Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae)

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Abstract: Growth of larvae of the eastern tree-hole mosquito, *Aedes triseriatus* (Say), measured as survival to adulthood, development time, and body mass at adult emergence, was significantly better when larvae were provided with fresh rather than senescent beech leaves as food substrate. Leaf type affected larval growth performance independently of ration of leaf available per larva when the ration level was high and larvae were not densely crowded, but leaf type and ration level had interactive effects on larval growth performance when ration was more limiting and larvae denser. Fresh leaves contained about twice as much nitrogen, had a lower carbon:nitrogen ratio, leached more mass into water, and contained significantly more soluble protein and carbohydrate than did senescent leaves. Thus, the observed growth responses could be explained on the basis of variation in nutrient content of, and greater leaching from, the fresh leaves. Larval growth was also significantly better on intact senescent beech leaves than on leaves that had been leached to remove soluble protein and carbohydrate; it was also significantly better on leaves whose surfaces had flourishing bacterial growth than on leaves with disinfected surfaces. Growth of female larvae fit well a pupation window model where larvae attained a minimum mass to emergence past a minimum development time across a range of leaf substrates varying in quality and quantity.

Résumé : La croissance des larves du moustique *Aedes triseriatus* (Say), une donnée tenant compte de la survie jusqu'à l'âge adulte, de la durée du développement et de la masse au moment de l'émergence, est significativement meilleure lorsque les larves sont nourries de feuilles fraîches que lorsqu'elles sont élevées sur des feuilles sénescentes de hêtre. Le type de feuille affecte la performance de la croissance indépendamment de la quantité disponible par larve lorsque la quantité est élevée et la densité des larves, faible, mais le type de feuille et la quantité ont des effets interactifs sur la performance de la croissance lorsque la quantité est plus limitée et la densité des larves, plus élevée. Les feuilles fraîches contiennent environ deux fois plus d'azote, le rapport carbone : azote y est plus faible, elles lessivent une plus grande partie de leur masse dans l'eau et contiennent significativement plus de protéines et d'hydrates de carbone solubles que les feuilles sénescentes. Les résultats obtenus peuvent donc s'expliquer par les différences dans le contenu en éléments nutritifs et le lessivage plus importants des feuilles fraîches. La croissance larvaire est aussi significativement plus rapide sur les feuilles sénescentes entières que sur les feuilles qui ont été lessivées de leurs protéines et hydrates de carbone solubles; elle est aussi plus rapide sur les feuilles dont la surface est couverte de colonies bactériennes que sur les feuilles à surface désinfectée. La croissance des larves femelles correspond bien à un modèle de fenêtre de nymphose où les larves atteignent une masse minimale à l'émergence après un développement de durée minimale, sur des substrats de feuilles de qualité et de quantité variées.
[Traduit par la Rédaction]

Introduction

Many macroinvertebrates inhabiting freshwater ecosystems utilize allochthonous leaf detritus as food. Some invertebrates consume leaf detritus directly by shredding coarse particulate detritus or by gathering finer detritus, but for these guilds, the efficiency of conversion of leaf material into invertebrate tissue is usually low (Berrie 1976; Slansky and Scriber 1985). Other invertebrates, including mosquito larvae, exploit leaf detritus by filtering, scraping, or browsing microorganisms in the biofilm on the leaf surface (Cummins and Klug 1979; Fish and Carpenter 1982; Walker and Merritt 1991; Merritt et al. 1992). Invertebrate growth on leaf detritus varies with feeding mode, quantity of leaf detritus, chemical composition of leaf detritus, decomposition rate of leaves, and microbial conditioning of the leaf material (Kaushik and Hynes 1971; Berrie 1976; Anderson and Sedell 1979; Cummins and Klug 1979; Merritt et al. 1984).

Water-filled containers, both natural ("phytotelmata") and anthropogenic (e.g., discarded tires), are common habitats of mosquitoes and other insects in temperate and tropical areas

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(Frank and Lounibos 1983). Developmental success of mosquitoes in containers tends to be highly variable, partly because of food limitations (Carpenter 1982*b*, 1983; Copeland and Craig 1992; Fish and Carpenter 1982; Fisher et al. 1990; Frank et al. 1985; Hawley 1985; Leonard and Juliano 1995; Livdahl 1982; Lounibos et al. 1993; Mogi 1984; Walker and Merritt 1988; Walker et al. 1991). Water-filled tree holes harbor a specialized community of mosquitoes and other insects that share heterotrophic food resources, especially leaf detritus, through competitive or facilitative interactions (Kitching 1971, 1987). Tree-hole ecosystems are supported by decomposition of leaf detritus, which generates microorganisms and particulate and dissolved organic matter that are consumed by the insects (Kitching 1983; Walker et al. 1988). A common inhabitant of water-filled tree holes in eastern North America is the eastern tree-hole mosquito, *Aedes triseriatus* (Say) (Craig 1983). Larval growth of *A. triseriatus* is highly sensitive to the ration of leaf litter available per larva (Fish and Carpenter 1982; Carpenter 1983; Leonard and Juliano 1995). The microbial lawn exposed on leaf surfaces can be rapidly grazed to depletion by mosquito larvae, while microorganisms flourish on leaf surfaces not exposed to larval feeding (Fish and Carpenter 1982; M. Kaufman, unpublished data). Variation in litter quality also affects growth performance of *A. triseriatus* (Fish and Carpenter 1982; Lounibos et al. 1993). However, the relative importance of variation in litter quantity, litter quality, and microbial colonization to larval growth remains poorly understood. We tested the hypothesis that detritus quality and microbial populations will interact with food ration to affect the survival, development time, and adult mass of *A. triseriatus*. Experiments involved factorial manipulations of detritus quality, microbial populations, and food ration level in replicated laboratory microcosms.

Methods and materials

Microcosm experiments

Laboratory microcosms (plastic dishes, 15.5 cm in diameter × 6.5 cm deep, with friction-fitting lids) were used as simulated tree-hole habitats for growth experiments. Newly hatched first instars of *A. triseriatus*, taken from a laboratory colony started in 1988 and supplemented annually with wild mosquitoes, were stocked into the microcosms at the beginning of each experiment. Leaf detritus was provided as a resource for larvae. We conducted three microcosm experiments. In experiment 1, the effect of two rations of fresh or senescent leaves on mosquito growth was studied. Leaves of American beech (*Fagus grandifolia* Ehr.) were removed in August (fresh and green) and October (senescent and brown) from the same 15 saplings and stored at room temperature in plastic bags. Prior to the experiment, leaves were dried at 55°C for 7 days, weighed into leaf packs of 0.3 or 0.6 g, and placed in the microcosms. Distilled water (400 mL) was added and then 20 larvae were added by gentle pipetting, yielding the equivalent of 15 or 30 mg of initial leaf mass per larva. Thus, experiment 1 formed a 2 × 2 factorial design. Each treatment combination was replicated with 6 microcosms. The microcosms were arranged randomly with respect to treatment in a constant-temperature (22 ± 2°C) incubator. The microcosms were examined daily and emerged adult mosquitoes were removed, killed by freezing, dried, and weighed (±0.001 mg).

