

2021

Oviposition Response of Monarch Butterfly (Lepidoptera: Nymphalidae) to Imidacloprid-Treated Milkweed

Alexander N. Mullins
Iowa State University

Steven P. Bradbury
Iowa State University, sbrad@iastate.edu

Thomas W. Sappington
U.S. Department of Agriculture, tsapping@iastate.edu

James S. Adelman
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/nrem_pubs



Part of the [Agriculture Commons](#), [Behavior and Ethology Commons](#), [Entomology Commons](#), and the [Natural Resources Management and Policy Commons](#)

The complete bibliographic information for this item can be found at https://lib.dr.iastate.edu/nrem_pubs/373. For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

This Article is brought to you for free and open access by the Natural Resource Ecology and Management at Iowa State University Digital Repository. It has been accepted for inclusion in Natural Resource Ecology and Management Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Oviposition Response of Monarch Butterfly (Lepidoptera: Nymphalidae) to Imidacloprid-Treated Milkweed

Abstract

Monarch butterfly (*Danaus plexippus*) populations have declined over the last two decades, attributable in part to declines in its larval host plant, milkweed (*Asclepias* spp.), across its breeding range. Conservation efforts in the United States call for restoration of 1.3 billion milkweed stems into the Midwestern landscape. Reaching this goal will require habitat establishment in marginal croplands, where there is a high potential for exposure to agrochemicals. Corn and soybean crops may be treated with neonicotinoid insecticides systemically or through foliar applications to provide protection against insect pests. Here, we investigate whether ovipositing monarchs discriminate against milkweed plants exposed to the neonicotinoid insecticide imidacloprid, either systemically or through foliar application. In our first experiment, we placed gravid females in enclosures containing a choice of two cut stems for oviposition: one in 15 ml of a 0.5 mg/ml aqueous solution of imidacloprid and one in 15 ml water. In a second experiment, females were given a choice of milkweed plants whose leaves were treated with 30 μ l of a 0.825 mg/ml imidacloprid-surfactant solution or plants treated with surfactant alone. To evaluate oviposition preference, we counted and removed eggs from all plants daily for 3 d. We also collected video data on a subset of butterflies to evaluate landing behavior. Results indicate that neither systemic nor foliar treatment with imidacloprid influenced oviposition behavior in female monarchs. The implications of these findings for monarch conservation practices will be informed by the results of ongoing egg and larval toxicity studies.

Keywords

monarch butterfly, conservation, egg laying, neonicotinoid insecticide, milkweed

Disciplines

Agriculture | Behavior and Ethology | Ecology and Evolutionary Biology | Entomology | Natural Resources Management and Policy

Comments

This article is published as Mullins, Alexander N., Steven P. Bradbury, Thomas W. Sappington, and James S. Adelman. "Oviposition Response of Monarch Butterfly (Lepidoptera: Nymphalidae) to Imidacloprid-Treated Milkweed." *Environmental Entomology* (2021). doi:10.1093/ee/nvab024.

Behavioral Ecology

Oviposition Response of Monarch Butterfly (Lepidoptera: Nymphalidae) to Imidacloprid-Treated Milkweed

Alexander N. Mullins,^{1,2,6,✉} Steven P. Bradbury,^{1,2,3} Thomas W. Sappington,⁴ and James S. Adelman^{1,2,5}

¹Department of Natural Resource Ecology and Management, Iowa State University, 339 Science Hall II, 2310 Pammel Drive, Ames, IA 50011, USA, ²Ecology and Evolutionary Biology Interdepartmental Program, Iowa State University, 1009 Agronomy, 716 Farmhouse Lane, Ames, IA 50011, USA, ³Department of Entomology, Iowa State University, 339 Science Hall II, 2310 Pammel Drive, Ames, IA 50011, USA, ⁴Corn Insects and Crop Genetics Research Unit, USDA Agricultural Research Service, 503 Science Hall II, 2310 Pammel Drive, Ames, IA 50011, USA, ⁵Department of Biological Sciences, The University of Memphis, Life Sciences 239 Ellington Hall, 3700 Walker Avenue, Memphis, TN 38152, USA, and ⁶Corresponding author, tel: +1 719-688-6519, e-mail: mullinsa@iastate.edu

Subject Editor: Matthew Ginzel

Received 11 December 2020; Editorial decision 12 February 2021

Abstract

Monarch butterfly (*Danaus plexippus*) populations have declined over the last two decades, attributable in part to declines in its larval host plant, milkweed (*Asclepias* spp.), across its breeding range. Conservation efforts in the United States call for restoration of 1.3 billion milkweed stems into the Midwestern landscape. Reaching this goal will require habitat establishment in marginal croplands, where there is a high potential for exposure to agrochemicals. Corn and soybean crops may be treated with neonicotinoid insecticides systemically or through foliar applications to provide protection against insect pests. Here, we investigate whether ovipositing monarchs discriminate against milkweed plants exposed to the neonicotinoid insecticide imidacloprid, either systemically or through foliar application. In our first experiment, we placed gravid females in enclosures containing a choice of two cut stems for oviposition: one in 15 ml of a 0.5 mg/ml aqueous solution of imidacloprid and one in 15 ml water. In a second experiment, females were given a choice of milkweed plants whose leaves were treated with 30 μ l of a 0.825 mg/ml imidacloprid-surfactant solution or plants treated with surfactant alone. To evaluate oviposition preference, we counted and removed eggs from all plants daily for 3 d. We also collected video data on a subset of butterflies to evaluate landing behavior. Results indicate that neither systemic nor foliar treatment with imidacloprid influenced oviposition behavior in female monarchs. The implications of these findings for monarch conservation practices will be informed by the results of ongoing egg and larval toxicity studies.

Key words: monarch butterfly, conservation, egg laying, neonicotinoid insecticide, milkweed

Over the last 25 yr, the monarch butterfly (*Danaus plexippus*) population in eastern North America has declined (Brower et al. 2012, Oberhauser et al. 2017) and is currently being considered for listing under the Endangered Species Act (ESA) (U.S. Fish and Wildlife Service [U.S. FWS] 2016). Various factors contribute to this decline (Flockhart et al. 2015, Oberhauser et al. 2017, Wilcox et al. 2019), but the loss of milkweed (*Asclepias* spp.), the monarch's obligate larval host plant, in agricultural landscapes across its Midwestern breeding range is suspected as a leading cause (Pleasants and Oberhauser 2013, Flockhart et al. 2015, Pleasants 2017, Pleasants

et al. 2017). Conservation efforts to reestablish milkweed across the Midwest call for the strategic use of marginal croplands and available crop-adjacent lands to reach habitat targets (Thogmartin et al. 2017). However, due to pesticide exposure, these areas may serve as ecological traps that threaten recovery of monarch populations (U.S. FWS 2017). Effects of several insecticide classes used in corn (*Zea mays*) and soybean (*Glycine max*) production on monarch survival, growth, and development are available (Krishnan et al. 2020, 2021), but the degree to which monarch butterflies can perceive and avoid such chemicals remains unclear.

