

## DISTANCE DIMINISHES THE EFFECT OF DELTAMETHRIN EXPOSURE ON THE MONARCH BUTTERFLY, *DANAUS PLEXIPPUS*

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**ABSTRACT.** The monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae), is threatened by substantial loss of habitat, extreme weather events linked to global climate change, and nontarget impacts of broad-spectrum insecticides. To investigate the impact of chronic ingestion of pyrethroids on monarchs, wild-type Florida *D. plexippus* were reared on milkweed (*Asclepias curassavica*) that was exposed to ultra-low volume applications of DeltaGard by a truck-mounted fogger, at distances of 25 and 50 m. We observed significant negative impacts on monarchs reared on milkweed at 25 m from the DeltaGard spray route, including significant decreases in survival, and significantly longer development times, compared with untreated controls. Larvae reared on host plants closest to the truck spray route were 3 times more likely to experience a mortality event than the control cohort in trial 1 and 6 times in trial 2. Survival of monarch caterpillars reared on milkweed sprayed at 50 m was not significantly different from controls. For monarchs that survived to adulthood, we did not observe statistically significant differences among cohorts for variables measured. These data demonstrate that ultra-low volume treatments of pyrethroids can result in significant mortality in monarchs, but that the effects diminish with distance from the spray route.

**KEY WORDS** *Danaus plexippus*, DeltaGard, deltamethrin, pest control, nontarget effects

### INTRODUCTION

Management of mosquito vectors and agricultural pests through outdoor space applications of aerosolized chemical insecticides is important for protecting humans, crops, livestock, and wildlife. Balancing this critical service with preserving natural diversity and nontargets, especially pollinators, should also be a goal of an integrated pest management program. It is estimated that approximately 30% of all pyrethroid use in the USA is in an agricultural setting (approximately 4% mosquito control) (USEPA 2009). However, growing concerns of local mosquito-borne transmission of Zika virus in the continental USA have resulted in increased budgets for public health mosquito abatement programs, with Florida and Texas receiving \$22 million in 2016 to strengthen their mosquito surveillance and control programs (CDC 2016). Despite their ubiquitous presence in mosquito abatement programs, little is known of the potential acute and chronic effects of pyrethrins and pyrethroids on nontarget insects. Understanding the role insect control activities play in the persistence of nontarget organisms is imperative to ensure applications are effectively reducing target populations while causing minimal harm to beneficial insect species.

*Danaus plexippus* L. (monarch butterfly) has gained much attention from the general public and scientific community due to its striking and easily recognizable appearance, its remarkable migration biology, and declining populations. The eastern population, which overwinters in central Mexico and travels approximately 4,000 km to breeding grounds in southeastern Canada and the northeastern

USA, has seen a decline of approximately 80% over the last 2 decades (Vidal and Rendón-Salinas 2014). Major factors contributing to its decline are widespread reduction in available milkweed (*Asclepias* spp.) along migratory pathways, loss of breeding habitat, destruction of overwintering sites, and extreme weather events (Hartzler 2010, Brower et al. 2012, Thogmartin et al. 2017, Pelton et al. 2019). Agricultural and surrounding habitats, which have already seen a 58% decline in milkweed, are an important source of host plants and compose the majority of the eastern population's migration route (Pleasants and Oberhauser 2012).

*Danaus plexippus* is susceptible to predation, parasites, bacterial and viral diseases (e.g., “black death” disease, in which infected caterpillars display black or brown discoloration and often liquefy at death), and the effects of herbicides and insecticides (Brewer and Thomas 1966, Oberhauser et al. 2007, Geest et al. 2019). *Danaus plexippus* larvae reared on milkweed previously exposed to permethrin and resmethrin exhibited decreased survival, longer development times, and a reduction in adult weight compared with larvae raised on control plants (Oberhauser et al. 2006, 2009). When exposed to resmethrin by field application doses, larvae exhibited increased mortality and development times (Oberhauser et al. 2009). Permethrin is also acutely toxic to several other Florida native pollinator butterfly species (Hoang et al. 2011, Hoang and Rand 2015).

