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Leaf species identity and combination affect performance and oviposition choice of two container mosquito species

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Abstract

1. Resource diversity can be an important determinant of individual and population performance in insects. Fallen parts of plants form the nutritive base for many aquatic systems, including mosquito habitats, but the effect of plant diversity on mosquito production is poorly understood.

2. To determine the effects of diverse plant inputs on larval mosquitoes, experiments were conducted that examined how leaves of *Vitis aestivalis*, *Quercus virginiana*, *Psychotria nervosa*, and *Nephrolepis exalta* affected the container species *Aedes triseriatus* and *Aedes albopictus*.

3. The hypothesis that leaf species have different effects on larval survival, growth, population performance, and oviposition choice of the two mosquito species was tested. The hypothesis that larval performance of *A. albopictus* responds additively to combinations of the four plant species was also tested.

4. Larval survival and growth differed among the four leaf species, and oviposition preference differed among the two leaf species examined. Measurements of population performance demonstrated significant variation between leaf treatments. Larval outcomes for *A. albopictus* were significantly affected by leaf combination, and the hypothesis of additivity could be rejected.

5. These results indicate that individual leaf species are important in determining the performance of container dwelling mosquitoes, which grow larger and survive better on mixed-species resource than expected based on an additive model of resource utilisation.

Keywords

Additivity model; *Aedes albopictus*; *Aedes triseriatus*; container habitats; Florida; invasive species; optimal oviposition theory

Introduction

Resource diversity of nutritive substances can have important implications for the population performance of many animals. Superior performance (e.g. greater growth of individuals, increased fecundity) with diverse resources compared to less diverse resources has been observed when an arthropod can actively control its diet (Greenstone, 1979; Waldbauer & Friedman, 1991). Even organisms that do not seem to actively choose their diet may have post-ingestion mechanisms for acquiring ideal ratios of resources (Raubenheimer & Simpson, 2004). However, the effect of resource diversity on organisms in aquatic environments that have limited control over their intake, such as filter feeding mosquito larvae, is less well

understood. The specific qualities of each resource are crucial to integrate the overall effect of resource diversity, as some sources of nutrition may be complimentary while others may be redundant or even deleterious (Raubenheimer & Simpson, 2004). Recent advances in nutrition theory suggest optimal ratios of nutrients are ideal, such that intake of too few or too many essential nutrients will result in lower fitness (Raubenheimer & Simpson, 2004). Organisms that can make active choices for foods of differing quality have been shown to optimise species-specific needs, and, when forced to deviate from their optimal intake of nutrients, have lower fitness (Behmer & Joern, 2008).

Allochthonous plant material provides the nutritive base of many aquatic systems that have low exposures to sunlight, including common mosquito habitats such as tree holes and artificial containers (Kitching, 2001). The impact of leaf material can be direct, as for detritivorous invertebrates, or mediated through microbial intermediates, as for browsing or filter feeding mosquito larvae. The importance of leaf quantity for mosquito growth has been well documented (Jenkins & Carpenter, 1946; Carpenter, 1982a, b, 1983; Fish & Carpenter, 1982), as has leaf species in field and laboratory studies. Barrera *et al.* (2006) noted leaf species diversity in rain-filled containers and associated individual leaf species with variation in larval growth of *Aedes aegypti* (L.). We know of no other field studies that have examined leaf species as a determinant of mosquito productivity in container systems.

In controlled laboratory studies, leaf species has been shown to be important for mosquito production from microcosms simulating artificial containers (Fish & Carpenter, 1982; Sota, 1993; Yanoviak, 1999; Dieng *et al.*, 2002). Differences in larval performance by leaf species have been often ascribed to differences in decay rate, with higher decay rate associated with better larval growth and survival (Fish & Carpenter, 1982; Yanoviak, 1999; Yee & Juliano, 2006). Growth of *Aedes triseriatus* (Say) is also affected by leaf condition (fresh versus senescent), and other factors impacting the microbial environment, as shown in a multifactorial experiment with beech leaves (*Fagus grandifolia* Ehrl.) (Walker *et al.*, 1991). Sota (1993) demonstrated that the closely related *Aedes albopictus* (Skuse) and *Aedes riversi* Bohart and Ingram survived better on the leaves of *Camellia japonica* (Theaceae) relative to the leaves of *Elaeocarpus sylvestris* (Elaeocarpaceae) with no differences between the mosquito species, nor between senescent versus fresh leaves. In growth studies of other aquatic insects, leaf condition has not been consistently predictive of responses, with growth and survival sometimes higher on green leaves, sometimes higher on senescent leaves, and sometimes showing no difference (Yeates & Barmuta, 1999; Kochi & Kagaya, 2005; Kochi & Yanai, 2006).

