Biology and Feeding Requirements of Larval Hunter Flies *Coenosia attenuata* (Diptera: Muscidae) Reared on Larvae of the Fungus Gnat *Bradysia impatiens* (Diptera: Sciaridae)

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ABSTRACT The larval feeding requirements and biology of the generalist predatory muscid hunter fly *Coenosia attenuata* Stein 1903 (Diptera: Muscidae) were investigated at 25°C. Larval *C. attenuata* were fed second-, third, and fourth-instar (L_2, L_3, and L_4) larvae of the fungus gnat *Bradysia impatiens* (Johannsen) (Diptera: Sciaridae) at variable rates to determine minimum and optimum numbers of these prey required for normal development. The proportion of *C. attenuata* larvae surviving to pupation differed significantly as a function of L_2 and L_3 prey numbers. When the number of prey/d was increased from 10 to 15 L_2 and from 5 to 7 L_3 per day, the respective percentages of pupation increased from 0 to 77% and from 0 to 48%. In contrast, all numbers of L_4 prey (1–7 prey per d) supported pupation, and the pupation rate did not vary with prey number. At the highest prey numbers tested, mortalities of *C. attenuata* larvae fed L_2, L_3, and L_4 fungus gnat larvae were 7, 30, and 75%, respectively. The higher mortality of larvae fed L_4 prey was clearly the result of lethal wounds inflicted by the fungus gnat larvae in defensive strikes against the predators. At prey numbers supporting maximum rates of adult emergence, larval development required 12–14 d, and duration of the pupal stage was ≈10 d. *C. attenuata* larvae killed large numbers of prey during their development (means of up to 232 L_2, 144 L_3, or 87 L_4 fungus gnats), and larvae provided with marginally inadequate numbers of prey survived for long periods (mean 14–22 d, maximum 34 d) before succumbing to apparent starvation. These are favorable attributes with respect to use of *C. attenuata* as a biological control agent, suggesting a strong potential to substantially impact high-density pest populations and to survive in low-density pest populations.

KEY WORDS biocontrol, predator, *Coenosia attenuata*, *Bradysia*, development

The hunter fly *Coenosia attenuata* Stein 1903 (Diptera: Muscidae) is a generalist predator originally described as being native to the Palearctic region of southern Europe (Hennig 1964). The range of this species has expanded worldwide to include parts of Africa, Asia, and Australia (Pont 1986) and, most recently, South America (Martinez-Sánchez 2002) and North America (Hoebeke et al. 2003). This predator has been described from greenhouses throughout its range, where it has been observed preying on many common greenhouse pests, including fungus gnats (Sciaridae), shore flies (Ephydridae), leafmining flies (Agromyzidae), leafhoppers (Cicadellidae), and whiteflies (Aleyrodidae) (Freidberg and Gijswijt 1983, Schrameyer 1991, Kühne et al. 1997, Moreschi and Süss 1998, Martinez-Sánchez 2002, Hoebeke et al. 2003, Sensenbach 2004). These observations have stimulated interest in the manipulation of these predators as biological control agents (Tellez et al. 2009).

During the last decade, a small number of authors have published observations of the biology of the immature stages of *C. attenuata.* The egg, larva, and pupa of the species are all soil-dwelling, and, like the adults, larvae are generalist predators (Schrameyer 1991). Some life history data for these stages are now available, but much about their ecology, behavior, and development remains unknown. The predatory nature of larval *C. attenuata* suggests potential for biological control of soil-dwelling greenhouse crop pests such as larvae of fungus gnats and shore flies. Increased understanding of the basic ecology and survival requirements of the soil-inhabiting life stages of this predator would enable better estimation of the potential for control and suggest ways in which growers could enhance the activity of hunter flies in the greenhouse.

Recent work describes some of the basic biology of immature *C. attenuata.* Moreschi and Süss (1998) calculated egg hatch rates at a variety of temperature regimes. They observed the highest rate of hatching at 21°C and a minimum incubation period of 3 d at 40°C.

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The number of instars for *C. attenuata* has been hypothesized, on the basis of morphology, to be three, with only the third and final instar living freely outside of the egg (Kühne 2000). Muscid larvae with three instars have amphipneustic tracheal systems (Hennig 1964). *Coenosia dubiosa* Hennig seems to eclose as a third instar, having such an amphipneustic tracheal system, a very elastic cuticle, and mouthparts that remain a constant length until pupariation (Waitzbauer 1976). Kühne (2000) noted that *C. attenuata* also exhibits these features, and extensive monitoring of *C. attenuata* larvae reported by Sensenbach (2004) and Sensenbach et al. (2005) also revealed no change in the size of the mouthparts of posteclosion larvae and no evidence of molting.

Moreschi and Colombo (1999) reared *C. attenuata* on larvae of the sciarid fungus gnat *Bradysia difformis* Frey [=*B. paupera* (Tuomikoski 1960)]; at 25°C and reported a posteclosion larval developmental period of ≈15 d; pupal development required a similar length of time, for a total of 30–35 d from egg hatch to adult eclosion. In another experiment; however, Moreschi and Süss (1998) reported a mean pupal period of 20 d at 25°C. Kühne et al. (1997) observed substantially shorter developmental periods of 10.4 d for *C. attenuata* larvae and 10.5 d for pupae reared on the same host and at the same temperature.

Feeding behavior and nutritional requirements of larval *C. attenuata* are poorly understood. Although the larvae, like adults of the species, seem to be polyphagous, attempts at rearing them on a variety of diets have resulted in very different degrees of success. Kühne (2000) reported successful rearing of larval *C. attenuata* on larvae of the fungus gnat *B. difformis* and the scavenger midge *Scatopse transversalis* Lowe (Scatopsidae). Moreschi and Colombo (1999) also reared *C. attenuata* on mixed age populations of *B. difformis* larvae and reported that the optimal prey density was four to five larvae per cm^3 of substrate. Nondipterous prey also have been used to rear immature *C. attenuata,* albeit with lower success. Rearing on slices of the earthworm *Eisenia fetida* (Sav.) resulted in very high mortality (98%) before pupation and a protracted larval developmental period of 23 d, possibly because of rapid decomposition of the food (Moreschi and Süss 1998). Work by Yahnke and George (1972) and Morris and Cloutier (1987) with *Coenosia tigrina* (F.) suggests that live, immature *E. fetida* may be a better food source. Rearing on prekilled European corn borer, *Ostrinia nubilalis* (Hübner), was only slightly more successful, yielding a 94.5% mortality rate and a larval developmental period averaging 20 d (Moreschi and Süss 1998).

