#### POPULATION AND COMMUNITY ECOLOGY

# Malathion Influences Competition Between Aedes albopictus and Aedes japonicus

BANUGOPAN KESAVARAJU, 1,2 BARRY ALTO, ALI AFIFY, AND RANDY GAUGLER<sup>2</sup>

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ABSTRACT Competitive interactions may facilitate or repel invaders into new communities, and these interactions may depend on other environmental conditions such as the presence of pesticides. Malathion is widely used in controlling agricultural pests and mosquitoes worldwide. Small amounts of malathion, previously considered inconsequential, may in fact increase in lethality when combined with biotic stressors in aquatic systems. We tested whether low concentrations of malathion (0.11 ppm) that are often detected in aquatic systems, affect competition between two invasive mosquito species Aedes albopictus (Skuse) and Aedes japonicus Theobald. There were no survivors of Ae. japonicus larvae in malathion. There was a significant negative effect of Ae. japonicus density on Ae. albopictus survival, but this effect was absent in the presence of malathion. There was also a moderate negative effect of Ae. japonicus density on Ae. albopictus female size, but this effect was absent in the presence of malathion. These findings indicate that pesticide-mediated alterations in competition and species-specific differences in susceptibility to pesticides could play a role in enhancing invasive potential of Ae. albopictus.

KEY WORDS malathion, competition, Aedes albopictus, Aedes japonicus, pesticides

Competition has been recognized as an important phenomenon of community ecology, and it plays a vital role in determining species distribution patterns and shaping the structure of communities. Interspecific competition in many instances is asymmetrical where one species is clearly the superior competitor (references in Juliano 2009, Twomey et al. 2008). However, these interspecific competitive interactions may be modified by other environmental influences such as biotic (e.g., predation; reviewed by Chase et al. 2002) and abiotic factors (e.g., temperature, pH, salinity, humidity; e.g., Costanzo et al. 2005). Some species that are superior competitors under normal conditions may be relatively disadvantaged under more stressful abiotic conditions that may, in part, explain the coexistence between species under those stressful conditions (Juliano et al. 2002, Leisnham and Juliano 2009). These disadvantages may be due to relative differences in tolerance to stressful conditions among species. Several studies have shown that these differences in tolerances could be for tannins (Sota 1993), salinity (Gomez-Mestre and Tejedo 2003), and manmade pollution from copper (Piola and Johnston 2005). Thus, inferior competitors may be able to es-

Pesticides are used widely to control agricultural pests and mosquitoes that are vectors of disease agents. The direct impact of pesticides on nontarget organisms is mostly reported as the lethal dose required to kill 50% of the tested individuals ( $LD_{50}$ ). However, pesticides may affect species directly (e.g., physiology and behavior) or indirectly by altering other community components such as competition and predation (Relyea 2004). These effects may be attributable to both lethal and sublethal concentrations of pesticides. Consider an aquatic community where two species are stressed due to competition for resources, if an additional stress in the form of pesticides is induced into the system then the relative mortality of the individuals may increase compared with a situation where they are subjected to stress

cape extinction through trade-offs between competitive ability and environmental tolerance. Furthermore, in some instances, abiotic factors and physiological responses to those factors can be primary determinants to community composition. Only recently have ecotoxicology studies begun to investigate how abiotic factors such as environmental contaminants (e.g., pesticides) mediate interspecific competition (Relyea and Hoverman 2006). Agudelo-Silva and Spielman (1984) showed larviciding could be counterproductive and could increase the number of adults emerging from containers due to release from competition, whereas Alto et al. (2008) showed that size of the emerging adult mosquitoes from larval competition could alter their susceptibility to infection by viruses.

<sup>&</sup>lt;sup>1</sup> Corresponding author: Salt Lake City Mosquito Abatement District, 2020 N. Redwood Rd., Salt Lake City, UT 84116 (e-mail: banu@slcmad.org).