Immatures of container dwelling mosquitoes are placed in their habitat by ovipositing females, and optimal oviposition theory suggests that gravid insects lay their eggs in habitats that maximise the performance of their offspring (Jaenike, 1978; Scheirs & De Bruyn, 2002). If this hypothesis is correct, then gravid female mosquitoes prefer containers with high quality leaf material, relative to containers with no leaf material or low quality leaf material. Previous examination of oviposition preference has shown that *A. albopictus* and *A. triseriatus* oviposit in water infused with oak leaves in preference to water without leaves, although *A. triseriatus* was less discriminating in field trials (Trexler *et al.*, 1998). However, a direct correlation between ovi-position preference for a leaf infusion and mosquito larval performance on that leaf substrate has not been examined.

Aedes albopictus is an invasive mosquito in North America (Hawley *et al.*, 1987), and may encounter a diverse array of larval resources over its expanding range. The ability of this species to thrive on a diversity of resources may facilitate its rapid expansion and competitive superiority at the larval stage, shown in common garden experiments with *A. triseriatus* and *A. aegypti* (Livdahl & Willey, 1991; Braks *et al.*, 2004). In spite of the competitive superiority

of *A. albopictus* in laboratory experiments, it appears to coexist with both *A. triseriatus* and *A. aegypti* under natural conditions (Juliano *et al.*, 2004; Costanzo *et al.*, 2005; Griswold & Lounibos, 2005). One mechanism for the coexistence of *A. albopictus* and *A. triseriatus* or *A. aegypti* may be resource diversity in terms of plant versus animal detritus (Yee *et al.*, 2007; Murrell & Juliano, 2008). Leaf diversity may also be important, but little is known about how larval mosquitoes respond to mixtures of leaf species.

Three hypotheses were tested in this study. First, leaf species common in subtropical Florida hammocks were hypothesised to differ in their suitability for larval growth and survival. To test this, survival, growth and intrinsic rate of population growth were predicted to differ among larval cohorts of *A. triseriatus* and *A. albopictus* provided with different leaf species in the laboratory. Based upon previous studies (Fish & Carpenter, 1982; Yanoviak, 1999; Dieng *et al.*, 2002; Yee & Juliano, 2006) higher survivorship and faster larval growth were predicted for leaves that lost more mass. The second hypothesis was that larval *A. albopictus* respond to mixtures of leaf species additively based upon their responses to single species. Previous studies on leaf litter decay have suggested deviations from additivity are due to increased microbial activity on one leaf species affecting less microbially active leaf species, a process that may be important in this system (Swan & Palmer, 2006; Lecerf *et al.*, 2007; Ostrofsky, 2007). To test this, the survival, growth, and intrinsic population growth rate of *A. albopictus* in microcosms with a mixture of leaf species were predicted to be a linear combination of these same variables measured in single leaf species microcosms. The final hypothesis was that both species of mosquitoes oviposit in habitats that provide their offspring the best environment for growth. To test this, both mosquito species were predicted to prefer to oviposit in water infused with a leaf species known to be more favourable for larval growth than an alternative leaf species or water without leaves.

Materials and methods

Mosquitoes

For all experiments, *A. albopictus* mosquitoes were grown from F₁ eggs from a colony established in December, 2006 from 30 sampling sites throughout Palm Beach County, Florida and added to weekly. Colony conditions were L:D 14:10 h at $\approx$ 80% relative humidity and 28°C, with 20% sucrose *ad libitum*. To generate F₁ eggs, adults were fed on restrained chickens (UF IACUC protocol VB-17). For all experiments, eggs were hatched at 28°C in tap water 24–36 h prior to use.

Aedes triseriatus eggs came from a long maintained colony from field material originally collected in southern Florida, U.S.A., and occasionally replenished with mosquitoes collected in the field. Egg hatching and adult mosquitoes were treated identically as for *A. albopictus*.