The Midwestern United States serves as a primary summer breeding ground for monarch butterflies (Wassenaar and Hobson 1998, Flockhart et al. 2017), and neonicotinoids are a class of insecticide widely used throughout this region to control corn and soybean crop pests. Consequently, monarch breeding cycles in this area overlap with planting of neonicotinoid-treated seeds early in the growing season and late in the summer following foliar applications (Krishnan et al. 2020). Neonicotinoids are acetylcholine agonists that overstimulate acetylcholine-mediated neurotransmission in the insect central nervous system (CNS), resulting in mortality and sublethal effects on growth and development (Tomizawa and Casida 2005). Neonicotinoids are applied as foliar or, more commonly, seed treatment formulations (Jeschke et al. 2011, Douglas and Tooker 2015, Tooker et al. 2017). Aerial applications of neonicotinoids may drift offsite, contaminating nearby vegetation and potentially impacting pollinators and other beneficial insects (Koppert Biological Systems 2005, Braak et al. 2018). Although seed treatment reduces the potential for direct (e.g., cuticular) exposure of nontarget insects, neonicotinoids are highly soluble in water and readily move off-field via surface runoff and groundwater leaching (PPDB 2012; Hladik et al. 2014, 2017; Bonmatin et al. 2015). Indeed, neonicotinoid residues originating from both systemic and foliar applications have been detected in nontarget plants (Greatti et al. 2006, Krupke et al. 2012, Botias et al. 2016, Mogren and Lundgren 2016), including milkweed surrounding crop fields (Olaya-Arenas and Kaplan 2019, Hall et al. 2020, Halsch et al. 2020). Lethal and sublethal effects of topical and dietary neonicotinoid exposure to monarch larvae have been reported (Krischik et al. 2015, Pecenka and Lundgren 2015, Krishnan et al. 2020, Olaya-Arenas et al. 2020a); however, effects on adult monarch behavior, particularly female oviposition behavior, remain largely unknown (Halsch et al. 2020, Olaya-Arenas et al. 2020b). A better understanding of the extent to which oviposition behavior is modified by exposure to neonicotinoid-exposed milkweed will help in evaluating the conservation risks and benefits of establishing habitat near corn and soybean fields.

As a narrowly oligophagous species, monarchs have evolved specialized sensory perception of compounds found in host plant species (Cohen and Brower 1982, Malcolm and Brower 1986, Oyeyele and Zalucki 1990, Haribal and Renwick 1996, 1998). Female monarchs likely detect, locate, and assess milkweed plants using a variety of sensory mechanisms, several of which may be influenced by the presence of neonicotinoids. Receptors located on the butterfly's antennae, tarsi, proboscis, and ovipositor detect plant compounds that provide information of the host plant's quality (Roessingh et al. 1991, Renwick and Chew 1994, Briscoe et al. 2013). Likely indicators of plant quality are compounds reflecting morphological and physiological perturbations due to herbivore damage, environmental stress, and pathogen exposure and infection (Holopainen 2004, Raguso 2004, Peñuelas and Munné-Bosch 2005). Compounds indicative of plant health are important cues for gravid adult insects producing phytophagous larvae, given that larval survival is often affected by natal host plant chemistry (Zucker 1982, Haribal and Feeny 2003). Accordingly, a link between female host selection site and larval performance is suspected (Jaenike 1978, Gripenberg et al. 2010). Thus, active plant compounds can act as attractants or repellants during the searching phase of oviposition and may subsequently induce or inhibit egg laying (Bruce et al. 2005). Consequently, although establishing habitat near crop fields presents an opportunity to regain essential milkweed acres, there is a possibility that compounds found in insecticide formulations may interfere with monarch host plant selection behavior and subsequent oviposition. Thus, if neonicotinoids interfere with host plant detection or selection,

this scenario could ultimately result in negligible or reduced reproductive output of monarchs in agricultural habitats. Alternatively, if egg-laying females fail to discriminate neonicotinoid-contaminated plants, increased larval exposure to this insecticide could impact survival, growth, and development (Krishnan et al. 2020). Therefore, the behavioral response of egg-laying monarchs to the presence of neonicotinoids in or on milkweed may have a dramatic effect on the use and benefits of conservation habitat established near crop fields.

Insect avoidance behavior can be genetic or acquired by learning (Després et al. 2007), and plant aversion is often species and context specific. For example, neonicotinoids repel pollinating flies and beetles at field-realistic rates (Easton and Goulson 2013). In contrast, bees appear to show an affinity to feed on neonicotinoid-contaminated forage (Kessler et al. 2015, Thompson et al. 2015, Arce et al. 2018; but see Muth and Leonard 2019, Muth et al. 2020). An oviposition choice study on monarchs suggests that they are not likely to avoid laying eggs on milkweed plants treated with the pyrethroid insecticide permethrin (Oberhauser et al. 2006). In addition, Olaya-Arenas et al. (2020b) investigated the behavioral response of nine adult monarchs to agriculturally employed pesticides. They found that a mixture of six different pesticides including herbicides, fungicides, and the neonicotinoid clothianidin at their mean field concentrations caused no change in egg-laying behavior; however, at maximum field concentrations, fewer eggs were laid on treated common milkweed hosts. While these findings suggest monarchs detected the presence of the pesticide mixture and subsequently discriminated against treated plants for oviposition, the specific compound(s) that elicited the deterrence response were not identified.

To evaluate if egg-laying monarchs discriminate against neonicotinoid-treated milkweed plants, we conducted behavioral choice experiments to assess oviposition under conditions that mimicked systemic uptake and foliar exposure to a model neonicotinoid, imidacloprid. We tested the hypothesis that there would be a difference in oviposition response toward neonicotinoid-treated milkweed. However, given that encounters of monarchs with neonicotinoids are evolutionarily recent, and there is no obvious toxicological mechanism by which neonicotinoids are expected to alter oviposition behavior, we suspected that monarchs would not discriminate against milkweed plants treated with imidacloprid. The number of eggs deposited on treated and untreated milkweed plants in cage studies was used to quantify oviposition preference. In addition, aversion behavior was assessed by evaluating the physical contact time on treated versus untreated milkweed for a subset of butterflies.

Materials and Methods

Tropical Milkweed (*Asclepias curassavica*)

Production

Tropical milkweed plants were grown from seed (Johnny's Selected Seeds, Winslow, ME) in an Iowa State University greenhouse (see Krishnan et al. 2020 for full procedures). Milkweed plants selected for oviposition trials were nonflowering and approximately 40–45 cm tall.

Monarch Butterflies

Adult monarchs were supplied from colonies maintained by the U.S. Department of Agriculture (USDA), Agriculture Research Service (ARS), Corn Insects and Crop Genetics Research Unit (CICGRU). All butterflies in this study were free of the protozoan parasite *Ophryocystis elektroscirrha* (see Krishnan et al. 2020 for rearing methods). *In-copula* mated pairs were selected from colony

populations initiated from wild monarchs in 2016 and 2017 and held overnight in an environmental chamber (I-35VL, Percival Scientific, Perry, IA) at 15.5°C, 60% relative humidity (RH), 25% light intensity, and a 16:8-h light:dark cycle for approximately 16 h to ensure completion of mating. Upon disjoining, female butterflies were assigned a unique identification number and used for testing. Males were returned to their colonies.

Oviposition Choice Experiments

Experimental Design

Oviposition preference experiments were conducted indoors in a 8.8-m × 4.5-m room with a 16:8 light:dark cycle. Mean daytime (08:00–18:00) and nighttime (18:15–07:45) conditions were 28.6°C and 56.9% RH, and 24.8°C and 60.8% RH, respectively. Monarchs were housed individually inside translucent enclosures (1.2-m L × 1.2-m W × 1.4-m H Pop-Up Greenhouse; FlowerHouse, Clio, MI) made of Gro-tec rip-stop fabric (FlowerHouse, Clio, MI). Enclosures had mesh entrances (1-mm weave, 25.4 mesh count) and were oriented to ensure neighboring monarchs were not visible to each other through the entrances (see Fig. 1). Enclosures contained a centrally positioned artificial nectar feeder consisting of a 0.24-L paper food container (Solo Cup Company, Lake Forest, IL) holding a circular sponge (Scrub Daddy Inc., Folcroft, PA) saturated with Fruit Punch Gatorade (The Gatorade Company, Inc., Pryor, OK).

