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1 **How to stand the heat? Post-stress nutrition and developmental**  
2 **stage determine insect response to a heat wave**

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22 **Abstract**

23 Organisms are increasingly confronted with intense and long-lasting heat waves. In insects,  
24 the effects of heat waves on individual performance can vary in magnitude both within (e.g.  
25 from one larval instar to another) and between life stages. However, the reasons underlying  
26 these stage-dependent effects are not fully understood. There are several lines of evidence  
27 suggesting that individual ability to withstand a heat stress depends on mechanisms based on  
28 nutrition and supporting energetically physiological stress responses. Hence, we tested the  
29 hypothesis that the efficiency of these food-based buffering mechanisms may vary between  
30 different larval instars of a phytophagous insect. Using larvae of the moth *Lobesia botrana*,  
31 we examined the importance of post-stress food quality in insect response to a non-lethal heat  
32 wave at two distinct larval instars. Three major conclusions were drawn from this work. First,  
33 heat waves induced an overall decline in larval performance (delayed development, depressed  
34 immunity). Second, food quality primarily mediated the insect's ability to respond to the heat  
35 stress: the reduction in performance following heat wave application was mostly restricted to  
36 individuals with access to low-quality food after the heat stress. Third, larval instars differed  
37 in their susceptibility to this combination of thermal and food stressors, but conclusions about  
38 the instar being the most vulnerable differed in a trait-specific manner. In a global warming  
39 context, this study may shed additional light on the combination of direct and indirect  
40 (through alteration of plant nutritional value) effects of rising temperatures on the ecology and  
41 the evolution of phytophagous insects.

42 **Keywords**

43 Climate change; European grapevine moth; food quality; heat wave; larval nutrition; stage-  
44 specific effects.

## 45 **1. Introduction**

46 Ongoing climate change is recognised as a major threat imperilling biodiversity, from  
47 individual survival to ecosystem structure and functioning (Bellard et al., 2012; Parmesan,  
48 2006; Walther et al., 2002). This complex phenomenon cannot simply be reduced to an  
49 overall rise in mean temperature, but also involves more subtle changes in temperature  
50 variability at different temporal scales (Easterling et al., 1997; IPCC, 2014; Meehl and  
51 Tebaldi, 2004). Specifically, a widely recognised feature of the predicted climate alterations  
52 for the coming century is the considerable increase in the frequency, duration and magnitude  
53 of extreme thermal events, especially heat waves (Easterling et al., 2000; Meehl and Tebaldi,  
54 2004; Perkins et al., 2012). Although there is no consensual climatological definition of a heat  
55 wave (Meehl and Tebaldi, 2004; Smith et al., 2013), it often refers to as extended periods of  
56 exposure to abnormally high temperatures (for definitions and indexes, see Smith et al.,  
57 2013).

58 As ectothermic animals, insects have only a limited capacity to keep their body  
59 temperature constant in a changing thermal environment (Angilletta et al., 2010; Huey and  
60 Stevenson, 1979). Therefore, they are particularly vulnerable to the threat posed by extreme  
61 high temperatures, the effects of which span across communities, populations and individuals  
62 (González-Tokman et al., 2020; Ma et al., 2021; Stoks et al., 2017). At the community level,  
63 heat waves might affect the species assemblage through changes in the relative dominance of  
64 competing species and alteration of interspecific relationships (Ma et al., 2015b; Sentis et al.,  
65 2013). At the population level, they might lead to a decline in demographic rates and  
66 modification of the genetic constitution of natural populations (Chiu et al., 2015; Rodriguez-  
67 Trelles et al., 2013). Finally, at the individual level, they may unleash a wide diversity of  
68 biological responses (González-Tokman et al., 2020; Ma et al., 2021). Most of the time, this  
69 consists of an overall reduction in several traits related to individual performance, such as

70 growth and survival of immature stages (Chiu et al., 2015; Ma et al., 2015a; Potter et al.,  
71 2011), adult lifespan and reproductive success (Zhang et al., 2015a, 2015b, 2013), immunity  
72 (Fischer et al., 2014) and the accumulation of fat reserves (Fischer et al., 2014).

73         Within a single insect species, individual responses to a heat wave can vary from a  
74 permanent decrease in performance to a total recovery from the physiological damage and  
75 fitness costs arising from exposure to high temperature spikes (Zhang et al., 2015a, 2015b;  
76 Zhao et al., 2019). This variability can in part be explained by the timing of when the heat  
77 wave occurs during insect ontogeny and, subsequently, the life stage affected by this heat  
78 event. Indeed, the available body of knowledge indicates ontogenetic variation in heat  
79 tolerance both within (e.g. from one larval instar to another) and between developmental  
80 stages (Chiu et al., 2015; Klockmann et al., 2017; Zhang et al., 2015b, 2015a). For instance,  
81 heat tolerance increases exponentially in larvae of the tropical butterfly *Bicyclus anynana*  
82 (Lepidoptera: Nymphalidae) as they develop and get closer to metamorphosis (Klockmann et  
83 al., 2017). In the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae), a brief  
84 larval exposure to 40°C for a few hours caused detrimental effects on adult reproduction, and  
85 the magnitude of these negative effects was increasingly greater as the heat stress occurred at  
86 a developmental stage closer to the adult stage (Zhang et al., 2015a). The modularity of insect  
87 development could explain these stage-dependent effects of heat wave (Potter et al., 2011).  
88 This hypothesis lies on the discrepancies between insect life stages in terms of morphological  
89 (e.g. body size, morphology), physiological (e.g. accumulated resources, heat shock response)  
90 and behavioural traits (e.g. thermoregulation) to account for the ontogenetic variation in heat  
91 tolerance (Bowler and Terblanche, 2008; Klockmann et al., 2017; Ma and Ma, 2012).

92         The individual ability to recover from heat injuries should hinge on the post-stress  
93 acquisition of energetic resources essential for physiological stress responses to take place  
94 (Klockmann et al., 2017; Van Dievel et al., 2017; Zhang et al., 2015a). For example, feeding

95 responses (e.g. increased food intake and growth rate) may specifically occur following  
96 exposure to heat waves and determine their consequences in terms of energy storage (fat  
97 content) and immunity (Van Dievel et al., 2017). In this regard, it is generally understood that  
98 food restriction can exacerbate the negative impacts of a heat stress on insect performance  
99 (e.g. survival, immunity, reproductive success): performance drops when food is limited while  
100 needed the most (Adamo et al., 2012; Karl et al., 2011). Nonetheless, whether or not the  
101 efficiency of such buffering mechanisms based on nutrition may differ across ontogenetic  
102 stages to account for the stage-dependency of heat wave impacts on individual performance is  
103 still an open question. One could expect the efficiency of these mechanisms to vary in a stage-  
104 dependent fashion because physiological requirements and feeding activity should be highly  
105 variable within and between developmental stages (Zhang et al., 2015a). Furthermore,  
106 postulating that recovering from heat stress through nutrition is a time-consuming process,  
107 there should be less and less time available to express an appropriate feeding response when  
108 the heat wave happens later and later during insect development.

109         Here, we propose an experimental study to examine the role played by nutrition in the  
110 heat stress response at different steps of larval development in a phytophagous insect. Our aim  
111 was to test the hypothesis of ontogenetic variation in the efficiency of food-based buffering  
112 mechanisms following application of a non-lethal heat wave. This was done by means of a  
113 full factorial design, enabling to quantify the fitness impacts of the heat wave relative to a  
114 control thermal treatment for different combinations of larval instar and food quality available  
115 for post-stress recovery. Indeed, we manipulated the ability of the larvae to recover from heat  
116 loads by means of feeding through the variation in the larval instar exposed to the heat stress  
117 (determining the time allowed for recovery) and the variation in the nutritional quality of the  
118 food available after the heat wave (determining the time needed to regenerate from the heat  
119 damage). We used larvae of the European grapevine moth *Lobesia botrana* (Lepidoptera:

120 Tortricidae), one of the most destructive insect pests for winemaking activities in the  
121 Palearctic region (Thiéry et al., 2018). This species is a capital breeder, meaning that its  
122 feeding activity is restricted to larvae. As such, feeding during the larval stage represents the  
123 unique opportunity to repair the physiological damage induced by exposure to a heat wave.  
124 To adopt an integrative approach of individual larval performance, we measured: (i) the body  
125 amounts of four major energetic compounds stocked before pupation (proteins, lipids, soluble  
126 carbohydrates, glycogen), and (ii) the basal levels of different key immune effectors –  
127 haemocyte load and activity of the phenoloxidase/prophenoloxidase system – in the context of  
128 host-parasitoid interactions (Carton et al., 2008; Poyet et al., 2013). Indeed, the encapsulation  
129 of parasitoid eggs is ensured by a cellular response involving the recruitment of haemocytes  
130 (immune cells of invertebrates) (Lavine and Strand, 2002). Then, parasitoid eggs are killed  
131 through a melanotic response triggered by the enzyme phenoloxidase (PO), which is produced  
132 from its inactive precursor – the prophenoloxidase (PPO) – stored in the haemocytes and the  
133 haemolymph (González-Santoyo and Córdoba-Aguilar, 2012).

134         We expect complex patterns of response to heat wave conditions, depending on the  
135 combination of larval instar being affected and the quality of the nutritional diet being  
136 provided after the heat wave (i.e. depending on the duration of exposure to the different diets  
137 after the heat wave). We expect young larvae (characterised by small body size, low mobility,  
138 few accumulated resources) to be the most vulnerable to the heat wave and subsequent heat-  
139 driven decline in performance. Indeed, upper lethal temperature increases with ontogeny in *L.*  
140 *botrana*: from 32°C for eggs to 34°C and 36°C for early (first and second) and late larval  
141 instars (third to fifth), respectively (Briere and Pracros, 1998). Moreover, survival rates of *L.*  
142 *botrana* cohorts decrease more steeply during the first days of larval development at 30°C by  
143 comparison with cooler conditions (20°C), thereby indicating the high susceptibility to heat  
144 conditions of larvae at their early steps of development in this species (Moshtaghi Maleki et

145 al., 2016). As such, their response to the heat wave should highly depend on the nutritional  
146 value of the food available post-stress, especially since they are exposed to post-stress food  
147 treatments for a relatively long duration. Specifically, the negative impacts of the heat wave  
148 on larval performance should display a lower magnitude for larvae fed with a high-quality  
149 diet after the heat wave (most likely to fully or at least partially recover from heat damage)  
150 than those provisioned with a low-quality diet. Inversely, we expect larvae closer to pupation  
151 to be the most resistant to the heat wave and to be exposed to post-stress food treatments for a  
152 relatively short duration, such that larval nutrition should not significantly modulate their  
153 response to the heat wave.

