Journal of the American Mosquito Control Association, 24(2):270–280, 2008

EFFECTS OF SINGLE AND MULTIPLE APPLICATIONS OF MOSQUITO INSECTICIDES ON NONTARGET ARTHROPODS

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ABSTRACT. Mosquito management plans have been implemented in the United States and globally to manage mosquito vectors of West Nile virus and many other diseases. However, there is public concern about ecological risks from using insecticides to manage mosquitoes. Two studies were conducted during the late summers of 2004 through 2006 at Benton Lake National Wildlife Refuge near Great Falls, MT. The first experiment was conducted in 2004 and 2005 to assess acute impacts of mosquito adulticides (permethrin and d-phenothrin) and larvicides (*Bacillus thuringiensis israelensis* and methoprene) on nontarget aquatic and terrestrial arthropods after a single application. The second experiment was conducted in 2005 and 2006 to assess longer-term impacts of permethrin on nontarget terrestrial arthropods after multiple repeated applications. For aquatic samples, in the first study, no overall treatment effects were observed despite a potentially deleterious effect on amphipods on sample date 1 in 2004. During the same study, 1 of 54 responses had a significant overall treatment effect for sticky-card samples. Many of the responses for sticky-card samples suggested significant time effects and time × treatment effects. Three response variables were associated with fewer individuals present in the insecticide-treated plots in a multivariate analysis. For the multiple-spray study conducted in 2005 and 2006, 6 of the response variables collected via sticky cards exhibited significant overall treatment effects, but none was associated with fewer individuals in the insecticide-treated plots. None of the responses collected using sweep-net sampling suggested overall treatment effects. Time and time × treatment effects were prevalent in 2005, but no discernable pattern was evident. In general, nearly all of the responses evaluated for either study indicated few, if any, deleterious effects from insecticide application.

KEY WORDS Mosquito control, adulticide, larvicide, ecological risk, nontarget organisms, West Nile virus

INTRODUCTION

West Nile virus (WNV) has been a concern for people across the United States since the disease was initially observed in North America during the summer of 1999. Since that year, WNV has caused the largest arboviral encephalitis epidemic in US history (Huhn et al. 2003). The disease has resulted in thousands of human morbidity cases and hundreds of deaths (Huhn et al. 2003). Many people are concerned about the risks associated with managing mosquitoes that vector WNV using insecticides (Peterson et al. 2006). This concern is related to the perception that ecological and human exposure to the insecticides will lead to risks that are more severe than from WNV itself.

Many of the studies conducted to measure effects of mosquito management chemicals on arthropods have focused on honey bees (*Apis mellifera* L.). Coldburn and Langford (1970) found high bee mortality from applications of naled, malathion, and pyrethrum; although this study did not use ultra-low volume (ULV) applications, it suggests that these chemicals may cause nontarget arthropod mortality. Caron (1979) exposed caged honey bees and beehives to ULV applications of malathion, naled, and pyrethrum. Bee mortality decreased as distance increased from the margin of the insecticide application. Pankiw and Jay (1992) found that honey bees in cages experienced significant mortality from malathion spray drift. Hester et al. (2001) observed significant bee mortality in hives that were exposed to malathion both in open fields and in a forested environment. Zhong et al. (2003) found that aerially applied naled had a negative effect on honey bees and reduced their honey production over a season. Tietze et al. (1996) used sentinel crickets, rather than bees, to measure malathion deposition in a peri-domestic environment. Cricket mortality varied from 12.5% to 48.7%, depending on the location of the crickets in residential yards.

Other researchers have focused on aquatic invertebrates. In laboratory studies, Siegfried (1993) found permethrin to be toxic at low concentrations (2.9–5.9 μg/liter) to several aquatic insects, including black flies, caddisflies, mayflies, and damselflies. Milam et al. (2000) found that when permethrin was applied at 219 ml/ha over test chambers (50-ml beakers) in the field, the nontargets *Daphnia pulex* de Geer, *Ceriodaphnia dubia* Richard, and *Pimephales promelas* Rafinesque had 90% survival in 8 of 10 experiments. Few experimental data are available for d-phenothrin.

Risk assessments have been conducted to predict mosquito insecticide risk to humans and

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to ecological receptors. Peterson et al. (2006) found that risks from adulticides to humans was most probably negligible, especially in the context of a WNV outbreak. Davis et al. (2007) developed a screening-level ecological assessment. Results from this assessment predicted that adulticide risks to terrestrial vertebrates and aquatic organisms would also be negligible.

The predominant larvicides now used in the United States are *Bacillus thuringiensis israelensis* (*Bti*) and the insect growth regulator methoprene. The *Bti* endotoxins are almost nontoxic to mammals and birds (Mittal 2003), although toxic to some aquatic receptors. Milam et al. (2000) found that toxicity of *Bti* was much greater to *Daphnia pulex* than to *Anopheles quadramiculatus* Say when applied as a liquid formulation.

Ecological effects have been monitored for *Bti* used for black fly and mosquito management. Merritt et al. (1989) found few changes in indices used to measure treatment effects of *Bti* used for black fly management in a Michigan river. Drift samples taken at a control and treatment site did not differ for chironomids, baetids, gammarids, or hydropsychids, but did have some treatment effects on perlid stoneflies and elmid beetles. Similar results were observed in 10 stream trials measuring stream insect density of selected taxa. Molloy (1992) observed that *Bti* applied for black fly control within a New York stream affected filter-feeding chironomids, but not surface-dwelling or tube-dwelling members of the same family. Caddisflies and mayflies also showed no response to *Bti* treatments.

Although methoprene toxicity to terrestrial vertebrates is very low, fish are susceptible to methoprene and *Bti* exposure. Methoprene degrades quickly in soil, groundwater, exposed water, and on vegetation (USEPA 1991). Methoprene degrades rapidly in water; its reregistration eligibility document suggests that 80% will degrade within 13 days after application (USEPA 1991). Methoprene has adverse effects on aquatic arthropods, including the freshwater amphipod *Gammarus* sp. (Breaud et al. 1977), the mayfly *Callibaetis pacificus* Seemann, the dytiscid beetle *Laccophilus* sp., and chironomids (Norland and Mulla 1975).