Numerous questions remain with regard to the dietary requirements of *C. attenuata* larvae, particularly the minimum numbers of prey needed for development and the capacity of this predator to survive prolonged periods with limited food. The principal objectives of the current study were to determine the minimum and optimum numbers of three instars of *Bradysia impatiens* (Johannsen) (Diptera: Sciaridae) larvae required by *C. attenuata* larvae to complete development to the adult stage. *B. impatiens* is a ubiquitous pest of greenhouse crops in North America. Adults of this species have been reported as a highly favored prey of adult *C. attenuata* (Sensenbach 2004), and the soil-inhabiting larvae of this fungus gnat are undoubtedly among the most important prey taken by larval *C. attenuata* in greenhouses over much of its range in North America and probably worldwide (see Discussion).

Materials and Methods

Fungus Gnat Rearing. Experiments were conducted from October 2006 through May of 2007 to investigate the development of *C. attenuata* provided constant daily numbers of a specific fungus gnat instar. A colony of fungus gnats was initiated in October 2006 with ≈300 *B. impatiens* adults aspirated from a greenhouse at Cornell University (Ithaca, NY). Fungus gnats were reared using the methods of Sensenbach (2004), based on techniques developed by Gillespie (1986). Several additional modifications were made (by T.A.U.) to simplify the processes of rearing and collecting fungus gnats for use in bioassays, as follows.

Production of Adult Fungus Gnats. Fresh rearing medium (pinto bean mix) was prepared daily and new cultures were set up daily. To set up a culture, 300 ml of a peat-based potting mix (ProMix research quality control grade, Premier Horticulture Inc., Red Hill, PA), was placed in a 470-ml polystyrene, friction-lid canister (Pioneer Plastics, North Dixon, KY). Thirty to 40 g of dry ground pinto beans (whole pinto beans ground in a blender until no whole beans remained) was mixed into the peat. The mix was then moistened with 80–100 ml of tap water. Twenty to 30 unsexed newly emerged adult fungus gnats (<3 d old) were chilled at 4°C for ≈5 min to prevent their escape into the laboratory and added to each container, which was then covered with the container lid. Lids were modified to include a 5-cm-diameter hole covered with fine screen (0.2-mm mesh openings) for ventilation and a small (5-mm-diameter) hole fitted with a cork stopper to enable aspiration of adults from the containers. The added adults deposited eggs onto the media in the containers that developed into an abundance of adults after 12–14 d. The colony was maintained in a laboratory incubator set at 25°C, with a photoperiod of 14:10 (L:D) h. Actual temperatures ranged from 23 to 26°C.

Production of Larval Fungus Gnats for Bioassays. A single disk (pad) of cotton (85 mm in diameter), cut from a sheet of absorbent cotton (Thermo Fischer Scientific, Waltham, MA), was placed in an inverted 90-mm-diameter petri dish lid. The cotton was then saturated with tap water, and excess water was poured off. A single piece of black filter paper (90 mm in diameter) was positioned on top of the cotton so that there were no large gaps between the filter paper and the sidewall of the petri dish lid. Pinto bean flour (0.5–0.8 g) was then sprinkled onto the filter paper, and the dishes were placed into a cage containing 50–200 unsexed fungus gnats (<3 d old; replenished

daily) for oviposition. Petri dishes with eggs were removed from the cage after 24 h and placed on a cafeteria tray; a second tray was inverted overtop, creating a domed cover. Trays were incubated at 28 ± 2°C, and additional beans and water were added after 5–6 d. Fungus gnats of the desired instar for use in bioassays were readily identified on the filter paper among the bean flour under a low-power microscope and transferred using a fine paintbrush (size 00).

Hunter Fly Egg Production. *C. attenuata* eggs were collected using a method adapted from Kühne (2000). Petri dish lids containing water-saturated cotton pads were lined with black filter paper, sprinkled with coir, and placed into cages with greenhouse-collected adult female *C. attenuata.* The eggs deposited on the filter paper were collected daily using a 00-sized paintbrush. They were then kept until they hatched (4 d at 25°C) in 60-mm-diameter petri dishes containing a single 55-mm-diameter filter paper disk, moistened with water, sealed with Parafilm, and inverted to keep the eggs moist. Filter papers in petri dishes that were not inverted dried out and all the water condensed on the lids resulting in poor egg hatch.

Effects of Prey Instar and Density on Hunter Fly Survival and Development. Experiments to measure predation rates were conducted in which specific numbers of second-instar (L_2), third-instar (L_3), or fourth-instar (L_4) fungus gnat larvae were offered daily to *C. attenuata* larvae. Newly hatched (<24-h-old) *C. attenuata* larvae were transferred onto 1.5% water agar (unsterile and 1–7 d old) in 35- by 10-mm petri dishes (one larva per dish). Previous studies in our laboratory revealed that *C. attenuata* larvae fed *B. impatiens* L_1 larvae or pupae did not survive to pupation (Sensenbach 2004, Sensenbach et al. 2005; see Discussion). Therefore, these prey stages were not included in the testing. *Coenosia attenuata* larvae were provided with 0.5, 1, 5, 10, 15, 20, and 25 L_2; 0.5, 1, 3, 5, 7, 10, and 15 L_3; and 1, 2, 4, and 7 L_4 *B. impatiens* larvae per day (0.5 prey per d = one larva provided every 2 d). Hereafter, the numbers of prey provided to the *C. attenuata* larvae is referred to as the prey density treatments. After provisioning, each dish was sealed with Parafilm. *Coenosia* were maintained in this way until death or pupation; pupae were held until adult emergence. Throughout the experiment, insects were kept in their arenas in a constant-temperature chamber (25°C ± 1°C and a photoperiod of 14:10 [L:D] h). The numbers of surviving and dead prey were recorded daily, and dead prey were removed and replaced. The day of pupation and the day of adult emergence or death also was recorded, and adult flies were sexed. Fresh prey were added daily. In those cases where less than one prey was offered per day, dead prey were removed and replaced with fresh prey every 48 h; any fungus gnat larva surviving to the midpoint of a 48-h period was replaced with a fresh individual of the appropriate instar.