Experiment 1: single leaf species effects

To examine the effects of single leaf species on larval mosquitoes, a 4 × 2 factorial experiment was conducted with four leaf and two mosquito species, *A. albopictus* and *A. triseriatus*, for a total of eight treatments. Four plants common to southern Florida coastal hammocks, an ecosystem with tropical and sub-tropical characteristics (Myers & Ewel, 1990), were used: summer grape ['G' or 'Grape', *Vitis aestivalis* Michaux (Vitaceae), a liana], live oak ['O' or 'Oak', *Quercus virginiana* Miller (Fagaceae), a tree], wild coffee ['C' or 'Coffee', *Psychotria nervosa* Swartz (Rubiaceae), a shrub], and Boston fern ['F' or 'Fern', *Nephrolepis exalta* Schott (Nephrolepidaceae), a herb]. These species were chosen because of their abundance in leaf litter surveys in discarded tyres in a Florida coastal hammock (M. H. Reiskind, pers. obs.). Fresh leaves of grape, fern, and coffee were used because none of these plants are fully deciduous in Florida, studies from the tropics suggest fresh leaves constitute a substantial part

of leaf litter (Stout, 1980), and fresh leaves are commonly encountered in artificial container habitats in southern Florida (M. H. Reiskind, pers. obs.).

Five microcosms per treatment were established for a total of 40 replicates in covered 500 ml food grade plastic containers. Each container held 250 ml water, 2 g of leaves, including petioles, oven dried for 48 h at 48 °C, and 17 first-instar larvae. The leaf amount was chosen based upon preliminary experiments with oak leaves that produced $\geq 80\%$ larval survival. Containers were placed in an insectary with constant temperature ($28^\circ \pm 1^\circ \text{C}$) and a light:dark cycle (LD 14:10 h). After 4 days, containers were checked daily for pupation. Pupae were transferred to tubes in which adults emerged. Two days after emergence adults were killed and dried at 48° C for at least 48 h. The wings from each adult were then removed, photographed, and measured with IMT I-Solution Lite 6.1 software (Image and Microscope Technology, Daeduk-gu, South Korea) as an index of body size (Blackmore & Lord, 2000; Armbruster & Hutchinson, 2002; Lounibos *et al.*, 2002). Mean wing length, survival, and days to emergence were calculated for each replicate for each species and sex. In addition, mean female wing length, days to emergence and number surviving to adulthood were used to calculate a composite measure of population performance for each replicate, λ' , as described elsewhere (Braks *et al.*, 2004), using fecundity–growth relationships and days to first egg-laying parameters also reported elsewhere (Livdahl & Willey, 1991; Lounibos *et al.*, 2002). In the interest of space, only results for female mosquitoes are shown for the single leaf species experiment. Leaf mass lost from each replicate was measured by drying all leaf material remaining after 40 days, by which time all but a single *A. triseriatus* larva had pupated or died.

Experiment 2: effects of leaf combinations on *Aedes albopictus*

A separate experiment was conducted using the same plant material to examine the combinatorial effects of leaf species. Examining only *A. albopictus*, a 15×1 factorial design with all possible combinations of the four leaf types was used with five replicates of the following treatments: single leaf types (four: C, F, G, O), 50:50% (six: CG, FC, FG, OC, OF, OG), 33:33:33% (four: FCG, OCG, OFC, OFG), and 25:25:25:25% (one: OFCG). A total of 2 g of leaf material was placed in 500 ml food grade plastic containers with 250 ml of tap water and 17 first-instar *A. albopictus*. As previously described, containers were monitored daily for pupation, and pupae were removed and adults allowed to emerge. Outcomes measured were time to emergence, wing length, and per cent survival as described for experiment 1, except both males and females were included in the analysis of additivity in experiment 2. Population performance (λ') of each replicate was also determined.

The expected additive effect was calculated based upon the per cent of the total 2 g of leaf material made up by each component species as follows:

$$E = \frac{\sum_{i=1}^n LR_i}{n}$$

where E is the expected larval response (wing length, survival, or days to emergence) for a given leaf combination, LR_i is the average larval response to leaf species i from the five single species microcosm replicates, and n is the number of species in the mixed litter microcosm.