We assessed some of the potential ecological effects associated with mosquito management agents, both adulticides and larvicides, at a field site in central Montana. Two pyrethroid adulticides with the synergist piperonyl butoxide (PBO), permethrin (Aquareslin®) and d-phenothrin (Anvil®), and 2 larvicides, *Bti* (Vectobac®) and methoprene (Altosid®), were evaluated for acute effects in an environmental setting that includes a permanent water body. A study also was conducted to determine the effects on terrestrial arthropods potentially exposed to permethrin and PBO (Biomist®) after multiple applications.

MATERIALS AND METHODS

Two studies (representing 2 replications of 2 separate experiments) were conducted during the late summers of 2004 through 2006 at Benton Lake National Wildlife Refuge near Great Falls, MT. The first experiment was conducted in 2004 and 2005 to assess acute impacts of mosquito adulticides and larvicides on nontarget aquatic and terrestrial arthropods after a single application. The second experiment was conducted in 2005 and 2006 to assess longer-term impacts of a mosquito adulticide on nontarget terrestrial arthropods after multiple applications.

Terrestrial and aquatic single-spray study, 2004–2005

The site for the first experiment was near a road that parallels Pond 2 within the wildlife refuge (47°41′34.43″N, 111°20′47.69″W–47°41′5.68″N, 111°20′54.01″W). The experiment was arranged in a randomized complete block design (RCBD) with 5 treatments replicated 3 times. The blocking factor was location along the length of the experimental site. Experimental units were 30.48 m in length, 22.86 m in width, with a buffer zone of 15.24 m between each unit. The total length of the site was approximately 686 m, and the area was approximately 15,678 m^2. Treatments consisted of 2 adulticides applied at their maximum labeled rates, d-phenothrin (4 g/ha) + PBO (39.2 g/ha) (Anvil 10+10; Clark Mosquito Control, Roselle, IL) and permethrin (7.8 g/ha) + PBO (39.2 g/ha) (Aquareslin; Wellmark, Jayhawk, KS), 2 larvicides applied directly to water, *Bti* (302.6 g/ha) (Vectobac, Valent BioSciences, Walnut Creek, CA) and methoprene (14 g/ha) (Altosid Wellmark International, Schaumburg, IL), and an untreated control. Adulticides were applied via ULV sprayer downwind to the pond at dusk to represent a reasonable worst-case acute exposure scenario. Larvicides were applied as liquids directly to water.

Samples were taken from each plot 1, 7, 14, and 28 days after the treatments were applied, using a variety of techniques to capture both terrestrial and aquatic invertebrates. For terrestrial arthropods, 2 Olson yellow sticky cards (Olson Products, Medina, OH) (7.62 × 12.7 cm) were placed in each plot at 1 m high, 1 upwind and 1 downwind of the spray zone, to survey flying insects (in 2004, 2 sticky cards were used on the same stake facing in perpendicular directions). Sticky-card samples were gathered 1, 2, and 4 wk after treatments, but not the first sample date (1 day after treatment). Samples were taken to make weekly estimates of individuals captured. The sticky-card counts for the last sample date were divided by 2 to represent average weekly counts.

For aquatic arthropods, a 500-μm D-shaped net (Bioquip Products, Rancho Dominguez, CA)

was used to capture sediment-dwelling aquatic invertebrates. Dipper samples were taken to collect free-swimming aquatic invertebrates (e.g., *Daphnia magna* Straus). Bottom dredge samples were taken (2004 only) to capture benthic invertebrates that live in the pond sediment. Fauna captured in D-nets in 2004 was similar in number and type to those captured in the dredge samples; thus, we took only D-net samples the second year. At each sampling date, 2 D-net, dipper, and dredge subsamples were taken from each experimental unit at random locations approximately 3 m from the water's edge. Each sample from the aquatic environment was preserved in 95% ethyl alcohol, sorted, and counted.

Arthropod counts were recorded for each sampling type. Insects were classified into orders and families. Noninsects (such as amphipods and spiders) were classified to order. The sum of 2 samples from dipper and the D-net was used to estimate abundance within each plot. Family diversity, richness, and evenness were calculated for each sticky-card sample using the following equations:

$$\text{if } a_i = 0 \text{ then } D_i = 0, \text{ if } a_i > 0$$
$$\text{then } D_i = -(a/t) \times \ln(a/t) D = \sum_{i=1}^{n} D_i, \quad (1)$$

where D is the diversity index for the treatment, n is the number of families observed, a is the abundance of a family within the sample, and t is the total of all the organisms within the sample (Magurran 1988);

$$\text{if } a_i = 0 \text{ then } r_i = 0, \text{ if } a_i > 0$$
$$\text{then } r_i = 1 \; R = \sum_{i=1}^{n} r_i, \quad (2)$$

where a is the abundance of the family within the sample, r is the marker if the species is present, and R is the richness, counted as the overall number of families within the sample; and

$$E = D/\ln R, \quad (3)$$

where E is the evenness index from the sample, D is the diversity index from the sample, and R is the richness of the sample. These indices capture relevant changes within the community structure. Significant treatment effects on these indices may indicate changes in community structure that may not be apparent from treatment effects on individual taxa (Magurran 1988).

Arthropods from sticky-card samples were classified into functional guilds and size classes. Functional guilds were arranged by the most probable feeding habits of the adults and included nectar/pollen foragers, predators, parasitoids, general scavengers, phloem feeders, blood feeders, and leaf feeders. These are not exclusive groups; for example, some parasitoids supplement their diet by obtaining plant carbohydrates and may exhibit some feeding characteristics of nectar/pollen foragers. Insect sizes were categorized by medium–small insects (<5 mm), medium-sized insects (5–10 mm), and large insects (>10 mm).

A linear model was fit to each of the response variables within the measurement types of the form:

$$Y = (Block + Treatment) \times Time, \quad (4)$$

where Y is the predicted family, order, total count, or community index present in the sample, *Block* and *Treatment* are the experimental design elements of the model, and *Time* is the variable included for repeated measures over each of the time periods.

The Shapiro–Wilk test was used to verify normality of the response data, for all of the samples and at each measurement date. A Type III analysis of variance (ANOVA) was then performed on data that fit the assumptions of the linear model to estimate effects of the predictor variables on the response, and to check for interaction terms among the predictors.

Pairwise contrasts were calculated to detect significant differences ($\alpha = 0.05$) between the control plots and insecticide treatment plots on specific measurement days. Retrospective power analyses were done to identify the probability of making a Type II error within a multivariate repeated-measures test.