Statistical Analysis. *C. attenuata* larvae were only randomly assigned to prey density treatments within a given fungus gnat instar, and all instars were not tested at the same time. Thus, statistical comparisons across the three fungus gnat instars were not testable, and analyses were limited to within-fungus gnat instar effects. Time of egg hatch, pupation, adult emergence, or death was estimated as the midpoint of the observation interval (e.g., with daily observations, larvae found dead on day 7 were reported as having died on day 6.5).

Statistical analyses, including analysis of variance (ANOVA), analysis of frequencies, and linear regression, were performed using JMP version 5 (SAS Institute 2002). Linear regression was used to investigate trends in duration of life stages, age at pupation or adult emergence, and prey kill rates as a function of prey density. Life stage duration data were log $(x + 1)$ transformed to normalized variances. Because rate data are typically not normally distributed, prey kill rates were subjected to Box-Cox transformation, and residuals were examined for marked departures from normality. Analysis of interactions associated with nonnormal rate data are also problematic (Zar 1999). Results from standard two-way ANOVAs were therefore compared with results after rank transformation of the data as recommended by Conover (1999). In all cases, the significance test results were the same from each analysis, and results from the standard ANOVA are reported.

Chi-square (contingency table) analyses were conducted to compare frequencies (percentages) of successful pupation and emergence of adults among treatments. When chi-square analysis revealed significant treatment effects, means were compared using the sequential Bonferroni test described by Sokal and Rohlf (1995), with an experimentwise error rate of 0.05.

Results

Effects of Prey Instar and Density on Survival and Developmental Times of Hunter Fly Larvae. Data concerning the survival and developmental time of *C. attenuata* larvae are summarized in Table 1. There was a strong effect of prey density on larval survival; larvae survived to pupation only when provided a minimum of 15 L_2, 7 L_3, or 1 L_4 fungus gnats daily. Larvae receiving the lowest densities of L_2 and L_3 prey (0.5 or 1 prey per d) survived ≤6 d and in most cases <3 d). Larvae subsisting on marginally inadequate numbers of prey survived for long periods (14–22 d) before succumbing to apparent starvation. Across the L_2, L_3, and L_4 prey densities that supported pupation, weighted mean larval mortality was 29.8 ± 11.5, 37.5 ± 7.1, and 73.4 ± 4.5%, respectively. There was a significant effect (regression) of prey density on the number of days required for completion of development by larvae fed L_2 and L_3 prey, with duration of the larval stage tending to decrease with increasing prey density (in both cases $P < 0.0001$; Table 1). No trend was evident, however, for larvae fed L_4 prey ($P = 0.28$). The minimum times for completion of larval development (excluding the 4 d required for egg incubation at 25°C) occurred on a daily diet of 25 second- and 15

Table 1. Effects of fungus gnat prey instar and density on developmental times (in days) and survival rates of *C. attenuata* larvae, with standard errors and linear regression statistics[a]

No. *C. attenata* larvae tested	Treatment[b]	% pupation[c]	Larval age at premature death in days (n)[d]	Duration of posteclosion larval stage in days (n)[e]	Duration of pupal stage in days (n)[e]	% adult emergence[c,f]	% pupal eclosion[c,h]	Age at emergence (d)[g]
10	L_2–0.5	0	2.7 ± 0.2 (10)			0	0	
10	L_2–1	0	2.9 ± 0.3 (10)			0	0	
13	L_2–5	0	22.0 ± 2.4 (13)			0	0	
10	L_2–10	0	18.6 ± 3.2 (10)			0	0	
13	L_2–15	76.9ab	8.0 ± 3.2 (3)	16.6 ± 0.4 (10)	8.0 ± 0.3 (5)	38.5a	50.0a	23.8 ± 0.5
29	L_2–20	55.2a	7.2 ± 1.0 (13)	14.1 ± 0.4 (16)	10.3 ± 0.5 (10)	34.5a	62.5a	23.8 ± 0.7
15	L_2–25	93.3b	12.0 ± 0 (1)	12.3 ± 0.3 (14)	10.1 ± 0.2 (13)	86.7b	92.9a	22.2 ± 0.2
				$r^2 = 0.580$	$r^2 = 0.262$			$r^2 = 0.204$
				$F_{[1,38]} = 52.5$	$F_{[1,26]} = 9.2$			$F_{[1,26]} = 6.65$
				$P < 0.0001$	$P = 0.005$			$P = 0.016$
15	L_3–0.5	0	2.9 ± 0.4 (15)			0	0	
15	L_3–1	0	6.1 ± 1.4 (15)			0	0	
15	L_3–3	0	19.5 ± 1.8 (15)			0	0	
11	L_3–5	0	14.5 ± 3.9 (11)			0	0	
29	L_3–7	48.3a	6.5 ± 1.2 (15)	15.5 ± 0.4 (14)	10.2 ± 0.3 (6)	20.7a	42.8a	25.3 ± 0.8
29	L_3–10	69.0a	9.3 ± 1.9 (9)	13.5 ± 0.5 (20)	10.7 ± 0.2 (16)	55.2b	80.0a	24.1 ± 0.4
30	L_3–15	70.0a	4.7 ± 1.4 (9)	12.2 ± 0.4 (21)	9.7 ± 0.2 (15)	50.0b	71.4a	21.6 ± 0.4
				$r^2 = 0.279$	$r^2 = 0.170$			$r^2 = 0.461$
				$F_{[1,53]} = 20.5$	$F_{[1,35]} = 7.17$			$F_{[1,35]} = 29.9$
				$P < 0.0001$	$P = 0.011$			$P < 0.0001$
36	L_4–1	36.1a	3.3 ± 0.4 (23)	16.5 ± 0.5 (13)	10.1 ± 0.2 (8)	22.2a	61.5a	26.1 ± 0.7
54	L_4–2	31.5a	2.9 ± 0.6 (37)	14.2 ± 0.4 (17)	10.5 ± 0.3 (10)	18.5a	58.8a	24.9 ± 0.6
59	L_4–4	16.9a	2.0 ± 0.2 (49)	14.0 ± 0.6 (10)	10.6 ± 0.4 (7)	11.9a	70.0a	24.0 ± 0.9
20	L_4–7	25.0a	4.7 ± 1.2 (15)	15.4 ± 0.7 (5)	11.0 ± 0.6 (3)	15.0a	60.0a	26.0 ± 1.0
				$r^2 = 0.0285$	$r^2 = 0.083$			$r^2 = 0.011$
				$F_{[1,43]} = 1.26$	$F_{[1,26]} = 2.36$			$F_{[1,26]} = 0.30$
				$P = 0.28$	$P = 0.014$			$P = 0.59$

