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Survival and Development of *Amblypelta nitida* Stål, *A. l. lutescens* Distant (Hemiptera: Coreidae) and the Egg Parasitoid, *Anastatus* sp. (Hymenoptera: Eupelmidae) at Constant Rearing Temperatures

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ABSTRACT.

The effects of constant rearing temperatures on the development and survival of *Amblypelta nitida*, *A. lutescens lutescens* (Hemiptera: Coreidae) and their egg parasitoid, *Anastatus* sp. (Hymenoptera: Eupelmidae), were studied in the laboratory. *Amblypelta nitida* and *A. l. lutescens* survival and development was studied at 10, 15, 20, 25, 30 and 35°C. The development rate of both species increased linearly with increasing temperature but insects only developed to adults at 20, 25 and 30°C; at these temperatures, mean development times for *A. nitida* were 87, 64 and 29 days and for *A. l. lutescens* they were 93, 65 and 31 days respectively. No eggs of either species hatched at 10°C and only *A. l. lutescens* eggs hatched at 35°C. At all temperatures at which insects developed beyond the first instar, mortality rates were highest in the second instar for both species. Lower developmental threshold temperatures to complete development were 15.9°C and 17.1°C for *A. nitida* and *A. l. lutescens* respectively; *A. nitida* required 421 degree-days and *A. l. lutescens* required 404 degree-days to complete development. *Anastatus* sp. completed development at all six study temperatures and development times decreased from 54 days at 17.5°C to 16 days at 30°C; similarly *Anastatus* sp. survival increased with increasing temperature. The lower developmental threshold temperature and degree-days required for *Anastatus* sp. to complete development were 15.0°C and 234 degree-days respectively. Results are discussed with respect to the different geographical distributions of *A. nitida* and *A. l. lutescens* and likely interactions with *Anastatus* sp..

KEY WORDS Fruitspotting bugs, developmental threshold, degree-days, parasitoid

Introduction

The fruitspotting bug, *Amblypelta nitida* Stål and the banana-spotting bug, *A. lutescens lutescens* Distant (Hemiptera: Coreidae) are native polyphagous insect pests of a wide range of tropical and subtropical fruit and nut crops on the east coast of Australia (*A. nitida* ≈23°S 150°E- ≈34°S 151°E and *A. l. lutescens* ≈10°S 142°E- ≈27°S 153°E; Danne et al. 2014). Collectively they are referred to as fruitspotting bugs and they are economically damaging to horticultural crops including avocado, custard apple, papaya, cashew, cocoa, durian, guava, kiwifruit, lychee, low-chill stone fruit, persimmon and macadamia (Fay et al. 2009). The life cycles of both *A. nitida* and *A. l. lutescens* consist of an egg and five nymph stages before the development of the imago (Ironside 1978). Both *A. nitida* and *A. l. lutescens* feed on flowers and fruit, and *A. l. lutescens* also feeds on the terminal growth of papaya, mango, cashews and macadamia plants (Fay 2002). When feeding, fruitspotting bugs inject salivary sucrase into the plant tissue; this generates an osmotically driven outflow from the surrounding cells and leaves sunken lesions from the collapsed tissue (Miles 1987, Miles and Taylor 1994). Fruitspotting bugs damage plant tissue both as nymphs and adults so, to reduce crop damage, reducing the numbers of early instar nymphs is desirable. Insecticides remain the primary method of control for fruitspotting bugs in Australia (Danne et al. 2014), however, the possibility of developing integrated pest management strategies, including biological control, is now being explored (Danne et al. 2014).

Despite the significance of fruitspotting bugs as pests, little is known about the effect of temperature on their growth and development. Waite (2000) showed that *A. l. lutescens* took longer to develop than *A. nitida* at three constant temperatures (20, 25 and 30°C), but investigation of a wider range of temperatures is required to more accurately estimate lower developmental threshold temperatures and the thermal constants (degree-days, DD) for development through specific stages for the two species.