In the current work, we observed the effects of deltamethrin, a 4th-generation pyrethroid, exposure on *D. plexippus* by route of ingestion of contaminat-

ed foliage. Deltamethrin is one of many pyrethroids utilized in mosquito abatement and agricultural pest management programs in Florida and is known to persist in the environment for several days, with literature values of half-life on foliage ranging from 5.9 to 17 days (Ruzo and Casida 1979, Hill and Johnson 1987). The half-life of deltamethrin after ultra-low volume (ULV) applications on foliage has not been described. *Pieris brassicae* L. and *P. rapae* L. (Lepidoptera: Pieridae), Florida native pollinator species, that ingested deltamethrin exhibited increased mortality and decreased larval weight (Cilgi and Jepson 1995). To our knowledge, there are currently no data that describe the risks associated with deltamethrin exposure to *D. plexippus*.

Florida provides an opportunity to study nontarget effects due to its well-established mosquito abatement programs that contribute to the health and quality of life of Florida residents. Florida's subtropical and humid environment favor year-round flowering milkweed, which has allowed monarchs to continue to breed during the winter months (Brower 1961, Knight and Brower 2009). This population, much like the eastern population, is estimated to have declined 80% in abundance since the early 1990s and may serve as an important reservoir for the eastern population (Brower et al. 2018).

## MATERIALS AND METHODS

### Monarch collection and rearing

Tropical milkweed plants (*Asclepias curassavica* L.; Gentianales: Apocynaceae) were purchased from a local nursery and washed with warm soapy water to facilitate the removal of spider mites and aphids. The nursery does not use any systemic insecticides. Milkweed plants were kept in a portable greenhouse (model No. HGC-1219-FDW; Walmart, Bentonville, AR) prior to and after treatments. Control plants were housed in a separate greenhouse. Plants were watered every other day with tap water applied at the base of the stem.

Wild-type adult female and male *D. plexippus* were collected from the grounds of the Florida Medical Entomology Laboratory (FMEL) by aerial net and maintained in the FMEL roundhouse (a 15-m-diam screened-in gazebo) in large bug dorms (BioQuip, Rancho Dominguez, CA). To obtain monarch caterpillars, adult butterflies were presented flowering milkweed plants (that had not been exposed to insecticides) and cotton soaked with 10% sucrose solution ad libitum. Milkweed plants were checked daily for the presence of eggs. When eggs were observed, the plants were transferred to a new bug dorm and replaced with a new plant.

More than 70% of monarchs in southern Florida are observed to be heavily infected with *Ophryocystis elektroscirrha* McLaughlin and Myers (Neogregarinida: Ophryocystidae), an obligate protozoan para-

site. A single spore ingested by a 1st instar is enough to produce a detectable spore load by the time it reaches adulthood (Leong et al. 1997, Altizer et al. 2000). In adults, *O. elektroscirrha* infections have been linked to decreased flight and in many cases render the animal incapable of escaping the chrysalis (Leong et al. 1992, Altizer and Oberhauser 1999). All wild-type adult *D. plexippus* were checked for presence of *O. elektroscirrha* spores by sampling the abdominal scales. Samples were obtained by gently pressing a small piece of clear tape on the dorsal side of the abdomen (Altizer et al. 2000). Samples were fixed to a glass slide and visually inspected with a compound microscope at 100× magnification for spores, which are dark brown and ovoid (Leong et al. 1992). All wild-caught adults used to establish the colony were found to be infected with *O. elektroscirrha* ( $n = 20$ ). We followed the aseptic techniques outlined in Altizer and Oberhauser (1999) to avoid accidental contamination of larvae. We used nitrile gloves when handling the monarchs and disinfected all surfaces and materials that encountered the monarchs with a 10% bleach solution followed by 95% ethanol.

### Outdoor field space spray

Exposure of deltamethrin, formulated as DeltaGard (lot NP65GX8660; Bayer Environmental Science, Clayton, NC), was examined by rearing monarch caterpillars on DeltaGard-treated milkweed plants. Indian River County Mosquito Control District performed field spray applications on June 25, 2019 (trial 1), and July 25, 2019 (trial 2), at approximately 1900 h (Eastern Time) at the Indian River County Fairgrounds and Expo Center. Previously untreated milkweed plants were placed 25 m ( $n = 15$ ) and 50 m ( $n = 15$ ) downwind of the spray truck path. Control plants ( $n = 15$ ) were set 100 m upwind from the truck path. Plants were aligned parallel to the truck path and spaced 1 m apart from each other. Aerosols of DeltaGard were produced using a truck-mounted ULV fogger calibrated to release droplets of  $15 \pm 2 \mu\text{m}$  at a maximum rate of 0.00134 lb AI/acre, consistent with the product label (droplet size range 8–30  $\mu\text{m}$ ; application rate not to exceed 0.00134 lb AI/acre). To estimate the amount of DeltaGard coverage on milkweed plants, rotary impingers were used to determine if the spray traveled through the target area. Spinning at approximately 600 rpm, they are intended to harvest droplets and provide an estimate of what a leaf would experience as the plume travels through the target zone. Droplets were collected on 2 Teflon-coated glass slides fixed to the rotary impingers located in the center of the plot at –100 (upwind), 25, and 50 m from the spray line. Mosquito Control staff recorded droplet counts, density (droplets/ $\text{mm}^2$ ), and volume median diameter (VMD) using the DropVision® AG System version 3.0 (Leading Edge Associates, Inc., Fletcher, NC). The optimal droplet size for mosquito abatement