[a] Coefficient of determination (r^2) and F-test statistics from significance test of the regression coefficient.
[b] Fungus gnat instar–number of fungus gnat larvae provided per day (prey density); tests conducted at 25°C.
[c] Means within a treatment followed by a common letter are not significantly different (experimentwise $\alpha = 0.05$; sequential Bonferroni test).
[d] Age in days of *C. attenuata* larvae that died before pupation, excluding the egg incubation period.
[e] Duration of posteclosion larval stage of insects that survived to pupation or pupal stage of insects that survived to adult emergence.
[f] Percentages based on total number of insects tested.
[g] Age in days of *C. attenuata* at time of adult emergence, excluding the egg incubation period.
[h] Percentages based on the number of insects that pupated.

third-instar fungus gnats (12.3 ± 0.3 and 12.2 ± 0.4 d, respectively).

Effects of Prey Instar and Density on Pupation. The proportion of *C. attenuata* larvae that survived to pupation differed significantly as a function of L_2 and L_3 prey density ($\chi^2_6 = 56.6$, $P < 0.0001$; $\chi^2_6 = 60.3$, $P < 0.0001$). When the number of prey per day was increased from 10 to 15 L_2 and from 5 to 7 L_3 per day, the respective percentages of pupation increased from 0 to 77% and from 0 to 48% (Table 1). In contrast, all densities of L_4 prey supported pupation, and the rate of pupation (17–36%) did not vary significantly with prey density ($\chi^2_3 = 5.2$, $P = 0.16$). Despite the above-reported inverse relationships between the three highest densities of L_2 and L_3 prey (those supporting pupation) and duration of the larval stage, there were few significant differences among the ultimate rates of pupation observed at these prey densities (L_2: $\chi^2_2 = 7.2$, $P = 0.03$; L_3: $\chi^2_2 = 3.7$, $P = 0.15$; Table 1).

Regressions of pupal-stage duration on L_2, L_3, and L_4 prey density (for those densities that supported pupation) were statistically significant ($P = 0.005$, 0.011, and 0.014, respectively; Table 1); but, in general, pupal-stage duration did not differ markedly among treatments and trends were not consistent.

Effects of Prey Instar and Density on Hunter Fly Survival to Adulthood. Excluding treatments where percentage of adult emergence was zero, the proportion of insects that survived to adulthood with respect to all insects tested was significantly affected by prey density for *C. attenuata* larvae provided L_2 and L_3 fungus gnats ($\chi^2_2 = 11.5$, $P = 0.003$; $\chi^2_2 = 8.3$, $P = 0.02$) but not for larvae fed L_4 fungus gnats ($\chi^2_3 = 2.0$, $P = 0.58$). When percentage of adult emergence was considered with respect to only those insects that successfully pupated, there were marginally significant effects of prey density on emergence of *C. attenuata* provided L_2 and L_3 but not L_4 fungus gnats (L_2: $\chi^2_2 = 5.8$, $P = 0.057$; L_3: $\chi^2_2 = 5.5$, $P = 0.068$; L_4: $\chi^2_3 = 0.4$, $P = 0.95$). Similarly, regression analysis revealed an inverse relationship between prey density and total developmental time (age at adult emergence) when *C. attenuata* were fed L_2 ($P = 0.016$) and L_3 ($P < 0.0001$) fungus gnats (Table 1) but not when fed L_4 ($P = 0.59$). ANOVA indicated no effect of *C. attenuata* sex on developmental time, regardless of prey instar (L_2: $F_{[1,22]} = 0.4$, $P = 0.54$; L_3: $F_{[1,31]} = 0.2$, $P = 0.64$; L_4: $F_{[1,20]} = 0.005$, $P = 0.95$), and there were no prey density by sex interactions (L_2: $F_{[2,22]} = 3.1$, $P = 0.066$; L_3: $F_{[2,31]} = 0.68$, $P = 0.52$; L_4: $F_{[3,20]} = 0.88$, $P = 0.47$).

Effects of Prey Instar and Density on Rates of Predation. Table 2 shows the numbers of prey killed by *C. attenuata* larvae in the constant prey density experiment. With respect to all *C. attenuata* larvae that pupated, there was a significant relationship between L_3 and L_4 prey density and total prey killed (in both cases $P < 0.0001$), but not between L_2 prey density and total prey killed ($P = 0.16$). The number of prey killed per day by *C. attenuata* that pupated was significantly affected by prey density for all fungus gnat instars (in all cases $P < 0.0001$). These relationships also held true when considering total prey killed (L_2: $P = 0.069$; L_3 and L_4: $P < 0.0001$) and prey killed per day (in all cases $P < 0.0001$; Table 2) by larvae of *C. attenuata* individuals that survived to the adult stage. Larvae of *C. attenuata* that ultimately reached adulthood killed, on average, a minimum of 213.1, 95.6 and 15.2 L_2, L_3, and L_4 larvae in their lifetime.

