

Growth and Survival of Invasive *Aedes albopictus* Larvae on *Diospyros virginiana* Leaves

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ABSTRACT Studies on the interactions of exotic species with their invaded environment are imperative in understanding their invasion biology. Larvae of container mosquitoes such as the invasive *Aedes albopictus* (Skuse) feed on microorganisms that subsist on allochthonous inputs like leaves. *Ae. albopictus* are vectors for many diseases including West Nile virus and are rapidly expanding their distribution in the United States. We tested the larval performance of *Ae. albopictus* at different larval densities in maple, oak, American elm, and persimmon. Survival was significantly lower and days to pupation were significantly higher with persimmon leaves compared with all others. In a follow-up experiment, we compared the performance of *Ae. albopictus* in different amounts of oak and persimmon and different ratios of persimmon + oak. The linear model for the growth rate (defined by larval head width) showed a positive slope as the amount of oak leaves increased in oak treatment, but there was no significant slope for persimmon. In the persimmon + oak combination, as the ratio of persimmon to oak increased, the growth rates of the larvae decreased. Lack of a significant slope for survival rate in combination with the results from the growth rate indicated that persimmon was a poor nutritional resource for *Ae. albopictus*.

KEY WORDS *Aedes albopictus*, persimmon, detritus

Invasion by exotic species has increased in the past decade (Kolar and Lodge 2001). Successful invasion mostly depends on how well the invasive species can adapt to the new environment. Eradicating invasive species such as Argentinian ant [*Linepithema humile* (Mayr)] in the United States has largely been difficult (Menke et al. 2007), but understanding the interaction of invasive species with its novel environment may help in predicting what might limit its range. Argentine ants are invasive in California and have caused local extinctions of native species. Studies on their biology and ecology have shown that their distribution and invasive success is dependent on physical factors including moisture availability (Menke et al. 2007). In another study, Hazel et al. (2008) attribute rapid invasion of the polyphagous insect *Aleurodicus disperses* (Russell) to larger proportions of exotic plant species in Seychelles.

Larvae of the invasive mosquito *Aedes albopictus* (Family: Culicidae) inhabit natural container habitats like tree holes, which are cavities in trees filled with rain water (Kitching 2000). *Ae. albopictus* are vectors for arboviruses such as dengue and chikungunya (Ibanez-Bernal et al. 1997), and West Nile virus has been isolated from wild-caught *Ae. albopictus* in the United States (Turell et al. 2005). Many container-inhabiting mosquitoes including *Ae. albopictus* have

also adapted to colonize artificial container habitats including water filled tires and cemetery vases (Vez-zani 2007). These containers receive nutrients in the form of allochthonous inputs such as plant and animal detritus (Yee et al. 2007a). Mosquitoes that colonize these systems feed on the bacteria, fungi, and protozoa that colonize these detritus (Merritt et al. 1992). The type of detritus input is related to the productivity of the system, which in turn affects interactions among the different trophic levels (Yee et al. 2007b). Dynamics of detritus input affect the mosquito larval growth in these small container systems. Some leaves like sugar maple decompose at a faster rate compared with black oak and therefore support higher larval growth (Fish and Carpenter 1982). Carpenter (1983) showed that mosquito larval growth is determined by the amount of detritus input than inhibitory chemicals produced by conspecifics larvae. Hence, the community structure and the interactions among different trophic levels are dependent on the external input in the system.

Aedes albopictus are invasive container mosquitoes that were introduced into the United States in the 1980s (Hawley et al. 1987), and after the introduction, they have expanded their distribution to many areas in the United States (Juliano and Lounibos 2005). Although *Ae. albopictus* was first recorded in New Jersey in 1995 (Crans et al. 1996), their abundance and distribution did not increase significantly until the past 5

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yr (Farajollahi et al., unpublished data). The importance of external inputs in determining the community composition in this small container system is probably best explained with studies on competitive interactions between *Ae. albopictus* and *Ae. aegypti*. *Ae. albopictus* have displaced *Ae. aegypti* in many areas in Florida (O'Meara et al. 1995). Competitive superiority over *Ae. aegypti* is considered to be one of the important factors facilitating displacement but the type of detritus in a container determines the outcome of competition between *Ae. albopictus* and *Ae. aegypti* (Juliano and Lounibos 2005). In an interspecific larval competition experiment, the negative effects of interspecific competition on *Ae. aegypti* survival was absent in grass compared with other detritus, whereas *Ae. albopictus* survival was not affected by detritus type (Murrell and Juliano 2008). The importance of detritus type in container mosquito communities has been explored only recently (Yee et al. 2004, Yee and Juliano 2006, Kesavaraju et al. 2007), with oak, elm, grass clippings, pine needles, and insect carcasses being some of the detritus types that have been studied (Yee et al. 2007a, Murrell and Juliano 2008).