## 154 **2. Materials and methods**

### 155 *2.1. Insect stock and rearing methods*

156 This study was conducted using a laboratory stock of *L. botrana* maintained without diapause  
157 under standard controlled conditions (temperature:  $20 \pm 0.5^\circ\text{C}$ , relative humidity:  $60 \pm 5\%$ ,  
158 photoperiod: L17:D6 and 1 h of dusk, luminosity: 600 lx during photophase and 100 lx at  
159 dusk). All individuals used in the experiments originated from a large stock with a high  
160 density of adults (several thousands of caged moths per week) supplemented annually with  
161 wild individuals sampled in nearby vineyards (Semillon cultivar:  $44.46^\circ\text{N}$ ;  $0.32^\circ\text{E}$ ) to enhance  
162 the genetic diversity. In this regard, it has been shown that larvae from this laboratory stock  
163 display similar patterns of investment in basal immunity and ability to respond to a bacterial  
164 infection compared with field-collected individuals (Vogelweith et al., 2014).

165 During a one-month period, a total of 2843 larvae were continuously collected from  
166 the insect stock. Throughout the experiments, these newly born individuals (age < 24 h) were  
167 regularly and haphazardly assigned to one of the eight experimental blocks (each defined by  
168 the combination of one thermal treatment, larval instar, and food treatment) constituting the



169 full factorial design involved in this work (see the subsection 2.4.). Using a fine paintbrush,  
170 they were delicately transferred to microtubes filled with 1.5 ml of rearing medium for which  
171 the recipe was developed from Thiéry and Moreau (2005) (Table 1). Hereafter, this nutritional  
172 diet will be referred to as the augmented medium (AM) considering its high nutritive value for  
173 *L. botrana* larvae, since it provides higher larval and adult performance compared with semi-  
174 synthetic food derived from plant material (grapevine or alternative hosts) (Moreau et al.,  
175 2006; Muller et al., 2015; Thiéry and Moreau, 2005; Vogelweith et al., 2011). Feeding the  
176 freshly collected larvae with AM was a way to limit larval mortality and possible artificial  
177 selection. Moreover, it ensured that individuals assigned to the heat wave treatment were in  
178 good health before being subjected to the heat wave. Larvae were raised individually in  
179 microtubes to avoid larval intraspecific competition for food (Thiéry et al., 2014). Once  
180 deposited in their rearing tube, all larvae were immediately transferred to the normal thermal  
181 regime described below (see the subsection 2.2.). Thermal regimes were run with climate  
182 chambers (ST 2/2 BASIC, Pol-Eko Aparatura). Abiotic conditions inside these devices were  
183 controlled (temperature  $\pm 0.1^{\circ}\text{C}$ ,  $50 \pm 10\%$  relative humidity, L18: D6, 650 lx). Temperatures  
184 were checked once a week with independent data loggers (Hobo, Onset Computer  
185 Corporation) to ensure they conformed to the daily thermal cycle programmed during the  
186 entire experiments.

187 **Table 1** Composition of the two larval diets used in the experiments (for a final volume of  
 188 1000 ml). Freshly collected larvae were fed with the augmented medium (AM) as a highly  
 189 nutritive food until they were distributed among the two food treatments (at a larval age of 7  
 190 days or 14 days). One subset was kept on AM, and another group of larvae was transferred to  
 191 depleted medium (DM) as a poorly nutritive food until the end of the experiments. The recipe  
 192 of DM was elaborated by halving the quantity of plant material (maize flour and wheat germs)  
 193 by comparison with AM, without further modification regarding the other ingredients.

	<b>Augmented medium (AM)</b>	<b>Depleted medium (DM)</b>
Water (ml)	<b>1000</b>	<b>1000</b>
Agar (g)	<b>15</b>	<b>15</b>
Maize flour (g)	<b>86.6</b>	<b>43.3</b>
Wheat germs (g)	<b>41.3</b>	<b>20.6</b>
Beer yeast (g)	<b>45.5</b>	<b>45.5</b>
Ascorbic acid (g)	<b>6</b>	<b>6</b>
Mineral salt (g)	<b>3.4</b>	<b>3.4</b>
Pyrimethanil (mg)	<b>128</b>	<b>128</b>
Benzoic acid (mg)	<b>2.7</b>	<b>2.7</b>
Nipagin® (mg)	<b>2.8</b>	<b>2.8</b>
95% ethanol (ml)	<b>5</b>	<b>5</b>

194

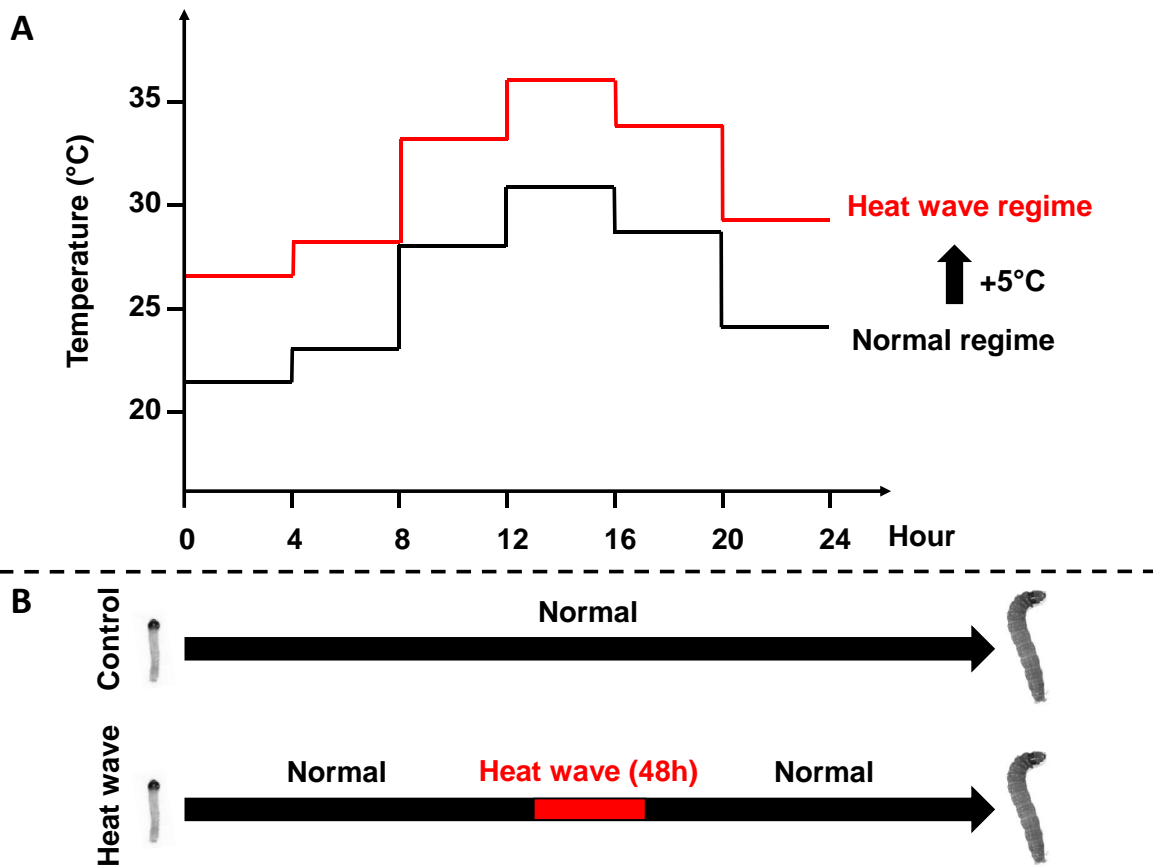
195 2.2. *Thermal treatment: temperature regimes*

196 Thermal treatment was comprised of two levels labelled as control and heat wave. As a first  
197 step, all individuals used in the experiments were placed in the normal thermal regime  
198 immediately after their initial deposition in rearing tubes. This daily fluctuating temperature  
199 regime simulated a mean summer day (from 15 July to 15 August) likely to be observed in  
200 Burgundy (Eastern France, 47.27°N; 5.09°E; altitude = 219 m) by the end of the century  
201 (2081-2100) (for more details, see Iltis et al., 2019). It was constituted by a daily thermal  
202 cycle comprised of six temperature segments (each lasting 4 h) and included a photoperiod of  
203 L16:D8 to reproduce natural day and night durations during summertime (Fig. 1A).

204 Individuals assigned to the control thermal treatment were not exposed to the heat  
205 wave and therefore left developing in the normal regime until the measurements were taken.  
206 Individuals undergoing the heat wave treatment were transferred from incubators running the  
207 normal regime to incubators running the heat wave regime at a given standardised time, and  
208 maintained in the heat wave regime for 48 h precisely, after which they returned to the normal  
209 regime (Fig. 1B). The heat wave regime was built based on the normal regime with a +5°C  
210 increment in mean temperature (i.e. a constant increase of 5°C over all temperature segments)  
211 (Fig. 1A). From a climatological perspective, our experimental simulation of a heat wave  
212 matches the definition provided by Tan et al. (2006), namely a period of at least one day  
213 during which the daily maximum temperature exceeds 35°C. From an ecological perspective,  
214 the heat wave regime included temperatures exceeding by more than 5°C the thermal  
215 optimum for development in *L. botrana* larvae (28-30°C), thus highlighting the stressful  
216 nature of these extreme (though non-lethal) thermal conditions for *L. botrana* larvae  
217 (Gutierrez et al., 2012). By comparing larval performance under expected ‘normal’ and heat  
218 wave conditions associated with climate change projections, our study enables to explore the  
219 consequences of future heat events on *L. botrana* in the context of anthropogenic climate

220 disturbance, as these heat waves are anticipated to become an increasingly important  
221 component of future climates.

