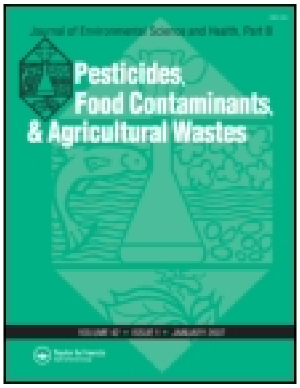

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Sublethal contaminant exposure alters behavior in a common insect: Important implications for trophic transfer

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Sublethal contaminant exposure alters behavior in a common insect: Important implications for trophic transfer

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This study examined the effects of sub-lethal exposure of the ubiquitous pesticide malathion on the behavior of the model orthopteran species, the house cricket (*Acheta domesticus*). Increasing concentrations of malathion caused male crickets to increase periods of non-directional movement, such as twitching and grooming, directional movement, and to seek out shelter less. These are all behavioral alterations that may increase the cricket's chances of being preyed upon, and thus have the potential for serious ecological consequences through trophic transfer. This study also revealed that female crickets appeared to be less affected by malathion than their male conspecifics, indicating a potential sex-bias in both susceptibility and possible predator attack.

Keywords: Predator, house cricket, *Acheta domesticus*, malathion, behavior.

Introduction

Behavior is rapidly becoming an established method of determining the impact of sub-lethal levels of contamination on organisms.[1,2] The study of an organism's behavior in response to a specific contaminant is critical to understanding if and how the contaminant is spread throughout the environment.[1] While organisms can come into direct contact with the chemical or chemical residues,[3–5] they can also come into contact with other organisms that have been exposed through a wide variety of modes, such as predation.[6,7] Because predation of target organisms, such as insects, is a common way contaminants move through the ecosystem, understanding how sub-lethal doses affect the behavior of the prey is critical.[8] If, for example, an organism's exposure to contamination makes it more likely to be preyed upon, there may be an increased probability of trophic transfer and/or bioaccumulation.[9–11]

It is important to not only focus on the lethal properties of contamination, but also these sub-lethal effects.[12] The ramifications of sub-lethal effects can be more detrimental to the ecosystem than one group of organisms being lethally dosed due to the tendency of transfer among other organisms in the environment.[1,13] Sub-lethal effects are often seen in non-target organisms, including insects, humans, birds, reptiles, amphibians, and fish.[14–17]

The ubiquitous cholinesterase inhibitor malathion has been shown to adversely affect a wide variety of non-target organisms, such as non-target insects,[18] fish,[19,20] reptiles,[21] mammals,[22] and amphibians,[23–25] but how sub-lethal doses affect the behavior of target insects, such as orthopteran species, is still largely unknown.[26]

To help elucidate this gap in the relationship between behavior and ecotoxicology, the non-toxic effects of malathion on the house cricket (*Acheta domesticus*) were examined. House crickets are distributed worldwide, are an agricultural pest in some areas,[27,28] and have been found to be excellent bioindicators of malathion contamination and spray distribution.[29] In addition, house crickets are a model orthopteran species widely used in physiological, ecological and behavioral studies.[27,28,30] Within this system, sub-lethal doses may affect behavior in such a way that will cause these insects to become more susceptible to predation.

To fully examine this, two experiments were conducted. The first experiment observed basic movement behavior using several parameters (distance moved, average velocity, duration and frequency of directional movement, duration and frequency of non-directional movement such as grooming, convulsing, or twitching). The second experiment analyzed the crickets' preference between three habitat zones (food, shelter, and neutral). These two experiments allowed for further understanding of how sub-lethal levels of malathion affect cricket behavior and thus the likelihood of exposure to potential predators.

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Materials and methods

Experimental insects

All insects used in experiments were commercially bred 4–5 week old house crickets (*Acheta domesticus*) obtained from Fluker Farms Inc. Crickets were communally housed in a 37.9 L glass terrarium and fed commercially prepared "Cricket Quencher with Calcium" and "High Calcium Cricket Diet" (Fluker Farms Inc.), *ad libitum*.