Experiment 2 was similar to experiment 1, except that microcosms were provisioned with 0.5- and 1.0-g leaf packs and stocked with 40 larvae per microcosm, yielding a leaf ration of 12.5 or 25 mg per larva.

Experiment 3 was designed to test simultaneously the effect on mosquito growth of (*i*) removing the soluble fraction of leaf material from leaves and (*ii*) variation in microbial colonization of leaf surfaces. Dried, senescent beech leaves were formed into 1.0-g packs. Half of the leaf packs were leached for 24 h in microcosms with 400 mL of distilled water. Then leaves from half of the leached and nonleached packs were disinfected by individually dipping them for 5 s into a solution of 5.25% chlorine bleach, followed by rinsing in a series of three containers of distilled water. All leaves were redried and the leaf packs were placed in clean microcosms with 400 mL distilled water and 20 mosquito larvae. Microcosms were arranged randomly on a laboratory bench at room temperature (27 ± 4°C). Each treatment combination in this 2 × 2 factorial included 6 replicate microcosms.

For statistical analyses, microcosms were treated as the experimental unit. We tested for treatment effects on survival, average development time, and average adult mass with two-way factorial analysis of variance (ANOVA) using SYSTAT (Wilkinson 1989). For comparison of means after ANOVA, pooled standard errors were calculated (Steel and Torrie 1980) and plotted on the figures. Survival data were transformed with arcsine of the square root prior to analysis.

We conducted an experiment to test if the disinfectant treatment impacted larvae directly (i.e., was toxic) and if the treatment reduced bacterial densities on leaf surfaces. Senescent beech leaves were either leached or not leached. Half of the leaves were disinfected as before, then individual leaves were placed in vials (20 mL) filled with distilled water and containing 10 first-instar larvae. After 24 h, vials were emptied and the surviving larvae counted. Leaves treated the same way were swabbed thoroughly on one side with cotton-tipped dowels that had been dipped in a liquid bacterial culture medium (R2A without agar, Difco, Detroit, Mich.; Reasoner and Geldreich 1985). The dowels were then streaked onto the surface of R2A agar medium in petri dishes. Dishes were incubated for 24 h at 30°C. Bacterial colonies were counted along the last 1 cm of the streaked area, where individual colonies could easily be differentiated. Larval survivorship and numbers of bacterial colonies were compared among treatments with two-way ANOVA and individual means were separated by comparing least significant differences (Steel and Torrie 1980).

Leaf decomposition

We compared decomposition rates of fresh and senescent beech leaves. Individual leaves were dried, weighed (±1 mg), placed in tubes with 20 mL of water, and capped. At intervals of 2, 6, 13, 19, 36, and 43 days, three replicate leaves were removed from tubes, redried, and reweighed. The proportion of original leaf mass remaining was calculated, transformed with arcsine of the square root, and compared among leaf types over time with a two-way ANOVA. The decomposition rate, k, of the leaf types was taken as the slope from linear regression of the natural logarithm of the percentage of initial mass remaining over time, approximating the negative exponential model (Carpenter 1982*a*). Homogeneity of the slopes of the two regression lines was determined with the t-test method of Steel and Torrie (1980).

Chemical analysis of leaves and leachate

We compared the chemical composition of fresh and senescent beech leaves and the leachate from them. Dried leaves were leached for 24 h in 300 mL of distilled water containing 150 ppm each of the antibiotics streptomycin sulfate, tetracycline hydrochloride, and cycloheximide (Sigma Chemical, St. Louis, Mo.) to minimize microbially mediated oxidation of soluble compounds in the leachate. A 5-mL sample of the leachate was frozen (−70°C) and later analyzed for total protein (Lowry et al. 1951; Van Handel 1986) and total soluble carbohydrate (Dubois et al. 1965). Results were compared by means of t tests.

Table 1. Summary of ANOVAs testing for effects of leaf senescence and leaf ration level on mosquito growth and survival (experiment 1, Fig. 1).

		F statistic		
Response variable	Error mean square	Leaf senescence	Ration level	Leaf senescence × ration level
Male development time	12.44	3.95	3.29	1.06
Female development time	125.90	5.46*	0.49	0.26
Male mass	0.002	18.35***	4.63	1.93
Female mass	0.007	23.59***	8.79*	3.85
Survival	0.075	9.62**	7.07*	2.56

Note: Degrees of freedom for error terms were 13, 14, and 20 for males, females, and percent survival, respectively.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

Dried subsamples of fresh and senescent leaves that had been either leached or not leached were ground with a dental amalgamator (Wiglbug™, Crescent Dental Manufacturing Co., Chicago, Ill.) and analyzed with a Carlo Erba nitrogen analyzer (No. 1500, Series 2, Carlo Erba, Milan, Italy). Carbon and nitrogen concentrations and carbon:nitrogen ratios were subjected to two-way ANOVA where the factors were leaf type and leaching treatment. Individual means were separated by comparing least significant differences. Data were transformed with arcsine of the square root prior to analysis.

Pupation window model

It has been hypothesized that mosquito pupation occurs when the larval growth trajectory intersects a pupation threshold that is defined by development time and adult mass (Gilpin and McClelland 1979; Dye 1982; Carpenter 1984). In this "pupation window model," litter quality and litter ration are thought to influence the larval growth trajectory, and therefore to influence development time and adult mass, but the pupation threshold function is thought to be an attribute of the genotype that is independent of litter quality and litter ration. Equation 1 represents the pupation threshold function,

$$[1] \quad (T - h_1)(W - h_2) = h_3$$

where T is development time, h_1 is minimum development time, W is adult mass, h_2 is minimum adult mass, and h_3 indicates the curvature of the function.

We used nonlinear modeling (Marquardt's algorithm, NLIN procedure; SAS Institute Inc. 1985) to fit the coefficients h_1, h_2, and h_3 to the development time and adult mass data (microcosm means) from experiments 1, 2, and 3, respectively.

