Growth and Survival of Monarch Butterflies (Lepidoptera: Danaidae) After Exposure to Permethrin Barrier Treatments

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ABSTRACT We assessed the toxicity of permethrin residues from barrier treatments for mosquito control on monarch butterfly (*Danaus plexippus* L.) larvae and adults. The motivation for this study was an absence of work on the effects of this commonly used insecticide on nontarget herbivorous insects and the fact that monarchs' host plant, milkweed (*Asclepias* spp.), is often found in treated areas. In one experiment, larvae fed leaves from naturally growing milkweeds in operationally treated areas were much less likely to survive than control larvae, even when treated leaves were collected up to 21 d after spraying. In a second experiment, larvae fed leaves from potted milkweeds sprayed with 0.5 and 0.1% dilutions of the operational dose had lower survival and longer development times than control larvae. In a third experiment, ovipositing females did not discriminate against permethrin-treated plants. Female survival in 0.66-m^3 cages with plants sprayed 1 d earlier was very low, whereas survival was greater if plants were assayed 8 or 15 d later. In a fourth experiment, neither frequent overhead watering (versus watering from below) nor outdoor sunlight (versus in a glasshouse) over a 14-d period lessened the toxicity of sprayed plants to larvae. These findings indicate that monarch larvae and adults are likely to be killed if exposed to residues of permethrin after barrier treatments for mosquito control. Extent of mortality in a field population of monarch butterflies will depend, among other things, on the proportion of host plants that are treated in a given area.

KEY WORDS nontarget impacts, Monarch butterfly, *Danaus plexippus*, permethrin, mosquito control

Permethrin is a synthetic pesticide in the pyrethroid group of insecticides. Pyrethroids act in a similar manner to pyrethrins, which are derived from chrysanthemum flowers, but have been modified to increase their photostability and toxicity to insect pests (Mueller-Beilschmidt 1990). Estimates of the half-life of permethrin range from 7 to 21 d (analysis of data presented by Hameed and Allen 1976, Ohkawa et al. 1977, Gaughan and Casida 1978, Southwick et al. 1986).

Permethrin is commonly used for adult mosquito control in residential areas. While integrated mosquito control programs emphasize larvicidal treatments as the primary means of mosquito control, adult control is used to combat outbreaks of mosquito-borne disease or heavy nuisance infestation of mosquitoes (U.S. EPA 2003). The insecticide is applied by trained public health officials and mosquito control district personnel as a foliar treatment along edges of woodlots and other densely vegetated habitats that are thought to harbor adult mosquitoes, although its use is not restricted and it can be applied by the public as well. These "barrier treatments" are intended to reduce densities of mosquitoes that emigrate from the harborage areas by killing mosquitoes that come into contact with treated foliage. It is also applied as an ultra-low volume (ULV) spray, which produces fine aerosol droplets that kill adult mosquitoes on contact. While it is thought that the formulations of permethrin used for mosquito control do not pose unreasonable risks to wildlife or the environment (U.S. EPA 2003), few studies have examined the nontarget effects of barrier applications of permethrin on foliage-inhabiting arthropods.

The magnitude and duration of adverse effects of permethrin on nontarget species are likely to depend on taxon, habitat, and method of application. Permethrin is toxic to honey bees (Morse 1987, Ray 1991) and is excluded from use near apiaries. Jensen et al. (1999) applied permethrin as a ULV fog to plots in California wetlands and found no reductions in the biomass or abundance of macro-invertebrates swept from the water. Abundance of flying insects (Diptera, Lepidoptera, Coleoptera, and Hemiptera) caught in blacklight traps was temporarily depressed but re-

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Table 1. Details of bioassays of field-collected milkweed plant leaves with monarch butterfly larvae, by leaf collection date, source, and barrier treatment history, Twin Cities Metropolitan Area, MN, 2003

Date collected	Days on original leaf	Double blind?	Source	Treatment	Days since spraying	Initial stadia	No. larvae
7/28/2003	2	No	SMPP	Treated	7	1,2,3	20
			KG	Control			20
			LL	Control			20
			LW	Treated	3		20
			LS	Treated	3		20
7/31/2003	5	Yes	SMPV	Treated	10	1,2,3	15
			SMPP	Treated	10		15
			KG	Control			15
			LS	Treated	6		15
8/4/2003	1	Yes	SMPP	Treated	14	1,2,3	15
			KG	Control			15
8/5/2003	2	Yes	CAP	Control		2	15
			COL	Treated	1		15
8/8/2003	3	Yes	COL1	Treated	1	1,2	45
			COL2	Control			45
8/9/2003	5	Yes	SMPP	Treated	19	1	10
			SMPV	Treated	19		10
			KG	Control			6
			LOR	Control			4
8/11/2003	5	No	JY	Control		2	7
			SMPV	Treated	19		7
			SMPP	Treated	21		7
			LOR	Control			7
8/11/2003	3	Yes	COL1	Treated	4	2	45
			COL2	Control			45
8/13/2003	4	No	L	Control		1	15
			JY	Control			15
8/15/2003	4	Yes	COL1	Treated	8	1,2	45
			COL2	Control			45
8/23/2003	2	Yes	COL1	Treated	16		45
			COL2	Control			45