We conducted two separate experiments to test oviposition preferences: one using systemic imidacloprid application, and the other using foliar imidacloprid application. In the systemic imidacloprid application experiment, four rounds of 3-d trials were conducted during October of 2018. Each of 12 enclosures contained a single mated female, and one imidacloprid-treated and one untreated tropical milkweed stem. In the foliar imidacloprid application trials, three rounds of 3-d trials were conducted during October of 2019. Ten enclosures, each containing a single mated female, were used to compare oviposition on imidacloprid-treated and surfactant-treated control potted plants. The remaining two enclosures were used to compare untreated control and surfactant-treated control plants. No differences in egg laying were detected between untreated control and surfactant-treated plants and will not be discussed further here.



Fig. 1. Mated monarch females were placed individually in one of 12 translucent enclosures, each containing two tropical milkweed plants and a centrally located feeder. The enclosures were orientated in the chamber, so that neighboring monarchs were not visible to one another through the mesh door.

Oviposition preference, measured by number of eggs oviposited on treatment and control plants, was assessed over three consecutive days. A Go-Pro Hero 6 Black video recorder (GoPro Inc., San Mateo, CA) was mounted inside 6 of the 12 enclosures. Video data were collected from approximately 08:00 to 11:00 on all days of the trial. From 13:00 to 14:00 each day, milkweed plants were removed from all enclosures and eggs were counted and removed to mitigate possible density-dependent oviposition behavior (Drury and Dwyer 2005) and to prevent larval feeding damage, which could also influence egg laying (Agrawal 2017). Video data captured during oviposition experiments were used to quantify alighting behavior and contact time as a proxy for discrimination and preference between treatments. In the wild, monarchs show a preference for laying eggs near the top of milkweed plants (Zalucki and Kitching 1982). To understand if oviposition choice behavior across treatments was influenced by spatial preference, we quantified within-plant spatial preference by visually separating plants into three zones (top, middle, and bottom). Time data are reported as the proportion of total time that individuals spent on plants (not the proportion of total video duration). To prevent cross-contamination, control plants were handled before treated plants, or they were handled by different investigators. After eggs were counted and removed, plants were returned to their respective enclosures and the locations of the control and treated plants were switched to control for any effects of spatial preference. At the end of each trial, monarchs were removed and frozen. Milkweed leaves were wrapped in aluminum foil and stored at –80°C for future imidacloprid residue analysis.

Imidacloprid and Control Treatments

Systemic Application

Imidacloprid (N-[1-[(6-chloropyridin-3-yl)methyl]-4,5-dihydroimidazol-2-yl]nitramide; CAS number: 138261-41-3; purity:100%; Sigma-Aldrich, St. Louis, MO) was dissolved in deionized water by mixing on a magnetic stir plate for 10 min at 20°C to obtain a nominal stock solution concentration of 0.50 µg/µl (solubility limit in water is 0.61 µg/µl at 20°C; Gervais et al. 2010). We chose this high concentration to evaluate whether there was any potential for systemic uptake to alter oviposition behavior, even though field concentrations for systemic exposure are likely to be considerably lower. Fifty-milliliter self-standing centrifuge tubes (Corning Inc., New York, NY) were filled with 15 ml of the imidacloprid stock solution or 15 ml of deionized water (control treatment) and placed inside 177-ml mason jars (Ball Corp., Broomfield, CO) for support. Parafilm M (Bemis Company Inc., Neenah, WI) was stretched over the mouth of the jar and secured with the jar's metal screw band to ensure monarchs were not directly exposed to the imidacloprid solution. A 25-cm milkweed stem was cut and immediately inserted through a small incision in the parafilm into the centrifuge tube containing the treatment or control solutions. Milkweed donor plants were not watered for 2 d prior, to promote uptake of solution by the cut stems. Stems were left 18–24 h in the centrifuge tubes to allow solution uptake before placement in the enclosures. Based on the number of leaves per plant, the weight of leaves at the end of a trial, and the volume of imidacloprid solution taken up by the plant, we estimated that nominal leaf concentrations ranged from 400 to 1,800 µg imidacloprid per gram of leaf.

Foliar Application

The target range of nominal concentrations of foliar imidacloprid exposure was based on results of spray drift modeling using AgDRIFT version 2.1.1 (U.S. Environmental Protection Agency [U.S. EPA]

2003). Imidacloprid drift concentrations were selected by modeling the maximum field-label application rate of 0.112 kg of imidacloprid per hectare to manage armyworm, *Mythimna unipuncta* (Lepidoptera: Noctuidae) in cornfields following a high-boom ground application (medium-course spray nozzle; 16 kph wind speed) of Swagger (R) (Loveland Products Inc., Loveland, Colorado) (active ingredients: imidacloprid and bifenthrin; EPA registration number: 34704-1045). At the field edge, concentrations of 1.1 $\mu\text{g}/\text{cm}^2$ were predicted. The estimated mean leaf surface area was $22.5 \pm 7.3 \text{ cm}^2$, determined using NIH's ImageJ software (Schneider et al. 2012). To achieve predicted field-edge imidacloprid concentration on our experimental plants, imidacloprid was mixed with a surfactant (Silwet L-77, Fisher Scientific, Hampton, NH) to obtain a stock suspension with a nominal concentration of 0.825 $\mu\text{g}/\mu\text{l}$. A stock suspension of 0.1% surfactant-water was prepared for control treatments. Immediately after solution preparation, milkweed leaves were surface-treated with 30 μl of the 0.825 $\mu\text{g}/\mu\text{l}$ imidacloprid-surfactant suspension or 30 μl of the 0.1% surfactant-water suspension using a micropipette. Monarch oviposition trials began 24 h after treatment of leaves. Mean nominal imidacloprid concentration on each leaf was calculated to be 25 $\mu\text{g}/\text{cm}^2$. The fresh weight of a subset of leaves collected at the end of the trials ranged from 0.4 to 0.7 g. Consequently, the nominal imidacloprid leaf concentration on a per-gram of leaf basis was estimated to range from 35 to 62 $\mu\text{g}/\text{g}$. Milkweed leaves were collected at the end of each 3-d trial and analyzed for imidacloprid concentrations to confirm the exposure levels (see *Analytical Chemistry*).

Analytical Chemistry

Nominal concentrations of imidacloprid for systemic and foliar applications of imidacloprid ranged from 400 to 1,800 and 35 to 62 $\mu\text{g}/\text{g}$ leaf, respectively. A subsample of leaves were analyzed to confirm actual concentrations in the systemic- and foliar-treated plants. A total of 8 leaves from four systemically treated plants and 12 leaves from three foliar-treated plants were analyzed. A 0.2-g portion of a leaf was placed into a 2-ml prefilled tube kit containing 1.5-mm-diameter high-impact zirconium beads (catalog# D1032-15; Benchmark, Sayreville, NJ). Acetonitrile (1.5 ml) was added to all samples followed by shaking on a multi-tube shaker for 5 min at 2,500 rpm. The samples were then centrifuged for 5 min at 6,000 rpm. After centrifugation, the supernatant was transferred into a 2-ml dSPE tube (catalog# 03150625; Fisher Scientific) containing 150 mg MgSO_4 , 50 mg PSA, and 50 mg C18 followed by shaking on a multi-tube shaker for 2 min at 2,500 rpm. The sample tubes were centrifuged for 5 min at 6,000 rpm. The supernatant (100 μl) was transferred into an amber autosampler vial with an insert prior to high-performance liquid chromatography (HPLC) analysis. Samples were diluted as needed with 75/25 water/acetonitrile (V/V). Imidacloprid concentrations were quantified by HPLC with UV detection (271 nm). A Thermo Sycronis C18 column (250 \times 4mm, 5 μM) was used with a mobile phase comprised of 75% water and 25% acetonitrile run isocratically with a flow rate of 1 ml/min. HPLC analysis had a 97.5% recovery rate.

