

# Pyrethroid Exposure Reduces Growth and Development of Monarch Butterfly (Lepidoptera: Nymphalidae) Caterpillars

Annie J. Krueger,<sup>1</sup> Kathryn Hanford,<sup>2</sup> Thomas J. Weissling,<sup>1</sup> Ana M. Vélez,<sup>1</sup> and Troy D. Anderson<sup>1,3,✉</sup>

<sup>1</sup>Department of Entomology, University of Nebraska, Lincoln, NE 68583, USA, <sup>2</sup>Department of Statistics, University of Nebraska, Lincoln, NE 68583, USA and <sup>3</sup>Corresponding author, e-mail: [tanderson44@unl.edu](mailto:tanderson44@unl.edu)

Subject Editor: Phyllis Weintraub

Received 25 September 2020; Editorial decision 11 December 2020

## Abstract

Insecticide exposure has been identified as a contributing stressor to the decline in the North American monarch butterfly *Danaus plexippus* L. (Lepidoptera: Nymphalidae) population. Monarch toxicity data are currently limited and available data focuses on lethal endpoints. This study examined the 72-h toxicity of two pyrethroid insecticides, bifenthrin and  $\beta$ -cyfluthrin, and their effects on growth and diet consumption. The toxicity of bifenthrin to caterpillars was lower than  $\beta$ -cyfluthrin after 72 h. Survival was the most sensitive endpoint for bifenthrin, but diet consumption and caterpillar growth were significantly reduced at sublethal levels of  $\beta$ -cyfluthrin. Using AgDRIFT spray drift assessment, the aerial application of bifenthrin or  $\beta$ -cyfluthrin is predicted to pose the greatest risk to fifth-instar caterpillars, with lethal insecticide deposition up to 28 m for bifenthrin and up to 23 m for  $\beta$ -cyfluthrin from treated edges of fields. Low boom ground applications are predicted to reduce distances of lethal insecticide exposure to 2 m from the treated field edge for bifenthrin and  $\beta$ -cyfluthrin. Growth and survival of fifth-instar monarch caterpillars developing within the margins of a treated field may be significantly impacted following foliar applications of bifenthrin or  $\beta$ -cyfluthrin. These findings provide evidence that pyrethroid insecticides commonly used for soybean pest control are a potential risk to monarch caterpillars in agricultural landscapes.

**Key words:** monarch butterfly, pyrethroid, toxicity, growth, development

The monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Nymphalidae), is a globally distributed species, primarily in the Americas and Oceania. In North America, it has become an icon for extensive migration across the continent. Monarchs east of the Rocky Mountains overwinter in vast numbers in Mexico and travel north in the spring, covering most of the Midwest and east coast, advancing farther north with every generation (Oberhauser and Solensky 2004). By the fourth generation, the adults make the 1,000-km flight to return to overwintering grounds in Mexico (Alonso-Mejia et al. 1997). This unique life history has made the North American population more susceptible to multiple stressors, both in their overwintering grounds and breeding habitat. The monarch is a charismatic flagship for invertebrate conservation more broadly (Oberhauser and Guiney 2009) and the conservation of the monarch butterfly has been valued upwards of \$4 billion according to a survey of U.S. households (Diffendorfer et al. 2014). An understanding of the threats to and conservation opportunities for the monarch butterfly is critical for securing further public engagement for invertebrate conservation.

In the United States, the increased use of glyphosate and expansion of farmland over the past 40 yr has greatly diminished the presence of milkweed in the breeding grounds and removed it almost

entirely within fields (Pleasants and Oberhauser 2013, Pleasants 2017, Thogmartin et al. 2017). Pleasants and Oberhauser (2013) documented a ca. fourfold difference between egg densities on milkweed in agricultural fields compared with milkweed on roadsides or in pastureland. To make up for this loss of preferred oviposition habitat, researchers have set a 1.8 billion milkweed stem goal to restore and stabilize the overwintering monarch population (Thogmartin et al. 2017). While the number of milkweed stems on the landscape has been the focus of conservation efforts, the location of these stems and their proximity to commercial agriculture has raised concerns over the risk of agrochemicals to monarchs. In Europe, several studies have shown decreased butterfly abundance in margins of fields treated with foliar applied insecticides (Çilgi and Jepson 1995, Longley et al. 1997, Rundlöf et al. 2008). In the 1990s, *Bacillus thuringiensis* (*Bt*) (Bacillales: Bacillaceae) crops and pollen expressing *Bt* Cry1 proteins targeting lepidopteran pests were heavily investigated for the risk to developing monarchs; however, the risk of most varieties on the market was considered negligible (Sears et al. 2001). Although the risk of *Bt* crops was heavily studied, toxicity data detailing the risk of other insecticide products to monarchs is limited. Braak et al. 2018 report insecticide data for a number of lepidopteran species and found only three available toxicity studies for monarchs using permethrin

(Oberhauser et al. 2006), imidacloprid (Krischik et al. 2015), and clothianidin (Pecenka and Lundgren 2015). While more exhaustive toxicity (Krishnan et al. 2020) and exposure data (Olaya-Arenas and Kaplan 2019) are becoming available for different life stages and classes of insecticides, data are lacking on the sublethal effects of insecticide exposures to monarchs.

Pyrethroid insecticides are commonly used to control insect pests of corn and soybean across the United States (Ragsdale et al. 2011). These broad-spectrum insecticides are acutely neurotoxic, targeting the voltage-gated Na<sup>+</sup> channel and disrupting neurological function (Clements and May 1977). Pyrethroids are classified as type I or type II based on their chemical structure, effects on the central nervous system and subsequent symptomology (Gammon et al. 1981). Pyrethroid studies in butterfly species have focused on compounds largely used for mosquito management, including permethrin and deltamethrin (Braak et al. 2018). However, in agriculture, pyrethroids like bifenthrin and  $\beta$ -cyfluthrin are used in much larger quantities but toxicity data has only recently become available for  $\beta$ -cyfluthrin (Krishnan et al. 2020). Because of their acute toxicity, pyrethroids have been used to control a variety of insect outbreaks. For example, soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae) outbreaks and subsequent foliar applications of pyrethroids often occur in mid-July and again in mid-September when monarch caterpillars are present on the landscape (Nail et al. 2015, Bradbury et al. 2017). In Iowa, true armyworm populations *Mythimna unipuncta* Haworth (Lepidoptera: Noctuidae) can exceed economic thresholds in mid-May and late-June, prompting foliar insecticide applications at a time when monarchs are first beginning to colonize the Midwest United States (Dunbar et al. 2016). AgDRIFT is a model for estimating near-field spray drift from aerial applications and has been used as a modeling tool for risk assessment when residue data are unavailable (Teske et al. 2002). This model can be used as a screening tool at the Tier 1 level to provide a conservative assessment of off-field pesticide risk and has been used for nontarget plant assessments (Brain et al. 2019). Krishnan et al. 2020 reported the application of AgDRIFT for the evaluation of pesticide risk to nontarget insect communities, including monarch caterpillars.

