Effect of Malathion on Larval Competition Between *Aedes albopictus* and *Aedes atropalpus* (Diptera: Culicidae)

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ABSTRACT *Aedes albopictus* (Skuse) and *Aedes atropalpus* (Coquillett) (Diptera: Culicidae) are container-dwelling mosquito species that are well established in the eastern United States. Interspecific larval competition studies have shown *Ae. albopictus* to be a superior competitor over many species. A laboratory experiment was conducted in artificial containers to evaluate the effects of malathion on larval interactions between *Ae. albopictus* and *Ae. atropalpus*. The survivorship of *Ae. albopictus* increased with increasing *Ae. atropalpus* densities in control but decreased with increasing *Ae. atropalpus* densities in the presence of malathion. Alternatively, *Ae. atropalpus* survivorship did not differ between control and malathion treatments. Developmental times were not affected by interspecific competition in both treatments for either species. These results show that malathion could facilitate coexistence between *Ae. albopictus* and *Ae. atropalpus*. This demonstrates how sublethal concentrations of malathion (and perhaps other pesticides with similar modes of action) can enable an inferior competitor to coexist in the same habitat with a superior competitor. This is the first report of synergistic survival of a weaker mosquito competitor in the presence of a pesticide due to condition-specific competition.

KEY WORDS *Aedes albopictus*, *Aedes atropalpus*, malathion, competition, pesticide

Competition is important in structuring communities (Paine 1974). Asymmetry is common in interspecific competition wherein one species is a superior competitor that most often displaces the inferior competitor. In some instances, interspecific competition may be affected by environmental factors that may reduce asymmetry, or reverse competitive advantages that may lead to coexistence (Costanzo et al. 2005) and hence are referred to as condition-specific competition. Investigations on condition-specific competition have focused on biotic factors such as predatory cues (Kesavaraju et al. 2008) and abiotic factors such as temperature, pH, salinity, and desiccation (Taniguchi and Nakano 2000, Costanzo et al. 2005). Relatively little is known about how abiotic factors such as pesticides may effect interspecific competition but studies on amphibians have shown that small doses of pesticides could have a significant impact on mortality and competitive interactions (Relyea 2004).

Artificial containers such as garbage cans, toys, tires, cemetery vases, and natural containers such as tree holes collect rainwater and detritus. A specialized group of insects including a few species of mosquitoes, such as *Aedes albopictus* (Skuse), the Asian tiger mosquito, colonize and proliferate in these container habitats. *Ae. albopictus* is native to Asia but was detected in the southern United States during the mid-1980s (Hawley et al. 1987) and has since spread to many other states, including the Northeast (Moore 1999, Farajollahi and Nelder 2009). Larvae of *Ae. albopictus* are competitively superior to many mosquitoes and have caused local extinctions of some species (O'Meara et al. 1995, Juliano and Lounibos 2005). *Ae. albopictus* is a medically important pest because of its capacity to vector arboviral diseases that include chikungunya, eastern equine encephalitis, West Nile virus, and La Crosse encephalitis (Mitchell et al. 1992; Ibañez-Bernal et al. 1997; Gerhardt et al. 2001; Turell et al. 2001, 2005). The indigenous mosquito *Aedes atropalpus* (Coquillett) usually colonizes rock pools but reports indicate that they also have adapted to colonize container habitats such as tires, enabling them to expand their range and invade new areas (Lounibos 2002). *Ae. albopictus* and *Ae. atropalpus* co-occur in container habitats across New Jersey, but there have been no reports on competition between these species. Although *Ae. atropalpus* is not recognized as an important vector for major diseases, laboratory experiments have shown *Ae. atropalpus* to be a potential vector for La Crosse virus (Freier and Beier 1984).