Statistical Analyses

Data were analyzed using R version 3.6.1 (R Development Core Team 2019). In our analyses of egg laying, we excluded monarchs that did not survive all 3 d of systemic or foliar oviposition trials ($N = 8$ and 2, respectively), and those that did not lay at least one egg over the course of the trial period, i.e., 'nonlayers' ($N = 14$ systemic and 6 foliar treatments). This left a total of 26 and 28 monarch females in

systemic and foliar experiments, respectively. These sample sizes were, per experiment, roughly three times that used in Olaya-Arenas et al. (2020b), where differential oviposition was detected. We considered the individual butterfly as the unit of replication across all analyses.

Within each experiment, we tested whether eggs laid on untreated versus treated plants differed across trial days using a linear mixed-effects model (lme4 package) (Bates et al. 2015), with the number of eggs laid square root transformed for normality. Fixed effects in this model were day of trial, imidacloprid treatment, and the interaction between the two. We tested the inclusion of different random effects using Akaike information criterion (AICc) adjusted for small sample sizes (Burnham and Anderson 2002). This analysis revealed that models with a random intercept for each individual were more parsimonious than those with either random effects of both intercept and day, or intercept and imidacloprid treatment. P -values for linear mixed effect models were calculated using Satterthwaite's method.

We analyzed the proportion of time spent between untreated and treated plants as well as the proportion of time butterflies spent within the top, middle and bottom of milkweed plants using linear mixed-effects models with fixed effects for treatment, location and the interaction between the two. As above, we included a random intercept for each individual. In addition to excluding the individuals described above (nonlayers and those that did not survive all 3 d), here we also excluded any monarchs that spent no time on either plant during the video. This left a total of 20 and 15 monarchs in systemic and foliar experiments, respectively. Proportion data were arcsine square root transformed. Finally, we tested for any differences in which plant (untreated or treated) was visited first using a chi-squared test.

Results

The mean cumulative egg counts (± 1 SE) for monarchs in the systemic versus foliar experiments were 133.6 ± 19.5 and 224.6 ± 26.4 , respectively (Wilcoxon rank sum test: $W = 203.5$, $P = 0.01$). In both experiments, however, the number of eggs was not significantly different between untreated and treated plants (see below).

Systemic Exposure

Systemic treatment of cut milkweed stems yielded leaf concentrations ranging from 118 to 5,890 $\mu\text{g}/\text{g}$ leaf with a mean concentration of $1,620 \pm 540 \mu\text{g}/\text{g}$ leaf for stems which absorbed 6–15 ml (40–100%) of the provided 15 ml solution. Imidacloprid leaf concentrations were positively associated with the total volume of solution taken up ($F_{1,8} = 8.9$, $P = 0.01$). Sampled control leaves had no detectable imidacloprid residues.

Egg counts show that female monarchs did not discriminate between milkweed stems systemically treated with imidacloprid and untreated control stems for oviposition. The mean number of eggs laid did not differ based on imidacloprid treatment ($F_{1,78} = 0.37$, $P = 0.54$), day of trial ($F_{2,26} = 0.23$, $P = 0.23$), or their interaction ($F_{2,78} = 1.64$; $P = 0.19$) (Fig. 2). Further, there was no difference in the total number of eggs laid on either untreated or treated stems based on the total volume of solution absorbed (volume absorbed: $F_{1,24.5} = 1.96$, $P = 0.17$; treatment: $F_{1,16} = 0.02$, $P = 0.89$; uptake \times treatment: $F_{1,14} = 0.40$, $P = 0.53$), suggesting that the volume of water (imidacloprid solution or control) translocated into leaf tissues did not influence oviposition preference.

Female monarchs spent similar proportions of time on untreated (0.64 ± 0.08) versus treated plants (0.52 ± 0.08) during the recording period, even when accounting for location of landing (top, middle,

bottom) within plants (treatment: $F_{1,126} = 0.32, P = 0.58$; location: $F_{2,126} = 1.26, P = 0.29$; treatment \times location: $F_{2,126} = 2.02, P = 0.14$) (Fig. 3). Additionally, females did not show a preference for which plant, treated or untreated, they visited first ($\chi^2 = 0.27, df = 1, P = 0.60$).

Foliar Exposure

Foliar treatment of milkweed leaves yielded leaf concentrations ranging from 33.7 to 99.5 $\mu\text{g/g}$ leaf, with a mean of $63.34 \pm 6.59 \mu\text{g/g}$. The imidacloprid stock solution had a mean concentration of 0.88 mg/ml.

Egg counts show that female monarchs did not discriminate between plants whose leaves were treated with imidacloprid versus

those treated with surfactant only. The mean number of eggs laid did not differ based on imidacloprid treatment ($F_{1,106} = 0.0002, P = 0.98$), day of trial ($F_{2,111} = 1.75, P = 0.17$), or their interaction ($F_{2,106} = 0.41, P = 0.66$) (Fig. 4). However, the number of eggs was positively associated with individual leaf area across both treatments ($F_{1,51} = 16.9, P < 0.001$).

Female monarchs spent similar proportions of time on untreated (0.45 ± 0.10) versus treated plants (0.62 ± 0.095), even when accounting for location of landing (top, middle, bottom) within plants (treatment: $F_{1,66} = 1.61, P = 0.21$ location: $F_{1,63} = 1.89, P = 0.16$; treatment \times location: $F_{2,62} = 0.29, P = 0.75$) (Fig. 5). Females did not show a preference for which plant, untreated or treated, they visited first ($\chi^2 = 0.18, df = 1, P = 0.67$).

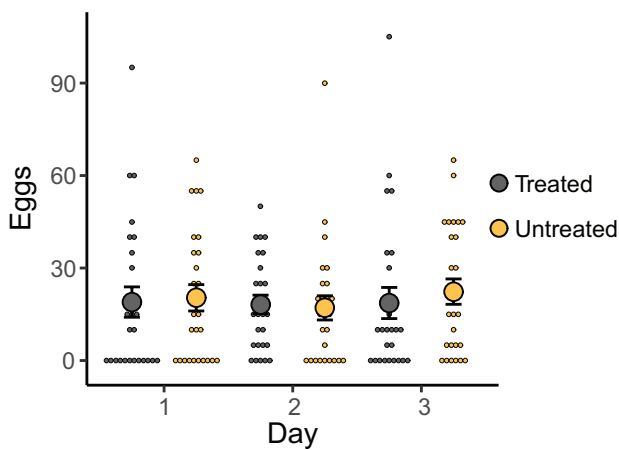


Fig. 2. Daily eggs laid by female monarch butterflies on paired milkweed plants in a laboratory cage either systemically treated or untreated (control) with imidacloprid. Large dots represent daily means with SE bars. Each small dot is an individual butterfly.

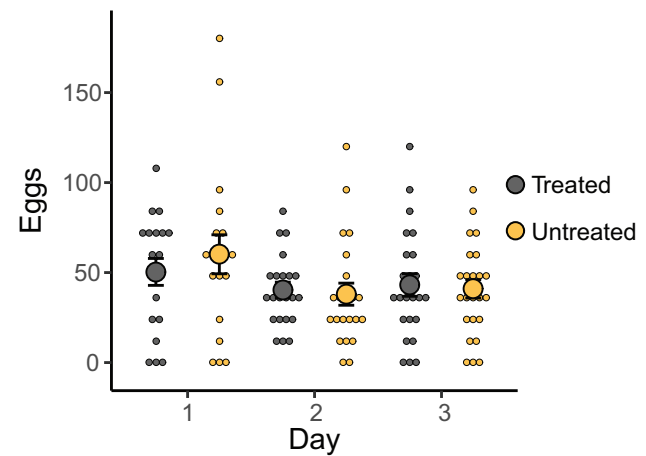


Fig. 4. Daily eggs laid by female monarch butterflies on paired milkweed plants in a laboratory cage either foliar-treated or untreated (control) with imidacloprid. Large dots represent daily means with SE bars. Each small dot is an individual butterfly.

