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The Effects of Temperature, Diet, and Other Factors on Development, Survivorship, and Oviposition of *Aethina tumida* (Coleoptera: Nitidulidae)

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ABSTRACT Developmental rate and survivorship of small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), life stages were measured across different temperatures (21, 25, 28, 32 and 35°C) and diets, which included natural and artificial pollen, honey, and bee pupae. Temperature affected hatch success, time to hatching, and larval growth. Eggs hatched in 61 h at 21°C but in <22 h at 35°C. Larvae achieved peak weight in <8 d at 35°C but needed 17 d at 21°C. Diet had comparatively little effect on larval survivorship or maximum weight, although larvae fed only bee pupae had lower survivorship. Access to soil influenced pupation success. Duration of the life stage spent in the soil, during which pupation occurs, was also affected by temperature: adults emerged after 32.7 d at 21°C but after only 14.8 d at 35°C, albeit with high mortality. Minimum temperature for development was estimated at 13.5°C for eggs, and 10.0°C for larvae and pupae. Temperature influenced adult longevity and oviposition: on a honey and pollen diet average adult lifespan was 92.8 d at 24°C but only 11.6 d at 35°C. Beetles lived longer at 28°C or lower but produced the most eggs per female, regardless of diet, at 32°C. Beetle density influenced fecundity: beetles kept at three pairs per vial laid 6.7 times more eggs per female than those kept as single pairs. Overall, beetles fared best at 28–32°C with mortality of all stages highest at 35°C.

KEY WORDS *Aethina tumida*, temperature, diet, survivorship, oviposition

Small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), is native to sub-Saharan Africa (Lundie 1940), where it is found associated with *Apis mellifera* L. (Hymenoptera: Apidae). It was first detected in the United States in 1998 (Elzen et al. 1999, Arbogast et al. 2009). *A. tumida* feed on pollen, honey, and brood (Lundie 1940) and can contaminate honey and pollen with a yeast, *Kodamaea ohmeri*, that commonly occurs naturally on its cuticle (Torto et al. 2007). In large numbers of *A. tumida* can cause hive collapse (Schmolke 1974, Neumann and Elzen 2004, Ellis and Delaplane 2008). *A. tumida* can transmit American foulbrood, *Paenibacillus larvae* (Schäfer et al. 2009) and be infected by honey bee sacbrood virus (Eyer et al. 2009).

A. tumida spend most of their lives in bee hives, which in healthy hives has a largely controlled temperature. Although usually more constant than ambient temperature, hive temperatures can vary with respect to location and within the hive itself (Southwick and Heldmaier 1987, DeGrandi-Hoffman et al. 1993). Human et al. (2006) reported pollen store temperatures from 14.6 to 38.1°C, whereas brood temperatures remained a relatively constant 35°C, and Southwick and Heldmaier (1987) observed that bee hive core temperatures can get as low as 18.5°C when exterior temperatures are low and no brood are present. Larvae and adults of *A. tumida* are mobile, but not the pupae, and because *A. tumida* pupate in soil outside the hive, pupae are subjected to very different conditions than the other life stages. One objective of this study was to refine our estimate of the relationship between *A. tumida* development with temperature by subjecting eggs, larvae, pupae, and adults to a series of controlled temperature studies. The range of temperatures examined here would be within those typically experienced by *A. tumida* in the southern United States, where the beetle is a serious problem (Neumann and Ellis 2008).

Another important factor affecting *A. tumida* growth and survival is diet. *A. tumida* can be raised on many different foods, including pollen and honey (Ellis et al. 2002); bee brood (de Guzman and Frake 2007), and fresh and rotten apples, oranges, cantaloupe, and grapes (Ellis et al. 2002, Arbogast et al. 2009). Ellis et al. (2002) found a significant role of diet in oviposition, adult survivorship, and pupation success. Arbogast et al. (2009, 2010) reported significant diet effects on beetle reproduction. Less is known about other factors that might affect *A. tumida* lifespan and oviposition, such as the presence of conspecifics or the role of oviposition site. Here, we studied the

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effects of different combinations of common diet components on the duration and survivorship of *A. tumida* larvae, pupae, and adults.

This work is intended to help estimate *A. tumida* population growth based on the kind and amount of food available to them in the hive. That information can be used to help estimate the impact of control strategies for *A. tumida.* Furthermore, it can help understand the impact of the bees themselves on *A. tumida* dynamics as they interact with beetles and perform hygienic activities such as cleaning out eggs and young larvae. Also, information on *A. tumida* development, growth, and survivorship can be used to develop a population simulation models (Stone and Gutierrez 1986, Meikle et al. 1999), which are useful in understanding pest dynamics, for estimating the potential range of a pest, and for developing and testing integrated management practices, such as biological control (Meikle et al. 2002).

Methods and Materials

Insect Rearing. To rear *A. tumida,* 40–50 adult beetles taken from laboratory cultures (founded with beetles caught in the vicinity of Weslaco, TX) were placed in an "oviposition chamber," a 1-liter (8.5- by 30- by 21-cm) plastic container containing ≈230 g of standard pollen patty (see below) and a 10- by 10-cm piece of brood comb with 10 ml of honey spread on it. The beetles were kept in the chamber at 32°C for 3 d and then removed. After 14 d this produced ≈5,000 late-instar larvae seeking a pupation site. Larvae were then placed into a 2-liter, 15-cm-diameter jar filled with moist, sandy soil (moisture content, 5%). The pupation jars were kept at 22°-26°C until the adults emerged 4 wk later. Experiments on larval, pupal, and adult stages were conducted using 120-ml polypropylene specimen vials with screw-on lids (Kendall vials, Tyco Healthcare, MA). For larval experiments, a 2-cm^2 hole was cut in each lid, over which a piece of nylon gauze was glued to facilitate respiration. For experiments on pupae, five to six pinholes were made in the lid to allow respiration but prevent excessive drying.

Diet. Protein cake, hereafter referred to as standard pollen patty (SPP), was prepared in batches of 10 kg, consisting of granulated sugar (44.2% by weight); Bee-Pro artificial pollen (Mann Lake Ltd, Hackensack, MN) (32.2% by weight), bee-collected pollen (5.4% by weight), and water (18.3% by weight). Pollen and honey patties (HP) were prepared in smaller batches (usually 1–2 kg) and consisted of bee-collected pollen (61% by weight) and honey (39% by weight). Bee pupae, collected from local hives and stored at −15°C, were ground into the HP diet at two concentrations: one pupa per gram of diet (HP1P) and one pupa per 10 g of diet (HP.1P). Honey and pollen diet combined with brood comb containing at least two pupae is referred to as HPB. Brood alone consisted of a 4-cm^2 piece of brood comb with at least two bee pupae.