Results

Microcosm experiments

With respect to survival, development time, and adult mass, mosquito growth was greatest when larvae were provided with fresh leaves at the highest ration level and least with sensescent leaves at the lowest ration level (Fig. 1). Growth and survival responses were intermediate to these two treatment combinations, and similar to each other, for the fresh leaf / low ration level and the senescent leaf / high ration level treatments. Survival, average female mass, average male mass, and female development time were significantly affected by the type of leaf material provided (Table 1). Survival and female mass were also significantly affected by leaf ration level. There were no significant interactions (Table 1).

In experiment 2, as in experiment 1, mosquito growth performance was best when larvae were provided with fresh leaves at the highest ration level and worst with senescent leaves at the lowest ration level (Fig. 2). Again, growth responses were similar between the fresh leaf / low ration level and senescent leaf / high ration level treatments, except that development time of females from the senescent leaf / high ration level treatment was slower than that of females from the fresh leaf / low ration level treatment (Fig. 2). All growth responses were significantly affected by leaf type and ration level (Table 2). Interactions between leaf type and ration level were significant for survival and male development time (Table 2).

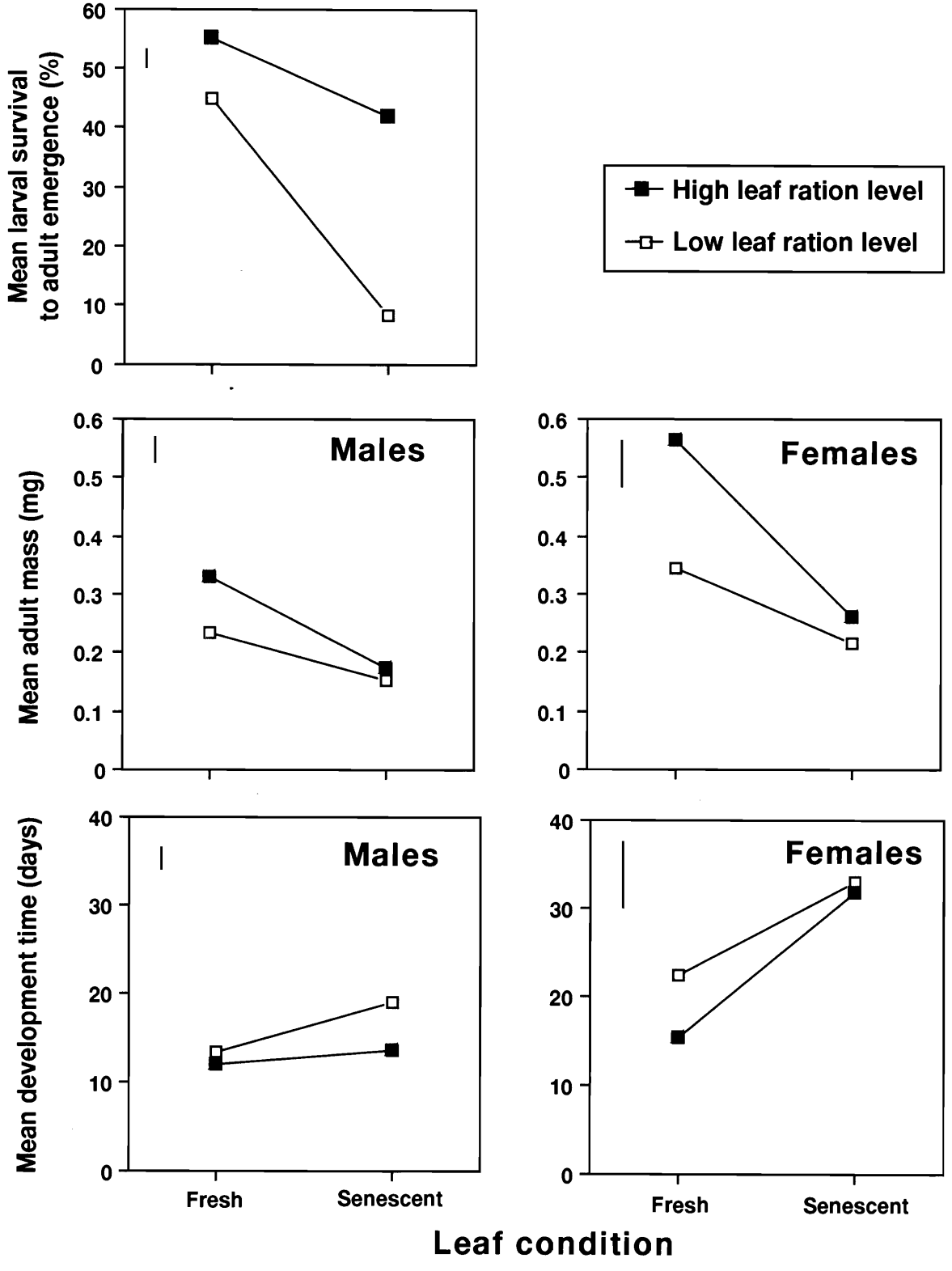
In experiment 3, survival was greatest on non-disinfected leaves regardless of leaching effects (Fig. 3). Male and female development time was increased by both disinfection and leaching. Adult mass was reduced by leaching. Leaching significantly affected male and female development time and male and female mass, but not survival (Table 3). Disinfection of leaf surfaces significantly affected male and female development time and percent survival, but not average male and female mass. Male mass was significantly affected by the interaction between leaching and disinfection treatments.

Disinfection had no direct effect on survival of first instars ($F = 0.80$, df = 1,16, $P > 0.05$) (Table 4). Leaching had a slight positive effect on larval survival ($F = 4.98$, df = 1,16, $P < 0.05$). There was a highly significant effect of disinfection ($F = 91.73$, df = 1,16, $P < 0.001$), leaching ($F = 26.87$, df = 1,16, $P < 0.001$), and the interaction of these treatments ($F = 33.79$, df = 1,16, $P < 0.001$) on the number of bacterial colonies recovered from leaf swabs onto agar plates (Table 4). Very few bacterial colonies resulted from swabs on non-soaked leaves and soaked, disinfected leaves compared with soaked, non-disinfected leaves (Table 4).