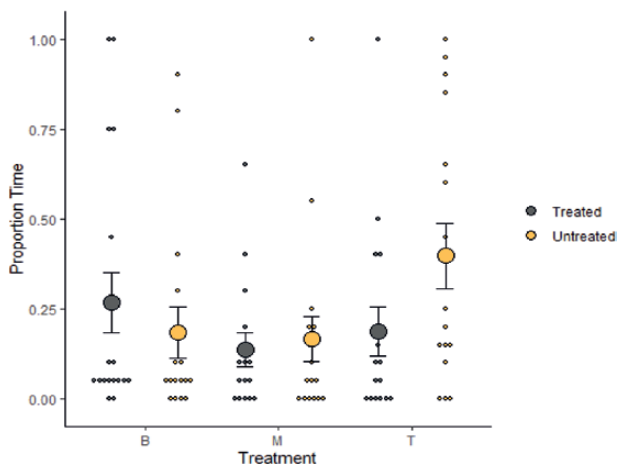


Fig. 3. Proportion time spent by a mated female monarch butterfly on milkweed plants systemically treated or untreated (control) with imidacloprid by location within plant. B = bottom, M = middle, T = top thirds of plants. Large dots represent location means with SE bars. Each small dot is an individual butterfly.

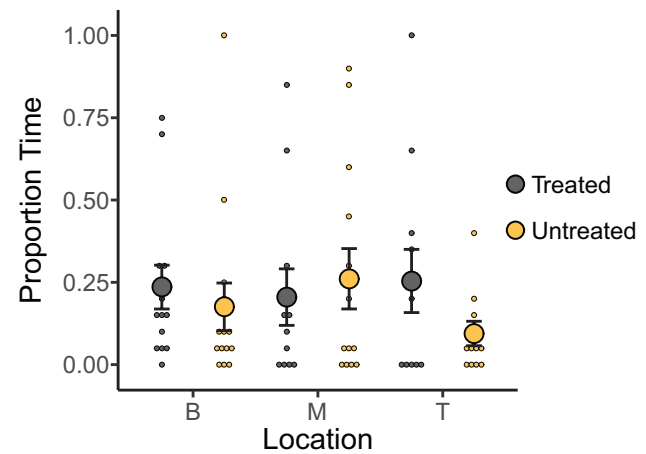


Fig. 5. Proportion time spent by mated female monarch butterfly on milkweed plants foliar-treated or untreated (control) with imidacloprid by location within plant B = bottom, M = middle, T = top thirds of plants. Large dots represent location means with SE bars. Each small dot is an individual butterfly.

Discussion

In this study, we investigated whether egg-laying monarchs avoid milkweed plants treated systemically or foliarly with the neonicotinoid imidacloprid. Our oviposition choice experiments indicate that monarchs do not avoid or discriminate against milkweed for oviposition based on presence of imidacloprid residues. Neither systemic nor foliar application of imidacloprid resulted in a mean difference in contact time or the number of eggs laid on milkweed plants. The mean imidacloprid concentration of 1,618 $\mu\text{g/g}$ in the systemic study was approximately 1,000-fold higher than concentrations reported in monitoring studies (Olaya-Arenas and Kaplan 2019). The mean foliar imidacloprid concentration of 63 $\mu\text{g/g}$ represents the predicted upper level of modeled contamination due to imidacloprid spray drift onto milkweed immediately adjacent to treated crop fields. Imidacloprid residues at this level exceed oral LC50s by 12-fold for second instar (5.1 $\mu\text{g/g}$), at least 3-fold for third instar (17 $\mu\text{g/g}$ leaf) and 6-fold for fifth instar (9.4 $\mu\text{g/g}$ leaf) (Krishnan et al. 2020). Overall, these results suggest that neither the method of application nor the concentration of imidacloprid deters oviposition in monarch butterflies, even at levels exceeding field-realistic rates. While the primary milkweed host species in the Midwest is common milkweed, there is no reason to expect the lack of discrimination between imidacloprid-treated and nontreated tropical milkweed plants is a unique milkweed species-specific response. As noted previously, Olaya-Arenas et al. (2020b) did not observe discriminatory egg-laying behavior when common milkweed leaves were treated with a mixture of six pesticides, including clothianidin, at concentrations consistent with mean residue values reported for common milkweed collected in agricultural settings (Olaya-Arenas and Kaplan 2019).

Approximately 100% of U.S. corn and 50% of soybean acres are planted with neonicotinoid-treated seeds (Tooker et al. 2017). Given their high water solubility, neonicotinoids can move off-field via surface runoff and groundwater leaching (Hall et al. 2020). Several neonicotinoids are also registered in the United States for foliar treatment of corn and soybeans. Neonicotinoid concentrations on milkweed leaves downwind of aerial or ground boom applications are estimated to range from 0.3 $\mu\text{g}/\text{cm}^2$ at the edge of a treated field to 9.8×10^{-4} $\mu\text{g}/\text{cm}^2$ at 60 m downwind (Krishnan et al. 2020). Thus, larval and adult monarchs may be exposed to neonicotinoids in and on milkweed plants located downwind or downslope of treated crop fields. Modeled foliar applications of imidacloprid to control soybean aphid in soybean crops predicted monarch larval exposures expected to cause 80% second-instar mortality at the field edge and 13% mortality 60 m further downwind (Krishnan et al. 2020). Exposure of monarchs to insecticides could be mitigated behaviorally through avoidance of contaminated milkweed by larvae or adults. However, previous exposure studies generally suggest that monarch larvae do not avoid consuming neonicotinoid-treated milkweed (Krischik et al. 2015, Bargar et al. 2020, Krishnan et al. 2020) and thus may be unable to compensate for maternal oviposition on a contaminated plant. There are few studies addressing the behavioral responses of gravid monarch females to neonicotinoid-contaminated milkweed plants. Olaya-Arenas et al. (2020b) reported that a pesticide cocktail containing a neonicotinoid (clothianidin), two herbicides (atrazine and S-metolachlor), and three fungicides (azoxystrobin, pyraclostrobin, and trifloxystrobin) at their mean reported field concentrations did not elicit oviposition avoidance. However, females ($N = 9$) did lay, on average, four fewer eggs when the pesticide components of the mixture were applied at their maximum reported field concentrations. Although statistically significant, the

biological significance of this difference may be negligible, but regardless, the extent to which, if any, clothianidin contributed to the behavioral response is unknown.

Although monarchs in the systemic treatment experiments laid significantly fewer total eggs than in the foliar treatment experiments, there was no significant difference in the number of eggs laid between treated and untreated milkweed. The difference in eggs laid across foliar and systemic exposures is likely not a function of the imidacloprid application method, but rather attributed to a decline in quality of the cut stems over the course of systemic trials that did not occur in planted stems used in the foliar trials and/or annual variation in rates of egg production within the monarch colonies.