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Pesticides are widely used across the world for controlling both agricultural pests and disease-causing vectors such as mosquitoes. Lethality of a pesticide is usually measured in amounts needed to kill 50% of the individuals (LD_{50} values). Typically, if the LD_{50} values are higher, the amount of pesticide needed to kill 50% of individuals will be higher. But most of the experiments that estimate LD_{50} values consider the direct impact of the pesticides. Recent work has shown that pesticide doses that are much lower than the LD_{50} values can cause significant mortality if they are combined with an ecological stress such as predation pressure or competition (Relyea 2004, 2005). Alternatively, pesticides also can affect interspecific interactions where one species is more tolerant to a pesticide than the other (Relyea and Hoverman 2006). Although there are no direct comparisons in susceptibility to malathion between *Ae. albopictus* and *Ae. atropalpus*, a study comparing LD_{50} values for three *Aedes* species has shown *Ae. atropalpus* to be most tolerant to malathion (Cilek et al. 1995).

Malathion is a broad-spectrum organophosphate insecticide that is commonly used as an adulticide for mosquitoes and other agricultural pests. Application rates of malathion for mosquito control range from 0.2 to 4.3 kg/ha (Relyea 2004). Malathion is mostly applied as an adulticide in terrestrial areas but is present at 0.001–0.6 mg/liter in aquatic environments (CDFG 1982, USDA 1997). Although malathion is not directly applied to water bodies, other organophosphates such as temephos are directly applied to water as a larvicide. Low concentrations of malathion, once thought to be nonlethal in aquatic ecosystems, have been shown to cause high mortality among amphibians when combined with natural stressors (Relyea 2004). Malathion sprayed from ultralow volume sprayers in residential areas may collect in container habitats such as garbage cans, toys, tires, and flower vases in low doses (Tietze et al. 1996). The objective of our research was to test whether malathion alters competition between *Ae. albopictus* and *Ae. atropalpus.*

Materials and Methods

Two treatments, malathion or no malathion (control), were established with 14 different density combinations of *Ae. albopictus:Ae. atropalpus*: 0:20, 0:40, 0:60, 20:0, 40:0, 60:0, 20:20, 30:30, 15:45, 45:15, 10:30, 30:10, 10:0, and 0:10. Each treatment and density combination was replicated three times, making 84 replicated units in total (2 by 14 by 3). *Ae. albopictus* larvae used in the experiment were from an F2 laboratory colony whose base population was collected as larvae from cemeteries and tire sites in Mercer County, NJ. *Ae. atropalpus* used in this experiment were from a laboratory colony maintained at the Center for Vector Biology, Rutgers University, since 1995. Eggs of *Ae. albopictus* and *Ae. atropalpus* were hatched by immersion in 1,000 ml of distilled water (DI) and 0.15 g of lactalbumin and brewers yeast (1:1).

The experiment was conducted in 400-ml containers with lids, and each container received 350 ml of DI, 1 ml of microbial inoculum, and 1.0 g of oak (*Quercus* spp.) leaf detritus 3 d before first-instar larvae were added to allow sufficient microbial productivity in the containers (Murrell and Juliano 2008). Containers were supplemented with the same amount of oak leaf detritus on days 11, 22, and 32 to maintain larval food supply. The containers were randomized and placed in an incubator and held at 25°C and a photoperiod of 16:8 (L:D) h. The malathion solution was prepared by mixing 2 μl of ortho-malathion plus (The Scotts Company, Marysville, OH) with 1 liter of DI water. Three days after first-instar larvae were added, 20 ml of malathion solution (0.11 ppm) was added to treatment beakers, and 20 ml of DI water was added to control beakers (Kesavaraju et al. 2010). Preliminary studies showed that younger instars were more susceptible to malathion than older instars; hence, malathion was added 3 d after first-instar larvae were added to the cups. Pupae were removed daily and placed in 1.5-ml microcentrifuge tubes for eclosion. Adults were identified to sex and species, and their date to eclosion was recorded. We ended the experiment on day 73, when we collected and identified the remaining larvae. Pupae collected on day 73 were allowed to eclose and included in the adult data set.

Proportion surviving and developmental time (days to pupation) for males and females for each species were analyzed following Murrell and Juliano (2008) by using PROC GLM (SAS 9.1, SAS Institute, Cary, NC). Proportion surviving was arcsine square-root transformed to satisfy assumptions of normality and homogeneity of variances. A significant interaction between treatment (control and malathion) and densities (*Ae. albopictus* and *Ae. atropalpus*) would indicate that the treatments affected inter or intraspecific competition.