<sup>&</sup>lt;sup>2</sup> Center for Vector Biology, 180 Jones Ave., Rutgers University, New Brunswick, NJ 08901-8536.

<sup>&</sup>lt;sup>3</sup> Illinois Natural History Survey, Institute of Natural Resource Sustainability, University of Illinois, 1816 S. Oak St., Champaign, IL 61820.

from pesticides alone (Relyea 2005). Similarly mortality is relatively higher where stress due to predation is combined with pesticide compared with predation or pesticide alone (Relyea and Mills 2001, Relyea 2004). These interactions are synergistic effects because the impact of pesticides is greater when combined with other ecological stressors (e.g., competition and predation) compared with the ecological stress by itself. Dynamics of interspecific competition also might change if one competing species is more sensitive to a pesticide than the other, as demonstrated in aguatic systems (Relyea and Hoverman 2006). This becomes more interesting when considering invasive species, a problem that causes significant economical and biological losses annually (Kolar and Lodge 2001) and is accelerating due to globalization (Lee 2002).

Malathion is an organophosphate that is globally applied and a frequently used pesticide in the United States for controlling agricultural pests and mosquitoes (Kiely et al. 2004). Malathion is usually used for controlling the adult stages of insects, but it does get into aquatic systems (Kiely et al. 2004, Relyea and Hoverman 2006) indirectly, and it has been detected in concentrations up to 0.6 ppm (Relyea 2004, and the references within). Malathion is applied on truckmounted ultralow volume sprayers for mosquito control in residential areas also could result in containers such as garbage cans, toys, tires, flower pots, and vases receiving varying amounts of the pesticide (Tietze et al. 1996). Likewise, other organophosphates (e.g., Temephos) are applied directly to aquatic habitats for mosquito control.

Container mosquitoes specialize in colonizing small containers. The larvae feed on microorganisms that grow on the surface of detritus that fall in to the containers (Merritt et al. 1992). Most of these container mosquitoes lay eggs just above the water level that hatch upon flooding. Aedes albopictus (Skuse) is a container mosquito native to Asia that has invaded many countries across the globe (Benedict et al. 2007, Enserink 2008). Ae. albopictus was first reported in Texas in the 1980s but now it has spread to 26 states in the continental United States (Hawley 1988, Moore 1999). Used tires that contain desiccation-tolerant eggs being transported globally is claimed to be the leading cause of introduction (Hawley et al. 1987). Ae. albopictus is an important vector of dengue and chikungunya viruses and in the United States this species has been isolated with West Nile (Holick et al. 2002, Kitron 2003), LaCrosse (Gerhardt et al. 2001), and Eastern equine encephalitis viruses (Mitchell et al. 1992).

Ae. japonicus Theobald from Japan was first discovered in container habitats in the northeastern United States in 1998. Similarly to Ae. albopictus, introduction of Ae. japonicus is suspected to be facilitated by the used tire trade (Peyton et al. 1999, Lounibos 2002). This species now has a geographic range that includes parts of the east coast, central, and foci in western United States (Peyton et al. 1999, Roppo et al. 2004, Gallitano et al. 2006). Although Ae. japonicus has not been recognized as a major disease vector, it is com-

petent for several arboviruses, including West Nile, Eastern equine, LaCrosse, St. Louis encephalitis, and Japanese encephalitis viruses (Turell et al. 2001). Ae. albopictus invasion was from the south to the northeast (Juliano and Lounibos 2005), whereas Ae. japonicus invasion was from northeast to the south (Bevins 2007). The distribution of these species started overlapping only recently, and interspecific competition occurs among the larval stages (Armistead et al. 2008). Both species occupy water-filled artificial containers around residential areas, and competition among the larval stages is asymmetric, with Ae. albopictus being the superior competitor (Armistead et al. 2008). Superior competitive ability of Ae. albopictus is projected to be one of the important factors facilitating its invasion (Juliano and Lounibos 2005). If malathion alters the competitive interactions between Ae. albopictus and other species, it could indirectly affect the invasive potential of Ae. albopictus. For example, low sensitivity to malathion by Ae. albopictus relative to other species could favor Ae. albopictus. We tested whether malathion alters the competitive interactions between Ae. albopictus and Ae. japonicus.