Collection sources: SMPP, St. Mary's Park in Lakeland, MN, treated 7/21; KG, private garden in Roseville, MN; LW, LL, and LS, wooded area, edge of lake, and edge of soccer field in Langton Park in Roseville MN, LW and LS treated 7/25, LL is nearby control; SMPV, vacant lot near St. Mary's Park in Lakeland, MN, treated 7/21; CAP, Capp Road in St. Paul, MN, control; COL, Columbia Park in White Bear Lake, MN, treated 8/4; COL1, treated area of Chain of Lakes Regional Park in Lino Lakes, MN, treated 8/7; COL2, untreated area of Chain of Lakes Regional Park in Lino Lakes, MN; JY, private garden in St. Paul, MN; LOR, garden in St. Paul, MN; L, laboratory milkweed mixed from several control sites.

bounded 48 h after application. Shires (1985) studied agricultural applications of permethrin and found that it decreased the abundance of flying and vegetation-inhabiting arthropods (including predatory beetles), but not soil-dwelling arthropods. Kingsbury and Kreutzweiser (1980) found that permethrin treatments in forests in northern Ontario suppressed nontarget invertebrate populations immediately after spraying, but these populations recovered to control area levels by 2 wk after treatment. Species inhabiting litter and soil were less affected than flying or tree-dwelling species.

The absence of studies of effects of barrier applications of permethrin on herbivorous insects prompted us to study effects on monarch butterfly (*Danaus plexippus* L.) larvae and adults. This charismatic butterfly occurs during summer in most of North America. Adults feed and oviposit on milkweed plants (*Asclepias* spp.), and larvae feed on the same plants. Milkweeds occur naturally in a variety of habitats, including some that are likely to receive barrier applications of permethrin for adult mosquito control. We conducted a series of experiments that considered possible effects of barrier applications of permethrin on survival and development times of monarch butterfly larvae, as well as effects on survival and oviposition by females.

Materials and Methods

Experiment I: Survival on Milkweed Collected in Treated Areas. We collected and assayed leaves of *A. syriaca* L. from sites that had received barrier treatments of permethrin, applied by staff of the Metropolitan Mosquito Control District (MMCD) in the Twin Cities Metropolitan Area of Minnesota (Table 1). The operational solution (OS) contained 0.23 kg (0.5 lb) permethrin per 3.78 liter (1 gal) of 2:1 mineral oil:soybean oil (vol:vol), and was applied at a rate of 0.109 kg active ingredient/ha (MMCD 2003). In some cases (sites SMPP, SMPV, LW, and LS), applicators did not know that milkweed would be collected for study, whereas pending assays were known in other cases (COL, COL1). All spray dates and locations were verified by MMCD staff. We also collected control leaves from unsprayed sites. We picked naturally occurring milkweed ramets and used most

leaves on each ramet, omitting any with herbivore damage.

Individual leaves were bioassayed with monarch larvae 2–4 h after collection. Larvae were F_2 descendants of ≈80 wild eggs and larvae collected in Missouri, Minnesota, and Wisconsin. Experimental larvae were housed individually in 0.5-liter plastic deli containers with porous lids and moistened filter papers to retard leaf drying. For each assay, we prepared sets of larvae with leaves and used a double-blind technique in most cases so that the milkweed source was unknown to the experimenter (Table 1). Larval stadia and sample sizes varied among assays, depending on the amount of milkweed at each site.

After each bioassay was begun, all leaves were replaced with control leaves when any leaf in the assay became too dry to provide adequate nutrition or when any leaf had been completely consumed. We monitored larvae and cleaned the containers daily, recording dates when larvae died. Leaves and larvae were handled with latex gloves to prevent cross-contamination. We recorded the day when surviving adults eclosed and weighed them on a Mettler semimicroanalytical balance (±0.001 g) the next day, after their wings had expanded and they had expelled the meconium. In a few cases, we discontinued assays when all survivors had pupated, and we ascertained that only control individuals were still alive.

Experiment II: Sublethal Effects of Low Permethrin Doses. To further assess effects of permethrin residues on monarch larvae, we applied dilutions of the operational solution to greenhouse-grown *A. currasavica* L. We used the same oil diluent used by the MMCD (2:1 mineral oil:soybean oil) to make 0.5 and 0.1% dilutions by volume of the operational solution (OS). These dilutions were chosen after >95% mortality within 48 h was observed in larvae exposed to 50, 10, 5, 2, and 1% OS dilutions. Application rates were calibrated to match the operational method, using methods described by Rathburn (1970). We estimated the volume of solution applied to a 1-cm square on a Teflon-coated slide, measured the diameter of all droplets in the square, calculated their volumes ($\pi/6 \times d^3$), and summed them to get a total volume on the square. We conducted this procedure with $n = 10$ slides at several spraying distances to find the distance that delivered the same volume per square centimeter as the operational method. We used the calibrated procedure to spray three sets of plants: one with a 0.5% dose, one with a 0.1% dose, and one with oil diluent alone. A fourth group was left unsprayed.