Malathion preparation

Malathion (99% pure; Chem Service, Inc., West Chester, PA, USA) was mixed with corn oil (Sigma-Aldrich) to make three malathion: corn oil mixtures: 1 μg/L, 10 μg/L, and 100 μg/L. A control mixture of corn oil only was used. Prior to application, this mixture was vortexed to ensure equal distribution of malathion.

Experiment 1: Effects on movement and activity level

Experimental set-up. One hundred and twenty 4–5 week old crickets (m = 48, f = 72) were used in this experiment. Twelve 14 cm × 14 cm × 4.5 cm plastic containers were set up in a grid pattern to serve as experimental arenas. One cricket was placed in each arena. White paper dividers were placed between adjacent containers to prevent the possibility of intraspecific interactions from confounding the behavioral results. A piece of white poster-board paper was placed under all containers to help increase contrast for video analysis purposes.

One of three different treatments of malathion (100 μg/L, 10 μg/L, 1 μg/L) and a control (corn oil) were randomly assigned to each animal using a random number generator. Insects were cooled to make handling easier by placing them in a plastic container on ice for approximately 5 minutes. After cooling, each insect was placed in the center of their experimental arena and 1 μL of experimental mixture was pipetted onto their dorsal abdomen. This cuticular absorption method has been used to effectively dose insects with a variety of chemical pesticides,[31–33] including malathion.[34] This procedure resulted in three replicates of each treatment group per experimental trial. A total of 10 experimental trials were conducted for this experiment, with a total of 30 replicates per treatment. The trials were stratified by sex.

Filming procedure. A Canon® VIXIA HFS10 HD digital video camera was mounted on a tripod directly above the 12 experimental arenas and when all insects were treated, a large piece of glass was placed over all arenas to prevent the escape of experimental insects. The camera was then set to begin recording, with all 12 arenas and their insects in the video frame. A total of 50 minutes of video was recorded per trial.

Video analysis. Video analysis was conducted using EthoVision XT behavioral video analysis software (Noldus Information Technology Inc.). Analysis began after a 5 min acclimation period, so a total of 45 min of video footage was behaviorally analyzed per trial, resulting in total analysis of 450 min of video for this experiment (approximately 7.5 hours).

The following statistics were calculated for each animal: directional movement, velocity (cm/s), distance moved (cm), and non-directional movement (s). For non-directional movement, only values when > 60% of the cricket's body was moving were considered.

Experiment 2: Effects on shelter use versus foraging activity

Experimental set-up. Fifty-six 4–5 week old crickets (m = 24, f = 32) were used in this experiment. Four 32.5 cm × 16 cm × 11 cm plastic containers were set up in a grid pattern to serve as experimental arenas. Each container was divided into three zones: a "shelter zone," which was 11.6 cm long and consisted of a sheet of black cardboard curved to form a "cave" on one end of the container, which was further covered with a sheet of a white paper; a "food zone" which contained a small circular petri dish lid (3 cm diameter, 0.5 cm deep) filled with 5.0 g of "Cricket Quencher" nutritional supplement and a "neutral zone," covering the rest of the arena, which did not contain a shelter or stimulant. One cricket was placed in each arena, and white paper dividers were placed between adjacent containers to prevent the possibility of intraspecific interactions from confounding the behavioral results. A piece of white poster-board paper was placed under all containers to help increase contrast for video analysis purposes.

One of three different treatments of malathion (100 μg/L, 10 μg/L, 1 μg/L) and a control (corn oil) were randomly assigned to each animal using a random number generator. Insects were cooled to make handling easier by placing them in a container on ice for approximately 5 minutes. After cooling, each insect was placed in the center of their experimental arena and 2 μL of experimental mixture was pipetted onto their dorsal abdomen. This procedure resulted in one replicate of each treatment group per experimental trial. A total of 14 experimental trials were conducted for this experiment, with a total of 14 replicates per treatment. The trials were stratified by sex.