Terrestrial study multiple-spray events, 2005–2006

The multiple-spray, terrestrial study was conducted adjacent to a 4.83-km portion of Bootlegger Trail Road (47°39′52.56″N, 111°16′55.17″W–47°37′39.39″N, 111°16′55.68″W) on the eastern border of the wildlife refuge. Two treatments were arranged in an RCBD with 3 replicates. The blocking factor was location along the length of the experimental site. Experimental units were 0.4 km long, with 0.4-km buffers between them. Treatments consisted of permethrin (7.8 g/ha) + PBO (39.2 g/ha) (Aquareslin) at the maximum labeled rate and an untreated control. Spray applications were made via truck-mounted ULV sprayer 4 separate times in 1-wk intervals in August of 2005 and 2006.

For arthropod sampling, 3 Olson yellow sticky cards, at 7.62 m, 15.24 m, and 45.72 m from the road were placed in each plot on both the east (2006 only) and west side of the road. Also, sweep-net samples (50 sweeps) were taken at the same distances within each plot. These samples were taken the day after the treatment (sweep only), 1, 2, and 4 wk posttreatment. Taxonomic groups, size classes, and functional guilds were counted from abundance, and richness, diversity, and evenness were calculated from each sample as shown above (Eqs. 1–3). Each group from each sample was put into functional guilds and size classes as mentioned from the single-spray study.

Table 1. *P*-values for overall treatment effects, aquatic counts, 2004–2005, for single-spray study.

			P				
Samples	Predictor	df	Amphipoda	Coleoptera	Hemiptera	Odonata	Total of all taxa
D-net							
2004	Treatment	4, 8	0.2	0.9	0.2	-	0.4
	Time	3, 24	0.6	0.5	0.01	-	0.03
	Time × Treatment	12, 24	0.7	0.05	0.6	-	0.2
2005	Treatment	4, 8	0.9	-	0.5	0.3	0.9
	Time	3, 24	0.2	-	0.1	0.006	0.2
	Time × Treatment	12, 24	0.5	-	0.7	0.3	0.6
Sediment	Predictor	df	Amphipoda	Coleoptera	Diptera	Total	
2004	Treatment	4, 8	0.42	0.5	0.88	0.67	
	Time	3, 24	0.66	0.01	0.39	0.74	
	Time × Treatment	12, 24	0.27	0.84	0.55	0.23	
Dipper	Predictor	df	*Daphnia* sp.	Rotifer			
2004	Treatment	4, 8	0.28	-			
	Time	3, 24	<0.0001	-			
	Time × Treatment	12, 24	0.66	-			
2005	Treatment	4, 8	0.93	0.94			
	Time	3, 24	0.85	0.69			
	Time × Treatment	12, 24	0.64	0.27			

A model was fit to the data of the form:

$$Y = (Block + Treatment + Treatment \times Distance) \times Time, \quad (5)$$

where *y* is the predicted family, order, total count, or community index present in the sample, *Block* and *Treatment* are the experimental design elements of the model, *Distance* is the distance from the road, and *Time* is the variable included for repeated measures throughout the time periods. The *Treatment* × *Distance* interaction term was included in the model as it is likely an interaction could occur between those predictors along with a *Treatment* × *Distance* × *Time* interaction. The Shapiro–Wilk test was utilized to check the normality of the response data, for all of the total counts, and at each measurement date. A Type III ANOVA was performed on data that fit the assumptions of the linear model to estimate effects of the predictor variables on the observed responses, utilizing both multivariate and univariate tests.

For sampling dates that showed significant treatment effects, pairwise contrasts were calculated to detect significant differences ($\alpha = 0.05$) between the control and insecticide-treated plots. Retrospective power analyses were done to identify the probability of making a Type II error.

RESULTS

Terrestrial and aquatic single-spray study, 2004–2005

D-net samples collected from the pond during the summers of 2004–2005 captured several aquatic invertebrates, including amphipods (*Gammarus* sp.), water boatmen (Hemiptera: Corixidae), beetles (Coleoptera: Dytiscidae), flies (Diptera: Chironomidae and Culicidae), and dragonflies and damselflies (Odonata). The model above was fitted to each group, along with a total count. No overall treatment effects were identified for any receptor (df = 4, 8; $P = 0.2$–0.9) for 2004 or 2005 (Table 1). Other significant predictors for models fitted to both years' response variables included a block effect for both coleopterans and hemipterans (df = 2, 8; $P = 0.03$, 0.001). Also, there was a time effect for those groups (df = 3, 24; $P = 0.01$) (Table 1), as well as a block × time interaction effect (df = 12, 24; $P = 0.02$). Overall, multivariate treatment effects were significant in 2004 for amphipods on the first sampling date (df = 4, 8; $P = 0.01$.). Multivariate pairwise contrasts detected significant differences between the control and *Bti*, methoprene, and d-phenothrin on date 1 ($P = 0.001$, 0.01, 0.02). A significant time × treatment interaction was observed for coleopterans (df = 12, 24; $P = 0.05$) (Table 1) related to a pairwise contrast that detected a significant difference between the control plots and the permethrin-treated plots on date 2. On average, more beetles were observed in the permethrin-treated plots ($P = 0.03$). The same experiment conducted in 2005 revealed no overall treatment effects for amphipods on date 1 (df = 4, 8; $P = 0.4$–0.7) (Fig. 1). Total D-net counts in 2004 had overall treatment effects that mimicked treatment effects found in the analysis of the amphipods because they contributed most of the individuals for each sample. Power to detect overall multivariate

