIs), beta-cyfluthrin and chlorantraniliprole were the most toxic insecticides (across all instars, LD50 values range from 9.2×10^{-3} to 4.8×10^{-2} and from 1.2×10^{-2} to 0.19 μg/g larva, respectively). Chlorpyrifos was the least toxic to first instars (LD50 of 79 μg/g), and thiamethoxam was the least toxic to fifth instars (35 μg/g; Figure 2). When LD50 values are expressed on a μg/cm^2 larva and μg/larva basis, the first instars tend to be the most sensitive (typically 95% CIs do not overlap with CIs of older instars), followed by third and fifth instars (Table 2; Supplemental Data, Table S6 and Figures S1 and S2). A subset of bioassays were undertaken with common milkweed and the results compared to tropical milkweed bioassay toxicity values; LD50 values and associated 95% CIs are provided in Supplemental Data, Table S10. Responses were similar between the plant species. Except for imidacloprid, ratios of tropical milkweed to common milkweed LD50 values ranged from 0.91 to 1.9, with overlapping 95% CIs. The tropical milkweed imidacloprid LD50 value was 2.3-fold higher (upper bound common milkweed 95% CI = 2.0 μg/larva and lower bound tropical 95% CI = 2.2 μg/larva; this difference is not considered biologically significant).

For all insecticide exposure levels that caused <70% larval mortality, there were no differences in final weights between control and surviving insecticide-treated larvae at a $p = 0.01$

TABLE 1: Cuticular study: Acute toxicity of 5 insecticides to monarch first-, third-, and fifth-instar larvae fed tropical milkweed leaves[a]

		96-h LD values and 95% CIs (μg insecticide/g larva)[b]		
Insecticide	Instar	LD10	LD50	LD90
BCF	First	2.1×10^{-3} (7.4×10^{-5}–4.2×10^{-3})	9.2×10^{-3} (5.2×10^{-3}–1.3×10^{-2})	4.0×10^{-2} (1.7×10^{-2}–6.3×10^{-2})
	Third	2.8×10^{-3} (7.5×10^{-4}–1.0×10^{-2})[c]	1.8×10^{-2} (9.7×10^{-3}–3.4×10^{-2})[c]	0.12 (5.7×10^{-2}–0.32)[c]
	Fifth[d]	1.5×10^{-2} (3.1×10^{-3}–2.7×10^{-2})	4.8×10^{-2} (2.7×10^{-2}–6.8×10^{-2})	0.15 (8.7×10^{-2}–0.22)
CTR	First	1.1×10^{-3} (1.4×10^{-4}–4.2×10^{-3})[c]	1.2×10^{-2} (5.1×10^{-3}–2.8×10^{-2})[c]	0.14 (5.4×10^{-2}–0.60)[c]
	Third	1.3×10^{-2} (4.0×10^{-3}–3.7×10^{-2})[c]	9.5×10^{-2} (5.2×10^{-2}–0.17)[c]	0.68 (0.34–1.7)[c]
	Fifth[d]	5.8×10^{-2} (1.7×10^{-2}–0.10)	0.19 (0.12–0.26)	0.62 (0.31–0.93)
CFS	First	40 (17–62)	79 (55–100)	150 (100–200)
	Third	8.5 (2.7–14)	22 (15–30)	58 (32–84)
	Fifth[d]	8.6 (7.9–9.3)	18 (15–21)	38 (30–45)
IMI	First	2.6 (0.99–4.3)	6.7 (4.5–8.8)	17 (9.9–24)
	Third	1.3 (0.30–4.0)[c]	8.4 (4.4–16)[c]	56 (30–140)[c]
	Fifth[d]	1.0 (0.33–1.7)	3.0 (2.0–4.0)	9.0 (3.5–15)
TMX	First	1.4 (0.27–2.5)	6.1 (3.0–9.3)	27 (6.6–47)
	Third	1.8 (0.58–3)	8.8 (5.6–12)	43 (19–67)
	Fifth[d]	17 (7.2–27)	35 (28–41)	71 (39–100)

[a]Based on combined mortality data from triplicate or quadruplicate toxicity bioassays for each insecticide–instar combination. Larvae were treated with acetone and 5 insecticide–acetone solutions.
[b]The micrograms of insecticide per gram of larva were calculated by dividing the nominal concentrations and volume of insecticide solution applied to each larva by the average weights of control larvae before treatment. Respective control larval weights for each insecticide–instar combination were used (Supplemental Data, Table S1). Except as noted in the table, LD values were estimated using a nonlinear least square estimate model (see *Statistical analyses*). Adjustment for control (acetone) mortality was made using Abbott's formula.
[c]The LD values were calculated using a maximum likelihood estimate model (see *Statistical analyses*).
[d]Observations until pupation (usually 72 or 96 h after treatment).
BCF = beta-cyfluthrin; CFS = chlorpyrifos; CI = confidence interval; CTR = chlorantraniliprole; IMI = imidacloprid; LD10, LD50, and LD90 = lethal doses that kill 10, 50, and 90% of a treated population, respectively; TMX = thiamethoxam.

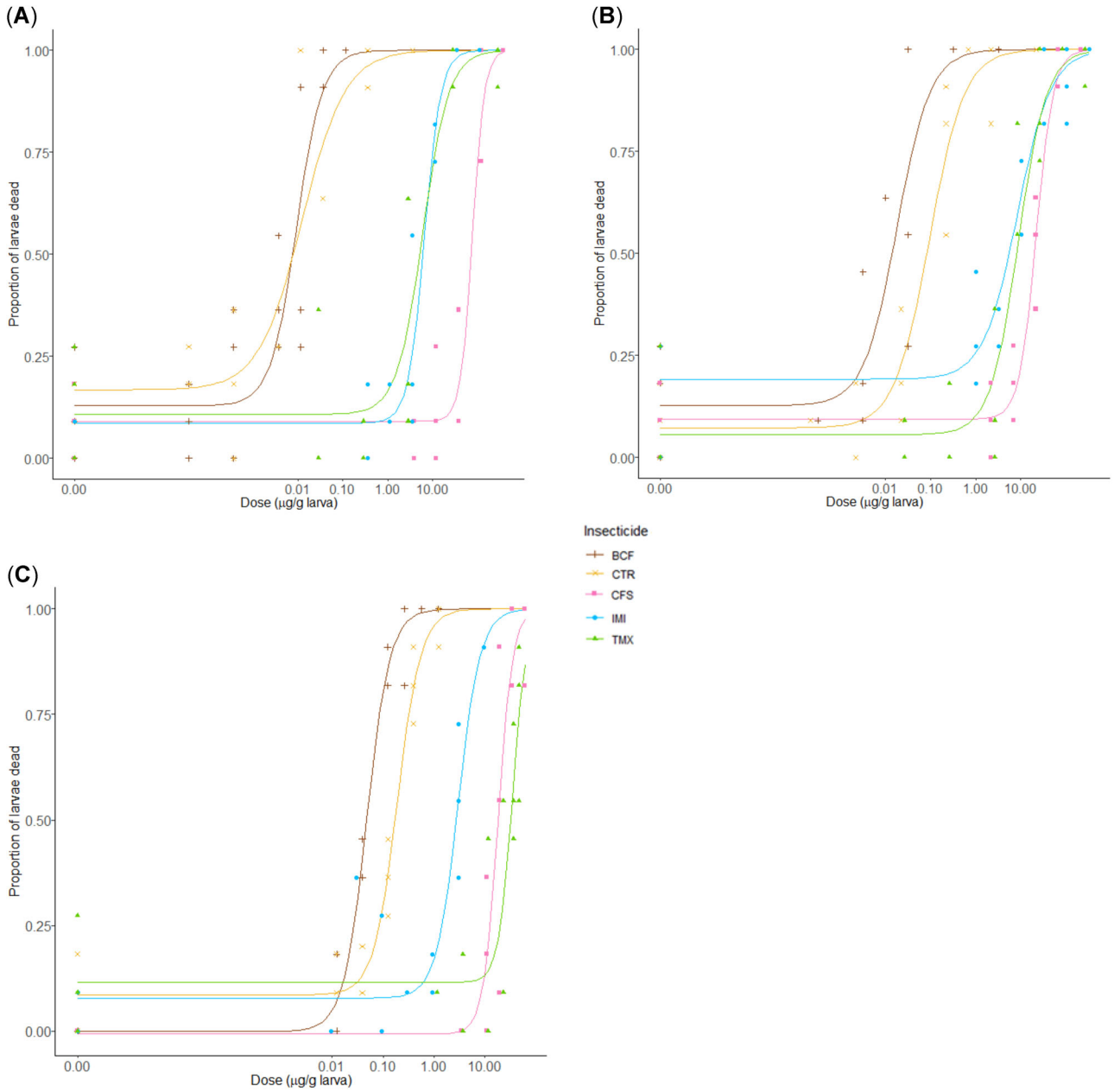

FIGURE 2: Mortality dose–response curves for first- (**A**), third- (**B**), and fifth-instar (**C**) monarch butterfly larvae following cuticular application of 5 insecticide solutions in acetone. For the first and third instars, observations were made daily through 96-h postapplication. For the fifth instars, observations were made through pupation (usually 72 or 96 h after treatment). BCF = beta-cyfluthrin; CFS = chlorpyrifos; CTR = chlorantraniliprole; IMI = imidacloprid; TMX = thiamethoxam.

level of significance; however, at $p = 0.05$, third instars treated with chlorantraniliprole weighed less than controls ($p = 0.0092$ for 2.21×10^{-2} µg/g chlorantraniliprole, based on Dunnett's multiple comparison test; Supplemental Data, Table S7). A slight delay in development was observed when third instars were treated with 2.21×10^{-3} and 2.21×10^{-2} µg/g chlorantraniliprole; at 96 h, the majority of treated larvae (52–54%) were third or fourth instars, whereas the majority of control larvae (60%) were fifth instars. Adult eclosion rates for insecticide-treated and control fifth instars were not significantly different ($p > 0.54$; Supplemental Data, Table S8).

Most insecticide-treated first and third instars died within 0 to 48 h after treatment. When fifth instars were treated with beta-cyfluthrin and chlorantraniliprole, mortality generally occurred 0 to 72 h postexposure and before ecdysis. However, mortality in fifth instars treated with neonicotinoids, and to a lesser extent chlorpyrifos, typically occurred during ecdysis (72–96 h after application) and was characterized by a cessation in pupa formation. Larvae died in transition to the pupal stage (suspended in a "J" shape) or after excreting molting fluid. Before onset of pupation, treated larvae rarely showed signs of intoxication. This symptomology was observed with 92, 87,

TABLE 2: Cuticular study: Acute toxicity of 5 insecticides to monarch first-, third-, and fifth-instar larvae fed tropical milkweed leaves[a]