Egg Developmental Time. *A. tumida* egg developmental rate was measured at 21, 25, 28, 32, and 35°C. First, eggs were collected by placing oviposition slides similar to those described by de Guzman and Frake (2007) in an oviposition chamber (see above). Oviposition slides were constructed by first affixing one glass coverslip (18 by 18 mm) at each end of a standard glass microscope slide (75 by 25 by 1 mm), by using Superglue (Henkel Corp. Avon, OH) and then affixing a second slide on top, the oviposition site being the narrow space between the slides. Eggs <2 h old were counted using a dissecting microscope. To accommodate a digital camera to record egg hatching, experiments at 28°, 32° and 35°C were conducted in a Plexiglas box (40 by 40 by 20 cm) in which was placed an open plastic container holding 1.0 liter of water. The Plexiglas box was kept in a fume hood which was itself wrapped in plastic and warmed with a thermostat-controlled space heater (model LH-873/G, Soleil Heaters, Shanghai Limach Manufacturing Co., Shanghai, China). Temperature and humidity were monitored in this experiment and in the following experiments by using an electronic thermometer (model 06-662-4, Thermo Fisher Scientific, Waltham, MA), and humidity was maintained between 40 and 70%. A slide was propped against a dark piece of paper and photographed with a digital camera (model D300S, Nikon Corporation, Tokyo, Japan) with a macro lens, programmed to take one photo every 15 min. The experiment was conducted at least five times at each temperature.

For experiments at 21 and 25°C, egg hatch times were estimated using a "bracketing" technique similar to that of de Guzman and Frake (2007). Several slides containing freshly laid eggs were labeled with the time and date, and placed in a 0.5-liter plastic container, which was placed on distilled water inside a closed (not airtight) 2.0-liter plastic container placed in a controlled-temperature cabinet (Percival Scientific, Perry, IA; and Humidaire Incubator Co., New Madison, OH). Slides were inspected every 12 h. When the eggs of a given slide hatched, time and date were noted. Time of hatching was estimated for a each slide as the midpoint between the last observation with unhatched eggs and the observation with hatched eggs. Egg stage duration was calculated as the difference in time between the estimated hatching time and the known time of oviposition.

Larval Growth and Survivorship. Larval growth and survivorship were measured at 21, 25, 28, 32, and 35°C in controlled-temperature cabinets (Percival Scientific). Eggs were collected by placing oviposition slides in oviposition chambers (see Egg Developmental Time). Slides with eggs were removed, the eggs counted, the slide placed in a 120-ml vial with 2 g of diet, and the vial placed in a controlled temperature cabinet (Percival Scientific). The cabinet had a water reservoir to maintain humidity >50%. At 5, 8, 11, and 14 d after oviposition, the larvae were counted and weighed as a group on an electronic balance (OHaus Corp., Pine Brook, NJ). These days were chosen because at most temperatures larvae were too small to manipulate without damage before day 5, and weighing every t3 d thereafter did not seem to disturb the

Table 1. Repeated measures analysis of larval weight data for *A. tumida* kept on different diets and under different temperature regimes

Analysis	Factor: Temp (°C)	Factor: Diet	Factor: Observation days	Significant factor[a]	*F* ratio	df	*P*
I	35, 32, 28, 25	SPP, HP, HP.1P	5, 8, 11, 14	Temp	15.61	3,107	<0.0001
				Temp × diet	3.03	6,548	0.0064
				Day	337.81	3,547	<0.0001
				Temp × day	43.69	9,547	<0.0001
II	21	SPP, HP, HP.1P	11, 14, 17, 20	Day	162.96	3,103	<0.0001
III	28	SPP, HP, HP.1P, HP1P	5, 8, 11, 14	Diet	5.29	3, 24	0.0062
				Day	268.1	3,114	<0.0001
IV	32	SPP, HP, HP.1P, HP1P, HPB	5, 8, 11, 14	Day	50.92	3,174	<0.0001

[a] Temperature was included as a factor only in analysis I; all analyses included diet as a factor, with weight measured on the observation days.

larvae. Experiments were conducted twice at 25 and 35°C and three times at all other temperatures; at least three diets were examined at every temperature but at 28 and 32°C more diets were included (Table 1). Each diet group had at least five replicates. In one experiment at 25°C, and in all experiments at 21°C, larvae were too small to be weighed on day 5, so larvae were weighed as soon as they were deemed large enough. The numbers of dead eggs remaining on each slide were counted on day 5, and those data were used to calculate hatch success. Hatchling survivorship was calculated as the number of live larvae after 8 d (14 d at 21°C) divided by the number of hatched eggs. Diet was replenished or replaced as needed.

Pupation Rate. By pupation, we mean the period of time the insect spends in the soil to pupate, that is, the time from entry into the soil until emergence from the soil as an adult. Pupation rate was measured at 21, 25, 28, 32, and 35°C in controlled temperature cabinets. One to five 2-wk-old larvae, raised on standard pollen patty, 50 g of brood comb, and honey were placed on 90–100 g of moist, sandy soil in 120-ml vials with punctured lids and reared at 22–26°C. Soil moisture content was measured by weighing six 1-g samples in small plastic containers, drying the samples for 2 wk in a crystallizing dish containing silica gel, and then reweighing. The pupation containers were monitored daily until all beetles had emerged. In eight of the 10 experiments, containers with unaccounted-for beetles were sifted for cadavers. Two to three experiments were conducted at each temperature with 45–100 larvae per experiment, except at 32°C, in which a single experiment was conducted with 200 larvae. To measure the effect of diet on pupation success and adult size, larvae from two of the 4-diet experiments at 28°C (trials 1 and 2 below) described above were placed in pupation vials after 14 d, separated according with respect to replicate, and kept at 28°C until emergence. Emerged adults were weighed by replicate each day.