Results

Survivorship. *Ae. albopictus.* There was a significant interaction between *Ae. atropalpus* density and treatment (control and malathion) for the proportion surviving, indicating that treatment affected interspecific competition (Table 1.). However, the interaction between *Ae. albopictus* density and treatment was marginally significant indicating that treatment also might have affected intraspecific competition (Table 1). *Ae. albopictus* proportion surviving increased with increase in *Ae. atropalpus* density in the control but it decreased with increase in *Ae. atropalpus* density in the presence of malathion (Fig. 1A). Proportion surviving decreased with increase in *Ae. albopictus* density both in control and malathion treatments (Fig. 1B).

Ae. atropalpus. There was a significant interaction between treatment and *Ae. atropalpus* density but no interaction between treatment and *Ae. albopictus* density indicating that the intraspecific competition was affected by treatment but not interspecific competition. *Ae. atropalpus* proportion surviving decreased with increase in *Ae. atropalpus* density both in control and malathion (Fig. 2).

Table 1. Linear model results for *Ae. albopictus* and *Ae. atropalpus* from the interspecific competition experiment in the presence and absence of malathion

| Variable	Survival			Developmental time					
				Female			Male		
	df	F	P	df	F	P	df	F	P
Ae. albopictus									
Treatment	1	9.86	**0.0027**	1	0.53	0.4691	1	0.01	0.9423
Ae. albopictus density	1	25.09	**<0.0001**	1	46.12	**<0.0001**	1	42.46	**<0.0001**
Ae. atropalpus density	1	2.23	0.1415	1	9.88	**0.0030**	1	3.98	0.0518
Treatment × *Ae. albopictus* density	1	3.16	0.0809	1	8.85	**0.0047**	1	12.81	**0.0008**
Treatment × *Ae. atropalpus* density	1	4.77	**0.0333**	1	1.40	0.2427	1	0.60	0.4406
Error	59			50			53		
Ae. atropalpus									
Treatment	1	13.07	**0.0007**	1	0.49	0.4876	1	0.07	0.7875
Ae. albopictus density	1	87.78	**<0.0001**	1	4.40	**0.0448**	1	2.85	0.0998
Ae. atropalpus density	1	51.75	**<0.0001**	1	18.49	**0.0002**	1	10.64	**0.0024**
Treatment × *Ae. albopictus* density	1	0.01	0.9265	1	0.02	0.8956	1	0.00	0.9769
Treatment × *Ae. atropalpus* density	1	8.29	**0.0057**	1	1.53	0.2255	1	0.71	0.4033
Error	59			34			42		

Numbers in bold are significant.