		96-h LD values and 95% CIs (μg insecticide/cm^2 larva)[b]		
Insecticide	Instar	LD10	LD50	LD90
BCF	First	3.4×10^{-5} (1.0×10^{-6}–6.7×10^{-5})	1.5×10^{-4} (8.4×10^{-5}–2.1×10^{-4})	6.5×10^{-4} (2.7×10^{-4}–1.0×10^{-3})
	Third	1.4×10^{-4} (3.6×10^{-5}–5.0×10^{-4})[c]	8.7×10^{-4} (4.7×10^{-4}–1.7×10^{-3})[c]	5.6×10^{-3} (2.8×10^{-3}–1.6×10^{-2})[c]
	Fifth[d]	1.8×10^{-3} (9.8×10^{-4}–2.9×10^{-3})[c]	6.5×10^{-3} (4.7×10^{-3}–8.7×10^{-3})[c]	2.3×10^{-2} (1.6×10^{-2}–3.7×10^{-2})[c]
CTR	First	1.7×10^{-5} (2.3×10^{-6}–6.9×10^{-5})[c]	2.0×10^{-4} (8.3×10^{-5}–4.6×10^{-4})[c]	2.3×10^{-3} (8.9×10^{-4}–9.8×10^{-3})[c]
	Third	9.3×10^{-4} (2.8×10^{-4}–2.6×10^{-3})[c]	6.6×10^{-3} (3.6×10^{-3}–1.2×10^{-2})[c]	4.7×10^{-2} (2.4×10^{-2}–0.12)[c]
	Fifth[d]	6.6×10^{-3} (2.0×10^{-3}–1.1×10^{-2})	2.2×10^{-2} (1.4×10^{-2}–2.9×10^{-2})	7.1×10^{-2} (3.5×10^{-2}–0.11)
CFS	First	0.60 (0.26–0.94)	1.2 (0.83–1.5)	2.3 (1.6–3.1)
	Third	0.60 (0.19–1.0)	1.6 (1.1–2.1)	4.1 (2.3–5.9)
	Fifth[d]	1.1 (1.0–1.2)	2.3 (1.9–2.7)	4.9 (3.6–6.1)
IMI	First	4.3×10^{-2} (1.6×10^{-2}–7.0×10^{-2})	0.11 (7.5×10^{-2}–0.15)	0.28 (0.16–0.40)
	Third	5.9×10^{-2} (1.4×10^{-2}–0.19)[c]	0.39 (0.21–0.74)[c]	2.6 (1.4–6.7)[c]
	Fifth[d]	0.15 (5.0×10^{-2}–0.25)	0.45 (0.30–0.59)	1.3 (0.52–2.2)
TMX	First	2.9×10^{-2} (5.5×10^{-3}–5.2×10^{-2})	0.13 (6.0×10^{-2}–0.19)	0.55 (0.13–0.96)
	Third	0.10 (3.4×10^{-2}–0.17)	0.51 (0.32–0.70)	2.5 (1.1–3.9)
	Fifth[d]	2.1 (0.87–3.3)	4.2 (3.4–5.0)	8.6 (4.7–12)

[a]Based on combined mortality data from triplicate or quadruplicate toxicity bioassays for each insecticide–instar combination. Larvae were treated with acetone and 5 insecticide–acetone solutions.
[b]Larvae were assumed to be cylinders. Surface area in square centimeters was estimated by measuring the height (h; or length) and radius (r; or half the thickness) of 10 individuals for each larval instar using the following formula: $2\pi rh + 2\pi r^2$. Estimated surface areas of first, third, and fifth instars were 0.17 ± 0.05, 0.65 ± 0.12, and 7.1 ± 1.3 cm^2, respectively. Except as noted in the table, LD values were estimated using the nonlinear least square estimate model (see *Statistical analyses*). Adjustment for control (acetone) mortality was made using Abbott's formula.
[c]The LD values were estimated using a maximum likelihood estimate model (see *Statistical analyses*).
[d]Observations until pupation (usually 72 or 96 h after treatment).
BCF = beta-cyfluthrin; CFS = chlorpyrifos; CI = confidence interval; CTR = chlorantraniliprole; IMI = imidacloprid; LD10, LD50, and LD90 = lethal doses that kill 10, 50, and 90% of a treated population, respectively; TMX = thiamethoxam.

and 18% of moribund fifth instars treated with imidacloprid, thiamethoxam, and chlorpyrifos, respectively (Supplemental Data, Table S9). Dissected fifth instars that exhibited arrested ecdysis had pupal cuticle with adult features; however, the wing buds were not expanded. We also observed melanization in the hemolymph. In subsequent experiments, third instars were treated with the same doses as used in the fifth-instar bioassays, and the surviving larvae successfully pupated. Arrested ecdysis also was observed in the imidacloprid fifth-instar bioassays with common milkweed. Although arrested ecdysis was observed occasionally in control larvae and in the colony-reared larvae, the rates are much lower than what was observed with the neonicotinoid treatments (Supplemental Data, Table S9).

At imidacloprid doses of 0.944, 2.98, and 9.44 μg/g larva, all mortality was associated with arrested ecdysis. Prior to ecdysis, most of the treated larvae did not exhibit signs of intoxication and maintained feeding. The 9.44 μg/g dose elicited 91% mortality, all through arrested ecdysis. However, in range-finding assays, all 10 fifth instars treated with approximately 100 μg/g larva showed signs of intoxication at 24 h and died prior to ecdysis. These observations indicate that doses that elicited nearly 100% mortality associated with arrested ecdysis are 10 times lower than doses that caused 100% mortality prior to ecdysis, suggesting that there may be 2 modes of action associated with neonicotinoids.

Though clothianidin is not registered for foliar uses in maize and soybean, we undertook range-finding bioassays to compare responses to the other neonicotinoids (Supplemental Data, Table S11). Clothianidin was more toxic (non-overlapping 95% CIs) than imidacloprid and thiamethoxam, with LD50 values of 0.19, 0.83, and 1.3 μg/g larva for first, third, and fifth instars, respectively (Supplemental Data, Table S12 and Figure S3). Clothianidin-treated fifth instars also exhibited arrested ecdysis.

Dietary bioassays

Acute dietary concentrations that kill 10, 50, and 90% of a treated population (LC10, LC50, and LC90, respectively) and associated 95% CIs for second-, third-, and fifth-instar larvae are provided in Table 3. Chlorantraniliprole was the most toxic insecticide (95% CIs do not overlap with other insecticide CIs) for second (LC50 of 8.3×10^{-3} μg/g leaf) and third (LC50 of 4.6×10^{-2} μg/g leaf) instars. Chlorpyrifos, imidacloprid, and thiamethoxam were similarly toxic to second (LC50 values range 3.5–8.4 μg/g leaf) and fifth (LC50 values range 9.4–33 μg/g leaf; Table 3 and Figure 3) instars. When toxicity values were reported on a μg/cm^2 leaf basis, 95% CIs also overlapped with these insecticides (Table 4; Supplemental Data, Figure S4). Results of select bioassays with common milkweed leaves are provided in Supplemental Data, Table S16. Leaf concentrations expected to elicit 50% mortality, based on results of tropical milkweed bioassays, caused 42 to 70% larval mortality. These rates of mortality are within the ranges expected based on the tropical milkweed 95% CIs.

At insecticide concentrations that caused <70% larval mortality, the final weights of surviving larvae were significantly lower than larvae fed control leaves in several instances. Reduced weight was typically seen in third instars, where it was often associated with delayed development (Table 5). Adult eclosion rates for treated and control fifth instars were not

TABLE 3: Dietary study: Acute toxicity to monarch second-, third-, and fifth-instar larvae following exposure to tropical milkweed leaves treated with 5 insecticides[a]

		96-h LC values and 95% CIs (μg insecticide/g leaf)[b]		
Insecticide	Instar	LC10	LC50	LC90
BCF	Second	2.1×10^{-2} (6.1×10^{-3}–5.5×10^{-2})	0.21 (0.12–0.35)	2.1 (1.1–5.0)
	Third	0.20 (4.1×10^{-3}–0.39)[c]	0.94 (0.45–1.4)[c]	4.5 (1.2–7.8)[c]
	Fifth[d]	3.6×10^{-2} (5.9×10^{-3}–0.15)	0.62 (0.27–1.4)	11 (3.8–52)
CTR	Second	4.9×10^{-4} (7.3×10^{-5}–1.8×10^{-3})	8.3×10^{-3} (3.8×10^{-3}–1.6×10^{-2})	0.14 (6.0×10^{-2}–0.49)
	Third	6.0×10^{-4} (6.8×10^{-5}–2.9×10^{-3})	4.6×10^{-2} (1.8×10^{-2}–0.11)	3.6 (1.1–21)
	Fifth[d]	1.7×10^{-2} (1.8×10^{-3}–0.10)	0.97 (0.36–3.0)	55 (13–580)
CFS	Second	0.68 (0.14–6.4)	8.4 (4.0–19)	100 (24–530)
	Third	0.31 (4.4×10^{-2}–1.6)	6.0 (2.7–14)	120 (40–630)
	Fifth[d]	0.74 (0.16–2.3)	10 (5.0–23)	140 (48–820)
IMI	Second	1.4 (0.57–2.1)[c]	5.1 (3.3–6.8)[c]	19 (7.5–30)[c]
	Third	3.7 (0.48–6.9)[c]	17 (9.4–24)[c]	77 (22–130)[c]
	Fifth[d]	0.27 (1.4×10^{-2}–2.3)	9.4 (3.0–27)	330 (92–3100)
TMX	Second	1.4 (0.36–3.6)	3.5 (2.2–5.0)	8.8 (NC–26)
	Third	1.1 (0.48–2.1)	5.6 (3.7–8.9)	29 (15–69)
	Fifth[d]	4.2 (NC–13)[c]	33 (4.5–62)[c]	270 (NC–550)[c]

[a]Based on combined mortality data from triplicate or quadruplicate bioassays for each insecticide–instar combination. Larvae were fed leaf tissue treated with 0.1% Silwet:water and 5 insecticide suspensions in 0.1% Silwet:water.
[b]The micrograms of insecticide per gram of leaf tissue were calculated by dividing the concentrations and volume of insecticide solution pipetted on each leaf tissue by the known weights of the leaf tissue. The average weights of leaves provided to larvae in each insecticide, instar, bioassay run, and concentration are available in the Supplemental Data (*Weights and surface areas of leaves*). Except as noted in the table, LC values were estimated using the maximum likelihood estimate model (see *Statistical analyses*). Adjustment for control (0.1% Silwet:water) mortality was done using Abbott's formula.
[c]The LC values were calculated using the nonlinear least square estimate model (see *Statistical analyses*).
[d]Observations until pupation (usually 72 or 96 h after treatment).
BCF = beta-cyfluthrin; CFS = chlorpyrifos; CI = confidence interval; CTR = chlorantraniliprole; IMI = imidacloprid; LC10, LC50, and LC90 = lethal concentrations that kill 10, 50, and 90% of a treated population, respectively; NC = not calculable or a negative lower bound CI value; TMX = thiamethoxam.

significantly different ($p > 0.19$; Supplemental Data, Table S14). In 2 of the 15 bioassays, the eclosion rates were suppressed, in part because of pupal infection observed in both control and treated fifth instars.

With dietary exposure, the rate of arrested ecdysis was less than that observed following cuticular exposure. Monarch fifth instars treated with chlorantraniliprole, beta-cyfluthrin, and chlorpyrifos had low rates of arrested ecdysis (10, 5, and 2%, respectively). The rate of arrested ecdysis was 16 and 21% with imidacloprid and thiamethoxam treatments, respectively (Supplemental Data, Table S15). The dietary bioassays, like the cuticular bioassays, were carried out with early fifth instars (approximately 24 h old). However, when late fifth instars (approximately 72 h old) were exposed to neonicotinoids through their diet, the rate of arrested ecdysis and corresponding mortality increased. For example, when early fifth instars fed on a concentration of 0.78 μg of imidacloprid/g leaf, 10% died (Table 5). However, when this concentration was provided to late fifth instars, 82% of the larvae died, with 89% of the mortality attributable to arrested ecdysis.

Results of dietary bioassays with clothianidin were similar to those with imidacloprid and thiamethoxam for second and third instars (overlapping 95% CIs), with LC50 values of 4.2 and 7.8 μg/g leaf, respectively. Clothianidin-treated fifth instars were more sensitive than thiamethoxam-treated fifth instars, producing an LC50 value of 0.80 μg/g leaf (Supplemental Data, Table S19 and Figure S5). These values were calculated using measured clothianidin stock solution concentrations and estimated leaf concentrations (Supplemental Data, Tables S17 and S18). As with the other neonicotinoids, treated larvae showed reduced larval growth and development in a few instances; there was no effect on adult eclosion (Supplemental Data, Table S20).