Experiment 3: oviposition response of *Aedes triseriatus* and *Aedes albopictus* to leaf species

Adult mosquitoes were generated from larvae reared under conditions of generous non-plant food (50 first-instar larvae in 1 litre of water with 0.3 g of 1:1 yeast albumin in $22.8 \times 30.5 \times 7.5$ cm enamel pans). Adults were given 20% sucrose and water *ad libitum* for 3 days prior to

being blood fed. Mosquitoes were allowed to feed on a restrained chicken (UF IACUC protocol VB-17), and blood fed mosquitoes were aspirated into cardboard containers (total volume = 1litre). For each of 10 replicates (five for *A. albopictus*, five for *A. triseriatus*) three gravid mosquitoes were placed in a screened enclosure (total volume $\approx 2 \text{ m}^3$), which were inside a screened building in a mesic, coastal hammock on the campus of the Florida Medical Entomology Laboratory (FMEL) in Vero Beach, Florida. Inside each screened enclosure were three black plastic 475 ml oviposition cups. Two of the oviposition cups contained 2 g of leaf material (grape or oak) with 250 ml of tap water (infused for 3 days prior to use), with the other 250 ml cup containing 3-day-old tap water.

Once released, the mosquitoes were allowed to oviposit on seed germination paper (Seedburo Co., Chicago, Illinois) placed into each cup. The number of eggs laid on each paper was enumerated after 72h. Due to differences in day of emergence from the pupal stage and to ensure all ovipositing mosquitoes were the same age post pupation, the experiment was conducted over 5 days.

Statistical analysis

For experiments 1 and 2, the effects of each species of leaf on the various outcomes (average days to emergence, per cent survival, and wing length) for females of each species (experiment 1) or female *A. albopictus* alone (experiment 2) were analysed using multivariate analysis of variance (MANOVA) (Scheiner, 2001). Per cent survival was square-root transformed (+1) to achieve normality. After detection of significant effects by MANOVA, standardised canonical coefficients (SCCs) generated by the MANOVA procedure were examined to elucidate the relationships between the outcomes and leaf treatment. Replicates that yielded no female mosquitoes were not included in the MANOVA. After a significant MANOVA, ANOVA was used to examine biologically relevant univariate comparisons (a 'protected ANOVA', Scheiner, 2001). As estimates of population rate of increase (λ') could not be transformed to achieve normality, the distributions of λ' values among treatments were compared using Kruskal–Wallis tests (Sokal & Rohlf, 1995).

For experiment 2, the hypothesis of additivity in larval response was initially tested using methodology from Ball *et al.* (2008) and Kominoski *et al.* (2007) on both male and female *A. albopictus*. Dummy variables for the presence/absence of each leaf species were entered into a linear model, followed by species number and leaf combination. By using type I sums of squares with the presence/absence of each species entered first, a significant leaf combination term nested in species number rejects the hypothesis of additivity. A significant species number term would mean there was a significant effect of richness above the non-additive effect of each species combination. As richness was never significant, and in the interest of space, only the leaf combination term (the test of additivity) is reported for each outcome.

In order to examine general trends for synergy or antagonism, the differences between the observed effect and the expected, additive effect (O–E) were plotted by leaf combination with 95% confidence intervals. Deviations from the additive model were considered significant when error bars did not include zero. Each outcome was examined separately for male and female mosquitoes.

Numbers of eggs laid were analysed by negative binomial regression with infusion type and replicate as factors in PROC GENMOD (SAS 9.1, SAS Institute, Cary, North Carolina). The negative binomial distribution was chosen because of its biological appropriateness in entomological studies of oviposition (Bliss & Fisher, 1953; Candy, 2000) and better goodness of fit measurements (deviance and Pearson χ^2 closer to 1) compared to Poisson or Gaussian distributions. *Post hoc* tests of individual pairwise comparisons were made comparing differences in least square means estimated by maximum likelihood to a χ^2 distribution.

Results

Experiment 1: single leaf species effects

Different leaf species lost different amounts of mass, but total loss of mass was not influenced by the mosquito species present (Fig. 1; two-way ANOVA: $F_{7,32} = 51.43$, $P < 0.0001$; leaf: $F_{3,32} = 119.78$, $P < 0.0001$; mosquito: $F_{1,32} = 0.001$, $P = 0.9503$; mosquito $\times$ leaf: $F_{3,32} = 0.21$, $P = 0.8905$).

The full MANOVA model yielded significant leaf, species and interaction effects on larval outcomes (Table 1). When species were analysed separately, leaf species explained significant variation in larval female *A. triseriatus* and *A. albopictus* performance (Table 2). The coffee treatment did not produce any female mosquitoes of either species and is not included in the MANOVA.