Daily cohort-specific rates of kill (Fig. 1) are based on the total number of *C. attenuata* larvae used in the study and thus reflect the time-dependent predation rate by the cohort as a whole, due to both increasing age and mortality of the individuals comprising the cohort (each reported average incorporates zero values for deceased individuals). Cohort specific predation generally increased for the first few days as the larval *C. attenuata* grew large enough to take all the prey introduced into the chamber. For fungus gnat instars and densities insufficient for completion of larval *C. attenuata* development, the *C. attenuata* generally killed all the prey available up to the day of death, which occurred after 30 d in some instances. Age-specific rates of predation per day (i.e., average number of fungus gnat larvae killed per day with averages not incorporating zero values for deceased individuals) were plotted for each day for all treatments (Fig. 2). The C. *attenuata* larvae grew rapidly. At the highest densities of L_2 and L_3 prey, predation peaked within 5 or 6 d posteclosion and declined rapidly beyond day 11. At the highest density of L_4 prey, the feeding rate remained stable for a long period (days 4–14), before declining.

Discussion

Kühne (2000) noted that the mouthhooks of larval *C. attenuata,* and three other species of the *tigrina* group, did not change in size over the course of larval development, but that part of the basal region of the maxillary frame became increasingly sclerotized with age. The observations of Sensenbach (2004), Sensenbach et al. (2005) and those of the current study confirm these findings. We observed no evidence of molting (i.e., exuviae or mouthhooks), even when larvae were kept individually with prey on water agar in clear petri dishes. It should be noted that when *C. attenuata* died and were scavenged upon by fungus gnat larvae the *C. attenuata* mouthhooks were not difficult to find. This and the above-cited previous studies provide strong evidence that this species emerges from the egg in its final larval instar.

The phenomenon of larval cannibalism has been directly observed by Morris and Cloutier (1987) for *C. tigrina.* Kühne (2000) did not report direct observation of this behavior in *C. attenuata* but attributed a drastic decrease in a larval population to this behavior with scarcity of prey. Newly hatched *C. attenuata* larvae in the egg-holding petri dishes did not exhibit aggressive behavior toward other members of their cohorts; however, no observations were attempted of older *C. attenuata* larvae inhabiting the same petri dish. *C. attenuata* in the colony were occasionally found feeding on conspecific cadavers; however, it is unclear how the *C. attenuata* larvae died. The circumstances conducive to cannibalism of conspecifics are not known, though for mosquito larvae (Culicidae) it has been found that cannibalism is related to prey density (Dennehy et al. 2001) and size differences among larvae (Yasuda and Hashimoto 1995). Cannibalism of adult male *C. attenuata* by females has been observed in cages (T.A.U., unpublished observation).

Kühne (2000, 1998) hypothesized that *C. attenuata* larvae may produce a paralyzing toxin, which they inject into their prey. Although the current study did not address the presence or absence of a toxin, the speed with which *C. attenuata* larvae were able to subdue their prey supports this hypothesis. All prey, even much larger L_4 fungus gnats, ceased movement within 1–5 s after being attacked.

Sensenbach (2004) and Sensenbach et al. (2005) reported that daily feeding of up to 14 L_1 or five pupal fungus gnats was inadequate to support larval development. *C. attenuata* larvae fed fungus gnat pupae failed to develop (did not grow) and died within 3 d. The reason for the early death of the *C. attenuata* larvae in the latter case was clearly an inability to penetrate the sclerotized exoskeleton of the fungus gnat pupae. For those larvae fed L_1 prey, however, successful kill was observed, although only at low rates (average of five prey per day). Feeding on these small prey supported limited growth, but all individuals died prematurely without pupating and within an average of <9 d. These observations suggest that the *C. attenuata* larvae searching for prey in the test arenas may not have encountered sufficient numbers of L_1 larvae to support normal development. To confirm the inability of *C. attenuata* larvae to subsist on L_1 prey, we conducted a preliminary test in which newly eclosed *C. attenuata* larvae ($n = 10$) were fed up to 100 L_1 fungus gnat larvae per day. Despite large numbers of prey being killed, none of the larvae successfully pupated. A few individuals grew to nearly normal size before death, but this was over an extended period (up to 22 d). It may be that the efficiency of searching for these small prey items in relation to their nutritive value was too low to support completion of larval development. However, we cannot rule out that mortality might also have resulted from stress associated with the highly unnatural rearing conditions in which the predators were forced to continually interact with large (potentially overwhelming) numbers of prey over their entire larval life spans.

Table 2. Effects of fungus gnat prey instar and density on the duration of the larval stage (in days) and predation rates of *C. attenuata* larvae, with standard errors and linear regression statistics[a]