Leaf decomposition

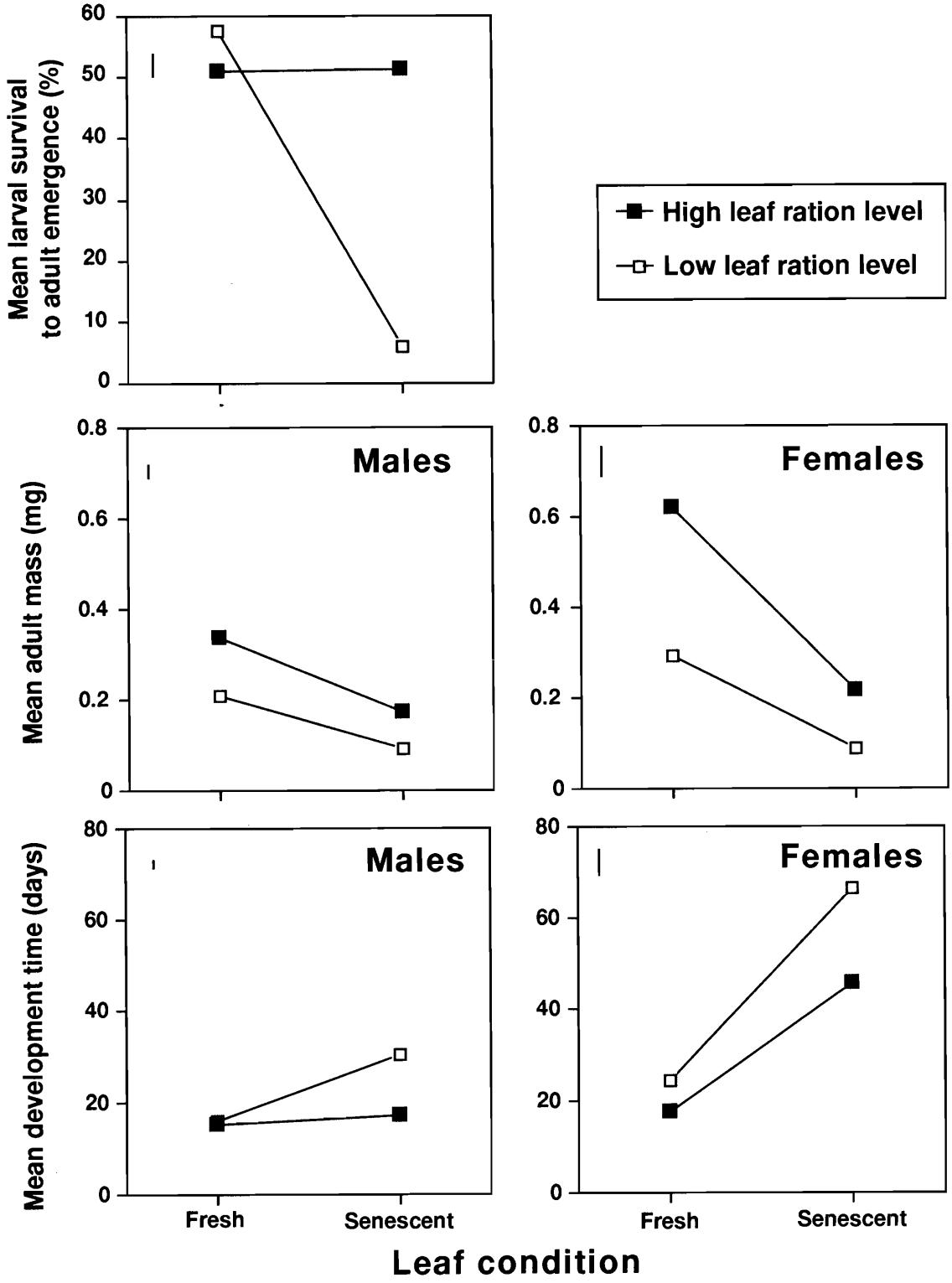
Leaf mass decreased rapidly after 2 days in water, especially for fresh leaves, and decreased at a lower rate from 2 to 43 days (Fig. 4). There were significant main effects of leaf type ($F = 166.04$, df = 1,24, $P < 0.001$) and day after

Fig. 1. Survival, adult mass, and development time of *Aedes triseriatus* provided fresh or senescent beech leaves at two ration levels (experiment 1, Table 1). Vertical lines represent pooled standard errors from ANOVA.



immersion (F = 3.10, df = 5,24, $P < 0.05$) on the proportion of leaf mass remaining; however, there was no significant interaction (F = 2.39, df = 5,24, $P > 0.05$). Regression of $\log_e$ (initial leaf mass remaining) on day after immersion provided estimates of decomposition rates of fresh and senescent leaves, 0.005 and 0.002 mg · mg^{-1} · d^{-1}, respectively. The slopes of the regression lines were significantly different from 0 (fresh leaves: r = 0.57,

Fig. 2. Survival, adult mass, and development time of *Aedes triseriatus* provided fresh or senescent beech leaves at two ration levels (experiment 2, Table 2). Vertical lines represent pooled standard errors from ANOVA.



df = 19, $P < 0.01$; senescent leaves: $r = 0.53$, df = 19, $P < 0.05$) and were not significantly different from each other ($t = 0.005$, df = 34, $P > 0.05$).

Leaf and leachate chemistry

Carbon content ranged between 44 and 45.2% of leaf mass (Table 5), and did not differ between fresh and senescent leaves ($F = 1.64$, df = 1,15, $P > 0.05$) or between leached and non-leached leaves ($F = 0.57$, df = 1,15, $P > 0.05$). Nitrogen content ranged between 1.0 and 2.3% of leaf mass; fresh leaves had about twice as much nitrogen as senescent leaves, therefore the carbon:nitrogen ratio was lower for

Table 2. Summary of ANOVAs testing for effects of leaf senescence and leaf ration level on mosquito growth and survival (experiment 2, Fig. 2).

Response variable	Error mean square	*F* statistic: Leaf senescence	*F* statistic: Ration level	*F* statistic: Leaf senescence × ration level
Male development time	12.72	31.14***	23.39***	17.22***
Female development time	86.98	37.11***	5.83*	1.56
Male mass	0.002	58.12***	32.82***	1.96
Female mass	0.007	103.66***	62.62***	3.64
Survival	0.024	24.75***	24.93***	15.23**

Note: Degrees of freedom for error terms were 20, except for female development time, for which the number was 15.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

fresh leaves (Table 5). There were significant differences between fresh and senescent leaves in nitrogen content (F = 45.07, df = 1,15, $P < 0.001$) and carbon:nitrogen ratio (F = 29.34, df = 1,15, $P < 0.001$). There was no significant effect of leaching on nitrogen content (F = 0.78, df = 1,15, $P > 0.05$) or on the carbon:nitrogen ratio (F = 0.15, df = 1,15, $P > 0.05$) of leaves. There were no significant interactions of leaf type and soaking treatment ($F < 2.58$, df = 1,15, $P > 0.05$). There was significantly more protein and carbohydrate in leachate from fresh leaves than in leachate from senescent leaves (t = 3.7 and t = 11.3, df = 9, $P < 0.01$).