Adult Longevity and Oviposition. Newly emerged adults obtained from laboratory cultures were sexed, by gently depressing the ventral abdomen and noting the genitalia, and placed in pairs in 120-ml polypropylene specimen vials with screw-on lids (Kendall vials, Covidien, Mansfield, MA). A 2-cm^2 hole was cut in each lid, and a piece of nylon gauze was glued over the hole to provide ventilation. For each experiment, one oviposition slide was placed in the vial with 2 g of diet and one pair of beetles. Vials were placed in controlled temperature chambers at either 25, 28, 32, or 35°C with 1–2-liter reservoirs of water to maintain relative humidity at least 40%. A summary of the temperature × diet experiments is shown in Table 2. Vials were inspected daily for adult mortality, any eggs were counted using a dissecting microscope, and a new slide was placed in the vial. Dead adults were sexed or, if the cadaver was too dry, the remaining beetle was sexed. Diet was inspected at the same time, and diet that was dry was replaced; widowed males were moved to vials with widowed females.

Table 2. Longevity (days) of adult *A. tumida* at different diets and temperatures

Beetle sex	Temp (°C)	Experimental units	Diet[a]: SPP	Diet[a]: HP	Diet[a]: HP.1P
Females	25	30		92.8 ± 7.5x	
	28	50	94.2 ± 3.8x		
	32	20	81.1 ± 9.1a,x	43.8 ± 7.0b,y	61.9 ± 7.5ab
	35	50 (SPP), 20 (HP)	33.2 ± 3.4a,y	11.6 ± 0.9b,z	
Males	25	30		72.7 ± 6.3x	
	28	50	74.3 ± 5.3x		
	32	20	61.8 ± 12.3a,xy	34.7 ± 7.4a,y	47.1 ± 5.3a
	35	50 (SPP), 20 (HP)	30.0 ± 3.0a,y	8.8 ± 0.7b,z	

Letters a or b after a value indicate significant differences ($P \leq 0.05$) with respect to diet within temperature (comparisons within rows); likewise letters x, y, and z indicate significant differences with respect to temperature within diet (comparisons within columns).

[a] SPP, HP, and HP.1P are beetle diets (see text for details). Longevities were compared across diets at 32 and 35°C and across temperatures for SPP and HP diets.

In an experiment on the effects of different beetle densities per vial, 25 vials were prepared as described above with SPP diet. In 10 of those vials we placed one pair of beetles, in 10 vials we placed three pairs of beetles, and in five vials we placed five pairs of beetles. In the three- and five-pair treatments, beetles escaped too readily for accurate measurement of survivorship, so beetles were counted and sexed only at the beginning and end of the trial.

In an experiment on the effects of oviposition site on oviposition, two beetle pairs were placed in each cup and five diets were presented: HP, brood comb, pollen + brood comb, HP + brood comb, and bee pupae alone. Oviposition slides were not used in cups containing brood comb. Instead, females were allowed to oviposit on the comb for 2 d, after which the females were moved to a new vial with fresh diet. Eggs in the old vial were allowed to hatch and grow for 3 d, when the larvae were counted, similar to Arbogast et al. (2009).

Statistics. Data were analyzed using SAS software (SAS Institute, Cary NC). Multiple regression analyses and repeated measures analyses ($\alpha = 0.05$) were conducted for linear mixed models using PROC MIXED (Littell et al. 1996), with experiment number as the random effect where appropriate. All response variables expressed as proportions (e.g., hatch rate) were arcsine square-root transformed, as is recommended for percentages that cover a large range of values (Steel and Torrie 1960). In all analyses of larval and pupal data, the experimental units were specimen vials and average values per vial were used in the statistical analyses. Larval weights (log transformed) were evaluated using repeated measures with temperature, diet, day, and all two-way interactions as fixed effects; experiment number as a random effect; and with larvae number per replicate as a covariate to control for crowding effects. Oviposition data were analyzed either as weekly egg production per female and evaluated using repeated measures or evaluated as total, rather than weekly, egg production using standard analysis of variance (ANOVA) and regressions. Egg numbers were transformed as log(egg number + 0.5). For all PROC MIXED analyses, degrees of freedom were calculated using the Satterthwaite method, type III sums of squares were used where applicable, and residual plots were assessed visually for variance homogeneity. Post hoc contrasts of the least squares means differences were conducted for all significant factors, by using the Bonferroni adjustment for the *t*-value probability. Test-of-effect slices were used to evaluate significant interaction effects. Insect longevity was evaluated using Kaplan–Meier log rank analysis (SigmaPlot 11.0, Systat Software, Inc., San Jose, CA) with pairwise comparisons. Developmental rate was calculated for each life stage at each of the five temperatures, and those rates regressed across temperature to estimate minimum temperatures for development.

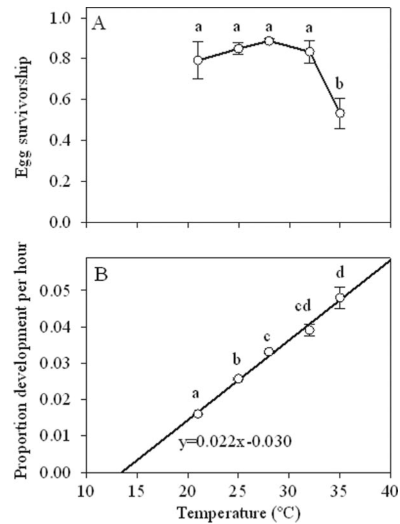

Fig. 1. Egg survivorship and proportion development per hour for *A. tumida* across five temperatures. (A) Egg survivorship. (B) Proportion development per hour (regression equation shown). Within each graph, points with no common letters are significantly different at $\alpha < 0.05$.

Results

Egg Developmental Time. Egg survivorship was significantly affected by temperature ($F = 18.80$; df = 4, 189; $P < 0.0001$) (Fig. 1). Mean proportion hatched (±SE) peaked at 0.89 ± 0.01 at 28°C and was significantly lower at 21, 25, and 35°C. Egg duration at the three highest temperatures, 28, 32, and 35°C, were 21.0 ± 1.3, 25.8 ± 1.2, and 30.2 ± 0.4 h, respectively. Three of six experiments at 35°C resulted in the death of all eggs (those experiments not included in the statistical analysis), indicating that 35°C is near the upper threshold. Egg duration at 21 and 25°C, estimated with the bracket method, was 62.6 ± 0.4 and 39.0 ± 0.5 h, respectively. Temperature had a significant effect on egg duration ($F = 308.6$; df = 4, 38; $P < 0.0001$) and most pairwise comparisons of temperature groups were significant (min. $P < 0.0001$; max $P = 0.0294$), although not those between 32°C and either 28 or 35°C. Average egg developmental rate, calculated as the inverse of duration and representing the proportion of the development completed per unit time (in this case hours), was regressed on temperature and was significant ($F = 360.1$; df = 1, 3; $P < 0.001$; adjusted $r^2 = 0.99$). Assuming the relationship is linear and extrapolating the regression line to the point at which the rate was zero, i.e., the x-intercept, the minimum temperature for egg development was 13.5°C. According to this analysis, eggs would take 12–13 d to hatch at 15°C.