#### **Materials and Methods**

Ae. albopictus larvae were collected at a junkyard in Mercer Co. and a cemetery in Monmouth Co., NJ, in 2007. Larvae were reared in enamel pans with brewers yeast and lactalbumin (50:50), and the adults were reared in a 30- by 30-cm cage. Adults were provided with 10% sucrose solution, weekly bloodmeals from quails, and a black cup lined with germination paper for egg laying. Eggs on paper were harvested weekly and stored in containers at high humidity. The offspring of these mosquitoes (F2 generation) were used to start the experiment. Ae. japonicus do not readily colonize under laboratory conditions, so their eggs were obtained from a laboratory colony established in 2005 at the Center for Vector Biology, Rutgers University. The generations of Ae. japonicus have not been recorded, but we estimate 20.

Malathion and control treatments were crossed with 14 different density combinations of Ae. albopictus and Ae. japonicus, respectively (0:10, 0:20, 0:40, 10:0, 20:0, 40:0, 10:10, 20:20, 20:10, 10:20, 40:10, 10:40, 20:40, and 40:20), and each unique combination was replicated three times for a total of 84 replicated units  $(2 \times 14 \times 3)$ . The experiment was conducted in 400-ml polypropylene cups in which 0.7 g of oak (Quercus spp.) leaves, 350 ml of filtered tap water, and 1 ml of microbial inoculum were added. The cups were setup 4 d before larvae were added to allow sufficient microbial productivity that served as the nutritional resource for larvae (Merritt et al. 1992). Microbial inoculums were obtained by filtering water (0.149-mm sieve) from 7.6-liter plastic containers that had been naturally colonized by mosquitoes at the Center for Vector Biology campus, Rutgers University. Mosquito eggs were hatched by immersion in water with 0.15 g of lactalbumin and brewers yeast mix (50:50) for 24 h. The experiment was conducted at  $25^{\circ}C \pm 1$  and a

Table 1.	Linear model results for survival and developmental time from the interspecific competition experiment in the presence and
absence of r	nalathion

	Survival			Developmental time					
Variable				Female			Male		
	df	F	P	df	F	P	df	F	P
Ae. albopictus									
Treatment	1	76.55	< 0.0001	1	0.17	0.6822	1	0.11	0.7470
Ae. japonicus density	1	1.21	0.2754	1	0.55	0.4625	1	1.43	0.2392
Ae. albopictus density	1	6.60	0.0127	1	4.52	< 0.0418	1	2.87	0.0993
Treatment $\times$ Ae. japonicus density	1	4.38	0.0405	1	2.81	0.2749	1	1.28	0.2651
Treatment $\times$ Ae. albopictus density	1	7.01	0.0103	1	1.24	0.2749	1	0.44	0.5092
Error	66			35			40		
Ae. japonicus									
Ae. japonicus density	1	8.52	0.0066	1	32.98	< 0.0001	1	4.74	0.0406
Ae. albopictus density	1	15.6	0.0004	1	28.95	< 0.0001	1	10.53	0.0037
Error	30			26			24		

Numbers in bold are significant. Because there were no Ae. japonicus survivors in malathion, the interactions were dropped from the model.

