



This is a pre-peer review version of an article published in *Physiological Entomology* and which will be made available in final form at: [10.1111/phen.12310](https://doi.org/10.1111/phen.12310). Note that as of 17/01/2020, this article is in press, and not yet published.

Hill, S., Silcocks, S., & Andrew, N. Impacts of temperature on metabolic rates of adult *Extatosoma tiaratum* reared on different host plant species. *Physiological Entomology*. doi: [10.1111/phen.12310](https://doi.org/10.1111/phen.12310)

This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

1
2 **Impacts of temperature on metabolic rates of adult *Extatosoma tiaratum* reared on**
3 **different host plant species.**

4 Authors: Sarah J. Hill¹, Sarah C. Silcocks^{1,2}, Nigel R. Andrew^{1*}

5
6 ¹ Zoology, University of New England, Armidale, NSW, 2351, Australia.

7 ² Melbourne Zoo, P.O. Box 74, Parkville, Victoria 3052, Australia.

8
9 *Corresponding author: nigel.andrew@une.edu.au

10
11 Running title: Temperature impacts on metabolic rates of stick insects.

12
13 **ABSTRACT**

14 Access to balanced nutrition enables optimum health and development, body repair,
15 fat storage, increased fecundity and longevity. In this study we assessed the responses of a
16 generalist leaf feeder (the phasmid *Extatosoma tiaratum*) reared continuously on one of three
17 host plants: tree lucerne (*Chamaecyclus palmensis*), bramble (*Rubus fruticosus*) and
18 *Eucalyptus* species in a low fluctuating temperature environment until adulthood. Once all
19 individuals reached adulthood, we exposed each individual to a ramping temperature event
20 (starting at 25°C and ramping the temperature at 0.25°C min⁻¹ and assessed their metabolic
21 rates ($\dot{V}CO_2$) responses at specific temperature ‘bins’(25°C, 30°C, 35°C, 40°C and 42°C). Sex
22 but not diet influenced respiration and metabolic rate. Male individuals had, on average, a
23 higher $\dot{V}CO_2$ than females. Sex and diet were significant influences on $\dot{V}CO_2$ at different
24 temperatures. Metabolic rates at lower temperatures were not affected by sex or diet type. At
25 35°C, metabolic rates were influenced by sex and diet with males reared on bramble and tree
26 lucerne having a higher metabolic rate than females reared on the same foodplant, but
27 Eucalypt reared animals showing an opposite trend. Lifetime egg production by females was
28 150% higher on bramble compared to the other host plants. Incorporating fluctuating
29 temperature ranges into experiments will further help understand the impact thermal stress
30 will have on the growth, development, performance and survival of insects in a more variable
31 climatic and nutritional landscape.

32
33 Keywords: metabolic rates; diet, temperature; climate change; sex; thermal stress; temperature
34 stress point; stick insect; phasmid.

35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68

INTRODUCTION

Host plants influence the life history of insect herbivores in terms of time taken to reach maturity, longevity, and fecundity (Clissold et al., 2009). For herbivorous insects to feed on plants, they have adapted to deal with sub-optimal nutrition, and resist and overcome plant defences; however, this comes at the cost of body repair, fat storage, survival, development and reproductive output (Arnó et al., 2009; Fürstenberg-Hägg et al., 2013). Generalist insect feeders (i.e. those insects that can feed on a range of plants across multiple families) may rely on one host plant once they have started feeding, becoming specialised on a single host plant, and even preferring specific aged leaves (e.g. older leaves) when feeding (see Blüthgen and Metzner, 2007).

In this study, we assessed the responses of a generalist leaf feeder, the phasmid *Extatosoma tiaratum* (Macleay, 1826; Phasmatodea; Phasmatidae) reared on one of three host plants since birth: tree lucerne (*Chamaecyisus palmensis*), bramble (*Rubus fruticosus*) and *Eucalyptus* species. These plant species have several chemical (e.g. macro and micro-nutrients) and morphological differences which can influence foliage digestibility, insect herbivore growth and physiology if the insects are allowed to specialise feeding on a specific host plant throughout their lives. These species are also commonly used to rear *E. tiaratum*, even though they have very different native ranges: *Eucalyptus* is native to Australia, tree lucerne and bramble are both introduced but commonly available.

Eucalyptus species (Myrtaceae) are native to Australia and are one component of *E. tiaratum* diet from eastern coastal forests (Brock and Hasenpusch, 2009). They have tough adult leaves and a chemical profile based on terpene compounds (Moore et al., 2004) as well as phenols and tannins (Macauley and Fox, 1980; Ohmart and Edwards, 1991). Exposure to these chemicals when insects are feeding can decrease the food intake, and the ability to digest proteins and cell-wall carbohydrates. Tree lucerne (Fabaceae) is native to the Canary Islands and was introduced into Australia as fodder for livestock due to its high leaf protein content (Borens and Poppi, 1990; Lambert et al., 1989; Lindeque and Rethman, 1998). As a food source for insect herbivores, it does have mechanical feeding deterrents on the leaves (hairs), and a chemical deterrent is the alkaloid sparteine (Ventura et al., 2000). The introduction of brambles (Rosaceae) from the British Isles into Australia occurred in the 1840s; primarily for their fruit (Blood, 2001). Brambles quickly escaped into the wild, becoming a significant weed, particularly in south-eastern Australia, and invaded forests where *E. tiaratum* was naturally found (CRC for Australian Weed Management, 2003). It is

69 now considered an invasive weed. Brambles are highly nutritious but have extensive
70 mechanical defences, including spikey leaves as well as thorns and prickles (Bazely et al.,
71 1991; Pellissier, 2013). Their leaves contain secondary metabolites such as flavonoids,
72 tannins and ellagic acid (Buřičová et al., 2011; Gudej and Tomczyk, 2004) which can deter
73 some insects from feeding (War et al., 2012), or may reduce their metabolic efficiency.