Few studies have investigated the effects of temperature on the development and survival of Coreidae. In Tasmania, Australia, Steinbauer (1997) found that the minimum developmental threshold temperatures for eggs of two *Eucalypt*-feeding coreids, *Amorbius obscuricornis* (Westwood) and *Gelonus tasmanicus* (Le Guillou), were 11.8 and 10.8°C respectively, and that the corresponding degree-days required to complete development were 147 and 136. Egwuatu and Ajibola Taylor (1977) found that mean immature development times of the legume pest *Acanthomia* (= *Clavigralla*) *tomentosicollis* Stål (Hemiptera: Coreidae) decreased with increasing temperature (43 days at 20°C and 12 days at 36°C). Total immature mortality of *A. tomentosicollis* ranged between 23% and 63% at the eight study temperatures (20-36°C), with highest immature mortality occurring at the upper and lower temperatures studied (20°C, mortality=63% and 36°C, mortality=56%) (Egwuatu and Taylor 1977). Similarly, Fargo and Bonjour (1988) showed that mean immature development times for the cucurbit pest *Anasa tristis* DeGeer (Hemiptera: Coreidae) decreased from 79 days at 20°C to 23 days at 37.8°C. Total immature mortalities at temperatures between 23.3 and 37.8°C were very low, but at 20°C only 3% of the initial cohort survived to adult and 40°C no adults developed. The lower temperature threshold for *A. tristis* was 15.6°C, and it was estimated that it would take 376.5 degree-days to complete neonate to adult development (Fargo and Bonjour 1988).

The hymenopteran parasitoid *Anastatus* sp. (Eupelmidae) was among a suite of parasitoids collected from eggs of *A. l. lutescens* in north Queensland in 1993 (Fay and Huwer 1993). In glasshouse conditions parasitism rates of *A. l. lutescens* eggs by *Anastatus* sp. can be high (>90%) and the parasitoid can be reared in factitious hosts (Fay and De Faveri 1997). As a first step in exploring the potential of this parasitoid for biological control, its thermal requirements for development need to be understood relative to those of its hosts.

This is the first study on the developmental biology of *Anastatus* sp. attacking *A.*

nitida in Australia, although a previous study investigated the temperature relationships between *Anastatus biproruli* and its host, *Biprorulus bibax* (Hemiptera: Pentatomidae) (James 1993). Understanding the effects of temperature on the developmental biology of *Anastatus* sp. is a fundamental step for mass rearing and release programs for biological control of *Amblypelta* spp.. Temperature significantly affected the longevity and fertility of adult *Anastatus semiflavidus* Gahan, an egg parasitoid of the range caterpillar, *Hemileuca oliviae* Cockerell (Lepidoptera: Saturnidae) (Mendel et al. 1987). *Anastatus semiflavidus* did not oviposit at temperatures ≤ 15°C, but the intrinsic rate of increase increased with increasing temperature between 20 and 35°C (Mendel et al. 1987). The effectiveness of *A. semiflavidus* as a biological control agent of *H. oliviae* is thus likely to be affected by cold temperatures. In New Mexico, peak *H. oliviae* oviposition occurs between September and November; *A. semiflavidus* parasitism rates are likely to be higher earlier in this period and may be significantly depressed in years experiencing cold autumn temperatures (Mendel et al. 1987).

The objectives of this study were to determine the relative temperature requirements for development of *A. nitida, A. l. lutescens* and the parasitoid *Anastatus* sp. This was achieved by investigating the effects of a range of constant rearing temperatures that was commensurate with the temperatures experienced by these species across their distributions (Danne et al., 2014). The development and survival rates of *Amblypelta nitida* and *A. l. lutescens* were measured between 10 and 35°C while the effects of a narrower range of temperatures (17.5- 30°C) was investigated on their egg parasitoid, *Anastatus* sp. The data were then used to estimate the stage-specific lower developmental thresholds and the thermal constants (degree-days) for *A. nitida* and *A. l. lutescens* and the lower developmental thresholds and the thermal constants (degree-days) required for both pest species and the parasitoid to complete egg-adult development. These important parameters are necessary for the development of simulation models to investigate likely spatial and temporal host-

parasitoid relationships between *Anastatus* sp. and *A. nitida* and *A. l. lutescens* throughout their distributions in Australia. This is an essential next step in assessing the potential of *Anastatus* sp. as a biological control agent for fruitspotting bugs.