Average days to emergence was strongly affected by leaf species in the *A. triseriatus* MANOVA, with per cent survival and wing length less important. As there was no production of female *A. triseriatus* from grape or coffee treatments, the MANOVA only generated one canonical. For *A. albopictus*, standardised canonical coefficients suggest all outcomes contributed to the explanatory power of the first and only significant canonical. Coffee leaves produced no female *A. albopictus*, so the MANOVA only produced two canonicals, of which only the first canonical was significant and explained 98.54% of the variation. The non-significant, second canonical is not shown.

After the significant MANOVA, ANOVAS for each outcome were examined. Per cent survival was significantly affected by leaf species for both species (Fig. 2a; *A. albopictus*: $F_{3,16} = 9.64$, $P < 0.001$; *A. triseriatus*: $F_{3,16} = 12.55$, $P < 0.001$). Wing length was significantly affected by leaf species for *A. albopictus* (Fig. 2b, $F_{2,8} = 5.89$, $P < 0.05$), but not *A. triseriatus* (Fig. 2b, $F_{1,7} = 0.78$, $P = 0.4117$). Average days to emergence was significantly affected by leaf species for both mosquito species (Fig. 2c, *A. albopictus*: $F_{2,8} = 160.47$, $P < 0.0001$; *A. triseriatus*: $F_{1,7} = 50.63$, $P < 0.001$).

Non-parametric tests comparing ranked λ 's for each species separately found a significant leaf species effect for both mosquitoes (Kruskal–Wallis, d.f. = 3; for *A. triseriatus*, $\chi^2 = 16.95$, $P < 0.001$; for *A. albopictus*, $\chi^2 = 13.93$, $P < 0.001$).

Experiment 2: effects of leaf combinations on *Aedes albopictus*

The overall effect of leaf combination treatment on female *A. albopictus* larval outcomes was significant when all outcomes were considered simultaneously (MANOVA: Pillai's trace = 1.687, $F_{42,132} = 4.04$, $P < 0.001$). Factor loadings demonstrated the importance of all three measures of larval performance in the first three standardised canonical coefficients from the MANOVA (Table 3). The first canonical explained almost half of the variation and was overwhelmingly influenced by average days to emergence, with per cent survival and average wing length leading to much smaller factor loadings in the same direction as days to emergence. The second canonical explained 29.54% of the variation and was strongly influenced by per cent survival and wing length, which acted in opposite directions. The final canonical explained the remaining 25% of the variation and was most heavily influenced by wing length, followed by average days to emergence and per cent survival. Indeed, individual ANOVAS for each outcome demonstrated that leaf treatment affected days to emergence ($F_{14,44} = 5.71$, $P < 0.0001$), wing length ($F_{14,44} = 3.96$, $P < 0.001$), and per cent survival ($F_{14,44} = 3.63$, $P < 0.005$).

A significant effect of leaf treatment on λ' was detected by non-parametric analysis (Fig. 3, Kruskal–Wallis, d.f. = 14, $\chi^2 = 37.32$, $P < 0.001$).

The number of leaf species *per se* had no significant effect on larval outcomes (data not shown: Pillai's trace = 0.2365, $F_{9,165} = 1.57$, $P = 0.1285$). However, the null hypothesis of additivity could be rejected for female survival, female wing length, and male days to emergence at $\alpha = 0.05$, and there was a trend towards an effect ($0.10 > \alpha > 0.05$) in male wing length and female days to emergence (Table 4; Fig.4a–f). The hypothesis of additivity could not be rejected for lambda (data not shown) or male survival (Fig. 4d). In agreement with the results of the MANOVA on number of leaf species, no additivity analyses showed the number of leaf species to be a significant variable (all $P > 0.15$; data not shown). In general, females had higher survival (although the combination of grape and coffee had significantly lower survival) and longer wings (although there was a trend in the fern–grape combination towards shorter wings) in leaf combinations (Fig.4a,c), and showed a trend towards longer development time than expected from the additive model (Fig.4b). Discerning a clear association with any particular leaf is difficult, although the presence of oak leaves seemed associated with superior performance. Males were more likely to have emerged earlier and had longer wings than expected under additivity for most leaf combinations (Fig.4e,f). Again, any clear association with one versus another leaf species was difficult to determine.

Experiment 3: oviposition response of *Aedes triseriatus* and *Aedes albopictus* to leaf species

Infusion type significantly affected oviposition choice for both species of mosquitoes (Fig.5). *Aedes triseriatus* avoided ovipositing in grape leaf infusion, but did not discriminate between oak leaf infusion and water without leaves. *Aedes albopictus* preferentially oviposited in oak leaf infusion, while avoiding water without leaves and grape leaf infusion equally.