222            Provided that *L. botrana* larvae require 17-18 days on average to complete all five  
223 larval instars within the normal regime (Iltis et al., 2019), the duration of exposure to the heat  
224 wave regime was set to two days (approximately 10% of the total larval development  
225 duration) for two main reasons. The first was to maximise the age difference between ‘young’  
226 (second instar) and ‘old’ larvae (fourth instar) at the time of the heat wave application while  
227 avoiding temporal overlap in the exposure to the heat wave between these two larval age  
228 groups. The second reason was to avoid measurements on larvae that had initiated pupation  
229 after the occurrence of the heat wave, because *L. botrana* larvae may respond to an  
230 environmental stress by accelerating their development and reaching earlier pupation  
231 (Vogelweith et al., 2013a).



232

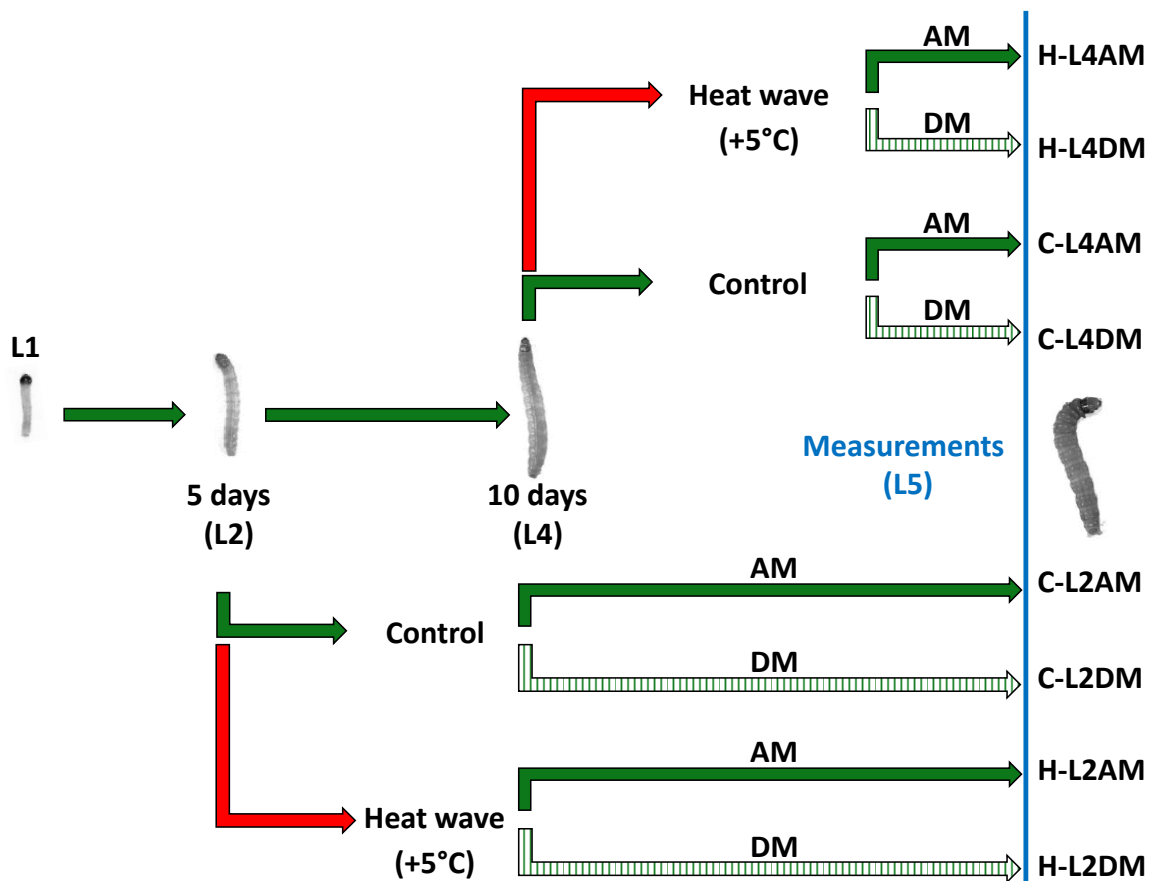
233 **Fig. 1.** Description of the thermal treatments (temperature regimes) applied. Two daily  
 234 fluctuating regimes were used in the experiments: the normal regime (black) and the heat  
 235 wave regime (red), built by translating the normal regime according to a constant +5°C  
 236 increment over the six thermal segments (A). Larvae assigned to the control thermal treatment  
 237 were simply kept within the normal regime during the whole course of the experiments (14  
 238 days), while those undergoing the heat wave treatment were first placed in the normal regime,  
 239 then subjected at one particular larval age to a heat wave extending over 48h, before returning  
 240 to the normal regime until the experiments ended (B).

241 2.3. Larval instar and food treatment: diet compositions

242 Larvae were subjected to heat wave conditions at two distinct larval ages: 5 days,  
243 corresponding to the second larval instar (L2), or 10 days, corresponding to the fourth larval  
244 instar (L4). These two larval ages were selected to minimise variability in developmental  
245 stage within each group, based on available information about the relative durations of the  
246 different larval instars in *L. botrana* at different temperatures (Moshtaghi Maleki et al., 2016;  
247 Thiéry and Moreau, 2005). During the heat wave, larvae were supplied with AM as they had  
248 not been changed from rearing tube since the start of the experiments. Once the heat wave  
249 ended (i.e. 7 days after the initial deposition for L2 or 12 days after the initial deposition for  
250 L4), the larvae were gently manipulated with a fine paintbrush to be extracted from their  
251 rearing tube and placed into a new tube filled with either AM as a high nutritional quality diet  
252 or depleted medium (DM) as a low nutritional quality diet. The depleted medium recipe was  
253 designed based on a previous work (Thiéry and Moreau, 2005) and was defined by a 50%  
254 decrease in plant material by comparison with AM (Table 1). This resulted in an alteration of  
255 diet nutritional value from both quantitative (i.e. dilution effect, since less plant material was  
256 added to the same volume of medium) and qualitative perspectives (i.e. reduction in the  
257 carbohydrate-to-protein ratio, since the plant material is the main source of carbohydrates in  
258 the diet recipe). Importantly, such an alteration of diet nutritional value is known to severely  
259 depress *L. botrana* larval performance, ranking DM as a particularly poorly nutritive food for  
260 the insect (Moreau et al., 2006; Thiéry and Moreau, 2005). In order to standardise the stress  
261 associated with the manipulation, all larvae were transferred to a new rearing tube – either at 7  
262 or 12 days – including individuals that did not change thermal regime (i.e. individuals  
263 maintained in the normal thermal regime all throughout the experiments) and those that did  
264 not experience a shift in diet quality (i.e. individuals raised on AM all throughout the  
265 experiments).

266 *2.4. Overall experimental design*

267 To summarise, we compared the performance of larvae continuously reared in normal thermal  
268 conditions (control thermal treatment) to larvae briefly exposed to a non-lethal heat wave  
269 (heat wave treatment), and manipulated both the stage (larval instar: L2 or L4) at which the  
270 heat wave occurred during larval development and the nutritional quality of the food available  
271 after the heat wave (food treatment: AM or DM). Hence, this work was performed using a full  
272 factorial design with eight experimental blocks resulting from the combination of three factors  
273 (thermal treatment, larval instar and food treatment) of two levels each (Fig. 2).



274

275 **Fig. 2.** General design of the experiment with the eight experimental blocks resulting from the  
 276 combination of thermal treatment (control versus heat wave), larval instar (L2 versus L4) and  
 277 food treatment (augmented medium versus depleted medium). First instar larvae (L1, age < 1  
 278 day) were placed in the normal thermal regime (green) and fed with augmented medium (AM,  
 279 solid arrow) until they were split between the different food treatments. At a larval age of 5  
 280 days (corresponding to the second larval instar, L2) or 10 days (fourth larval instar, L4), they  
 281 were either kept in the normal regime (control thermal treatment) or briefly exposed to the  
 282 heat wave regime (red) for two days before returning to the normal regime (heat wave  
 283 treatment). Subsequently, at a larval age of either 7 days (L2) or 12 days (L4), all individuals  
 284 were placed in the normal regime, isolated in new rearing tube and either kept supplied with  
 285 AM or transferred to depleted medium (DM, hatched arrow) until the measurements were  
 286 taken. All measurements were carried out at a larval age of 14 days (corresponding to the fifth  
 287 and last larval instar, L5).



288 *2.5. General procedure for measurements*

289 All energetic and immune measurements were taken at a larval age of 14 days after initial  
290 deposition in rearing tubes, corresponding to the fifth and last larval instar in this species. For  
291 standardisation purposes, individuals displaying a developmental stage that was too advanced  
292 (larvae starting to pupate) were discarded for the subsequent measurements. As a measure of  
293 development, the pupation rate at 14 days was then calculated for each of the eight  
294 experimental blocks. It was expressed as the ratio of the number of individuals removed from  
295 the experiments following the above selection procedure to the total number of larvae alive at  
296 14 days. Of note, larval survival up until the measurements was very high (90-96%) across the  
297 eight experimental blocks. At the time of the measurements, larvae were haphazardly  
298 distributed among two experimental groups, because the energetic and immune traits could  
299 not have been measured on the same individuals. In Group 1, larvae were flash-frozen and  
300 thereafter their energetic budgets were quantified through colorimetric assays. In Group 2,  
301 haemolymph samples were collected to assess the basal levels of different immune parameters  
302 (haemocyte load and activity of the PO/PPO system).