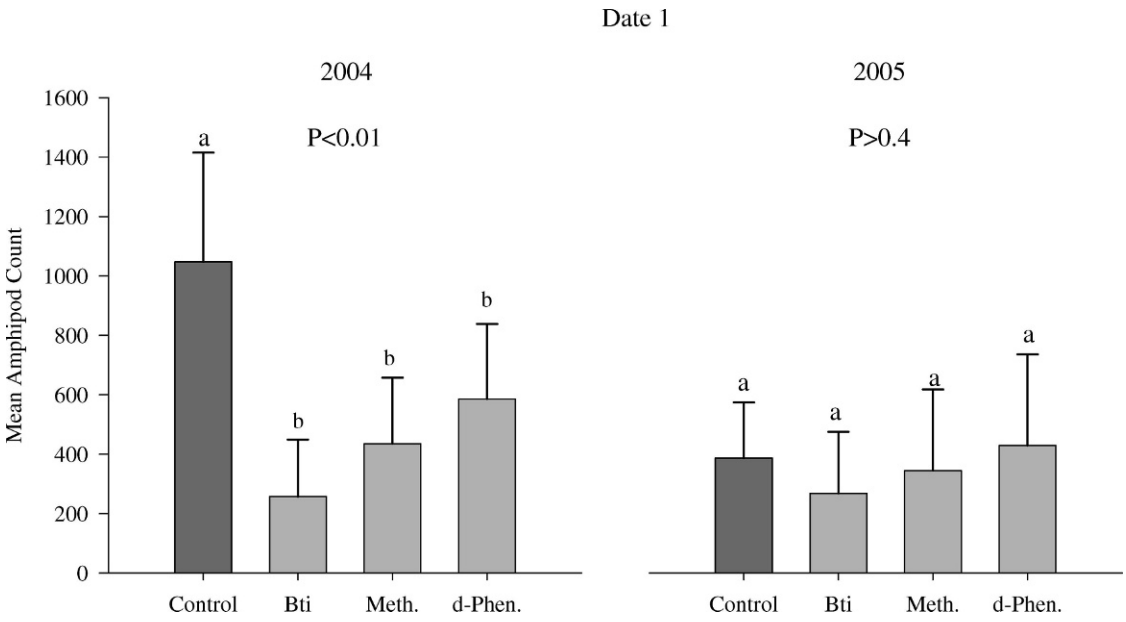

Fig. 1. Amphipods per D-net sample, date 1, 2004–2005, single-spray study.

treatment effects among any taxa during either year was low (<0.5), except for dipterans on date 2 in 2004 (0.99).

Sediment samples were taken in the pond during 2004. Taxa captured in these samples were amphipods, beetles (Coleoptera: Dytiscidae), and nonbiting midge larvae (Diptera: Chironomidae). No overall treatment effects were detected for either of these 3 groups (df = 4, 8; P = 0.4–0.9) (Table 1). Block was a significant predictor for amphipods and diving beetles (df = 2, 8; P = 0.03, 0.04). Time was also a significant predictor for diving beetles (df = 3, 24; P = 0.01), and more beetles were observed in the methoprene-treated plots on date 3 (P = 0.03). Multivariate tests for treatment effects in this experiment had low power (<0.221).

Dipper samples were taken in the same locations during 2004 and 2005. Two taxa were collected in the dipper samples, including *Daphnia* sp. and rotifers (phylum Rotifera). No overall treatment effects were observed during either year (df = 4, 8; P = 0.3–0.9) (Table 1). Time was significant in the univariate analysis during 2004 (df = 3, 24; P = <0.001) as was block on date 3 in 2004 (df = 2, 8; P = 0.01). Fewer daphnids were collected in 2004 in the permethrin-treated plots (P = 0.05). Power to detect treatment effects was reasonable on dates 1, 2, and 3 in 2004 (0.8–0.999) but was low in 2005 (<0.5).

Sticky-card samples were taken in 2004 to survey flying insects in each treatment plot. Samples close to the pond (≈1 m, West) were analyzed separately from those farther from the pond (≈25 m, East). Each sample type came from different environments, one directly on the edge of the pond, the other on dry prairie. Approximately 50 families of insects were identified in 2004. Of the families identified in 2004, 30 had sufficient numbers to be analyzed in at least 1 of the environments. Individuals were categorized into 8 orders, 6 functional guilds, 3 size classes, and 3 ecological indices. Univariate tests did not suggest any overall treatment effects for any family group (df = 4, 8; P = 0.08–0.96), order group (P = 0.09–0.93), size class (P = 0.2–0.9), or ecological index (P = 0.2–0.6) for either data set (Table 2). A significant overall treatment effect was observed for parasitoids for the eastern data set (P = 0.05). Pairwise contrasts indicated a greater numbers of parasitoids in the control plots than the d-phenothrin–treated plots on date 2. No significant overall treatment effect was observed for the 5 remaining functional guilds (P = 0.08–0.8) (Table 2). Total arthropod counts showed no significant overall treatment effect at either distance from the pond (P = 0.1, 0.8).

Multivariate analysis suggested more Ceratopogonidae within methoprene- and permethrin-treated plots vs. the control on date 2, farther from the pond (P = 0.02) (Fig. 2), and pairwise contrasts detected significantly fewer Calliphoridae for the same plots on date 1 for the West samples (df = 1, 8; P = 0.04, 0.02) (Fig. 3). Relatively more predators were observed in the *Bti* plots close to the pond on date 1 (P = 0.01) (Fig. 4). Fewer scavengers were observed on dates 1 and 2 close to the pond for d-phenothrin as detected by pairwise contrasts (P = 0.04, 0.04) (Fig. 5). A significant time effect was observed

Table 2. *P*-values for overall treatment effects, sticky-card counts, 2004–2005, for single-spray study, both East and West plots.

Year	Variables	df	Families	Orders	Functional guilds	Size classes	Indices	Total of all taxa
2004	Total no. analyzed		30	8	6	3	3	
	Predictors							
	Treatment	4, 8	0.08–0.96	0.09–0.93	0.05–0.8	0.2–0.90	0.2–0.6	0.1
	Time	2, 16	<0.0001–0.2	<0.001–0.1	<0.0001–0.1	<0.0001–0.001	<0.0001–0.01	0.003–0.007
	Time × Treatment	8, 16	0.03–0.99	0.004–0.96	0.005–0.98	0.31–0.7	0.003–0.5	0.2–0.5
2005	Total no. analyzed		7	5	3	3	3	
	Predictors							
	Treatment	4, 8	0.03–0.8	0.03–0.8	0.5–0.7	0.2–0.9	0.2–0.9	0.8
	Time	2, 16	0.0001–0.7	0.002–0.98	0.002–0.3	0.0002–0.6	0.2–0.9	0.003–0.2
	Time × Treatment	8, 16	0.2–0.9	0.09–0.9	0.2–0.6	0.2–0.9	0.1–0.98	0.2–0.5

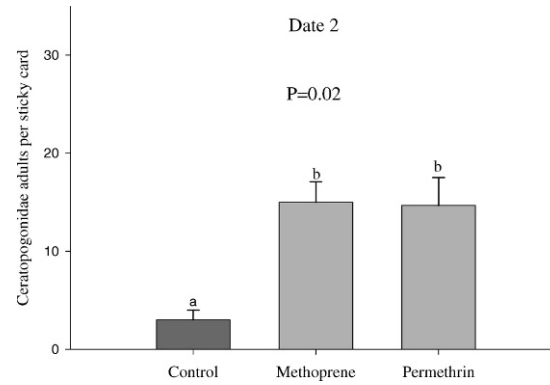

Fig. 2. Ceratopogonidae adults per sticky card, East, date 2, 2004, single-spray study.

for nearly all of the responses studied (df = 2, 16; P = <0.0001–0.2) (Table 2). Interactions between treatment and time were significant for Odonata. Samples had relatively lower counts of odonates for the larvicide-treated plots on the first sampling date followed by a slight increase on date 2. Each of the adulticide-treated plots and the control plots started with relatively more odonates on date 1, followed by a decrease on date 2. Power to detect multivariate overall treatment effects was generally low for sticky-card samples during 2004 (0.05–0.717), with some exceptions within certain dates, including Araneae, Coleoptera, and the large size class in the eastern plots, as well as Bombyliidae, Ceratopogonidae, Chironomidae, Hymenoptera, Odonata, and predators in the western plots (>0.85).

Sticky-card samples were collected in 2005; fewer individual taxa were observed in these samples. Only 7 families had sufficient individuals to be analyzed. Five orders, 3 ecological indices, 3 size classes, and 3 functional guilds were also analyzed. None of the response variables showed an overall treatment effect (df = 4, 8; P = 0.1–

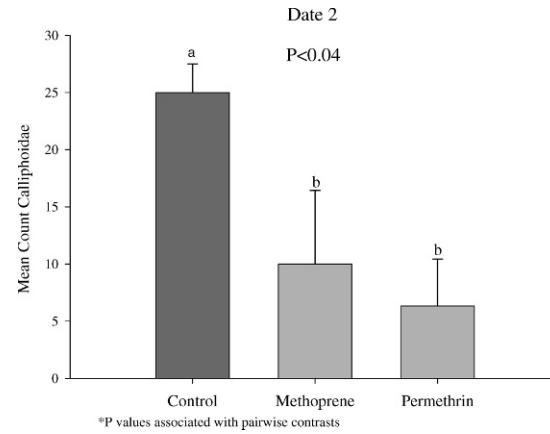

Fig. 3. Calliphoridae per sticky card, West, date 2, 2004, single-spray study.

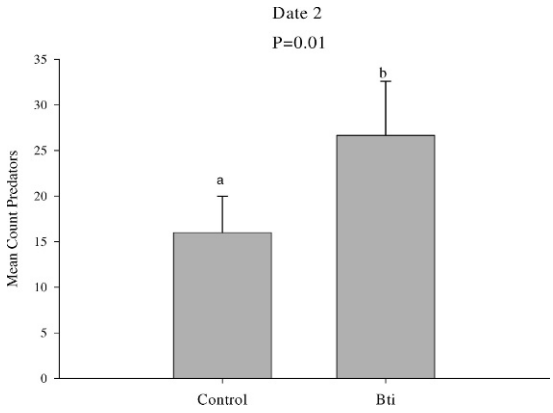

Fig. 4. Predators per sticky card, West, date 1, 2004, single-spray study.

0.9) (Table 2). Six of the analyzed groups were associated with a significant time effect (Table 2). Multivariate power was low to moderate (<0.85), with a few exceptions on certain dates, including Lestidae, Limnephilidae, Syrphidae, Araneae, Diptera, Odonata, Trichoptera, and the medium size class in the eastern plots, and Lesitidae and Odonata in the western plots (>0.85).

Terrestrial study multiple-spray events, 2005–2006

Few arthropods were collected from each sticky-card sample in 2005 because of low overall abundance. The greatest number of identified arthropods was 17 on 1 card. Family richness was low. The greatest number of families sampled for 1 card was 7. Three families, (Pieridae, Bombyliidae, and Formicidae), 3 orders (Hymenoptera, Lepidoptera, and Diptera), 3 ecological indices, 3 size classes, and 1 functional guild (nectar/pollen foragers) were included. Two indicators suggested overall treatment effects: total lepidopterans and the "largest" size class were associated with more individuals in permethrin-treated plots (df = 1, 12; $P = 0.03$). The remaining groups did not have significant overall treatment effects ($P = 0.05$–0.8). Distance × treatment interactions occurred for each of these groups along with Pieridae and the nectar/pollen foraging guild (df = 1, 12; $P = 0.02$–0.05) (Table 3). Multivariate power to detect treatment effects was generally low, with some exceptions on specific dates, including lepidopterans and the large size category (>0.8), with other groups having power <0.8 on each date.

Sticky-card samples were gathered the same way in 2006 as in 2005. Overall total counts were low, as in 2005. One sample had 53 identified individuals; the rest had fewer than 21. Five families had enough individuals for analysis (Bombyliidae, Formicidae, Muscidae, Pieridae, and Syrphidae). Four orders were included (Araneae, Diptera, Hymenoptera, and Lepidoptera) as well as 3 size classes, 3 ecological indices, 4 functional guilds (nectar/pollen foragers, predators, parasitoids, and scavengers), and total counts. No treatment effects were seen within

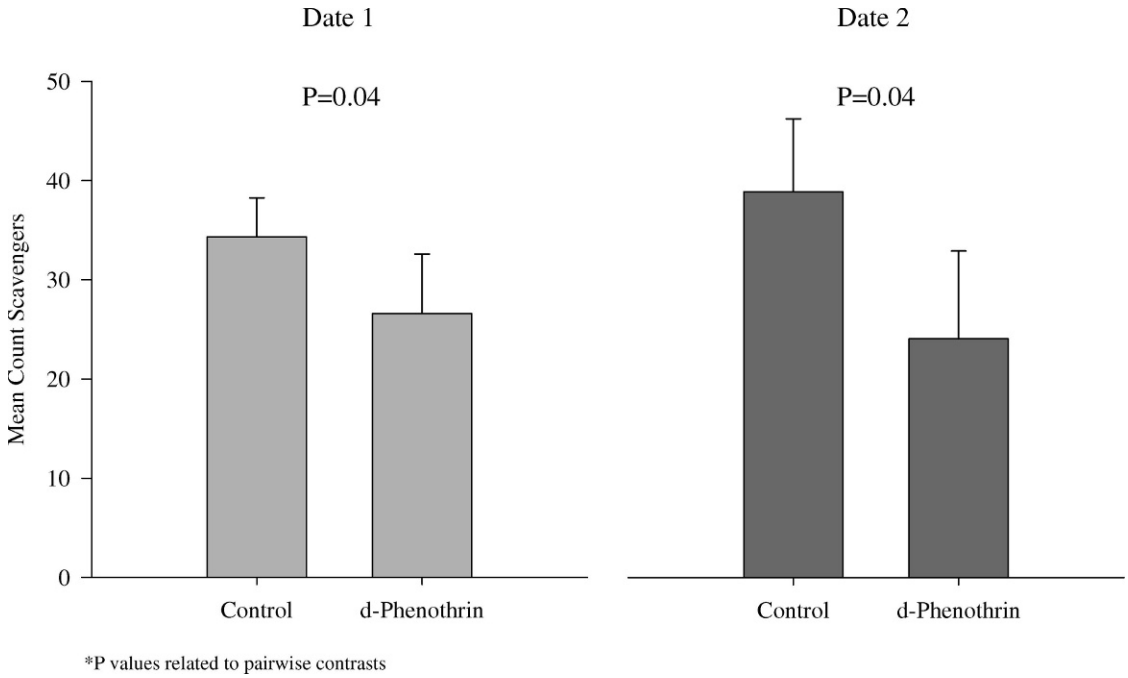

Fig. 5. Scavengers per sticky card, West, dates 1 and 2, 2004, single-spray study.

Table 3. *P*-values for overall treatment effects for sticky-card and sweep-net samples, 2005–2006, multiple-spray study.

Samples	df	Families	Orders	Functional guilds	Size classes	Indices	Total of all taxa
Sticky-card samples							
2005							
Total no. analyzed		3	3	1	3	3	
Predictors							
Treatment	1, 12	0.07–0.8	0.03–0.4	0.05	0.03–0.6	0.1–0.2	0.2
Time	2, 24	0.1–0.2	0.04–0.2	0.01	0.03–0.9	0.4–0.9	0.05
Time × Treatment	2, 24	0.05–0.7	0.07–0.5	0.3	0.08–0.7	0.04–0.1	0.08
Distance × Treatment	1, 12	0.05–0.5	0.03–0.5	0.02	0.03–0.98	0.09–0.1	0.09
2006							
Total no. analyzed		5	4	4	3	3	
Predictors							
Treatment	1, 28	0.4–0.9	0.1–0.6	0.05–0.8	0.3–0.7	0.003–0.005	0.9
Time	2, 56	0.004–0.6	0.0002–0.3	0.07–0.3	<0.0001–0.3	0.0006–0.09	0.2
Time × Treatment	2, 56	0.03–0.7	0.1–0.5	0.03–0.7	0.2–0.7	0.07–0.4	0.3
Distance × Treatment	1, 28	0.4–0.99	0.2–0.8	0.1–0.9	0.4–0.6	0.02–0.4	0.96
Sweep-net samples							
2005							
Total no. analyzed		5	4	3	3	3	
Predictors							
Treatment	1, 12	0.06–0.6	0.2–0.9	0.3–0.9	0.2–0.6	0.3–0.99	0.8
Time	2, 24	0.02–0.3	0.01–0.2	0.09–0.3	0.01–0.4	0.1–0.98	0.02
Time × Treatment	2, 24	0.002–0.8	0.009–0.9	0.007–0.9	0.2–0.7	0.8–0.97	0.7
Distance × Treatment	1, 12	0.06–0.99	0.3–0.6	0.2–0.9	0.2–0.7	0.3–0.6	0.95
2006							
Total no. analyzed		7	5	4	3	3	
Predictors							
Treatment	1, 28	0.3–0.97	0.2–0.8	0.4–0.7	0.6–0.9	>0.8	0.8
Time	2, 56	<0.0001–0.3	<0.0001–0.03	<0.0001–0.002	<0.0001–0.3	<0.0001	0.02
Time × Treatment	2, 56	0.08–0.9	0.3–0.9	0.3–0.7	0.06–0.6	0.4–0.7	0.7
Distance × Treatment	1, 28	0.3–0.9	0.2–0.97	0.3–0.91	0.3–0.97	0.5–0.8	0.95

the family groups (df = 2, 29; P = 0.4–0.9) or order groups (P = 0.1–0.6) (Table 3). The parasitoid guild showed a significant treatment effect (P = 0.05) as did each of the ecological indices (P = 0.003–0.05) that were associated with higher abundance of individuals within permethrin-treated plots. Significant treatment effects were not observed for any of the other functional guilds (P = 0.7–0.8) or the total arthropod count (P = 0.9) (Table 3). Multivariate power for these analyses was generally low, with some exceptions on specific dates, including nectar/pollen foragers and the small size class. Most groups observed had power of <0.9 on each date.

Five families (Acrididae, Cercopidae, Cicadellidae, Formicidae, and Membracidae), 4 orders (Araneae, Coleoptera, Diptera, and Hymenoptera), 3 functional guilds (nectar/pollinators, plant feeders, and predators), 3 ecological indices, and 3 size classes and total arthropod counts were analyzed from sweep-net samples in 2005. No overall treatment effect was noted for any of the response variables (df = 1, 12; P = 0.06–0.99) (Table 3). Six groups had significant time effects (Acrididae, Formicidae, Membracidae, Diptera, the medium size class, and total counts). In general, fewer individuals were collected later in the season (df = 1, 24; P = 0.01–0.05). Five of the responses showed time × treatment effects (Cercopidae, Formicidae, Araneae, Hymenoptera, and predators) (df = 2, 24; P = 0.002–0.05) (Table 3). In general, average counts were low for these groups and a discernable pattern was not evident. The multivariate test revealed more Araneae in the permethrin-treated plots on date 1 (df = 1, 12; P = 0.01); low counts of Coleoptera revealed a similar result (df = 1, 12; P = 0.01). Multivariate power for sweep-net samples was generally moderate to low, with all of the samples having power <0.8.