Interestingly, we observed eggs laid on all parts of the plant including the stems and both the upper and lower surface of leaves, which is not in accordance with field observations of oviposition mainly on the underside of leaves (Urquhart 2019, Zalucki and Kitching 1982, Zalucki et al. 1990). This observation may suggest unnatural oviposition behavior by colony butterflies or may be a response to the limited number of host plants provided, resulting in egg dumping. Nevertheless, if such dumping occurred, it was not completely indiscriminate because the monarchs still preferred laying eggs on host plants over inanimate surfaces such as the cage screen or feeders, where no eggs were observed. Furthermore, eggs were removed from the plants daily to mitigate density-dependent effects of oviposition. Video data suggest that monarchs did not discriminate among the plants, at least within the first 2–3 h of testing, when plant egg density was low.

Olaya-Arenas et al. (2020b) found monarchs laid fewer eggs on plants treated with a mixture of the maximum concentrations of component pesticides, including clothianidin at approximately 55 ng/g. While this suggests that monarch females have the potential to avoid pesticide-treated host plants, the extent to which clothianidin (a nitro-neonicotinoid) contributed to this behavioral response could not be discerned. Our results suggest that imidacloprid, also a nitro-neonicotinoid, does not elicit an aversion response at concentrations well above those used by Olaya-Arenas et al. (2020b). The lack of discrimination by ovipositing female monarchs against imidacloprid-treated milkweed could be due to 1) an inability to detect imidacloprid residues, or 2) successful detection of imidacloprid residues without alteration of behavioral responses.

Oviposition behavior in butterflies involves CNS processing of chemosensory inputs. Potential detection of imidacloprid residues derived from systemic or foliar applications would involve olfactory and/or tactile chemosensory pathways. While olfactory perception plays an important role in the initial detection of potential host plants, final selection of a host plant likely occurs via the contact chemosensory pathway after alighting on the plant's surface (Renwick 1989, Bernays and Chapman 1994).

Electrophysiological studies show spikes in neural activity when host or nonhost extracts are presented to the chemosensory sensillum of lepidopteran species (Ramawamy 1988, Roessingh et al. 1991), including monarchs (Baur et al. 1998). However, to our knowledge, a study addressing the electrophysiological responses of monarchs to neonicotinoids has not been conducted. In many cases, a specific combination and ratio of compounds is necessary to induce oviposition. For example, in swallowtail butterfly (Papilionidae) species that specialize on citrus plants, individual components of oviposition stimulants elicited weak or no response compared to a strong response when presented in mixtures resembling the natural ratios produced by host plants (Nishida 2005). Flavonoids, which are suspected to serve as the primary oviposition stimulants in monarchs, also elicit a stronger response as blends (Haribal and Renwick 1996,

1998). Thus, if the correct suite and ratio of host plant compounds is present, ancillary compounds, such as neonicotinoid insecticides, may be ignored or may not elicit a signal sufficient to alter oviposition response in monarchs. In summary, the adoption of an aversion response to neonicotinoid-compounds may be limited by the neurophysiological processing of chemical compounds during host plant selection (Cunningham 2012).

Since neonicotinoids are not volatile (imidacloprid: 9×10^{-7} mPa [7×10^{-12} mm Hg] at 25°C; clothianidin: 1.3×10^{-7} mPa [9.8×10^{-10} mm Hg] at 25°C) (National Center for Biotechnology Information 2020a,b), it is unlikely that the olfactory sensory pathway plays a significant role in monarch discrimination between treated and untreated milkweed. The lack of discrimination also suggests that imidacloprid (and potentially clothianidin) do not readily bind to chemoreceptor sites in the female's tarsi, antennae, or ovipositor. Consequently, it seems unlikely that imidacloprid disrupts the peripheral nervous system. It is possible that oviposition behavior could be influenced after monarchs consume neonicotinoid-contaminated nectar or are directly exposed to neonicotinoid spray drift, with subsequent disruption of acetylcholine pathways in the CNS that process sensory inputs. In our study design, however, dietary exposure was not possible and cuticular absorption was very unlikely. It should be noted that disruption of bee behavior following dietary or cuticular exposure to neonicotinoids (Decourtye et al. 2004; Yang et al. 2008, 2012; Han et al. 2010; Henry et al. 2012) is presumably due to CNS effects. Therefore, while our results are relevant for monarchs, the exposure pathways likely differ for other species, particularly after pollen or nectar consumption. Consequently, we caution against extrapolating these results to other insect species and routes of exposure.

In addition to the mechanistic explanation above, limited selection pressure may help explain the lack of discrimination toward neonicotinoid-treated milkweed. Assuming that neonicotinoid compounds can be detected by monarch chemoreceptors, inconsistent exposure across the landscape may not impose strong selection for an aversion response in wild monarch populations. For example, although there is evidence that neonicotinoids may persist across the agricultural landscape for extended periods of time, milkweed near crop fields shows considerable spatio-temporal variability in neonicotinoid prevalence and concentration (Olaya-Arenas et al. 2020a, Hall et al. 2020). Furthermore, the monarch is a highly vagile species and individuals move readily from patch to patch across the landscape (Zalucki et al. 2016). Oviposition is expected to occur over a wide spatial scale (Grant et al. 2018), with females effectively following a diversified bet-hedging strategy, limiting impacts of individual stressors such as neonicotinoid exposure while optimizing fitness over the larger landscape.

While previous studies have found adverse effects of neonicotinoids on larval survival and development, our results help extend the knowledge of potential effects of neonicotinoids to adult oviposition behavior. We found that ovipositing females do not discriminate between imidacloprid-treated and untreated milkweed plants, and thus eggs and larvae will likely be subject to exposure in habitats immediately adjacent to treated crop fields. On the other hand, any monarch habitat established in crop margins will likely be used for oviposition, resulting in greater overall egg numbers in the landscape (Grant et al. 2018). Ultimately, it appears that increasing habitat availability, regardless of its proximity to crop fields, is likely to provide a net benefit to monarchs (Grant et al. 2021). Further research investigating the oviposition response of monarch butterflies toward other commonly employed neonicotinoids such as clothianidin and thiamethoxam—also found in milkweed within agroecosystems (Pecenka

and Lundgren 2015, Olaya-Arenas and Kaplan 2019)—is warranted. Finally, electrophysiological studies investigating the individual and synergistic effects of pesticides, including neonicotinoids, on monarch behavior, would provide useful insights in planning future establishment of milkweed habitat in agricultural landscapes.

Acknowledgments

The authors thank R. Ritland and M. A. Bennett for technical support, K. Bidne for rearing the monarch butterflies and milkweed used in the experiments, and N. Krishnan for assistance with toxicological methods development. We thank D. Schrunck and the ISU College of Veterinary Medicine, Veterinary Diagnostic Laboratory for quantifying insecticide concentrations on leaves. The present study was supported by the Agriculture and Food Research Initiative Pollinator Health Program (grant 2018-67013-27541) from the U.S. Department of Agriculture's (USDA) National Institute of Food and Agriculture, the College of Agriculture and Life Sciences, Iowa State University, the Iowa Monarch Conservation Consortium, and the Graduate Minority Assistantship Program. This research was also supported in part by the USDA, Agricultural Research Service, projects #5030-22000-018-00D and 5030-22000-019-00D. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy. USDA is an equal opportunity provider and employer.

Data Availability

Data and metadata pertaining to this article are publicly available through GitHub: <https://github.com/Mullinsa/Oviposition-response-of-monarch-butterfly-to-imidacloprid-treated-mlkweed>.