Materials and Methods

Insects

Amblypelta nitida and *A. lutescens lutescens* were obtained from BioResources Pty. Ltd., Samford, Queensland, Australia and originated from colonies established from adult insects collected in northern New South Wales and northern Queensland respectively. Separate colonies were prepared by placing approximately 30 pairs of adults of each species into a different wooden framed cage that consisted of three separate sections; a bottom and an upper frame (each 40 cm × 40 cm × 4 cm), each supporting a single layer of fiberglass mesh (mesh size 0.25 mm^2) (Cyclone Pty. Ltd., Dandenong South, Victoria, Australia) and a middle section (40 cm × 40 cm × 10 cm). The three sections were held in place by rubber bands that passed through hooks on the external sides of each section. The fiberglass mesh provided ventilation and an oviposition substrate. Insects were fed commercially sourced green beans (*Phaseolus vulgaris* L.) that had been washed in detergent and rinsed in tap water. Green beans were secured within twisted rubber bands attached to hooks inside the cages and changed daily. Colonies of both species were maintained in an incubator at 27 ± 1°C, 80 ± 2% RH, 12: 12 (L: D) h and freshly eclosed adults were added to each colony regularly to maintain egg production. *Amblypelta nitida* and *A. l. lutescens* tend to lay eggs on the upper fiberglass mesh surface in the cages and eggs were harvested by turning rearing cages upside down under a white light source to attract bugs to the new upper mesh surface. The lower frame was then removed and replaced with a clean frame containing fresh green beans. Eggs were removed from the cage by levering them off the fiberglass mesh, beans or wooden frame

using a small piece of flexible plastic. Eggs were harvested in this manner daily and were used immediately in experiments. Frame sections were cleaned using a dilute bleach solution and warm water before being reused.

Anastatus sp. was originally collected from parasitized *A. l. lutescens* in coastal areas of northern Queensland. They were supplied in parasitized *Antheraea pernyi* Guérin-Méneville (Lepidoptera: Saturniidae) eggs glued to cardboard sheets (BioResources Pty. Ltd. Samford, Queensland, Australia). Rearing cages consisted of a wooden, glass-topped box (30 × 40 × 120 cm) with fiberglass mesh sides for ventilation. The culture was maintained in a temperature-controlled room at 25 ± 1°C, 80% RH, 12: 12 (L: D) h and adult parasitoids were fed honey and a saturated solution of sucrose.

Development and Survival of A. nitida *and* A. l. lutescens *at Constant Temperatures*

Immature development and mortality rates for *A. nitida* and *A. l. lutescens* were investigated at six constant temperatures: 10, 15, 20, 25, 30 and 35°C. Eggs of each species were collected between 08:00 and 10:00 daily and transferred individually into labeled 70 ml plastic containers, the open ends of which were covered by fine nylon mesh was that was secured in place with a rubber band. After each daily collection of eggs, the eggs and containers were randomly divided into 6 groups and each group was allocated to one of the six test temperatures. Containers were labeled with the date that the egg was laid and transferred to an incubator set at the appropriate temperature, RH ≥ 40%, 12: 12 (L: D) h (fluorescent light, 120 microEinsteins); this process was repeated daily until approximately 120 eggs of each species were set up per temperature. Upon egg hatch, each neonate nymph was provided with a portion of a washed green bean and a water-saturated dental wick. Individual eggs/ nymphs were monitored daily until they died or developed to adults; beans and wicks were changed

every 3-4 days. Mortality and molting time data were recorded and used to construct life tables for each species at each rearing temperature.

Development and Survival of Anastatus sp. at Constant Temperatures

Immature development and mortality rates of *Anastatus* sp. developing in eggs of *A. nitida* were recorded at six constant temperatures: 17.5, 20, 22.5, 25, 27.5 and 30°C. Newly emerged male and female *Anastatus* sp. were allowed to mate overnight in a mesh cage (30 × 30 × 40 cm) (25 ± 2°C°C, 80% RH, 12: 12 (L: D) h) and provided with water-saturated dental wicks and honey as a food source. The next day, female *Anastatus* sp. were individually transferred into labeled 70 ml plastic containers, the open ends of which were covered by fine nylon mesh was that was secured in place with a rubber band. A 1-2 day old *A. nitida* egg was placed into each container with the female *Anastatus* sp. and the insects incubated at 25 ± 1°C, 80% RH, 12: 12 (L: D) h for oviposition. After 24 hours, female *Anastatus* sp. were removed from containers. The parasitoid-exposed eggs produced in this way were then randomly allocated to one of the constant temperatures, RH ≥ 40%, 12: 12 (L: D) h (fluorescent light, 120 microEinsteins). Approximately 200 *Anastatus* sp.-exposed *A. nitida* eggs were placed at each temperature; they were monitored daily and the time of adult *Anastatus* sp. emergence from each was recorded. Approximately one month after the first adult *Anastatus* sp. eclosed from eggs at a given temperature all remaining *A. nitida* eggs that had not hatched or yielded an adult parasitoid were dissected. *Anastatus* sp. eggs could not be found within host eggs but dead parasitoid larvae, pupae and pharate adults were recorded. The immature *Anastatus* sp. mortality data recorded in eggs incubated at each test temperature were combined with adult *Anastatus* sp. emergence data at that temperature to construct

partial life tables for immature *Anastatus* sp. developing in *A. nitida* eggs at each test temperature.