Table 1. Summary of successful emergence and mortality events summed over repeated measures ( $n = 2$ ), beginning with 2nd-stage larvae.

Cohort ( $n$ )	Survived to pupal stage	Healthy adults	Black death	Failure to thrive	Failed eclosions
Upwind (40)	20	13	12	8	7
50 m (40)	23	15	5	0	5
25 m (40)	5	2	9	0	1

activities performed at ground level ranges from 10 to 30  $\mu\text{m}$  (WHOPES 2003, Bonds 2012).

Ambient temperature, RH, and wind speed and direction were recorded with an agricultural weather meter (model No. Kestral 5500AG; KestrelMeters.com, Boothwyn, PA). The wind direction was identified before each application so that the plot and truck path could be set perpendicular to the wind direction. Applications began when the wind speed was between 3 and 15 mph.

### Bioassays

Each bioassay commenced 12 h after the field spray application. Second-stage larvae (120 total, 40 per cohort) were placed on plants in either the control, 25-m, or 50-m cohorts. Thereafter, larvae were reared to adulthood or mortality on milkweed clippings from plants in their assigned cohort. Caterpillars were housed individually in paper cups (model No. 760SOUP16WPA; Webrestaurantstore, Lititz, PA) with mesh lids in the FMEL roundhouse. Milkweed bouquets of 4 leaves were secured in a 2.0-ml microcentrifuge tube (model No. 19201; Qiagen, Hilden, Germany) filled with tap water to reduce wilting. Microcentrifuge tube openings were covered with Parafilm (Bemis™ Parafilm™, Neenah, WI) to keep larvae from drowning. The tubes were pushed approximately halfway through the bottom of the paper cups to secure them in place.

Each day the housing cups were cleared of frass, caterpillars were given fresh leaves, and the developmental stage was recorded. Housing cups were replaced every other day. Day and cause of mortality, time to pupation, mass of chrysalis, malformation(s) of adults, successful emergence, and mass of adult were recorded. Twenty-four hours after pupation, chrysalides were carefully removed from the housing cups using forceps to pull the silk pad from the substrate. After weighing, the chrysalides were resuspended by string in a new housing cup by tying a knot at the base of the cremaster. The string knot was secured with a small drop of nontoxic Elmer's glue (Newell Office Brands, Atlanta, GA), which was left to dry for 30 min before the chrysalides were hung. Twenty-four hours postecdysis, adult butterflies were checked for presence of *O. elektroscirra* spores, as previously described, and then killed in a freezer ( $-20^{\circ}\text{C}$ ). Each adult specimen was dried at  $60^{\circ}\text{C}$  for 48 h before weight measurements.

Deceased larvae or pupae that displayed black or brown discoloration or liquefied at death were

classified as “black death.” We attributed the deaths of caterpillars that wandered away from their milkweed bouquet and died without showing any signs of black death (i.e., no discoloration and did not liquefy at death) to “failure to thrive” syndrome (Oberhauser et al. 2007).

### Data analysis

We analyzed the effects of distance from the spray line and trial on monarch mortality using a mixed analysis of variance (ANOVA). We assessed each data set for normality using the Shapiro–Wilks test and visual inspection of quantile–quantile plots. Means between cohorts were assessed with a Student's  $t$ -test for normally distributed data and Mann–Whitney  $U$ -test with Bonferroni correction if normality was violated.

We conducted a survivorship analysis using the “survival” and “survminer” packages downloaded directly from R (R Core Team 2019). Differences in the survival distribution among treatment groups was assessed with a log-rank test and Cox proportional-hazards model.