Sweep-net samples, 2006

Sufficient individuals were collected in 7 families (Acrididae, Bombyliidae, Cicadellidae, Formicidae, Staphylinidae, Syrphidae, and Tachinidae), 5 orders (Araneae, Coleoptera, Diptera, Hemiptera, and Hymenoptera), 4 functional guilds (parasitoids, plant feeder, nectar/pollinators, and predators), 3 size classes, and total arthropod count from sweep-net samples in 2006 for analysis. No response variables showed an overall treatment effect (df = 1, 23; P = 0.2–0.97). Sixteen of the observed responses had observed significant time effects related to decreasing abundance later in the season (df = 3, 69; P = <0.0001–0.03) (Table 3). Power for these tests was generally low to moderate, with some groups having high power (>0.9) on particular dates, including Acrididae, Cicadellidae, and the large size class. Other groups had lower power on all dates of the multivariate tests (<0.9).

DISCUSSION

The aquatic effects observed for the acute spray experiment conducted in 2004 and 2005 are consistent with those found by previous researchers who examined similar single-treatment scenarios. The responses evaluated during our study found few, if any, deleterious effects in insecticide-treated plots.

Of the 5 response variables examined for D-net samples in either year of the single-application study, none showed overall treatment effects. This was also true for the 3 analyzed responses for sediment samples in 2004, and the 2 responses analyzed in dipper samples in 2004 and 2005 (Table 1). Multivariate analysis considering pairwise contrasts coupled with repeated-measures analysis revealed 2 overall treatment effects out of a total of 14 response variables among all 3 sample types and both years; these were associated with fewer individuals in the insecticide-treated plots (Fig. 1). These potential reductions in aquatic nontarget populations did not suggest any trends or persistent deleterious biological effects following a single adulticide or a single larvicide application. Benthic invertebrates that are mostly static in the pond would be the best indicators to measure effects of adulticides drifting into the pond and liquid larvicides applied directly to water. Data from D-net samples showed possible impact on amphipods that may have represented relatively stable populations within the treatment plots (Fig. 1). Significant differences for the pond study were found on the dates closest to the spray event when treatment effects were most likely observable. Detection of significant differences between treatments and control plots were minimal and may have been observed based only on the α-level and the relatively large number of recorded response variables. Additionally, no significant treatment effects were observed in 2005 (Table 1).

Terrestrial sampling via Olson yellow sticky cards for the same experiment revealed similar results for terrestrial organisms in both 2004 and 2005. For both years, only 1 out of 54 observed response variables exhibited a significant overall treatment effect (Table 2). A time effect was observed for >30 of the responses and a time × treatment effect was observed for 6 of the responses (Table 2). Three of the responses were associated with a mean reduction in specific nontarget responses on particular dates (Figs. 2, 3, 5). Time × treatment interactions did not exhibit any identifiable pattern. Like the aquatic samples, potential reductions in terrestrial nontarget populations did not exhibit trends across

dependent groups and persistent biological effects were not observed.

Sticky-card results for the terrestrial multiple-spray experiment conducted in 2005–2006 did not seem to indicate any potential deleterious effects. Six of the 20 analyzed responses exhibited a significant overall treatment effect (Table 3). None of the significant treatment effects in either year was associated with a reduction of the given response in the permethrin-treated plots. Treatment × distance effects were present in responses where an overall treatment effect occurred but did not result in fewer numbers closer to where the spray truck applied the insecticide.

None of the 23 groups collected via sweep net showed overall treatment effects. Six of 19 groups collected in 2005 and 16 of 22 groups collected in 2006 exhibited significant time effects. There was a general decrease in individuals collected, particularly later in the season for most of the groups observed. Five of 19 groups in 2005 exhibited time × treatment effects, although no discernable patterns were associated with these effects (Table 3). This finding was not repeated in 2006. However, multivariate test results did not signify deleterious effects on any particular date. In general, the terrestrial study did not measure deleterious effects on nontarget arthropods. No persistent or biological patterns were noticeable in this study.

Type I error rates were set at 5% for each response variable in each year and measurement. With >200 responses examined between both studies, Type I errors would not be uncommon. Conversely, power to detect departures from the null hypothesis of "no treatment effects" was low in most cases and had the potential to mask potential deleterious trends within the data sets. For most sample types except for dipper samples in 2004, the average power was <0.2. This is not surprising because of the small sample sizes but could represent confounding variables between treatment plots, especially in the first study, where flying insects may have moved throughout treatment plots.

Our results are similar to those of Lawler et al. (1999), who did not find a decrease in amphipods when *Bti* and methoprene were applied to seasonal pools in Florida mangrove swamps. Although our study covered only 2 years, it yielded similar results when compared to a long-term study conducted by Niemi et al. (1999) in Minnesota wetlands; they found no significant decrease in zooplankton when methoprene or *Bti* was applied in granular formulations to 9 permanent ponds over a 3-year period. Charbonneau et al. (1994) found that, although *Bti* caused high mortality of chironomids in a lab environment, a much smaller mortality was observed in the field. Hershey et al. (1998) conducted the most rigorous nontarget organism study on larviciding. Throughout a full summer season of numerous treatments, they found that *Bti* was effective at killing target dipterans and the food chain was disrupted. Predator numbers dropped as prey became less prominent. Ali (1981) found that *Bti* treatments in 4 × 6-m experimental ponds significantly lowered nontarget chironomid numbers. At the highest treatment rate of 4 kg AI/ha, there was a 54–92% reduction in chironomid numbers. But the same product used in golf course ponds had no significant effect on chironomid numbers; in this setting at a treatment of 3 kg/ha, there was only a 30–67% chironomid reduction. Numbers returned to normal 14 days posttreatment. Davis (2007) found that it is unlikely that risks from the larvicides applied directly to water will cause significant mortality to sentinel nontarget invertebrates. Exposure of methoprene to lenthic invertebrates may be mitigated by its tendency to bind to organic compounds. It is unclear if methoprene remains bioavailable when bound to sediment.

In a study similar to ours, Jensen et al. (1999) found no treatment effects on nontarget aquatic invertebrates downwind from ULV applications of pyrethrins, malathion, and permethrin. Further, they found no impacts on species diversity within seasonally impounded ponds. Studies for terrestrial nontarget receptors other than honey bees are lacking.