photoperiod of 16:8 (L:D) h. The first instars that hatched after 24 h were counted and added to the experimental cups. Preliminary studies on susceptibility of larvae showed that first instars were more susceptible to malathion than higher instars, so malathion solution was added 3 d after the larvae were added to the cups. Malathion solution was prepared by mixing 2 µl of Ortho malathion plus (The Scotts company, Marysville, OH) in 1 liter of filtered tap water. Malathion treatment cups received 0.11 ppm malathion (20 ml of malathion solution), whereas the control cups received 20 ml of filtered tap water. The amount of malathion used was based on concentrations of malathion in aquatic systems in nature and experiments on the impacts of sublethal doses of malathion on larval amphibians (Tietze et al. 1996; Relyea 2004, and references therein). Cups were monitored daily and pupae were collected and individually held in water-filled vials sealed with cotton. Upon eclosion the adults were identified, sexed, and dried at 50°C. Wings of females were dissected, and their lengths were measured on a microscope (Media Cybernetics, Silver Spring, MD) and used as an indicator of adult female size.

Data Analysis. A composite index of population performance ( $\lambda$ ') analogous to finite rate of population increase was calculated for each cup by first estimating the instantaneous rate of increase r' (Livdahl and Sugihara 1984) for each cup:

$$\mathbf{r}' = \left[ \frac{\ln\left[ (1/N_0) \sum_{x} A_x f(w_x) \right]}{D + \left[ \sum_{x} x A_x f(w_x) \middle/ \sum_{x} A_x f(w_x) \right]} \right]$$

where  $N_0$  is the initial number of females in the cohort (assumed to be 50%);  $A_x$  is the number of females eclosing on day x; D is the time from eclosion to reproduction taken as 14 d for  $Ae.\ albopictus$  (Livdahl and Willey 1991) and 12 d for  $Ae.\ japonicus$  (Armistead et al. 2008);  $f(w_x)$  is a function based on the relationship between size and fecundity in female mosquitoes

estimated from the mean wing length on day x,  $w_x$  of female mosquitoes (Juliano 1998). For Ae. Albopictus,  $f(w_x) = 78.02 w_x - 121.240$  (Lounibos et al.2002) and for Ae. japonicus,  $f(w_x) = 53.078 w_x - 113.91$ (Armistead et al. 2008). Finite rate of increase was calculated from the r' as  $\lambda' = \exp(r')$  (Juliano 1998). We express population performance based on  $\lambda$ ' because it is estimable even if no individuals survive to reproduction. Survivorship (arcsine square roottransformed values of proportion surviving to satisfy assumptions), developmental time and lambda  $(\lambda')$ per cup were analyzed using a linear model with densities of Ae. albopictus and Ae. japonicus as continuous variables and treatment (control and malathion) as discrete variables (PROCGLM, SAS 9.1, SAS Institute, Cary, NC) following Murrell and Juliano (2008). We tested for the equality of slopes and for a significant interaction between treatment and mosquito densities (Ae. albopictus or Ae. japonicus) that would indicate that the treatments affected the competitive effect on one another (Murrell and Juliano 2008).

#### Results

There were no survivors of Ae. japonicus in the presence of malathion; hence, the interaction between treatment and density was not included in the model. However, survival was affected by Ae. albopictus (interspecific competition) and Ae. japonicus densities (intraspecific competition) (Table 1). Ae. japonicus survival decreased with increase in Ae. albopictus and Ae. japonicus densities (Fig. 1A and B). Developmental times of both male and female Ae. japonicus were affected by Ae. japonicus and Ae. albopictus densities (Table 1). Ae. japonicus female and male developmental times increased with increase in Ae. japonicus and Ae. albopictus densities (Fig. 2A and B). The interaction between treatment (control and malathion), and Ae. japonicus density was significant for Ae. albopictus survivorship, indicating that the number of survivors differed in the presence of malathion. There was also a significant interaction between treatment and Ae. albopictus density (Table 1)

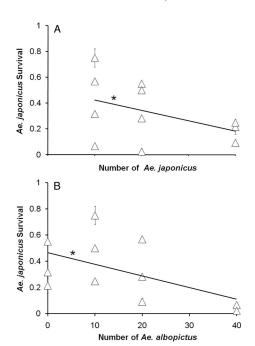


Fig. 1. (A) Ae. japonicus mean  $\pm$  SE proportion surviving and Ae. japonicus density (back transformed values). (B) Ae. japonicus mean  $\pm$  SE proportion surviving and Ae. albopictus density (back transformed values). Symbols show mean estimates for each experimental container. An asterisk (\*) shows significant effects.