No. *C. attenuata* larvae tested	Treatment[b]	Duration of larval stage of insects surviving to pupal vs. adult stage		Kills per day by insects surviving to pupal vs. adult stage		Total prey killed by insects surviving to pupal vs. adult stage		Kills per day by larvae surviving to pupal stage (n)	Total prey killed by larvae surviving to pupal stage
		Pupal stage (n)	Adult stage (n)	Pupal stage	Adult stage	Pupal stage	Adult stage		
13	L_2–15	17.4 ± 0.4 (5)	15.8 ± 0.6 (5)	13.0 ± 0.3	12.6 ± 0.3	227.0 ± 9.7	199.2 ± 10.0	13.2 ± 0.2 (10)	213.1 ± 8.0A
29	L_2–20	15.0 ± 0.7 (6)	13.5 ± 0.5 (10)	16.9 ± 0.2	16.8 ± 0.3	253.3 ± 13.2	227.2 ± 9.8	17.5 ± 0.2 (16)	237.0 ± 8.3A
15	L_2–25	14.0 ± — (1)	12.2 ± 0.3 (13)	18.8 ± —	18.9 ± 0.3	263.0 ± —	229.8 ± 7.6	19.7 ± 0.3 (14)	232.1 ± 7.4A
		$r^2 = 0.540$	$r^2 = 0.528$	$r^2 = 0.875$	$r^2 = 0.823$	$r^2 = 0.245$	$r^2 = 0.122$	$r^2 = 0.865$	$r^2 = 0.050$
		$F_{[1,10]} = 18.7$ $P = 0.007$	$F_{[1,26]} = 29.1$ $P < 0.0001$	$F_{[1,10]} = 70.1$ $P < 0.0001$	$F_{[1,26]} = 121$ $P < 0.0001$	$F_{[1,10]} = 3.2$ $P = 0.102$	$F_{[1,26]} = 3.6$ $P < 0.069$	$F_{[1,38]} = 244$ $P < 0.0001$	$F_{[1,38]} = 2.0$ $P = 0.16$
29	L_3–7	15.7 ± 1.0 (8)	15.2 ± 0.7 (6)	6.1 ± 0.1	6.2 ± 0.1	96.8 ± 7.9	94.2 ± 5.5	6.4 ± 0.1 (14)	95.6 ± 4.9A
29	L_3–10	14.3 ± 1.3 (4)	13.1 ± 0.5 (16)	8.7 ± 0.1	8.6 ± 0.1	123.8 ± 12.2	114.1 ± 4.7	8.9 ± 0.1 (20)	116.1 ± 4.4B
30	L_3–15	13.0 ± 0.9 (6)	11.9 ± 0.04 (15)	11.7 ± 0.4	11.7 ± 0.1	152.2 ± 12.5	140.2 ± 5.5	12.2 ± 0.1 (21)	143.6 ± 5.3C
		$r^2 = 0.244$	$r^2 = 0.281$	$r^2 = 0.938$	$r^2 = 0.942$	$r^2 = 0.546$	$r^2 = 0.464$	$r^2 = 0.957$	$r^2 = 0.487$
		$F_{[1,16]} = 26.9$ $P = 0.037$	$F_{[1,35]} = 13.7$ $P = 0.0007$	$F_{[1,16]} = 241$ $P < 0.0001$	$F_{[1,35]} = 573$ $P < 0.0001$	$F_{[1,16]} = 19.2$ $P = 0.0005$	$F_{[1,35]} = 30.3$ $P < 0.0001$	$F_{[1,53]} = 1191$ $P < 0.0001$	$F_{[1,53]} = 50.4$ $P < 0.0001$
36	L_4–1	17.2 ± 0.5 (5)	16.0 ± 0.7 (8)	0.9 ± 0.002	0.9 ± 0.01	16.2 ± 0.5	14.6 ± 0.6	1.0 ± 0.01 (13)	15.2 ± 0.4A
54	L_4–2	14.0 ± 0.7 (7)	14.4 ± 0.5 (10)	1.8 ± 0.03	1.8 ± 0.02	24.9 ± 1.5	26.2 ± 1.1	1.9 ± 0.02 (17)	25.6 ± 0.9B
59	L_4–4	15.3 ± 0.9 (3)	13.4 ± 0.7 (7)	3.6 ± 0.04	3.5 ± 0.05	56.0 ± 3.8	46.7 ± 2.8	3.7 ± 0.04 (10)	49.5 ± 2.6C
20	L_4–7	16.0 ± 2.0 (2)	15.0 ± 0.6 (3)	5.6 ± 0.4	5.7 ± 0.4	90.0 ± 17.0	84.7 ± 3.2	5.8 ± 0.02 (5)	86.8 ± 5.8D
		$r^2 = 0.003$	$r^2 = 0.057$	$r^2 = 0.937$	$r^2 = 0.936$	$r^2 = 0.920$	$r^2 = 0.957$	$r^2 = 0.983$	$r^2 = 0.935$
		$F_{[1,15]} = 0.05$ $P = 0.83$	$F_{[1,26]} = 1.6$ $P = 0.22$	$F_{[1,15]} = 222$ $P < 0.0001$	$F_{[1,26]} = 379$ $P < 0.0001$	$F_{[1,15]} = 172$ $P < 0.0001$	$F_{[1,26]} = 577$ $P < 0.0001$	$F_{[1,43]} = 2435$ $P < 0.0001$	$F_{[1,43]} = 615$ $P < 0.0001$

[a] Coefficient of determination (r^2) and F-test statistics from significance test of the regression coefficient.
[b] Fungus gnat instar–number of fungus gnat larvae provided per day (prey density); tests conducted at 25°C.