We assessed correlations between the quantity and density of DeltaGard droplets recorded at each distance from the spray line against *D. plexippus* mortality. Associations were analyzed by logistic regression and chi-square test. All statistical analyses were performed using the “stats” package, and graphics were created using the “ggplot2” package downloaded directly from R (Wickham 2016, R Core Team 2019).

## RESULTS

### Survival analyses

Monarch caterpillars that had fed on leaves exposed to DeltaGard exhibited increased mortality compared with control insects, with generally greater impacts on larvae at closer spray distances. Third, 4th, and 5th instars from the control and 50-m cohorts would completely defoliate the milkweed bouquets each day, while larvae in the 25-m cohort would rarely eat enough to defoliate the bouquets. Larvae fed from plants at 25 m had loose or runny frass and decreased activity. Summaries of pupation, successful emergence, and mortality events are listed in Table 1. More than 50% of the mortality events in the control cohort in trial 2 were associated with black death. All surviving adults were infected with

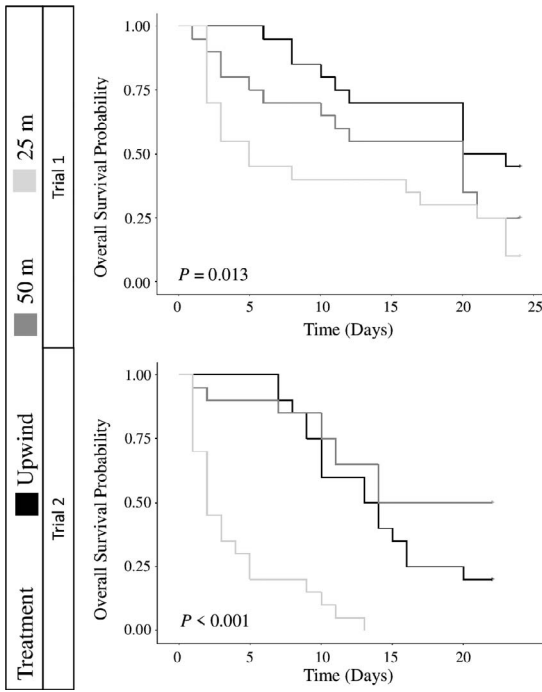


Fig. 1. Kaplan–Meier estimated survival curves of *Danaus plexippus* reared to adulthood or mortality on milkweed leaves exposed to ultra-low volume (ULV) DeltaGard applications. Curves were compared with a log-rank test. The null hypothesis was rejected.

*O. elektroscirra*, with exception of 2 individuals in the 50-m cohort.

A significant overall effect of cohort on survival was observed in trial 1 ( $\chi^2(2) = 8.7, P = 0.013$ ) and trial 2 ( $\chi^2(2) = 44.5, P < 0.001$ ), with lowest survival observed in caterpillars reared on milkweed sprayed with DeltaGard at the closer distance (25 m) (Fig. 1). We observed a significant overall decrease in survival of *D. plexippus* reared on leaves exposed to DeltaGard at the 25-m-distant spray line (trial 1: hazard ratio [HR] = 2.9 [1.38–6.2,  $P = 0.005$ ]; trial 2: HR = 6.22 [2.94–13.2,  $P < 0.001$ ]), compared with the control. Survival of *D. plexippus* reared on leaves exposed to DeltaGard at the 50-m spray line was not

statistically significant from the control (trial 1: HR = 1.8 [0.82–3.90,  $P = 0.146$ ]; trial 2: HR = 0.54 [0.24–1.20,  $P = 0.126$ ]). The results of the mixed ANOVA indicate that trial had no significant effect on mortality, but significant difference was observed between the cohorts in each trial (Table 2 and Fig. 2).

***Danaus plexippus* development**

Overall, ingestion of DeltaGard had a weak negative effect on monarch larval development (Table 2). In the 1st trial, oral ingestion of DeltaGard prolonged the development times of larvae (Fig. 2). The time to pupation was significantly lower in the control ( $1.25 \pm 0.55$  days) compared with the 50-m cohort ( $2.47 \pm 1.18$  days,  $P = 0.027$ ) and 25-m cohort ( $3.25 \pm 1.86$  days,  $P = 0.027$ ). Time to pupation did not significantly differ in the control and 50-m cohort ( $P = 0.513$ ). Ingestion of DeltaGard had no statistically significant effect on pupal weight, time to eclosion, and adult weight (Table 2 and Fig. 2).