Field-scale mortality assessments

Larval cuticular exposure. When aerial applications for beta-cyfluthrin and chlorantraniliprole were modeled for soybean aphid management, predicted monarch larval mortality was between 100 and 32% at all modeled distances (0, 15, 30, and 60 m downwind from the field). Chlorpyrifos, imidacloprid, and thiamethoxam were estimated to cause 99, 91, and 67% mortality, respectively, to the most sensitive larval instar at the edge of field. There was 0 to 2% mortality predicted for these insecticides at 60 m downwind (Figure 4A). Similar trends were seen with insecticide applications using a high-ground boom. However, because of reduced off-site drift, lower mortality was predicted at 15, 30, and 60 m downwind compared to aerial applications; but greater larval mortality was observed at 0 m (Figure 4B). Modeled high- and low-ground boom applications to manage true armyworm infestations produced similar mortality patterns (Figure 4C,D). Ninetieth percentile results for ground applications, to capture worse-case drift scenarios, are provided in Supplemental Data, Figure S6. Over all the scenarios, the mortality rate was generally highest for the first instars and lowest for fifth instars.

Larval dietary exposure. When beta-cyfluthrin and chlorantraniliprole exposures were modeled for aerial applications to manage soybean aphids, predictions for monarch larval

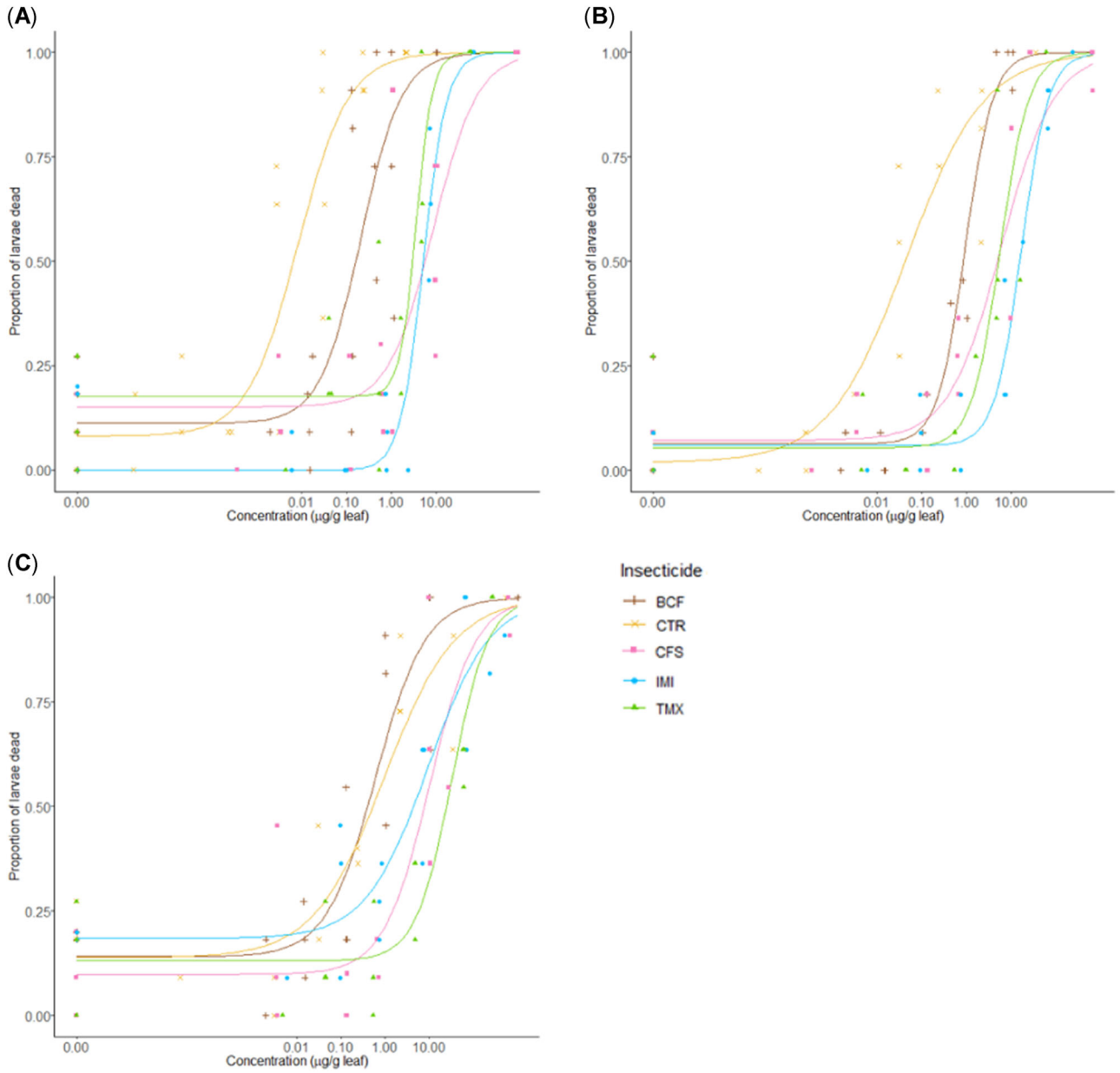

FIGURE 3: Mortality concentration–response curves for second- (**A**), third- (**B**), and fifth-instar (**C**) monarch butterfly larvae following dietary exposure to tropical milkweed leaves treated with 5 insecticide suspensions in 0.1% Silwet:water. For the second and third instars, observations were made daily through 96-h postapplication. For the fifth instars, observations were made through pupation (usually 72 or 96 h after treatment). BCF = beta-cyfluthrin; CFS = chlorpyrifos; CTR = chlorantraniliprole; IMI = imidacloprid; TMX = thiamethoxam.

mortality were between 100 and 10% at all modeled distances downwind from the field (0, 15, 30, and 60 m). Chlorpyrifos, imidacloprid, and thiamethoxam were estimated to cause 96, 80, and 83% mortality, respectively, to the most sensitive larval instar at the edge of field. They caused 64, 13, and 3% mortality to the most sensitive larval instar at 60 m downwind (Figure 5A). Similar trends were seen with insecticide applications using a high-ground boom; however, because of reduced off-site drift, lower mortality was predicted compared to aerial applications (with the exception of 0 m; Figure 5B). High- and low-ground boom applications to manage true armyworm infestations produced similar mortality patterns (Figure 5C,D). Ninetieth percentile results for ground applications are provided in Supplemental Data, Figure S7. Over all the scenarios, mortality rates were generally highest for the second instars and lowest for third or fifth instars.

DISCUSSION

Foliar insecticide applications to manage late- and early-season pests can occur when monarch larvae are found in significant numbers in the north central states (Figure 1). In Iowa, mid- to late-season pests that can require foliar

TABLE 4: Dietary study: Acute toxicity to monarch second-, third-, and fifth-instar larvae following exposure to tropical milkweed leaves treated with 5 insecticides[a]

		96-h LC values and 95% CIs (μg insecticide/cm^2 leaf)[b]		
Insecticide	Instar	LC10	LC50	LC90
BCF	Second	6.3×10^{-4} (2.1×10^{-4}–1.6×10^{-3})	5.0×10^{-3} (3.0×10^{-3}–8.2×10^{-3})	4.0×10^{-2} (2.2×10^{-2}–9.0×10^{-2})
	Third	5.9×10^{-3} (9.4×10^{-5}–1.2×10^{-2})[c]	2.6×10^{-2} (1.3×10^{-2}–4.0×10^{-2})[c]	0.12 (3.0×10^{-2}–0.21)[c]
	Fifth[d]	8.6×10^{-4} (1.4×10^{-4}–3.7×10^{-3})	1.7×10^{-2} (7.5×10^{-3}–4.0×10^{-2})	0.34 (0.11–1.8)
CTR	Second	9.8×10^{-6} (1.0×10^{-6}–4.0×10^{-5})	1.9×10^{-4} (7.4×10^{-5}–3.8×10^{-4})	3.5×10^{-3} (1.5×10^{-3}–1.3×10^{-2})
	Third	1.3×10^{-5} (1.2×10^{-6}–7.5×10^{-5})	1.2×10^{-3} (4.3×10^{-4}–2.9×10^{-3})	0.11 (3.0×10^{-2}–0.64)
	Fifth[d]	4.1×10^{-4} (4.5×10^{-5}–2.4×10^{-3})	2.3×10^{-2} (8.6×10^{-3}–7.2×10^{-2})	1.3 (0.30–14)
CFS	Second	1.5×10^{-2} (3.4×10^{-3}–0.15)	0.17 (8.6×10^{-2}–0.39)	2.0 (0.47–9.9)
	Third	7.4×10^{-3} (1.0×10^{-3}–3.9×10^{-2})	0.14 (6.2×10^{-2}–0.33)	2.7 (0.92–15)
	Fifth[d]	1.9×10^{-2} (4.2×10^{-3}–6.0×10^{-2})	0.25 (0.13–0.57)	3.4 (1.2–19)
IMI	Second	3.4×10^{-2} (2.2×10^{-2}–4.6×10^{-2})[c]	0.13 (8.1×10^{-2}–0.17)[c]	0.48 (0.30–0.66)[c]
	Third	8.8×10^{-2} (1.0×10^{-2}–0.16)[c]	0.41 (0.23–0.60)[c]	1.9 (0.53–3.4)[c]
	Fifth[d]	7.8×10^{-3} (4.1×10^{-4}–6.4×10^{-2})	0.25 (7.7×10^{-2}–0.71)	7.8 (2.2–70)
TMX	Second	2.8×10^{-2} (7.8×10^{-3}–7.1×10^{-2})	8.7×10^{-2} (5.5×10^{-2}–0.13)	0.27 (0.16–0.80)
	Third	2.8×10^{-2} (6.4×10^{-3}–5.0×10^{-2})[c]	0.17 (9.0×10^{-2}–0.24)[c]	0.99 (0.22–1.8)[c]
	Fifth[d]	0.13 (NC–0.39)[c]	1.1 (0.14–2.0)[c]	8.8 (NC–18)[c]

[a]Based on combined mortality data from triplicate or quadruplicate bioassays for each insecticide–instar combination. Larvae were fed leaf tissues treated with 0.1% Silwet:water and 5 insecticide suspensions in 0.1% Silwet:water.
[b]The square centimeters of leaf tissue provided to each larvae (see *Dietary toxicity studies*) were used to estimate dietary insecticide concentrations. The average surface areas of leaves given to larvae in each insecticide, instar, bioassay run, and concentration were used (see Supplemental Data, *Weights and surface areas of leaves*). Except as noted in the table, LC values were calculated using the maximum likelihood estimate model (see *Statistical analyses).* Adjustment for control (0.1% Silwet:water) mortality was done using Abbott's formula.
[c]The LC values were calculated using the nonlinear square estimate model (see *Statistical analyses*).
[d]Observations until pupation (usually 72 or 96 h after treatment).
BCF = beta-cyfluthrin; CFS = chlorpyrifos; CI = confidence interval; CTR = chlorantraniliprole; IMI = imidacloprid; LC10, LC50, and LC90 = lethal concentrations that kill 10, 50, and 90% of a treated population, respectively; NC = not calculable or a negative lower bound CI value; TMX = thiamethoxam.

applications include soybean aphids, European corn borers (*Ostrinia nubilalis* [Hodgson and Rice 2017]), adult western and northern corn rootworms (*Diabrotica virgifera virgifera* and *Diabrotica barberi* [Gassmann and Weber 2016]), and corn aphids (*Rhopalosiphum maidis* [Hodgson 2018]). The true armyworm is an example of a reemerging early-season pest that is associated with the increased use of cover crops (Dunbar et al. 2016). Although pyrethroids and organophosphates are the most commonly used foliar insecticides in soybean fields, neonicotinoids and diamides also are being used (Hodgson et al. 2012; Whalen et al. 2016). Potential risk of foliar insecticide applications to monarch larvae is a function of insecticide toxicity and exposure. Exposure is a function of habitat proximity to treated maize or soybean fields, wind speed and direction at time of foliar application, and the nature and extent of insecticide use patterns within and across growing seasons.