303 *2.6. Group 1: Energetic reserves*

304 The energetic budgets were quantified for a subsample comprised of 327 individuals: 162  
305 larvae assigned to the control thermal treatment ( $n = 43$  for L2AM,  $n = 45$  for L2DM,  $n = 37$   
306 for L4AM,  $n = 37$  for L4DM) and 165 larvae assigned to the heat wave treatment ( $n = 41$  for  
307 L2AM,  $n = 42$  for L2DM,  $n = 33$  for L4AM,  $n = 49$  for L4DM). Fourteen day-old larvae were  
308 placed in empty microtubes and instantaneously frozen by immersion in liquid nitrogen.  
309 These samples were kept at  $-80^{\circ}\text{C}$  until the energetic assays were run. Larvae were weighed  
310 with a microbalance (Quintix 35-15, Sartorius) to measure fresh body mass ( $\pm 0.1$  mg), before  
311 being crushed with stainless steel beads (tubes shaken for 90 s at 25 Hz) in 180  $\mu\text{l}$  of aqueous  
312 lysis buffer solution (composition: 100 mM  $\text{KH}_2\text{PO}_4$ , 1 mM dithiothreitol DTT, 1 mM

313 ethylenediaminetetraacetic EDTA, pH = 7.4). The energetic budgets of the larvae were  
314 estimated through quantification of the body amounts of the four major energetic  
315 compartments in insects (proteins, lipids, soluble carbohydrates and glycogen) using  
316 spectrophotometric methods (for a detailed description of these methods, see Iltis et al., 2019).  
317 This procedure enables to simultaneously evaluate, in the same individual, the body levels of  
318 the four nutrients (Iltis et al., 2019). For the proteins, a Bradford approach involving a DC  
319 Protein Assay kit (Bio-Rad) was used. For the three other energetic compounds, biochemical  
320 measurements were based on the protocol developed by Foray et al. (2012), which has been  
321 successfully used to evaluate the energetic state of *L. botrana* larvae in a recent work (Iltis et  
322 al., 2019). Absorbance readings were carried out at room temperature in a microplate reader  
323 (Versamax, Molecular Devices), with the SoftMax Pro software (version 4.0, Molecular  
324 Devices). The protein and lipid measurements were taken in two replicates, and individuals  
325 for whom the coefficient of variation calculated over these two values exceeded 30% were  
326 excluded from the experiments (Iltis et al., 2019). The measurements displayed a high  
327 repeatability overall, as suggested by the low percentages of individuals removed from the  
328 protein dataset ( $1/327 = 0.3\%$ ) and the lipid dataset ( $17/327 = 5.2\%$ ).

### 329 2.7. Group 2: Immune parameters

330 Haemolymph samples were taken on a subset of 314 larvae: 161 individuals assigned to the  
331 control thermal treatment ( $n = 43$  for L2AM,  $n = 45$  for L2DM,  $n = 35$  for L4AM,  $n = 38$  for  
332 L4DM), and 153 individuals assigned to the heat wave treatment ( $n = 38$  for L2AM,  $n = 37$   
333 for L2DM,  $n = 32$  for L4AM,  $n = 46$  for L4DM). Larvae were chilled on ice for 15 min to  
334 facilitate manipulation, before being photographed using a stereomicroscope at a  
335 magnification of 12.5x (Stemi 508, Zeiss). These photographic data were subsequently  
336 analysed with the ZEN imaging software (version 2.3, Zeiss) to measure the distance between  
337 the most distant lateral sides of the head capsule (precision:  $\pm 1 \mu\text{m}$ ) as a proxy of individual

338 body size and to control for potential allometric effects on larval immunity (Vogelweith et al.,  
339 2013b). For this experimental group, larval body size was measured rather than larval body  
340 mass because alive larvae – particularly fifth instar ones – are particularly active when  
341 manipulated and they are difficult to accurately weigh (Muller et al., 2015). Immediately  
342 following the acquisition of the photographic data, larvae were placed on a cold surface and  
343 pricked in the dorsal part of the abdomen to collect a 1  $\mu$ l sample of haemolymph with a cold  
344 sterile glass capillary (Hirschmann Laborgeräte). This sample was gently mixed with 20  $\mu$ l of  
345 filtered, cold phosphate-buffered saline (PBS, 10 mM, pH 7.4). A volume of 10  $\mu$ l of this  
346 solution was pipetted into a Neubauer improved haemocytometer placed under a phase  
347 contrast microscope at a magnification of 400x (Primo Star, Zeiss) in order to evaluate the  
348 haemocyte load. The remaining 11  $\mu$ l of diluted haemolymph samples were supplemented  
349 with 10  $\mu$ l of filtered PBS, flash-frozen in liquid nitrogen and stored at -80°C for later  
350 measurements of PO/PPO system activity.

351         The enzymatic activity of PO and PPO was extrapolated spectrophotometrically  
352 following the method described by Vogelweith et al. (2011). A clear distinction was made  
353 between the activity of the functional PO naturally present in the haemolymph sample (PO  
354 activity), and the potential activity of the whole PO/PPO system, i.e. the activity of both  
355 functional PO and proenzyme stock (total PO activity). This distinction allowed to assess the  
356 levels of PO activity naturally expressed by larvae at a given time alongside with the overall  
357 maximal enzymatic activity that can be potentially mobilised upon an actual infection. In  
358 addition, PO and total PO activities in *L. botrana* can exhibit different responses to a change  
359 occurring in thermal conditions, thereby providing different information about the effects of  
360 temperature on the immune function of this species (Iltis et al., 2019, 2018). The  
361 determination of the total PO activity required the transformation of PPO into PO by means of  
362 chymotrypsin addition. Frozen samples were put on ice until completely thawed and were

363 centrifuged (4000 g, 15 min, 4°C) before 2 x 5 µl of supernatant were pipetted into microplate  
364 wells. One of these two aliquots was mixed with 160 µl of diluted PBS solution (35 ml  
365 ultrapure water, 5 ml filtered PBS) to measure the PO activity, while the other was  
366 supplemented with 160 µl of chymotrypsin solution (35 ml ultrapure water, 5 ml filtered PBS,  
367 2.45 mg trypsin) to measure the total PO activity. Finally, 20 µl of L-Dopa solution (40 ml  
368 ultrapure water, 160 mg L-Dopamine) was deposited in each well as a colorimetric substrate.  
369 The enzymatic reaction was allowed to proceed at 30°C for 40 min in a microplate reader  
370 (Versamax, Molecular Devices). Absorbance readings were taken every 15 s at 490 nm.  
371 Absorbance curves were subsequently analysed with the SoftMax Pro software (version 4.0,  
372 Molecular Devices) to extract the maximum enzymatic activity  $V_{\max}$  (the maximum speed of  
373 conversion of L-Dopamine into dopachrome). This value was calculated as the maximum  
374 slope of the absorbance curve during the starting linear phase of the reaction. All immune  
375 measurements were recalculated in order to refer to 1 µl of pure haemolymph.

376 2.8. *Statistical analyses*

377 Since our aim was to quantify the impacts of the heat wave relative to the control thermal  
378 treatment taking into consideration both the time left to recover from heat damage  
379 (determined by the timing of heat wave application during ontogeny) and the nutritional value  
380 of the food provided post-stress, we merged the larval instar and food treatment into one  
381 variable with four modalities (L2AM, L2DM, L4AM, or L4DM) for the statistical analyses.  
382 This allowed us to test whether the duration of exposure to the different diets after the heat  
383 stress is a key determinant of the larval ability to stand the heat, as initially postulated.  
384 Besides, larval stage and food treatment are interrelated as they both define the different  
385 experimental conditions (i.e. duration of exposure to the different diets) faced by larvae after  
386 the heat event. Therefore, in all the statistical tests carried out, the models involved the simple  
387 effects of thermal treatment (control or heat wave), the combination of larval instar and food  
388 treatment, and the interaction between thermal treatment and instar/food combination. In  
389 addition, one covariate was included in the models to control for potential allometric effects  
390 on energetic reserves (larval body mass) or immune parameters (larval body size).

391 The pupation rate at 14 days was compared among groups using a Generalised Linear  
392 Model (GLM) with a binomial error structure coupled with a logit link function. The body  
393 amounts of the four energetic compounds (proteins, lipids, soluble carbohydrates and  
394 glycogen) were normally distributed and satisfied the assumption of homoscedasticity  
395 (homogeneity of variances) among thermal treatments and pairs of larval instar and food  
396 treatment. Hence, all these data were studied with analyses of covariance (ANCOVAs). As  
397 overdispersed count data, haemocyte load was processed using a GLM with negative binomial  
398 error structure and a log link function. Data for PO and total PO activities were not normally  
399 distributed and were best fitted by GLMs with Gamma error structure and a log link function.  
400 To tease out significant differences among the groups, Cohen's  $d$  (or Cohen's  $h$  in case of data

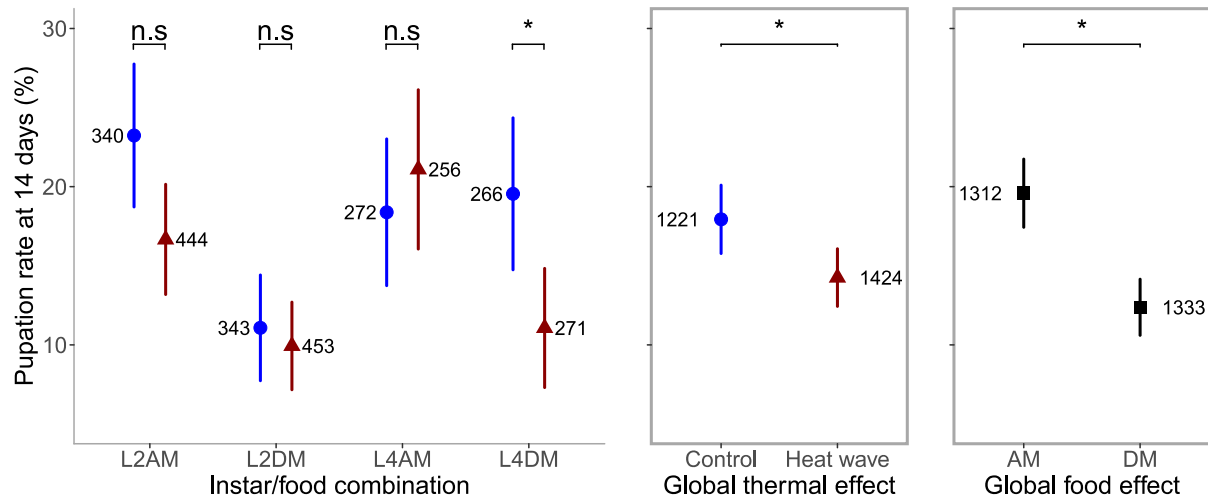
401 expressed in percentages: pupation rate at 14 days) with their bootstrapped confidence  
402 intervals (CI<sub>95%</sub>, 10,000 iterations) were calculated and reported (Nakagawa and Cuthill,  
403 2007). Congruently with our initial goal, these standardised measures of effect size enabled us  
404 (i) to quantify the overall effect of the heat wave on larval performance and (ii) to assess how  
405 the timing of when this hot event occurred (larval instar) combined with the quality of the  
406 food available for recovery (nutritional diet) might influence the importance of the heat wave  
407 effect. All statistical analyses were carried out using the R software (version 4.0.3),  
408 implemented with the following packages: *ggplot2*, *ggsignif*, *cowplot* (for drawing figures),  
409 *stats* and *CRAN* (for model analyses).