Leaves from the four sets were bioassayed with sets of larvae as in experiment I, with the exception that larvae were fed either control or treated leaves throughout their development. Assays of each treatment group were conducted with 10 larvae in each of three stadia (second, third, and fourth), for a total of 120 larvae ([2 control + 2 permethrin] treatments × 3 stadia/treatment × 10 larvae/stadium). Larvae received an appropriate new leaf daily, and date of death, pupation, and emergence were recorded. We weighed adults 24 h after eclosion, with the exception of a few that were mistakenly discarded before masses had been recorded.

Experiment III: Oviposition Choice and Adult Survival. We grew potted specimens of *A. currasavica* in a glasshouse until 20–30 cm tall and haphazardly designated them to receive no spray, diluent oil only, or operational permethrin. Permethrin plants were sprayed as part of a regular MMCD operation in a city park in Roseville, MN. We placed them along a spraying transect, and the insecticide was applied to the experimental plants and natural vegetation by a trained applicator. After treatment, we transported plants to the glasshouse (16:8 L:D cycle) for assays with female butterflies. Diluent plants were treated as described above.

We conducted assays in 12 0.66-m^3 cages, screened on all sides, and arranged ≈8 cm apart on a single glasshouse bench. Cage treatments differed in the kinds of plants placed in a cage and the time of assay after spraying. "Control" cages contained three nonsprayed plants, arranged ≈25 cm apart in an equilateral triangle, with no contact between plants. "Choice" cages contained one nonsprayed plant, one sprayed with oil only and one sprayed with operational permethrin. "Permethrin" cages contained three permethrin-treated plants. There were four replicate cages for each treatment, treatments were assigned to cages randomly, and positions of permethrin-treated plants in choice cages were rotated among replicates. After assembly, three haphazardly chosen mated female butterflies were introduced into each cage and fed a 20% honey solution ad libitum. After 48 h, we removed females from the cages, recorded whether each was alive or dead, and counted eggs on each plant. The same experiment was conducted with plants 1, 8, and 15 d after sprays were applied. We kept plants in the glasshouse until they were used.

Experiment IV: Environmental Persistence. We bioassayed the persistence of permethrin residues using 96 potted 70- to 80-cm-tall *A. currasavica* plants. We used a 2 × 2 × 2 × 3 factorial treatment design replicated four times. Plants were (1) sprayed with the operational permethrin dose or were unsprayed controls; (2) exposed outdoors to UV light in full sun or protected inside a glasshouse; (3) watered daily with a hose from above to simulate rain or given the same amount of water but from below in dishes under the pots; and (4) assayed the day of spraying or 7 or 14 d after spraying. Permethrin-treated plants were sprayed as part of a regular MMCD operation in a city park in Roseville, MN, where plants were placed along a spraying transect as described for experiment III. After treatment, the plants were relocated to the University of Minnesota campus, where one half were housed outdoors in a location receiving full sunlight all day, and the other half were in a glasshouse, which presumably reduced UV light exposure. All plants received approximately equal amounts of water, measured by level of filling of trays underlying all pots.

We bioassayed leaves from plants in the 24 treatment groups by feeding them to larvae for the duration of their development, beginning as first instars on the

designated day after spraying. Each assay involved four larvae, started together in a 0.5-liter deli container, and moved as a group to a 25 by 6 by 5-cm plastic container with a screen top when they reached third instars. Each group was fed leaves from the same plant for their entire lives or until all of the leaves from the plant were used, when they were fed leaves from extra control plants. We recorded the date when each larva died and discontinued all larvae from a particular starting date when all larvae fed permethrin treated leaves had died in all exposure treatments.

Statistical Analyses. We used logistic regression (Analytical Software 2003) to assess how survival was affected by permethrin treatment and other independent variables in experiment I, because the dependent variable was binomial. Variables considered were starting stadium, duration of exposure to assayed leaves, number of days between permethrin application and larval exposure, and site from which the leaves were picked. Stadium and site were coded as categorical indicator variables, whereas the remaining ones were continuous. To estimate survival caused by exposure to permethrin, we adjusted for survival among matching control groups, where adjusted survival = 100 × [(no. alive in control group before treatment × no. in treated group after treatment)/(no. in treated group before treatment × no. in control group after treatment)] (Henderson and Tilton 1955).