Discussion

There was support for the hypothesis that larval outcomes differed among leaf types for both species. However, no positive correlation between mass loss and population performance was observed in the four individual leaf species, providing no evidence to support the first hypothesis that leaf mass lost determines larval growth. There were no significant differences between mosquito species in population performance estimates (λ'), suggesting that growth and survivorship of the two mosquito species responded similarly to different leaf species. Field studies of invasive *A. albopictus* have noted its ability to colonise a wide variety of container habitats, an observation that suggests an ability to produce pupae in a variety of leaf environments (Sota, 1993). There was some production of female *A. albopictus* on grape leaves, but not of *A. triseriatus*, suggesting that *A. albopictus* may be able to achieve pupation in some leaf environments that *A. triseriatus* cannot. This may be due to the lower nutritional requirements of *A. albopictus* relative to *A. triseriatus*, the former species being a generally smaller mosquito (Livdahl & Willey, 1991).

Leaf species combination had significant effects on *A. albopictus* larval performance although the number of species present, regardless of identity, had no impact on larval performance. This result means that species identity of leaf is important, whether as the only available resource or in combination with other leaf species. The hypothesis of strict additivity can be rejected, as there were significant deviations in female survival (females survived better than expected), female growth (females had longer wings than expected from the additive model) and male development rate (males took shorter than expected from the additive model). Male growth showed a trend towards rejecting additivity (males generally grew larger than expected from the additive model), as did female time to emergence (females generally took longer than expected from the additive model). Theoretical and empirical investigations into *Aedes sierrensis* (Ludlow), and empirical studies of *A. aegypti*, aspecies closely related to *A. albopictus*, suggest females should take longer to develop if that can lead to increased size, while male mosquitoes should favour faster development (Kleckner *et al.*, 1995; Bedhomme

et al., 2003). This is because of the correlation between size and female fitness, and between early reproduction/emergence and male fitness. In light of these observations, the slower than expected growth of female mosquitoes in leaf combinations may be a response to leaf diversity that results in greater female fitness. Indeed, differences in female size reflected the proposed development time tradeoff with growth, although there was no significant change in population growth rate estimates (λ'). On balance, there is more evidence to reject the additivity model in favour of a synergistic model in which mosquito larvae provided with diverse resources have higher fitness than expected based upon their response to individual resources. However, leaf identity is important, and the particular combinations of leaves favoured improved performance in mosquito larvae. Indeed, monospecific microcosms solely consisting of oak resulted in as good or better larval performance than any combination of leaves, and oak in combination with the other leaves was often associated with superior larval performance.

There may be several reasons why mosquitoes performed better than expected by strict additivity in several of the outcomes measured. Multiple leaf species may act as a more diverse resource base for micro-organisms in aquatic (Lecerf *et al.*, 2005; Cardinale *et al.*, 2006) and terrestrial systems (Cardinale *et al.*, 2006), and therefore may provide either increased abundance or diversity of microbes, resulting in a superior diet for mosquito larvae. This may allow faster larval growth through complimentary or self-selecting feeding, in which a diversity of resources provides better nutrition for a consumer (Greenstone, 1979; Waldbauer & Friedman, 1991). The mechanism by which mosquito larvae may be acquiring complimentary resources is unclear, but may involve changes in amount of time in different feeding behaviours or location, as was observed in studies comparing responses to animal versus plant detritus (Kesavaraju *et al.*, 2007). Mosquitoes are active filter feeders, and can make choices about where to forage and on what substrates, even in relatively small containers. Furthermore, there is the possibility that selection for complimentary resources occurs post ingestion, by varying processing time of resources, as has been observed in mosquitoes and other insects (Rashed & Mulla, 1989; Merritt *et al.*, 1992; Raubenheimer & Simpson, 2004). Post ingestion selection could occur by varying the amount of time a bolus of food is passed through the various portions of the gut, with each portion of the gut responsible for the absorption of different nutrient components (Clements, 1999). Movement of the food bolus through the larval gut can vary in time and may even involve backwards, anti-peristaltic movement (Dadd, 1970; Clements, 1999), although this behaviour was not observed in *A. aegypti* consuming toxic leaf litter (David *et al.*, 2000). The adjustment of feeding behaviour to plant material quality has been shown to involve both chemical and physical aspects, and may result in a counterintuitive movement towards areas that contain deleterious leaf litter (David *et al.*, 2002). Changes in feeding behaviour and post-ingestion processing are testable as mechanisms for larval complimentary feeding in future experiments.

The response of larval mosquitoes to leaf litter is mediated through microbial production via the decay of the base leaf material (Merritt *et al.*, 1992; Walker *et al.*, 1997; Kaufman *et al.*, 2001), and therefore may not reflect a direct, stoichiometric relationship between leaf chemistry and mosquito biomass. Synergistic deviations from an additive model for decay processes have been attributed to nutrient flow (particularly nitrogen) from one leaf species to another, thereby allowing for increased microbial decay in leaf material that would be resistant to decay in monospecific conditions (Lecerf *et al.*, 2005). A similar process may be occurring here. However, the lack of correlation between mass loss and larval growth and survival in the monospecific treatments suggests mass loss may not be important in this system. It is also possible that some monospecific treatments are toxic, while mixtures of leaf material moderate this toxicity through undescribed processes (e.g. altering the pH of the water). There has been some evidence for toxicity from decaying plant material having a direct effect on mosquito larvae (David *et al.*, 2000), although we know of no studies that show alterations in this toxicity due to the presence of other leaf species.

The use of fresh leaves in the present study may also impact the growth of larvae. For aquatic macroinvertebrates, fresh leaves can be a superior resource (Walker *et al.*, 1997), an inferior resource (Kochi & Kagaya, 2005), or show little difference from senescent leaves (Sota, 1993; Kochi & Yanai, 2006). Fresh leaves may provide more nitrogen and other necessary nutrients, but may also contribute plant secondary metabolites that could have deleterious properties. Fresh leaves that are more nutrient rich than their senesced counterparts may allow increased microbial decay of leaf species more resistant to decay, leading to greater than additive larval responses. Alternatively, toxic plant secondary metabolites found in fresh but not senesced leaves may lead to negative deviations from the additive model. Although we recognise the potential importance of fresh versus senescent leaves, the question of leaf condition was not addressed in this study.

There was support for the hypothesis that both species of mosquitoes preferentially oviposit in containers with leaf material likely to result in greater larval growth and survival. The pattern of oviposition in these two species is similar to results reported from a previous oviposition study of these two species (Trexler *et al.*, 1998), and may be due to differences in development rate. *Aedes triseriatus* takes longer to develop than *A. albopictus*, and therefore habitat permanence may be an important consideration for ovipositing females utilising container habitats. Other researchers have suggested that tap water may signal a more permanent habitat than oak infused water (Edgerly *et al.*, 1998; Trexler *et al.*, 1998), and may explain *A. triseriatus*'s preference for tap water. *Aedes albopictus*, which develops more quickly, may not consider habitat permanence as important as *A. triseriatus* does, and choose habitats only with high quality nutritional signals.

In addition to generating larval performance measurements on three plant species previously unexamined (coffee, fern, and grape), this study represents the first investigation into how mosquito larvae perform in combinations of leaf species. The response to combinations of leaf material was better than additive for several outcomes, suggesting that a diversity of leaf material may benefit mosquito larvae. Many previous studies have examined the effects of single leaf detritus types on mosquitoes (Fish & Carpenter, 1982; Sota, 1993; Yanoviak, 1999; Dieng *et al.*, 2002; Yee & Juliano, 2006), but containers often contain a diversity of leaf species (Barrera *et al.*, 2006; M. H. Reiskind, pers. obs.). This study examined the more realistic situation of a diversity of plant material in containers. This study also demonstrates the ability of both species of mosquito to discriminate between high and low quality leaf habitats in making oviposition choices. Several unanswered questions remain. First, what is the variation in leaf diversity in containers in the field, and does this leaf diversity influence competition (both inter- and intraspecific)? Second, how do combinations of leaves influence oviposition choice? Finally, how do leaf diversity, the microbial community, and mosquito larval behaviour interact to determine the productivity of container habitats?

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Fig. 1.

Average mass loss (± 1 SE) of leaves by leaf and mosquito species. Similar letters denote homogeneous groups ($P < 0.05$) of leaf species by *post hoc* comparisons with Bonferroni's correction.