Statistical Analysis

Overall development times for *A. nitida* and *A. l. lutescens* at different temperatures were analyzed by two-way ANOVA with *Amblypelta* species and temperature as the main effects. The effect of rearing temperature on the pre-imaginal development rates of *A. nitida, A. l. lutescens* and *Anastatus* sp. was analyzed by linear regression. All statistical analyses were performed using GraphPad Prism version 6.00 for Mac (GraphPad Software Inc., 2015).

Results

Development and Survival of A. nitida and A. l. lutescens at Constant Temperatures.

Amblypelta nitida did not complete pre-imaginal development at temperatures below 20°C or above 30°C and no *A. nitida* eggs hatched at either 10 or 35°C (Table 1). At the remaining test temperatures, 44, 82, 89 and 88% of *A. nitida* eggs hatched at 15, 20, 25 and 30°C respectively (Table 1). Some *A. nitida* individuals (n = 9; 7% of test insects) developed to the 2nd instar at 15°C, but none completed development to the third instar (Table 1; Fig 1a). Total immature *A. nitida* survivorship was highest at 30°C and 47% of the initial cohort completed pre-imaginal development at this temperature. At the three temperatures at which *A. nitida* completed development, mortality was highest during the 2nd instar (Table 1; Fig 1b-d).

Similarly *A. l. lutescens* did not complete pre-imaginal development at temperatures below 20°C or above 30°C and no *A. l. lutescens* eggs hatched at 10°C (Table 1). At the remaining test temperatures, 5, 95, 92, 88 and 37% of *A. l. lutescens* eggs hatched at 15, 20, 25, 30 and 35°C respectively (Table 1). All of the *A. l. lutescens* that hatched at 15°C died as neonate larvae (Table 1; Fig 2a). Total immature survivorship was highest at 30°C for *A. l.*

lutescens and 62% of the initial cohort completed pre-imaginal development at this temperature. At the three temperatures at which *A. l. lutescens* completed development, mortality was highest during the 2nd instar (Table 1; Fig 2b-d).

Temperature significantly affected the total development time (egg to adult) for both *A. nitida* and *A. l. lutescens* ($F_{2, 212=}$ 1180; P<0.001; Table 2) and *A. l. lutescens* took longer to complete development than *A. nitida* ($F_{1, 212=}$ 8.14; P=0.005; Table 2) but there was no significant interaction between *Amblypelta* species and temperature with respect to total development time ($F_{2, 212=}$ 1.71; P=0.312; Table 2).

There was a significant linear relationship between temperature and development rate of immature insects at specific stages of development and for egg- adult development for both *A. nitida* and *A. l. lutescens* (Fig 3; Table 3). The estimated lower developmental threshold (95% CI) for *A. nitida* eggs (11.3°C (11.0-11.5)) was significantly lower than that for *A. l. lutescens* eggs (14.1°C (13.6-14.5)) (Table 3) but, based on overlapping 95% confidence intervals, there were no differences between the estimated lower developmental thresholds at any of the other developmental stages (Table 3). Overall, the lower developmental threshold for *A. nitida* was 15.9°C (15.0- 16.7) and it was estimated that 421 degree-days were required for it to complete development (Table 3) while the lower developmental threshold for *A. l. lutescens* was 17.1°C (16.1- 17.9) and it was estimated that 404 degree-days were required for it to complete development (Table 3).

Development and Survival of Anastatus sp. at Constant Temperatures.

Anastatus sp. completed pre-imaginal development at all six constant temperatures investigated (Table 4). *Anastatus* sp. eggs could not be found in the dissected host eggs but dead *Anastatus* sp. that had developed to the larval, pupal and pharate adult stages were recorded and partial life tables constructed (Table 4). Absolute parasitism rates of eggs

incubated at each test temperature could not be determined due to the undetectability of parasitoid eggs but the number of adult wasps developing from eggs at 17.5°C and 20°C was much lower than at higher temperatures (Table 4). Similarly, the proportion of larvae, pupae and pharate adults that died within host eggs was greater at temperatures ≤ 22.5°C than at higher temperatures (Table 4).

The time required for *Anastatus* sp. to complete development from egg to adult (mean (± SE)) was 53.6 (±2.1), 39.6 (±0.4), 33.0 (±0.4), 21.5 (±0.3), 17.9 (±0.3) and 15.5 (±0.2) days at 17.5, 20, 22.5, 25, 27.5 and 30°C respectively. The development rate of pre-imaginal *Anastatus* sp. increased linearly with increasing temperature ($F_{1, 455}$= 2561; $P < 0.001$; Fig. 4) and the lower threshold for development was estimated to be 14.3°C (13.8- 14.7)) and 238 degree-days were required for it to complete pre-imaginal development (Fig. 4).