We used multiple linear regressions (Analytical Software 2003) to determine how the independent variables of treatment (included in the models as indicator variables) and initial stadium (included as an ordered variable) affected adult mass and development time in our study of sublethal impacts of diluted permethrin treatments (experiment II). In the oviposition study (experiment III), we used ANOVA models to test the effects of cage and plant treatment, and start date on the number of eggs laid on individual plants. We used Tukey's honestly significant difference (HSD) post hoc test procedure to compare mean levels of adult survival and oviposition among treatment groups. We used ANOVA to test the effects of spray treatment, exposure, watering, and days after treatment in the persistence study (experiment IV). The dependent variables were the arcsine-transformed proportion surviving and the mean time to death within each rearing container.

Results

Experiment I: Survival on Milkweed Collected in Treated Areas. Figure 1 shows overall larval survival in all experiment I assays. Odds of surviving were significantly lower on leaves from permethrin-treated sites than from nontreated sites and lower for first instars than later instars (Table 2). The odds ratios in Table 2 indicate that larvae fed treated leaves were 2% as likely to survive as control larvae and that first instars were approximately one third as likely to survive. The estimated overall mortality caused by being exposed to permethrin, using the Henderson-Tilton correction for mortality in the control group, was 92, 89, and 71% for first, second, and third stadia, respectively. Larvae killed accidentally during handling or trapped in milkweed latex were removed from the data set. A total of 13 larvae were removed from the data set for this reason: 9 control larvae and 4 larvae fed milkweed from treated areas.

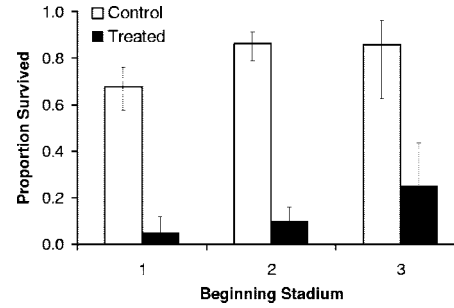

Fig. 1. Overall survival on leaves from permethrin-treated and nontreated milkweed in experiment I, combining all experimental and control monarchs. Error bars represent 95% CIs.

When we restricted analysis to the six treated sites (Table 2), survival rates were significantly lower for first instars, lower with increasing numbers of days on treated leaves (Fig. 2a), and higher with increasing days after spraying (Fig. 2b). After adjusting for differences in stadium and exposure time, larvae were more likely to survive if fed leaves from three of the treated sites (SMPV, LW, and LS) than from the remaining three sites (Fig. 2c). Because there was no trend toward higher survival on milkweed from sites at which the applicators knew that the milkweed would be collected (COL and COL1), it is unlikely that this knowledge affected our results.

Development times of monarchs fed control leaves were not significantly different from those of survivors on leaves from the six treated sites when initial stadium was included in the ANOVA model ($F = 1.96$; df = 1; $P = 0.167$), nor were masses of the resulting adults (mean mass ± SE for control and treated were 0.527 ± 0.010 and 0.536 ± 0.010 g, respectively; $T = 0.37$; df = 101; $P = 0.69$). Note that the survivors were all fed control leaves after their initial 1–5 d on treated leaves.

Experiment II: Sublethal Effects of Permethrin-Treated Milkweed. Larvae fed leaves treated with either the 0.5 or 0.1% OS were less likely to survive than those fed leaves treated with the control solution of oil only or untreated leaves (Fig. 3). Survival of larvae fed the 0.5 and 0.1% dilutions were different from each other and from both control groups (two sample proportion tests, all $P < 0.01$). Survival rates on leaves sprayed with oil alone were equivalent to those on untreated controls (two sample proportion test, $P = 0.65$). There was a tendency for larvae that received leaves with the 0.5% dose to die sooner than those that received 0.1% dose, although this difference was not statistically significant (mean times to death ±

Table 2. Final binomial regression models of survival probabilities in experiment I

Predictor	Coefficient	SE	Coef/SE	*P*	Odds ratio (95% CI)
Treated and nontreated sites combined					
Constant	1.81	0.21	8.54	<0.0001	
Treated leaves	−3.74	0.27	−13.81	<0.0001	0.02 (0.01–0.04)
First instar	−1.06	0.26	−4.00	0.0001	0.35 (0.25–0.58)
Deviance	441.87				
df	546				
Cases included	549				
Treated sites only					
Constant	−1.13	0.83	−1.37	0.170	
First instar	−1.33	0.61	−2.19	0.028	0.26 (0.08–0.87)
Days on orig leaf	−1.00	0.24	−4.24	<0.0001	0.37 (0.23–0.58)
Days since spraying	0.173	0.054	3.30	0.001	1.19 (1.07–1.32)
Site SMPV	2.69	0.99	2.72	0.006	14.68 (2.12–101.7)
Site LW	2.93	0.81	3.62	0.0003	18.57 (3.81–90.5)
Site LS	1.80	0.70	2.57	0.010	6.01 (1.53–23.5)
Deviance	126.60				
df	276				
Cases included	283				

Model in Treated and nontreated sites combined only tested the effects of treatment and beginning stadium (included in the model as indicator variables). Odds ratios give the change in odds for an increase in one unit of the independent variable.