Developmental Time. *Ae. albopictus.* There was a significant interaction between treatment and *Ae. albopictus* density for both sexes indicating that treatment affected intraspecific competition (Table 1). Developmental time for both males and females increased with an increase in *Ae. albopictus* density in both control and malathion (Fig. 3A and B). There was no significant interaction between treatment and *Ae. atropalpus* density but both female and male developmental times were affected by *Ae. atropalpus* density. Female developmen-
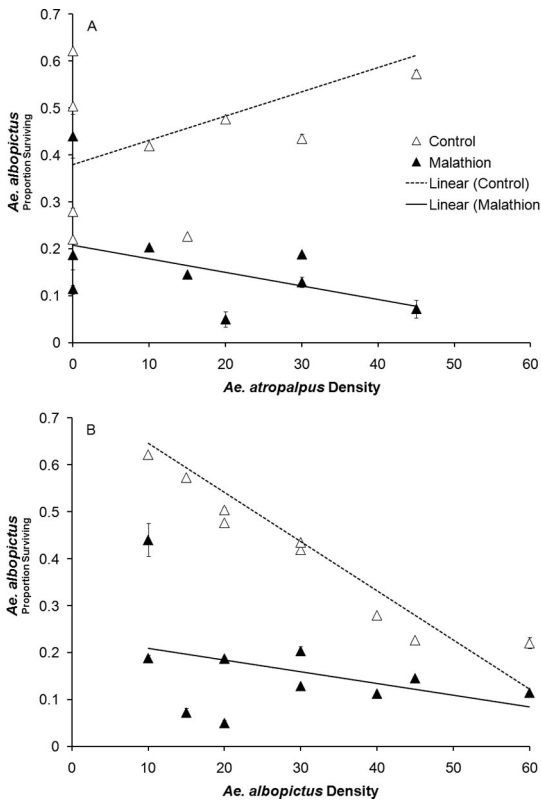
Fig. 1. Mean survivorship (back-transformed means ± SE) of *Ae. albopictus* in interspecific (*Ae. atropalpus*) (A) and intraspecific (*Ae. albopictus*) (B) competition in the presence and absence of malathion.
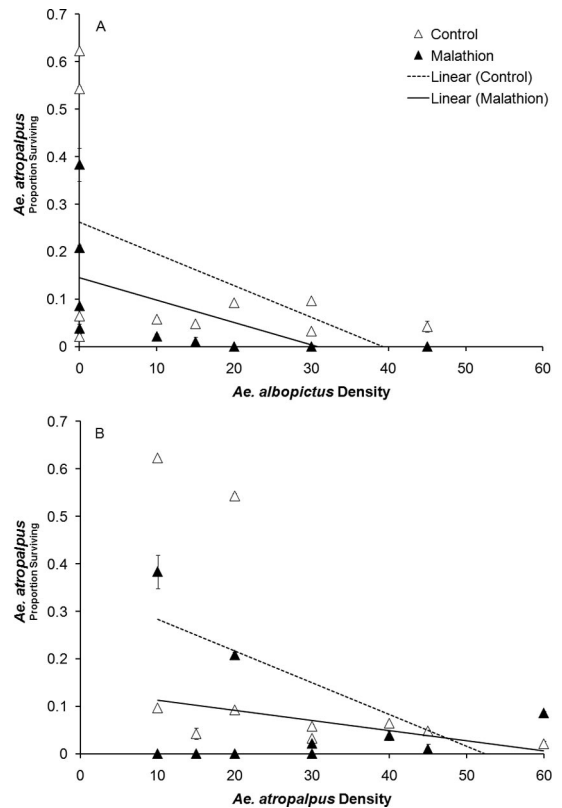
Fig. 2. Mean survivorship (back-transformed means ± SE) of *Ae. atropalpus* in interspecific (*Ae. albopictus*) (A) and intraspecific (*Ae. atropalpus*) (B) competition in the presence and absence of malathion.
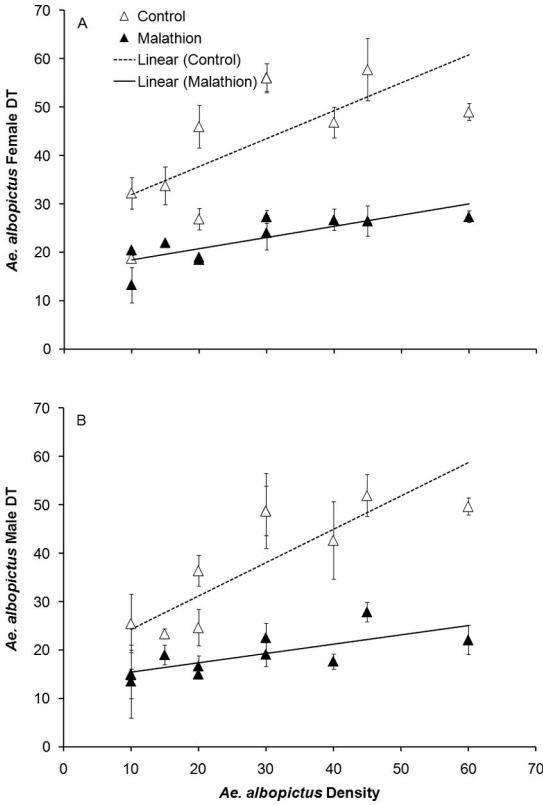
Fig. 3. Mean ± SE developmental time (DT) for *Ae. albopictus* females (A) and males (B) in intraspecific (*Ae. albopictus*) competition in the presence and absence of malathion.

Fig. 5. Mean ± SE developmental time (DT) for *Ae. atropalpus* females and males in interspecific (*Ae. albopictus*) (A) and intraspecific (*Ae. albopictus*) (B) competition.

tal times increased and male developmental times decreased with increase in *Ae. atropalpus* density (Fig. 4).