The common persimmon (*Diospyros virginiana* L.; Family: Ebenaceae) is a dioecious tree found primarily in deciduous hardwood forests in the eastern United States (Iverson et al. 2004). Persimmon trees are also grown ornamentally, and they are found throughout New Jersey, especially around the campus of the Center for Vector Biology campus in Rutgers University. American persimmon trees are commonly found in some areas of New Jersey and their leaves have been collected from containers that were breeding mosquitoes (B.K., personal observations). Mosquito larval performances in elm (*Ulmus americana* L.), red oak (*Quercus rubra* L.), and maple (*Acer* spp.) have been compared, but there have been no studies on the mosquito larval performances in American persimmon leaves (*Diospyros virginiana*). Persimmon fruits are not consumed widely in the Americas, but horses ingesting too many of these fruits have developed stomach poisoning (Kellam et al. 2000). Persimmon fruits and leaves are used in Japanese cuisine, and there are several reports on the antibacterial and odorant properties of the tannins in the Japanese persimmon (*Diospyros kaki* L.) (Hara-Kudo et al. 2004, Gu et al. 2008, Tsurunaga et al. 2008).

Our objectives were to test whether (1) growth and survival of *Ae. albopictus* larvae are affected in persimmon leaves compared with other leaf types, (2) the amount of persimmon leaves will increase or decrease the survival of *Ae. albopictus*, and (3) supplementing oak leaves alleviates the negative effects of persimmon on growth and survival of *Ae. albopictus*.

Materials and Methods

Aedes albopictus were collected as larvae from cemetery vases in Monmouth County and from tires in Mercer County, NJ. The adults were propagated in cages and bloodfed with guinea pigs [*Cavia* sp. (Erleben), IACUC protocol 86-129D] to obtain eggs. *Ae.*

albopictus eggs from the F₁ progeny were hatched by immersion in 1 liter of filtered tap water with 0.15 g of lactalbumin and brewers yeast combination (50:50) in enamel pans for 24 h at 25 ± 1°C and a 16:8 D:L cycle. The first-instar larvae were filtered, counted, and added to the experiments 24 h after the egg papers were immersed in the hatching stimulus. The experiments were conducted in 946-ml (32 fl oz) plastic disposable cups with 400 ml filtered water. Water from 7.6-liter plastic containers that had been naturally colonized by mosquitoes at the center for vector biology campus, Rutgers University, was filtered with a U.S. standard sieve (no. 100, mesh size 0.0059 in; W. S. Tyler Incorporated, Mentor, OH), and 1 ml of this filtrate (microbial inoculum) was added to each treatment cup. The filtration removes macro invertebrates and detritus but retains microorganisms such as bacteria, fungi, and protozoa. Microbial inoculums have been collected and used this way in many experiments on mosquito communities (Murrell and Juliano 2008). The center for the vector biology campus is surrounded by trees of multiple species including persimmon, oak, elm, and maple. For both experiments, treatment cups containing leaves, filtered tap water, and microbial inoculum were prepared 5 d in advance of first-instar *Ae. albopictus* larval addition, thus allowing time for microbial productivity (Yee et al. 2007a). The experiments were conducted at 25 ± 1°C and a 16:8 D:L cycle. The cups were monitored daily, and the emerging pupae were transferred to individual vials. On eclosion, the adult mosquitoes were sex typed and dried individually in an oven at 50°C.

Experiment 1. Performance in Different Detritus. Four leaf treatments, American elm (*Ulmus americana* L.), red oak (*Quercus rubra* L.), Norway maple (*Acer platanoides*), and American persimmon (*Diospyros virginiana*), were crossed with two larval density combinations (20 and 40 individuals), and each combination was replicated five times resulting in 40 replicated units. After abscission in the fall, the leaves of each of the four species were collected from the Center for Vector Biology campus in Rutgers University. The leaves were washed and dried in the oven at 50°C for at least 24 h. To each cup, 0.5 g of leaves was added, corresponding to the subjected leaf treatment. The experiment was conducted for 40 d, and the cups were replenished with 0.5 g of the leaves on the 18th day of the experiment. Proportion surviving and days to pupation were analyzed with analysis of variance (ANOVA) and multiple comparisons were corrected with Tukeys method. Proportion surviving was arcsine square root transformed to meet the assumptions of normality and homoscedasticity.