### 410 **3. Results**

#### 411 *3.1. Pupation rate at 14 days*

412 The pupation rate at 14 days was impacted by the thermal treatment (GLM binomial error  
413 structure:  $\chi^2_1 = 6.62, P = 0.01$ ) and the combination of larval instar and food treatment ( $\chi^2_3 =$   
414  $31.9, P < 0.001$ ), but not by the interaction between thermal treatment and instar/food  
415 combination ( $\chi^2_3 = 7.74, P = 0.05$ ). The simple effect of thermal treatment indicates that the  
416 heat wave delayed the development of *L. botrana* larvae: the pupation rate at 14 days after  
417 egg hatching was higher overall for individuals constantly held in the normal regime than for  
418 individuals that experienced the heat wave (Fig. 3) (Cohen's  $h = 0.101, CI_{95\%} = [0.024;$   
419  $0.178]$ ). Interestingly, this detrimental effect of the heat wave on larval development was only  
420 apparent for one modality of instar/food combination: L4DM (Figs 3, 4). This indicates that  
421 the heat wave delayed development mostly if it occurred late during larval development and if  
422 individuals were subsequently supplied with low-quality food. The simple effect of the  
423 instar/food combination on pupation rate at 14 days was mainly driven by food treatment:  
424 individuals kept on high-quality food developed faster (displayed a higher pupation rate at 14

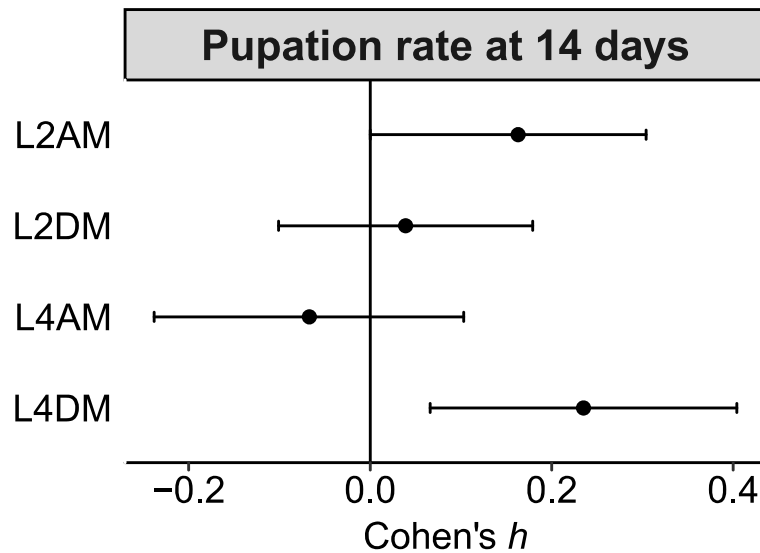
425 days after egg hatching) than those transferred to low-quality food at any larval instar (Fig. 3)  
426 (Cohen's  $h = 0.197$ ,  $CI_{95\%} = [0.121; 0.274]$ ).



427

428 **Fig. 3** Responses of the pupation rate at 14 days to the thermal treatment (blue circles:  
 429 control, red triangles: heat wave) and the instar/food combination (defined by the larval instar  
 430 at which larvae were transferred to a new rearing tube and the nutritional quality of the food  
 431 provided after the tube change), to the global effect of the thermal treatment alone, and to the  
 432 global effect of the food treatment alone. The represented values are the means  $\pm$  95% c.i.  
 433 Asterisks indicate a significant difference (Cohen's *h* significantly different from zero), n.s.  
 434 stands for non-significant and the numbers refer to the sample sizes.





435

436 **Fig. 4** Cohen's  $h$  and their 95% bootstrapped confidence intervals (10,000 iterations)  
 437 reflecting the effect size of the thermal treatment (control versus heat wave) on pupation rate  
 438 at 14 days for each of the four modalities of instar/food combination (defined by the larval  
 439 instar at which larvae were transferred to a new rearing tube and the nutritional quality of the  
 440 food provided after the tube change). Effect size is considered to be significantly different  
 441 from zero when the associated confidence interval does not bracket the zero value.

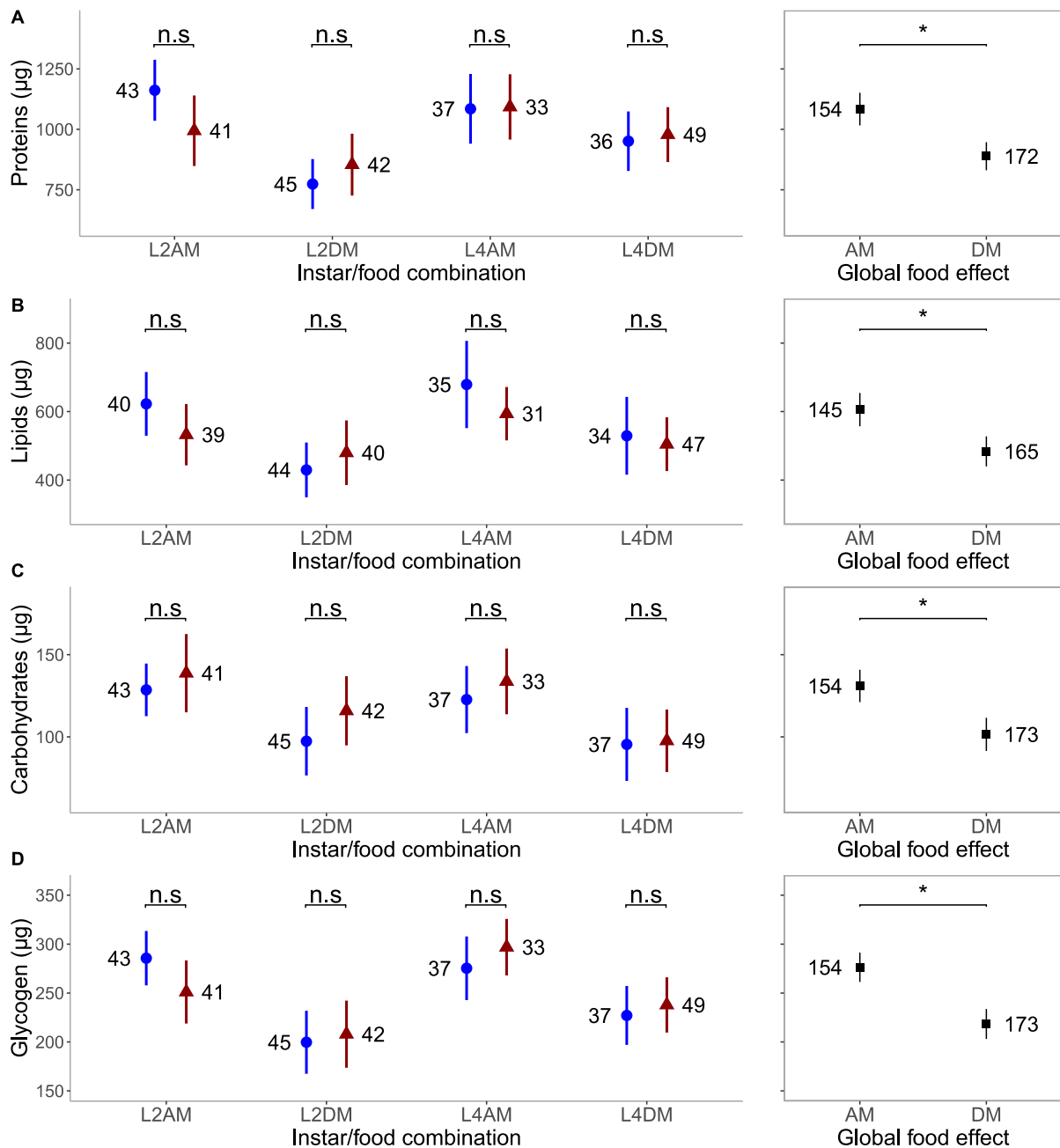
442 3.2. Group 1: Energetic reserves

443 The body levels of the four energetic compounds displayed similar patterns of response to the  
444 different variables included in the models: they were affected by the combination of larval  
445 instar and food treatment, but not by thermal treatment or by the interaction between thermal  
446 treatment and instar/food combination (Table 2). The effect of instar/food combination on the  
447 reserves was explained by food treatment: individuals provided with high-quality medium all  
448 throughout their development possessed higher body amounts of proteins, lipids, soluble  
449 carbohydrates and glycogen than individuals fed with low-quality food at any larval instar  
450 (Fig. 5A–D). Cohen’s  $d$  values remain in accordance with these statistical conclusions drawn  
451 when comparing high-quality versus low-quality food treatments (proteins:  $d = 0.484$ ,  $CI_{95\%} =$   
452  $[0.267; 0.713]$ ; lipids:  $d = 0.421$ ,  $CI_{95\%} = [0.203; 0.649]$ ; soluble carbohydrates:  $d = 0.456$ ,  
453  $CI_{95\%} = [0.234; 0.692]$ ; glycogen:  $d = 0.590$ ,  $CI_{95\%} = [0.370; 0.820]$ ). Measures of effect size  
454 did not indicate any difference in energetic reserves between the control and heat wave  
455 treatments for any of the four modalities of instar/food combination (Fig. 6). For all the  
456 energetic compounds examined, the body amounts were positively influenced by larval body  
457 mass: heavier larvae possessed higher reserves than lighter ones (Table 2) (slope values  $\pm$   
458 s.e.m., proteins:  $67.2 \pm 3.6$ ; lipids:  $42.2 \pm 3.0$ ; soluble carbohydrates:  $6.8 \pm 0.8$ ; glycogen:  
459  $16.0 \pm 0.9$ ).