74 Insects reared on different host plants throughout their life may exert different natural
75 history preferences. For example, the apple maggot fly (Diptera: Tephritidae) females had a
76 higher oviposition rate on either apple or hawthorn depending on prior exposure to the
77 particular fruit (Papaj and Prokopy, 1988). Highly polyphagous species also show a host plant
78 preference once they start developing. For example, the caterpillars of *Colias philoice*
79 (Lepidoptera: Pieridae) feed on a range of Fabaceae host plants but show a feeding preference
80 for a host that it has prior experience with (Karowe, 1989).

81 Here, we examined variations in the metabolic rate ($\dot{V}CO_2$) of adults when individuals
82 feed on one of three host plants from birth until adulthood in a low fluctuating temperature
83 environment. We used adult male and female individuals of *Extatosoma tiaratum* as our
84 model to investigate whether diet affects the metabolic rate of these animals reared in
85 captivity. Phasmids, or stick and leaf insects, occur worldwide, mostly in tropical regions.
86 There are 200 known phasmid species in Australia, out of the 3000 species identified globally
87 (Brock and Hasenpusch, 2007), are primarily herbivorous and have a hemimetabolous life
88 cycle. The study species are a sexually dimorphic species with the spiny wingless females
89 considerably larger and fatter than the winged males (Zborowski and Storey, 2003). The
90 females also have abdominal margins with flattened plates and legs, which resemble leaves
91 with spines, whereas the males are mottled in colour to mimic lichen (Brock, 2001). *E.*
92 *tiaratum* can reproduce both sexually and asexually via parthenogenesis. Their eggs resemble
93 seeds with an elaiosome which are attractive to ants for this lipid-rich appendage, and this egg
94 feature appears to be an adaptation for burial by ants which protects the egg from
95 environmental hazards and predation by wasps (Hughes and Westoby, 1992). The first instar
96 nymphs are ant mimics, which allow them to escape the ant nest after hatching (Bedford,
97 1978). *E. tiaratum* flick away their eggs so there is less certainty which plants will be
98 accessible when the nymph hatches. This species of insect has been kept in captivity in
99 Australia since the 1960s (Hadlington, 1966; Korboot, 1961) and Europe since the 1970s
100 (Brock, 1992), so we know that they gain all their nutritional requirements from feeding on
101 the leaves of a variety of host plants including native species of *Acacia*, *Callicoma*,
102 *Eucalyptus*, *Melaleuca*, and *Leptospermum*. Introduced species such as holme oak, rose,

103 bramble and guava are also palatable to these insects (Brock and Fry, 1999). Being able to
104 adapt to feeding on a variety of plant species is determined by host plant exposure at an early
105 age. It may be challenging to transfer individuals to a different plant species in the later stages
106 of their development.

107 Metabolic rate is a measure of performance for all organisms. It measures the rate at
108 which an organism transforms energy and resources changes with temperature exposure and
109 body mass (Clarke, 2006; Gillooly et al., 2001). Understanding the impact of temperature on
110 an animal is vital to know, and methods such as thermolimit respirometry have been devised
111 to assess changes in metabolic rates with a constant increase in the temperature ramping rate
112 (Lighton and Turner, 2004). For insects, temperature exposure assessments have most
113 commonly measured the critical endpoint when an animal loses muscular control (Critical
114 Thermal Maximum, CT_{max}) ignoring the changes in metabolic rate up until this point.
115 Understanding how the metabolic rate of insects changes with exposure to increasing
116 temperatures is vital: metabolic rate can be used as a measure of stress resilience (Krams et
117 al., 2018), and to determine more realistic CT_{max} endpoints using thermolimit respirometry
118 techniques (Lighton and Turner 2004). As the temperature rise, an insects metabolic rate
119 (measured as $\dot{V}CO_2$ ml/h) also rises, until reaching a premortal plateau (Andrew et al., 2016;
120 Lighton and Turner, 2004). Metabolic rate responses to temperature have been measured for a
121 range of insects including ants (Andrew et al., 2016; Lighton and Turner, 2004), *Helicoverpa*
122 caterpillars (Betz and Andrew, in review 5vi19), silkworms (Boardman and Terblanche,
123 2015), and beetles (Verberk and Bilton, 2015) among others (Neven, 2000).

124 We are interested in if host plant diets affect the metabolic rate of insects at different
125 temperatures, and female fecundity. Specifically, in this study, we addressed the questions:

126

127 How do metabolic rates vary between an adult male and female *Extatosoma tiaratum*
128 exposed to increasing temperatures, after being reared on different host plant species
129 throughout their lives?

130 Does host plant influence stick insect fecundity as measured by egg production per
131 female?

132

133 MATERIAL AND METHODS

134 Stick insect's and their feeding treatments

135 Adult *Extatosoma tiaratum* feeding on a mixed diet of tree lucerne, *Eucalyptus* spp,
136 *Acacia* spp., *Agonis* and holme oak laid the eggs used in this experiment. Nymphs hatched

137 from eggs laid in May-June 2015 with emergence occurring in October 2015. Water was
138 provided twice daily via hand-misting of each cage. Eighteen individuals (nine males and nine
139 females) were reared from their first-instar to adulthood for one generation on one of three
140 different host plants (three replicate individuals per sex per host plant): bramble (*Rubus*
141 *fruticosus*), *Eucalyptus* sp. and tree lucerne (*Chamaecytisus palmensis*) in separate plastic
142 insect cages (245 x 245 x 630mm). Food was made available *ad-libitum* to each individual
143 used in this study before the sampling period and included a variety of leaf ages on freshly cut
144 foliage. All animals in the trial were reared simultaneously at varying seasonal room
145 temperatures (ranging from 12°C to 25°C), but in a similar environment of enclosure size,
146 temperature, humidity and lighting over four months.