Insecticide toxicity

Cuticular and dietary LD50 and LC50 values for third-instar monarchs found beta-cyfluthrin and chlorantraniliprole to be approximately 10- to 1000-fold more toxic than chlorpyrifos, imidacloprid, and thiamethoxam. Cuticular LD50 values across larval instars for a given insecticide were generally within a factor of 10. For all the insecticides, except chlorantraniliprole, dietary LC50 values across larval instars were within a factor of 10. Fifth instars were approximately 100 times less sensitive to chlorantraniliprole than second instars. Following cuticular exposure to all the insecticides and dietary exposure to chlorpyrifos, minimal to no adverse effects on growth and development in surviving larvae were observed at doses or concentrations that caused <70% larval mortality. Following dietary exposure to the other insecticides, surviving third-instar larvae frequently weighed significantly less than controls (1.1- to 2.9-fold lower) and developed slower. There were no adverse effects on adult eclosion for surviving larvae following cuticular or dietary exposures.

Larvae responded similarly when bioassays were conducted with tropical and common milkweed, which suggests, at least with routes of exposures, endpoints, and insecticides examined in the present study, that differences in milkweed species did not confound interpretation of results. However, the condition of milkweed used in bioassays, regardless of the species, is an important consideration. Milkweed reared in our greenhouses can be infested with western flower thrips and oleander aphids if cultural and biological pest-management practices are not employed. Milkweed reared with significant insect feeding can increase the plant's cardenolide concentrations (Rasmann et al. 2009; Agrawal et al. 2014). Monarchs feeding on stressed milkweed with elevated cardenolide concentrations are smaller than monarchs feeding on unstressed milkweed with lower cardenolide concentrations (Agrawal et al. 2014).

Following cuticular exposure, arrested ecdysis was observed with neonicotinoid- and chlorpyrifos-treated fifth instars. Neonicotinoids also caused arrested ecdysis via the dietary route of exposure, though the rates were lower. The effect seems to be unique to fifth instars. Third instars exposed to imidacloprid at doses that cause arrested ecdysis in fifth instars developed

TABLE 5: Dietary study: Growth and development of surviving monarch second-, third-, and fifth-instar larvae following exposure to tropical milkweed leaves treated with 5 insecticides[a]

Insecticide	Instar	Concentration[b] (μg insecticide/g leaf)	Larval percent mortality[c]	Number of surviving larvae/pupae (no. of replicate bioassays)[d]	Instar/stage at 96 h after application[e]	Mean final weights[f] (±SD)	Statistical analysis
BCF	Second						$F_{(3,98)} = 2.373$; $p = 0.0749$[g]
		0	0%	38 (4)	Fourth	171 (±85)	
		1.5×10^{-2}	0%	38 (4)	Fourth	165 (±77)	
		0.13	45%	21 (4)	Fourth	160 (±55)	
		0.45	68%	9 (2)	Fourth	137 (±65)	
	Third						$F_{(4,117)} = 10.97$; $p = 1.383 \times 10^{-7}$, [h]
		0	0%	30 (3)	Fifth	410 (±126)	
		1.8×10^{-3}	0%	21 (2)	Fifth	407 (±136)	$df = 87$; t ratio = 0.039; $p = 0.9998$
		1.4×10^{-2}	0%	32 (3)	Fifth	407 (±147)	$df = 117$; t ratio = 0.028; $p = 0.9999$
		0.12	7%	28 (3)	Fifth	342 (±125)	$df = 117$; t ratio = 1.907; $p = 0.1845$
		0.93	35%	13 (2)	Fourth[j]	140 (±106)***	$df = 87$; t ratio = 5.784; $p < 0.0001$
	Fifth						$F_{(3,85)} = 2.615$; $p = 0.05632$[g]
		0	0%	26 (3)	Pupa	1156 (±137)	
		2.0×10^{-3}	0%	20 (2)	Pupa	1264 (±131)	
		1.5×10^{-2}	0%	27 (3)	Pupa	1158 (±160)	
		0.13	14%	23 (3)	Pupa	1129 (±202)	
CTR	Second						$F_{(4,119)} = 6.415$; $p = 1.04 \times 10^{-4}$, [h]
		0	0%	42 (4)	Fourth	207 (±110)	
		1.9×10^{-6}	5%	20 (2)	Fourth	243 (±103)	$df = 70$; t ratio = 0.139; $p = 0.9924$
		2.2×10^{-5}	14%	18 (2)	Fourth	148 (±63)	$df = 48$; t ratio = 0.717; $p = 0.6911$
		2.5×10^{-4}	5%	20 (2)	Fourth	192 (±126)*	$df = 70$; t ratio = 2.982; $p = 0.0112$
		2.9×10^{-3}	36%	27 (4)	Fourth	162 (±111)***	$df = 119$; t ratio = 4.262; $p = 0.0002$
	Third						$F_{(2,64)} = 25.1$; $p = 8.973 \times 10^{-9}$, [h]
		0	0%	32 (3)	Fifth	377 (±131)	
		2.6×10^{-4}	1%	21 (2)	Fifth	240 (±117)**	$df = 46$; t ratio = 3.504; $p = 0.0020$
		3.1×10^{-2}	50%	16 (3)	Fourth[j]	142 (±95)***	$df = 64$; t ratio = 6.913; $p < 0.0001$
	Fifth						$F_{(3,84)} = 3.722$; $p = 0.01445$[g]
		0	0%	26 (3)	Pupa	987 (±237)	
		3.1×10^{-3}	0%	21 (2)	Pupa	1062 (±158)	$df = 61$; t ratio = 0.584; $p = 0.8580$
		3.2×10^{-2}	8%	24 (3)	Pupa	938 (±188)	$df = 84$; t ratio = 1.397; $p = 0.3716$
		0.24	21%	20 (3)	Pupa	851 (±193)*	$df = 84$; t ratio = 2.520; $p = 0.0374$
CFS	Second[i]						$F_{(4,97)} = 1.705$; $p = 0.1551$[g]
		0	0%	27 (3)	Fourth	176 (±133)	
		0.12	0%	27 (3)	Fourth	185 (±119)	
		0.63	7%	26 (3)	Fourth	155 (±91)	
		1.1	39%	11 (2)	Fourth	83 (±38)	
		9.8	45%	13 (3)	Fourth	98 (±89)	

(*Continued*)

TABLE 5: (*Continued*)

Insecticide	Instar	Concentration[b] (μg insecticide/g leaf)	Larval percent mortality[c]	Number of surviving larvae/pupae (no. of replicate bioassays)[d]	Instar/stage at 96 h after application[e]	Mean final weights[f] (±SD)	Statistical analysis
	Third						$F_{(4,103)} = 0.6175$; $p = 0.651$[g]
		0	0%	31 (3)	Fifth	422 (±160)	
		3.6×10^{-3}	8%	19 (2)	Fifth	417 (±220)	
		0.13	3%	20 (2)	Fifth	426 (±130)	
		0.66	23%	24 (3)	Fifth	434 (±141)	
		10	48%	16 (3)	Fifth	373 (±170)	
	Fifth						$F_{(4,117)} = 2.149$; $p = 0.07907$[g]
		0	0%	29 (3)	Pupa	1048 (±172)	
		3.6×10^{-3}	10%	27 (3)	Pupa	1031 (±153)	
		0.14	0%	32 (3)	Pupa	987 (±180)	
		0.69	6%	28 (3)	Pupa	987 (±127)	
		10	63%	11 (2)	Pupa	947 (±192)	
IMI	Second						$F_{(4,117)} = 12.42$; $p = 1.890 \times 10^{-8}$, [h]
		0	0%	28 (3)	Fourth	251 (±70)	
		6.0×10^{-3}	0%	21 (2)	Fourth	258 (±71)	$df = 82$; t ratio = 1.636; $p = 0.3038$
		9.5×10^{-2}	0%	33 (3)	Fourth	260 (±69)	$df = 117$; t ratio = 0.197; $p = 0.9924$
		0.76	0%	30 (3)	Fourth	247 (±75)	$df = 117$; t ratio = 0.737; $p = 0.8341$
		7.0	58%	12 (3)	Fourth	145 (±103)***	$df = 117$; t ratio = 5.794; $p < 0.0001$
	Third						$F_{(4,120)} = 16.35$; $p = 1.027 \times 10^{-10}$, [h]
		0	0%	31 (3)	Fifth	437 (±121)	
		6.1×10^{-3}	0%	22 (2)	Fifth	418 (±133)	$df = 93$; t ratio = 0.847; $p = 0.7761$
		9.4×10^{-2}	3%	30 (3)	Fifth	377 (±126)	$df = 120$; t ratio = 2.036; $p = 0.1419$
		0.75	3%	20 (2)	Fifth	354 (±69)*	$df = 93$; t ratio = 2.741; $p = 0.0265$
		7.2	23%	24 (3)	Fourth[j]	199 (±102)***	$df = 120$; t ratio = 7.604; $p < 0.0001$
	Fifth						$F_{(3,76)} = 1.685$; $p = 0.1773$[g]
		0	0%	26 (3)	Pupa	1010 (±109)	
		9.7×10^{-2}	14%	23 (3)	Pupa	959 (±110)	
		0.78	10%	24 (3)	Pupa	959 (±174)	
		7.3	44%	15 (3)	Pupa	990 (±137)	
TMX	Second						$F_{(4,97)} = 1.944$; $p = 0.1092$[g]
		0	0%	29 (3)	Fourth	160 (±81)	
		4.2×10^{-2}	14%	25 (3)	Fourth	144 (±77)	
		0.52	14%	25 (3)	Fourth	143 (±90)	
		1.6	17%	16 (2)	Fourth	143 (±91)	
		4.7	69%	9 (2)	Fourth	120 (±70)	
	Third						$F_{(4,110)} = 9.216$; $p = 1.848 \times 10^{-6}$, [h]
		0	0%	30 (3)	Fifth	439 (±161)	
		4.7×10^{-3}	0%	19 (2)	Fifth	411 (±181)	$df = 82$; t ratio = 0.594; $p = 0.8978$
		4.5×10^{-2}	0%	22 (2)	Fifth	467 (±109)	$df = 82$; t ratio = 0.635; $p = 0.8809$
		0.54	0%	32 (3)	Fifth	430 (±134)	$df = 110$; t ratio = 0.170; $p = 0.9947$
		4.8	53%	14 (3)	Fourth[j]	175 (±143)***	$df = 110$; t ratio = 5.328; $p < 0.0001$

(*Continued*)

TABLE 5: (*Continued*)

Insecticide	Instar	Concentration[b] (µg insecticide/g leaf)	Larval percent mortality[c]	Number of surviving larvae/pupae (no. of replicate bioassays)[d]	Instar/stage at 96 h after application[e]	Mean final weights[f] (±SD)	Statistical analysis
	Fifth	0	0%	28 (3)	Pupa	1146 (±163)	$F_{(4,101)} = 5.779$; $p = 3.142 \times 10^{-4}$,[h]
		4.5×10^{-2}	0%	28 (3)	Pupa	1133 (±212)	$df = 101$; t ratio = 1.031; $p = 0.6658$
		0.55	0%	29 (3)	Pupa	1138 (±185)	$df = 101$; t ratio = 0.590; $p = 0.8994$
		4.9	18%	23 (3)	Pupa	1015 (±173)**	$df = 101$; t ratio = 3.464; $p = 0.0030$
		62	54%	13 (3)	Pupa	957 (±244)**	$df = 101$; t ratio = 3.724; $p = 0.0012$

[a]Based on combined mortality data from triplicate or quadruplicate bioassays for each insecticide–instar combination. Larvae were fed leaf tissues treated with 0.1% Silwet:water and 5 insecticide suspensions in 0.1% Silwet:water.
[b]Concentrations (averaged over runs) that caused equal to or fewer than 70% larval or pupal mortality (i.e., ≥30% survival) after adjusting for control (0.1% Silwet:water) mortality using Abbott's formula. Only data with concentrations that were used at least twice are provided in the table and analyzed for difference in final weights and development with respect to controls of the same bioassay run.
[c]Larval mortality calculated after setting control mortality to zero and adjusting for it in other concentrations (Abbott's formula).
[d]Eleven larvae were treated per concentration per run.
[e]Most common larval instar/stage observed at 96 h after application.
[f]Final weights of larvae were recorded 96 h after application. Final weights of pupae were recorded prior to adult eclosion.
[g]No significant concentration effect on larval weights based on ANOVA.
[h]Significant concentration effect on larval weights based on ANOVA. Post hoc analyses were conducted using Dunnett's test for multiple comparison with control larval weights from the same bioassay runs.
[i]One of the 4 runs excluded because of a hormesis effect (i.e., larval weight gain with increasing concentration).
[j]Of treated larvae, 79 to 92% were third or fourth instars; and 66 to 90% of control larvae were fifth instars.
*Treated larvae had significantly lower weights than control larvae at $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.
BCF = beta-cyfluthrin; CFS = chlorpyrifos; CTR = chlorantraniliprole; IMI = imidacloprid; SD = standard deviation; TMX = thiamethoxam.

normally. We also observed that the rate of arrested pupal ecdysis depends on the timing of fifth-instar exposure, particularly in dietary bioassays. Based on an experiment in which 72-h-old fifth instars were fed imidacloprid-treated leaves and results from our preliminary chronic dietary studies with imidacloprid, thiamethoxam, and chlorpyrifos, 24-h-old fifth instars are 10- to 100-fold less sensitive. Higher mortality rates in older fifth instars are associated with arrested ecdysis.