Larval Growth and Survivorship. The average number of eggs per oviposition slide was 38.4 ± 1.8 (n = 332 slides). The regression of larval weight on day 11 (day 17 for larvae at 21°C) in SPP, HP, and HP.1P diet treatments on the number of eggs per cup was significant ($F = 48.39$; df = 1, 226; $P < 0.001$), indicating that

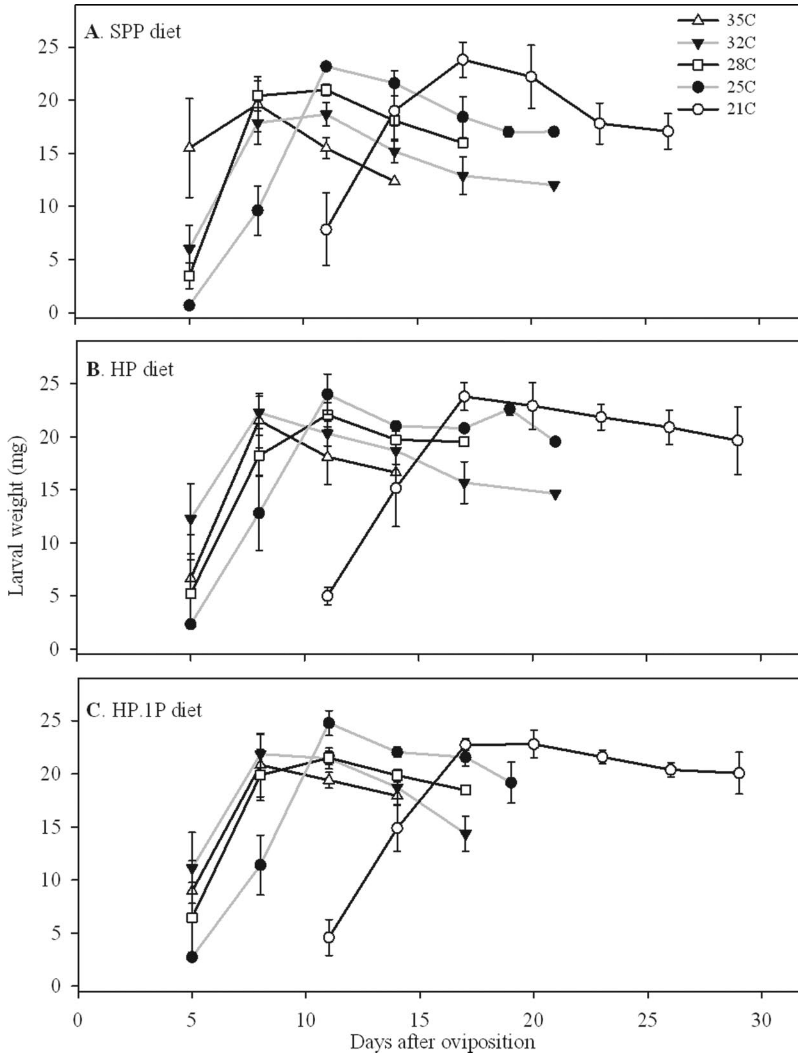

Fig. 2. Weight changes of *A. tumida* larvae over five temperatures with three different diets. Each point represents the mean of two to four experiments. (A) SPP diet (see text for details). (B) HP diet. (C) HP.1P diet. Error bars show SEs across experiments.

competition affected larval weight. Removing data for vials with 60 or more eggs ($n = 27$) removed a large part of the competition effect (resulting $F = 3.56$; df = 1, 199; $P = 0.06$). Larval number per cup on day 8 (day 14 for larvae at 21°C) also was used as a covariate in all analyses. Cannibalism was never observed.

In general, larvae grew rapidly in the first 8 d, then either less rapidly or started to lose weight (Fig. 2). Larval weights were examined in four repeated measures analyses (Table 1). Analysis I only involved larvae kept at 25 to 35°C because larvae kept at 21°C were too small to weigh without damage until at least 11 d after oviposition and were analyzed separately. All post hoc pairwise temperature contrasts for analysis I were significant, with the exception of 32 versus 35°C. The tests of effect slices showed that diet was significant at 25 and 32°C ($P = 0.0244$ and 0.0140, respectively). Weight loss after peak weight was probably due feeding cessation before finding a pupation site. Post hoc contrasts for analysis II showed significant diet effects on day 11 ($P = 0.0278$). Diet was a significant factor in analysis III, with larvae fed HP1P growing larger than those fed HP ($P = 0.0088$). Brood alone had been included in the experimental design for analysis IV but was not included in the statistics because of low survivorship; only four of the 12 replicates had any live larvae by day 14 (90–98% larval mortality) compared with 57 of 61 replicates for the other five diets. Brood was always entirely consumed.

Very young larvae occasionally died even in the presence of food, but older larvae seldom did. Therefore, larval survivorship was divided into two parts: hatchling survivorship, the survivorship from the day of oviposition to day 8 (day 14 for larvae kept at 21°C); and late-instar survivorship, survivorship between day 8 and day 14 (day 20 for larvae kept at 21°C). To

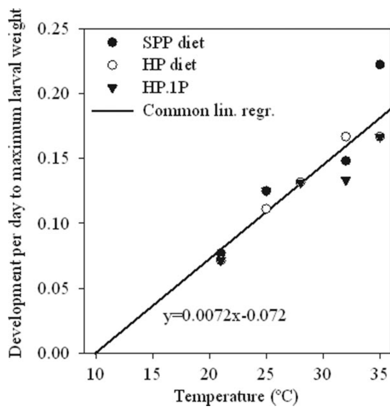

Fig. 3. Developmental rates of *A. tumida* larvae over five temperatures with three diets. Developmental time was calculated from the estimated day of egg hatch to the day of maximum observed weight. Each data point represents the mean value for two to four experiments. (A) SPP diet (see text for details). (B) HP diet. (C) HP.1P diet. Line shows common regression line for the data (equation for regression line shown).