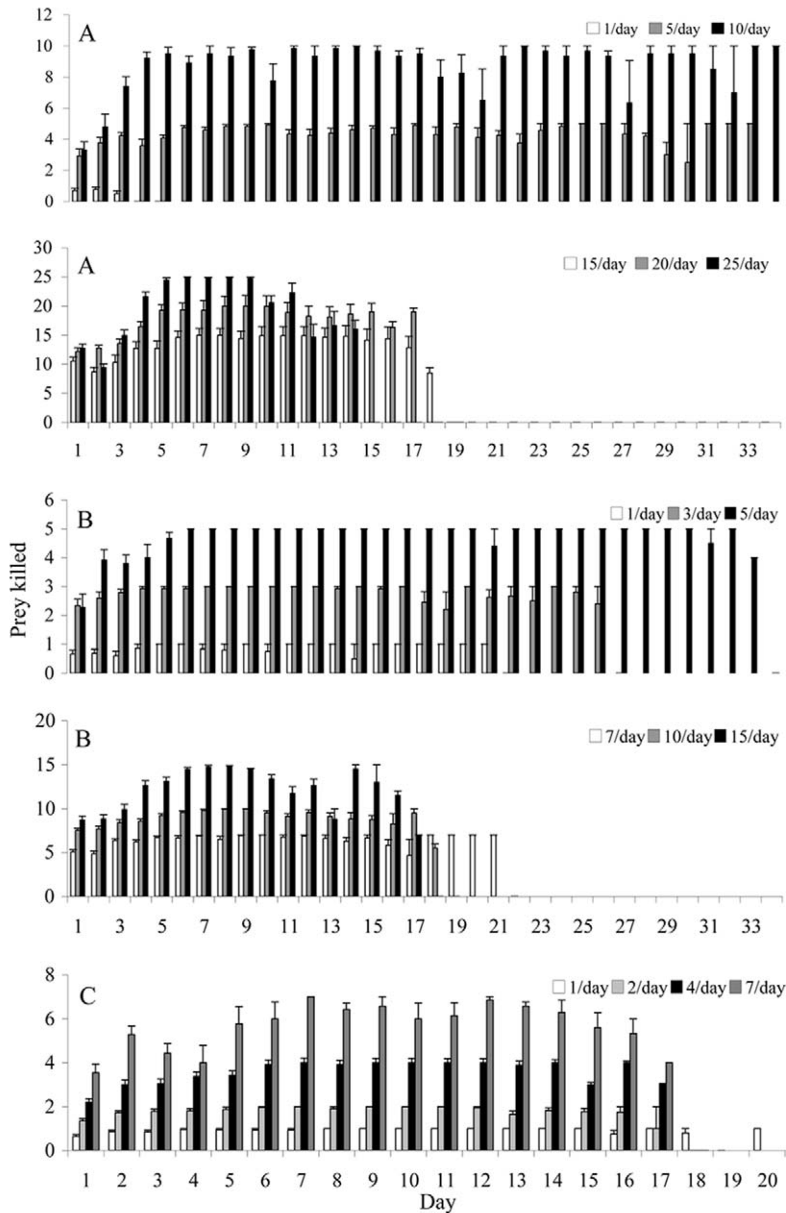

Fig. 1. Cohort-specific mean kills per day of six densities of second (A), third (B), and four (C) densities of fourth-instar fungus gnats by *C. attenuata* larvae. Day 1 represents the first day after egg hatch.

L_4 fungus gnats are capable of killing *C. attenuata* larvae. Under the conditions of our laboratory test, L_4 fungus gnat larvae were frequently observed to inflict lethal wounds with their mandibles in defensive strikes against the predator. We would expect less success in defending against attack in the natural habitat of these insects (soil or potting medium substrates) where prey movements might be restricted. The smaller L_2 and L_3 fungus gnat larvae were rarely observed to injure *C. attenuata* larvae. Sublethal injuries might account for the anomalously long period required for larval development under the highest L_4 prey density regime. In this treatment, development required nearly 15 d, >3 d longer than development at the highest densities of L_2 and L_3 prey (Table 1). L_4 fungus gnats at densities of one, two, four, and seven fungus gnats per day killed 50, 60, 76, and 55% of *C. attenuata* larvae within the first 5 d of initiating the test. Mortal attacks on *C. attenuata* larvae seemed to decrease after the first 3–4 d, after which time the *C. attenuata* larvae was one third to one fourth the length of an L_4. Fungus gnat-induced mortality of *C. attenuta* also occurred during the pupal stage. Killed pupae, both callow and tanned individuals, were frequently

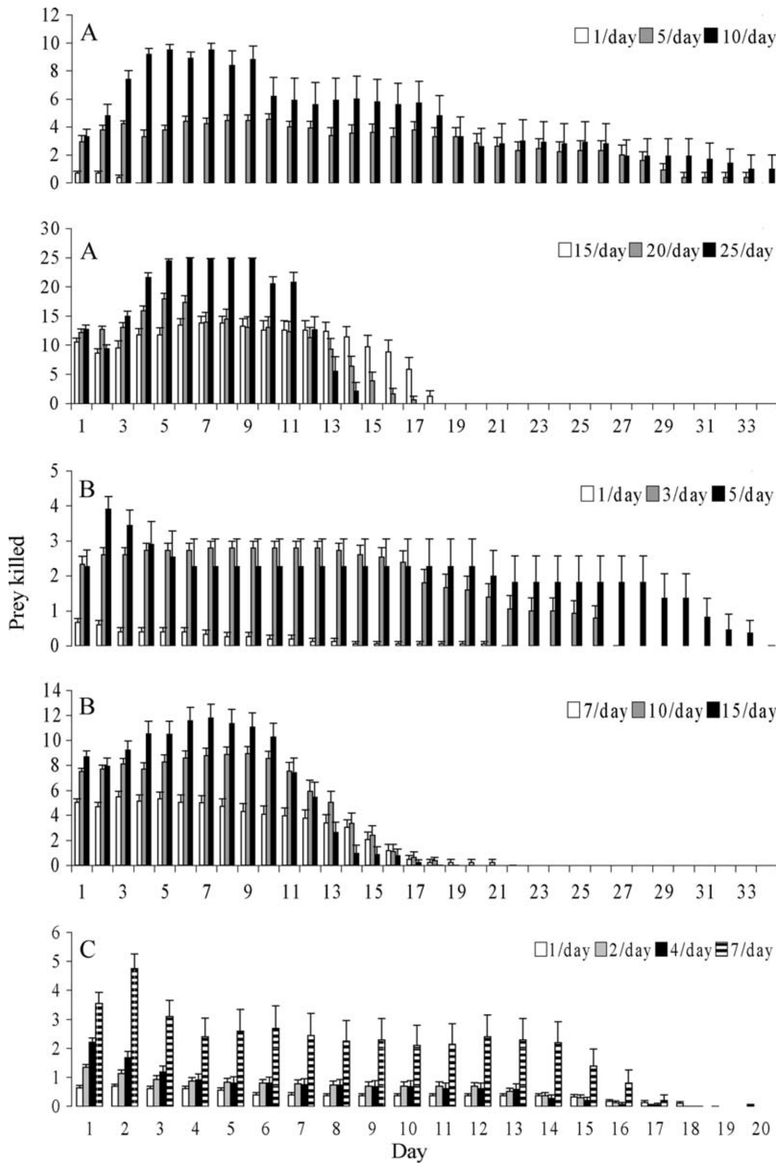

Fig. 2. Age-specific mean kills per day of six densities of second (A), third (B), and four (C) densities of fourth-instar fungus gnats by *C. attenuata* larvae. Day 1 represents the first day after egg hatch.

observed with melanized wounds that likely resulted in sepsis. Fungus gnat wounding of *C. attenuata* pupae certainly depressed percentage of emergence values in our tests, although by what magnitude we cannot say, as this phenomenon was not noticed until later in the study. We noted wounding of *C. attenuata* pupae by all instars of fungus gnats tested. However, the occurrence of this phenomenon in natural environments may be infrequent compared with the artificial conditions of this study.