Discussion

As expected, temperature had a significant effect on the development and survival of fruitspotting bugs, *A. nitida* and *A. l. lutescens*, and their egg parasitoid, *Anastatus* sp.. Both species of fruitspotting bug only completed immature development at temperatures between 20 and 30°C and survival rates were maximized at 30°C for each species (Table 1). At temperatures outside of this range, both species suffered considerable mortality as eggs and early instars (Table 1). At temperatures between 20 and 30°C, most mortality was suffered by second instar nymphs of both species; this demonstrates the sensitivity of this stage and the detrimental impact that unfavorable conditions at this stage of development can have on generational survival.

Overall, *A. l. lutescens* took longer to complete development than *A. nitida* (Table 2), a similar finding to that reported previously (Waite et al. 2000). However, in the current study development times for *A. nitida* and *A. l. lutescens* at 20 and 25°C were much longer than

those recorded by Waite et al. (2000), while at 30°C the development time for *A. l. lutescens* was much shorter. Differences in methodologies probably explain these apparent disparities. The current study used much larger sample sizes than those used by Waite et al. (2000), cf. n≥ 120 per temperature treatment in this study and n= 28-87 per temperature treatment in the previous study). Further, Waite et al., (2000) introduced additional insects, that were previously reared at unspecified temperatures, into the experiment to supplement the numbers in treatments that had suffered high mortality. Introducing an insect to a modified rearing temperature will affect its development rate compared with insects reared under constant temperatures (Bahar et al. 2012) and introduction of an insect that has already entered a given instar will incorrectly estimate the time that it typically takes to complete development through that instar at the new test temperature.

There were clear contrasts between the developmental and survival rates in response to high and low temperatures between *A. nitida* and *A. l. lutescens* (Tables 1-3, Figs. 1-3). For example, at 15°C some *A. nitida* individuals (7% of initial cohort) were able to complete development to the 2nd instar but no *A. l. lutescens* were able to do so (Table 1) and at 35°C no *A. nitida* eggs hatched but more than a third of *A. l. lutescens* eggs hatched at this temperature and some individuals (2% of initial cohort) completed development to the 3rd instar (Table 1). Further, the estimated overall lower developmental threshold for *A. nitida* was 15.9 °C, while it was 17.1°C for *A. l. lutescens* (Table 3, Fig 3). Differences in temperature requirements for egg development were also detected at low temperatures for the two species; the lower threshold for development of *A. nitida* eggs was estimated at 11.3°C, while it was estimated at 14.1°C for *A. l. lutescens* (Table 3, Fig 3). These differences are consistent with the lower temperatures experienced by *A. nitida* in its more southerly geographical distribution (≈34°S 151°E- ≈ 23°S 150°E) and the higher lower temperatures experienced by *A. l. lutescens* in its more northerly distribution (≈27°S 153°E- ≈10°S 142°E)

(Donaldson 1983; Danne et al. 2014), but it would be useful to investigate the thermal requirements of further populations of each species collected from different locations within their range.

Anastatus sp. completed pre-imaginal development within *A. nitida* eggs at all six study temperatures (17.5- 30°C) (Table 4). Adult emergence rates were much higher at temperatures ≥22.5°C than at lower temperatures (Table 4). It was not possible to find *Anastatus* eggs in *A. nitida* hosts and consequently it is not possible to estimate absolute parasitism rates; however, as all host eggs were exposed to parasitism in the same manner prior to allocation to different test temperatures it can be assumed that parasitism rates were comparable between rearing temperatures. At 17.5°C and 20°C the number of adults that eclosed and the number of dead larvae, pupa and pharate adults dissected from eggs was very low compared with higher temperatures (Table 4). This indicates that *Anastatus* sp. eggs and/ or small larvae suffered significant mortality at these temperatures, but the remains could not be found in dissected eggs. Similarly, *Anastatus* sp. developing at 22.5°C suffered significant mortality as larvae and pupae, while mortalities suffered by these stages at temperatures of ≥25°C were much lower (Table 4). Thus, although *Anastatus* sp. could develop at all temperatures between 17.5°C and 30°C, immature stages suffered significant mortality at temperatures ≤ 22.5°C. This is likely to preclude its establishment and limit its effectiveness as a biological control agent of *A. nitida* at more southerly latitudes within its distribution, where temperatures in this range occur frequently, particularly in winter.