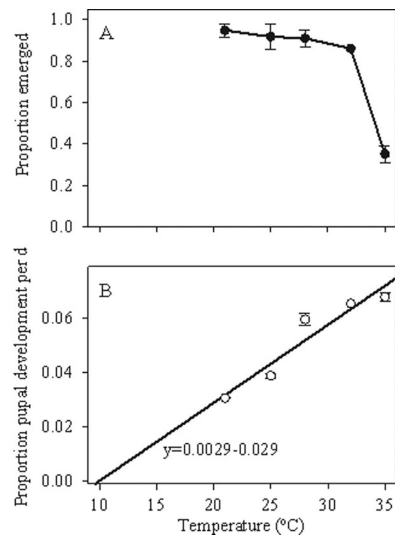

Fig. 4. Proportion of emerged pupae and developmental rate of pupal stage for *A. tumida* across five temperatures. (A) Pupal survivorship. (B) Proportion pupal development per day. Error bars show SEs across experiments. Line shows common regression line for the data (equation for regression line shown).

examine competition effects, egg number per oviposition slide was included as a covariate in the hatchling survivorship analysis, and number of 8-d-old larvae as a covariate in the late instar analysis. Proportion surviving hatchlings varied from an overall (across diets) mean of 0.73 ± 0.05 at 25°C to 0.26 ± 0.08 at 35°C, and it was significantly affected by temperature ($F = 10.99$, df = 4, 58; $P < 0.0001$) but not diet ($P = 0.52$) or the number of eggs on the oviposition slide ($P = 0.13$). Late-instar larval survivorship was affected by diet ($F = 24.39$; df = 2, 182; $P < 0.0001$) and temperature × diet ($F = 6.49$; df = 8, 182; $P < 0.0001$), but not temperature ($P = 0.09$). Survivorship was lower in SPP diet than in either of the other diets ($P < 0.0001$). Survivorship of late instar larvae was high, ranging from 0.93 with SPP diet to 0.99 with the other two diets. Maximum larval weight was not significantly affected by diet ($P = 0.08$) or temperature ($P = 0.24$), but it was affected by the number of 8-d-old larvae ($F = 6.14$; df = 47, 187; $P < 0.0001$), even though records with ≥60 larvae per vial had been removed.

The duration of the larval stage was calculated as the difference between the day of hatching (estimated using the data above) and observed maximum weight. Larval developmental rate data, calculated in the same manner as with eggs described above, for three diets (SPP, HP, and HP.1P) were regressed on temperature. The regression was significant ($F = 68.55$; df = 1, 13; $P < 0.001$; adjusted $r^2 = 0.84$) (Fig. 3), and the line was extrapolated to estimate the minimum temperature for larval development: 10.0°C. Larvae were estimated to need 27 d for development at 15°C and 68 d at 12°C.

Pupal Duration and Survivorship. Pupal stage duration was strongly affected by temperature ($F = 1935.4$; df = 4, 373; $P < 0.0001$; $r^2 = 0.95$), and temperature groups differed significantly from one another ($P \leq 0.002$ for all comparisons). Pupal duration was 14.8 d at 35°C, 15.3 d at 32°C, 16.8 d at 28°C, 25.8 d at 25°C, and 32.7 d at 21°C. Experiment-wide survivorship also varied with temperature ($F = 14.50$; df = 4, 5; $P = 0.0059$; $r^2 = 0.92$); survivorship at 35°C was lower than at any other temperature ($P \leq 0.0447$), but there were no other significant differences (Fig. 4). The regression of average developmental rate on temperature was significant ($F = 31.26$; df = 1, 3; $P = 0.011$; adjusted $r^2 = 0.88$). Extrapolating the line showed a minimum temperature for pupal development of 10.0°C (same as for larval development), and an estimated pupal duration of 70 d at 15°C and 174 d at 12°C. Most cadavers of unemerged beetles were recovered; the proportion of larvae neither emerged nor recovered ranged from none at 25°C to 8% at 21°C, but at 35°C 40% of the cadavers were not recovered. Soil moisture content was maintained at 5–8% by weight.

Pupal duration was similar between the two experiments at 28°C: 17.8 ± 0.1 d for trial 1 ($N = 350$) and 17.3 ± 0.6 d for trial 2 ($N = 312$), and diet had no effect ($P = 0.32$). No effect of diet was observed on survivorship (Kruskal–Wallis one-way test on ranks, $P = 0.54$), but the power of the test was low. Neither diet ($P = 0.22$), days to emergence ($P = 0.13$), nor larval weight (P from 0.09 to 0.62) significantly affected adult weight. Overall, the range of average adult weights per group was smaller (12.3–14.1 mg) than that of larval weights on day 8 (19.7–25.9 mg), day 11 (19.3–22.4 mg) or day 14 (17.2–20.6 mg).

Adult Longevity. Both diet (Fig. 5) and temperature (Fig. 6) affected beetle longevity (Table 2). The experiment at 32°C was replicated and neither female ($P = 0.54$) nor male longevity ($P = 0.58$) differed between the two trials. At 32°C, the maximum lon-

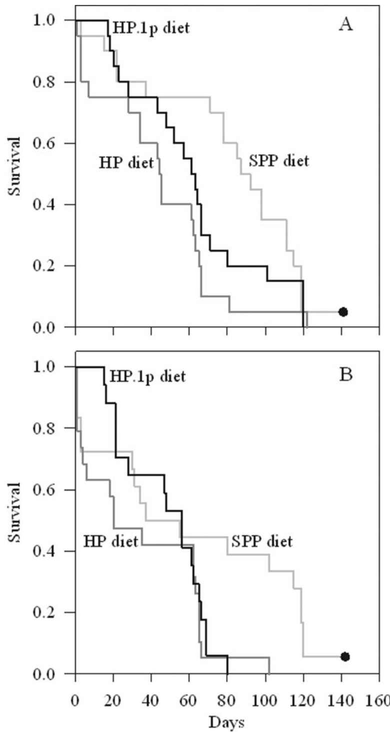

Fig. 5. Longevity of *A. tumida* adults kept at 32°C with one of three diets: SPP diet, HP diet, or HP.1P diet (see text for details). Beetles emerged on day 0. (A) Female longevity. (B) Male longevity.