Pupation window model

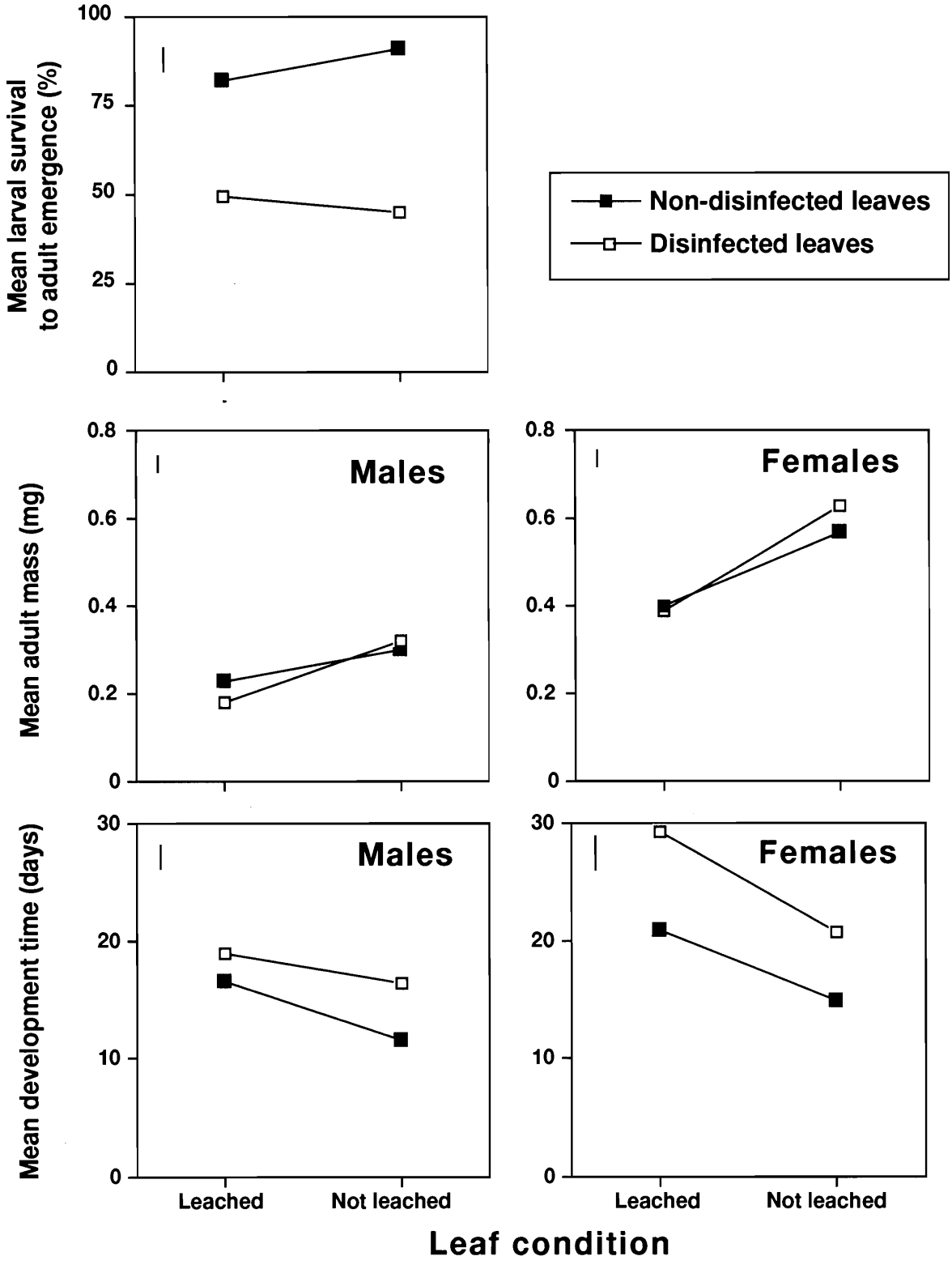
Results were generally consistent with the pupation window model in that (*i*) litter ration level and litter quality influenced both adult mass and development time, (*ii*) the relationship between adult mass and development time across treatments could be described by a single function, and (*iii*) the pupation threshold function was similar in form across replicate experiments (Fig. 5). The fit to eq. 1 was quite good for experiments 1 and 2 (r^2 = 0.55, $F_{[2,16]}$ = 9.80, $P < 0.01$, and r^2 = 0.88, $F_{[2,17]}$ = 62.66, $P < 0.01$) and the functions were nearly overlapping. The fit to experiment 3 was not as good as to experiments 1 and 2 (r^2 = 0.21, $F_{[2,22]}$ = 2.94, P = 0.08), and the function indicates a higher minimum mass for pupation (h_2 = 0.335 vs. 0.185 and 0.135 mg). Experiment 3 was conducted at a higher temperature than experiments 1 and 2 and produced less variation in development times.

Discussion

Variation in both quality and quantity of beech leaf substrate affected growth of *A. triseriatus* larvae in microcosms. In experiments 1 and 2, mosquitoes consistently grew better on fresh than senescent leaves when the two types were provided in equal rations. In experiment 1, which had higher leaf ration levels than experiment 2, litter quality had a much greater effect on larval growth than did ration level (compare F statistics in Table 1). In experiment 2, where less leaf ration per larva was provided relative to experiment 1, there were pronounced effects of both ration level and leaf type on growth responses, and highly significant interactions between these factors on survivorship and male development time (see F statistics in Table 2). Apparently, within experiments, the effects of litter quality became greater as litter quantity decreased. These effects may have been exacerbated by the higher density of larvae in experiment 2, possibly resulting in greater competition amongst larvae for food resources available from the leaves. When leaf ration was less limiting to larval growth within experiments, leaf quality affected larval growth more independently of ration level. Carpenter (1983) observed that the ration level of senescent beech detritus was the single most important factor in the success of *A. triseriatus* larvae, regardless of larval density, concentration of ammonium ions in the water, volume of water in microcosms, provision of stemflow water versus distilled water in microcosms, or "chemical interference" among larvae (Carpenter 1982*b*; Dye 1982, 1984). Here we show that variation in leaf quality at the same ration level can result in equally dramatic effects on the growth of mosquito larvae. In the only other study we know of that directly compared mosquito growth responses when fresh or senescent leaves were provided as food substrate, there was no consistent effect of leaf condition on mosquito growth (Sota 1993). However, rations of leaves were generous in that study compared with ours, which may explain the differences in results between the two studies.