147 Respirometry measurements

148 We used flow-through respirometry to measure the metabolic rate ($\dot{V}CO_2$) in adult *E.*
149 *tiaratum*. Atmospheric air was pumped via a HiBlow pump (HB40) through soda-lime and
150 Drierite (desiccant) columns to remove CO₂ and water (H₂O) from the air and then into two
151 mass flow control valves (Model 840, Sierra Side-Trak, Sierra Instruments Inc., Monterey,
152 USA) at a flow rate of 490 ml min⁻¹ which were regulated by a mass flow control unit (MFC-
153 2, Sable Systems). Air-flow was directed through the zero channel (cell A) of a calibrated (to
154 360 ppm CO₂ in Nitrogen) infrared CO₂-H₂O Analyzer (Li-7000, Li-Cor, Lincoln, NE, USA),
155 then over the test animal in its respirometry chamber. The respirometry chambers were put
156 into a double plastic bag and plunged into a programmable water bath (Grant, GP200-R4),
157 programmed using LABWISE software to ramp temperatures at a rate of 0.25°C min⁻¹. Air
158 continued through the animal chamber into the analyser through a second channel (Cell B)
159 which recorded the difference in CO₂ concentration of the air before and after it flowed
160 through the animal chamber, at 1-second intervals. The LI-7000 software (Version 2.0.0,
161 LiCor) records output from the CO₂-H₂O analyser.

162 Baseline air measurements were taken at the beginning and end of each trial for five
163 minutes by using an identical setup as described above, but without the test animal in the
164 respirometry chamber to correct for analyser drift. After each baseline recording, animals
165 were weighed using a Mettler Toledo XP404S balance to 0.1 mg and placed into a 500 ml
166 polypropylene chamber for flow-through respirometry. Animals were allowed to settle for ten
167 minutes (enough time for them to stop vigorously moving inside the chamber), and the CO₂
168 readings on the analyser were stabilised before recordings began. The animal chamber was
169 then submerged in a water bath, which was programmed to generate an equilibration period of
170 five minutes at 25°C, followed by a ramp at 0.25°C min⁻¹ to 42°C, followed by an

171 equilibration period at 42°C for five minutes. In total, each assay ran for 98 minutes. After
172 this time, animals were removed from the water bath and re-weighed. Diet treatments were
173 tested in a random sequence across six days. Three test animals were exposed to temperatures
174 up 50°C to determine thermolimit respirometry CT_{max} (Andrew et al., 2016; Lighton and
175 Turner, 2004). We found that CT_{max} was 46.36 ± 0.19 (s.e.) °C, and as we did not want to kill
176 the animals in this experiment, we pushed them to as close as possible to their CT_{max} without
177 death.

178 Data extraction

179 We used the program *ExpeData* Version 1.9.2 (Sable Systems Data acquisition and
180 analysis software) to extract our data. The rate of CO₂ release in ppm was corrected for
181 baseline analyser drift before been converted to ml CO₂ hour⁻¹ before any data analyses
182 occurred. We also calculated the rate of CO₂ release, $\dot{V}CO_2$, at specific temperature ‘bins’
183 (25°C, 30°C, 35°C, 40°C and 42°C with 0.5°C variation either side of the specific
184 temperature). We identified the temperature when each individual became stressed (the
185 ‘temperature stress point’) when a breakpoint was reached in the $\dot{V}CO_2$ curve: this was done
186 visually assessing each datafile in *Expedata* for a distinct change in the curve trend as the
187 temperatures were ramping.

188 Approximate digestibility of nutrients

189 The frass and representative leaf samples from each separate insect cage were collected
190 for a week over the same period when the respirometry measurements were made (see below)
191 and stored in the freezer. Both the leaf and frass samples for each diet were pooled together
192 and oven-dried at 80°C until a constant sample weight was obtained and then ground to a
193 particular size of <0.5mm. Macro- and micro-nutrient analysis were performed on the pooled
194 samples using a subsample of approximately 0.15-2.0g for each analysis. Carbon and nitrogen
195 were measured using a TruSpec Series Carbon and Nitrogen Analyser (LECO Corporation,
196 Michigan, USA). The other nutrients (Calcium, Copper, Iron, Potassium, Magnesium,
197 Manganese, Sodium, Phosphorus, Sulphur and Zinc) were measured using an Inductively
198 Coupled Plasma Optical Emission Spectrometer (ICP-OES, Model 725 Radical Viewed
199 ICPOES with a mass flow controller, Agilent Australia). Approximate digestibility of each
200 food type was calculated by subtracting the nutrients contained in the frass from the nutrients
201 contained in the leaves.

202 Egg production by females

203 We also calculated the lifetime production of eggs by females. Initially, five females
204 and four males were kept in each of three insect cages and fed consistently on one of the three

205 diets throughout their lives. Due to a few deaths the numbers in each reduced (but no less than
 206 two males and three females). Once they reached adulthood, we counted and removed eggs
 207 from the cages. The number of eggs produced/ female/ cage was used for analysis.

208 Statistical Analysis

209 A two-way ANOVA was carried out (using Datadesk 7, Data Description Inc) to test
 210 the effects of diet and sex on $\dot{V}CO_2$ (the rate of CO_2 released). As there was an effect of
 211 weight, $\dot{V}CO_2$ data was divided by the weight (mg) of each individual (units are ml/h/mg).
 212 Due to an effect of weight, an ANCOVA analysis with weight as a co-variable was
 213 inappropriate (Miller and Chapman, 2001). A two-way ANOVA was carried out (using
 214 Datadesk 7) to test the effects of diet and sex on the total $\dot{V}CO_2$. A two-way ANOVA (diet
 215 and sex) was also performed to test $\dot{V}CO_2$ of a 120 second period either side of each of five
 216 specific temperature 'bins' (25°C, 30°C, 35°C, 40°C, 42°C) and the temperature stress point
 217 (33.8±3.7 to 38.0±0.4°C). As the temperatures were ramped at 0.25°C min⁻¹, this is a 1°C
 218 temperature 'bin' with 0.5°C variation either side of the specific temperature.

219 For approximate digestibility: as the samples of frass and food were pooled for each
 220 diet. A χ^2 test was used to assess the differences among macronutrients (%) and
 221 micronutrients (ug/g) among the three host-plant diets.

222 For egg production by females, we analysed using a one-way ANOVA with the number
 223 of eggs produced/ female reared on each diet out using SigmaPlot 14 (Systat Software).