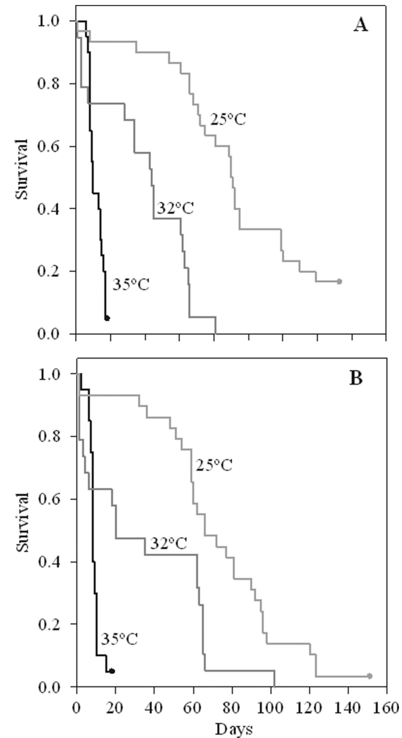

Fig. 6. Longevity of *A. tumida* adults kept on HP diet at 35, 32, and 25°C. Beetles emerged on day 0. For a description of the diet, please see text. (A) Female longevity. (B) Male longevity.

gevity was 141 and 142 d for females and males, respectively, on SPP diet. A replicate experiment diet conducted at 35°C with SPP and HP also showed that beetles fed SPP lived longer than those fed HP (log rank statistic = 26.37 for females and 37.78 for males, df = 1, $P < 0.001$), but longevities in the second trial were significantly lower than in the first: females on SPP and HP diets lived on average only 9.3 ± 1.4 and 2.9 ± 0.3 d, respectively, and males only 7.7 ± 0.5 and 2.7 ± 0.3 d (within-diet log rank statistic = 14.28 for females and 15.30 for males, df = 1, $P < 0.001$). Beetles clearly lived longer at lower temperatures, with the maximum longevity being >153 d for females on HP diet at 25°C.

Oviposition. Diet and temperature affected total egg production (Table 3). Although egg production at 32°C was not affected by diet ($P = 0.27$), the interaction of diet and week was significant ($F = 4.63$; df = 57, 776; $P < 0.0001$), in particular for week 4 (days 21–28) and weeks 12–15 (days 84–105) ($P \leq 0.0377$) (Fig. 7). Six of the 60 beetle pairs (at least one per diet treatment) produced no eggs. Total oviposition data from the SPP diet treatment were not significantly different ($P = 0.46$) from corresponding data from a replicate trial. Diet effects were observed at 35°C: total egg production was far lower than at 32°C but higher for females on HP diet than for those on SPP diet ($F = 22.71$; df = 1, 68; $P \leq 0.0001$), although females on SPP diet lived longer (see above). Temperature affected egg production regardless of diet (for HP diet: $F = 7.08$; df = 2, 67; $P = 0.0016$; and for SPP diet: $F = 74.96$, df = 2, 117; $P < 0.0001$) (Fig. 8). In general beetles laid the most eggs at 32°C and the least at 35°C. We observed varying levels of couple infertility: 20% with HP diet at 25°C, 20% with SPP diet at 28°C, 10% for all diets at 32°C, 21%, and 76% with SPP diet, and 30% with HP diet at 35°C.

The experiment involving different adult densities on oviposition was terminated after 56 d, at which point eight one-pair vials remained, the 10 vials in the three-pair group had an average of 2.2 females and 2.6 males each, and the five vials in the five-pair group had an average of 2.5 females and 2.6 males each. The numbers of females or males were not significantly different ($P = 0.51$ and 0.86, respectively) between the three-pair and five-pair groups after 56 d due to natural and accidental beetle losses during monitoring. Because the number of females per vial could not be regularly monitored during the experiment, the analysis was conducted using egg production per vial rather than per female. The original number of pairs per vial had a significant effect on egg production ($F = 11.54$; df = 2, 36; $P = 0.0001$), with the one-pair group was significantly lower than the three-pair ($P = 0.0006$) and five-pair ($P = 0.0011$) groups, but the three- and five-pair groups were not different (Fig. 9). The one-pair vials produced on average 37 ± 8 eggs

Table 3. Total egg production of *A. tumida* at different diets and temperatures

Temp (°C)	Experimental units	Diet[a]		
		SPP	HP	HP.1P
25	30		919 ± 175xy (max 1,525)	
28	50	114 ± 21x (max 672)		
32	20	1,449 ± 266a,y (max 3,955)	919 ± 175a,x (max 2,252)	1,347 ± 252a (max 3,586)
35	50 (SPP), 20 (HP)	6.4 ± 3a,z (max 143)	36 ± 11b,y (max 142)	

Letters a or b after a value indicate significant differences ($P \leq 0.05$) with respect to diet within temperature (comparisons within rows); likewise letters x, y, and z indicate significant differences with respect to temperature within diet (comparisons within columns).

[a] Egg production was compared across diets at 32 and 35°C and across temperatures for SPP and HP diets. SPP, HP, and HP.1P are beetle diets (see text for details), and max is maximum number of eggs per female for that treatment.

per week, compared with 250 ± 38 eggs in the three-pair group and 278 ± 49 eggs in the five-pair group.

The experiment on the role of oviposition site was analyzed with respect to progeny, rather than eggs, per female per week because, as preliminary trials showed, *A. tumida* much preferred to oviposit on brood comb rather than glass slides but eggs could not be counted accurately on comb. Progeny production is probably less than egg production because that number does not include either unhatched eggs or hatchlings that died very young. Of the 10 vials with *A. tumida* fed bee pupae without comb only two eggs were produced, so this treatment was not included in the statistics. Diet had a significant effect ($F = 30.99$; df = 3, 45; $P < 0.0001$) and the diet by week interaction was significant ($F = 6.53$; df = 8, 76; $P < 0.0001$) (Fig. 10). In post hoc contrasts, all pairwise comparisons were significant ($P \leq 0.0004$) except those between brood and HP diet and between HPB and P + brood.