**Droplet analysis**

The average temperature, RH, and wind speed recorded on June 25, 2019, were 85.1°F, 75.7%, and 2.6 mph and on July 25, 2019, were 88.9°F, 95.5%, and 3.4 mph. Droplet data are shown in Table 3. We detected 8 droplets at a single control impinger (Table 3). Droplet VMD was within the acceptable range (i.e., no droplets  $>30 \mu\text{m}$  were detected) (WHOPES 2003). We analyzed correlations between droplet counts and density and monarch mortality. No significant correlation was observed for droplet counts (trial 1:  $\chi^2 = 0.55, P = 0.460$ ) and droplet density (trial 1:  $\chi^2 = 1.00, P = 0.310$ ; trial 2:  $\chi^2 = 0.14, P = 0.700$ ) (Table 4). Although we observed a significant correlation for droplet counts and monarch mortality during the 2nd field trial ( $\chi^2 = 4.80, P = 0.028$ ), droplet count was not a significant predictor of mortality ( $P = 0.555$ ) (Table 4).

**DISCUSSION**

This is one of few studies to investigate nontarget effects of insecticides utilized by mosquito control

Table 2. Analysis of variance for development times, adult characteristics, and mortality of *Danaus plexippus* reared on DeltaGard-contaminated foliage beginning in 2nd instar. The F-statistic is presented with (numerator degrees of freedom, denominator degrees of freedom).

Variable	Distance		Replicate		Distance:Replicate	
	F-statistic	P-value	F-statistic	P-value	F-statistic	P-value
Percent survival to pupation <sup>1</sup>	12.52 (1, 57)	<0.001	6.19 (1, 57)	0.002	2.45 (1.53, 29.1)	0.095
Percent survival to eclosion <sup>1</sup>	8.08 (1, 57)	<0.001	0.05 (1, 57)	0.831	3.07 (2, 57)	0.054
Time to pupation (days)	0.287 (1, 9)	0.605	0.372 (1, 9)	0.557	4.02 (1, 9)	0.076
Time to eclosion (days)	0.25 (1, 2)	0.667	12.25 (1, 2)	0.073	6.25 (1, 2)	0.130
Pupal weight (g)	0.47 (1, 9)	0.051	72.77 (1, 9)	<0.001	0.56 (1, 9)	0.475
Adult weight (g)	0.00 (1, 2)	1.000	7.00 (1, 2)	0.118	0.01 (1, 2)	0.742

<sup>1</sup> Did not meet normality assumption.



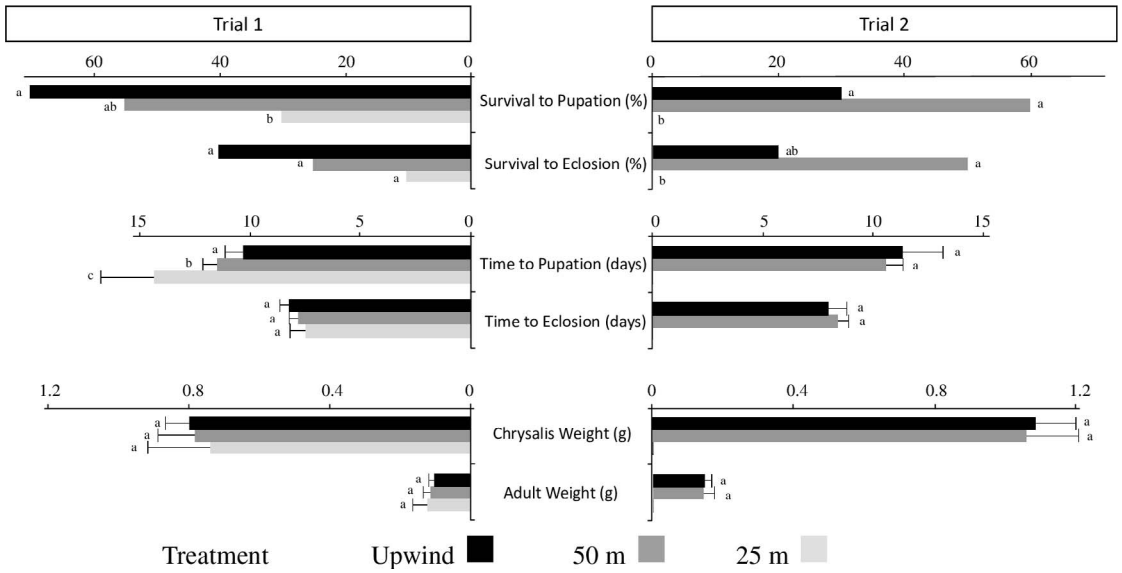


Fig. 2. Differences in larval development time, adult characteristics, and mortality of *Danaus plexippus* reared on DeltaGard-contaminated foliage beginning in 2nd instar. Average values with the same letter are not significantly different ( $P > 0.05$ ). All larvae began at the 2nd instar.

