The Effects of Mosquito-Control Applications of Permethrin on Monarch Butterfly (*Danaus plexippus*) Larvae

by Sara Brinda

Abstract

This project investigated the lethal and sublethal effects of permethrin, a pesticide widely used for mosquito control, on monarch butterfly larvae. Permethrin is an "adulticide," designed to kill adult mosquitoes immediately on contact. To test the lethality of mosquito-control applications of permethrin to monarchs, common milkweed leaves were collected from permethrin-treated areas, and then fed to monarch larvae. Mortality rates were significantly higher in larvae exposed to permethrin-treated leaves than in those exposed to control leaves. Larval mortality was affected by the larval instar when initially exposed to permethrin-treated leaves ($p = 0.0248$) and the number of days larvae consumed permethrin-treated leaves ($p = 0.0001$). The number of days between the spraying and larval exposure to the leaves did not significantly affect mortality ($p = 0.8578$).

To test sublethal affects of permethrin, the operational permethrin solution used for mosquito control was diluted with oil diluent (a 2:1 mineral oil: soybean oul solution) into 0.5% and 0.1% dilutions by volume of the operational solution nd sprayed on sets of milkweed leaves. In addition, some sets of leaves were treated with the oil diluent alone, and some were left untreated. Larval development time was significantly lengthened by 0.1% and 0.5% permethrin solutions ($p = 0.0000$ and 0.0002, respectively), but not by the oil diluent ($p = 0.1513$). Adult mass was not significantly affected by the untreated, the oil-treated ($p = 0.8935$), or the 0.1% dilution treatment ($p = 0.1913$), but it was affected by the 0.5% dilution treatment ($p = 0.0436$).

Introduction

The purpose of this project was to assess the effects of the insecticide permethrin on monarch butterfly (*Danaus plexippus*) larvae. Permethrin is part of the group of synthetic pesticides known as pyrethroids, and is widely used in the Minneapolis-St. Paul area by the Metropolitan Mosquito Control District (MMCD), which treated 5,734 acres with it in 2002 (1). Before spraying, permethrin is mixed with soybean and food-grade mineral oil. It is then applied to wooded public and residential areas as mist-like droplets, using the ultra-low volume (ULV) method. Due to public safety concerns about West Nile virus and other mosquito-borne illnesses, mosquito control applications of permethrin are expected to increase in future years (1).

Permethrin is an "adulticide," designed to kill adult mosquitoes immediately on contact with the insect. Adulticides are considered by the MMCD and other mosquito control experts to be a relatively ineffective form of mosquito control and are used only when the more effective larvicidal treatments are insufficient to control mosquito populations (1). According to MMCD Technical Services Coordinator Dr. Stephen Manweiler, permethrin has a measurable effect on adult mosquito populations for only four days after application (2).

Literature from the Environmental Protection Agency says that pyrethroids like permethrin, when used in mosquito control programs, "Do not pose unreasonable risks to wildlife or the environment" (3). Despite this assertion, little is actually known about the effects of ULV permethrin on non-target invertebrates. Only one peer-reviewed field study has investigated the effect of ULV permethrin on non-target insects, and it focused only on immediate mortality in aquatic and night-flying insects (4). The purpose of the study presented here was to investigate the short- and long-term effects of the residue from mosquito control-applications of permethrin on monarch larvae.

A monarch goes through four stages during its lifetime: egg, larva, pupa, and adult. The larva (caterpillar) goes through five larval growth stages, or instars. The first instar is the smallest and earliest, and the fifth the largest and the last. During this terrestrial larval stage, a monarch larva feeds only on milkweed plants (*Acslepias*). In the study presented here, monarchs were exposed to permethrin residue on common milkweed (*Acslepias syriaca*) during various larval instars.

The study presented here had two goals. The first was to assess mortality in monarch larvae fed common milkweed collected from areas treated for mosquitoes by the MMCD with ULV permethrin, as compared to larvae that had not been exposed to the spray. The hypothesis was that larvae fed milkweed from treated areas would have higher mortality rates than those on the control (unsprayed) treatments. It was also hypothesized that larval mortality rates would be higher in the following groups of larvae:

1. Those exposed to leaves treated more recently with permethrin.
2. Those exposed to treated leaves for greater periods of time.
3. Those exposed to permethrin during earlier instars.