This is the first study on the effect of temperature on the development and survival of *Anastatus* sp. in Australia. In a previous study James (1993) found that development rates of *A. biproruli* increased linearly between 17.5 and 35°C and in this study a similar relationship between development rate and rearing temperature was established for *Anastatus* sp. (Fig. 4). The lower developmental threshold temperature for *Anastatus* sp. was estimated to be 14.3°C

(Fig. 4), 3°C higher than the threshold temperature for host *A. nitida* egg development but only 0.2°C higher than the threshold temperature for egg development of *A. l. lutescens* (Table 3, Fig. 3). In order to complete pre-imaginal development *Anastatus* sp. requires 238 degree days at temperatures above 14.3°C. For biological control programs, knowledge of the lower developmental threshold temperature for parasitoids and the degree-day requirements for completion of pre-imaginal development above this temperature can be useful for selecting agents with similar climatic requirement to those of their hosts. Parasitoids and hosts with similar thermal requirements are more likely to remain synchronized throughout seasonal temperature fluctuations, while markedly different thermal requirements of host and parasitoids are likely to result in seasonal disruption of the host-parasitoid relationship.

Caution must be taken in extrapolating laboratory-generated data to field situations where temperature fluctuations at the scale of the micro-environment of insects are difficult to measure, especially for insects with mobile immature stages that may exhibit, for example, 'basking' behavior (Steinbauer and Clarke 1998). In addition, degree-day models assume that development rate is only a function of temperature, however, for polyphagous insects such as *A. nitida* and *A. l. lutescens* host plant can also significantly affect development. For example, preliminary findings suggest that *A. l. lutescens* nymphs may develop faster on papaya plants than when fed green beans (Huwer 1996). Photoperiod and humidity also affect insect development rates (Rahim et al. 1991, Steinbauer 1997, Sakashita et al. 1997, Wang et al. 2013, Zerbino et al. 2013). However, as the effects of these factors are difficult to quantify and as they are usually less important than temperature effects they are typically not considered in degree-day models.

This study provides important baseline data on the effect of temperature on the development of *A. nitida* and *A. l. lutescens,* and their egg parasitoid, *Anastatus* sp. and its likely effect on host-parasitoid relationships. As such it provides the basic information

required for the development of simulation models to investigate how *A. nitida*- *Anastatus* sp. and *A. l. lutescens*- *Anastatus* sp. relationships are likely to vary both geographically and vary seasonally. The development of such models, and further experimentation to refine and model parameters, will be important next steps in assessing the likely performance of *Anastatus* sp. in different fruit growing regions at different times of the year and as such will inform whether inoculative or inundative releases of *Anastatus* sp. for the biological control of *A. nitida* and *A. l. lutescens* are likely to be appropriate.

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Table 1. Life tables for *A. nitida and A. l. lutescens* at constant rearing temperatures.

	A. nitida				*A. l. lutescens*			
Temp. °C	Life stage	l_x [a]	d_x [b]	q_x [c]	Life stage	l_x [a]	d_x [b]	q_x [c]
10	Egg	125	125	1.000	Egg	123	123	1.000
	1st instar	0			1st instar	0		
15	Egg	126	70	0.556	Egg	123	117	0.951
	1st instar	56	47	0.839	1st instar	6	6	1.000
	2nd instar	9	9	1.000	2nd instar	0		
	3rd instar	0						
20	Egg	127	23	0.181	Egg	122	6	0.049
	1st instar	104	8	0.077	1st instar	116	10	0.086
	2nd instar	96	51	0.531	2nd instar	106	66	0.623
	3rd instar	45	16	0.356	3rd instar	40	19	0.475
	4th instar	29	2	0.069	4th instar	21	3	0.143
	5th instar	27	1	0.37	5th instar	18	1	0.056
	Adult	26			Adult	17		
25	Egg	140	16	0.114	Egg	122	10	0.082
	1st instar	124	6	0.048	1st instar	112	7	0.063
	2nd instar	117	88	0.752	2nd instar	105	47	0.448
	3rd instar	30	5	0.167	3rd instar	58	15	0.259
	4th instar	25	2	0.080	4th instar	43	9	0.209
	5th instar	23	3	0.130	5th instar	34	3	0.088
	Adult	20			Adult	31		
30	Egg	129	15	0.116	Egg	120	15	0.125
	1st instar	114	4	0.035	1st instar	105	9	0.086
	2nd instar	110	26	0.236	2nd instar	96	9	0.094
	3rd instar	84	6	0.071	3rd instar	87	5	0.057
	4th instar	78	9	0.115	4th instar	82	5	0.061
	5th instar	69	8	0.116	Adult	77	3	0.039
	Adult	61				74		
35	Egg	127	127	1.000	Egg	134	85	0.634
	1st instar	0			1st instar	49	21	0.429
					2nd instar	28	25	0.893
					3rd instar	3	3	1.000
					4th instar	0		