and the agricultural industry on *D. plexippus*. These data demonstrate significant increases in mortality and larval development times resulting from exposure to DeltaGard by route of ingestion, particularly for plants sprayed at closer distances. Larvae reared to adulthood on milkweed from the 25-m spray line were approximately 3 times less likely to survive compared with the reference control group and approximately 6 times less likely to survive in trial 2. These findings may have serious implications for *D. plexippus*, given that <10% of all eggs in the wild survive to adulthood (Oberhauser et al. 2001, Prysby 2004).

The current findings suggest that larval mortality is more likely to occur on host plants closest to the spray line, which supports findings of Oberhauser et al. (2009) that highest mortality rates were observed in larvae directly exposed to pyrethroids closest to the truck path. The relationship between mortality and distance from spray line is important because in Florida, and perhaps elsewhere, roadsides support plentiful *Asclepias tuberosa* L. (Gentianales: Apoc-

ynaceae) and *A. humistrata* L. (Gentianales: Apocynaceae) (Daniels et al. 2018). In fact, Daniels et al. (2018) observed that most milkweed plants were approximately 7–8 m from the road; therefore, there is an increased risk of them being exposed to truck-mounted fogging activities. We hypothesized that plants closest to the truck path would be exposed to a more concentrated plume and, therefore, contain a larger number of insecticide droplets when compared with plants placed farther from the truck path. However, the number of recorded droplets and the droplet density varied between the 2 applications and did not strongly correlate with mortality. The low number of recorded droplets may be attributed to the average wind speed of 2.6 mph, which was slightly below the optimal range. Variations in the observed droplet counts could be explained by differences in the temperature, humidity, wind speed and direction, and air pressure. These variables affect droplet density and determine how the droplet plume moves through the target zone (WHOPES 2003). Our results highlight the variability associated with outdoor field

Table 3. Droplet measurements ( $\pm$  SD) recorded during ultra-low volume DeltaGard applications.

Date	Distance from spray line (m)	Droplet counts	Droplet density (drops/mm <sup>2</sup> )	VMD <sup>1</sup> ( $\mu$ m)
Jun 25, 2019	Upwind (-100)	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	50	93.5 $\pm$ 10.6	2.1 $\pm$ 0.2	14.7 $\pm$ 0.5
	25	60.0 $\pm$ 5.7	1.3 $\pm$ 0.1	12.2 $\pm$ 0.2
Jul 25, 2019	Upwind (-100)	4.0 $\pm$ 5.7	1.0 $\pm$ 1.5	1.9 $\pm$ 2.8
	50	73.5 $\pm$ 16.3	1.6 $\pm$ 0.4	11.4 $\pm$ 0.7
	25	202.0 $\pm$ 3.5	11.6 $\pm$ 0.4	14.9 $\pm$ 0.3

<sup>1</sup> VMD, volume median diameter.

Table 4. Estimated regression parameters for associations of droplet counts, droplet density, and mortality from 2 outdoor space sprays conducted during June and July of 2019 in Indian River County, FL.

Date	Model	Variable	Estimate	SE	z-value	P-value
Jun 25, 2019	1	Intercept	0.24	0.32	0.74	0.458
	1	Droplet count	0.29	0.10	2.98	0.002
	2	Intercept	0.32	0.32	1.01	0.315
	2	Droplet density	1.17	0.43	2.69	0.007
Jul 25, 2019	1	Intercept	0.96	0.43	2.20	0.028
	1	Droplet count	0.07	0.11	0.59	0.555
	2	Intercept	0.14	0.36	0.38	0.705
	2	Droplet density	0.91	0.29	3.16	0.002

space sprays and the complexity of plume dynamics. The 8 droplets captured at the control impinger station were small (VMD approximately 3  $\mu\text{m}$ ) and unlikely to have contributed to high mortality (WHOPES 2003). We are unable to confirm their origin but speculate contamination derived from airborne diesel or aircraft exhaust particulate (Britch et al. 2018).