The second goal was to assess sublethal effects of permethrin on monarchs. The hypothesis for this goal was that larvae on the sprayed milkweed leaves would have slower development rates and be smaller as adults than larvae consuming untreated milkweed. It was also hypothesized that these effects would be more pronounced in the following groups of larvae:

1. Those exposed to higher concentrations of permethrin.
2. Those exposed to permethrin during earlier instars.

Materials and Methods

Part I: Lethality from milkweed treated by the MMCD

Common milkweed leaves were collected from that had been treated with a typical ULV application of permethrin by the MMCD sites (see Tables 1 and 2). The operational solution used by the MMCD contains 0.50 pounds permethrin per 128 fluid ounces (1). All dates and places where spraying had occurred were verified by the MMCD, but the employees who were applying the permethrin were not told that milkweed would be collected from the sites for this study. Leaves were collected in plastic bags between one day and twenty-one days after spraying and fed to larvae within two to four hours of collection. For most rounds, a "double-blind" technique was used so that the source of the milkweed was unknown to the experimenter; the rounds that were double-blind are indicated in Table 1.

Table 1. Data for each round: start date, days larvae spent on original leaf, whether or not the round was double blind, the sites from which leaves were collected, the number of days since spraying, and the number of larvae exposed to leaves from each site.

Round	Date started	Days on original leaf	Double blind?	Source of leaves	Days since spraying	Number of larvae
1	7/28/03	2	No	SMPP	7	20
				KG	N/A	20
				LW	3	20
				LS	3	20
2	7/31/03	5	Yes	SMPV	10	15
				SMPP	10	15
				KG	N/A	15
				LS	6	15
3	8/4/03	1	Yes	SMPP	14	15
				KG	N/A	20
4	8/5/03	2	Yes	CAP	N/A	15
				CL	1	15
5	8/8/03	3	Yes	COL1	1	45
				COL2	N/A	45
6	8/9/03	5	Yes	SMPP	19	10
				L	N/A	10
				KG	N/A	10
7	8/11/03	5	No	JY	N/A	10
				SMPP	21	9
				L	N/A	10
8	8/11/03	3	Yes	COL1	4	45
				COL2	N/A	45
9	8/13/03	4	No	L	N/A	15
				JY	N/A	15
10	8/15/03	4	Yes	COL1	8	45
				COL2	N/A	45

Table 2. Key for site abbreviations.

Abbreviation for site	Site	Date Treated
SMPP	St. Mary's Park in Lakeland, MN	7/21/03
KG	Private garden in Roseville, MN	N/A
LW	Wooded area of Langton Park in Roseville, MN	7/25/03
LS	Area near soccer field of Langton Park in Roseville, MN	7/25/03
SMPV	Vacant lot near St. Mary's Park in Lakeland, MN	7/21/03
CAP	Capp Park in MN	N/A

COL	Columbia Park in MN	8/4/03
COL1	Treated area of Chain of Lakes Regional Park in Lino Lakes, MN	8/7/03
COL2	Untreated area of Chain of Lakes Regional Park in Lino Lakes, MN	N/A
JY	Private garden in St. Paul, MN	N/A
L	Control milkweed in lab from various locations in MN	N/A

In the laboratory, each milkweed leaf was placed in a deli container with a porous lid. A filter paper was moistened with deionized water and placed in the bottom of the container to keep the leaf from drying. One monarch larva was placed in each container. A control group of larvae was prepared simultaneously with each sprayed group, kept on milkweed leaves that had been picked from the wild and had not been treated with permethrin. Throughout the process, latex gloves were used and changed frequently to keep larval containers as sterile as possible, and to prevent any permethrin-treated leaves from contaminating the control leaves.