[a] l_x, number of individuals living at beginning of a stage

[b] d_x, number of individuals dying by end of a stage

[c] q_x, proportion individuals entering a stage that died in that stage

Table 2. Mean development times (±SE) in days for *Amblypelta nitida* and *A. l. lutescens* at constant rearing temperatures.

			Time (days) to complete development of stage (±SE)						
	Temp. °C	n	Egg	1st instar	2nd instar	3rd instar	4th instar	5th instar	Total[a]
A. nitida	15	126	31.9 (±0.4)	11.4 (±0.3)	-	-	-	-	-
	20	127	12.6 (±0.1)	5.0 (±0.04)	21.9 (±1.0)	14.7 (±0.9)	14.5 (±0.8)	17.7 (±0.5)	86.7 (±2.6)
	25	140	8.3 (±0.1)	3.0 (±0.03)	20.0 (±2.0)	12.7 (±1.6)	9.9 (±0.6)	10.3 (±0.5)	63.5 (±2.1)
	30	129	6.0 (±0.1)	2.0 (±0.01)	5.3 (±0.2)	5.2 (±0.2)	5.1 (±0.2)	5.9 (±0.1)	29.4 (±0.4)
A. l. lutescens	15	123	31.3 (±1.0)	-	-	-	-	-	-
	20	122	17.0 (±0.2)	6.0 (±0.05)	17.2 (±0.6)	18.3 (±1.4)	16.6 (±0.7)	17.5 (±0.4)	92.5 (±2.3)
	25	122	9.3 (±0.1)	3.5 (±0.06)	14.7 (±0.8)	12.2 (±0.5)	11.8 (±0.8)	12.2 (±0.5)	64.7 (±1.6)
	30	120	5.3 (±0.1)	2.2 (±0.05)	6.1 (±0.2)	5.4 (±0.2)	5.6 (±0.2)	6.5 (±0.1)	30.8 (±0.5)
	35	134	5.1 (±0.1)	2.2 (±0.09)	5.7 (±1.8)	-	-	-	-

[a] Total development time was significantly affected by temperature ($F_{2, 212=}$ 1180; P<0.001) and *A. l. lutescens* took longer to complete development than *A. nitida* ($F_{1, 212=}$ 8.14; P=0.005) but there was no significant interaction between *Amblypelta* species and temperature ($F_{2, 212=}$ 1.71; P=0.312).

Table 3. Linear regression parameters for stage-specific and egg-adult development of *A. nitida* and *A. l. lutescens* over a range of constant rearing temperatures.

Stage of development	Species	Linear regression parameters				Slope differences		
		F (df); P	R^2	y- intercept (±SE)	Slope (±SE)	F (df); P	DD[a]	T_{min}[b] (95% CI)
Egg	*A. nitida*	13300 (1, 395); <0.001	0.971	-0.101 (±0.002)	0.0089 (±0.0001)	62 (1,782); <0.001	111	11.3 (11.0 - 11.5)
	A. l. lutescens	2860 (1, 387); <0.001	0.881	-0.149 (±0.005)	0.0106 (±0.0002)		91	14.1 (13.6- 14.5)
1st instar	*A. nitida*	8839 (1, 395); <0.001	0.964	-0.388 (±0.008)	0.0293 (±0.0003)	30 (1,662); <0.001	34	13.3 (13.0- 13.5)
	A. l. lutescens	1302(1, 395); <0.001	0.796	-0.323 (±0.018)	0.0248 (±0.0007)		40	13.0 (12.2- 13.7)
2nd instar	*A. nitida*	204 (1, 156); <0.001	0.567	-0.304 (±0.032)	0.0170 (±0.0011)	6.4 (1,342); =0.012	59	17.9 (16.4- 19.1)
	A. l. lutescens	230 (1, 186); <0.001	0.552	-0.227 (±0.024)	0.0133 (±0.0009)		75	17.0 (15.5- 18.2)
3rd instar	*A. nitida*	198 (1, 130); <0.001	0.604	-0.226 (±0.028)	0.0142 (±0.0010)	1.1 (1,274); =0.289	70	15.9 (14.0- 17.3)
	A. l. lutescens	260 (1, 144); <0.001	0.643	-0.280 (±0.027)	0.0157 (±0.0010)		64	17.8 (16.4- 18.9)
4th instar	*A. nitida*	258 (1, 116); <0.001	0.690	-0.218 (±0.024)	0.0140 (±0.0009)	0.3 (1, 243); =0.585	71	15.5 (13.9- 16.8)
	A. l. lutescens	169 (1, 127); <0.001	0.571	-0.254 (±0.031)	0.0148 (±0.0011)		67	17.1 (15.3- 18.6)
5th instar	*A. nitida*	707 (1, 105); <0.001	0.871	-0.183 (±0.012)	0.0118 (±0.0004)	1.5 (1,225); =0.231	85	15.5 (14.5-16.3)
	A. l. lutescens	456 (1, 120); <0.001	0.792	-0.175 (±0.014)	0.0110 (±0.0005)		91	15.9 (14.7-17.0)
Egg- adult	*A. nitida*	743 (1, 105); <0.001	0.876	-0.038 (±0.002)	0.0024 (±0.0001)	0.6 (1,225); =0.450	421	15.9 (15.0- 16.7)
	A. l. lutescens	621 (1, 120); <0.001	0.838	-0.042 (±0.003)	0.0025 (±0.0001)		404	17.1 (16.1- 17.9)