Discussion

The main objective of this study was to evaluate the effects of temperature, diet, and other factors on the different parts of the *A. tumida* life cycle. Beetles were exposed to conditions that they would probably encounter in bee hives in the southern United States. Artificial pollen diet was included because it is available to *A. tumida* when fed to bees. Evaluating stage-specific effects was intended to aid development of an *A. tumida* population model; the more information we have on factors driving the developmental rates for each stage, the more accurate such a model can be. Although some data are available, additional work was seen as necessary. de Guzman and Frake (2007), for example, observed that *A. tumida* egg and larvae develop faster at 34°C than at 24–28°C but more data are needed to define the relationship mathematically. Likewise, Arbogast et al. (2009) measured the period between the introduction of adults into cages and the emergence of wandering larvae ready for pupation, but that same period can be considered to have several parts: the time between adult introduction and first oviposition, the duration of the egg stage, and the duration of the larval stage to final instar. Although the larvae in these studies could not exit the vials to seek a pupation site, we estimated that point in development by weighing larvae, under the assumption that larval development was largely over by the time they were at peak weight or just afterward (larvae invari-

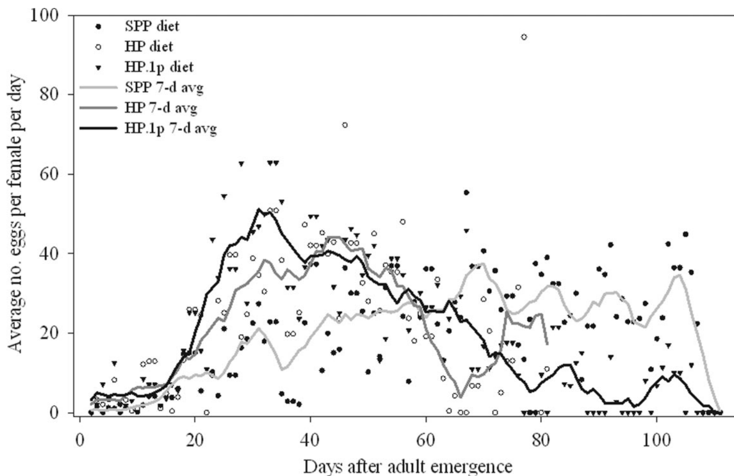

Fig. 7. Average daily egg production per *A. tumida* female at 32°C with one of three diets: SPP, HP, or HP.1P (see text for details). Each diet treatment initially had 20 pairs of beetles. Points show average egg production across replicates within treatment. Lines show 7-d running average, including the 3 d before and after each day, for each treatment with at least two replicates.

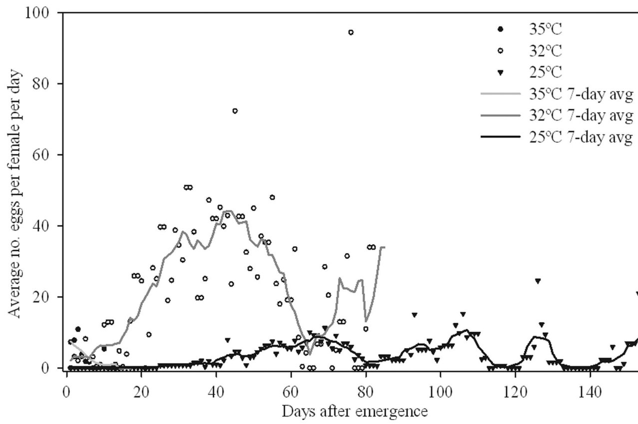

Fig. 8. Average daily egg production per female *A. tumida* with HP diet (see text for details) and kept at either 35, 32, or 25°C. Treatments at 35 and 32°C initially had 20 pairs of beetles, whereas treatments at 25°C had 30. Points show average egg production across replicates within treatment. Lines show 7-d running average, including the 3 d before and after each day. The experiment at 32°C when the number of replicates fell below two.

ably burrowed when placed on soil at that point). Also, we considered the part of the entire time the insect spent in the soil, from burrowing in to emergence as adult, to be one "stage," which we feel should serve most purposes.

Diet Effects. Beetle diet, although restricted to items found in bee hives, was a significant main effect in only one of the four larval growth analyses. Ellis et al. (2002) and Arbogast et al. (2009) included fruit diets in their studies. Ellis et al. (2002) noted that larvae fed pollen, honey and/or brood had higher pupation success than those fed diets not found in the hive. Arbogast et al. (2009) reported longer developmental period for larvae fed *K. ohmeri* inoculated diet but no effect on survivorship. Larvae did as well with pollen as the only protein source as when bee pupae was included; those fed only bee pupae grew as large as those fed other diets but mortality was much higher. de Guzman and Frake (2007) observed that *A. tumida* larvae grew well on brood alone but there were some differences in protocol with experiments presented here. de Guzman and Frake (2007) placed one *A. tumida* larva on each bee pupa, whereas in our study several larvae were presented with pieces of capped brood comb (usually previously frozen). Two diets examined here, HP.1P and HP1P, were intended to simulate a diet for *A. tumida* living in bee colonies where they would have limited access to brood. Diet did have a significant effect on pupation at 28°C, but the magnitude of the effect was small (<1 d).

Diet affected adult longevity and egg production. Beetles with SPP diet tended to live longer than those with natural pollen diet, but egg production was not significantly different, at least at temperatures <35°C. At 35°C both adult longevity and egg production were low and were affected by diet. Adding bee pupae to diet did not affect either longevity or egg production, and adults did not thrive on a brood alone. Ellis et al. (2002) observed that adults fed only brood comb lasted on average only 9 d, compared with those fed

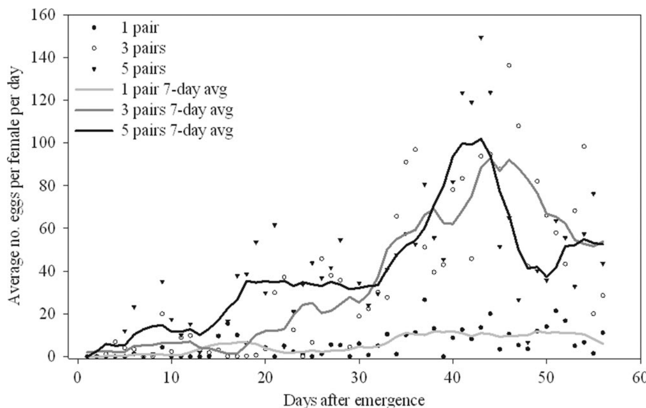

Fig. 9. Average daily *A. tumida* egg production per replicate with SPP diet (see text for details) and kept at 32°C with either one, three or five beetle pairs per oviposition vial. The treatments with one and three pairs per vial had 10 replicates and the treatment with five pairs had five replicates. Points show average egg production across replicates within treatment. Lines show 7-d running average, including the 3 d before and after each day.