Experiment 2. Performance in Varying Amounts of Detritus. The performance of *Ae. albopictus* was tested in varying amounts of oak and persimmon and a combination of persimmon + oak in different ratios. For oak treatment, five amounts of oak leaves (0.1, 0.2, 0.3, 0.4, and 0.5 g) were added. For persimmon treatment, five amounts of persimmon leaves (0.1, 0.2, 0.3, 0.4, and 0.5 g) were added. For persimmon (P) + oak (O) combination treatment, four different ratios of per-

Table 1. ANOVA for the effect of detritus on survival, female days to pupation, and male days to pupation of *A. albopictus*

Variables	Survival			Female days to pupation			Male days to pupation		
	df	F	P	df	F	P	df	F	P
Detritus	3	34.86	<0.0001	3	7.40	0.0005	3	10.15	<0.0001
Density	1	12.81	0.0008	1	5.17	0.0283	1	0.38	0.5433
Detritus × density	3	0.27	0.8471	3	1.02	0.3943	3	1.19	0.3263
Error	48			41			42		

Significant effects are in bold.

simmon + oak leaves (in grams: 0.4 P + 0.1 O, 0.3 P + 0.2 O, 0.2 P + 0.3 O, and 0.1 P + 0.4 O) were added such that amount of persimmon decreases as amount of oak increases. Each treatment was replicated five times (oak alone $5 \times 5 = 25$, persimmon alone $5 \times 5 = 25$, and persimmon + oak $4 \times 5 = 20$), resulting in 65 replicated units. The experiment was stopped at 30 d, and the surviving larvae were frozen at -20°C . The frozen larvae were later thawed, and head widths were measured using Image Pro MC Version 6 software (Media Cybernetics, Bethesda, MD). The wing lengths of the female adult mosquitoes were measured using a stereo microscope with Image Pro MC Version 6 software (Media Cybernetics). Proportion surviving and pupating, larval head width, and adult wing lengths were analyzed using a linear model (PROC GLM; SAS 9.1) with amount as a continuous variable and leaf type as a class variable. Proportion surviving and pupating was arcsine square root transformed to meet the assumptions of normality and homoscedasticity. If the persimmon leaves had any lethal effects, we predicted a negative slope with proportion of *Ae. albopictus* that are surviving in the persimmon alone treatment. Alternatively, if persimmon leaves are a poor nutritional resource for *Ae. albopictus*, we predicted that the growth rate measured by the head width of larvae would increase with decrease in persimmon leaves in persimmon + oak treatment.

Results

Experiment 1. The proportion of *Ae. albopictus* surviving was significantly different between detritus (American elm, red oak, maple, and persimmon); however, larval density and its interaction with detritus were not significant (Table 1). Multiple comparisons showed that the proportion surviving was lowest in persimmon, whereas the proportion surviving between the other detritus types were not different (Fig. 1). Female days to pupation were different between detritus type and larval density (Table 1). Male days to pupation differed only between detritus type but not larval densities. Furthermore, there was no interaction between detritus type and larval density in both sexes (Table 1). Multiple comparisons showed that both females and males took a longer time to pupate in persimmon leaves compared with others (Fig. 2).

Experiment 2. There was no significant interaction between leaf type (oak, persimmon, persimmon + oak) and amount (0.1, 0.2, 0.3, 0.4, and 0.5 g) for the proportion surviving ($df = 2, F = 1.57, P = 0.2166$), indicating that the slopes for each leaf type were not different from each other, but there was a significant interaction for proportion pupating and head width of larvae (Table 2). The interaction between leaf and amount for wing lengths was not significant ($df = 2, F = 2.53, P = 0.0891$), indicating the wing lengths were not different among detritus types (mean wing length,

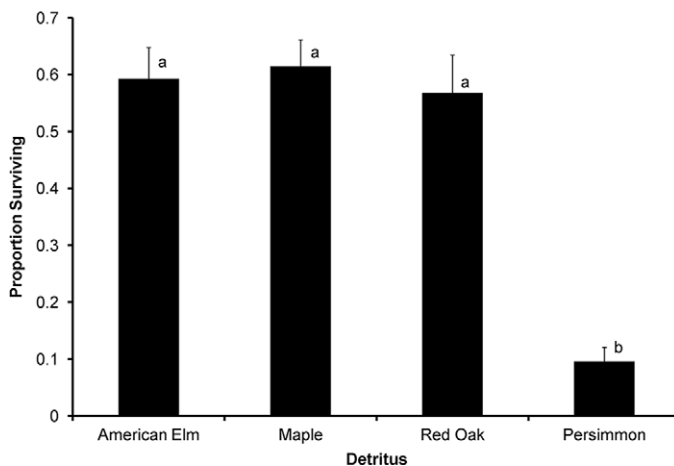


Fig. 1. Mean \pm SE proportion of *Ae. albopictus* surviving in the four detritus types. Means with similar letters are not significantly different from each other.