SE for 0.5 and 0.1% doses were 6.0 ± 1.1 and 9.1 ± 2.2 d, respectively; T = 1.37; df = 32; $P = 0.18$). The likelihood of mortality was not affected by beginning stadium (tested with logistic regression using indicator variables for stadia and controlling for leaf treatment; all $P > 0.30$). Of the 60 larvae exposed in the two permethrin treatment groups, 37 died, including 33 as larvae and 4 as pupae.

Development times of survivors, after adjusting for initial stadium, were significantly longer in the two permethrin groups than in the oil and nontreated groups (Fig. 4; linear regression coefficients for the 0.1 and 0.5% indicator variables were 1.97 [$P < 0.0001$] and 2.79 [$P < 0.0001$], respectively, suggesting increased times of ≈2 and 3 d). Sex did not affect development time ($P > 0.3$).

The adult masses of survivors in the control, diluent only, 0.1% OS, and 0.5% OS treatments were 0.420 ± 0.013, 0.423 ± 0.013, 0.397 ± 0.013 and 0.336 ± 0.040 (SE) g, respectively. The two monarchs that consumed the 0.5% dilution and for which we obtained masses were among the lightest 10 of the 59 monarchs that survived to adulthood and were weighed; this difference was marginally significant (linear regression coefficient for the 0.5% indicator variable was −0.08 [$P = 0.061$], suggesting decreased mass of ≈0.08 g). Adult masses were otherwise independent of the remaining spray treatments and initial stadium (all $P > 0.2$ in regression model).

Experiment III: Oviposition Choice and Female Survival. Among cages set up 1 d after spraying, only 1 of 12 females survived in the four cages with three permethrin-treated plants; 2 of 12 survived in the four choice cages that contained a mix of permethrin-treated, oil-treated, and nontreated plants; and 11 of 12 survived in cages with three nontreated plants (Fig. 5a). In contrast, survival rates were consistently higher in all three cage treatments that were assembled with plants sprayed 8 and 15 d earlier. An analysis of variance (ANOVA) of the number of females alive in each cage showed that the effects of start day ($F = 13.29$; df = 2; $P < 0.0001$), cage treatment ($F = 3.86$; df = 2; $P = 0.036$), and the interaction between start day and cage treatment ($F = 5.57$; df = 4; $P = 0.0021$) shown in Fig. 5a were all significant.

The number of eggs laid on a plant was affected by the start day ($F = 15.74$; df = 2; $P < 0.0001$), with fewer eggs laid 1 d after spraying, and the interaction between start day and cage treatment ($F = 4.34$; df = 4; $P = 0.0029$), with fewer eggs laid on plants in permethrin and choice cages than in control cages 1 d after spraying (Fig. 5b). When cage treatment was included in the model, there was no effect of plant type itself ($F = 1.54$; df = 2; $P = 0.219$), nor was there an effect of the interaction between start day and plant type ($F = 1.25$; df = 4; $P = 0.296$). Figure 5b shows the sum of all of the eggs on the three plants within a cage. Figure 5c shows the mean number of eggs on each of the plant types within the choice cages only to illustrate the lack of a plant effect in this treatment.

Experiment IV: Environmental Persistence. Of the 192 larvae in all permethrin treatments, only 3 survived to adulthood (Table 3), and all of these were fed leaves from the same plant (kept inside and watered from above). ANOVA showed survival was significantly lower if plants were sprayed with permethrin ($F = 265.2$; df = 1; $P < 0.001$), if they were assayed the day of spraying ($F = 5.39$; df = 2; $P = 0.0062$), and if they were kept outdoors ($F = 6.74$; df = 1; $P = 0.011$). Effects of time after spraying and location were both caused by differences within the nonpermethrin treatment groups (Table 3; $P < 0.05$, Tukey's HSD test). Larvae on permethrin-treated leaves died sooner if on freshly treated leaves than on leaves treated 7 or 14 d before exposure (Fig. 6; $F = 9.87$; df = 2; $P = 0.0003$). Neither exposure to UV light ($F = 0.64$; df = 1; $P =$