Fig. 2. Performance outcomes for females reared with different leaf species: (a) per cent survival, (b) wing length, and (c) average days to emergence. The same letters within each case (upper or lower) denote homogeneous groups within species ($P < 0.05$) by *post hoc* comparisons with Bonferroni's correction. ND, no data because of a lack of females.

Fig. 3.

Average λ' values (± 1 SE) of *Aedes albopictus* for all leaf combinations. (C, *Psychotria nervosa* 'coffee'; G, *Vitis aestivalis* 'grape'; F, *Nephrolepis exalta* 'fern'; O, *Quercus virginiana* 'oak'). White bars are single species combination, light grey bars are two-species combinations, dark grey bars are three-species combinations, and the hatched bar is the combination of all four leaf species.

Fig. 4. Deviations from null hypothesis of strict additivity in larval outcomes by leaf combination (± 1 95% CI) (see Table 4 for statistical tests of all outcomes). (a) Female survival, (b) female development time, (c) female growth (wing length), (d) male survival, (e) male development time, (f) male growth (wing length).

Fig. 5.

Average number of eggs of five replicates, oviposited into each infusion type (± 1 SE), by mosquito species. Similar letters within case denote homogeneous groups within species by *post hoc* comparisons (*Aedes triseriatus*: negative binomial regression: water type: $\chi^2_{df=2}=6.24$, $P < 0.05$; replicate: $\chi^2_{df=4}=1.06$, $P = 0.9$; *post hoc* pairwise comparisons: grape < oak: $\chi^2_{df=1}=5.42$, $P < 0.05$; grape < tap: $\chi^2_{df=1}=7.39$, $P < 0.01$; oak = tap: $\chi^2_{df=1}=0.01$, $P = 0.93$; *Aedes albopictus*: negative binomial regression: water type: $\chi^2_{df=2}=8.12$, $P < 0.05$; replicate: $\chi^2_{df=4}=4.07$, $P = 0.4$; *post hoc* pairwise comparisons: grape < oak: $\chi^2_{df=1}=4.31$, $P < 0.05$; grape = tap: $\chi^2_{df=1}=0.77$, $P = 0.4$; oak > tap: $\chi^2_{df=1}=9.69$, $P < 0.01$).

Table 1

MANOVA comparing larval outcomes by leaf and mosquito species for experiment 1.

Source	Pillai's trace	$F_{(\text{NumDF}, \text{DenDF})}$	<i>P</i> -value
Mosquito	0.972	114.76 _(3,10)	<0.0001
Leaf	1.505	11.15 _(6,22)	<0.0001
Mosquito $\times$ Leaf	0.816	14.82 _(3,10)	0.0005

Table 2

MANOVA output for effects of leaf species on measures of performance of *Aedes albopictus* and *Aedes triseriatus* females separately with the standardised canonical coefficients (SCCs) of the first (and only significant) canonical.

First SCC						
Species	Pillai's trace	$F_{(NumDF, DenDF)}$	P	Days to emergence	Wing length	Per cent survival
<i>A. albopictus</i>	1.574	6.15 _(6,10)	0.0063	9.17	1.272	-0.669
<i>A. triseriatus</i>	0.921	15.47 _(3,4)	0.0115	3.10	0.337	-0.269

Table 3

The three significant canonicals explaining variation in effects of leaf combinations on *Aedes albopictus* females and their standardised canonical coefficients (SCCs).

SCCs						
Canonical	Eigenvalue	Explained variation	F/(NumDF, DenDF)	P	Days to emergence	Wing length
1	1.8445	0.4593	3.98(42,125.4)	<0.0001	1.6801	0.2763
2	1.1840	0.2948	3.58(26,86)	<0.0001	-0.2688	-0.8607
3	0.9873	0.2459	3.62(12,44)	0.0008	1.0536	1.5029
						Per cent survival
						0.1172
						1.1655
						0.6333

Table 4

F-tests for non-additivity by outcome by examining the leaf combination factor nested in species richness (one, two, three, or four species present) after accounting for the effects of the presence and absence of each leaf species and species richness. Significant ($\alpha = 0.05$) outcomes are in bold. See Fig. 4 for pattern of deviations from additive model.

Outcome	$F_{d.f. = 8, 60}$	<i>P</i> -value
Per cent female survival	2.13	0.0463
Female days to emergence	1.89	0.0861
Female wing length	2.89	0.0113
Per cent male survival	0.88	0.5416
Male days to emergence	3.14	0.0067
Male wing length	2.07	0.0597
Lambda	1.0	0.4913