Sets of larvae were prepared in several "rounds," with each round containing larvae on leaves with the following treatments:

1. One or more sets of leaves that had been collected from sprayed sites together.
2. One or more control groups that had been prepared at the same time.

All sets of leaves within a round were replaced with non-sprayed (control) leaves when any leaf in the round became too dry to provide adequate nutrition, or when any leaf had been completely consumed.

The instar and mass of each larva were monitored daily, as was mortality. The moist filter paper was replaced and the containers cleaned as needed.

Part II: Sublethal effects from milkweed treated in the laboratory

The second part of the study was designed to investigate sublethal effects of permethrin on monarch larvae. Since so few larvae survived the doses of permethrin used for mosquito control, it was necessary to use more dilute permethrin solutions to assess sublethal effects. The operational permethrin solution used by the MMCD was obtained and further diluted with the oil diluent used by the MMCD (a 2:1 mineral oil:soybean oil solution) into 0.5% and 0.1% dilutions by volume of the operational solution. These dilutions were chosen after 100% mortality within 48 hours was observed in larvae exposed to 50%, 10%, 5%, 2%, and 1% dilutions of the operational solution.

A spray bottle was used to apply each dilution to sets of milkweed leaves. For safety, the spraying was done in a box, and the experimenter wore latex gloves, to keep from breathing or coming into contact with the permethrin. As before, latex gloves were changed frequently to prevent contamination between milkweed treatments. Two control sets of leaves were also prepared: one with leaves sprayed only with oil diluent and one with unsprayed leaves. There were thus four treatments in total: the control unsprayed leaves, leaves treated with oil diluent only, leaves treated with the 0.5% dilution of the operational solution, and leaves that were treated with the 0.1% dilution of the operational solution. Ten larvae in each of three instars (2^{nd}, 3^{rd}, 4^{th}) were placed on milkweed from each treatment. Each leaf was placed in a deli container with a porous lid, and larvae were maintained and monitored as described above. New leaves were sprayed daily with each of the three solutions. Each larva was given a new leaf daily; in this part of the experiment larvae were maintained on laboratory-treated or control leaves throughout their development. The mass and sex of each adult monarch were recorded 24 hours after emergence, when the wings had dried and the meconium (waste products built up during the pupal stage) had been expelled.

Results

Part I: Lethality of MMCD-treated milkweed

Overall mortality rates were significantly higher for larvae exposed to permethrin-treated leaves. Figure 1 (see Appendix) shows overall larval mortality in all rounds of the first part of the study. A chi-square test found significantly higher mortality in larvae placed on treated compared to untreated milkweed leaves for all three instars ($p < 0.05$ for all instars).

Table 3 shows that earlier-instar larvae were more likely to die on treated leaves ($p = 0.0248$), as were those that remained on the treated leaves for longer periods of time ($p = 0.0001$). Days since spraying did not significantly affect mortality ($p = 0.8578$). A logistic regression (similar to a linear regression, but used when the response variable, such as mortality, is yes/no) was used to determine which variables affected larval mortality rates, and to determine the significance of the effects. The significance of each of the variables, determined by the logistic regression, is indicated by the p-values in Table 3 (see Appendix). Table 3 also shows the coefficient of significance and the standard error for each variable. Larvae killed accidentally were removed from all data sets.

Table 3. The results of the logistic regression analysis of variables affecting mortality. The total sample size was 278 larvae.

Variable	Coefficient	Std. error	p-value
Beginning instar	-0.8220	0.3661	0.0248
Days on treated leaf	-1.2589	0.3279	0.0001
Days since spraying	-0.0106	0.0595	0.8578