224

225 **RESULTS**

226 There was a significant effect of sex, diet and a significant interaction between sex and
 227 diet on *Extatosoma tiaratum* weight (Table 1a; Figure 1a). Females feeding on bBramble
 228 were 217% heavier than females on *Eucalyptus* sp. ($P<0.0001$), and 653% heavier than males
 229 feeding on Bramble ($P<0.0001$); Females feeding on tree lucerne were also 183% heavier
 230 than females feeding on *Eucalyptus* sp. ($P=0.0013$) and 661% heavier than males feeding on
 231 Tree Lucerne ($P<0.0001$); and females feeding on *Eucalyptus* sp. were 328% heavier than
 232 males feeding on *Eucalyptus* sp. ($P=0.0049$).

233 All individuals of the stick insect species *Extatosoma tiaratum* tested for their metabolic
 234 response displayed a cyclical gas exchange pattern. Overall as the temperature was ramped
 235 higher, there was a greater gas exchange for each individual.

236

237 Sex influences overall $\dot{V}CO_2$.

238 Male individuals had on average (\pm s.e.) a higher $\dot{V}\text{CO}_2$ ($4.13\pm 0.37 - 5.21\pm 0.52$
 239 ml/h/mg) than females ($3.17\pm 1.71 - 5.11\pm 1.32$ ml/h/mg) for all three food types (Figure 1b).
 240 However, sex and diet did not have a significant effect on $\dot{V}\text{CO}_2$ for the whole sampling
 241 period (see Table 1b).

242

243 Sex and diet influences on $\dot{V}\text{CO}_2$ at different temperatures

244 We tested $\dot{V}\text{CO}_2$ of both sexes of *E. tiaratum* at different ‘binned’ temperatures and
 245 found that at lower temperatures (25°C and 30°C) there was no significant difference (Table
 246 2a,b, Figure 2a,b). At 35°C, males feeding on bramble and tree lucerne had a higher $\dot{V}\text{CO}_2$
 247 than the females in the same diet type. There was a significant interaction (Figure 2c) between
 248 diet and sex for females feeding on *Eucalyptus* sp. compared with females feeding on tree
 249 lucerne ($P<0.0001$) and bramble ($P=0.009$). There were also significant differences found
 250 between male and female individuals feeding on bramble ($P<0.038$) and those feeding on tree
 251 lucerne ($P<0.001$). At the highest temperatures (40°C and 42°C), sex was found to be
 252 significantly different with $\dot{V}\text{CO}_2$ in male individuals significantly higher than those in female
 253 individuals ($P<0.0001$, Figures 2d and 2e). Temperature stress points ranged from (33.8 ± 3.7
 254 to $38.0\pm 0.4^\circ\text{C}$) and were not significantly higher for males than females and did not differ
 255 significantly between diet types (Figure 2f; Table 2).

256

257 Approximate digestibility of nutrients

258 Of the seven macronutrients assessed (Table 3), all showed relatively consistent
 259 changes among the diets, and there was no significant difference among diets and percentage
 260 of each macronutrient ($\chi^2 = 0.98$, d.f. = 12, $p = 0.99$). Of the five micronutrients assessed
 261 (Table 3) there was a significant difference between diet and micronutrient concentration (χ^2
 262 = 398.5, d.f. = 8, $p = <0.00001$). Approximate digestibility of magnesium was negative for
 263 tree lucerne but positive for *Eucalyptus* sp. and negligible for bramble; was highly positive for
 264 sodium, and but highly negative for bramble and *Eucalyptus* sp.; and zZinc positive for
 265 bramble, negative for *Eucalyptus* sp. and negligible for tree lLucerne.

266

267 Egg production by females

268 Stick insect females feeding on bramble produced nearly 150% more eggs per female
 269 than females feeding on *Eucalyptus* and tree lucerne ($F_{2,6} = 31.15$, $P < 0.001$; Figure 1c).
 270 Female stick insects reared on *Eucalyptus* and tree lucerne produced a similar number of eggs
 271 (574 ± 33.2 eggs).

272

273

DISCUSSION

274

275

276

277

278

279

280

281

282

283

We investigated the metabolic rate response ($\dot{V}\text{CO}_2$) of adult *Extatosoma tiaratum* when exposed to thermolimit respirometry, after being reared on three different host plant species in captivity throughout their lives. We found that males had a higher total $\dot{V}\text{CO}_2$ than females for all three host species. $\dot{V}\text{CO}_2$ at lower temperatures were not affected by sex or diet type. At higher temperatures, $\dot{V}\text{CO}_2$ was affected by the sex of the animal and the diet that they were reared on. At 35°C, $\dot{V}\text{CO}_2$ was affected by sex and diet. Males that fed on bramble, and tree lucerne, had a higher $\dot{V}\text{CO}_2$ than females reared on the same host plant diet. Whereas females had a higher $\dot{V}\text{CO}_2$ than males reared on the *Eucalyptus* sp. diet. We also found that all stick insect individuals in this study exhibited a continuous gas exchange cycle which supports previous work on the metabolic rates of *E. tiaratum* (Marais et al., 2005).

284

285

286

287

288

289

290

291

292

293

294

Metabolic rate ($\dot{V}\text{CO}_2$) is influenced by several variables including activity level, age, size, sex, feeding status and breeding status (Waters and Harrison, 2012). Identifying a difference in $\dot{V}\text{CO}_2$ between insects reared on different food sources may indicate that nutrition has an impact on morphology, fitness and energy budget availability (Terblanche et al., 2004, 2005). In crickets, males have a higher $\dot{V}\text{CO}_2$ as they have more demanding performance activities such as calling and aggressive behaviour (Kolluru et al., 2004). Male *E. tiaratum* have costs associated with flight and seeking out the wingless females to mate with. Metabolic rates at higher temperatures may be affected by other factors other than food nutrient content, such as reproductive costs in *E. tiaratum* females. However, egg production was higher for the females reared on bramble, identifying that it is a more nutritious food source out of the three tested, and that egg production does not influence metabolic rate.