for Ae. albopictus survivorship. Ae. albopictus survival was negatively affected by increasing densities of Ae. japonicus (interspecific competition) and Ae. albopictus (intraspecific competition) in the controls, but Ae. albopictus survivorship in the presence of malathion was not affected by the density of either Ae. japonicus or Ae. albopictus (Fig. 3A and B). For Ae. albopictus female wing lengths, the interaction between treatment and Ae. japonicus density was not significant but close to 0.05, whereas the interaction between treatment and Ae. albopictus density was significant (Table 2). Female wing lengths for Ae. albopictus decreased with increase in Ae. japonicus and Ae. albopictus densities in control but wing lengths were not affected in the presence of malathion (Fig. 3C and D). There was a significant treatment effect on Ae. albopictus  $\lambda$ ' (control, 1.0812  $\pm$  0.0476; malathion,  $0.2075 \pm 0.0778$ ), but there were no significant interactions between treatment and Ae. japonicus or Ae. albopictus density for  $\lambda$ , indicating that treatment did not affect the competitive effect (Table 2). There was no significant interaction between treatment and Ae. japonicus or Ae. albopictus density for Ae. albopictus developmental times, indicating that treatment did not affect the competitive effect. Aedes albopictus female developmental times, but not males, increased with Ae. albopictus densities (intraspecific competition) (Fig. 4). However, developmental times of females were unaffected by Ae. japonicus densities (Table 1).

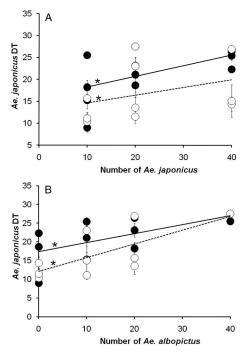


Fig. 2. (A) Ae. japonicus male and female mean ± SE developmental times (DT) and Ae. japonicus density. (B) Ae. japonicus male and female mean ± SE developmental times and Ae. albopictus density. Filled symbols are female and open symbols are male mean estimates for each experimental container. Solid and dashed lines drawn through means show the best fit for females and males, respectively. An asterisk (\*) shows significant effects.

## Discussion

The outcome of biotic interactions such as competition and predation may be condition-specific. Survival of Ae. albopictus was affected by interspecific competition from Ae. japonicus and intraspecific competition from conspecifics. Wing length of Ae. albopictus was affected by intraspecific competition from conspecifics. Although the overall number of survivors was much lower in malathion, the presence of malathion released Ae. albopictus from these negative competitive effects especially because none of Ae. japonicus survived the malathion treatment. Most of the Ae. *japonicus* that died in malathion died within the first 5 d of adding malathion to experimental containers. Hence, it is likely that Ae. albopictus that survived in the presence of malathion were exposed to little to no competitive effects from Ae. japonicus. Female wing lengths of Ae. albopictus decreased with higher Ae. japonicus and Ae. albopictus densities in control but were unaffected by Ae. japonicus and Ae. albopictus densities in malathion (Fig. 3C and D). So, it can be concluded that addition of malathion may favor Ae. albopictus in containers that have both species. However, these results on sizes must be interpreted with caution given that the trend for the treatment  $\times$  Ae. japonicus density interaction was only marginal. Overall, these results shows that Ae. albopictus benefits