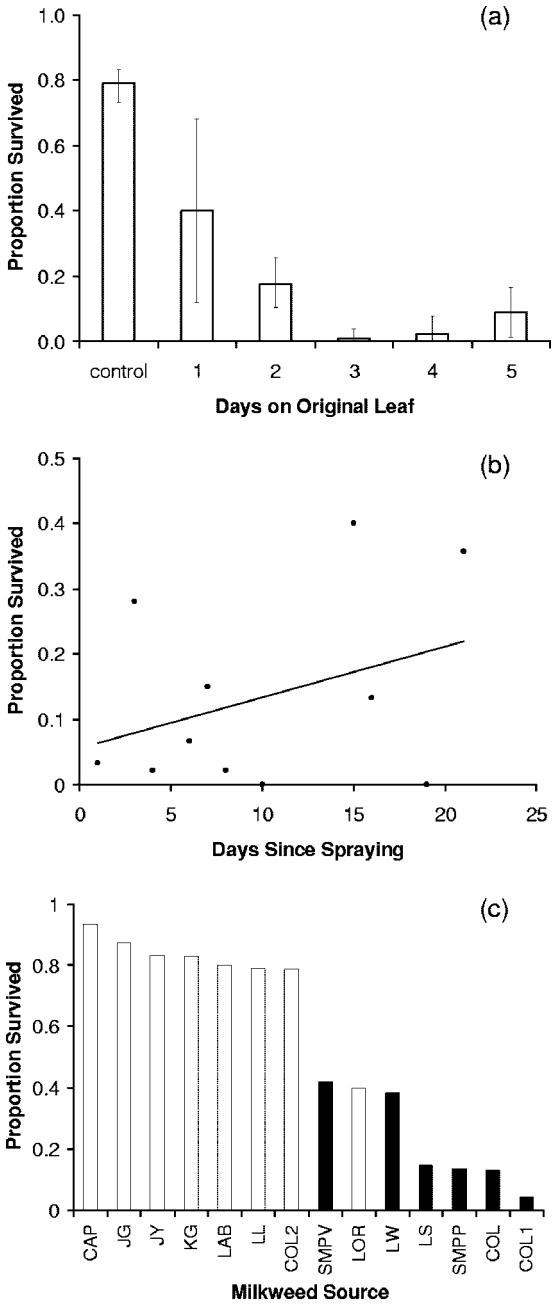

Fig. 2. Factors that affected larval survival in experiment 1. (a) Survival (with ±95% CIs) as a function of the amount of time that the larvae spent on the treated leaf. (b) Survival as a function of the time elapsed between spraying and larval exposure. Regression line shows fitted values from regression in Table 2. (c) Comparison of mortality of larvae from all sites (see Table 1 for site names and locations, and specifics of larval treatment). Open bars, control plants; solid bars, plants from sprayed areas.

0.427) nor watering regimen ($F = 2.53$; df = 1; $P = 0.119$) affected time of death on the permethrin-treated plants.

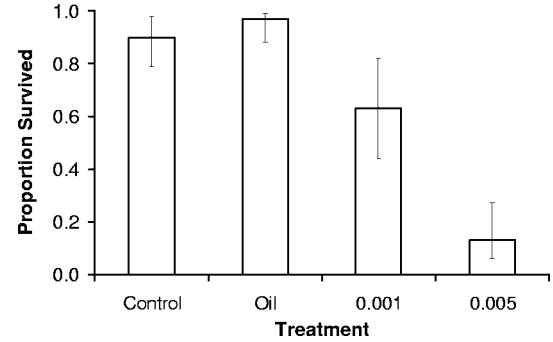

Fig. 3. Comparison of survival of larvae fed leaves in different experiment II treatments (±95% CIs).

Discussion

Risk Assessment Framework. Risk to a nontarget population is the combined probability of exposure to the toxic agent and the toxic effect of this agent (U.S. EPA 1998):

$$R = P_e \times P_t$$

The experiments reported here only address the latter part of the risk equation. To estimate exposure risk (P_e) to monarchs, it will be necessary to estimate the proportion of milkweeds with lethal amounts of the pesticide on their leaves. This will entail plant surveys and more detailed studies of pesticide fate. Because permethrin as a barrier treatment is usually applied in cities and suburbs, published data on landscape-level monarch and milkweed abundance, which focuses on rural areas (Oberhauser et al. 2001), will not be useful in assessing P_e for permethrin applied in this manner.

Toxic Effects of Permethrin. The toxic effect (P_t) of barrier treatments of permethrin for mosquito control

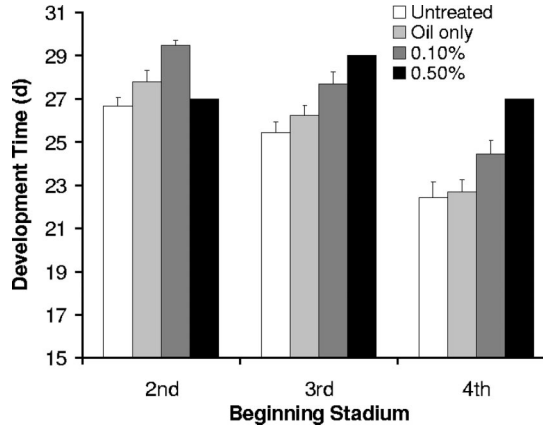

Fig. 4. Mean development time of monarchs (±SE) by initial stadium, where development time = the number of days between the start of experiment II and the emergence of the adult monarch (this varies between group because each group started at a different stadium). Note that there were only one, two, and one survivors in the 0.5% OS treatments for beginning stadia 2, 3, and 4, respectively.