460 **Table 2** Effects of thermal treatment (control or 48 h exposure to a heat wave), instar/food combination (defined by the larval instar at which  
 461 larvae were transferred to a new rearing tube and the nutritional quality of the food provided after the tube change), the interaction between these  
 462 two variables and larval body mass (energetic reserves) or body size (immune parameters) on the different larval traits measured.

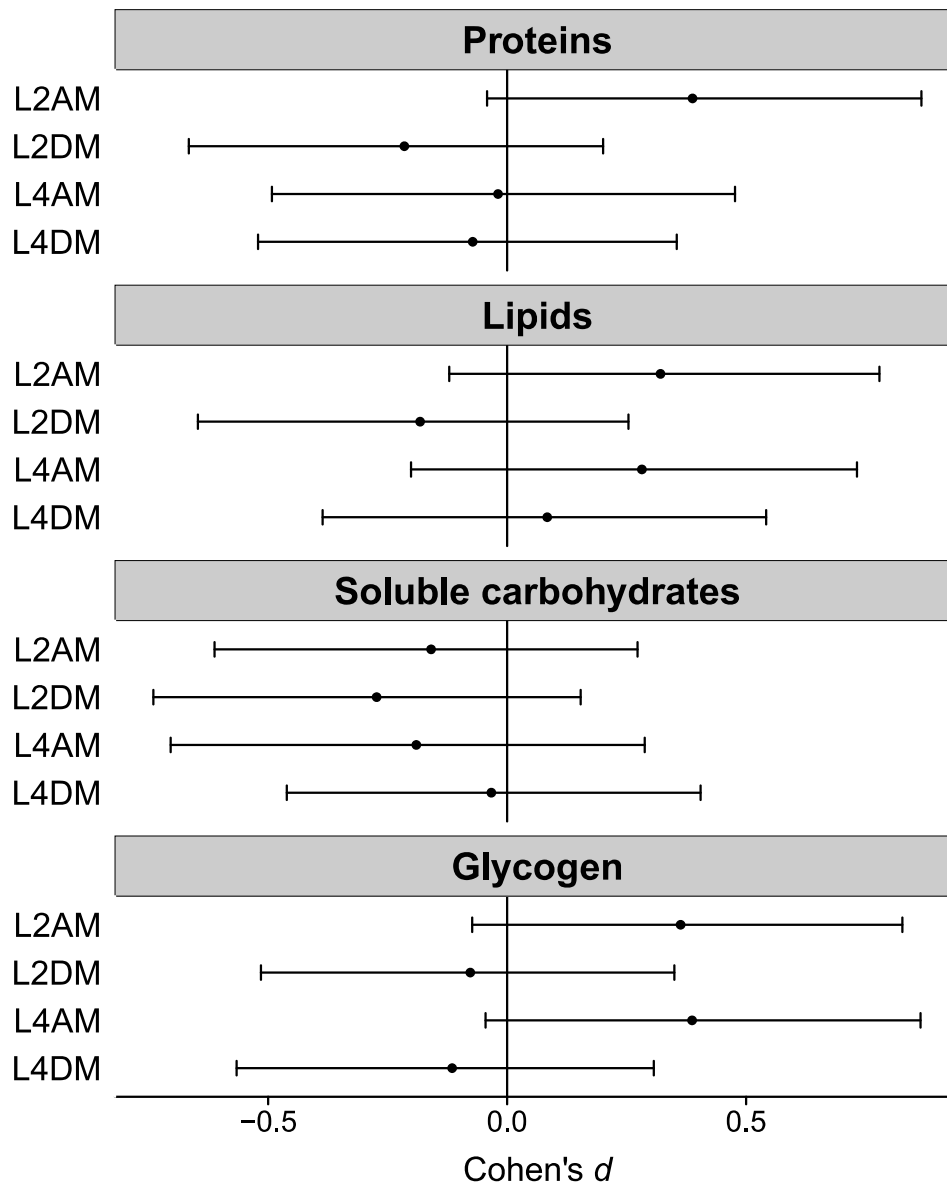
	Thermal treatment		Instar/food combination		Interaction		Covariate (mass or size)	
	<i>Test value</i>	<i>P</i>	<i>Test value</i>	<i>P</i>	<i>Test value</i>	<i>P</i>	<i>Test value</i>	<i>P</i>
<i>Energetic reserves</i>								
Proteins <sup>a</sup>	$F_{1,317} = 0.23$	0.63	$F_{3,317} = 17.6$	< <b>0.001</b>	$F_{3,317} = 2.47$	0.06	$F_{1,317} = 342$	< <b>0.001</b>
Lipids <sup>a</sup>	$F_{1,301} = 2.00$	0.16	$F_{3,301} = 9.29$	< <b>0.001</b>	$F_{3,301} = 1.08$	0.36	$F_{1,301} = 197$	< <b>0.001</b>
Soluble carbohydrates <sup>a</sup>	$F_{1,318} = 1.83$	0.18	$F_{3,318} = 7.75$	< <b>0.001</b>	$F_{3,318} = 0.63$	0.60	$F_{1,318} = 78.6$	< <b>0.001</b>
Glycogen <sup>a</sup>	$F_{1,318} = 0.01$	0.93	$F_{3,318} = 21.8$	< <b>0.001</b>	$F_{3,318} = 2.66$	0.05	$F_{1,318} = 307$	< <b>0.001</b>
<i>Immune parameters</i>								
Haemocyte load <sup>b</sup>	$\chi^2_1 = 4.24$	<b>0.04</b>	$\chi^2_3 = 4.49$	0.21	$\chi^2_3 = 4.65$	0.20	$\chi^2_1 = 1.76$	0.18
PO activity <sup>c</sup>	$\chi^2_1 = 4.82$	<b>0.01</b>	$\chi^2_3 = 0.69$	0.81	$\chi^2_3 = 1.46$	0.57	$\chi^2_1 = 1.13$	0.21
Total PO activity <sup>c</sup>	$\chi^2_1 = 4.37$	< <b>0.001</b>	$\chi^2_3 = 2.28$	0.13	$\chi^2_3 = 0.38$	0.81	$\chi^2_1 = 2.99$	<b>0.01</b>

463 <sup>a</sup> ANCOVA, <sup>b</sup> GLM with negative binomial error structure, <sup>c</sup> GLM with Gamma error structure



464

465 **Fig. 5** Responses of the total body amounts of proteins (A), lipids (B), soluble carbohydrates  
 466 (C) and glycogen (D) to the thermal treatment (blue circles: control, red triangles: heat wave)  
 467 and instar/food combination (defined by the larval instar at which larvae were transferred to a  
 468 new rearing tube and the nutritional quality of the food provided after the tube change), and to  
 469 the global effect of the food treatment alone. The represented values are the means  $\pm$  95% c.i.  
 470 Asterisks indicate a significant difference (Cohen's *d* significantly different from zero), n.s.  
 471 stands for non-significant and the numbers refer to the sample sizes.

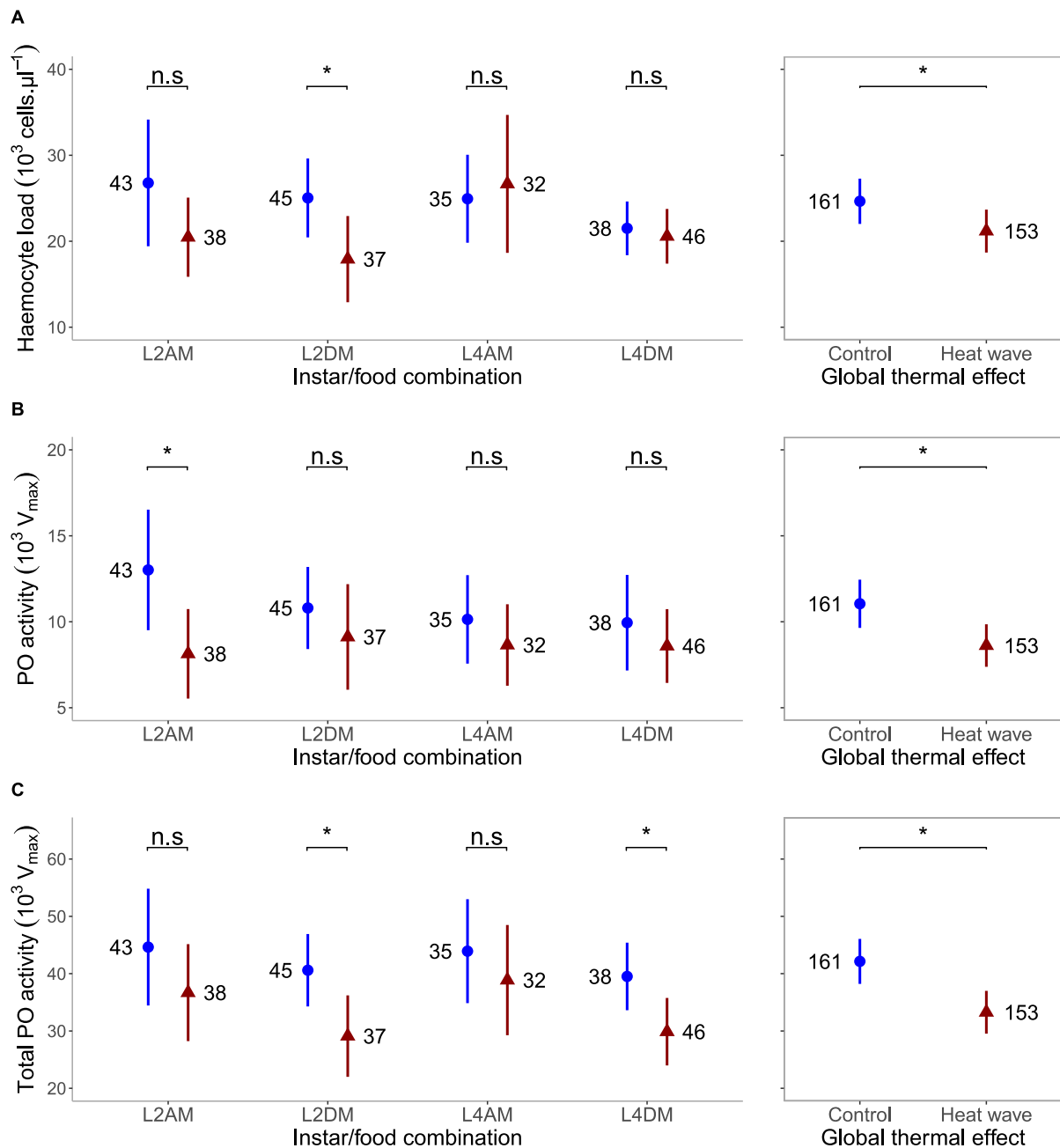


472

473 **Fig. 6** Cohen's *d* and their 95% bootstrapped confidence intervals (10,000 iterations)  
 474 reflecting the effect size of the thermal treatment (control versus heat wave) on the energetic  
 475 reserves for each of the four modalities of instar/food combination (defined by the larval  
 476 instar at which larvae were transferred to a new rearing tube and the nutritional quality of the  
 477 food provided after the tube change). Effect size is considered to be significantly different  
 478 from zero when the associated confidence interval does not bracket the zero value.