295

296

297

298

299

300

301

302

303

304

305

All animals in this trial were of a similar age (i.e. adults) and were reared in the same environment. The impact of nutrition availability was visibly more marked in females between groups than for males. While males attained final moult and maturity over the three diet groups before females, the females fed on bramble reached maturity faster than females fed on *Eucalyptus* sp (Silcocks *pers. obs.*). Weights of female individuals varied between each diet group, probably as a result of having different nutrient availability. Diet quality can have an impact on physiological functions and reproductive outputs (Naya et al., 2007; Niitepõld et al., 2014; Portman et al., 2015; Tan et al., 2013) as well as growth rates (Clissold et al., 2009). We found that nutrients varied between the different diets. While carbon amounts were similar across the diets, nitrogen was highest in the tree lucerne leaves. Food quality can impact on the physiological and life-history of some insects (Naya et al., 2007). High protein

306 diets improve body condition and fecundity whereas high carbohydrate diets can reduce
307 reproductive output due to the lack of nutrients available (Naya et al., 2007); and nutrient
308 restriction may increase survival (Naya et al., 2007; Nütepöld et al., 2014).

309 Although we did not measure leaf characteristics and physiology, we know that
310 herbivores make preference decisions of *Eucalyptus* leaf-feeding based on leaf age (Ohmart
311 and Edwards, 1991) and leaf toughness (Malishev and Sanson, 2015). Newly flushed foliage
312 lack physical defences that older leaves have and are generally higher in nitrogen (Ohmart
313 and Edwards, 1991). Leaf toughness and herbivore leaf preference can affect rates of growth,
314 development and performance of insects (Clissold et al., 2009; Clissold and Simpson, 2015;
315 Sanson et al., 2001). In this study, we did not assess individual leaf preferences among the
316 host plant types. For other host plant species, the age of the leaf is critical. Young leaves have
317 higher chemical defences against herbivores (Junker et al., 2008) and host plant preference
318 can also influence mating choices (Nosil et al., 2002; Papaj and Prokopy, 1988) and leaf
319 quality choices (Sandlin and Willig, 1993). Specialists feeding on specific host plants prefer
320 young leaves to old ones whereas generalist feeders prefer old leaves (Blüthgen and Metzner,
321 2007); *E. tiaratum* will eat most plant foliage offered to them (Brock and Hasenpusch, 2009).

322 Host plant usage will play a key role in enabling stick insects to adapt to a warmer and
323 more variable climate. For some species, populations that use different host plants may
324 diverge in morphology (body shape and size), and change their behaviour, as in the walking
325 stick insect *Timema cristinae* (Nosil et al., 2002). Increased metabolic rate leads to an
326 increased demand for energy resources (Dillon et al., 2010): here when male stick insects
327 become exposed to temperatures at 40°C and higher, metabolic rate increased significantly
328 across all food types. We know that elevated CO₂ levels reduce the nutrient value of leaves,
329 and this leads to higher consumption of foliage by herbivores (DeLucia et al., 2012). For the
330 Phasmatodea there has been a depauperate amount of research carried out on how they will
331 respond to climatic change: either directly via climate or indirectly via host plant chemistry
332 change (Andrew et al., 2013). We have demonstrated that higher temperatures can result in an
333 increase in $\dot{V}CO_2$ for male and female individuals of *E. tiaratum*. Incorporating fluctuating
334 temperature ranges into experiments (e.g. Ghaedi and Andrew, 2016; Holley and Andrew,
335 2019a, b) will help understand the impact that exposure to thermal extremes will have on the
336 growth, development, performance and survival of insects in a changing climate (Andrew,
337 2013; Andrew and Terblanche, 2013; Harris et al., 2018; Hoffmann et al., 2019).

338

339 **ACKNOWLEDGEMENTS**

340 We thank Melbourne Zoo for providing the eggs of *Extatosoma tiaratum* and the enclosures
341 used to rear them. Nicolas Meyer prepared leaf and frass samples for nutrient analysis. Partial
342 funding for this research came from the Australian Research Council grant DP160101561 to
343 N. R. A.

344

345 REFERENCES

- 346 Andrew, N.R., 2013. Population dynamics of insects: impacts of a changing climate, in:
347 Rohde, K. (Ed.), *The Balance of Nature and Human Impact*. Cambridge University
348 Press, pp. 311-323.
- 349 Andrew, N.R., Ghaedi, B., Groenewald, B., 2016. The role of nest surface temperatures and
350 the brain in influencing ant metabolic rates. *J Therm Biol* 60, 132-139.
- 351 Andrew, N.R., Hill, S.J., Binns, M., Bahar, M.H., Ridley, E.V., Jung, M.-P., Fyfe, C., Yates,
352 M., Khusro, M., 2013. Assessing insect responses to climate change: What are we
353 testing for? Where should we be heading? *PeerJ* 1, e11.
- 354 Andrew, N.R., Terblanche, J.S., 2013. The response of insects to climate change, in:
355 Salinger, J. (Ed.), *Living in a Warmer World: How a changing climate will affect our*
356 *lives*. David Bateman Ltd Auckland, pp. 38-50.
- 357 Arnó, J., Castañé, C., Riudavets, J., Gabarra, R., 2009. Risk of damage to tomato crops by
358 the generalist zoophytophagous predator *Nesidiocoris tenuis* (Reuter) (Hemiptera:
359 Miridae). *Bulletin of Entomological Research* 100, 105-115.
- 360 Bazely, D.R., Myers, J.H., da Silva, K.B., 1991. The Response of Numbers of Bramble
361 Prickles to Herbivory and Depressed Resource Availability. *Oikos* 61, 327-336.
- 362 Bedford, G.O., 1978. Biology and Ecology of the Phasmatodea. *Annual Review of*
363 *Entomology* 23, 125-149.
- 364 Betz, A., Andrew, N.R., in review 5vi19. Influence of non-lethal doses of natural
365 insecticides Spinetoram and Azadirachtin on *Helicoverpa punctigera* (native budworm,
366 Lepidoptera: Noctuidae) under laboratory conditions. *Austral Entomology*.
- 367 Blood, K., 2001. *Environmental Weeds: A field guide for SE Australia*. CH Jerram &
368 Associates-Science Publishers, Victoria Australia.
- 369 Blüthgen, N., Metzner, A., 2007. Contrasting leaf age preferences of specialist and
370 generalist stick insects (Phasmida). *Oikos* 116, 1853-1862.
- 371 Boardman, L., Terblanche, J.S., 2015. Oxygen safety margins set thermal limits in an insect
372 model system. *The Journal of Experimental Biology* 218, 1677-1685.