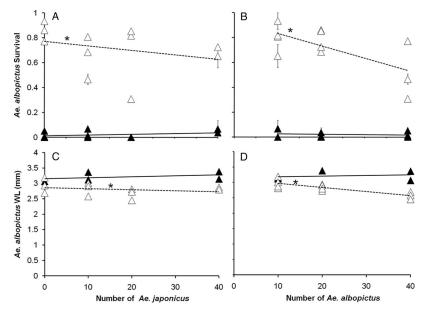


Fig. 3. (A) Ae. albopictus mean  $\pm$  SE proportion surviving and Ae. japonicus density (back transformed values). (B) Ae. albopictus mean  $\pm$  SE proportion surviving and Ae. albopictus density (back transformed values). (C) Ae. albopictus mean  $\pm$  SE female wing length (WL) and Ae. japonicus density. (D) Ae. albopictus mean  $\pm$  SE female wing length and Ae. albopicus density. Filled symbols (solid lines) are malathion and open symbols (dashed lines) are control and they show mean estimates for each experimental container. An asterisk (\*) shows significant effects.

from competitive release in the presence of malathion at high interspecific densities. Malathion was added 3 d after the addition of larvae to the cups, so it is possible there were competitive effects within that period that affected the final results. There are no studies on competition specific to the different instars of *Ae. albopictus* and *Ae. Japonicus*, so determining the consequences of such an effect is difficult. Because there were no *Ae. japonicus* survivors in the malathion treatment, irrespective of the competition (interspecific or intraspecific), we conclude that *Ae. japonicus* are less tolerant to malathion compared with *Ae. al-bopictus*.

Armistead et al. (2008) showed that Ae. albopictus is a superior competitor to Ae. japonicus and that they are more affected by intraspecific than interspecific

competition that is confirmed by the current study. Ae. albopictus are superior competitors to many species, explaining in part why Ae. albopictus has successfully invaded so many countries (Lounibos et al. 2001, Juliano and Lounibos 2005, Benedict et al. 2007). Because Ae. albopictus is more tolerant to pesticides, competitive asymmetry is further enhanced and there is a high probability that Ae. albopictus will have an advantage over Ae. japonicus, as well as other species, in areas where malathion is sprayed to control mosquitoes and other insects. Rapid invasion by Ae. albopictus may be attributable not only to being competitively superior but also by being less susceptible to malathion compared with some other species. If Ae. albopictus is relatively less susceptible to other pesticides, then we would predict that pesticides may fur-

Table 2. Linear model results for female wing lengths and  $\lambda'$  from the interspecific competition experiment in the presence and absence of malathion

v. :.11.		Wing length		λ'			
Variable	df	F	P	df	F	P	
Ae. albopictus							
Treatment	1	1	0.3236	1	16	0.0002	
Ae. japonicus density	1	0.06	0.8070	1	0.55	0.4611	
Ae. albopictus density	1	1.16	0.2900	1	2.16	0.1468	
Treatment × Ae. japonicus density	1	3.54	0.0691	1	0.03	0.8560	
Treatment $\times$ Ae. albopictus density	1	6.18	0.0183	1	0.18	0.6738	
Error	37			66			
Ae. japonicus							
Ae. japonicus density	1	3.33	0.0806	1	0.28	0.5970	
Ae. albopictus density	1	5.72	0.0249	1	1.08	0.3029	
Error	26			65			

Numbers in bold are significant. Because there were no Ae. japonicus survivors in malathion, the interactions were dropped from the model.

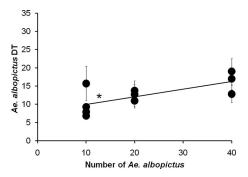


Fig. 4. Ae. albopictus female mean ± SE developmental times and Ae. albopictus density. Filled symbols are male mean estimates for each experimental container. Line drawn through means show the best fit. An asterisk (\*) shows significant effects.

ther facilitate the spread of *Ae. albopictus*, an unexpected consequence of control practices.