[a] DD= degree days

[b] T_{min}= lower temperature threshold for development

Table 4. Partial life tables for *Anastatus* sp. reared in *A. nitida* eggs at constant temperatures.

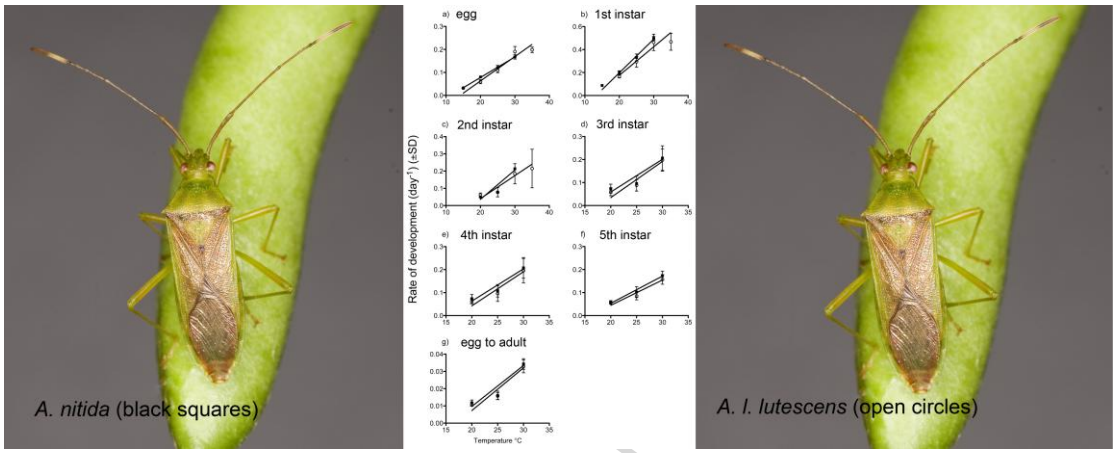
Temp. °C	Life stage	l_x [a]	d_x	q_x [c]
17.5	larva/ pupa	11	2	0.182
	pharate adult	9	4	0.444
	adult	5		
20	larva/ pupa	15	2	0.133
	pharate adult	13	7	0.539
	adult	6		
22.5	larva/ pupa	117	30	0.256
	pharate adult	87	7	0.081
	adult	80		
25	larva/ pupa	59	0	0
	pharate adult	59	3	0.051
	adult	56		
27.5	larva/ pupa	101	10	0.099
	pharate adult	91	5	0.055
	adult	86		
30	larva/ pupa	81	1	0.012
	pharate adult	80	0	0
	adult	80		

[a] l_x, number of individuals living at beginning of a stage

[b] d_x, number of individuals dying by end of a stage

[c] q_x, proportion individuals entering a stage that died in that stage.

Graphical abstract



Highlights

- Neither *Amblypelta nitida* nor *A. l*. lutescens completed development <20ºC or >30ºC
- *A. nitida*: lower development threshold= 15.9ºC; 421-DD to complete development
- *A. l. lutescens*: lower development threshold= 17.1ºC; 404-DD to complete development
- Both *Amblypelta* spp. suffered greatest mortality in 2nd instar at all temperatures
- *Anastatus* sp.: lower development threshold= 15.0ºC; 234-DD to complete development