The fifth larval instar is the longest larval development stage of monarch caterpillars that allows for changes in consumption and growth to be observed without confounding effects of molting (Zalucki 1982). The natural mortality rates of early instar caterpillars, in the field, are significantly higher than that of fifth-instar caterpillars surviving to adulthood (Nail et al. 2015). Therefore, this study was conducted to estimate the lethal and sublethal endpoints for fifth-instar monarch caterpillars exposed to the type I and type II pyrethroids bifenthrin and  $\beta$ -cyfluthrin, respectively, and the potential effect of these insecticide on the biological fitness of caterpillars. The AgDRIFT model was used to predict spray deposition and to provide a landscape perspective for toxicity endpoints.

## Materials and Methods

### Insects

Fifth-instar caterpillars of the monarch butterfly were used for all laboratory experiments. A laboratory colony was established in the Department of Entomology at the University of Nebraska-Lincoln using eggs received from Iowa State University (Ames, IA). The adults were maintained at 24°C on a 12:12 (L:D) h cycle, with an artificial nectar diet. For experiments, eggs were collected daily and stored at 16°C for up to 14 d. The eggs were moved to room temperature and hatched within 2–3 d. Neonates were then placed on artificial diet within 24 h of hatching.

### Artificial Diet

The monarch caterpillar diet was prepared using Southland multi-species Lepidoptera diet (Southland Products Inc., Lake Village, AR) with the addition of 15% (w/w) lyophilized tropical milkweed, *Asclepias curassavica* (Gentianales: Apocynaceae) leaf powder. The leaves were collected from plants grown in a greenhouse throughout the year, washed in a 10% (v/v) bleach solution, rinsed thoroughly with water and soaked in a 10% (v/v) Sonne's No. 7 clay (Sonne's Organic Foods Inc., Liberty, MO) solution. After washing, the leaves were air-dried and stored at -80°C before lyophilized and ground into a fine powder.

### Chemicals

Bifenthrin (CAS# 82657-04-3, 99.5%) and  $\beta$ -cyfluthrin (CAS# 1820573-27-0, 98.0%) were purchased from Chem Service Inc. (West Chester, PA) and stored at room temperature. All stock solutions and dilutions were prepared in acetone (Sigma-Aldrich, St. Louis, MO).

### Toxicity Assays

One-day-old fifth-instar monarch caterpillars were used to ensure individuals had fully finished their molt from the fourth instar and that insecticide residues on the cuticle remained for a 72-h observation period. In total, 50–60 individual 1-d-old fifth-instar caterpillars were weighed into preweighed diet cups. The caterpillars were stratified by weight and randomly assigned to treatment groups, 10 individuals per treatment group, to ensure an equal size distribution across all treatments. A 1- $\mu$ l aliquot of acetone (solvent control) or each insecticide at 0.025, 0.05, 0.1, 0.2, 0.4  $\mu$ g/ $\mu$ l bifenthrin or 0.0125, 0.025, 0.05, 0.1  $\mu$ g/ $\mu$ l  $\beta$ -cyfluthrin prepared in acetone was applied to the dorsal prothorax, between the anterior tentacles of each caterpillar. The mortality and behavior (i.e., normal, lethargic, immobile, loss of hemolymph) of the caterpillars was observed daily over a 72-h exposure period. Bifenthrin and  $\beta$ -cyfluthrin experiments were repeated in triplicate using caterpillars from two different generations for a total of 30 caterpillars per treatment.

### Diet Consumption and Growth Assays

The diet and frass of each monarch caterpillar were weighed at 24, 48, and 72 h. To correct for evaporative loss of diet, additional diet cups were prepared and weighed at the same time points. The individual caterpillars were weighed daily, with no adverse effects observed after handling caterpillars this frequently. The experiments were repeated in triplicate for a total of 30 caterpillars per treatment. The daily weight was not recorded for the 0.05  $\mu$ g/ $\mu$ l bifenthrin treatment, but the initial and final weight was recorded for each caterpillar. There were no behavioral changes observed at this treatment level and daily weights at 24 h and 48 h were estimated using a generalized linear mixed model.

### AgDRIFT Aerial and Ground Spray Drift Assessment

The AgDRIFT Tier 1 aerial and ground spray drift assessment (AgDRIFT ver. 2.1.1.1, U.S. Environmental Protection Agency 2016) was used as a conservative drift model to predict the spray deposition (mg/cm<sup>2</sup>) for agricultural applications of bifenthrin and  $\beta$ -cyfluthrin formulations (Teske et al. 2002). The point deposition ( $\mu$ g/cm<sup>2</sup>) of each insecticide estimated with AgDRIFT was multiplied by the total surface area of a caterpillar (ca. 7.1 cm<sup>2</sup>), as reported by Krishnan et al. (2020), to estimate the direct contact exposure of the insecticides to fifth-instar monarch caterpillars. The label rates from the common use pyrethroid formulations Brigade 2-EC (0.1 lb/ac bifenthrin) and Baythroid XL (0.022 lb/ac  $\beta$ -cyfluthrin) were used

for the AgDRIFT assessment. The spray deposition was modeled for low boom ground, high boom ground, and aerial applications at 0, 1, 3, 6, 12, and 24 m from the edge of a field. In accordance of the manufacturer's label instructions for each insecticide formulation, the Tier 1 ground application assessment was calculated using an ASAE fine to medium-coarse droplet size and an ASAE medium to coarse droplet size was used for the Tier 1 aerial application assessment. The distances were selected to predict insecticide deposition on milkweed in ditches and field margins where milkweed is commonly found in the U.S. Midwest (Pleasant and Oberhauser 2013, Pleasant 2017).