- 373 Borens, F.M.P., Poppi, D.P., 1990. The nutritive value for ruminants of tagasaste
374 (Chamaecytisus palmensis), a leguminous tree. Animal Feed Science and Technology
375 28, 275-292.
- 376 Brock, P.D., 1992. Rearing and Studying Stick and Leaf-Insects. The Amateur
377 Entomologists' Society, Feltham. The Amateur Entomologist 22.
- 378 Brock, P.D., 2001. Studies on the Australasian stick-insect genus *Extatosoma* Gray
379 (Phasmida: Phasmatidae: Tropoderinae: Extatosomatini). Journal of Orthoptera
380 Research 10, 303-313.
- 381 Brock, P.D., Fry, R., 1999. The amazing world of stick and leaf-insects. Amateur
382 Entomologist's Society 26.
- 383 Brock, P.D., Hasenpusch, J., 2007. Studies on the Australian stick insects (Phasmida),
384 including a checklist of species and bibliography. Zootaxa, 1-84.
- 385 Brock, P.D., Hasenpusch, J.W., 2009. The Complete Field Guide to Stick and leaf Insects of
386 Australia. CSIRO Publishing, Collingwood, Victoria.
- 387 Buřičová, L., Andjelkovic, M., Čermáková, A., Réblová, Z., Jurček, O., Kolehmainen, E.,
388 Verhé, R., Kvasnička, F., 2011. Antioxidant capacity and antioxidants of strawberry,
389 blackberry, and raspberry leaves. Czechoslovak Journal of Food Science 29, 181-189.
- 390 Clarke, A., 2006. Temperature and the metabolic theory of ecology. Functional Ecology 20,
391 405-412.
- 392 Clissold, F.J., Sanson, G.D., Read, J., Simpson, S.J., 2009. Gross vs. net income: How plant
393 toughness affects performance of an insect herbivore. Ecology 90, 3393-3405.
- 394 Clissold, F.J., Simpson, S.J., 2015. Temperature, food quality and life history traits of
395 herbivorous insects. Current Opinion in Insect Science 11, 63-70.
- 396 CRC for Australian Weed Management, 2003. Weed Management Guide: Blackberry
397 *Rubus fruticosus* aggregate.
398 [https://www.environment.gov.au/biodiversity/invasive/weeds/publications/guidelines/w](https://www.environment.gov.au/biodiversity/invasive/weeds/publications/guidelines/works/pubs/r-fruticosus.pdf)
399 [ons/pubs/r-fruticosus.pdf](https://www.environment.gov.au/biodiversity/invasive/weeds/publications/guidelines/works/pubs/r-fruticosus.pdf).
- 400 DeLucia, E.H., Nabity, P.D., Zavala, J.A., Berenbaum, M.R., 2012. Climate change:
401 Resetting plant-insect interactions. Plant Physiology 160, 1677-1685.
- 402 Dillon, M.E., Wang, G., Huey, R.B., 2010. Global metabolic impacts of recent climate
403 warming. Nature 467, 704-706.
- 404 Fürstenberg-Hägg, J., Zagrobelny, M., Bak, S., 2013. Plant defense against insect
405 herbivores. International Journal of Molecular Sciences 14, 10242-10297.

- 406 Ghaedi, B., Andrew, N.R., 2016. The physiological consequences of varied heat exposure
407 events in adult *Myzus persicae*: A single prolonged exposure compared to repeated
408 shorter exposures. PeerJ 2016.
- 409 Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size
410 and temperature on metabolic rate. Science 293, 2248-2251.
- 411 Gudej, J., Tomczyk, M., 2004. Determination of Flavonoids, Tannins and Ellagic acid in
412 leaves from *Rubus L.* species. Archives of Pharmacal Research 27, 1114-1119.
- 413 Hadlington, P., 1966. Parthenogenesis and diapause in the eggs of the phasmatid
414 *Extatosoma tiaratum* (MacLeay). The Journal of the Entomological Society of Australia
415 (N.S.W) 3, 59-65.
- 416 Harris, R.M.B., Beaumont, L.J., Vance, T.R., Tozer, C.R., Remenyi, T.A., Perkins-
417 Kirkpatrick, S.E., Mitchell, P.J., Nicotra, A.B., McGregor, S., Andrew, N.R., Letnic,
418 M., Kearney, M.R., Wernberg, T., Hutley, L.B., Chambers, L.E., Fletcher, M.S.,
419 Keatley, M.R., Woodward, C.A., Williamson, G., Duke, N.C., Bowman, D.M.J.S.,
420 2018. Biological responses to the press and pulse of climate trends and extreme events.
421 Nature CC 8, 579-587.
- 422 Hoffmann, A.A., Rymer, P.D., Byrne, M., Ruthof, K.X., Whinam, J., McGeoch, M.,
423 Bergstrom, D.M., Guerin, G.R., Sparrow, B., Joseph, L., Hill, S.J., Andrew, N.R.,
424 Camac, J., Bell, N., Riegler, M., Gardner, J.L., Williams, S.E., 2019. Impacts of recent
425 climate change on terrestrial flora and fauna: Some emerging Australian examples.
426 Aust. Ecol. 44, 1-23.
- 427 Holley, J., Andrew, N.R., 2019a. Experimental warming alters the relative survival and
428 emigration of two dung beetle species from an Australian dung pat community. Aust.
429 Ecol. 44, 800-811.
- 430 Holley, J., Andrew, N.R., 2019b. Experimental warming disrupts reproduction and dung
431 burial by a ball rolling dung beetle. Ecol. Entomol. 44, 206-216.
- 432 Hughes, L., Westoby, M., 1992. Capitula on stick insect eggs and elaiosomes on seeds:
433 convergent adaptations for burial by ants. Functional Ecology 6, 642-648.
- 434 Junker, R.R., Itioka, T., Bragg, P.E., Blüthgen, N., 2008. Feeding preferences of Phasmids
435 (Insecta: Phasmida) in a Bornean dipterocarp forest. Raffles Bulletin of Zoology 56,
436 445-452.
- 437 Karowe, D.N., 1989. Facultative monophagy as a consequence of prior feeding experience:
438 behavioral and physiological specialization in *Colias philodice* larvae. Oecologia 78,
439 106-111.