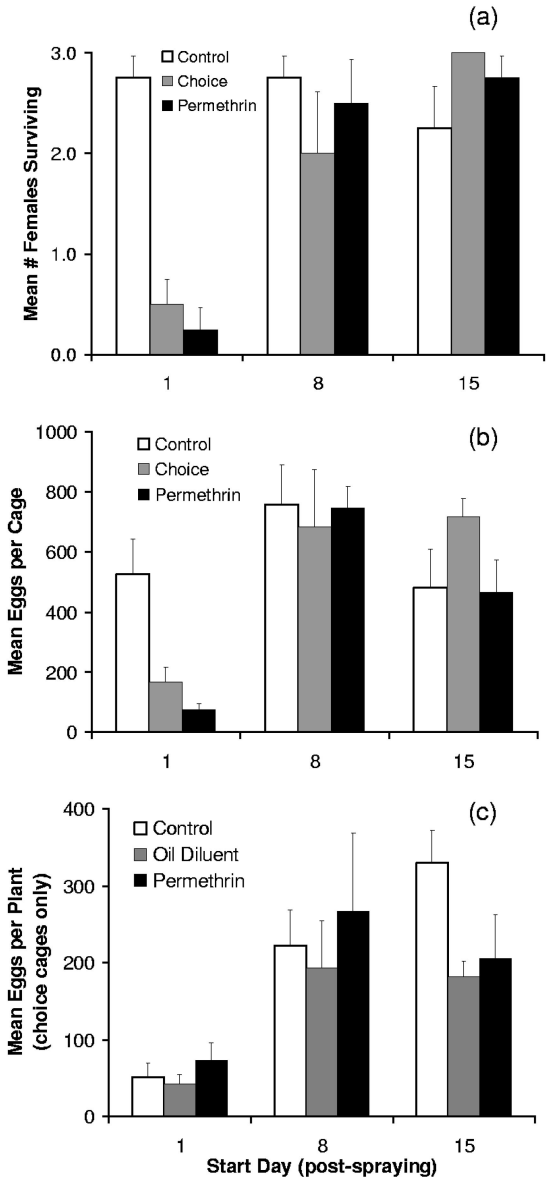

Fig. 5. Results of experiment III. (a) Mean number of females (±SE and of three) that survived in each cage treatment. (b) Mean number of eggs (±SE) summed over all plants within a cage ($n = 4$ cages per treatment per round). (c) Mean number of eggs (±SE) laid on plants of different types within the choice treatment cages only.

is high for monarch butterfly larvae, and possibly adults, that come into contact with milkweed plants in treated foliage. Dilutions down to 0.1% of the operational solution were as toxic as the full dose 1 d after application, and residues killed larvae for up to 21 d after application (experiments I and II). Furthermore, weathering by sunlight and simulated rain for 14 d after application did not reduce the apparent toxicity of the full-strength solution (experiment IV; see also Hameed and Allen 1976 and Gaughan and Casida 1978). However, larvae survived longer when they were fed plants that were watered from above, suggesting that rain does wash off some of the permethrin, just not enough to allow development to adulthood. We do not know what caused higher mortality in the control larvae that were fed leaves from plants kept outside in experiment IV. There are nearby agricultural fields and vehicle traffic, and contaminants associated with these factors may be responsible.

Table 3. Proportion of larvae surviving to adulthood in experiment IV as a function of permethrin exposure, location, and initial exposure date (d after application)

Treatment	Location	Watering	Survival by postspraying start day ($n = 16$ larvae per treatment)		
			0	7	14
Control	Glasshouse	Above	1	0.75	1
		Below	0.88	0.75	0.94
	Outside	Above	0.69	0.44	0.62
		Below	0.88	0.44	0.75
Permethrin	Glasshouse	Above	0	0.19	0
		Below	0	0	0
	Outside	Above	0	0	0
		Below	0	0	0

Experiment I provided insights on factors that could affect monarch survival in sprayed areas. Thirty-four larvae survived exposure to leaves collected in areas that had been sprayed; many of these crawled off of the leaves within their containers and did not eat until they were given new, unsprayed leaves. We did not determine if and to what extent this behavior occurs in the wild. It is also possible that some of the leaves were not in the path of the permethrin application, although the high mortality suggests that the application method effectively reached most of the leaves in a sprayed area.