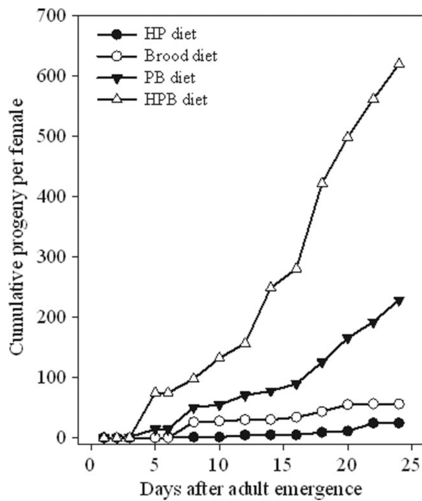

Fig. 10. Average cumulative progeny production per female for *A. tumida* with two pairs of beetles per vial and with one of four diets: honey and pollen; brood comb; pollen + brood comb; or honey, pollen, and brood comb.

pollen comb which last on average of 123 d. The interaction of diet and age (in weeks) in the oviposition analysis was significant, with more eggs being laid early among beetles fed natural pollen compared with SPP. As Arbogast et al. (2010) pointed out, high egg production earlier in life leads to shorter generation times and a higher potential for population growth, even if there is no difference in total lifetime fecundity.

Lundie (1940) measured the duration of *A. tumida* life stages using only pollen and honey as diet. He noted that beetles could eat bee larvae and eggs but doubted whether that caused much damage in the hive. Lundie (1940) did not provide the temperature of the experiments nor did he sex the beetles, but an analysis of his raw data showed an average egg duration (sample size) of 2.6 d (1299), larval duration of 15.2 d (1,137), pupal duration, as defined here, of 27.6 d (2143) and an average adult longevity of 77.2 d (68), corresponding to beetle development at ≈24–26°C according to our data. As with our study, Lundie (1940) noticed high mortality of young larvae and did not observe cannibalism but could not always rule it out.

Temperature Effects. Eggs hatched in <22 h at 35°C but took almost 3 times as long at 21°C. de Guzman and Frake (2007) reported longer average hatch times but our methods were different; we collected eggs within 2 h of oviposition and, at 28, 32, and 35°C, and we recorded hatching photographically to within 15 min. de Guzman and Frake (2007) collected eggs from an oviposition chamber after 20 h and inspected eggs twice a day until all had either hatched or died (similar to the approach of Lundie 1940). We also found higher egg mortality than that reported by de Guzman and Frake (2007). Some discrepancies may be due to errors in distinguishing dead from hatched eggs, particularly eggs grouped tightly in a clutch. A more reliable measure might be hatchling survivorship, because that represents the difference between the number of freshly laid eggs and that of hatchlings. Hatchling survivorship was ≈25% at 35°C, which suggests that *A. tumida* larvae probably do not survive well in the brood area of a healthy honey bee colony. When kept at 25 to 35°C, larvae grew rapidly, increasing their mass four- or five-fold during the first 6–8 d. Larval growth was significantly faster as the temperature treatments increased from 21 to 32°C, but 32°C was not different from 35°C. Maximum larval was not affected by temperature.

Pupal survivorship ranged from 86 to 95% between 21 and 32°C but dropped to 35% at 35°C. From 92 to 100% of pupal cadavers were recovered at 32°C or lower but few cadavers were recovered at 35°C, raising the question of whether the pupae were attacked by soil-borne organisms. Entomopathogenic nematodes (EPNs) are known to attack *A. tumida* (Cabanillos and Elzen 2006) but high EPN activity at 35°C is unlikely; Xu et al. (2010) observed that reproduction by two common genera of EPN was 75–100% on hosts at 25°C but <20% at 35°C. Cadaver disappearance also can be attributed to dehydration and to decomposition by saprophytic organisms. We estimated the pupal stage would take over three mos. at 15°C, without considering factors such as dehydration, disease or predation. Arbogast et al. (2010) found mean oviposition-to-adult duration of 28.5 d for beetles kept at 27.5°C and fed inoculated pollen dough. In our study, eggs took slightly more than a day to hatch at 28°C, larvae were ready to pupate at ≈7–9 d, and that pupal duration was ≈17.5 d, indicating an egg-to-adult developmental time of ≈26–28 d. de Guzman and Frake (2007) reported a first instar-to-adult developmental time of ≈21 d at 34°C, which, high mortality aside, is about the same as our observations at 35°C (6-d larval development followed by ≈15 d spent in soil).

Adult beetles kept on HP diet at 35°C had a lifespan of 12 d or less and produced far fewer eggs than those at 25°C or 32°C. Beetles on the same diet lived on average of 40 d at 32°C and 92 d at 25°C. Some infertility may also be attributed to temperature. Arbogast et al. (2010) reported an infertility rate of 8–10% at 27.5°C. Beetles on SPP diet in our study had an incidence of infertility ranging from 10% at 32°C to 76% at 35°C. As with larval survivorship, these results support the notion that 28–32°C are favorable temperatures for *A. tumida* and that they are poorly suited for life at a constant 35°C.

Several factors were found to influence egg production, including temperature, diet, oviposition site and density of conspecifics in the vial, as well as interactions among these factors. Comb pieces were found to be highly preferred over glass slides as oviposition sites, even when abundant diet with pollen and bee pupae was available in both cases, probably due to olfactory or physical cues of the comb. Small hive beetles presented with bee pupae but no comb produced only two eggs among 10 replicates. Strong substrate effects on oviposition have been observed in other beetles (Messina and Fry 2003). The analysis of

temperature, diet and other effects on *A. tumida* presented here was intended to shed light on behavior and ecology, and may be useful in evaluating control strategies. Knowledge of the effects of temperature and other factors will help in estimating population growth inside as well as outside hives.

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