Part II: Sublethal effects of laboritory-treated milkweed

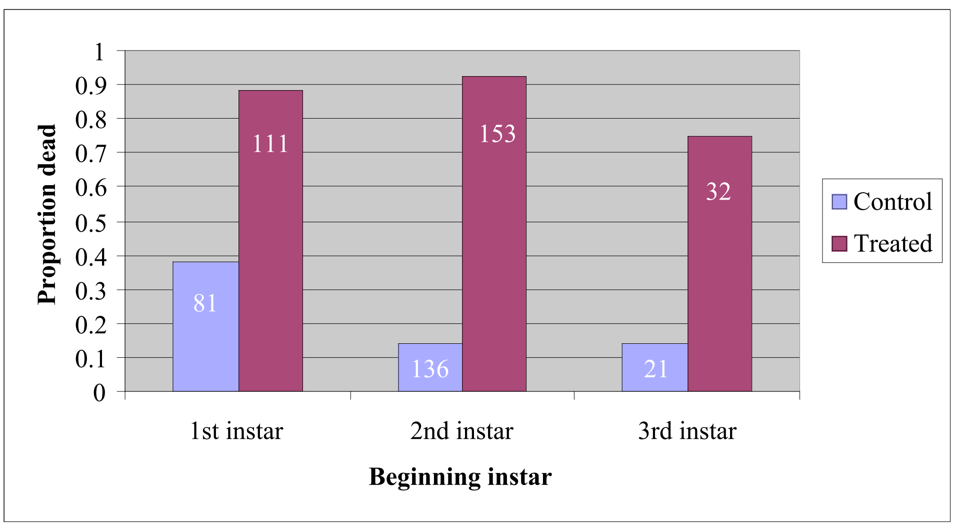
Development time was significantly lengthened by the 0.1% and 0.5% permethrin solutions. Figure 2 (see Appendix) shows the mean development time for larvae reared on the control leaves, leaves treated with oil, and leaves treated with two different dilutions of permethrin. A linear regression was used to determine the significance of the effect of milkweed treatment on the length of larval development time. The oil-treated leaves did not significantly affect development time ($p = 0.1513$), but the leaves treated with the 0.1% and 0.5% dilutions of the operational permethrin solution did ($p = 0.0000$ and 0.0002, respectively).

Neither the oil solution nor the 0.1% dilution of the operational permethrin solution had a significant effect on adult mass, although the 0.5% dilution did. The effects of these variables on adult mass were analyzed using a linear regression. The instar during which each larva began the experiment did not have an effect on adult mass. Results of the linear regression are included in Table 4 (see Appendix). Only data collected from larvae that survived into adulthood were included in the analyses of sublethal effects.

Discussion

This study demonstrated that the application of permethrin by mosquito control organizations caused mortality in a non-target insect, the monarch butterfly. In the first part of the study, larvae placed on milkweed from areas treated with permethrin had higher mortality rates than those on control leaves (Figure 1), supporting one hypothesis for this study.

Figure 1. Overall larval mortality on control and treated milkweed in Part I, illustrating the proportion dead from each group of larvae, separated by beginning instar and milkweed treatment (control or sprayed with permethrin). The sample size of each group is indicated by the number on each bar.