References Cited

- Agrawal, A. 2017. Hatching and defending, pp. 90–118. *In* Monarchs and Milkweed. Princeton University Press, Princeton. doi:10.1515/9781400884766-006
- Arce, A. N., A. R. Rodrigues, J. Yu, T. J. Colgan, Y. Wurm, and R. J. Gill. 2018. Foraging bumblebees acquire a preference for neonicotinoid-treated food with prolonged exposure. *Proc. R. Soc. B* 2852018065520180655. doi:10.1098/rspb.2018.0655.
- Bargar, T. A., M. L. Hladik, and J. C. Daniels. 2020. Uptake and toxicity of clothianidin to monarch butterflies from milkweed consumption. *PeerJ* 8: 19–24.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Soft.* 67: 1–48. doi:10.18637/jss.v067.i01
- Baur, R., M. Haribal, J. A. A. Renwick, and E. Städler. 1998. Contact chemoreception related to host selection and oviposition behaviour in the monarch butterfly, *Danaus plexippus*. *Physiol. Entomol.* 23: 7–19.
- Bernays, E. A., and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman & Hall, New York.
- Bonmatin, J. M., C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E. A. Mitchell, et al. 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environ. Sci. Pollut. Res. Int.* 22: 35–67.
- Botías, C., A. David, E. M. Hill, and D. Goulson. 2016. Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects. *Sci. Total Environ.* 566–567: 269–278.
- 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.
- Briscoe, A. D., A. Macias-Muñoz, K. M. Kozak, J. R. Walters, F. Yuan, G. A. Jamie, and C. D. Jiggins. 2013. Female behaviour drives expression and evolution of gustatory receptors in butterflies. *PLoS Genetics* 9: e1003620.
- Brower, L. P., O. R. Taylor, E. H. Williams, D. A. Slayback, R. R. Zubieta, and M. I. Ramirez. 2012. Decline of monarch butterflies overwintering

- in Mexico: is the migratory phenomenon at risk? *Insect Conserv. Divers.* 5: 95–100.
- Bruce, T. J. A., L. J. Wadhams, and C. M. Woodcock. 2005. Insect host location: a volatile situation. *Trends Plant Sci.* 10: 269–274.
- Burnham, K. P., and D. R. Anderson. 2002. A practical information-theoretic approach. Model selection and multimodel inference, 2nd ed. Springer, New York.
- Cohen, J. A., and L. P. Brower. 1982. Oviposition and larval success of wild monarch butterflies (Lepidoptera: Danaidae) in relation to host plant size and cardenolide concentration. *J. Kansas Entomol.* 55: 343–348.
- Cunningham, J. P. 2012. Can mechanism help explain insect host choice? *J. Evol. Biol.* 25: 244–251.
- Decourtye, A., C. Armengaud, M. Renou, J. Devillers, S. Cluzeau, M. Gauthier, and M. H. Pham-Delegue. 2004. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pestic. Biochem. Physiol.* 78: 83–92.
- Després, L., J. P. David, and C. Gallet. 2007. The evolutionary ecology of insect resistance to plant chemicals. *Trends Ecol. Evol.* 22: 298–307.
- Douglas, M. R., and J. F. Tooker. 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.
- Drury, K. L. S., and G. Dwyer. 2005. Combining stochastic models with experiments to understand the dynamics of monarch butterfly colonization. *Am. Nat.* 166: 731–750.
- Easton, A. H., and D. Goulson. 2013. The neonicotinoid insecticide imidacloprid repels pollinating flies and beetles at field-realistic concentrations. *PLoS ONE* 8: e54819.
- Flockhart, D. T. T., J.-B. Pichancourt, D. R. Norris, and T. G. Martin. 2015. Unravelling the annual cycle in a migratory animal: breeding-season habitat loss drives population declines of monarch butterflies. *J. Anim. Ecol.* 84: 155–165.
- Flockhart, D. T. T., L. P. Brower, M. I. Ramirez, K. A. Hobson, L. I. Wassenaar, S. Altizer, and D. R. Norris. 2017. Regional climate on the breeding grounds predicts variation in the natal origin of monarch butterflies overwintering in Mexico over 38 years. *Global Change Biol.* 23: 2565–2576.
- Grant, T. J., H. R. Parry, M. P. Zalucki, and S. P. Bradbury. 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.
- Grant, T. G., N. Krishnan, and S. P. Bradbury. 2021. Conservation risks and benefits of establishing monarch butterfly (*Danaus plexippus*) breeding habitat in close proximity to maize and soybean fields in the North Central U.S.: a landscape-scale analysis of foliar insecticide impacts on non-migratory monarch butterfly populations. *Integ. Environ. Assess. Manag.* doi:10.1002/ieam.4402
- Greatti, M., R. Barbattini, A. Stravisi, A. G. Sabatini, and S. Rossi. 2006. Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds. *Bull. Insectology* 59: 99–103.
- Gervais, J. A., B. Luukinen, K. Buhl, and D. Stone. 2010. Imidacloprid general fact sheet. National Pesticide Information Center, Oregon State University Extension Services. <http://npic.orst.edu/factsheets/imidacloprid>
- Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference–performance relationships in phytophagous insects. *Ecol. Lett.* 13: 383–393.
- Hall, M. J., V. Dang, S. P. Bradbury, and J. R. Coats. 2020. Optimization of QuEChERS method for simultaneous determination of neonicotinoid residues in pollinator forage. *Molecules* 25: 2732. doi:10.3390/molecules25122732
- Halsch, C. A., A. Code, S. M. Hoyle, J. A. Fordyce, N. Baert, and M. L. Forister. 2020. Pesticide contamination of milkweeds across the agricultural, urban, and open spaces of low-elevation Northern California. *Front. Ecol. Evol.* 8: 162. doi:10.3389/fevo.2020.00162
- Han, P., C. Y. Niu, C. L. Lei, J. J. Cui, and N. Desneux. 2010. Quantification of toxins in a Cry1Ac + CpTI cotton cultivar and its potential effects on the honey bee *Apis mellifera* L. *Ecotoxicology* 19: 1452–1459.
- Haribal, M., and P. Feeny. 2003. Combined roles of contact stimulant and deterrents in assessment of host-plant quality by ovipositing zebra swallowtail butterflies. *J. Chem. Ecol.* 29: 653–670.
- Haribal, M., and J. A. A. Renwick. 1996. Oviposition stimulants for the monarch butterfly: flavonol glycosides from *Asclepias curassavica*. *Phytochemistry* 41: 139–144.
- Haribal, M., and J. A. A. Renwick. 1998. Identification and distribution of oviposition stimulants for monarch butterflies in hosts and nonhosts. *J. Chem. Ecol.* 24: 891–904.
- Henry, M., M. Béguin, F. Requier, O. Rollin, J. F. Odoux, P. Aupinel, J. Aptel, S. Tchamitchian, and A. Decourtye. 2012. A common pesticide decreases foraging success and survival in honey bees. *Science* 336: 348–350.
- Hladik, M. L., D. W. Kolpin, and K. M. Kuivila. 2014. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environ. Pollut.* 193: 189–196.
- Hladik, M. L., S. Bradbury, L. A. Schulte, M. Helmers, C. Witte, D. W. Kolpin, J. D. Garrett, and M. Harris. 2017. Neonicotinoid insecticide removal by prairie strips in row-cropped watersheds with historical seed coating use. *Agric. Ecosyst. Environ.* 241: 160–167.
- Holopainen, J. K. 2004. Multiple functions of inducible plant volatiles. *Trends Plant Sci.* 9: 529–533.
- Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. *Theor. Popul. Biol.* 14: 350–356.
- Jeschke, P., R. Nauen, M. Schindler, and A. Elbert. 2011. Overview of the status and global strategy for neonicotinoids. *J. Agric. Food Chem.* 59: 2897–2908.
- Kessler, S. C., E. J. Tiedeken, K. L. Simcock, S. Derveau, J. Mitchell, S. Softley, J. C. Stout, and G. A. Wright. 2015. Bees prefer foods containing neonicotinoid pesticides. *Nature* 521: 74–76.
- Koppert Biological Systems. 2005. (<http://www.koppert.nl/e0110.html>) (accessed 23 October 2020).
- 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.
- Krishnan, N., Y. Zhang, M. E. Aust, R. L. Hellmich, J. R. Coats, and S. P. Bradbury. 2021. Monarch butterfly (*Danaus plexippus*) life cycle risks from foliar and seed-treatment insecticides used in maize and soybean production. *Environ. Toxicol. Chem.* doi:10.1002/etc.5016
- Krupke, C. H., G. J. Hunt, B. D. Eitzer, G. Andino, and K. Given. 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS ONE* 7: e29268.
- Malcolm, S. B., and L. P. Brower. 1986. Selective oviposition by monarch butterflies (*Danaus plexippus* L.) in a mixed stand of *Asclepias curassavica* L. and *A. incarnata* L. in South Florida. *J. Lepids. Soc.* 40: 255–263.
- Mogren, C. L., and J. G. Lundgren. 2016. Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee nutritional status. *Sci. Rep.* 6: 29608.
- Muth, F., and A. S. Leonard. 2019. A neonicotinoid pesticide impairs foraging, but not learning, in free-flying bumblebees. *Sci. Rep.* 9: 4764.
- Muth, F., R. L. Gaxiola, and A. S. Leonard. 2020. No evidence for neonicotinoid preferences in the bumblebee *Bombus impatiens*. *R. Soc. Open Sci.* 7: 191883.
- National Center for Biotechnology Information. 2020a. PubChem compound summary for CID 86287518, imidacloprid. (<https://pubchem.ncbi.nlm.nih.gov/compound/Imidacloprid>) (accessed 10 September 2020).
- National Center for Biotechnology Information. 2020b. PubChem compound summary for CID 86287519, clothianidin. (<https://pubchem.ncbi.nlm.nih.gov/compound/Clothianidin>) (accessed 10 September 2020).
- Nishida, R. 2005. Chemosensory basis of host recognition in butterflies—multi-component system of oviposition stimulants and deterrents. *Chem. Senses* 30: 293–294.