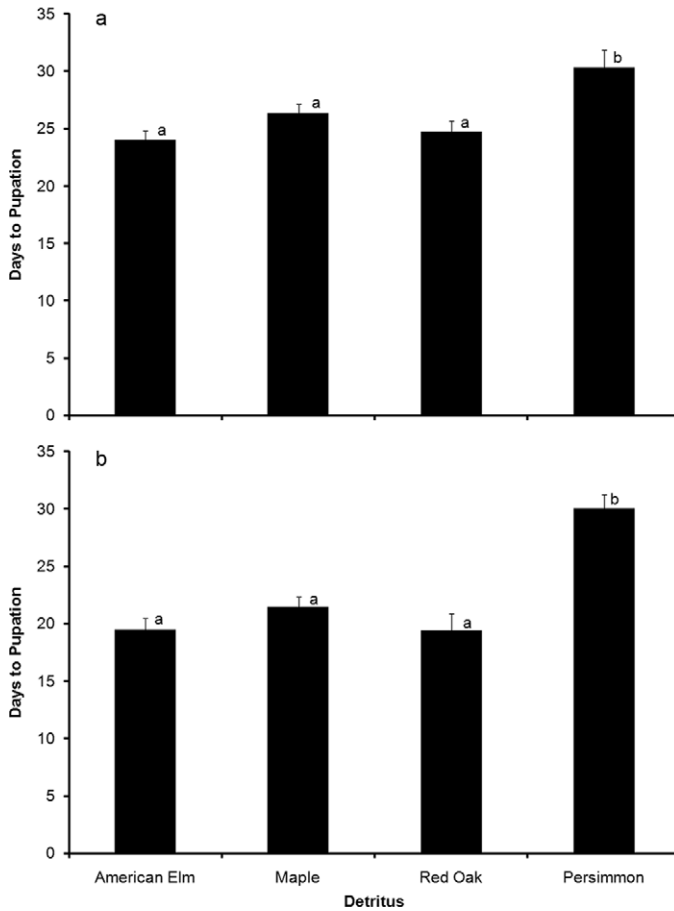


Fig. 2. (a) Mean \pm SE days to pupation of female *Ae. albopictus* in the four detritus types. (b) Days to pupation of male *Ae. albopictus* in the four detritus types. Means with similar letters are not significantly different from each other.

2.1154 \pm 0.0286 [SE] mm). Follow-up tests for proportion pupating showed a positive slope for oak, indicating that the number of pupae increased as the amount of oak leaves increased (Table 2; Fig. 3a). There was no slope for the persimmon and persimmon + oak combination, indicating that the different amounts had no effect on the proportion pupating (Table 2; Fig. 3b and c). For head width, oak and persimmon + oak had a positive slope, but there was no slope for persimmon (Fig. 3d-f). The positive slope of oak indicated that the average size of the larvae increased with increase in the amount of oak leaves.

The positive slope of persimmon + oak indicated that, as the ratio of persimmon to oak decreased (Fig. 3f), the size of the larvae increased.

Discussion

Aedes albopictus larval survival decreased and their developmental time increased in the presence of persimmon compared with red oak, maple, and American elm leaves (Figs. 1 and 2). Fruits and leaves of the persimmon have high concentrations of tannins, which could have attributed to low survival of larvae, but when the amount of persimmon decreased, the proportion surviving did not decrease, which suggests that tannins could not have been the cause for the lower survival. Moreover, oak leaves also have high tannin content (Shimada and Saitoh 2006), but the proportion pupating and growth rate (head width) of larvae increased with increase in oak leaf (Fig. 3). No difference among the slopes in proportion surviving also indicates lack of larvicidal effect of persimmon. Varying amounts of persimmon leaves did not have an impact on the growth rate of the larvae, but supple-

Table 2. Linear model table and slopes for proportion pupating and head width

Variables	Proportion pupating			Head width		
	df	F	P	df	F	P
Leaf	2	2.58	0.0838	2	6.96	0.0020
Amount	1	3.87	0.0536	1	1.59	0.2127
Leaf \times amount	2	3.00	0.0571	2	9.37	0.0003
Error	64			55		

Significant effects are in bold.