Ae. atropalpus. Developmental time for both females and males showed no significant interaction between species density combinations (intra and interspecific competition) and treatment (Table 1). De-
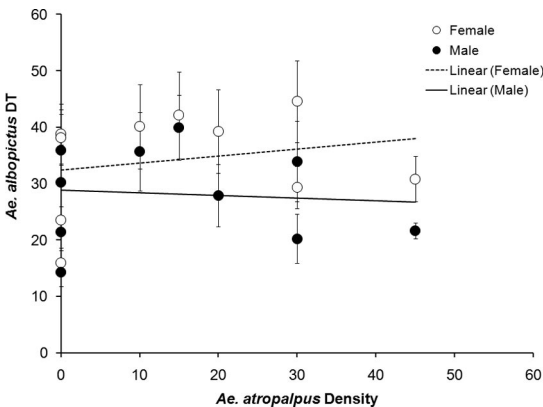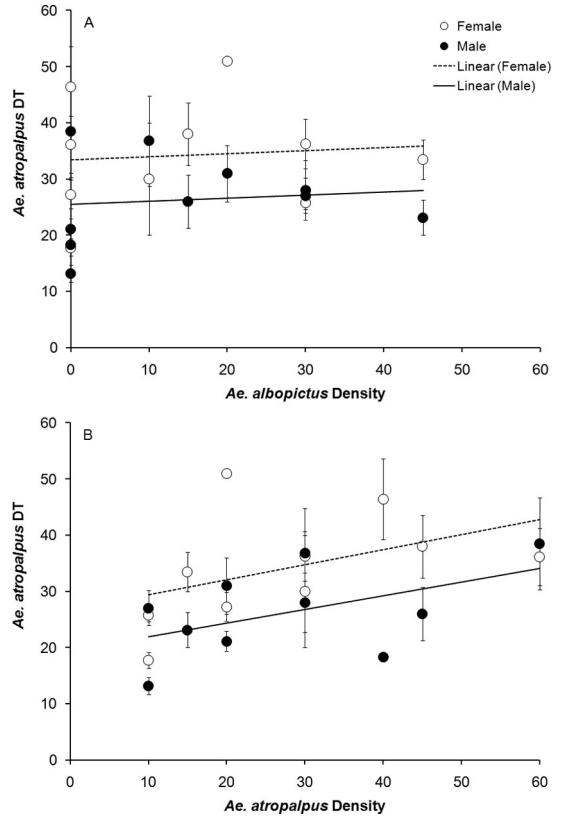

Fig. 4. Mean ± SE developmental time (DT) for *Ae. albopictus* females and males in interspecific (*Ae. atropalpus*) competition.

velopmental times for *Ae. atropalpus* females and males were affected by *Ae. atropalpus* density, but only females were affected by *Ae. albopictus* density. Both female and male developmental times increased with increase in *Ae. atropalpus* and *Ae. albopictus* density (Fig. 5).

Discussion

Our results demonstrate that interspecific competition between larvae of *Ae. albopictus* and *Ae. atropalpus* in the presence of malathion is strong and asymmetrical, with *Ae. albopictus* survivorship clearly at a disadvantage when competition from *Ae. atropalpus* and malathion are combined. In the absence of malathion, *Ae. albopictus* survivorship was positively affected by increasing densities of *Ae. atropalpus* but negatively affected with increasing densities of conspecifics indicating that in the absence of malathion, *Ae. albopictus* is more affected by intraspecific competition than interspecific competition. In control, *Ae. albopictus* developmental times were more affected by intraspecific than interspecific competition and they took longer times to pupate in the presence of conspecifics, indicating that they are superior competitors in the absence of malathion (Figs. 3A and B and 4). In the presence of malathion, the trend was reversed and

Ae. albopictus survivorship was negatively affected with increasing densities of *Ae. atropalpus* indicating that interspecific competition is affected by malathion. Interestingly, *Ae. atropalpus* survivorship when competing with *Ae. albopictus* was similar between control and malathion, indicating that survival was not different between the two treatments (Fig. 2A). So it can be concluded that malathion could facilitate coexistence between the two species, in the absence of which *Ae. albopictus* might outcompete *Ae. atropalpus. Ae. atropalpus* larvae usually colonize riverine rock pools and this could have contributed to their pesticide resistance due to decades of exposure to agricultural run-offs that may be contaminated with pesticides (e.g., Richards and Baker 1993).