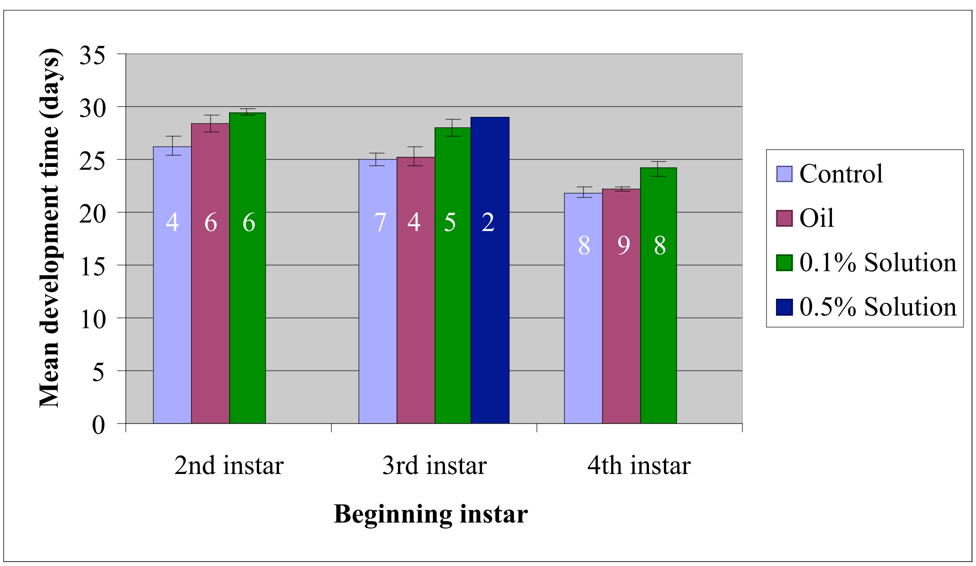


The results of this study also revealed which factors affected the lethality to monarchs of milkweed in sprayed areas. The instar during which the larva was exposed affected mortality; larvae exposed during later instars had a significantly better chance of surviving exposure to permethrin (Table 3). The number of days a larva spent on a treated leaf also significantly affected its chances of surviving; larvae exposed to permethrin-treated leaves for longer periods of time had higher mortality rates (Table 3). Both of these conclusions supported hypotheses generated for this study.

A variable that did not significantly affect larval mortality was the number of days between the spraying and the collection of the milkweed, which refuted one of the hypotheses of this study (Table 3). This was unexpected because permethrin loses its toxicity when exposed to water and UV light. A possible explanation for the continuing lethality of the toxin, even twenty-one days after spraying, was the unusually low exposure the permethrin-sprayed leaves had to rainwater. During July and August of 2003, when the permethrin treatments and milkweed collection occurred for this study, precipitation levels in the Minneapolis-St. Paul area were 4.91 inches below average (5).

In the second part of the study, development times of larvae were affected by the treatment of milkweed leaves on which they were raised. There was no significant difference in development times between larvae reared on control leaves and on oil-treated leaves, but monarchs in both the low and high dose treatments developed more slowly than both controls (Figure 2). This is yet another consequence of permethrin spraying that is detrimental to monarch fitness. Even when permethrin is allowed to decay to toxicity levels that are not lethal to monarchs, this harmful effect on development rate could still exist, although this study did not explicitly address permethrin degradation that occurs under outside conditions.

Figure 2. Mean development time of monarchs beginning experiment during 2nd, 3rd, and 4th instar comparing rates on control leaves, leaves treated with oil, leaves treated with the 0.1% dilution of the operational solution, and leaves treated with the 0.5% dilution of the operational solution. The white numbers on each bar represent the sample size. Development time is defined as the number of days between the start of the experiment and the emergence of the adult monarch. The error bars represent standard error. (High mortality levels were observed in the larvae on the milkweed treated with the 0.5% solution. The only ones which survived to adulthood were initially exposed during the third instar, which is why the only data on development time for larvae on the 0.5% treatment are in the third instar set.)



Adult mass, however, was not significantly affected by the treatment of leaves to which larvae were exposed and on which they fed, except in the monarchs exposed to milkweed treated with the 0.5% dilution (Table 4). The "trade-off" of slower development time for normal adult mass is not uncommon for monarch larvae under unfavorable conditions and is not unique to this study (7). The larvae raised on 0.1%-solution-treated milkweed leaves did achieve normal adult masses but took significantly longer to do so.

Table 4. The results of the linear regression analysis of variables affecting adult mass.

Variable	Coefficient	Std. Error	p-value
Beginning instar	-0.0101	0.0314	0.2639
Oil	0.0024	0.0089	0.8935
0.1% Solution	-0.0242	0.0182	0.1913
0.5% Solution	-0.0864	0.0418	0.0436

This study demonstrated the highly lethal effects of mosquito-control applications of permethrin on monarch butterfly larvae and the sublethal developmental effects of milkweed sprayed in the lab using the same permethrin mixture as is used for mosquito control but more dilute and applied differently. Mosquito-control applications of permethrin may kill non-target larvae, like monarchs, more effectively than adult mosquitoes, since the larvae cannot escape a toxin that covers their host plant, while mosquitoes can fly away to avoid harmful effects. Continued use of permethrin by mosquito control organizations should be seriously reconsidered until further research of its non-target effects is conducted.

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