- 440 Kolluru, G.R., Chappell, M.A., Zuk, M., 2004. Sex differences in metabolic rates in field
441 crickets and their dipteran parasitoids. *Journal of Comparative Physiology B:*
442 *Biochemical, Systemic, and Environmental Physiology* 174, 641-648.
- 443 Korboot, K., 1961. Observations on the Life Histories of the Stick Insects *Acrophylla*
444 *tessellata* Gray and *Extatosoma tiaratum* Macleay. *University of Queensland Papers* 1,
445 161-169.
- 446 Krams, I., Trakimas, G., Kecko, S., Elferts, D., Krams, R., Luoto, S., Rantala, M.J., Mänd,
447 M., Kuusik, A., Kekäläinen, J., Jöers, P., Kortet, R., Krama, T., 2018. Linking
448 organismal growth, coping styles, stress reactivity, and metabolism via responses
449 against a selective serotonin reuptake inhibitor in an insect. *Scientific reports* 8, 8599-
450 8599.
- 451 Lambert, M.G., Jung, G.A., Harpster, H.W., Lee, J., 1989. Forage shrubs in North Island hill
452 country 4. Chemical composition and conclusions. *New Zealand Journal of Agricultural*
453 *Research* 32, 499-506.
- 454 Lighton, J.R.B., Turner, R.J., 2004. Thermolimit respirometry: an objective assessment of
455 critical thermal maxima in two sympatric desert harvester ants, *Pogonomyrmex rugosus*
456 and *P-californicus*. *Journal of Experimental Biology* 207, 1903-1913.
- 457 Lindeque, J.P., Rethman, N.F.G., 1998. The nutritive value of Tasgasate, a leguminous
458 fodder tree, in marginal summer rainfall areas of South Africa. *Southern African*
459 *Forestry Journal* 182, 51-54.
- 460 Macauley, B.J., Fox, L.R., 1980. Variation in total phenols and condensed tannins in
461 *Eucalyptus*: leaf phenology and insect grazing. *Australian Journal of Ecology* 5, 31-35.
- 462 Malishev, M., Sanson, G.D., 2015. Leaf mechanics and herbivory defence: How tough
463 tissue along the leaf body deters growing insect herbivores. *Austral Ecology* 40, 300-
464 308.
- 465 Marais, E., Klok, C.J., Terblanche, J.S., Chown, S.L., 2005. Insect gas exchange patterns: A
466 phylogenetic perspective. *J Exp Biol* 208, 4495-4507.
- 467 Miller, G.A., Chapman, J.P., 2001. Misunderstanding analysis of covariance. *Journal of*
468 *Abnormal Psychology* 110, 40 - 48.
- 469 Moore, B.D., Wallis, I.R., Palá-Paúl, J., Brophy, J.J., Willis, R.H., Foley, W.J., 2004.
470 *Antiherbivore Chemistry of Eucalyptus--Cues and Deterrents for Marsupial Folivores.*
471 *Journal of Chemical Ecology* 30, 1743-1769.

- 472 Naya, D.E., Lardies, M.A., Bozinovic, F., 2007. The effect of diet quality on physiological
473 and life-history traits in the harvestman *Pachylus paessleri*. *Journal of Insect Physiology*
474 53, 132-138.
- 475 Neven, L.G., 2000. Physiological responses of insects to heat. *Postharvest Biology and*
476 *Technology* 21, 103-111.
- 477 Nütepöld, K., Perez, A., Boggs, C.L., 2014. Aging, Life span, And energetics under adult
478 dietary restriction in lepidoptera. *Physiological and Biochemical Zoology* 87, 684-694.
- 479 Nosil, P., Crespi, B.J., Sandoval, C.P., 2002. Host-plant adaptation drives the parallel
480 evolution of reproductive isolation. *Nature* 417, 440-443.
- 481 Ohmart, C.P., Edwards, P.B., 1991. Insect Herbivory on Eucalyptus. *Annual Review of*
482 *Entomology* 36, 637-657.
- 483 Papaj, D.R., Prokopy, R.J., 1988. The effect of prior adult experience on components of
484 habitat preference in the apple maggot fly (*Rhagoletis pomonella*). *Oecologia* 76, 538-
485 543.
- 486 Pellissier, F., 2013. Early physiological responses of *Abies alba* and *Rubus fruticosus* to
487 ungulate herbivory. *Plant Ecology* 214, 127-138.
- 488 Portman, S.L., Kariyat, R.R., Johnston, M.A., Stephenson, A.G., Marden, J.H., 2015.
489 Cascading effects of host plant inbreeding on the larval growth, muscle molecular
490 composition, and flight capacity of an adult herbivorous insect. *Functional Ecology* 29,
491 328-337.
- 492 Sandlin, E.A., Willig, M.R., 1993. Effects of age, sex, prior experience, and intraspecific
493 food variation on diet composition of a tropical folivore (Phasmatodea: Phasmatidae).
494 *Environmental Entomology* 22, 625-633.
- 495 Sanson, G., Read, J., Aranwela, N., Clissold, F., Peeters, P., 2001. Measurement of leaf
496 biomechanical properties in studies of herbivory: Opportunities, problems and
497 procedures. *Austral Ecology* 26, 535-546.
- 498 Tan, X.L., Wang, S., Zhang, F., 2013. Optimization an Optimal Artificial Diet for the
499 Predatory Bug *Orius sauteri* (Hemiptera: Anthocoridae). *PLoS ONE* 8.
- 500 Terblanche, J.S., Klok, C.J., Chown, S.L., 2004. Metabolic rate variation in *Glossina*
501 *pallidipes* (Diptera: Glossinidae): Gender, ageing and repeatability. *Journal of Insect*
502 *Physiology* 50, 419-428.
- 503 Terblanche, J.S., Klok, C.J., Chown, S.L., 2005. Temperature-dependence of metabolic rate
504 in *Glossina morsitans morsitans* (Diptera, Glossinidae) does not vary with gender, age,
505 feeding, pregnancy or acclimation. *J Ins Phys* 51, 861-870.