To the best of our knowledge, no previously published studies report neonicotinoids or organophosphates causing arrested pupal ecdysis in insects. Neonicotinoid and organophosphate insecticides increase acetylcholine signaling in the central nervous system of insects. Neonicotinoids act as acetylcholine agonists, whereas organophosphates, and their activated oxon metabolites, inhibit acetylcholinesterase (AChE), which increases synaptic concentrations of endogenous acetylcholine. Thany (2011) reported that thiamethoxam may bind to mixed nicotinic/muscarinic receptors in cercal afferent giant interneuron synapses of the American cockroach (*Periplaneta americana*). Aizono et al. (1997) suggested that muscarinic, cholinergic transmission may directly regulate prothoracicotropic hormone (PTTH) release from neurosecretory cells in the brain–corpus cardiacum–corpus allatum of the silkworm (*Bombyx mori*). Altered timing or levels of PTTH secretion attributable to neonicotinoid- or organophosphate-based stimulation of muscarinic receptors could perturb production and release of ecdysone from the prothoracic gland. In turn, the timing of ecdysis triggering hormone (ETH) production and secretion and/or expression of ETH receptors (ETHRs) in central nervous system neurons could be disrupted and impact subsequent steps in the signaling cascade that regulates ecdysis behavior, including the production of kinins and diuretic hormones (Kim et al. 2006; Lenaerts et al. 2017). These hormones regulate secretion of fluids in insects (Diao et al. 2016). Premature activation of neurons releasing these hormones could cause fluid loss that interferes with the molting process, consistent with our observation of fluid loss preceding arrested pupal ecdysis.

Notably, we did not observe arrested larval ecdysis. Kim et al. (2006) and Diao et al. (2016) described 2 ETHRs (ETHR-A and ETHR-B) that are expressed in distinct neurons of *Drosophila* and the hawkmoth, *Manduca*. Diao et al. (2016) showed that ETHR-A-expressing neurons are required for ecdysis at all developmental stages, whereas ETHR-B-expressing neurons are only required for pupal and adult ecdysis. The initiation of ecdysis behavior is regulated, in part, by the "disinhibition" of descending inhibitory ETHR-B neurons by segmental interneurons expressing ETHR-A and -B (Zitnan and Adams 2012). Diao et al. (2016) demonstrated that suppression of a subset of cholinergic ETHR-expressing neurons can block ecdysis. Exposure of acetylcholine-expressing neurons to acetylcholine agonists (e.g., neonicotinoids) or inhibitors of AChE (e.g., organophosphate insecticides) could alter the timing and/or degree of "disinhibition" and disrupt ecdysis. These hypotheses remain to be tested.

Although there are no monarch larval cuticular toxicity studies reported in the literature, Pecenka and Lundgren

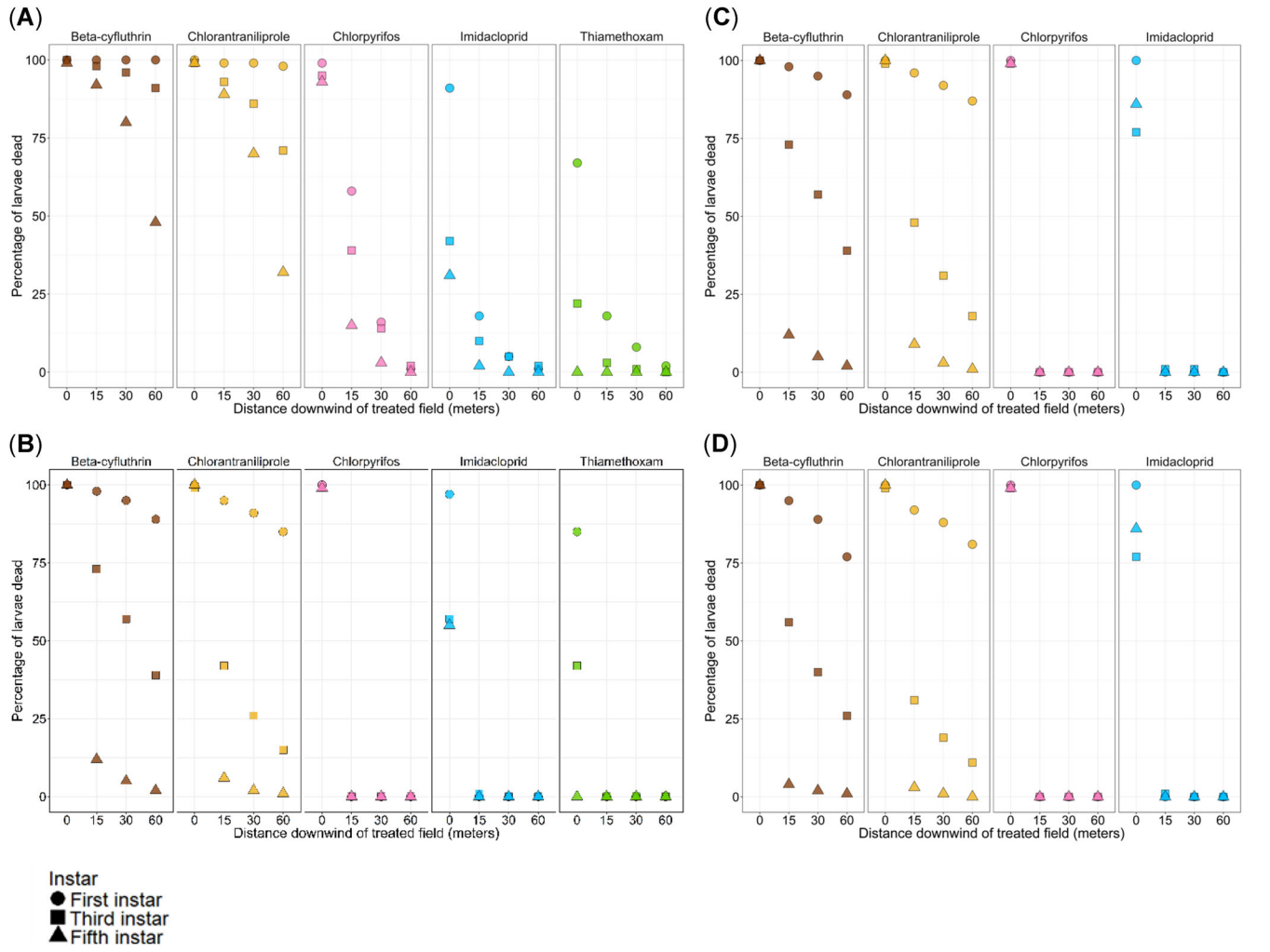

FIGURE 4: Estimated monarch larval mortality from cuticular exposure attributable to insecticide spray drift at increasing distances downwind from a treated crop field. Modeled spray drift scenarios using AgDRIFT (US Environmental Protection Agency 2003) include (**A**) aerial applications to manage soybean aphids, (**B**) high-ground boom applications to manage soybean aphids, (**C**) high-ground boom applications to manage true armyworms, and (**D**) low-ground boom applications to manage true armyworms. Mortality rates were estimated using active ingredient (a.i.)–specific larval dose–response curves (Supplemental Data, Figure S1) and estimated 50th percentile a.i.-specific exposures using the AgDRIFT model for ground boom applications (Supplemental Data, Table S5). Representative formulated products used to derive a.i.-specific exposures can also be found in Supplemental Data, Table S5. Thiamethoxam is not registered for use on true armyworms in maize or soybean fields. Note the *x*-axes are not proportionally spaced.

(2015) and Krischik et al. (2015) reported results from dietary bioassays with clothianidin and imidacloprid, respectively. Krischik et al. (2015) exposed early-instar larvae to tropical milkweed plants that were grown in imidacloprid-treated soil. Over a 7-d period, nearly 100% mortality occurred when larvae were reared on tropical milkweed with 10.4 µg imidacloprid/g leaf. In our 2-d dietary exposures, we observed a similar response, with 90% mortality for second instars feeding on tropical milkweed leaves with 19 µg of imidacloprid/g leaf (Table 3). Pecenka and Lundgren (2015) treated 1-cm-diameter discs of swamp milkweed (*Asclepias incarnata*) with 10 µL of aqueous solutions of clothianidin. A first-instar 36-h LC50 of 15.6 µg clothianidin/L of water was determined. This corresponds to an LC50 value of 2×10^{-4} µg of clothianidin/cm^2 swamp milkweed leaf. Our second-instar 96-h LC50 value is 9.7×10^{-2} µg clothianidin/cm^2 tropical milkweed leaf (Supplemental Data, Table S19). Differences in these LC50 values may be attributable to the source of larvae or experimental conditions.

To compare insecticide sensitivity of monarch larvae to other butterfly species, we primarily relied on the review conducted by Braak et al. (2018) and restricted our evaluation to those studies that reported LC or LD values based on mass of insecticide per gram of larva, per larva, per gram of diet, or per surface area of diet. Although there is a limited data set of comparable studies, results to date do not suggest a large range of species sensitivity to pyrethroid, organophosphate, and neonicotinoid insecticides. Hoang et al. (2011) estimated fifth-instar 24-h LD50 values of pyrethroid and organophosphate insecticides following cuticular exposure to larvae of 5 butterfly species: *Anartia jatrophae* (white peacock), *Eumaeus atala* (Atala butterfly), *Heliconius charitonius* (zebra longwing), *Junonia coenia* (common buckeye), and *Vanessa cardui* (painted lady). Permethrin (a pyrethroid) 24-h LD50 values