Filming was completed in a similar manner as in Experiment 1.

Video analysis. All video analysis was conducted using EthoVision® XT behavioral video analysis software (Noldus Information Technology Inc.). Analysis began after a 5 min acclimation period, so a total of 45 min of video footage was behaviorally analyzed per trial, resulting in total analysis of 630 min of video for this experiment (approximately 10.5 hours).

EthoVision® XT allows for the creation of specific zones within an arena, and records the time an animal spends in each zone. "Shelter," "food," and "neutral zones," were defined and EthoVision® XT calculated the amount of time(s) each animal spent in each zone.

Statistical analysis

For the movement experiment, trial was nested into sex and designated as a random effect because individuals of one sex were randomly assigned to each trial. This factor was then crossed with treatment, which was a fixed effect. Sex was analyzed as a fixed effect as well as its interaction with treatment. Each dependent variable (distance moved, time moving, time not moving, duration and frequency of non-directional movement, average velocity) was analyzed using a mixed model with the restricted maximum likelihood (REML) method for the random effects. The following variables were log-transformed to meet the assumption of normality and homoscedasticity: distance moved, duration and frequency of non-directional movement. The other variables did not require transformation. A contrast test was used to determine if the three treatments differed significantly from the control.

For the food experiment, the total time in the testing arena (2700 sec) was divided by the time each cricket spent in one of the three zones (designated neutral, food, or shelter). This proportion was arcsine transformed. Since there was no effect of trial on the experiment, it was removed from further analyses. The effect of sex and treatment was analyzed on the proportion of time spent in each of the zones using a nominal regression model and considered both effects fixed. There were not enough degrees of freedom to investigate the interaction of these two factors. There was, however, a significant effect of sex, so the sexes were split and analyzed separately using a contingency plot.

Results

There was a significant interactive effect of sex and treatment on the duration and frequency of high non-directional activity in crickets in Experiment 1 ($p < 0.05$; Table 1; Fig. 1). When separated by sex, it was found that male crickets exposed to the highest concentration (100 ug/L) spent more time stationary, but moving their body and or/limbs, compared to control animals (Table 1; Fig 1B), and the number of bouts of this non-directional activity were greater in 10 ug/L malathion exposed animals than control insects (Table 1; Fig 1A). Female crickets were not significantly affected by treatment ($P > 0.05$ in all cases). There was a nearly significant ($P = 0.06$) effect of treatment on time crickets spent moving vs. not moving, with the trend pointing toward increased movement with higher concentrations of malathion (Table 1). There was no effect of sex, treatment, nor the interaction of these two on distance moved, and average velocity ($P > 0.15$ for all factors).

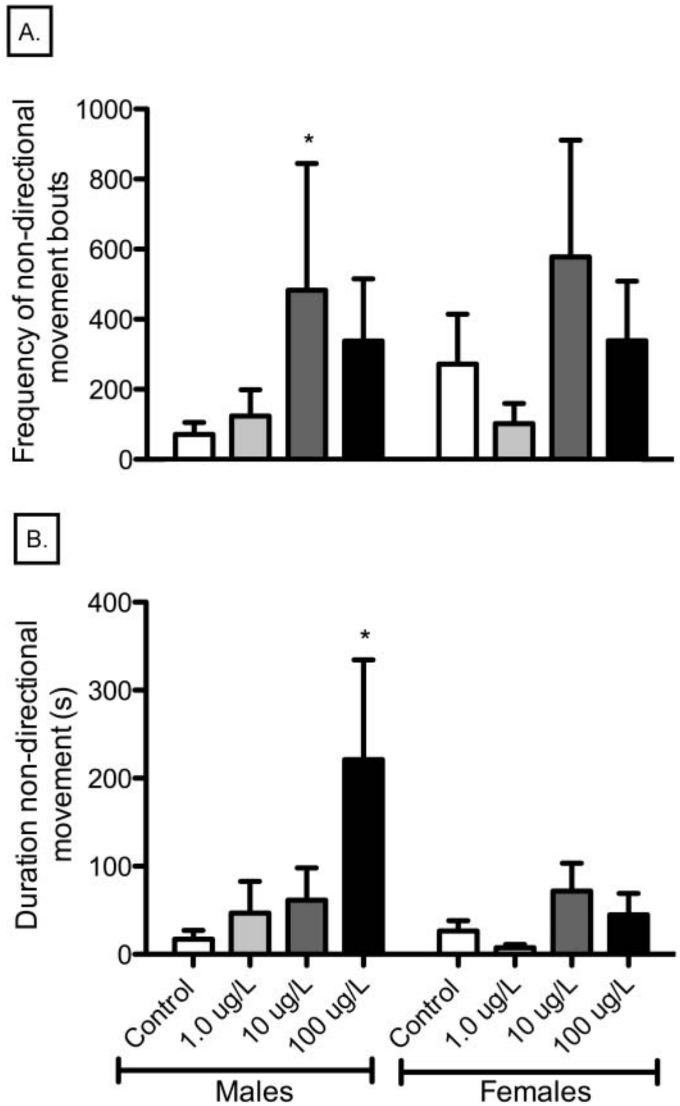

Fig. 1. (A) Frequency and (B) duration (sec) of non-directional movement in house cricket (*Acheta domesticus*) males and females when exposed to sublethal malathion treatments. Asterisks indicate significant differences from the control ($P < 0.05$).

The analysis of data in Experiment 2 revealed a strong effect of sex on the proportion of time spent in each of the three locations, with males spending more time in the neutral and food zones and females more time in the shelter zone (Neutral zone: $\chi^2_{(50,200)} = 70.72$ $P = 0.03$; Food $\chi^2_{(34,136)} = 51.94$ $P = 0.03$; Shelter: $\chi^2_{(50,200)} = 70.72$, $P = 0.03$). There was no significant effect of treatment with either of the sexes ($P > 0.1$). There was, however, a trend of decreased shelter use and increased time spent in the neutral, open zone of the arena with male crickets inoculated with 10 and 100 ug/L concentrations of malathion (Table 2).

Table 1. Mean values (±1 SE) of four metrics of cricket movement behavior after exposure to one of three concentrations of malathion or a control.

	Control	*1.0 ug/L*	*10 ug/L*	*100 ug/L*	*Trial(Sex) (REML)*	*Treatment*	*Sex*	*Treatment*Sex*
Duration of Non-directional movement (sec)	22.8 ± 8.01	21.9 ± 11.0	67.7 ± 18.8	119.1 ± 41.5	59.10	$F_{(3,3)} = 1.16$ $p = 0.36$	$F_{(1,1)} = 0.01$ $p = 0.93$	$F_{(3,3)} = 5.48$ **$p = 0.01$***
Frequency of Non-directional movement (sec)	196.1 ± 88.7	110.4 ± 42.1	406.5 ± 203.7	338.3 ± 122.6	55.32	$F_{(3,3)} = 0.91$ $p = 0.46$	$F_{(1,1)} = 0.18$ $p = 0.69$	$F_{(3,3)} = 4.98$ **$p = 0.015$***
Duration of Movement (sec)	452.4 ± 56.8	479.8 ± 59.9	468.6 ± 55.3	586.5 ± 68.8	48.1	$F_{(3,3)} = 2.80$ $p = 0.06$	$F_{(1,1)} = 1.60$ $p = 0.24$	$F_{(3,3)} = 0.60$ $p = 0.62$
Duration of Non-Movement (sec)	2297.3 ± 50.9	2271.8 ± 57.9	2288.4 ± 48.5	2167.5 ± 61.4	55.32	$F_{(3,3)} = 2.85$ $p = 0.06$	$F_{(1,1)} = 2.38$ $p = 0.16$	$F_{(3,3)} = 0.61$ $p = 0.62$

Table 2. Mean ± SE proportion of time spent in each zone by male and female crickets (*Acheta domesticus*) during behavioral trials in Experiment 2.

	Neutral Zone		*Food Zone*		*Shelter Zone*	
	Male	Female	Male	Female	Male	Female
Control	0.30 ± 0.09	0.52 ± 0.11	0.08 ± 0.04	0.07 ± 0.03	0.60 ± 0.10	0.41 ± 0.12
1.0 ug/L	0.38 ± 0.19	0.64 ± 0.11	0.01 ± 0.00	0.07 ± 0.05	0.62 ± 0.19	0.29 ± 0.11
10 ug/L	0.57 ± 0.10	0.38 ± 0.09	0.09 ± 0.05	0.07 ± 0.04	0.34 ± 0.09	0.55 ± 0.10
100 ug/L	0.54 ± 0.13	0.46 ± 0.12	0.05 ± 0.02	0.00 ± 0.00	0.41 ± 0.12	0.54 ± 0.12

Discussion

Sublethal doses of the pesticide malathion significantly affected the locomotory behavior of house crickets (*Acheta domesticus*) in this study. Periods of non-directional movement increased with the dose of administered malathion. There were also trends of increased movement and decreased use of shelters with increased malathion dose. In some cases, these behavioral effects were dependent on sex, indicating that malathion affects male and female crickets differently. These results provide evidence that malathion does disrupt the normal behavioral patterns of house crickets at sub-lethal doses and may render them more susceptible to predators. Many predators of insects, such as amphibians and fish, are visual hunters [35,36] and are more likely to successfully prey upon insects that are active. The increase in both directional movement and non-directional movement of contaminated animals may therefore make them more susceptible to predation.

Increasing sub-lethal malathion doses increased the amount of time and the frequency of which crickets in this study were engaged in periods of non-directional movement, which is motion of the organism independent of spatial movement. That is, an animal may not change its spatial coordinates but may still be mobile if it is engaged in grooming, fidgeting, convulsing, or other behaviors causing movement of body parts. These results, which indicate an increase in this type of behavior, are not surprising given that many other studies on insect exposure to pesticides reported similar findings.[37,38] For example, malathion residues have been implicated in increasing the mobility of hymenopterans.[18] Also, the pesticide chlordimeform has been shown to cause hyperreflexive and hypersensitive responses to stimuli in lepidopterans,[39] and sublethal doses of chlordane have similar effects on blattarians.[40] Cockroaches have also been shown to increase convulsions, leg movements, and lose balance control when exposed to sublethal concentrations of the pesticides DDT [41] and fipronil.[42]

While there was a significant effect of malathion dose on non-directional movement in crickets, this dose-response relationship was also dependent on sex. When the data are separated by sex it is apparent that the effect on the behavior of male crickets drives this relationship, because no dose-response relationship is evident with females (Fig. 1). These data suggest male crickets are more susceptible to sublethal behavioral effects of malathion than are females. This sex-biased susceptibility to pesticides has been observed in many insect orders, and indeed it has been proposed that male arthropods in general are less tolerant to pesticides and biological control agents than are females.[43,44] If the altered behavior of male crickets does indeed make them more susceptible to predation, this may affect the population and community ecology of the ecosystem.[45]

Male crickets appeared to hide in shelters less when exposed to medium and high doses of malathion and spent a larger proportion of their time in a neutral, open area of the behavioral arena (Table 2). While these trends were nonsignificant, they support the findings of increased activity in male crickets with increased malathion dose in Experiment 1. Crickets use shelter to hide from predators,[30,46] and this altered behavior may make male animals more susceptible to possible predation.

The alterations in cricket behavior in response to sublethal malathion exposure could also have wider ecological implications. For example, because these behaviors will likely increase the likelihood of being preyed upon, trophic transfer of malathion must be considered. Trophic transfer is a crucial issue that is being addressed in a multitude of contaminants.[10,11,36] Malathion has a half-life of 1–10 days,[47] which enables targeted species to transfer this contaminant to their predators. The findings of this study provide a potential pathway for non-target species to be exposed and affected by sub-lethal doses of malathion.

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