Condition specific competition is not uncommon in container mosquito systems. *Ae. albopictus* is a superior competitor to *Aedes triseriatus* (Say) under laboratory conditions, but in the field *Ae. triseriatus* continued to coexist with *Ae. albopictus* in tree hole habitats (Teng and Apperson 2000, Lounibos et al. 2001). *Corethrella appendiculata* (Grabham) is a midge predator that preys on *Ae. triseriatus* and *Ae. albopictus. Ae. triseriatus* shows antipredatory behavioral responses more so than *Ae. albopictus* in the presence of predation risk cues from *C. appendiculata* (Kesavaraju et al. 2007). Kesavaraju et al. (2008) showed that abundances of *C. appendiculata* were positively correlated with *Ae. triseriatus* and negatively correlated with *Ae. albopictus*. Incidentally, *C. appendiculata* and *Ae. triseriatus* abundances were higher in tree hole habitats compared with artificial container habitats, whereas *Ae. albopictus* abundances were higher in artificial container habitats compared with tree hole habitats. So, *C. appendiculata* serves as a keystone species facilitating coexistence between *Ae. albopictus* and *Ae. triseriatus* in the absence of which *Ae. albopictus* could outcompete and displace *Ae. triseriatus* in tree holes. Similarly Murrell and Juliano (2008) showed that grass as a detritus falling into containers could facilitate coexistence between *Ae. albopictus* and *Aedes aegypti* (L.), whereas other detritus types such as oak leaves could result in the competitive exclusion of *Ae. aegypti*. Biotic factors play an important role in most of these examples, but our results are the first to show that condition specific competition also could be influenced by pesticides in mosquitoes.

Ae. atropalpus in our experiments were from a laboratory colony, and so it is possible that laboratory selection increased their susceptibility to malathion. Collecting eggs from the wild for experiments may not be feasible because first-instar larvae especially in the container habitats are not easily identifiable. *Aedes japonicus* (Theobald), another invasive mosquito, is difficult to colonize in the laboratory, but Kesavaraju et al. (2010) conducted a similar interspecific competition experiment between *Ae. albopictus* and a laboratory colony of *Ae. japonicus*. Availability of field-collected individuals would be ideal for our studies, but in their absence using a laboratory colony still provides vital data. Moreover, studies on *Ae. aegypti*, a container mosquito species, have shown that laboratory selection may not affect their sensitivity to pesticides (Chaiyasit et al. 2006). Similarly, a comparison between an *Ae. albopictus* strain used in this experiment and a laboratory colony ($\approx$70 generations) has shown that their sensitivities to malathion do not differ (B.K., unpublished data).

Ae. japonicus, another invasive mosquito from Asia, has been stated to prefer similar rock pool habitats as *Ae. atropalpus* (Armistead et al. 2008). Armistead et al. (2008) showed that *Ae. japonicus* is a superior competitor to *Ae. atropalpus*, thus it is possible that *Ae. japonicus* will displace *Ae. atropalpus* in their rock pool habitats. But *Ae. japonicus* is more susceptible to malathion compared with *Ae. albopictus*, and in an interspecific competition experiment between the two species, none of the *Ae. japonicus* survived the malathion treatment at 0.11 ppm (Kesavaraju et al. 2010). Although a three-species competition study in the presence of malathion would provide more data, we predict that the presence of malathion would benefit *Ae. atropalpus* and prevent complete displacement by the two invasive species, *Ae. albopictus* and *Ae. japonicus*. Studies on amphibian communities have shown that a combination of pesticides may have higher lethality than just a single pesticide in the environment (Relyea 2009). Our study investigated the impacts of a single pesticide (malathion), but aquatic environments may get contaminated with multiple pesticides due to widespread application and availability of different pesticides. Future studies should focus on how multiple pesticides in low concentrations could impact mosquito communities.

Acknowledgments

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