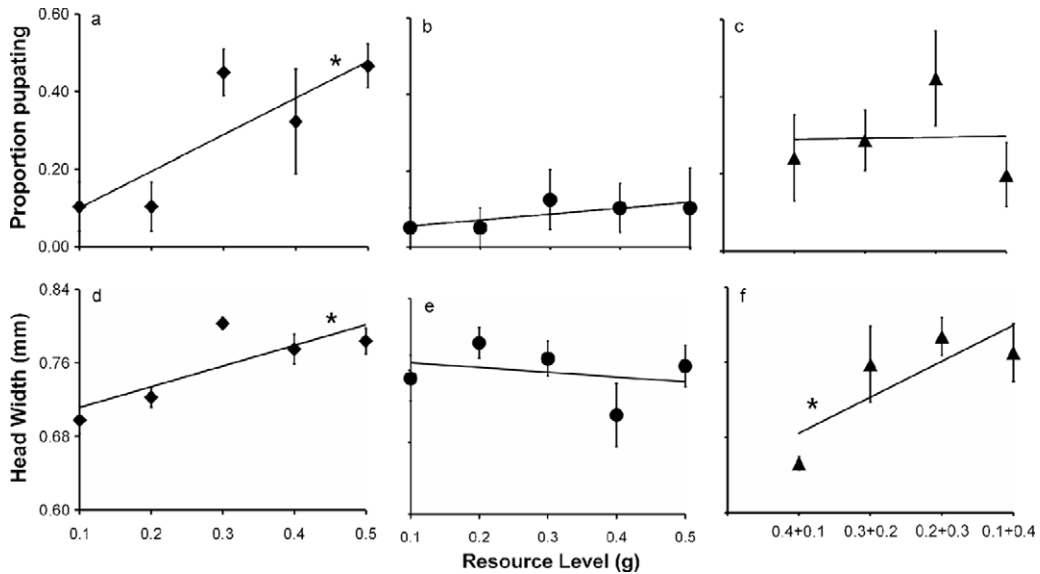


Fig. 3. Arcsine square-root transformed values of mean proportion pupating \pm SE (a-c). Mean head width (mm) of larvae \pm SE (d-f). Oak (a and d), persimmon (b and e), and persimmon + oak (c and f). Oak and persimmon treatments had five different amounts of leaves (0.1, 0.2, 0.3, 0.4, 0.5). Persimmon + oak treatment were all 0.5 g but in four different proportions: 0.4 + 0.1, 0.3 + 0.2, 0.2 + 0.3, and 0.1 + 0.4. *Slopes that are significant.

menting oak leaves to the persimmon leaves significantly affected the growth rate (persimmon + oak treatment). Larval growth rate decreased with the increase in proportion of persimmon leaves to oak leaves, which showed that persimmon leaves are a poor resource for *Ae. albopictus* larvae (Fig. 3f).

Mosquito larvae feed on microorganisms that are growing in the presence of plant and animal detritus that fall in to containers (Merritt et al. 1992). Bacterial productivity is an indicator of the overall microbial productivity in containers, with fluctuations affecting mosquito growth and development (Yee and Juliano 2006, Yee et al. 2007a, b). Persimmon extracts reduce growth of many species of bacteria and hence are widely used in some cuisines for this antibacterial property (Hara-Kudo et al. 2004). Although it is possible, the chances of microorganisms that break down persimmon leaves being absent in the microbial inoculum that were added to the experimental cups is low because they were obtained from multiple containers inhabited by mosquitoes. The lack of difference in larval survival and increase in growth rate with increase in proportion of oak leaves to persimmon leaves strongly suggests lower microbial productivity in persimmon leaves as the causal factor for poor performance of *Ae. albopictus* in the presence of persimmon leaves. Among the potential plant leaf species that thus far have been studied, growth and survival of mosquitoes (including *Ae. albopictus*) is lowest in oak leaves (Murrell and Juliano 2008), but our study shows that growth and survival of *Ae. albopictus* in persimmon leaves is even lower compared with oak leaves. Because *Ae. albopictus* is a superior competitor under low resource conditions compared with other container-dwelling mosquitoes, the growth and survival of

other mosquito species should also be lower in persimmon.

Importance of external inputs in small container systems has received considerable attention in the past decade (Juliano and Lounibos 2005, Yee and Juliano 2006, Yee et al. 2007a, b, Murrell and Juliano 2008). Leaves were thought to be the important external input until Yee and Juliano (2006) first showed that animal detritus could also influence the performance of mosquitoes. Yee et al. (2007a) showed that varying proportions of leaf and animal detritus could influence the performance and in turn competition in these systems. Murrell and Juliano (2008) showed that larval performances and the outcome of competition are dependent on the species of leaf detritus. Our results show that larval performances and potentially competition could also be influenced by varying proportions of different species of leaf detritus.

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