Other possible explanations for the observed high levels of pupal mortality include insufficient larval nutrition, pathogens, and unfavorable environmental conditions. With regard to nutrition, it is noteworthy that the duration of the larval stage in those larvae surviving only to pupation was ≈1 d shorter than in larvae surviving to adult emergence ($F_{[1,120]} = 8.7, P = 0.004$), with this response being independent of prey density treatment (interaction $F_{[9,120]} = 0.49, P = 0.88$; Table 2). The reason(s) for this result are unknown. An unidentified stress factor likely retarded larval development, yet it did not negatively impact daily rates of predation ($F_{[1,120]} = 0.08$, $P = 0.78$; interaction $F_{[9,120]} = 0.45$, $P = 0.90$; Table 2), and an apparent consequence was that the slower developing larvae actually killed significantly more prey than those larvae that ultimately survived to adulthood ($F_{[1,120]} = 7.2$, $P = 0.008$; interaction $F_{[9,120]} = 0.64$, $P = 0.76$).

It was noted in many cases of pupal death that the cuticles of puparia had split along a suture located on the anterior end, which always resulted in death. We hypothesize that this was due to the constant water-saturated (100% RH) environment and would expect pupal survival to be higher under more natural conditions (pupae in soil or potting media in open containers). Similarly, the chorion of eggs that were kept on water-saturated filter paper tended to split, exposing the yolk and rendering the egg inviable.

In the experiment that varied fungus gnat instar and density, we observed a significant effect of prey density on the percent pupation of *C. attenuata* larvae. Newly emerged larvae only reached pupation when provided a minimum of 15, 7, and 1 L_2, L_3, and L_4 fungus gnats daily, respectively. The finding of normal *C. attenuata* development when provided only a single fourth instar per day was surprising; we hypothesize that the presence of large amounts of fat body in L_4 larvae is the factor that makes this possible. When provided 10 L_2 or 5 L_3 fungus gnats, not a single *C. attenuata* successfully pupated, indicating a minimum dietary threshold. Above these thresholds within an instar, there was no clear correlation between percentage of pupation and prey density, although the numerically highest rates of pupation were recorded at the highest L_2 and L_3 prey densities; a clearer trend might be revealed by repeating the study with increased sample sizes. As prey density increased, the age at pupation (duration of larval stage) decreased; and although there was a significant effect of L_2 and L_3 prey density on the duration of the pupal stage, the differences were relatively small. The total time from newly emerged larvae to emerged adult was 21.1–25.6 d.

Moreschi and Colombo (1999) reported a larval developmental period of ≈15 d at 25°C. They also noted a 15-d pupal period at this temperature, but Moreschi and Süss (1998) reported a pupal period of 20 d at 25°C. These values are generally higher than the overall larval and pupal developmental periods we obtained when larvae were provided some of the higher densities of L_2 and L_3 larvae. Our minimum average larval and pupal times were 11.7 ± 0.4 and 8.0 ± 0.3 d, respectively. Kühne et al. (1997) reported substantially shorter developmental times than Moreschi and Süss (1998), namely, 10.4 d for larvae, and 10.5 d for pupae at 25°C, which are closer to the results we obtained.

The total and daily number of fungus gnats killed was directly related to the density of fungus gnats provided per day: the more fungus gnats provided, the more fungus gnats killed. This is a positive finding in considering *C. attenuata* for biological control of fungus gnats. *C. attenuata* larvae seem inclined to kill many more prey than are required to complete development. In our experimental arenas, the predator was observed to attack virtually all prey that it encountered, even if the contacts occurred in rapid succession. This is perhaps simply a defensive response to contact with other species of insect larvae. The ability of *C. attenuata* to live for long periods of time (20–35 d) when prey are scarce (not sufficient prey to support the completion of larval development), as in the 5 and 10 L_2 per day and one, three, and five L_3 per day treatments, is also encouraging. This suggests that *C. attenuata* larvae can live for extended periods of time contributing to biological control of fungus gnats and complete development when and if they enter a favorable dietary environment.

Our findings support those of Moreschi and Colombo that a density of four to five fungus gnat larvae per cm^3 of rearing substrate was optimal for rearing *C. attenuata.* This number seems reasonable, considering the average numbers of daily kills by the *C. attenuata* larvae observed in the current study (e.g., 6–12 L_3 larvae) in a small, more or less two-dimensional arena (9.6-cm^2 agar surface) and considering the direct observations of Sensenbach (2004) and Sensenbach et al. (2005) that prey taken by the *C. attenuata* larvae often were not completely consumed.

It is important to note that we have compared the original descriptions of *B. impatiens* (including male genitalia) provided by Johannsen (1912) and type specimens from the Cornell University Insect Collection with the redescription of *B. difformis* presented by Menzel et al. (2003) and specimens from our research colony. It is our impression that *B. impatiens* and *B. difformis* are synonymous; and, at the very least, sibling species. Thus, direct comparisons of our results with those of researchers who have reared *C. attenuata* on *B. difformis* is appropriate. And because *B. difformis* is a redescription of *B. paupera* (Menzel et al. 2003), our results are also comparable with results with *B. paupera.*

Interactions between prey and predator larvae will be much more complex in a greenhouse environment than described in this artificial system. The presence of mixed prey species, competing inter- and intraspecific predators and saprophytes, and a more complex environment will doubtless affect the performance and survival of *C. attenuata* larvae. Several of the results from these experiments, however, are very relevant to the role of *C. attenuata* as a potential biological control agent. Prey of susceptible life stages will be required in abundance for successful and rapid development of large *C. attenuata* populations, but this predator seems capable of subsisting for extended periods at prey densities that are marginal or insufficient for development. A total developmental time of ≈4 wk indicates that establishment/augmentation of this natural enemy will require maintenance of a stable soil environment within the greenhouse.

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