Ae. japonicus was less tolerant to pesticides, and no individuals in the pesticide treatment survived. But, it is possible that Ae. japonicus individuals from the laboratory colony lost their capability to tolerate pesticides. Collection of sufficient eggs from the wild to be used in mosquito competition experiments is seldom possible. It is difficult to identify species from eggs or first instars without destructive sampling. Rearing in the laboratory ensures ready supply of uncontaminated eggs that is achieved by collecting larvae from the wild, rearing them in the laboratory, and blood feeding the adults to obtain F1 generation eggs. Unlike Ae. albopictus, Ae. japonicus do not readily colonize in the laboratory, and we were aware of only two colonies of this species in the United States, including the colony used here. Hence, comparing the performance of laboratory and field strains of Ae. japonicus similar to Ae. albopictus would be difficult. There have been other studies that have used this Ae. japonicus strain and extrapolated the results to Ae. japonicus in general (e.g., Armistead et al. 2008). Although Ae. albopictus may have different susceptibility levels, studies indicate that laboratory rearing will have little impact on the pesticide susceptibility levels of Ae. albopictus (B.K., unpublished data). A laboratory strain of Ae. albopictus collected from the same area as the strain used in this study was propagated for >70 generations (in colony since 1995), but still their sensitivity to malathion was similar to the near wild strain used in the current study (B.K., unpublished data). A study on the pesticide sensitivities of another species, Aedes aegypti (L.), within the same genus has also shown that laboratory rearing does not increase sensitivity to pesticides strengthening our argument that laboratory rearing may not select against reduced pesticide sensitivity (Chaiyasit et al. 2006).

Laboratory animals are often used for experiments due to lack of readily available wild caught individuals, but it is likely that the survivors were selected for laboratory rearing conditions as demonstrated in other mosquito studies (Lorenz et al. 1984, Wallis et al. 1985). Crowding is a common side effect of laboratory rearing such that individuals that are less sensitive to crowding are selected. Hence intraspecific competition due to crowding may not have any negative fitness effects especially under nutrient-rich rearing conditions. Further research is warranted regarding the sensitivity of *Ae. japonicus* to malathion.

Adult female mosquitoes can detect potential dangers in egg-laying habitats. This has been well demonstrated in predator–prey studies where female mosquitoes avoid habitats that have cues from a feeding predator (Walton et al. 2009). It is suspected that chemical cues are the likely mechanism by which female mosquitoes avoid these habitats with predators (Blaustein and Chase 2005). Several pesticides repel ovipositing mosquitoes, and these pesticides have been reviewed by Bentley and Day (1989). Concentrations of most of these pesticides need to be above at least 50 ppm to repel ovipositing mosquitoes, and for malathion it has to be >125 ppm, which is much higher than the concentrations that we used in our experiments (Bentley and Day 1989).

The outcome of interspecific interactions for the species discussed here, and by extension other mosquitoes, may have consequences for determining mosquito distribution patterns and vector potential of diseases important for human health (Juliano and Lounibos 2005). Hence, malathion and other pesticides might affect the invasive potential of Ae. albopictus. For example Ae. albopictus is the superior competitor to Ae. aegypti. However, studies suggest that changes in basal food resources among larval stages as well as species differences in tolerance to abiotic factors may reduce competitive asymmetry given that these species coexist in some areas (e.g., Costanzo et al. 2005). Malathion is highly toxic to aquatic invertebrates and brings into question whether other biotic interactions may be modified by this ubiquitous environmental contaminant. Ae. albopictus is a superior competitor to native Aedes triseriatus, but the presence of dipteran predators promotes predator-mediated coexistence between these two species (Juliano and Lounibos 2005, Kesavaraju et al. 2008, Alto et al. 2009). Furthermore, Ae. triseriatus is known to be more susceptible to malathion than Ae. albopictus (PAN Pesticide database, http://www.pesticideinfo. org/). Future research will investigate whether malathion decreases the probability of predator-mediated coexistence between competing species due to detrimental effects on these dipteran predators. Overall, we conclude that low doses of pesticides can have indirect effects on insect communities there by altering the invasive potential of exotic mosquito species. Because impacts of invasive species are on the rise, more studies need to be directed toward the potential of pesticides in altering the interactions between invasive species and native communities.

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