- 506 Ventura, M.R., Castanon, J.I.R., Muzquiz, M., Mendez, P., Flores, M.P., 2000. Influence of
507 alkaloid content on intake of subspecies of *Chamaecytisus proliferus*. *Animal Feed*
508 *Science and Technology* 85, 279-282.
- 509 Verberk, W.C.E.P., Bilton, D.T., 2015. Oxygen-limited thermal tolerance is seen in a
510 plastron-breathing insect and can be induced in a bimodal gas exchanger. *The Journal of*
511 *Experimental Biology* 218, 2083-2088.
- 512 War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S., Sharma,
513 H.C., 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and*
514 *Behavior* 7.
- 515 Waters, J.S., Harrison, J.F., 2012. Insect metabolic rates, in: Sibly, R.M., Brown, J.H.,
516 Kodric-Brown, A. (Eds.), *Metabolic Ecology: A Scaling Approach*. Wiley-Blackwell,
517 pp. 198-211.
- 518 Zborowski, P., Storey, R., 2003. *A Field Guide to Insects in Australia*, 2nd ed. Reed New
519 Holland, Sydney.
- 520
- 521

522 **Table 1.** The results of a two-way ANOVA testing the effects of sex and diet on the (a)
 523 adult weight, and (b) total metabolic rate ($\dot{V}\text{CO}_2$). Significant factors in **bold**.

524

Factor	df	SS	MS	F-ratio	P-value
(a) Adult weight					
Diet	2	6.95 x 10⁷	3.47 x 10⁷	13.26	<0.0001
Sex	1	5.17 x 10⁸	5.17 x 10⁸	197.36	<0.0001
Diet*Sex	2	6.58 x 10⁷	3.29 x 10⁷	12.55	<0.0001
Error	12	3.14 x 10 ⁷	2.62 x 10 ⁷		
(b) Total volume of CO ₂ released					
Diet	2	5.08	2.54	1.82	0.20
Sex	1	1.70	1.70	1.23	0.29
Diet*Sex	2	2.43	1.21	0.87	0.44
Error	12	16.72	1.39		

525

526

527 **Table 2.** The results of a two-way ANOVA testing the effects of diet and sex on the
 528 metabolic response at different temperatures (a-e) and the temperature stress point (f).
 529 Significant factors in **bold**.

530

	Factor	df	SS	MS	F-ratio	P-value
(a) 25°C	Diet	2	12.08	6.04	1.53	0.26
	Sex	1	18.03	18.03	4.56	0.054
	Diet*Sex	2	5.13	2.56	0.65	0.54
	Error	12	47.46	4.00		
(b) 30°C	Diet	2	2.60	1.30	1.09	0.37
	Sex	1	2.37	2.37	1.98	0.18
	Diet*Sex	2	6.95	3.47	2.91	0.09
	Error	12	14.31	1.19		
(c) 35°C	Diet	2	0.79	0.39	3.70	0.06
	Sex	1	2.33	2.33	21.89	<0.0001
	Diet*Sex	2	4.09	2.05	19.25	<0.0001
	Error	12	1.28	0.11		
(d) 40°C	Diet	2	2.71	1.36	0.73	0.50
	Sex	1	40.04	40.04	21.68	<0.0001
	Diet*Sex	2	7.61	3.81	2.06	0.17
	Error	12	22.16	1.85		
(e) 42°C	Diet	2	4.42	2.21	2.21	0.15
	Sex	1	33.07	33.07	33.07	<0.0001
	Diet*Sex	2	2.81	1.40	1.40	0.28
	Error	12	12.00			
(f) Temperature stress point	Diet	2	5.89	2.95	0.42	0.66
	Sex	1	32.54	32.54	4.67	0.052
	Diet*Sex	2	3.55	1.78	0.26	0.78
	Error	12	83.62	6.97		

531

532

533 **Table 3.** Approximate digestibility (subtraction of nutrients contained in the frass from
 534 nutrients contained in leaves) of macronutrients and micronutrients from three different diet
 535 plants (Bramble, *Eucalyptus* sp. and Tree Lucerne).

Sample type	Macronutrients							Micronutrients				
	C (%) [*]	N (%)	Ca (%)	K (%)	Mg (%)	P (%)	S (%)	Cu (ug/g)	Fe (ug/g)	Mn (ug/g)	Na (ug/g)	Zn (ug/g)
Bramble	-2.64	1.01	0.47	-0.32	-0.24	0.15	0	2.5	-144	9	-317.8	9.6
<i>Eucalyptus</i>	-3.02	0.28	0.17	-1.3	-0.12	0.1	-0.05	2.2	-51.6	228.1	-511.8	-16.7
Tree Lucerne	-2.52	1.32	0.06	-1.49	-0.08	0.1	-0.07	5.3	-61.3	-93.1	225.4	-1

536 *C=Carbon; N=Nitrogen; Ca=Calcium; K=Potassium; Mg=Magnesium; P=Phosphorus;
 537 S=Sulfur; Cu=Copper; Fe=Iron; Mn=Manganese; Na=Sodium; Zn=Zinc.

538

539

540

541

542

543

544

545 Figure 1 – *Extatosoma tiaratum* adult weight (a), adult rate of CO₂ production ($\dot{V}CO_2$) (b),
546 and average egg production/ female (c), fed on one of three different diets: Bramble,
547 *Eucalyptus* sp. or Tree Lucerne.

548

549 Figure 2 – The rate of CO₂ production ($\dot{V}CO_2$) by adult *Extatosoma tiaratum* at different
550 temperatures: 25, 30, 35, 40, and 42°C and the temperature stress point. Different letters
551 signify significant differences (ANOVA, $P > 0.050$).

552