The differences between fresh and senescent leaves in our first two experiments were likely due to differences in leaf chemistry. The fresh leaves had more nitrogen, protein, and soluble carbohydrates. Other studies comparing fresh and senescent leaves produced similar results (McArthur et al. 1986; Boulton and Boon 1991). The nitrogen content of leaves is a well-known determinant of growth success of insect folivores (e.g., Slansky and Scriber 1985; Mattson and Scriber 1987) and appears to be at least as important for aquatic detritivores (Iversen 1974; Stout and Taft 1985; Lawson et al. 1984; Garden and Davies 1988; but see Ward and Cummins 1979). Ours are the first data indicating a similar relationship for aquatic insects, such as larval *A. triseriatus*, that consume microorganisms on the surface of the detritus rather than by ingesting the leaf detritus directly (Slansky and Scriber 1985; Merritt et al. 1992). Apparently, elevated nitrogen and protein contents in fresh leaves stimu-

Fig. 3. Survival, adult mass, and development time of *Aedes triseriatus* provided senescent beech leaves that were leached or not leached and disinfected or not disinfected (experiment 3, Table 3). Vertical lines represent pooled standard errors from ANOVA.



lated microbial growth on leaf surfaces and accelerated transfer of nutrients to mosquito larvae through the microbial food. Support for this interpretation comes from the study by Kaufman et al. (1996) in which the respiration rate of microorganisms on the surface of leaf detritus was positively correlated with the nitrogen content in the detritus. Besides elevating the nitrogen content in leaf tissue, leached nutrients could stimulate microbial growth in the water column and

Table 3. Summary of ANOVAs testing for effects of leaf leaching and surface disinfection on mosquito growth and survival (experiment 3, Fig. 3).

Response variable	Error mean square	*F* statistic		
		Leached	Disinfected	Leached × disinfected
Male development time	3.93	19.61***	17.58***	1.86
Female development time	12.09	26.69***	24.91***	0.68
Male mass	0.001	53.85***	1.28	6.70*
Female mass	0.01	20.72***	0.41	0.40
Survival	0.063	0.40	34.39***	0.22

Note: Degrees of freedom for error terms were 18–20.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

Table 4. Bacterial density and mosquito survival after exposure to senescent beech leaves that had leached or not leached and were disinfected or not disinfected with bleach.

Variable	Leached leaves		Non-leached leaves	
	Disinfected	Not disinfected	Disinfected	Not disinfected
No. of bacterial colonies/cm	3.2±0.9*a*	186.0±18.6*c*	3.8±1.2*a*	9.0±1.4*b*
Percent survival of first instars	96.4±4.6*a*	94.0±6.0*a*	89.6±4.5*b*	80.0±5.5*b*

Note: Values are given as the mean ± SEM ($N = 5$ per group). Values in rows followed by different letters were significantly different in least significant difference comparisons ($P < 0.05$).

thereby provide food for larvae that filter the microorganisms from the water.

The nitrogen content in leaf detritus usually increases with time as leaves condition in the water, because the microorganisms associated with the leaves sequester exogenous nitrogen from nitrate and other sources in the water column (Rice 1982; Webster and Benfield 1986). Exogenous nitrogen, especially nitrate, is known to enhance mosquito growth performance (Carpenter 1982*b*; Walker et al. 1991). Our experiments demonstrated the importance of endogenous litter nitrogen on mosquito growth because we used distilled water in the microcosms.

Senescent leaves are often considered to be the primary form of allochthonous input of organic material into aquatic ecosystems. However, fresh leaves also provide nutritional input into aquatic ecosystems (Stout 1980; Stout and Taft 1985; Maloney and Lamberti 1995). Fresh oak flowers uncommonly fall into tree holes in Florida, along with senescent oak leaves in winter and spring; experimental additions of fresh oak flowers into laboratory microcosms and field habitats (water-filled tires) stimulated growth of *A. triseriatus* (Lounibos et al. 1992, 1993). We have observed fresh leaves falling into tree holes in Michigan on occasion, but it is irregular. The experiments we present are heuristic because of the analysis of the nutritive benefit to mosquito larvae of senescent leaves (the common source of organic substrate for tree hole fauna) relative to fresh leaves.

Decomposition of leaf detritus in water is a two-step process: an initial loss of soluble organic compounds that leach from the leaf during the first few days of immersion, followed by a much slower rate of decomposition of refractile leaf tissue (Kaushik and Hynes 1971; Carpenter 1982*a*). Our experiment with leaf mass loss reflected this pattern (Fig. 4). The leaching treatment in our third experiment had the consequence of removing the soluble material from the senescent leaves, corresponding to the first phase of decomposition. Although leaf mass loss after 24 h of leaching averaged only about 14%, mosquito production (total biomass per microcosm) was 28–38% less on leached than on non-leached, senescent leaves when the surfaces of both were disinfected. Apparently, the soluble fraction of leaf mass accounted for a great deal of mosquito growth in that experiment. Presumably, this fraction stimulated microbial growth early in the experiment, thus providing food for young larvae.

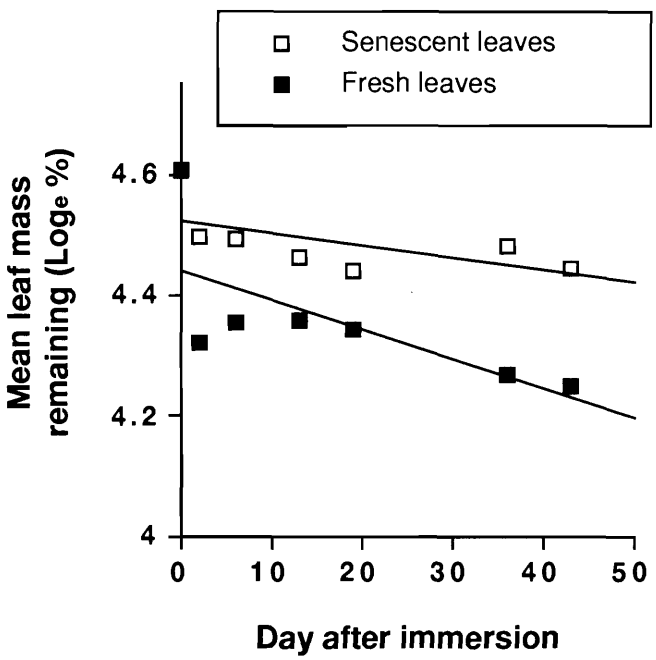
Microbial conditioning, i.e., colonization of the leaf surface and matrix with microorganisms, facilitates decomposition of the refractile component of detritus after the initial leaching losses (Kaushik and Hynes 1971; Anderson and Sedell 1979). Microorganisms greatly enhance the quality of detritus as food for detritivores by mineralizing it into assimilable forms (Cummins and Klug 1979). In our third experiment, leaching the leaves to remove the soluble fraction of leaf material simultaneously stimulated microbial colonization of leaf surfaces; thus, disinfection treatment was necessary to control for variation in microbial growth on leached and non-leached leaves. One explanation for the differences

Table 5. Carbon and nitrogen contents (as a percentage of mass) and the carbon:nitrogen (C:N) ratio of leached or non-leached fresh and senescent beech leaves; the carbohydrate and protein concentrations in leachate from fresh and senescent leaves are also given.

