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Interaction of a pesticide and larval competition on life history traits of *Culex pipiens*

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ABSTRACT

Mosquito larval development occurs in aquatic habitats that are directly or indirectly exposed to chemical contaminants. Little is known about how interaction of these chemicals with other biotic and abiotic stressors impact mosquito populations. We used two levels of nutrient (low and high) and four larval densities (10, 20, 30, 40) to examine the effects of low concentrations of insecticide malathion, on *Culex pipiens* L. mosquitoes experiencing stress from larval competition. Addition of malathion at the high nutrient condition enhanced survival with increasing larval densities, but this effect was not observed at low nutrient condition. Males exposed to malathion were significantly larger than those from control treatments while the effect of malathion on size of females varied with larval density and the level of nutrients. Larval exposure to malathion and low nutrient resulted in significantly larger females with increases in larval densities compared with other treatments. The effect of malathion on male longevity varied with larval density and amount of nutrients. At higher densities, male longevity was consistently higher in low nutrient than in high nutrient conditions and addition of malathion in high nutrient treatment increased male longevity at the highest density. These effects are most likely attributable to release from competition among survivors after mortality from malathion and density-dependent effects. We conclude that biotic conditions of the larval habitat can alter the impact of low concentrations of chemical contaminants on mosquito populations in ways that may influence the pattern of disease transmission and the outcome of vector control efforts.

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1. Introduction

Proper understanding of how density-dependent processes impact mosquito populations is critical to the design of mosquito control programs. This is because density-dependent processes among the immature larval stages can directly alter mosquito life history traits that are relevant to disease transmission such as the rate of mosquito development, survival to adulthood, propensity to blood feed, reproduction, and adult longevity (Macdonald, 1957; Grimstad and Haramis, 1984; Klowden, 1995; Juliano, 2007; Alto et al., 2008). Specifically, larval competition for limited resources have been shown to prolong development, reduce survival, and foster production of small and short-lived adults (Southwood et al., 1972; Lowrie, 1973; Costanzo et al., 2005a; Reiskind and Wilson, 2008; Reiskind and Lounibos, 2009) with low blood feeding success (Klowden, 1995). Also, density dependence can indirectly impact disease transmission by altering interactions between mosquitoes and pathogens (Alto et al., 2005; Bevins, 2008). These studies have shown that larval competition may be associated with either a reduction (Bevins, 2008) or enhancement (Alto et al., 2005, 2008) of susceptibility of adult mosquitoes to arbovirus infection and potential to transmit.

Density-dependent mechanisms can also alter the outcome of mosquito control strategies depending on the relationship between initial density at the stage when density dependence occurs and the final number of survivors (Legros et al., 2009). First, addition of other sources of mortality in a mosquito population where the final number of survivors is independent of initial density (compensatory) can simply replace one source of natural mortality (competition) with another source (control) with no net change in total mortality (Juliano, 2007). Second, control efforts may alleviate density-dependence and act to increase rather than decrease adult vector densities in populations experiencing strong negative density-dependent processes (overcompensatory, Juliano, 2007). Finally, control efforts may successfully reduce vector densities in populations where survivorship increases as initial density increases (undercompensatory, Legros et al., 2009).

Pesticides are anthropogenic environmental stressors whose impact on vector populations may be modified by density-dependent mechanisms. This is because chemical control is not

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only an important source of mortality but also the most widely used method of controlling agricultural and public health pests. On a global scale, more than 5 billion pounds of pesticides are used annually, with the United States accounting for at least 20% of this usage (Kiely et al., 2004). A large fraction of these chemicals are used in agricultural settings, and a fraction of the remainder for controlling public health pests (Ramade, 1988). Because mosquitoes breed within and around agricultural areas exposed to pesticides, and are direct targets of chemical control because of their role in disease transmission, they commonly experience target and non-target effects of pesticides. Temephos (Abate) and malathion, organophosphate pesticides which act by inhibiting acetylcholine esterase are some of the commonly used pesticides for controlling mosquitoes. Temephos is used as a mosquito larvicide while malathion is applied in wetlands as an ultra low volume (ULV) spray to control adult mosquitoes (Walker, 2000; Wheeler et al., 2009). Malathion is also used for several other purposes including insect eradication programs, head lice control, and control of agricultural pests in food crops, home yards and gardens (Cox, 2003). In the U.S. malathion is a commonly used insecticide and is commercially available to the public for residential application. Mosquito larvae may thus be exposed to these pesticides through direct overspray on the larval habitat or through agricultural runoff (Relyea, 2004). Malathion residues in small droplets from ULV aerial applications may also drift for long distances and enter waterways and induce mortality in non-target invertebrates. Existing data indicate that pesticides are major sources of water pollution around the world (U.S. Department of Agriculture, 1997; El Bakouri et al., 2007) and specifically, malathion has been detected in concentrations ranging from 0.001 to 1 part per million (U.S. Department of Agriculture, 1997; Relyea, 2004).