## Data Analysis

The dose–response calculations and associated statistical analyses were conducted using SAS 9.4 PROC PROBIT (SAS, Cary, NC). The monarch caterpillar weight and diet consumption were analyzed using SAS 9.4 PROC GLIMMIX (SAS). A Gaussian distribution was assumed for both outcomes. A repeated-measures analysis was conducted for weight and diet consumption on individual caterpillars over time. The treatments were analyzed as a continuous effect. The initial model included fixed linear, quadratic, and cubic treatment dose effects, time effect, interaction between linear, quadratic, and cubic treatment dose with time, and initial caterpillar weight as a covariate. Experiment was used as a significant blocking factor in all analyses. A first-order Antedependence pattern was chosen to model the covariance structure. The nonsignificant terms ( $P > 0.05$ ) were dropped from the initial model for the final analysis. Total diet consumption was analyzed with an initial model that included fixed linear, quadratic, and cubic treatment dose effects, and initial caterpillar weight as a covariate. The assessment estimates for each treatment level were compared with the control group at each time point using Scheffe's multiple comparison procedure (Scheffé 1953).

## Results

### Toxicity Assays

The results of the toxicity assays for bifenthrin and  $\beta$ -cyfluthrin are presented in Table 1. The toxicity of bifenthrin ( $LD_{50} = 0.44 \mu\text{g}/\mu\text{l}$  [0.32–0.65], slope = 1.86 [1.34–2.37]) was significantly less for the monarch caterpillars compared to  $\beta$ -cyfluthrin ( $LD_{50} = 0.14 \mu\text{g}/\mu\text{l}$  [0.12–0.19], slope = 3.59 [2.39–4.80]) 72 h after application of the insecticides based on nonoverlapping 95% CIs. There were symptoms of intoxication, including hemolymph bleeding and spasming, observed for the caterpillars treated with  $\geq 0.2 \mu\text{g}/\mu\text{l}$  bifenthrin and  $\geq 0.025 \mu\text{g}/\mu\text{l}$   $\beta$ -cyfluthrin at 0- to 1-h posttreatment. Caterpillars treated with 0.2  $\mu\text{g}/\mu\text{l}$  and 0.4  $\mu\text{g}/\mu\text{l}$  bifenthrin exhibit 27 and 36% mortality, respectively. There was no mortality observed for caterpillars treated with  $\beta$ -cyfluthrin at 0.025  $\mu\text{g}/\mu\text{l}$ , although there was

7% mortality observed for caterpillars treated with 0.05  $\mu\text{g}/\mu\text{l}$   $\beta$ -cyfluthrin, despite hemolymph bleeding and an upright posturing observed for these individuals.

### Diet Consumption and Growth Assays

The results of the daily diet consumed by monarch caterpillars after treatment with bifenthrin and  $\beta$ -cyfluthrin are presented in Fig. 1. The final model for the effect of bifenthrin and  $\beta$ -cyfluthrin on daily diet consumption, included the covariate of starting weight for individual caterpillars ( $P < 0.001$ ) in addition to significant linear ( $P < 0.001$ ) and quadratic ( $P < 0.005$ ) treatment by time interaction terms. Experiment was a significant ( $P < 0.0001$ ) blocking factor for bifenthrin diet consumption, but not for  $\beta$ -cyfluthrin ( $P = 0.22$ ). A significant 9, 33, 58, and 87% reduction in diet consumption was observed for caterpillars treated with 0.025, 0.1, 0.2, and 0.4  $\mu\text{g}/\mu\text{l}$   $\beta$ -cyfluthrin ( $P < 0.005$ ), respectively, at 24 h posttreatment compared with the solvent-treated individuals. Caterpillars treated with 0.4  $\mu\text{g}/\mu\text{l}$  bifenthrin also consumed significantly less diet after 48 h (91%,  $P < 0.0001$ ) and 72 h (75%,  $P = 0.0016$ ) compared with the solvent-treated individuals. The daily diet consumption was not significantly different than that of the untreated caterpillars for any other concentration or time-point. A significant 33, 59, 94, and 92% reduction in diet consumption was observed for caterpillars treated with 0.0125, 0.025, 0.05, and 0.1  $\mu\text{g}/\mu\text{l}$   $\beta$ -cyfluthrin ( $P < 0.0001$ ), respectively, at 24 h posttreatment compared to the solvent-treated individuals. A significant reduction in diet consumption for caterpillars in all treatment groups was observed at 48 h posttreatment ( $P < 0.0001$ ) compared with the solvent-treated individuals. However, at 72-h posttreatment, a significant decrease in diet consumption was observed for caterpillars treated with 0.1  $\mu\text{g}/\mu\text{l}$   $\beta$ -cyfluthrin (59%,  $P = 0.0034$ ) compared with the solvent-treated individuals.

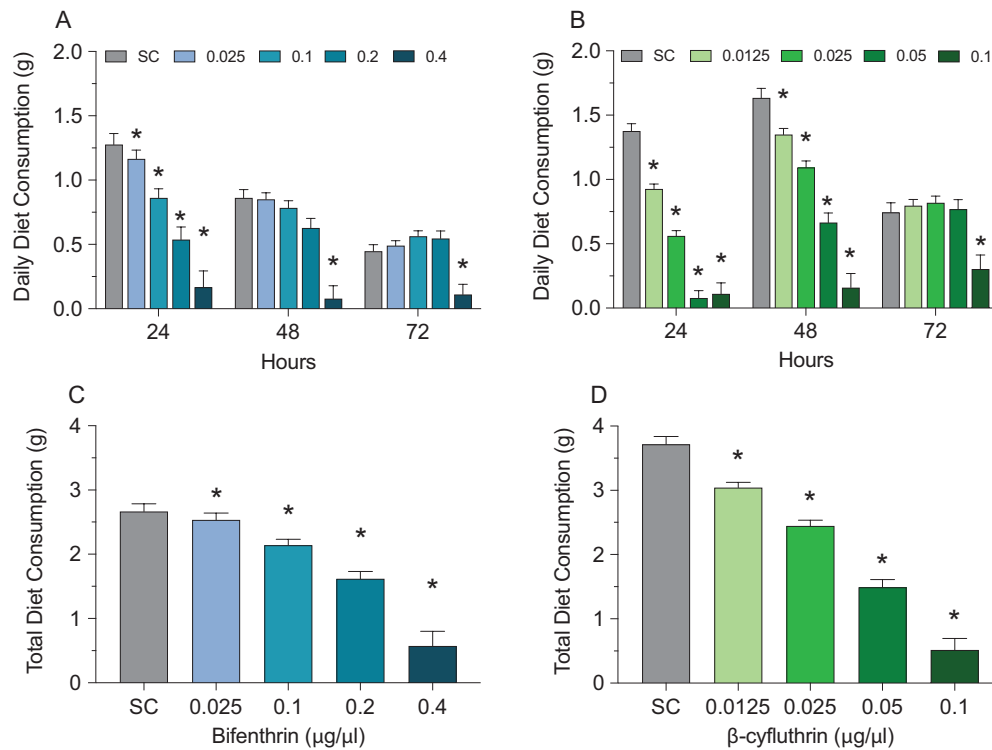
A model including a linear treatment effect ( $P < 0.0001$ ) and the individual starting weight covariate ( $P < 0.0001$ ) was used to predict total diet consumption for bifenthrin. A model including both a linear ( $P < 0.0001$ ) and quadratic ( $P = 0.0004$ ) treatment effect and the individual starting weight covariate ( $P = 0.0021$ ) was fit for  $\beta$ -cyfluthrin. Again, experiment was a significant ( $P < 0.005$ ) blocking factor for bifenthrin total diet consumption, but not for  $\beta$ -cyfluthrin total diet consumption ( $P = 0.88$ ) and was removed from the  $\beta$ -cyfluthrin diet models. The reduction in total diet consumed by caterpillars was 5, 20, 39, and 79% for caterpillars treated with bifenthrin at 0.025, 0.1, 0.2, and 0.4  $\mu\text{g}/\mu\text{l}$ , respectively, compared with the solvent-treated caterpillars (Fig. 1C). The total diet consumption was significantly reduced ( $P < 0.0001$ ) by 18, 34, 60, and 86% for caterpillars treated with  $\beta$ -cyfluthrin at 0.0125, 0.025, 0.05, and 0.1  $\mu\text{g}/\mu\text{l}$ , respectively, compared with the solvent-treated caterpillars (Fig. 1D). The total diet consumed between caterpillars

**Table 1.** Contact toxicity of bifenthrin and  $\beta$ -cyfluthrin to fifth-instar monarch caterpillars<sup>a</sup>

Insecticide	N	LD10	LD25	LD50	LD75	LD90	Slope	$\chi^{2b}$
		95% CI	95% CI	95% CI	95% CI	95% CI	95% CI	$Pr > \chi^2$
Bifenthrin	200	0.08 0.05–0.12	0.19 0.14–0.25	0.44 0.32–0.65	1 0.67–1.90	2.10 1.20–5.30	1.86 1.34–2.37	50.42 <0.0001
$\beta$ -Cyfluthrin	170	0.06 0.05–0.08	0.09 0.07–0.11	0.14 0.12–0.19	0.22 0.17–0.35	0.32 0.23–0.61	3.59 2.39–4.80	34.38 <0.0001

<sup>a</sup>Pyrethroid toxicity data are presented as  $LD_{10}$ ,  $LD_{25}$ ,  $LD_{50}$ ,  $LD_{75}$ , and  $LD_{90}$  and their 95% CIs in micrograms per microliter.

<sup>b</sup>Pearson's  $\chi^2$  and the probability of  $\chi^2$ . The probability of  $>0.05$  indicates that the observed regression model is not significantly different from the expected model.



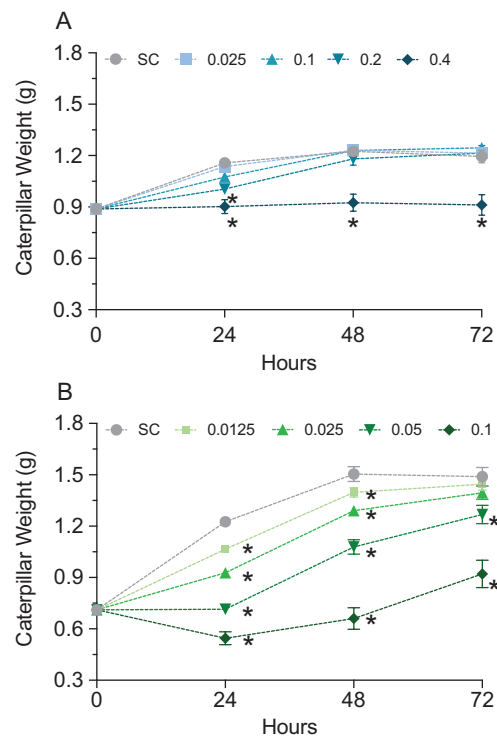
**Fig. 1.** Daily and total diet consumption of fifth-instar monarch caterpillars after topical exposure to bifenthrin (A and C) and  $\beta$ -cyfluthrin (B and D). Vertical bars represent the mean  $\pm$  standard error ( $n = 30$ ) and asterisks indicate significant differences between the solvent control (SC) and treatment means (SAS PROC GLIMMIX,  $P < 0.05$ ).

was variable for each experiment, but part of the variability was accounted for using the initial weight of each caterpillar.

The results of the caterpillar body weights after treatment with bifenthrin and  $\beta$ -cyfluthrin are shown in Fig. 2. The final model for the effect of bifenthrin and  $\beta$ -cyfluthrin on caterpillar weight included the covariate of individual starting weight ( $P < 0.0001$ ), blocking factor of experiment ( $P < 0.005$ ), and significant linear ( $P < 0.005$ ) and quadratic ( $P < 0.005$ ) treatment by time interaction. There was a significant reduction in body weight for caterpillars treated with 0.2  $\mu\text{g}/\mu\text{l}$  (13%,  $P = 0.0085$ ) and 0.4  $\mu\text{g}/\mu\text{l}$  bifenthrin (22%,  $P < 0.0001$ ) for 24 h, but only a significant reduction for caterpillars treated with 0.4  $\mu\text{g}/\mu\text{l}$  bifenthrin for 48 h (24%,  $P < 0.0001$ ) and 72 h (24%,  $P = 0.0003$ ) compared with the solvent-treated individuals (Fig. 2A). A significant decrease ( $P < 0.0001$ ) in body weight was observed for caterpillars treated with 0.0125, 0.025, 0.05, and 0.1  $\mu\text{g}/\mu\text{l}$   $\beta$ -cyfluthrin, respectively, after 24 and 48 h as compared with the solvent control individuals (Fig. 2B). At 72 h posttreatment, there was a significant 15% ( $P = 0.047$ ) and 45% ( $P < 0.0001$ ) reduction in body weight for caterpillars that were treated with, and survived, 0.05 and 0.1  $\mu\text{g}/\mu\text{l}$   $\beta$ -cyfluthrin, respectively, compared with the solvent-treated caterpillars.

#### AgDRIFT Aerial and Ground Spray Drift Assessment

The results of the AgDRIFT Tier 1 aerial and ground spray drift assessment are presented in Fig. 3. The aerial assessment predicted that bifenthrin deposition could exceed 0.44  $\mu\text{g}/\text{caterpillar}$ , the estimated  $\text{LD}_{50}$  for fifth-instar monarch caterpillars on milkweed up to 28 m from the treated edge of a field (Fig. 3). Additionally, the aerial assessment predicted  $\beta$ -cyfluthrin deposition could exceed 0.14  $\mu\text{g}/\text{caterpillar}$ , the estimated  $\text{LD}_{50}$  for fifth-instar caterpillars on milkweed



**Fig. 2.** Weight of fifth-instar monarch caterpillars after topical exposure to bifenthrin (A) and  $\beta$ -cyfluthrin (B). Symbols represent the mean  $\pm$  SE ( $n = 30$ ) and when absent the error bars are within the size of the symbol. Asterisks indicate significant differences between the solvent control (SC) and treatment means (SAS PROC GLIMMIX,  $P < 0.05$ ).

up to 23 m from the treated edge of a field (Fig. 3). These exposure distances are reduced in the ground assessment with the high boom deposition of bifenthrin and  $\beta$ -cyfluthrin predicted to be lethal at 3 and 2 m, respectively, from the treated edge of a field. For the low boom deposition of bifenthrin and  $\beta$ -cyfluthrin, these distances are reduced to 2 m from the treated edge of a field.

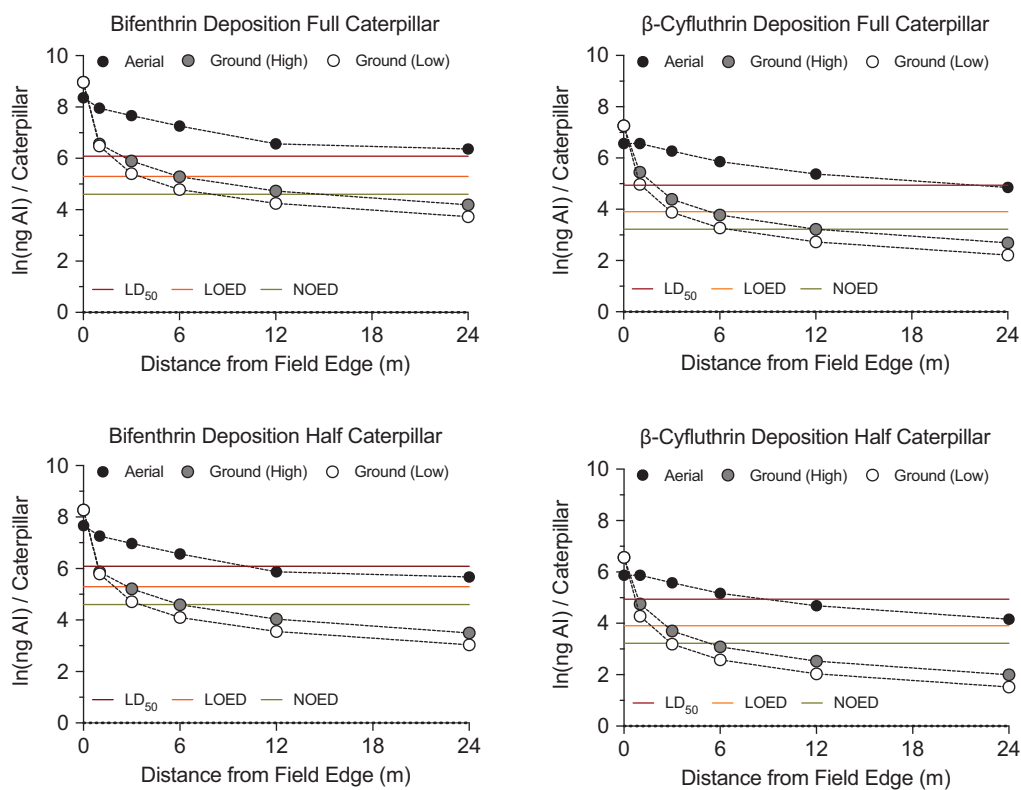
The most sensitive endpoint for bifenthrin was caterpillar survival and, thus, the NOED (0.10  $\mu\text{g}/\text{caterpillar}$ ) and LOED (0.20  $\mu\text{g}/\text{caterpillar}$ ) were estimated based on survival 72 h after insecticide treatment. However, the most sensitive endpoint for  $\beta$ -cyfluthrin was caterpillar weight and, thus, the NOED (0.025  $\mu\text{g}/\text{caterpillar}$ ) and LOED (0.05  $\mu\text{g}/\text{caterpillar}$ ) were estimated based on weight following 72 h of insecticide treatment. The aerial assessment predicts the deposition of bifenthrin on milkweeds at distances up to 60 m from the treated edge of a field to be lethal to caterpillars, but the insecticide would not be lethal at distances >105 m from the treated edge of a field. For  $\beta$ -cyfluthrin, the aerial assessment predicts deposition on milkweeds at distances up to 55 m from the treated edge of a field to affect caterpillar growth, but the insecticide would not affect growth at distances >94 m from the treated edge of a field. The low and high boom ground assessment predicts the deposition of bifenthrin to milkweeds at distances up to 4 and 6 m, respectively, from the treated edge of a field to be lethal to monarch caterpillars. Bifenthrin would not be lethal at distances beyond 8 m for low boom and 15 m for high boom applications. The low and high boom ground assessment predicts the deposition of  $\beta$ -cyfluthrin to milkweeds at distances up to 3 and 6 m, respectively, from the treated edge of a field to reduce caterpillar growth.  $\beta$ -Cyfluthrin deposition would not affect growth if deposition was >7 and 13 m from the

edge of a treated field for high boom and low boom applications, respectively. However, if the only dorsal side of the caterpillar is exposed to the insecticides, there would be a substantial decrease in these predicted distances.

## Discussion

This study not only provides the first report of bifenthrin toxicity to monarch caterpillars, but it also confirmed that pyrethroid insecticides affect the growth and development of caterpillars as reported by Oberhauser et al. (2006) and Krishnan et al. (2020). Bifenthrin was found to be less toxic than  $\beta$ -cyfluthrin to fifth-instar caterpillars as documented in other insect species (Clements and May 1977, Gammon et al. 1981). There were observations of caterpillar mortality 12 h after bifenthrin treatment, whereas caterpillar mortality was observed within 6 h of  $\beta$ -cyfluthrin treatment. Type II pyrethroids, such as  $\beta$ -cyfluthrin, can cause prolonged interference with the gating kinetics of the voltage-gated  $\text{Na}^+$ -channel leading to a greater influx of  $\text{Na}^+$  and more prolonged convulsions. Furthermore, alternative neuronal target sites have been identified with type II pyrethroids, which leads to the CS-syndrome observed with  $\beta$ -cyfluthrin and may explain the increased toxicity observed with the caterpillars (Soderlund et al. 2002, Davies et al. 2007).

Bifenthrin and  $\beta$ -cyfluthrin were observed to significantly affect monarch caterpillar growth and development throughout the 72-h exposure period. A reduction in body size and diet consumption can affect pupation success (Rhains et al. 1999), adult lifespan (McKay et al. 2016), and immune function (Adamo et al. 2016). Since reduced body size and diet consumption were observed at the



**Fig. 3.** Spray-drift exposure estimates of bifenthrin and  $\beta$ -cyfluthrin for fifth-instar monarch caterpillars using the AgDRIFT model. Deposition ( $\mu\text{g}/\text{cm}^2$ ) was multiplied by either the full caterpillar surface area (7.10  $\text{cm}^2$ ) or one-half caterpillar surface area (3.55  $\text{cm}^2$ ). Exposure values were log-transformed to account for orders of magnitude differences in deposition estimates. Effect thresholds,  $\text{LD}_{50}$  (red line), LOED (orange line) and NOED (green line), are overlaid for each insecticide.



final larval instar stage, it is likely the surviving individuals could have challenges with pupation success and, in turn, lead to higher mortality. While our study did not focus on pyrethroid effects to caterpillars infected with the protozoan *Ophryocystis elektroscirrha* (OE) (Neogregarinorida: Ophryocystidae), a challenged immune system in response to infection may affect the susceptibility of caterpillars to insecticide exposures. It should be noted that our adult monarchs are routinely checked for the OE, which has never been observed in the colony, and that field-collected adults are not introduced to our colony. However, further studies would be important for determining if the reduced weight observed from pyrethroid exposure not only affects pupation, adult emergence, and fitness but also if OE infection can increase susceptibility to pyrethroid insecticides.

The performance of a monarch butterfly colony can fluctuate throughout the year, and growth rates can be influenced by changes in humidity and ambient temperatures (Kingsolver 2007). Growth rates in solvent-treated caterpillars differed between the bifenthrin and  $\beta$ -cyfluthrin experiments. For the bifenthrin experiments, the solvent-treated caterpillars were 1.3-fold higher than their original starting weight at the end of the experiment. However, the solvent-treated caterpillars exposed to  $\beta$ -cyfluthrin were 2.1-fold higher than their original starting weight at the end of the experiment. The bifenthrin experiments were conducted prior to the  $\beta$ -cyfluthrin experiments and, thus, the natural variability in the caterpillar growth rate may explain the differences observed with each experiment. Despite this variability, there were statistically significant differences observed between the solvent and bifenthrin treatments for the three cohorts of caterpillars used in this study.

In this study, the 72 h LD<sub>50</sub> for  $\beta$ -cyfluthrin (0.15  $\mu$ g/caterpillar or 0.21  $\mu$ g/g) was found to be significantly higher than the 96 h LD<sub>50</sub> (0.048  $\mu$ g/g caterpillar) reported by Krishnan et al. (2020). However, there cannot be a direct comparison between the two studies due to differences between the experimental approach. Our study was designed to exclude postpupation observations due to the high pupation mortality observed within the monarch colony. There is ca. 20% pupation mortality observed with the monarch colony, which is often attributed to caterpillars in the J-state falling mid-pupation from the top of the test chamber (Greiner et al. 2019). Thus, in our study, the mortality of caterpillars that would have failed to initiate pupation (i.e., lagers) or would die during pupation is not captured in our 72-h mortality observations and, instead, these individuals are recorded as alive. In contrast, Krishnan et al. (2020) recorded mortality for fifth-instar monarch caterpillars after pupation, which includes this additional source of mortality. Similar to the study of Krishnan et al. (2020), the caterpillars treated with the highest three concentrations of  $\beta$ -cyfluthrin were observed to bleed (i.e., losing hemolymph) less than 1 h after treatment, which contributes to the weight loss recorded at 24 h. Caterpillars exposed to the LOED of  $\beta$ -cyfluthrin did recover from this loss of hemolymph and were observed to gain weight. Hemolymph is critical for molting, immunity, thermal regulation, maintaining turgor pressure, and a number of other physiological processes (Klowden 2008, Kanost 2009). A loss of hemolymph, and possibly turgor pressure, could significantly impact the molting and pupation success of the caterpillars. While it is unclear how hemolymph loss might affect pupation, McKay et al. (2016) reported monarch caterpillar hemolymph loss to reduce pupal mass and increase infection of OE. Additionally, a delay in development could increase the risk of predation or parasitism of monarch caterpillars in the field (Geest et al. 2019).

The AgDRIFT Tier 1 aerial spray drift assessment predicts the aerial application of bifenthrin and  $\beta$ -cyfluthrin to be a potential

risk for caterpillar development on the leaf surface of milkweeds that border pyrethroid-treated crops. This prediction is based on a worst-case scenario for the whole-body surface area of the caterpillar to be exposed to bifenthrin or  $\beta$ -cyfluthrin either by direct deposition or with the caterpillar walking across the pyrethroid-treated surface of a milkweed leaf. If a less conservative exposure scenario is considered for the deposition of the insecticides on the dorsal half of the caterpillar following a low ground boom application, the risk of lethal exposure is predicted to be within 2 m for a bifenthrin- or  $\beta$ -cyfluthrin treated crop. If the risk of exposure is based on the  $\beta$ -cyfluthrin LOED of 0.05  $\mu$ g/ $\mu$ l, then the deposition affecting caterpillar growth after a ground application is predicted to be 3 m for low boom and 6 m for high boom from the edge of the insecticide-treated field. The AgDRIFT Tier 1 aerial and ground deposition assessments are conservative assessments and other studies have found deposition estimates from this model to be 20–40 times higher than what is detected in spray drift residue trials (Brain et al. 2019). While the buffer distances calculated in this study would not be applicable for every field scenario, these distances provide a worst-case estimate for the risk of pyrethroid exposure and provide an opportunity to test laboratory toxicity data in an agricultural landscape. Krishnan et al. (2020) documented larger buffer distances and greater risk down-wind to fifth-instar monarch caterpillars near a  $\beta$ -cyfluthrin-treated field. However, the different estimates are due to the lower toxicity values determined in the earlier study (Krishnan et al. 2020). Aside from these two models, there is minimal pyrethroid residue data and minimal toxicity data for monarch butterflies, which provides a challenge for determining the actual risk of exposure to caterpillars. Additionally, application timing, frequency and resistance management programs further complicate exposure predictions for caterpillars and determining temporal and spatial overlap near agriculture. A recent study reports the residue levels of deltamethrin on milkweeds that border agricultural crops (Olaya-Arenas and Kaplan 2019), but there are no data collected for other pyrethroids, including bifenthrin and  $\beta$ -cyfluthrin. Additional studies are needed to examine the persistence and stability of these pyrethroids to determine the duration of exposure to caterpillars following the application of these insecticides. Previously, (Oberhauser et al. 2006) found the pyrethroid permethrin, used for mosquito control, to persist on milkweed leaves for 21 d following application. Terrestrial field dissipation studies have reported the half-life of bifenthrin and  $\beta$ -cyfluthrin to be 78–325 and 4–24 d, respectively (US EPA 2016). The dissipation half-life for  $\beta$ -cyfluthrin is less than that for bifenthrin and, according to the Baythroid XL label, there can be multiple applications of the insecticide to pest-infested soybean fields at 7-d intervals. Additionally, the deposition assessment with AgDRIFT and the field deposition reported in the ‘EPA Environmental Fate and Ecological Effect Assessment’ (US EPA 2016) raises concerns for the risk of bifenthrin and  $\beta$ -cyfluthrin to monarch caterpillars on milkweeds that border agricultural crops. Future work should focus on testing these drift assessments and the application of drift reduction technologies to reduce pyrethroid exposures to caterpillars.

Here, we report the significant effects that the pyrethroids bifenthrin and  $\beta$ -cyfluthrin, at field-relevant concentrations, have on the growth and survival of fifth-instar monarch caterpillars. These data are important for the ecological risk characterization of foliar-applied insecticides in agriculture-dominated landscapes. Our findings provide evidence that pyrethroids are a potential risk to caterpillars in these landscapes. However, this risk can be mitigated if prevailing wind direction is considered when establishing milkweed near conventional agricultural fields and, when possible, pyrethroids should be applied using

low boom ground applications. The conservation efforts to restore monarch butterfly populations require ca. 1.8 billion new milkweed stems on the landscape, a goal that can only be reached with the cooperation of agricultural land managers (Thogmartin et al. 2017).

## Acknowledgments

We thank Terence Spencer and Matthew Greiner for assistance maintaining the UNL monarch colony. We also thank Niranjana Krishnan and Steve Bradbury for technical guidance and sharing of toxicity data. The authors appreciate the assistance provided by Richard Hellmich and Keith Bidne from USDA-ARS, Corn Insects and Crop Genetics Unit, as well as Chip Taylor at Monarch Watch in establishing the UNL monarch colony and optimizing the artificial diet. This research was funded by a United States Department of Agriculture Hatch Grant NEB-28-116 awarded to A.M.V, T.J.K, and T.D.A.

## Author Contributions

A.J.K., T.J.W., A.M.V., and T.D.A. contributed to the conceptualization of this manuscript. A.J.K. and T.D.A. participated in the investigation and A.J.K., K.H., T.J.W., A.M.V., and T.D.A. contributed to the formal analysis of this manuscript. A.J.K. and T.D.A. contributed to the original draft preparation. A.J.K., K.H., T.J.W., A.M.V., and T.D.A. contributed in review and editing. All authors have read and agreed to the published version of the manuscript.

## References Cited

- Adamo, S. A., G. Davies, R. Easy, I. Kovalko, and K. F. Turnbull. 2016. Reconfiguration of the immune system network during food limitation in the caterpillar *Manduca sexta*. *J. Exp. Biol.* 219: 706–718.
- Alonso-Mejia, A., E. Rendon-Salinas, E. Montesinos-Patino, and L. P. Brower. 1997. Use of lipid reserves by monarch butterflies overwintering in Mexico: implications for conservation. *Ecol. Appl.* 7: 934–947.
- Braak, N., R. Neve, A. K. Jones, M. Gibbs, and C. J. Breuker. 2018. The effects of insecticides on butterflies: a review. *Environ. Pollut.* 242: 507–518.
- Bradbury, S., T. Grant, and N. Krishnan. 2017. Iowa monarch conservation, pest management and crop production. *Proc. Integr. Crop Manag. Conf.*
- Brain, R., G. Goodwin, F. Abi-Akar, B. Lee, C. Rodgers, B. Flatt, A. Lynn, G. Kruger, and D. Perkins. 2019. Winds of change, developing a non-target plant bioassay employing field-based pesticide drift exposure: a case study with atrazine. *Sci. Total Environ.* 678: 239–252.
- Çilgi, T., and P. C. Jepson. 1995. The risks posed by deltamethrin drift to hedgerow butterflies. *Environ. Pollut.* 87: 1–9.
- Clements, A. N., and T. E. May. 1977. The actions of pyrethroids upon the peripheral nervous system and associated organs in the locust. *Pestic. Sci.* 8: 661–680.
- Davies, T. G., L. M. Field, P. N. Usherwood, and M. S. Williamson. 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life.* 59: 151–162.
- Diffendorfer, J. E., J. B. Loomis, L. Ries, K. Oberhauser, L. Lopez-Hoffman, D. Semmens, B. Semmens, B. Butterfield, K. Bagstad, J. Goldstein, et al. 2014. National valuation of monarch butterflies indicates an untapped potential for incentive-based conservation. *Conserv. Lett.* 7: 253–262.
- Dunbar, M. W., M. E. O'Neal, and A. J. Gassmann. 2016. Increased risk of insect injury to corn following rye cover crop. *J. Econ. Entomol.* 109: 1691–1697.
- Gammon, D. W., M. A. Brown, and J. E. Casida. 1981. Two classes of pyrethroid action in the cockroach. *Pestic. Biochem. Physiol.* 15: 181–191.
- Geest, E. A., L. L. Wolfenbarger, and J. P. McCarty. 2019. Recruitment, survival, and parasitism of monarch butterflies (*Danaus plexippus*) in milkweed gardens and conservation areas. *J. Insect Conserv.* 23: 211–224.
- Greiner, M., A. Krueger, T. A. Spencer, T. D. Anderson, T. Weissling, and A. M. Velez. 2019. Evaluation of artificial diet on monarchs (*Danaus plexippus* L.) population growth parameters for pesticide bioassays. National of the Entomological Society of America, 17–20 November, St. Louis, MO. Entomological Society of America, Lanham, MD.
- Kanost, M. R. 2009. Hemolymph, pp. 446–449. In V. H. Resh and R. T. Cardé (eds.), *Encyclopedia of insects*, 2nd edn. Academic Press, San Diego, CA.
- Kingsolver, J. G. 2007. Variation in growth and instar number in field and laboratory *Manduca sexta*. *Proc. Biol. Sci.* 274: 977–981.
- Klowden, M. J. 2008. Circulatory systems, pp. 357–401. In M. J. Klowden (ed.), *Physiological systems in insects*, 2nd edn. Academic Press, San Diego, CA.
- Krischik, V., M. Rogers, G. Gupta, and A. Varshney. 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.
- Krishnan, N., Y. Zhang, K. G. Bidne, R. L. Hellmich, J. R. Coats, and S. P. Bradbury. 2020. Assessing field-scale risks of foliar insecticide applications to monarch butterfly (*Danaus plexippus*) Larvae. *Environ. Toxicol. Chem.* 39: 923–941.
- Longley, M., T. Çilgi, P. C. Jepson, and N. W. Sotherton. 1997. Measurements of pesticide spray drift deposition into field boundaries and hedgerows: 1. Summer applications. *Environ. Toxicol. Chem.* 16: 165–172.
- McKay, A. F., V. O. Ezenwa, and S. Altizer. 2016. Consequences of food restriction for immune defense, parasite infection, and fitness in monarch butterflies. *Physiol. Biochem. Zool.* 89: 389–401.
- Nail, K. R., C. Stenoien, and K. S. Oberhauser. 2015. Immature monarch survival: effects of site characteristics, density, and time. *Ann. Entomol. Soc. Am.* 108: 680–690.
- Oberhauser, K., and M. Guiney. 2009. Insects as flagship conservation species. *Terr. Arthropod Rev.* 1: 111–123.
- Oberhauser, K. S., and M. J. Solensky. 2004. *The monarch butterfly: biology & conservation*. Cornell University Press, Ithaca, NY.
- Oberhauser, K. S., S. J. Brinda, S. Weaver, R. D. Moon, S. A. Manweiler, and N. Read. 2006. Growth and survival of monarch butterflies (Lepidoptera: Danaidae) after exposure to permethrin barrier treatments. *Environ. Entomol.* 35: 1626–1634.
- Olaya-Arenas, P., and I. Kaplan. 2019. Quantifying pesticide exposure risk for monarch caterpillars on milkweeds bordering agricultural land. *Front. Ecol. Evol.* 7: 223. doi:10.3389/fevo.2019.00223
- Pecenka, J. R., and J. G. Lundgren. 2015. Non-target effects of clothianidin on monarch butterflies. *Naturwissenschaften.* 102: 19.
- Pleasants, J. 2017. Milkweed restoration in the Midwest for monarch butterfly recovery: estimates of milkweeds lost, milkweeds remaining and milkweeds that must be added to increase the monarch population. *Insect Conserv. Divers.* 10: 42–53.
- Pleasants, J. M., and K. S. Oberhauser. 2013. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. *Insect Conserv. Divers.* 6: 135–144.
- Ragsdale, D. W., D. A. Landis, J. Brodeur, G. E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. *Annu. Rev. Entomol.* 56: 375–399.
- Rhainds, M., G. Gries, and M. M. Min. 1999. Size- and density-dependent reproductive success of bagworms, *Metisa plana*. *Entomol. Exp. Appl.* 91: 375–383.
- Rundlöf, M., J. Bengtsson, and H. G. Smith. 2008. Local and landscape effects of organic farming on butterfly species richness and abundance. *J. Appl. Ecol.* 45: 813–820.
- Scheffé, H. 1953. A method for judging all contrasts in the analysis of variance. *Biometrika.* 40: 87–110.
- Sears, M. K., R. L. Hellmich, D. E. Stanley-Horn, K. S. Oberhauser, J. M. Pleasants, H. R. Mattila, B. D. Siegfried, and G. P. Dively. 2001. Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proc. Natl. Acad. Sci. U. S. A.* 98: 11937–11942.
- Soderlund, D. M., J. M. Clark, L. P. Sheets, L. S. Mullin, V. J. Piccirillo, D. Sargent, J. T. Stevens, and M. L. Weiner. 2002. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology.* 171: 3–59.

- Teske, M. E., S. L. Bird, D. M. Esterly, T. B. Curbishley, S. L. Ray, and S. G. Perry. 2002. AgDRIFT: a model for estimating near-field spray drift from aerial applications. *Environ. Toxicol. Chem.* 21: 659–671.
- Thogmartin, W. E., L. López-Hoffman, J. Rohweder, J. Diffendorfer, R. Drum, D. Semmens, S. Black, I. Caldwell, D. Cotter, P. Drobney, et al. 2017. Restoring monarch butterfly habitat in the Midwestern US: 'all hands on deck'. *Environ. Res. Lett.* 12: 074005.
- (USEPA) U.S. Environmental Protection Agency. 2016. Preliminary comparative environmental fate and ecological risk assessment for the registration review of eight synthetic pyrethroids and the pyrethrins (No. EPA-HQ-OPP-2010-0384-0045). USEPA, Washington, DC.
- Zalucki, M. P. 1982. Temperature and rate of development in *Danaus plexippus* L. and *D. Chrysippus* L. (Lepidoptera: Nymphalidae). *Aust. J. Entomol.* 21: 241–246.