	Fresh leaves		Senescent leaves	
Variable	Leached	Non-leached	Leached	Non-leached
Carbon (%)	44.0±0.8*a*	44.0±0.3*a*	44.3±0.7*a*	45.2±0.3*a*
Nitrogen (%)	1.8±0.1*a*	2.3±0.2*a*	1.1±0.2*b*	1.0±0.1*b*
C:N ratio	24.3±1.6*a*	20.0±2.1*a*	45.3±7.7*b*	46.8±3.2*b*
Carbohydrate (ppm)	123.0±3.0*a*	—	70.0±2.0*b*	—
Protein (ppm)	9.5±0.6*a*	—	6.5±0.5*b*	—

Note: Values are given as the mean ± SEM (N = 5 per group). Values in rows followed by the same letter were not significantly different in least significant difference comparisons or t tests ($P < 0.05$).

Fig. 4. Percentages of remaining dry mass of fresh and senescent beech leaves immersed in distilled water. Error bars are smaller than the symbols. N = 3 per leaf type × day combination. Linear regressions of $\log_e$ (percent dry mass remaining) on day after immersion to estimate k (coefficent of leaf decay, equal to the slope of the regression line) were as follows: senescent leaves: $Y = -0.002X + 4.52$, df = 19, $r = 0.60$, $P < 0.01$; fresh leaves: $Y = -0.005X + 4.44$, df = 19, $r = 0.69$, $P < 0.01$.



in mosquito growth and survival on non-disinfected and disinfected leaves is that microorganisms on leaves were readily available for consumption by those larvae that were provided leached, non-disinfected leaves as the experiment commenced.

Development of larval mosquitoes, like that of other insects, is characterized by feeding, molting, and growth to a critical mass in the final instar, allowing pupation (Nijhout 1975; Lounibos 1979; Chambers and Klowden 1990; Clements 1992). For mosquitoes, a negative correlation often exists between development time of larvae and mass at pupation or adult emergence across a range of food regimens, i.e., larvae with shorter development times are heavier at pupation than larvae with longer development times (Carpenter 1984; Bradshaw and Johnson 1995; this study). Two recent studies address factors affecting the propensity to pupate in mosquitoes. Chambers and Klowden (1990) observed that nutritional reserves of carbohydrate, lipid, and glycogen increased at a greater rate than did gain in body mass during the last instar of *Aedes aegypti* (L.); they suggested that accumulation of metabolic reserves influences the propensity to pupate separately from mass gain. Bradshaw and Johnson (1995) proposed a developmental model for the pitcher-plant mosquito, *Wyeomyia smithii* (Coquillett), where commitments to growth and molting and initiation of metamorphosis are flexible functions of food availability at critical points at different larval stages. The predicted and observed growth trajectories for this mosquito, under experimental conditions of decreasing food supply (see Figs. 1B and 2 of Bradshaw and Johnson 1995), are consistent with our results (Fig. 5).

The growth of aquatic insect detritivores, such as mosquitoes, is described by eq. 2 (from Gilpin and McClelland 1979; Dye 1982; and Carpenter 1984),

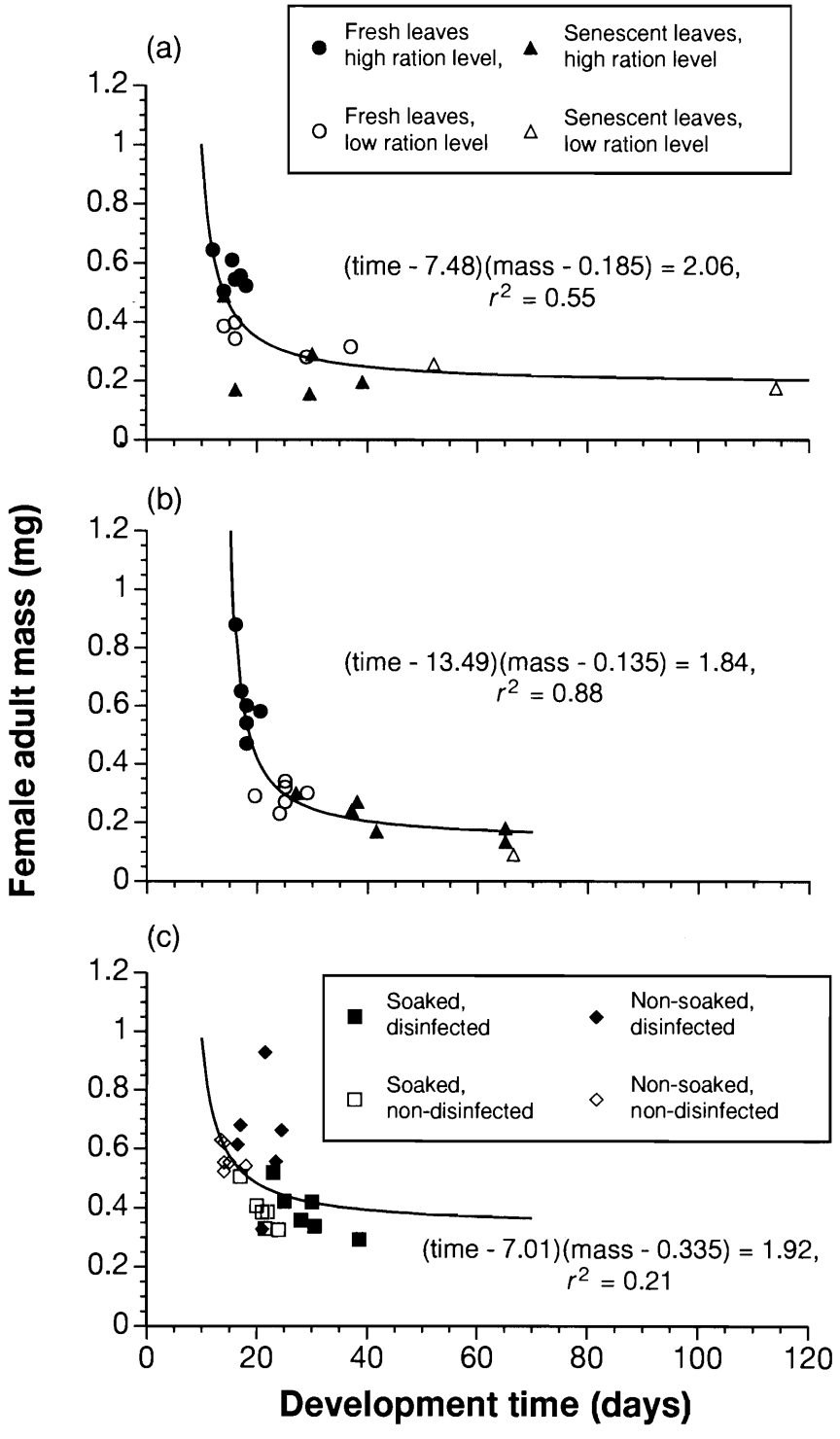
$$[2] \quad W_t = W_0 \exp\{(ak/m - k)(B_0/N_0)[\exp((m - k)t) - 1]\}$$