Leaves from some sprayed sites resulted in lower mortality than those from other sites, and those from one unsprayed site resulted in mortality similar to leaves from treated areas. It is possible that the lower mortality from some sprayed sites resulted from variation in application. The unsprayed site with high mortality (LOR) was adjacent to a busy city street,

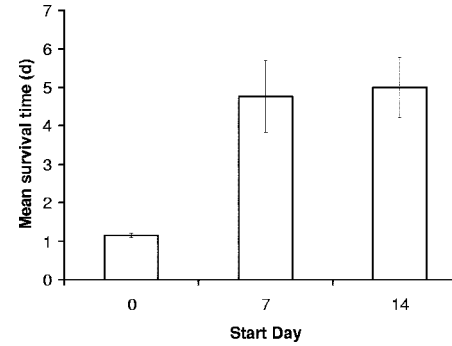

Fig. 6. Mean time to death (±SE) for larvae fed permethrin leaves as a function of experimental start day in experiment IV.

suggesting that factors associated with a road may have contaminated the leaves collected from this site.

Our measures of permethrin persistence in experiments I and IV were consistent with earlier studies (Hameed and Allen, 1976, Ohkawa et al. 1977, Gaughan and Casida 1978, Southwick et al. 1986), where residue half-lives on foliage of several different crop species ranged from 1 to 3 wk and were relatively unaffected by variation in exposure to direct sunlight and rainfall.

We did not attempt to calculate a larval LD_{50} from results in experiment II, because we had an insufficient number of dilutions within the active range. Nevertheless, an LD_{50} would appear to be within 0.1 and 0.5% OS, because ≈20 and 85% (adjusted for contol mortality) of exposed larvae died at these two concentrations, respectively. Results from experiment I suggest that LD_{50}s may differ slightly among stadia.

Survivors of both the 0.1 and 0.5% doses in experiment II developed more slowly than those in control groups, although sample size in the 0.5% group was too small to declare the differences significant. The 0.1% dose did not affect adult mass, but the few survivors of the 0.5% dose were marginally lighter. Slower development from egg to adult is likely to decrease fitness, largely by increasing exposure to larval predators (Borkin 1982, Lynch and Martin 1993, Calvert 1999, Prysby 2004). Furthermore, reduced adult mass could diminish realized fecundity (Oberhauser 1997) and ability to migrate successfully from northern natal sites in temperate latitudes to overwintering localities in California and Mexico (Masters et al. 1988, Alonso-Mejia et al. 1997, but see Borland et al. 2004).

Effects of permethrin were also evident among adults caged on operationally treated plants (experiment III). Residues 1 d after application were great enough to kill almost all females, but not after 8 or 15 d. Increased survival with time after application may have occurred because a female, having more mass than a larva in an early stadium, may have an LD_{50} that is closer to 100% OS, and residue levels probably declined after application. It is also possible that increased survival occurred because permethrin residues may have been transported internally from the milkweed leaf surfaces, as was found with two species of beans (Ohkawa et al. 1977, Southwick et al. 1986). Larvae, which consume both surface and internal leaf tissues, would be exposed to residues in both locations, whereas adults do not consume leaf tissue and would only be exposed to residues on leaf surfaces. Formal studies of dose responses of adults and larvae and of fate of residues on and inside milkweed leaves would be informative.

Equivalence in numbers of eggs laid on the different plant treatments in the choice cages (Fig. 5c) indicated that surviving females did not discriminate against permethrin-sprayed plants. This finding suggests that wild females are not likely to avoid laying eggs on treated milkweed plants. Consequently, for as long as residues from barrier treatments persist above levels toxic to monarch larvae, larvae will occur on milkweed with toxic levels of permethrin.

Potential Ecological Impacts of Pemethrin. Our results suggest that barrier applications of permethrin for mosquito control will have harmful effects on individual insect herbivores exposed to the pesticide. These effects are likely to persist for at least 3 wk.

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA, as amended through P.L. 108–199 in 2004) mandates pesticide registration and outlines a framework for rules of conduct, implementation, and decision-making. Registration data submitted by an applicant must assure that use of the pesticide as proposed will not cause unreasonable adverse effects on people, wildlife, or the environment. Adverse effects of barrier treatments with permethrin as used in our studies cannot yet be fully quantified because of a lack of information on the proportion of the monarch population that will be affected. However, the experiments presented here indicate that residues could be great enough to kill monarchs and possibly other nontarget herbivores that come into contact with treated vegetation. For a population of monarch butterflies in a given area, the magnitude of an effect will depend on the fraction of host plants that are treated, adult movement through the area, and when during the butterfly's reproductive season treatment occurs. Additionally, the extent to which permethrin barrier treatments might affect abundance of monarch butterflies is likely to be modified by a multitude of factors involving weather, natural enemies, and host plant availability and distribution.

Further studies will address the degree of overlap between permethrin barrier treatments, monarchs, and milkweed. Other studies that could help to assess the environmental effects of this form of mosquito control include impacts on other herbivores and the relative costs and benefits of this and other forms of mosquito control, such as ULV applications and more intensive larval control. Because larval control methods are generally more taxon specific, nontarget impacts are likely to be lower.

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