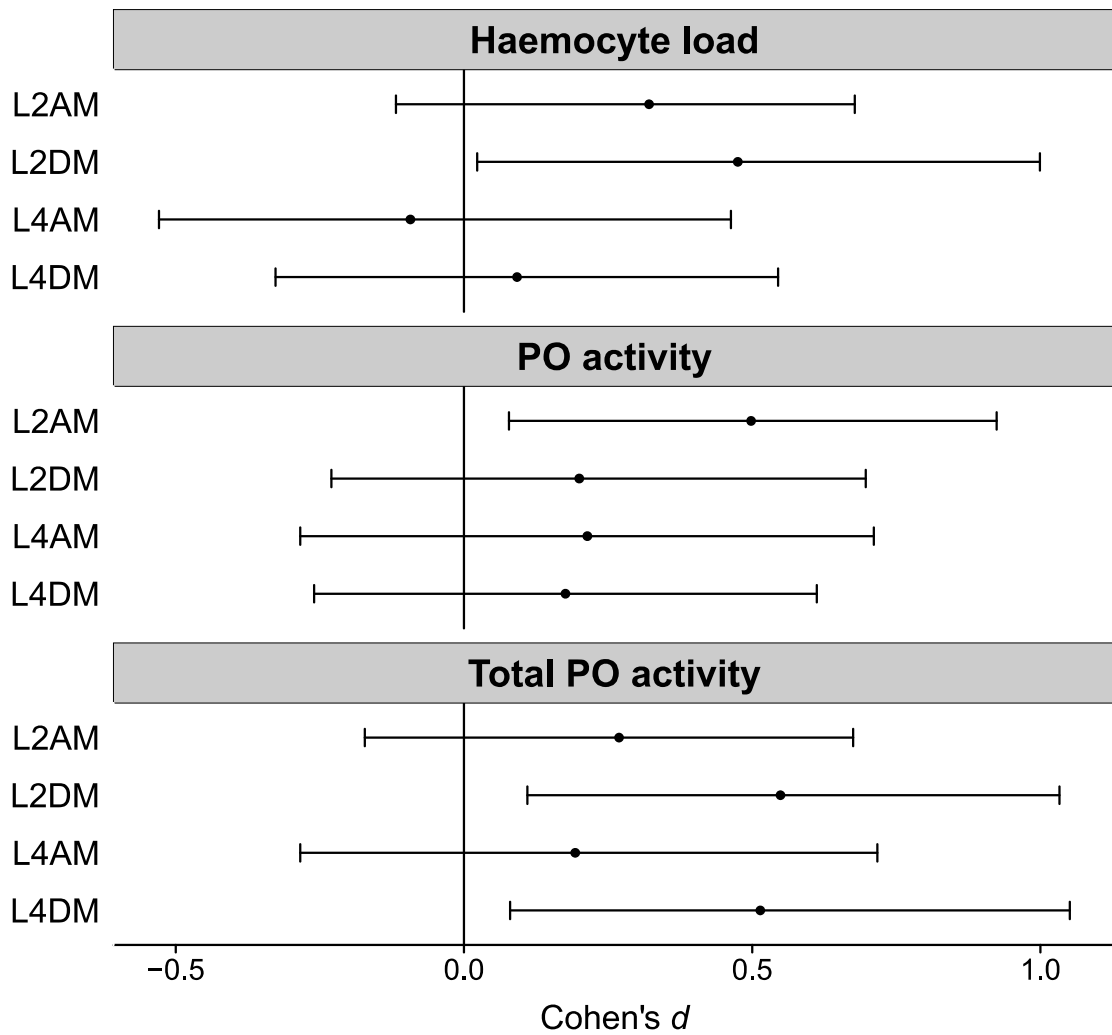
479 3.3. Group 2: Immune parameters

480 The three immune parameters inspected were all impacted by thermal treatment in the same  
481 way, but not by the combination of larval instar and food treatment or by the interaction  
482 between thermal treatment and instar/food combination (Table 2). Overall, the heat wave  
483 decreased larval basal immunity, as shown by the higher haemocyte load, PO and total PO  
484 activities for individuals continuously raised in the normal regime compared with individuals  
485 that experienced the heat wave (Fig. 7A–C). These conclusions are supported by measures of  
486 effect size calculated when comparing control versus heat wave treatments (haemocyte load:  
487  $d = 0.213$ ,  $CI_{95\%} = [0.002; 0.435]$ ; PO activity:  $d = 0.290$ ,  $CI_{95\%} = [0.068; 0.511]$ ; total PO  
488 activity:  $d = 0.366$ ,  $CI_{95\%} = [0.144; 0.594]$ ). Among the different modalities of instar/food  
489 combination, measures of effect size for the thermal treatment reveal complex patterns of  
490 immunological response to the heat wave (Fig. 8). For haemocyte load, the heat wave  
491 decreased basal immunity only if it occurred early during larval development and if  
492 individuals were fed with low-quality food after the heat stress (L2DM) (Fig. 8). For PO  
493 activity, the negative effect of the heat wave was detectible only if the heat wave happened  
494 early during larval development and if individuals were maintained on high-quality food after  
495 the heat stress (L2AM) (Fig. 8). For total PO activity, the heat wave exerted adverse effects  
496 on immunity only if individuals were subsequently provided with low-quality food,  
497 irrespective of the timing of the heat event during larval development (L2DM and L4DM)  
498 (Fig. 8). Finally, total PO activity was the only immune effector affected (positively) by larval  
499 body size: the larger the larvae, the higher the basal levels of total PO activity (slope =  $0.002$   
500  $\pm 0.0005$ ).



501

502 **Fig. 7** Responses of haemocyte load (A), phenoloxidase activity (B) and total phenoloxidase  
 503 activity (C) to the thermal treatment (blue circles: control, red triangles: heat wave) and  
 504 instar/food combination (defined by the larval instar at which larvae were transferred to a new  
 505 rearing tube and the nutritional quality of the food provided after the tube change), and to the  
 506 global effect of the thermal treatment alone. The represented values are the means  $\pm$  95% c.i.  
 507 Asterisks indicate a significant difference (Cohen's *d* significantly different from zero), n.s.  
 508 stands for non-significant and the numbers refer to the sample sizes.



509

510 **Fig. 8** Cohen's  $d$  and their 95% bootstrapped confidence intervals (10,000 iterations)  
 511 reflecting the effect size of thermal treatment (control versus heat wave) on the immune  
 512 parameters for each of the four modalities of instar/food combination (defined by the larval  
 513 instar at which larvae were transferred to a new rearing tube and the nutritional quality of the  
 514 food provided after the tube change). Effect size is considered to be significantly different  
 515 from zero when the associated confidence interval does not bracket the zero value.



#### 516 **4. Discussion**

517 Three major hierarchical conclusions can be drawn from this work, from the broadest to the  
518 most specific. First, the non-lethal heat wave applied was detrimental overall to *L. botrana*  
519 larvae, as witnessed by its pervasive negative effects on development and immunity. Second,  
520 for almost all fitness correlates impacted by the heat wave (except PO activity), the negative  
521 effect of the hot event on larval performance was only detected when larvae were exposed to  
522 low-quality food (DM) following the heat wave. However, no similar decline in performance  
523 was observed for individuals kept on high-quality food (AM), probably due to a complete  
524 recovery from the heat stress. This indicates that food quality played an important role in  
525 determining the consequences of the heat wave for larval performance (mostly development  
526 and immunity), thereby providing experimental support to the hypothesis that larval nutrition  
527 could mediate the insect response to heat wave episodes encountered during development, but  
528 with no consistent pattern across life stages. Third, clear evidence was indeed found that  
529 larval instars differed in their sensitivity to the combined thermal and food stresses above  
530 mentioned, as the negative fitness impacts of the heat wave followed by low-quality food  
531 were in most cases restricted to one larval instar only. Interestingly, conclusions regarding the  
532 larval instar being the most susceptible were highly trait-dependent: the heat wave delayed  
533 development only if it affected old larvae, while for immunity, the impacts of the heat wave  
534 were mostly detected for young larvae (haemocyte load and PO activity). Hence, contrary to  
535 our initial expectations, we did not find obvious experimental support for the assumption that  
536 larval nutrition should be more significant in the event of an early heat wave, affecting larvae  
537 at the very start of their development. In a rapidly changing climatic context, this study should  
538 contribute to increase our mechanistic understanding of how individuals and species could  
539 cope with temporary extreme heat events from physiological, ecological and evolutionary  
540 perspectives.