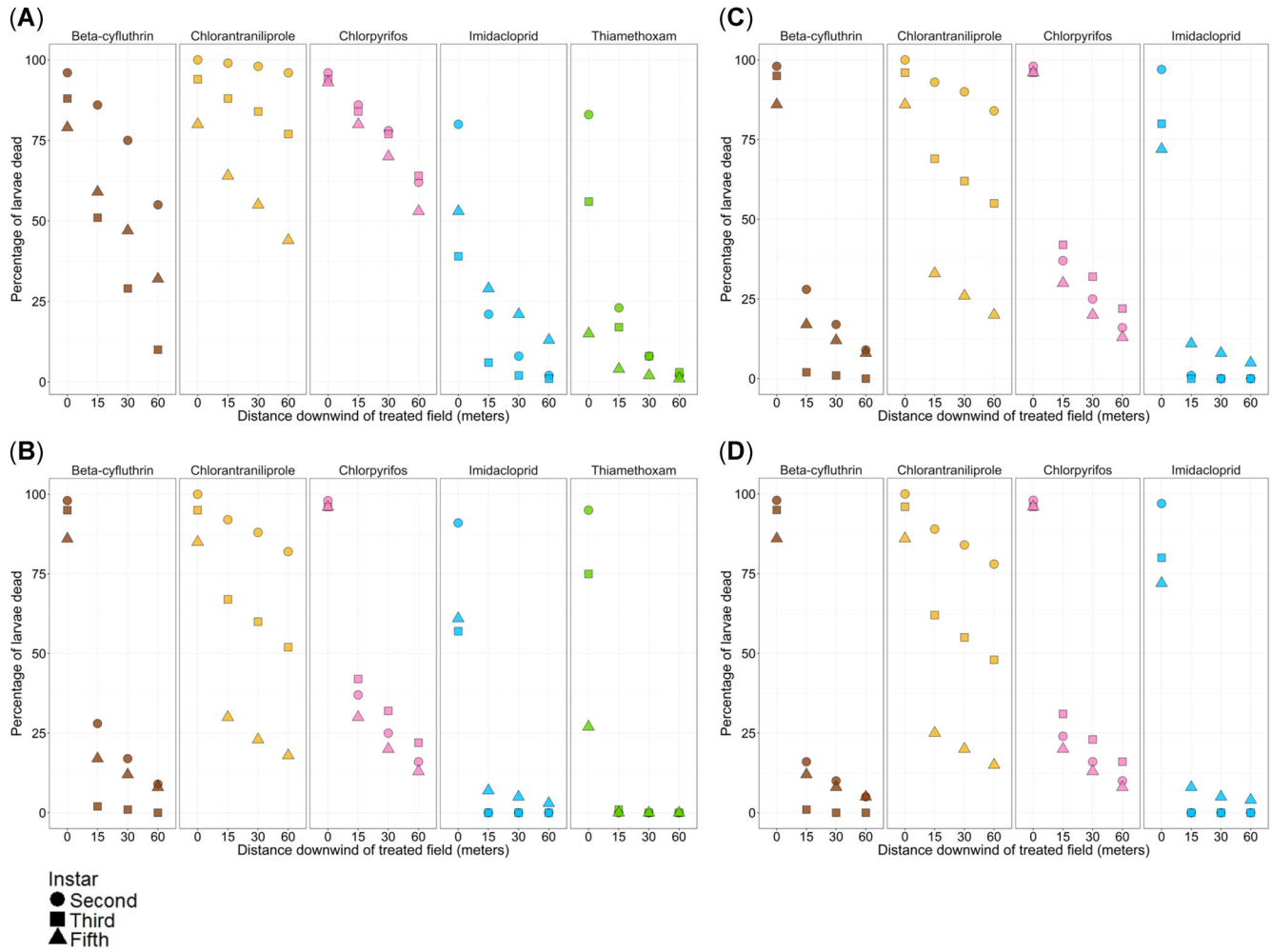

FIGURE 5: Estimated monarch larval mortality from dietary exposure attributable to insecticide spray drift at increasing distances downwind from a treated crop field. Modeled spray drift scenarios using AgDRIFT (US Environmental Protection Agency 2003) include aerial applications to manage soybean aphids (**A**), high-ground boom applications to manage soybean aphids (**B**), high-ground boom applications to manage true armyworms (**C**), and low-ground boom applications to manage true armyworms (**D**). Mortality rates were estimated using active ingredient (a.i.)–specific larval concentration–response curves (Supplemental Data, Figure S4) and estimated 50th percentile, a.i.-specific exposures using the AgDRIFT model for ground boom applications (Supplemental Data, Table S5). Representative formulated products used to derive a.i.-specific exposures can also be found in Supplemental Data, Table S5. Thiamethoxam is not registered for use on true armyworms in maize or soybean fields. Note the *x*-axes are not proportionally spaced.

ranged from 8×10^{-2} to 0.79 μg/g larva, whereas naled and dichlorvos (organophosphates) 24-h LD50 values ranged from 0.19 to 10.82 μg/g larva. Our fifth-instar monarch studies with beta-cyfluthrin and chlorpyrifos produced 96-h LD50 values of 4.8×10^{-2} and 18 μg/g larva, respectively (Table 1). Basley and Goulson (2018) reported 22% mortality (corrected for control mortality) with 7-d-old *Polyommatus icarus* (common blue butterfly) larvae reared on 0.439 μg clothianidin/g white clover leaves until pupation. Based on our clothianidin 96-h concentration–response curve, 4.9 μg/g milkweed leaf is expected to cause 22% mortality in third-instar monarchs (Supplemental Data, Figure S5).

Results of toxicity studies with the insecticides examined in the present study have also been reported for several pest moth species. Third-instar larvae of cotton bollworm (*Helicoverpa armigera*) topically treated with beta-cyfluthrin produced 72-h LD50s of approximately 4.7×10^{-2} and 9.9×10^{-2} μg/g larva (Martin et al. 2003; Tan and McCaffery 2007). The 96-h LD50 value of third-instar monarchs treated with beta-cyfluthrin is 1.8×10^{-2} μg/g. Following cuticular treatment with chlorpyrifos, third-instar common cutworm (*Spodoptera litura*) and cotton bollworm larvae produced 72-h LD50 values of 0.73 and 8.11 μg/g, respectively (Martin et al. 2003; Huang et al. 2006). Chlorpyrifos-treated monarch third instars produced a 96-h LD50 value of 22 μg/g (Table 1), suggesting that cotton bollworms and monarch butterflies have similar sensitivities to pyrethroids and organophosphates; however, the common cutworm is approximately 30-fold more sensitive to organophosphates. A dietary clothianidin toxicity study with fourth-instar black cutworm (*Agrotis ipsilon*) resulted in a 72-h LC50 of 27.8 μg/g artificial diet (Ding et al. 2018). The 96-h LC50 of third and fifth instar monarchs exposed to clothianidin is 7.8 and 0.80 μg/g leaf, making them approximately 4- to 35-fold more sensitive than fourth-instar black cutworm. He et al. (2019) reported a chlorantraniliprole 72-h LC50 of 0.187 μg/g artificial diet for third-instar black cutworms. The 96-h LC50 values

of third-instar monarchs exposed to chlorantraniliprole is approximately 4-fold lower (4.6×10^{-2} μg/g leaf).

A robust lepidopteran species sensitivity distribution could be used to estimate toxicity for insects of conservation concern and minimize, if not avoid, the time, costs, and challenges of rearing insects and host plants. Hoang and Rand (2015) carried out a probabilistic risk assessment for 3 insecticides encompassing 2 modes of action using toxicity data generated for 5 adult butterfly species. Developing an expanded lepidopteran sensitivity distribution with more insecticide modes of action requires clear description of dosimetry information to support a robust compilation of toxicity data. Screening bioassays used to identify candidate insecticides for lepidopteran pest species typically do not incorporate full dose–response curves, late-instar larvae, or extended observation periods, which limits their utility in developing models to support ecological risk assessments. Our observation of arrested pupal ecdysis and increased sensitivity of fifth-instar monarchs to neonicotinoid and organophosphate insecticides highlights the need to use standardized bioassay methods to generate well-defined data sets that can be used for species sensitivity modeling.

We also compared the cuticular toxicity values of monarch larvae to adult honey bees and found that monarch larvae are less sensitive to 3 of the 4 insecticide modes of action evaluated in the present study. As reviewed by Arena and Sgolastra (2014), cyfluthrin (mixed isomers), imidacloprid, thiamethoxam, and chlorpyrifos honey bee 24-h LD50 values range from 1×10^{-3}, 2.6×10^{-3} to 4×10^{-2}, 6.1×10^{-3}, and 5.9×10^{-2} μg/bee, respectively. Assuming an adult honey bee weighs 0.1 g (Thompson 2015), these values correspond to an LD50 range of 1×10^{-4} to 5.9×10^{-3} μg/g bee. Based on our first-instar monarch bioassays, beta-cyfluthrin, thiamethoxam, imidacloprid, and chlorpyrifos produced 96-h LD50 values of 9.2×10^{-3}, 6.1, 6.7, and 79 μg/g larva, respectively (Table 1), which suggests that honey bees are significantly more sensitive than monarch larvae to these insecticides following cuticular exposure. With the monarch, beta-cyfluthrin is approximately 700- to 9000-fold more potent than the neonicotinoids and chlorpyrifos; however, with the honey bee, cyfluthrin and the neonicotinoids are approximately 1- to 60-fold more toxic than chlorpyrifos. Wade et al. (2019) and Kadala et al. (2019) topically treated adult honey bees with chlorantraniliprole and reported 48- and 144-h LD50s of 0.706 and 0.250 μg/bee, respectively (or 7.06 and 2.50 μg/g bee, respectively); first-instar monarch larvae are approximately 200- to 600-fold more sensitive (Table 1; chlorantraniliprole 96-h LD50 is 1.2×10^{-2} μg/g larva). Differences in sensitivity to insecticide classes may reflect differences in susceptibility at the molecular sites of action and/or differences in rates of metabolic detoxification and sequestration.

Characterizing mortality risks

We provide estimates of larval mortality at varying distances downwind from treated fields under different application scenarios by integrating exposure estimates to larvae and milkweed with our cuticular and dietary dose– (or concentration–) response curves, respectively. Because there are no studies that measure insecticide residues on monarch larvae or milkweed leaves immediately following foliar applications, we estimated exposure using the AgDRIFT model (US Environmental Protection Agency 2003). With this model, insecticide exposure to surfaces up to 300 m downwind of an application are estimated based on droplet size, wind speed, and insecticide-specific application rate, as specified on the label of the formulated product. The formulated products we selected are illustrative of the types of products available to manage early- and late-season pests of maize and soybean in the north central states. We did not undertake an exhaustive evaluation of all registered products; however, the method we employed could be readily adapted to other foliar formulations.

The cuticular assessment indicated that aerial applications of formulated beta-cyfluthrin and chlorantraniliprole products at maximum label rates to manage soybean aphids would be expected to cause 100 to 32% mortality of all larvae at 0 and 60 m downwind from treated fields, respectively. Foliar applications of chlorpyrifos and the neonicotinoids were estimated to cause between 99 and 0% mortality at 0 and 60 m downwind. Because of chlorpyrifos' higher application rate, there is greater downwind deposition (5.6–0.3 μg/cm^2 at 0 and 60 m, respectively, following aerial application of Lorsban) compared to the other insecticides. Thus, this insecticide causes high mortality near the edges of field despite its comparatively low toxicity. The other insecticides had similarly lower application rates (Supplemental Data, Table S5). Consequently, beta-cyfluthrin and chlorantraniliprole, the most toxic insecticides, produced the highest downwind mortality rates, whereas the neonicotinoids produced the lowest mortality rates. Based on results of our toxicity studies, for insecticide exposures estimated to cause <70% larval mortality, negligible downwind effects on larval growth or development would be expected. In our analysis we assumed that all monarch larvae are exposed to the spray drift plume; however, larvae are most frequently found underneath milkweed leaves (Rawlins and Lederhouse 1981; Fisher et al. 2020). For example, Fisher et al. (2020) reported monarch larvae on the underside of the leaves during approximately 60% of their observations of development from neonate larvae to pupae. Consequently, our estimates of cuticular exposure and field-scale mortality are likely overestimated.

The dietary assessment indicated that aerial applications of formulated chlorantraniliprole and chlorpyrifos products at maximum label rates to manage soybean aphids would be expected to cause 100 to 44% mortality of all larvae at 0 and 60 m downwind from treated fields, respectively. Foliar applications of beta-cyfluthrin and the neonicotinoids were estimated to cause between 96 and 1% mortality at 0 and 60 m downwind. Beta-cyfluthrin is expected to cause greater mortality via the cuticular exposure route, whereas chlorpyrifos is expected to cause greater mortality via the dietary route. Downwind effects on monarch larval growth and development could be expected following dietary insecticide exposure.

Two published studies estimated monarch mortality rates from aerial applications of mosquito adulticides. Oberhauser et al. (2006) collected common milkweed leaves following

application of permethrin (application rate 0.109 kg active ingredient [a.i.]/ha). First, second, and third instars that fed on these leaves had >71% mortality. When larvae were directly exposed to resmethrin (application rate 0.0039 kg a.i./ha), >60% mortality was seen up to 23 m downwind (Oberhauser et al. 2009). Although droplet sizes are much smaller with mosquito adulticide formulations compared to formulations used for agricultural pests, the level of larval mortality observed in these field studies is qualitatively similar to the larval mortality we estimated with aerial beta-cyfluthrin applications.

Our mortality estimates based on dietary exposure are most relevant for a period of 1 to 2 d postapplication; however, for some of the insecticides, especially chlorantraniliprole, significant mortality may occur for several days postapplication. Length of dietary exposure is a function of an insecticide's photolysis, hydrolysis, and oxidation rates. In field and greenhouse studies conducted with growing plants, beta-cyfluthrin was found to have a half-life of 1 to 2 d (Banerjee et al. 2012), whereas chlorpyrifos, imidacloprid, and thiamethoxam had half-lives of 2 to 6 d (Galietta et al. 2011; Hassanzadeh et al. 2012; Rahman et al. 2015). Chlorantraniliprole has a reported half-life of 16 to 17 d (Szpyrka et al. 2017). Chronic studies to mimic longer-term dietary exposure to foliar insecticides are in progress. Our estimates also do not incorporate additional exposure episodes associated with multiple insecticide applications during the approximately 10 to 14 d of larval development. Label instructions for Baythroid, Admire Pro, Swagger, and Endigo require a minimum 7-d interval between the first and second applications; however, the minimum application interval is 5 d for Beseige and 14 d for Lorsban.

Although our risk assessments for individual insecticide applications at the field scale are conservative in that they employ upper-end exposure estimates, they could underestimate mortality to larvae simultaneously exposed to a mixture of insecticides. For example, with our representative formulated products, Beseige contains chlorantraniliprole and lambda-cyhalothrin, Endigo contains thiamethoxam and lambda-cyhalothrin, and Swagger contains bifenthrin and imidacloprid. Risks for formulated products with multiple active ingredients could be derived by adding the concentrations for insecticides with the same mode of action or by adding the responses (or mortality rates) for insecticides with different modes of action (National Research Council 2013). This approach would not capture any potential synergistic or antagonistic effects with insecticide–fungicide tank mixes, for example. We also did not assess the combined mortality rates from cuticular and dietary exposures. However, because larvae are typically found under milkweed leaves (Rawlins and Lederhouse 1981; Fisher et al. 2020), cuticular exposure to spray drift is likely low. Therefore, independently assessing mortality risks for the 2 routes of exposure is a reasonable approach.

Data and field-scale mortality estimates from the present study can augment expert opinion recently used to elucidate the potential impact of insecticide use on recovery of the monarch butterfly (Voorhies et al. 2019). We estimated high monarch larval mortality rates 0 to 15 m downwind of maize and soybean fields treated with foliar insecticide applications; however, these findings are not relevant for all monarch habitat that is in close proximity to crop fields. At the time of application, insecticide spray drift is deposited downwind of a treated field, with less or no insecticide deposition occurring on larvae or milkweed crosswind or upwind. Hence, similar levels of larval exposure and mortality will likely not occur on all sides of a treated field. In addition, across the north central states, insect pressure can vary widely within a given year, with some states having pest pressure above economic thresholds and other states with pest levels that do not require insecticide treatment. For example, from 2000 through 2012 soybean aphid pressure varied widely across the north central states (Bahlai et al. 2015). Variation also occurs within a state in a given year. Schmidt et al. (2008) reported a gradient of soybean aphid pressure that increased from southern to northern Iowa counties in 2005. Similarly, a small percentage of Iowa fields are being treated with foliar insecticides to manage true armyworms. In 2018, approximately 4% of the maize and soybean hectares had cover crops (Juchems 2019; US Department of Agriculture 2019). In addition, Dunbar et al. (2016) reported in their study that only half of the 6 maize fields with rye cover crops had true armyworm populations exceeding economic thresholds that warranted insecticide use.

Characterizing risks of foliar insecticides to nonmigratory monarch populations in agricultural ecosystems requires landscape-scale analyses (Uhl and Brühl 2019). Adult monarchs are vagile (Zalucki et al. 2016), which requires that attributes of their movement and reproductive behavior be integrated with spatial and temporal heterogeneity of monarch breeding habitat, agricultural fields, pastures, rural road rights-of-way, weather conditions, and pest pressure (Grant and Bradbury 2019). Results from the present study, ongoing acute contact exposures to egg and pupae from foliar insecticides, chronic larval dietary exposures to foliar and seed treatment insecticides, and acute adult oral exposures to seed treatment insecticides are being incorporated into an individual-based model (Grant et al. 2018) to obtain a more complete picture of landscape-scale risks. These analyses will evaluate the conservation risks and benefits of establishing new monarch habitat within agricultural landscapes of the north central United States.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4672.

Acknowledgment—The present study was supported by the Agriculture and Food Research Initiative Pollinator Health Program (grant 2018-67013-27541) from the US Department of Agriculture's National Institute of Food and Agriculture; the College of Agriculture and Life Sciences, Iowa State University (ISU); and the Iowa Monarch Conservation Consortium. Y. Zhang's 1-yr sabbatical at ISU was funded by the China Scholarship Council. V. Dang, D. Schrunk, and J. Peterson in the ISU College of Veterinary Medicine, Veterinary Diagnostic Laboratory, helped quantify insecticide concentrations in stock solutions. K. Goode and P. Dixon, ISU Statistics Department,

assisted with the statistical analyses. The authors gratefully acknowledge the technical support of current and former ISU undergraduate students A. Euken, A. Kindred, M. Aust, K. Weber, A. Kiehl, and T. Boysen. We also thank R. Jurenka for his helpful comments on arrested ecdysis and J. Pleasants for sharing his raw data on monarch phenology, which were used in developing Figure 1.

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://github.com/Niranjana296/Assessing-risk-of-insecticides-to-monarch-butterfly-larvae. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki.

Data Availability Statement—Data and metadata pertaining to this article are publicly available through GitHub (https://github.com/Niranjana296/Assessing-risk-of-insecticides-to-monarch-butterfly-larvae).

REFERENCES

Agrawal AA, Hastings AP, Patrick ET, Knight AC. 2014. Specificity of herbivore-induced hormonal signaling and defensive traits in five closely related milkweeds (*Asclepias* spp.). *J Chem Ecol* 40:717–729.

Aizono Y, Endo Y, Sattelle DB, Shirai Y. 1997. Prothoracicotropic hormone-producing neurosecretory cells in the silkworm, *Bombyx mori*, express a muscarinic acetylcholine receptor. *Brain Res* 763:131–136.

Altizer SM, Oberhauser KS, Brower LP. 2000. Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. *Ecol Entomol* 25:125–139.

Arena M, Sgolastra F. 2014. A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicology* 23:324–334.

Bahlai CA, Vander Werf W, O'Neal M, Hemerik L, Landis DA. 2015. Shifts in dynamic regime of an invasive lady beetle are linked to the invasion and insecticidal management of its prey. *Ecol Appl* 25:1807–1818.

Banerjee T, Banerjee D, Roy S, Banerjee H, Pal S. 2012. A comparative study on the persistence of imidacloprid and beta-cyfluthrin in vegetables. *Bull Environ Contam Toxicol* 89:193–196.

Basley K, Goulson D. 2018. Effects of field-relevant concentrations of clothianidin on larval development of the butterfly *Polyommatus icarus* (Lepidoptera, Lycaenidae). *Environ Sci Technol* 52:3990–3996.

Braak N, Neve R, Jones AK, Gibbs M, Breuker CJ. 2018. The effects of insecticides on butterflies—A review. *Environ Pollut* 242:507–518.

Brower LP, Taylor OR, Williams EH, Slayback DA, Zubieta RR, Ramírez MI. 2012. Decline of monarch butterflies overwintering in Mexico: Is the migratory phenomenon at risk? *Insect Conserv Divers* 5:95–100.

Carscallen GE, Kher SV, Evenden ML. 2019. Efficacy of chlorantraniliprole seed treatments against armyworm (*Mythimna unipuncta* [Lepidoptera: Noctuidae]) larvae on corn (*Zea mays*). *J Econ Entomol* 112:188–195.

Diao F, Mena W, Shi J, Park D, Diao Fengqiu, Taghert P, Ewer J, White BH. 2016. The splice isoforms of the *Drosophila* ecdysis triggering hormone receptor have developmentally distinct roles. *Genetics* 202:175–189.

Ding J, Zhao Y, Zhang Z, Xu C, Mu W. 2018. Sublethal and hormesis effects of clothianidin on the black cutworm (Lepidoptera: Noctuidae). *J Econ Entomol* 111:2809–2816.

Dixon PM, Goode KJ, Lay C. 2020. Profile likelihood confidence intervals for ECx, Statistics Technical Reports. Iowa State University Digital Repository, Ames, IA. [cited 2020 February 10]. Available from: https://lib.dr.iastate.edu/stat_las_reports/1

Douglas MR, Tooker JF. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. *Environ Sci Technol* 49:5088–5097.

Dunbar MW, O'Neal ME, Gassmann AJ. 2016. Increased risk of insect injury to corn following rye cover crop. *J Econ Entomol* 109:1691–1697.

Elmore R. 2012. Best corn planting dates for Iowa. Ames (IA): Iowa State University Extension. [cited 2019 October 18]. Available from: https://crops.extension.iastate.edu/cropnews/2012/03/best-corn-planting-dates-iowa

Fisher KE, Hellmich RL, Bradbury SP. 2020. Estimates of common milkweed (*Asclepias syriaca*) utilization by monarch larvae (*Danaus plexippus*) and the significance of larval movement. *J Insect Conserv*, in press. https://doi.org/10.1007/s10841-019-00213-2

Flockhart DTT, Brower LP, Ramirez MI, Hobson KA, Wassenaar LI, Altizer S, Norris DR. 2017. Regional climate on the breeding grounds predicts variation in the natal origin of monarch butterflies overwintering in Mexico over 38 years. *Glob Chang Biol* 23:2565–2576.

Galietta G, Egana E, Gemelli F, Maeso D, Casco N, Conde P, Nunez S. 2011. Pesticide dissipation curves in peach, pear and tomato crops in Uruguay. *J Environ Sci Health B* 46:35–40.

Gassmann A, Weber P. 2016. 2015 Iowa evaluation of insecticides and plant-incorporated protectants. Ames (IA): Iowa State University Extension. [cited 2019 October 18]. Available from: http://www.ent.iastate.edu/dept/faculty/gassmann/files/page/files/2015_Ent_Report.pdf

Grant TJ, Bradbury SP. 2019. The role of modeling in monarch butterfly research and conservation. *Front Ecol Evol* 7:197.

Grant TJ, Parry HR, Zalucki MP, Bradbury SP. 2018. Predicting monarch butterfly (*Danaus plexippus*) movement and egg-laying with a spatially-explicit agent-based model: The role of monarch perceptual range and spatial memory. *Ecol Modell* 374:37–50.

Hall MJ, Dang V, Zhang G, O'Neal M, Borts D, Bradbury SP, Coats JR. 2018. LC-MS/MS method for estimating the exposure to neonicotinoid residues in pollinator attractive habitat adjacent to corn and soybean fields. Iowa State Digital Repository. [cited 2020 February 10]. Available from: https://www.lib.dr.iastate.edu/ent_conf/7/

Hassanzadeh N, Sari AE, Bahramifar N. 2012. Dissipation of imidacloprid in greenhouse cucumbers at single and double dosages spraying. *J Agric Sci Technol* 14:557–564.

He F, Sun S, Tan H, Sun X, Qin C, Ji S, Li X, Zhang J, Jiang X. 2019. Chlorantraniliprole against the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae): From biochemical/physiological to demographic responses. *Sci Rep* 9:10328.

Hoang TC, Pryor RL, Rand GM, Frakes RA. 2011. Use of butterflies as nontarget insect test species and the acute toxicity and hazard of mosquito control insecticides. *Environ Toxicol Chem* 30:997–1005.

Hoang TC, Rand GM. 2015. Acute toxicity and risk assessment of permethrin, naled, and dichlorvos to larval butterflies via ingestion of contaminated foliage. *Chemosphere* 120:714–721.

Hodgson EW. 2018. Check your corn for aphids. Ames (IA): Iowa State University Extension. [cited 2019 October 18]. Available from: https://lib.dr.iastate.edu/cropnews/2484/

Hodgson EW, McCornack BP, Tilmon K, Knodel JJ. 2012. Management recommendations for soybean aphid (Hemiptera: Aphididae) in the United States. *J Integr Pest Manag* 3:E1–E10.

Hodgson EW, Rice M. 2017. Ecology and management of European corn borer in Iowa field corn. Ames (IA): Iowa State University Extension. [cited 2019 October 18]. Available from: https://store.extension.iastate.edu/Product/15141

Huang S, Xu J, Han Z. 2006. Baseline toxicity data of insecticides against the common cutworm *Spodoptera litura* (Fabricius) and a comparison of resistance monitoring methods. *Int J Pest Manag* 52:209–213.

Journey North. 2016. Milkweed sighted. Madison (WI): Journey North. [cited 2019 October 18]. Available from: https://journeynorth.org/sightings/querylist.html?map=milkweed&year=2016&season=spring

Juchems L. 2019. Cover crop acres increase but rate of growth declines in 2018. Ames (IA): Iowa State University Extension. [cited 2019 October 18]. Available from: https://www.extension.iastate.edu/news/cover-crop-acres-increase-rate-growth-declines-2018

Kadala A, Charreton M, Charnet P, Collet C. 2019. Honey bees long-lasting locomotor deficits after exposure to the diamide chlorantraniliprole are accompanied by brain and muscular calcium channels alterations. *Sci Rep* 9:2153.

Kaul RB, Rolfsmeier SB, Esch JJ. 1991. The distribution and reproductive phenology of the milkweeds (Asclepiadaceae: Asclepias and Cynanchum) in Nebraska. *Trans Nebr Acad Sci Affil Soc* 18:127–140.

Kim YJ, Žitňan D, Cho KH, Schooley DA, Misoguchi A, Adams ME. 2006. Central peptidergic ensembles associated with organization of an innate behavior. *Proc Natl Acad Sci USA* 103:14211–14216.

Krischik V, Rogers M, Gupta G, Varshney A. 2015. Soil-applied imidacloprid translocates to ornamental flowers and reduces survival of adult *Coleomegilla maculata*, *Harmonia axyridis*, and *Hippodamia convergens* lady beetles, and larval *Danaus plexippus* and *Vanessa cardui* butterflies. *PLoS One* 10:e0119133.

Lenaerts C, Cools D, Verdonck R, Verbakel L, Vanden Broeck J, Marchal E. 2017. The ecdysis triggering hormone system is essential for successful moulting of a major hemimetabolous pest insect, *Schistocerca gregaria*. *Sci Rep* 7:46502.

Malcolm SB, Cockrell BJ, Brower LP. 1993. Spring recolonization of eastern North America by the monarch butterfly: Successive brood or single sweep migration? *Biol Conserv* 253–267.

Martin T, Ochou OG, Vaissayre M, Fournier D. 2003. Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from West Africa. *J Econ Entomol* 96:468–474.

Monarch Watch. 2019. Monarch population status. *Monarch Watch Blog* (blog). 2019 January 30. [cited 2019 October 18]. Available from: https://monarchwatch.org/blog/2019/01/30/monarch-population-status-37/

Nail KR, Stenoien C, Oberhauser KS. 2015. Immature monarch survival: Effects of site characteristics, density, and time. *Ann Entomol Soc Am* 108:680–690.

National Research Council. 2013. *Assessing Risks to Endangered and Threatened Species from Pesticides*. National Academies Press, Washingtom, DC.

Natural Resources Conservation Service. 2016. Monarch butterfly wildlife habitat evaluation guide and decision support tool: Midwest edition 1.1. [cited 2020 February 10]. Available from: https://www.nrcs.usda.gov/wps/PA_NRCSConsumption/download?cid=nrcseprd895842&ext=pdf

Oberhauser KS, Brinda SJ, Weaver S, Moon RD, Read N, Manweiler SA. 2006. Growth and survival of monarch butterflies (Lepidoptera: Danaidae) after exposure to permethrin barrier treatments. *Environ Entomol* 35:1626–1634.

Oberhauser KS, Manweiler S, Lelich R, Blank M, Batalden RV, de Anda A. 2009. Impacts of ultra-low volume resmethrin applications on non-target insects. *J Am Mosq Control Assoc* 25:83–93.

Oberhauser KS, Wiederholt R, Diffendorfer JE, Semmens D, Ries L, Thogmartin WE, Lopez-Hoffman L, Semmens B. 2017. A trans-national monarch butterfly population model and implications for regional conservation priorities. *Ecol Entomol* 42:51–60.

Olaya-Arenas P, Kaplan I. 2019. Quantifying pesticide exposure risk for monarch caterpillars on milkweeds bordering agricultural land. *Front Ecol Evol* 7:223.

Pecenka JR, Lundgren JG. 2015. Non-target effects of clothianidin on monarch butterflies. *Naturwissenschaften* 102:19.

Pedersen P. 2007. Soybean planting date. Ames (IA): Iowa State University Extension. [cited 2019 October 18]. Available from: https://crops.extension.iastate.edu/files/article/PlantingDate_000.pdf

Pleasants JM. 2015. Monarch butterflies and agriculture. In Oberhauser KS, Nail KR, Altizer S, eds, *Monarchs in a Changing World: Biology and Conservation of an Iconic Butterfly*. Cornell University, Ithaca, NY, USA, pp 169–178.

Prysby MD, Oberhauser KS. 2004. Temporal and geographic variation in monarch densities: Citizen scientists document monarch population patterns. In Oberhauser KS, Solensky MJ, eds, *The Monarch Butterfly: Biology and Conservation*. Cornell University, Ithaca, NY, USA, pp 9–20.

Rahman MM, Farha W, Abd El-Aty AM, Kabir MH, Im SJ, Jung DI, Choi JH, Kim SW, Son YW, Kwon CH, Shin HC, Shim JH. 2015. Dynamic behaviour and residual pattern of thiamethoxam and its metabolite clothianidin in Swiss chard using liquid chromatography-tandem mass spectrometry. *Food Chem* 174:248–255.

Rasmann S, Agrawal AA, Cook SC, Erwin AC. 2009. Cardenolides, induced responses, and interactions between above- and below ground herbivores of milkweed (*Asclepias* spp.). *Ecology* 90:2393–2404.

Rawlins E, Lederhouse C. 1981. Developmental influences of thermal behavior on monarch caterpillars (*Danaus plexippus*): An adaptation for migration (Lepidoptera: Nymphalidae: Danainae). *J Kans Entomol Soc* 54:387–408.

Schmidt NP, O'Neal ME, Dixon PM. 2008. Aphidophagous predators in Iowa soybean: A community comparison across multiple years and sampling methods. *Ann Entomol Soc Am* 101:341–350.

Semmens BX, Semmens DJ, Thogmartin WE, Wiederholt R, López-Hoffman L, Diffendorfer JE, Pleasants JM, Oberhauser KS, Taylor OR. 2016. Quasi-extinction risk and population targets for the eastern, migratory population of monarch butterflies (*Danaus plexippus*). *Sci Rep* 6:23265.

Szpyrka E, Matyaszek A, Słowik-Borowiec M. 2017. Dissipation of chlorantraniliprole, chlorpyrifos-methyl and indoxacarb—Insecticides used to control codling moth (*Cydia pomonella* L.) and leafrollers (Tortricidae) in apples for production of baby food. *Environ Sci Pollut Res Int* 24:12128–12135.

Tan J, McCaffery AR. 2007. Efficacy of various pyrethroid structures against a highly metabolically resistant isogenic strain of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from China. *Pest Manag Sci* 63:960–968.

Thany SH. 2011. Thiamethoxam, a poor agonist of nicotinic acetylcholine receptors expressed on isolated cell bodies, acts as a full agonist at cockroach cercal afferent/giant interneuron synapses. *Neuropharmacology* 60:587–592.

Thogmartin WE, López-Hoffman L, Rohweder J, Diffendorfer J, Drum R, Semmens D, Black S, Caldwell I, Cotter D, Drobney P, Jackson LL, Gale M, Helmers D, Hilburger S, Howard E, Oberhauser K, Pleasants J, Semmens B, Taylor O, Ward P, Weltzin JF, Widerholt R. 2017. Restoring monarch butterfly habitat in the Midwestern US: "All hands on deck." *Environ Res Lett* 12:7.

Thompson H. 2015. Extrapolation of acute toxicity across bee species. *Integr Environ Assess Manag* 12:622–626.

Thrash B, Adamczyk JJ, Lorenz G, Scott AW, Armstrong JS, Pfannenstiel R, Taillon N. 2013. Laboratory evaluations of lepidopteran-active soybean seed treatments on survivorship of fall armyworm (Lepidoptera: Noctuidae) larvae. *Fla Entomol* 96:724–728.

Tripathy A. 2019. Calculating leaf surface areas using OpenCV. Madison (WI): MINDS@UW. [cited 2020 February 10]. Available from: http://digital.library.wisc.edu/1793/79417

Uhl P, Brühl CA. 2019. The impact of pesticides on flower-visiting insects: A review with regard to European risk assessment. *Environ Toxicol Chem* 38:2355–2370.

US Department of Agriculture. 2018. Quick stats. Washington, DC: National Agricultural Statistics Service. [cited 2019 October 18]. Available from: https://quickstats.nass.usda.gov/#59CB4138-B1CE-31CD-B29D-A71A0DF9BBE6

US Department of Agriculture. 2019. Iowa ag news—2018 crop production. Washington, DC: National Agricultural Statistics Service. Washington, DC. [cited 2019 October 18]. Available from: https://www.nass.usda.gov/Statistics_by_State/Iowa/Publications/Crop_Report/2019/IA-Crop-Production-Annual-01-19.pdf

US Environmental Protection Agency. 2003. AgDRIFT. Washington, DC. [cited 2019 October 18]. Available from: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#AgDrift

US Fish and Wildlife Service. 2016. Assessing the status of the monarch butterfly. Washington, DC: US Fish and Wildlife Service. [cited 2019 October 18]. Available from: https://www.fws.gov/savethemonarch/ssa.html

US Fish and Wildlife Service. 2017. Monarch butterfly species assessment update. [cited 2020 February 10]. Available from: https://www.fws.gov/savethemonarch/pdfs/Monarch.pdf

Voorhies KJ, Szymanski J, Nail KR, Fidino M. 2019. A method to project future impacts from threats and conservation on the probability of extinction for North American migratory nonarch (*Danaus plexxipus*) populations. *Front Ecol Evol* 7:384.

Wade A, Lin CH, Kurkul C, Regan ER, Johnson RM. 2019. Combined toxicity of insecticides and fungicides applied to California almond orchards to honey bee larvae and adults. *Insects* 10:E20.

Whalen RA, Herbert DA, Malone S, Kuhar TP, Brewster CC, Reisig DD. 2016. Effects of diamide insecticides on predators in soybean. *J Econ Entomol* 109:2014–2019.

Zalucki MP, Parry HR, Zalucki JM. 2016. Movement and egg laying in monarchs: To move or not to move, that is the equation. *Austral Ecol* 41:154–167.

Zitnan D, Adams ME. 2012. Neuroendocrine regulation of ecdysis. In Lawrence IG, ed, *Insect Endocrinology*. Academic, San Diego, CA, USA, pp 253–309.