In the current work, we did not observe any sublethal effects of DeltaGard on adult characteristics (Table 2). This contrasts with previously published reports of sublethal effects of pyrethroids on adult monarch butterflies (Oberhauser et al. 2009). Our findings could be attributed to sample size, natural variation of wild-type larvae, and sexual dimorphism. Given the relatively small number of surviving adults, we did not separate out males and females from our analysis. Further studies with a larger sample size are required to further investigate this observation. The effects of insecticide exposure on fecundity and adult life span could also be investigated with a larger sample size of surviving adults. Lethal and sublethal developmental impacts can have devastating effects for this migratory insect. Survival rates from egg to adult in the wild have been estimated to be <10%, with the highest mortality occurring in eggs and early instars (Oberhauser et al. 2001, Prysby 2004). A recent survey in Florida observed that 77.5% of eggs survived to 1st instar, of which only 2.9% survived to the 5th instar (Brower et al. 2018). Impacts on adult characteristics and fitness can lead to failure to reach overwintering grounds (Bradley and Altizer 2005).

Naturally occurring infections contributed to observed mortality in some cohorts. In the control cohort, 30% of dead larvae were observed with black death disease; most cases occurred during the 4th and 5th instars (Table 1). Despite the relatively high levels of mortality in our control cohort, we still observed significantly greater mortality and longer development times in larvae reared on DeltaGard-treated plants at 25 m. Causal agent of the black death was not determined. Unsuccessful emergences of adults from pupal stage were observed, a characteristic of high *O. elektroscirra* spore load. All but 2 surviving adults were infected with *O. elektroscirra*, as were all wild adults captured for eggs, higher than that (70%) observed by Altizer et

al. (2000) in southern Florida. Altizer and Oberhauser (1999) observed that *O. elektroscirra* parasitism had minimal sublethal effects on larval survival and development, except at the highest doses.

It is likely that our results somewhat overestimate actual mortality caused by DeltaGard exposure in nature. This experiment was conducted in an open field, with no obstructions (e.g., buildings, vegetation) that would otherwise impede insecticide drift (Taylor and Schoof 1971, Andis et al. 1987, Reddy et al. 2006). Seasonality of monarch breeding may also afford some protection against DeltaGard exposure. While monarchs are known to breed year-round in Florida, peak abundance occurs in early April in Florida (Brower et al. 2018). A 2nd generation emerges soon after in May, the majority of which migrate northward toward breeding grounds. Therefore, the preponderance of monarch reproduction occurs well in advance of peak mosquito season (June–September).

Other possible consequences of outdoor space spray applications, such as effects on adult survival, foraging behavior, and oviposition selection by females, were beyond the scope of this study. While gravid monarchs displayed a lack of selectivity when presented permethrin-treated and insecticide-free host plants (Oberhauser et al. 2006), oviposition selection for DeltaGard remains to be elucidated. As insecticide resistance builds in target populations, novel insecticides are entering the market and will need to be assessed for effects on pollinators as well. It is pertinent to note that the Indian River Mosquito Control District (IRMCD) only uses DeltaGard to treat the surrounding areas in response to reported travel-related human cases of chikungunya, dengue, or Zika viruses. The IRMCD has since switched to Malathion EW (FMC Global Specialty Solutions Corporation, Philadelphia, PA) because it is a much cheaper alternative and there is currently no evidence of resistance in the county (Michael Hudon, IRMCD, personal communication).

Our results shed light on yet another factor that may be contributing to monarch decline in North America. There is an increased risk of exposure when treating edge, roadside, and agricultural habitats, where host plants are abundant (Pleasants and Oberhauser 2012, Daniels et al. 2018, Olaya-Arenas and Kaplan 2019). Adult mosquito abatement

activities are typically targeted in residential areas, where host plants are less abundant. However, backyard milkweed gardens could become important monarch reproduction sites (Geest et al. 2019) and provide insecticide-free refuge if they are sufficient quality and distance from spray routes (>50 m). Our results suggest that no-spray refuges with suitable milkweed populations could help lower chronic impacts of DeltaGard on monarchs and other pollinators while providing effective pest control. Knowledge of historical abundance and trends in seasonality should be taken into consideration when planning insect abatement activities near known monarch breeding habitats. However, many insect species transmit disease and adversely affect agricultural productivity, causing significant morbidity in humans and economic losses in Florida, and the protection of public health and food security should remain a top priority.

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