where W_t is the mass of an individual at day t, W_0 is the mass of a neonate first instar, a is the efficiency of conversion of leaf mass into mosquito tissue, k is the decomposition rate of leaves, m is the larval mortality rate, B_0 is the initial mass of leaf detritus, and N_0 is the initial number of mosquito larvae. Larvae are predicted to pupate where eq. 2 intersects the pupation window described by eq. 1 (see Fig. 5).

Equation 2 suggests that phenotypic variation in the growth rate of mosquito larvae is strongly influenced by litter decomposition rate (Fig. 4). Fitting eq. 1 to data for *A. triseriatus* (Fig. 5) suggests that phenotypic variation in mosquito growth rates leads to effects on both development time and adult size. We used eqs. 1 and 2 to evaluate whether the difference in decomposition rates between fresh and senescent leaves was adequate to explain foliage effects on development time and adult size of *A. triseriatus*.

Substituting measured decomposition rates into eq. 2 indicated that the differences between fresh and senescent leaves are important to mosquitoes that exploit those leaves. For

Fig. 5. Fits of female development time – body mass plots to the pupation window model for experiments 1 (*a*), 2 (*b*), and 3 (*c*). Legends and symbols indicate mean data for individual microcosms from the treatment combinations in each experiment. Development time is time from larval hatch to emergence as an adult.



example, the predicted time for mosquito development to a nominal adult mass of 0.2 mg increases from 37 to 60 d for microcosms with leaf-decomposition rates of 0.005 vs. 0.002 mg · mg^{-1} · d^{-1} (Fig. 4), where W_0 = 0.011 mg and a = 0.389 mg/mg (Carpenter 1984), m = 0.029 larvae · larva^{-1} · d^{-1} (average for four treatments in experiment 2), B_0 = 1000 mg, and N_0 = 40 neonates (high leaf ration level in experiment 2). However, further analysis of eqs. 1 and 2 suggests that the differences in decomposition rate between fresh and senescent leaves were not adequate to explain the effects on adult mass and development time; indeed, these rates were not signficantly different in our experiment

(Fig. 4). For each of the eight treatments in experiments 1 and 2 (Fig. 5), we calculated the value of k (decomposition rate) that would be required in eq. 2 to predict the combination of development time and adult mass attained by an average female mosquito in that treatment. B_0 and N_0 were known for each treatment; m was calculated for each treatment on the basis of cumulative survival and average development time; estimates of W_0 and a follow Carpenter (1984). These indirect estimates of k for four treatments with senescent leaves averaged 0.0044 mg · mg^{-1} · d^{-1} (range 0.0015–0.0067 mg · mg^{-1} · d^{-1}), which is similar to the value of 0.0039 mg · mg^{-1} · d^{-1} for senescent beech leaves (Carpenter 1984), but somewhat more than our empirical estimate of 0.0020 mg · mg^{-1} · d^{-1}, based on mass loss of leaf packs in microcosms (Fig. 4). Indirect estimates of k for four treatments with fresh leaves averaged 0.0237 mg · mg^{-1} · d^{-1} (range 0.0194–0.0314 mg · mg^{-1} · d^{-1}), which is much greater than our empirical estimate of 0.0050 mg · mg^{-1} · d^{-1}, based on mass loss of leaf packs (Fig. 4). Thus, eq. 2 requires differences in leaf-decomposition rate greater than 5-fold to produce the effects on mosquito growth that we observed between fresh and sensecent leaves, but mass-loss measurements indicate a difference of only about 2.5-fold.

The only other parameter in eq. 2 that might reasonably be expected to vary with litter quality is conversion efficiency, a, i.e., milligrams of mosquito tissue produced per milligram of leaf mass ingested. Equation 2 would require a doubling of a (to 0.75 mg · mg^{-1}) to account for the effects of fresh leaves on mosquito growth that remain after allowing for an increase of 2.5-fold in decomposition rates. To our knowledge, even 0.61 mg · mg^{-1} is beyond the ingestion conversion efficiencies that have ever been reported for insect detritivores (Slansky and Scriber 1985), so it appears unlikely that the effects of fresh leaves on k and a could have been entirely responsible for the increased growth rate of the mosquito larvae. Direct measures of conversion efficiency of organic matter to mosquito tissue will be needed to further evaluate the importance of a in driving variation in mosquito growth rates. Bradshaw and Johnson (1995) showed that the efficiency of conversion (''yield'' in their study) of laboratory chow to mass of *W. smithii* increased with the amount of food available per larva; however, variation in conversion efficiency as a function of food quality was not addressed in their study.

In general, the pupation window model (eq. 1) appears to describe mosquito growth and development quite well across a range of environmental conditions. The similar fit of eq. 1 to experiments 1 and 2 is consistent with the premise that there is a genetically determined threshold for pupation that can be described as a function of development time and adult mass (Figs. 5*a* and 5*b*; experiment 3 was conducted at various temperatures and so the results were expected to differ). Furthermore, estimates of decomposition rate for senescent leaves that were based upon the assumptions of eq. 2 were comparable to independent measures of decomposition rate based on mass loss. However, our results also indicate that the pupation window model does not fully represent the effects of litter quality (e.g., fresh versus senescent leaves) on mosquito growth. Aspects of eq. 2 that may warrant refinement include (*i*) the assumption of a constant mosquito mortality rate, (*ii*) the assumption of a constant litter-decomposition rate, and (*iii*) the implicit assumption that microbial production rate is a constant fraction of decomposition rate for leaves varying in age and chemical content (Newell et al. 1995). We hypothesize that the primary and secondary chemistry of litter input impacts the survival, development time, and fecundity of tree-hole mosquitoes by influencing the release of biomass to the microbial community (decomposition rate), the efficiency with which microorganisms convert the decomposing fractions of the litter to new microbial tissue, and the nutritional quality of the microbial community for grazing mosquito larvae.

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