- 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.
- Oberhauser, K., R. Wiederholt, J. E. Diffendorfer, D. Semmens, L. Ries, L. Ries, W. Thogmartin, L. E. Lopez-Hofman, and B. Semmens. 2017. A trans-national monarch butterfly population model and implications for regional conservation priorities. *Ecol. Entomol.* 42: 51–60.
- 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
- Olaya-Arenas, P., K. Hauri, M. E. Scharf, and I. Kaplan. 2020a. Larval pesticide exposure impacts monarch butterfly performance. *Sci. Rep.* 10: 14490.
- Olaya-Arenas, P., M. E. Scharf, and I. Kaplan. 2020b. Do pollinators prefer pesticide-free plants? An experimental test with monarchs and milkweeds. *J. Appl. Ecol.* 57: 2019–2030.
- Oyeyele, S. O., and M. P. Zalucki. 1990. Cardiac glycosides and oviposition by *Danaus plexippus* on *Asclepias fruticosa* in south-east Queensland (Australia), with notes of the effect of plant nitrogen content. *Ecol. Entomol.* 15: 177–185.
- Pecenka, J. R., and J. G. Lundgren. 2015. Non-target effects of clothianidin on monarch butterflies. *Sci. Nat.* 102: 19. doi:10.1007/s00114-015-1270-y
- Peñuelas, J., and S. Munné-Bosch. 2005. Isoprenoids: an evolutionary pool for photoprotection. *Trends Plant Sci.* 10: 166–169.
- 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.
- Pleasants, J. M., M. P. Zalucki, K. S. Oberhauser, L. P. Brower, O. R. Taylor, and W. E. Thogmartin. 2017. Interpreting surveys to estimate the size of the monarch butterfly population: pitfalls and prospects. *PLoS ONE* 12: e0181245.
- PPDB. 2012. Pesticide properties database. (<http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm>) (accessed 16 March 2021).
- Raguso, R. A. 2004. Why are some floral nectars scented? *Ecology* 85: 1486–1494.
- Ramaswamy, S. B. 1988. Host finding by moths: sensory modalities and behaviours. *J. Insect Physiol.* 34: 235–249.
- R Development Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Renwick, J. A. A. 1989. Chemical ecology of oviposition in phytophagous insects. *Experientia* 45: 223–228.
- Renwick, J. A. A., and F. S. Chew. 1994. Oviposition behavior in Lepidoptera. *Annu. Rev. Entomol.* 39: 377–400.
- Roessingh, P., E. Städler, R. Schöni, and P. Feeny. 1991. Tarsal contact chemoreceptors of the black swallowtail butterfly *Papilio polyxenes*: responses to phytochemicals from host- and non-host plants. *Physiol. Entomol.* 16: 485–495.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9: 671–675.
- 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.
- Thompson, H. M., S. Wilkins, S. Harkin, S. Milner, and K. F. A. Walters. 2015. Neonicotinoids and bumblebees (*Bombus terrestris*): effects on nectar consumption in individual workers. *Pest Manag. Sci.* 71: 946–950.
- Tomizawa, M., and J. E. Casida. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu. Rev. Pharmacol. Toxicol.* 45: 247–268.
- Tooker, J. F., M. R. Douglas, and C. H. Krupke. 2017. Neonicotinoid seed treatments: limitations and compatibility with integrated pest management. *Agric. Environ. Lett.* 2: ael2017.08.0026.
- Urquhart, F. 2019. The monarch butterfly. University of Toronto Press, Toronto. doi:10.3138/9781487584252
- U.S. Environmental Protection Agency (U.S. EPA). 2003. AgDRIFT. Washington, D.C. (<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#AgDrift>) (accessed 16 March 2021).
- U.S. Fish and Wildlife Service (U.S. FWS). 2016. Assessing the status of the monarch butterfly. U.S. Fish and Wildlife Service, Washington, D. C. (<https://www.fws.gov/savethemonarch/ssa.html>) (accessed 16 March 2021).
- U.S. Fish and Wildlife Service (U.S. FWS). 2017. Monarch butterfly species assessment update. (<https://www.fws.gov/savethemonarch/pdfs/Monarch-SSA-report.pdf>) (accessed 16 March 2021).
- Wassenaar, L. I., and K. A. Hobson. 1998. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: new isotopic evidence. *Proc. Natl Acad. Sci. USA* 95: 15436–15439.
- Wilcox A. A. E., D. T. T. Flockhart, A. E. M. Newman, and D. R. Norris. 2019. An evaluation of studies on the potential threats contributing to the decline of eastern migratory North American monarch butterflies (*Danaus plexippus*). *Front. Ecol. Evol.* 7: 99.
- Yang, E. C., Y. C. Chuang, Y. L. Chen, and L. H. Chang. 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J. Econ. Entomol.* 101: 1743–1748.
- Yang, E. C., H. C. Chang, W. Y. Wu, and Y. W. Chen. 2012. Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage. *PLoS ONE* 7: e49472.
- Zalucki, M. P., and R. L. Kitching. 1982. Dynamics of oviposition in *Danaus plexippus* (Insecta: Lepidoptera) on milkweed, *Asclepias* spp. *J. Zool.* 198: 103–116.
- Zalucki, M. P., L. P. Brower, and S. B. Malcolm. 1990. Oviposition by *Danaus plexippus* in relation to cardenolide content of three *Asclepias* species in the southeastern U.S.A. *Ecol. Entomol.* 15: 231–240.
- Zalucki, M. P., H. R. Parry, and J. M. Zalucki. 2016. Movement and egg laying in monarchs: to move or not to move, that is the equation. *Austral Ecol.* 41: 154–167.
- Zucker, W. V. 1982. How aphids choose leaves, the role of phenolics in host selection by galling aphid. *Ecology* 63: 972–981.