In a risk assessment for aquatic organisms and terrestrial vertebrates, Davis et al. (2007) concluded that risks to the overall ecosystem dynamics from multiple applications of adulticides would most likely be minimal. Predicted risks to sentinel species from exposure to adulticides were low.

Although it is likely that collateral mortality occurs, especially for small flying insects that are active at the same time as mosquitoes, data from our study suggest that measurable and persistent biological effects on nontarget arthropods exposed to larvicides or adulticides applied via ULV sprayer would be small in this ecosystem.

ACKNOWLEDGMENTS

This study was funded by a grant from the Deployed War Fighter Protection Research Program of the US Armed Forces Pest Management Board, the Northwest Mosquito and Vector Control Association, and by the Montana Agricultural Experiment Station, Montana State University. We thank T. Bennett, C. Pariera Dinkins, L. Shama, M. Schat, J. Schleier, J. Marquez, and J. Wiese for technical support. We also thank D. Johnson and the Cascade County Mosquito and Vector Control District and B. Johnson and the staff at the Benton Lake National Wildlife Refuge.

REFERENCES CITED

Ali A. 1981. *Bacillus thuringiensis* serovar *israelensis* (ABG-61080) against chironomids and some nontarget aquatic invertebrates. *J Invertebr Pathol* 38:264–272.

Breaud TP, Farlow JE, Steelman CD, Schilling PE. 1977. Effects of the insect growth regulator methoprene on natural populations of aquatic organisms in Louisiana intermediate marsh habitats. *Mosq News* 37:704–712.

Caron DM. 1979. Effects of some ULV mosquito abatement insecticides on honey bees. *J Econ Entomol* 72:148–151.

Charbonneau CS, Drobney RD, Rabeni CF. 1994. Effects of *Bacillus thuringiensis* var. *israelensis* on nontarget benthic organisms in a lenthic habitat and factors affecting the efficacy of the larvicide. *Environ Toxicol Chem* 13:267–269.

Coldburn RB, Langford GS. 1970. Field evaluation of some mosquito adulticides with observations on toxicity to honey bees and house flies. *Mosq News* 30:519–522.

Davis RS. 2007. *An ecological risk assessment for mosquito insecticides* [M.Sc. thesis]. Montana State University: Bozeman, MT.

Davis RS, Peterson RKD, Macedo PA. 2007. An ecological risk assessment for insecticides used in adult mosquito management. *Integr Environ Assess Manag* 3:373–382.

Hershey AE, Lima AR, Niemi GJ, Regal RR. 1998. Effects of *Bacillius thuringiensis israelensis* (Bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecol Appl* 8:41–60.

Hester PG, Shaffer KR, Tietze NS, Zhong H, Griggs J Jr. 2001. Efficacy of ground applied ultra low volume malathion on honey bee survival and productivity in open and forest areas. *J Am Mosq Control Assoc* 17:2–7.

Huhn GD, Sejvar JJ, Montgomery SP, Dworkin MS. 2003. West Nile virus in the United States: an update on an emerging infectious disease. *Am Fam Physician* 68:653–660.

Jensen T, Lawler SP, Dritz DA. 1999. Effects of ultra-low volume pyrethrin, malathion, and permethrin on nontarget invertebrates, sentinel mosquitoes, and mosquitofish in seasonally impounded wetlands. *J Am Mosq Control Assoc* 15:330–338.

Lawler SP, Jensen T, Dritz DA, Wichterman G. 1999. Field efficacy and nontarget effects of the mosquito larvicides temephos, methoprene, and Bti in Florida mangrove swamps. *J Am Mosq Control Assoc* 15: 446–452.

Magurran AE. 1988. *Ecological diversity and its measurement*. Princeton, NJ: Princeton Univ. Press.

Merritt RW, Walker ED, Wizbach MA, Cummins KW, Morgan WT. 1989. A broad evaluation of Bti for black fly (Diptera: Simuliidae) control in a Michigan river: efficacy, carry, and nontarget effects on invertebrates and fish. *J Am Mosq Control Assoc* 5:397–415.

Milam CD, Farris JL, Wilhide JD. 2000. Evaluating mosquito control pesticides for effect on target and nontarget organisms. *Arch Environ Contam Toxicol* 39:324–328.

Mittal PK. 2003. Biolarvicides in vector control: challenges and prospects. *J Vector Borne Dis* 40:20–32.

Molloy DP. 1992. Impact of the black fly (Diptera: Simuliidae) control agent *Bacillus thuringiensis* var. *israelensis* on chironomids (Diptera: Chironomidae) and other nontarget insects: results of ten field trials. *J Am Mosq Control Assoc* 8:24–31.

Niemi GJ, Hershey AE, Shannon L, Hanowski JM, Lima A, Axler RP, Regal RR. 1999. Ecological effects of mosquito control on zooplankton, insects, and birds. *Environ Toxicol Chem* 18:549–559.

Norland RL, Mulla MS. 1975. Impact of Altosid® on selected members of an aquatic ecosystem. *Environ Entomol* 4:145–153.

Pankiw T, Jay SC. 1992. Aerially applied ultra-low volume malathion effects on caged honey bees (Hymenoptera: Apidae), caged mosquitoes (Diptera: Culicidae), and malathion residues. *J Econ Entomol* 85:687–691.

Peterson RKD, Macedo PA, Davis RS. 2006. A human-health risk assessment for West Nile virus and insecticides used in mosquito management. *Environ Health Perspect* 114:366–372.

Siegfried BD. 1993. Comparative toxicity of pyrethroid insecticides to terrestrial and aquatic insects. *Environ Toxicol Chem* 12:1683–1689.

Tietze NS, Hester PG, Shaffer KR, Wakefield FT. 1996. Peridomestic deposition of ultra-low volume malathion applied as a mosquito adulticide. *Bull Environ Contam Toxicol* 56:210–218.

USEPA. 1991. Reregistration Eligibility Document Isopropyl (2E, 4E)-11-Methoxy-3,7,11-Trimethyl-2, 4-Dodecadienoate (Referred to as Methoprene. Case No. 0030. U.S. Environmental Protection Agency, Washington, D.C.

Zhong HE, Latham M, Hester PG, Frommer RL, Brock C. 2003. Impact of naled on honey bee survival and productivity: aerial ULV application using a flat fan nozzle system. *Arch Environ Contam Toxicol* 45:216–220.