541 Climate projections for the coming century suggest that living organisms will face the  
542 challenge of more and more frequent, intense and increasingly longer periods of abnormally  
543 high temperatures (IPCC, 2014; Meehl and Tebaldi, 2004). In our study, we found that a 48-h  
544 exposure to a realistic heat wave elicited extensive and detrimental effects on several aspects  
545 of larval performance in the moth *L. botrana*. On one hand, the heat wave delayed larval  
546 development overall, as evidenced by a lower pupation rate at 14 days for individuals briefly  
547 subjected to a heat wave relative to those continuously reared in the normal regime. In insect  
548 species, an extended development appears to be a common pattern of individual response to a  
549 heat wave, and presumably results from the physiological damage sustained during periods of  
550 exposure to temperatures above the thermal optimum for developmental function (i.e. the  
551 temperature at which development is the fastest) (Chiu et al., 2015; Gillespie et al., 2012;  
552 Jeffs and Leather, 2014). Here, *L. botrana* larvae that experienced the heat wave regime spent  
553 12 h every day at supraoptimal temperatures, provided that the thermal optimum for  
554 development in this species lies between 28 and 30°C, at least for laboratory-reared strains  
555 (Briere and Pracros, 1998). This temporary exposure to stressful thermal conditions for two  
556 consecutive days could account for the differences in pupation rate at 14 days observed  
557 between the heat wave and control treatments. On the other hand, we found that larval  
558 immunity, including all three immune parameters examined, was depressed by the heat wave.  
559 This may arise from direct effects of temperature, whereby transient exposure to high  
560 temperature spikes might have compromised immune function in this species, as previously  
561 evidenced in case of a rise in mean temperature within rearing conditions (Iltis et al., 2019,  
562 2018). These immune differences might also be explained by indirect effects of temperature,  
563 mediated by the development rates of individuals. As stated above, individuals facing the heat  
564 wave displayed delayed development, which may have affected their immunity at time of  
565 measurements because immune parameters are known to vary throughout a given larval instar

566 in lepidopteran larvae (Beetz et al., 2008; Booth et al., 2015). Our results suggest that heat  
567 waves might have persistent implications for the defensive abilities of a host's vulnerable  
568 stage against its natural enemies. Such extreme thermal events could therefore predispose  
569 natural populations of *L. botrana* to infection by larval parasitoids, although the response of  
570 these natural enemies to high temperatures would also need to be considered for a deeper  
571 understanding of the consequences of heat waves at the level of interspecific interaction.

572         The negative effects of the heat wave on fitness-related traits were largely restricted to  
573 individuals with access to low-quality food after the heat exposure, while those maintained on  
574 high-quality medium did not exhibit a similar decline in larval performance. The importance  
575 of nutrition in modulating the thermal response of phytophagous insects is increasingly  
576 acknowledged and is thought to result from the impacts of temperature on metabolic rates and  
577 energy requirements (Clissold and Simpson, 2015; Diamond and Kingsolver, 2010; Lemoine  
578 et al., 2014; Triggs and Knell, 2012). In this regard, previous works have reported that  
579 individuals subjected to food deprivation concomitantly with a heat stress were more  
580 susceptible to the heat-related reduction of performance (Adamo et al., 2012; Karl et al.,  
581 2011). Our study is the first one to emphasise that ability of insects to cope with a temporary  
582 heat stress also hinges on the nutritional value of the food available after the heat wave. The  
583 synergism between temperature and food stresses here highlighted could result from  
584 quantitative (i.e. a lower nutrient intake when feeding upon DM because of dilution effects)  
585 and/or qualitative nutritional effects (i.e. an unbalanced nutrient intake because of the low  
586 carbohydrate-to-protein ratio of DM) (Andersen et al., 2010; Clissold and Simpson, 2015; Lee  
587 and Roh, 2010). Exploring the nutritional basis for the fitness costs incurred by heat-stressed  
588 individuals when subsequently raised on low-quality food (for instance by using several diets  
589 with different degrees of nutrient dilution and imbalance) would merit further investigations

590 to clarify whether these effects were driven by an insufficient supply with a particular nutrient  
591 (carbohydrates, proteins, or both).

592           Interestingly, individuals raised on low-quality and high-quality food after the heat  
593 wave differed in their energetic budgets, the former having lower reserves compared with the  
594 latter. This means that the negative effects of the heat wave on larval immunity were mostly  
595 detectible for individuals with relatively low energetic reserves due to poor food quality, a  
596 finding that could be attributable to the supposed existence of resource-based trade-offs  
597 among immune function and stress response (Karl et al., 2011). Exposure to stressful heat  
598 conditions is known to trigger a widespread intracellular response consisting of the expression  
599 of a set of heat shock proteins, which act as protective agents actively contributing to enhance  
600 the insect's ability to withstand a thermal stress and to recover from the stress injuries (Feder  
601 and Hofmann, 1999; King and MacRae, 2015). Similar to maintaining high levels of  
602 immunity, it is assumed that the activation of a heat stress response based on the production of  
603 heat shock proteins is energy-demanding, such that trade-offs between these two biological  
604 functions should be expected in an energy shortage context (Karl et al., 2011; Krebs and  
605 Loeschke, 1994). Under harsh environmental conditions such as those related to heat wave  
606 application, larvae constrained by a low-quality food may thus favour allocating resources to  
607 an appropriate heat shock response to enhance heat tolerance and maintain high survival rates,  
608 like those recorded in this study, at the expense of immune function. In this regard, it is  
609 worth-mentioning that PO activity did not follow a pattern of response consistent with this  
610 hypothesis of resource-based trade-offs, because it was reduced only for heat-stressed  
611 individuals fed with the most nutritive diet (AM) afterwards. We postulate that this could  
612 arise from the relatively high carbohydrate-to-protein ratio of this medium by comparison  
613 with DM, while several studies evidenced that investment in spontaneous functional PO is  
614 often rather limited by protein intake in lepidopteran larvae, especially for basal levels

615 expressed in the absence of an immune challenge (Cotter et al., 2011; Lee et al., 2006; Ponton  
616 et al., 2013).

617         We also found that the heat wave impacts varied substantially among the ontogenetic  
618 stages, in a trait-specific manner. In terms of development, the adverse impacts of the heat  
619 wave were specifically detected in the case of a late exposure (involving fourth instar larvae),  
620 whilst the heat wave applied to larvae at an earlier ontogenetic stage (second instar larvae) did  
621 not significantly influence this physiological process. These findings suggest that *L. botrana*  
622 larvae may be able to fully recover from the physiological costs associated with exposure to  
623 supraoptimal temperatures if enough time has elapsed to allow this recovery to occur (even  
624 with low-quality food). Congruently with previous experimental investigations, our results for  
625 development seem to indicate that the biological impacts of heat waves can be interpreted  
626 through the lens of a subtle balance between the damage sustained during hot, stressful days  
627 and the duration of the repair periods acting as buffer against the negative consequences of  
628 heat exposure for organisms (Ma et al., 2018; Zhang et al., 2015a). With regards to immunity,  
629 the heat wave elicited extensive negative effects on the three traits examined when it occurred  
630 relatively early during larval development, whilst the heat wave applied to older larvae only  
631 had minor immune consequences (lower total PO activity). Hence, in contrast with  
632 development, the time left to express recovery after the heat wave does not appear to be a  
633 good predictor of the consequences of the heat stress in terms of immunity. Rather, these  
634 results might more likely arise from differences in susceptibility to high temperatures between  
635 the two ontogenetic stages studied (Briere and Pracros, 1998). In this regard, it could be  
636 hypothesised that larval investment in a heat stress response might vary ontogenetically, as  
637 shown by a marked variation in heat tolerance within and between developmental stages in  
638 other butterfly models (Klockmann et al., 2017; Zhang et al., 2015a, 2015b). As a result, the  
639 above mentioned trade-off between heat tolerance and immunity could be age-dependent,

640 such that young larvae may invest the most in physiological buffering mechanisms (and,  
641 consequently, the least in immunity) to endure prevailing heat conditions because of their  
642 intrinsic vulnerability (e.g. limited ability to escape from thermal stress, small body size) to  
643 high temperatures and desiccation (Klockmann et al., 2017).

## 644 **5. Conclusion**

645 To conclude, this present work provides the first experimental evidence that post-stress  
646 nutrition (manipulated through variation in food quality after the heat wave) can mediate the  
647 effects of a heat wave for individual performance, in a stage- and trait-dependent manner.  
648 More direct measurements of larval nutrition, such as food intake and gain in body mass  
649 before and after the heat wave application, would help further support the existence of stage-  
650 dependent feeding responses potentially involved in recovery process. By stressing the  
651 importance of food quality with regards to the ability of individuals to cope with short periods  
652 of extreme temperatures, we suggest that insect pests may not necessarily benefit from global  
653 warming and a variability in the response of pest populations to climate change can be  
654 expected depending on the nutritional value of the plant tissues ingested (Adamo et al., 2012).  
655 To a certain extent, the abrupt drop in diet nutritional value experienced by larvae fed with  
656 poorly nutritive food after the heat wave could be deemed ecologically realistic. Indeed,  
657 grapevine undergoes profound physiological modifications when exposed to a heat stress,  
658 with immediate and often long-lasting consequences for berry quality including interruption  
659 of fruit maturation, impediment of sugar accumulation, and alteration of plant secondary  
660 metabolism (Greer and Weston, 2010; Liu et al., 2012). Further investigations are needed to  
661 clarify (i) the magnitude of the presumed decrease in plant nutritional value following heat  
662 wave application, (ii) whether this magnitude is strong enough to shape the insect's response  
663 to heat and (iii) the role played by local viticultural practices, especially grapevine cultivars,  
664 in the response of the plant-insect complex to heat. Lastly, another worth-investigating

665 research avenue could lie in testing for the generality about the importance of developmental  
666 stage for the insect response to heat. This raises the question as to whether ontogenetic  
667 variation in insect response to heat might also exist for species with overlapping development  
668 stages (e.g. heterometabolous), or if adults in non-capital breeders could further repair through  
669 feeding the heat injuries sustained during different immature stages.

#### 670 **Declaration of competing interest**

671 The authors declare they have no conflict of interests.

#### 672 **CRediT authorship contribution statement**

673 **Corentin Iltis:** Conceptualisation, Methodology, Investigation, Formal Analysis, Writing –  
674 Original Draft, Writing – Review & Editing, Visualisation. **Philippe Louâpre:**  
675 Conceptualisation, Methodology, Formal Analysis, Writing – Review & Editing,  
676 Visualisation, Funding acquisition. **Fanny Vogelweith:** