Lethal and Sub-lethal Effects of Resmethrin on Nontargeted Species

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Introduction

Due to the increased prevalence of West Nile virus in recent years, the insecticide resmethrin is being used more widely to control mosquito populations. However, the effects of resmethrin on many non-target species are not known. The purpose of my study was to determine the lethal and sub-lethal effects of resmethrin on monarch butterflies (*Danaus plexippus*), milkweed bugs (*Lygaeus kalmii)*, and houseflies (*Musca domestica*).

Resmethrin is a synthetic pyrethroid that mimics a substance produced by chrysanthemum flowers. It is a "contact poison which quickly penetrates the nerve system of the insect" (1). The Environmental Protection Agency has permitted the controlled spraying of resmethrin; however, it sets restrictions, as resmethrin has been shown to be extremely toxic to mosquitoes, fish, and bees (2). The oral LD50[1] for resmethrin in rats has been reported as being greater than 2500 mg/kg (3).

There was a pilot study done in 2004 by Lelich on the effects of resmethrin on monarch butterflies, which showed that exposure to resmethrin can be lethal, but there has been no previous research done on the persistence of resmethrin spray or on the effects of resmethrin on many other non-target insects. In 2003, a study by Oberhauser et al. was performed on the toxicity of permethrin, another pyrethroid, on monarch butterflies, which determined that adult mass of monarch butterflies was affected by as little as a 0.5% dilution treatment. In addition, mortality rates were significantly higher in monarch larvae exposed to permethrin-sprayed leaves (4).

The study presented here differed from the Lelich and Oberhauser studies by increasing the number of downwind stations on the resmethrin spray path, testing the

[1] The dose at which is lethal for 50% of the test subjects

effects of resmethrin on milkweed bugs and houseflies, and testing the persistence of resmethrin on monarch butterflies.

This study had four goals. The first goal was to determine whether monarch larvae exposed at closer downwind distances from the spray path would have a greater mortality rate. Based on the results of the Lelich study, which showed that 58% of larvae exposed downwind from the resmethrin spray died, 18% of the larvae exposed directly upwind died, and 9% of the control larvae died, it was hypothesized that monarch larvae exposed to resmethrin at closer downwind distances from the spray path would have a greater mortality rate than those exposed further downwind (5).

The second goal was to determine the sub-lethal effects of resmethrin on monarch butterflies by recording the mass of emergent adult monarchs that had been exposed directly and indirectly to resmethrin spray. Both the study by Lelich and the study by Oberhauser showed a trend towards smaller masses for resmethrin-exposed larvae (4, 5), but these results were not statistically significant. It was hypothesized that the monarch larvae that survived direct and indirect exposure to resmethrin into the adult stage would have smaller masses than control larvae.

The third goal was to ascertain if there were lethal effects of resmethrin on milkweed bugs and houseflies. There has been no previous research done on this, but, since the Environmental Protection Agency has shown that resmethrin is toxic to bees, it was hypothesized that milkweed bugs and houseflies would be adversely affected by resmethrin (2).

The fourth goal was to determine the persistence of resmethrin after spraying. It was hypothesized that survival rate of both directly and indirectly exposed monarch larvae would increase as the days since spraying increased. It was also hypothesized that the survival rate of exposed monarch larvae would increase as the distance from resmethrin spraying increased.

Methods

Preparing Milkweed Plants for Spray: To prepare for spraying, 60 milkweed plants (*Asclepias spp.*) were grown, one stem per pot. Each pot was labeled to reflect where it would be placed on the spray transect. Then, 45 milkweed pots were labeled for treatments, as shown in Table 1: 15 pots as "1-day", 15 pots as "2-day", and 15 pots as "4-day". Then, six second-instar monarch larvae and four fourth-instar monarch larvae were placed on each of the remaining 15 milkweed plants and labeled "0-day". Three pots from each treatment ("0-day", "1-day", "2-day", "4-day") were labeled as controls.

Table 1: Spray Treatments

Treatment Type	**Study Procedure**
0-day	2^{nd} and 4^{th} instar larvae were directly exposed to the resmethrin spray
1-day	2^{nd} and 4^{th} instar larvae were fed leaves from sprayed plants one day after spraying
2-day	2^{nd} and 4^{th} instar larvae were fed leaves from the sprayed plants two days after spraying
4-day	2^{nd} and 4^{th} instar larvae were fed leaves from the sprayed plants four days after spraying

Then, 30 cages of non-targeted species were labeled to reflect where they would be placed on the spray transect: 15 for milkweed bugs and 15 for houseflies. Of those 30 cages, three cages per species were labeled as controls.

Site Setup and Spraying: Resmethrin spraying took place on an outdoor soccer field at the National Sports Center in Blaine, Minnesota. Prior to spraying, two parallel transects, transect A and transect B, were set up with five stations in each transect on the downwind side of the spray path and one station in each transect on the upwind side of the spray path, as seen in Table 2. Downwind stations were located at 15 m, 30 m, 75 m, 120 m, and 170 m from the spray path. The upwind stations were located at 15 m from the spray path.

Table 2: Spray Transects

						Spray Path	
Transect A	**3 mw plants and 1 mw plant w/ larvae +2 cages**	**3 mw plants and 1 mw plant w/ larvae +2 cages**	**3 mw plants and 1 mw plant w/ larvae +2 cages**	**3 mw plants and 1 mw plant w/ larvae +2 cages**	**3 mw plants and 1 mw plant w/ larvae +2 cages**	↓	**3 mw plants and 1 mw plant w/ larvae +2 cages**
Transect B	**3 mw plants and 1 mw plant w/ larvae +2 cages**	**3 mw plants and 1 mw plant w/ larvae +2 cages**	**3 mw plants and 1 mw plant w/ larvae +2 cages**	**3 mw plants and 1 mw plant w/ larvae +2 cages**	**3 mw plants and 1 mw plant w/ larvae +2 cages**	↓	**3 mw plants and 1 mw plant w/ larvae +2 cages**
	170 m	**120 m**	**75 m Downwind**	**30 m**	**15 m**		**15 m Upwind**

Distance from Spray Path

As seen in Table 2, four milkweed (mw) plants, one with "0-day" monarch larvae and three without larvae, were placed at each downwind and upwind station. Then, two cages of non-targeted species were placed at each station: one cage held milkweed bugs and the other cage held houseflies. Control plants with and without larvae and control cages of milkweed bugs and houseflies were set far away from the spray path.

A Metropolitan Mosquito Control District employee drove a sprayer vehicle down the spray path and applied a normal dosage of resmethrin to the site. After waiting 100 m away from the spraying path for 30 minutes after spraying, the milkweed plants were retrieved. Gloves were worn to protect against the spray. All non-targeted cages and milkweed plants were collected, and mortality of the monarch larvae, milkweed bugs, and houseflies was recorded. Then, milkweed plants with monarch larvae on them were cut down at the bottom of the stem and placed into tubs equipped with breathable lids. Cages of milkweed bugs and houseflies, along with tubs of milkweed plants with larvae and sprayed milkweed plants without larvae were transported back to the lab.

Larvae Mortality: Three monarch larvae mortality studies were undertaken in the lab. In the first study, 2nd and 4th instar larvae were fed leaves of resmethrin-sprayed plants one day after the spraying and labeled “1-day” larvae. In the second study, 2nd and 4th instar larvae were fed leaves that had been sprayed two days previously and labeled “2-day” larvae. The third study involved 2nd and 4th instar larvae that were fed leaves that had been sprayed four days prior to feeding and labeled “4-day” larvae. For each study, mortality was recorded each day until remaining larvae emerged as adults. Upon emergence, each adult monarch was weighed.

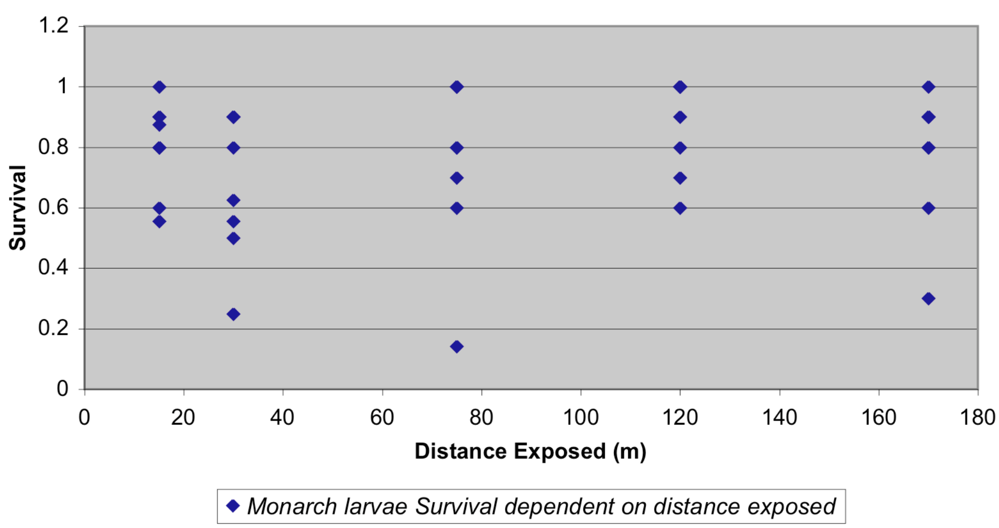
Milkweed Bugs and Houseflies: For each of the first four days after spraying, the mortality of the milkweed bugs and houseflies was recorded. Although they were not fed, water was given once each day.

Statistical Analysis: The final data were sorted into three Excel spreadsheets; one spreadsheet for the data on emergent adult monarchs, one for the monarch larval mortality study, and one for mortality of milkweed bugs and houseflies. The data were then analyzed in Statistix, using a one-way Anova and linear regression t-tests. A p-value of 0.05 was used to indicate significance.

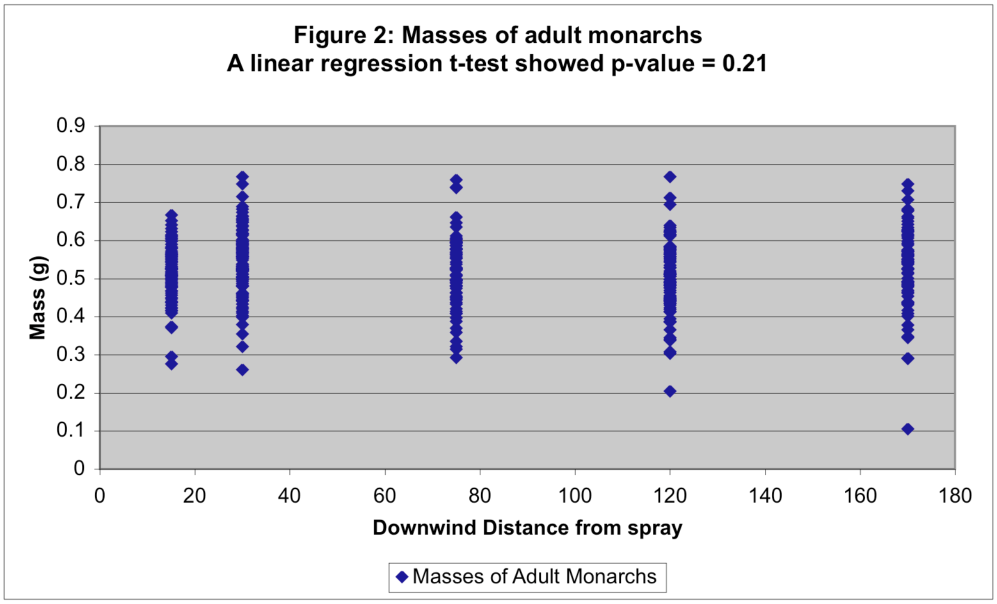
Results

The mortality for each treatment at downwind distances is shown in Figure 1. No statistically significant results were found when the survival rates of monarch larvae that were exposed directly to resmethrin spray at different downwind distances were compared, with a p-value of 0.16.

Figure 1: Monarch larvae survival dependent on distance exposed
A linear regression t-test showed a p-value = 0.16



Masses of adult monarchs exposed both indirectly and directly to resmethrin spray are shown in Figure 2. Downwind distance had no significant effect on mass of adult emergent monarchs, with a p-value of 0.21.



The lethal effects of resmethrin on milkweed bugs are shown in Table 3, and effects on houseflies are shown in Table 4. No statistically significant effects on milkweed bugs or houseflies were found, with p = 0.33 for milkweed bugs and p = 0.26 for houseflies.

Table 3: Mean Survival of Milkweed Bugs; linear regression t-test gave p = 0.33

Location	Number of Cages	Mean Survival	Standard Error
Transect A	6	0.9667	0.0333
Transect B	6	0.9111	0.0588
Control 1	1	0.4000	
Control 2	1	0.8000	
Control 3	1	1.000	

Table 4: Mean Survival of Tachinid Flies; linear regression t-test gave p = 0.26

Location	Numbers of Cages	Mean Survival	Standard Error
Transect A	6	0.1930	0.0797
Transect B	6	0.2080	0.0408
Control 1	1	0.2000	
Control 2	1	0.0000	
Control 3	1	0.6250	

As shown in Table 5, mean survival of monarch larvae is shown by exposure times. A statistically significant direct correlation was found between the mean survival of monarch larvae and the day that the larvae were exposed directly (“0-day”) or indirectly (“1-day” through “4-day”) to resmethrin sprayed leaves, shown by a p-value of 0.0022.

Table 5: Mean Survival Dependent on Day Exposed
A linear regression t-test gave p-value = 0.0022

Treatment Type	Mean Survival
0-day	0.6790
1-day	0.7333
2-day	0.8019
4-day	0.9000

Conclusion

The first hypothesis was not supported. No correlation was shown between the distance the monarch larvae were placed away from the spray path and mortality rate ($p = 0.16$). The second hypothesis was that resmethrin-exposed monarch larvae that survived to the adult stage would have smaller masses; however, this hypothesis was not proven. There was no significant difference in masses between resmethrin-exposed larvae and control larvae ($p = 0.21$). The third hypothesis was that milkweed bugs and houseflies would be affected by resmethrin-spray, however, there was no statistically significant effect of direct resmethrin spray on mortality of either milkweed bugs or houseflies ($p = 0.33$ for milkweed bugs and $p = 0.26$ for house flies). The final hypothesis was that the survival of exposed monarch larvae would be greater as the time after spraying increased. This hypothesis was supported, as there was a statistically significant direct relationship between exposure time to resmethrin and survival ($p = 0.0022$).

Resmethrin spraying was done only once under one set of weather conditions. On the day of the resmethrin spraying, the wind was gusting, possibly concentrating the spray. For future studies, spraying during a steady wind and measuring the wind speed and direction could be beneficial. Also, more rounds of resmethrin spraying could be done on days with different wind speeds, testing the effect of wind speed and direction on the toxicity of the resmethrin spray. Future studies on the effects of resmethrin spraying on non-targeted species at different summer temperature conditions should be done to see if higher temperatures affect mortality. Also, resmethrin studies should be done on other beneficial non-target species, such as ladybugs and spiders.

Few studies have been done on the effects of resmethrin on non-target species. Resmethrin is being used more widely to control mosquito populations, because of the increased prevalence of the West Nile virus. Although controlling disease-transmitting mosquitoes is both necessary and beneficial, its effects on non-targeted species should be known in order to preserve our interconnected environment.

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