The pesticide concentration that mosquito larvae are exposed to at any given time is dependent on the frequency of application and the rate of environmental degradation (Antonio et al., 2008). Therefore, mosquitoes may be frequently exposed to both lethal and sublethal pesticide concentrations, but most studies focus on the effect of lethal pesticide concentrations with little appreciation on the impact of sublethal pesticide concentrations despite their potential to alter mosquito population dynamics (Robert and Olson, 1989; Antonio et al., 2008). In addition, most studies ignore the potential of natural biotic (e.g. larval competition, predation) and abiotic (temperature) factors to alter the impact of low pesticide concentrations on mosquito populations. However, we can make some general predictions. As an important mortality factor, pesticides can eliminate negative density-dependent effects by killing some individuals and releasing the survivors from competition. Pesticides also can act additively or synergistically to increase the negative effects of larval competition where the latter refers to mortality due to pesticide and larval competition being greater than mortality due to pesticide or larval competition alone. Also, sublethal effects of pesticides may alter mosquito life history traits, other than survivorship, that are important to disease transmission (e.g. adult life span, size). Such effects have been documented in anuran populations (Boone and Semlitsch, 2002; Relyea, 2004).

To test the above predictions, we exposed different densities of *Culex pipiens* L. (a major vector of West Nile and Saint Louis Encephalitis viruses in the U.S.) larvae to malathion and examined how this affected life history traits including survivorship to adulthood, development time to adulthood, adult size, and adult life span (longevity).

2. Materials and methods

The experiment was conducted using field-collected samples of *C. pipiens* from Urbana-Champaign, IL. The samples were obtained by collecting *Culex* spp. egg rafts from 13 sites in Urbana-Champaign, IL using the standard infusion-baited ovitraps (Reiter, 1986). Egg rafts were individually hatched in Petri dishes in order to distinguish *C. pipiens* from *Culex restuans* first instars based on the presence of a clear scale anterior to the sclerotized egg-beaker (Crabtree et al., 1995; Reiskind and Wilson, 2008). This effort was discontinued because 18 h later when species identification was attempted, most larvae had molted to second instars and had lost this character. Because larvae were collected in September 2009 when *C. pipiens* was more abundant than *C. restuans* we assumed that a vast majority of individuals were *C. pipiens* (Lampman et al., 2006).

To test the effect of intraspecific competition and malathion on performance of mosquitoes, we maintained four larval densities (10, 20, 30, and 40) at two larval resources (low and high nutrients) and two malathion treatments (0 and 0.03 parts per million, ppm) at 25 ± 1° C. The high nutrient food consisted 360 ml of filtered grass infusion prepared by mixing approximately 1.5 kg of grass with 55 l of tap water and leaving the mixture to steep for 3 days (Reiter, 1986). The low food included the same grass infusion diluted with water in a 1:1 ratio. The larvae were supplemented with 0.01 g of Tetramin on the fifth and eighth day after experimental set up. One milliliter (1 ml) of de-ionized (DI) water and 1 ml of 5 ppm malathion concentration, were added as control and treatment, respectively at the time of experimental set up (day 0). Four days later, 0.25 ml of DI water or 5 ppm malathion were again added in respective containers. These pesticide treatments constitute exposing mosquitoes to low concentrations of malathion during larval development. The treatments were replicated five times resulting in a total of 80 containers. The larvae were allowed to grow and develop until all individuals had pupated or died. We also obtained estimates of *C. pipiens*:*C. restuans* ratio by identifying a random sample of dead third and fourth instar larvae from experimental containers (Darsie and Ward, 1981). Results showed that greater than 95% of larvae were *C. pipiens*. The remaining fraction was comprised of *C. restuans*, a species whose larvae are competitively similar to *C. pipiens* (Reiskind and Wilson, 2008). Thus, for the sake of simplicity, we will refer to the mosquitoes used in the current experiment as *C. pipiens*.

Each individual pupa was placed in a plastic tube with water and allowed to emerge to adult stage. Each adult was held individually in cages and maintained on DI water throughout their life at 25 ± 1° C. The water source was a moist cotton ball placed on top of the screen. The mosquitoes were monitored daily and dead adults were collected, recorded, and stored in microcentrifuge tubes. The adult mosquitoes were oven-dried at 50 °C for at least 24 h and their mass was determined by a weighing balance. So for each experimental replicate we measured adult longevity, survival to adulthood (proportion surviving), development time from egg-hatch to adult emergence, and mass of adult mosquitoes. We used a linear model for the analysis with malathion treatment and nutrient resource as the discrete variables and density as the continuous variable (PROC GLM, SAS 9.1). We used an additive approach following Kutner et al. (2005) and dropped non-significant interactions with density (continuous variable) from the model starting with the three-way interactions. We log transformed longevity and arcsine square root transformed survival data to meet the assumptions of normality and homogeneity of variance.

3. Results

There was a marginally significant three-way interaction ($P = 0.06$) for survival between density, resource, and malathion treatments (Table 1, Fig. 1). Mosquitoes had similar survival rates at the two larval resources and malathion conditions when reared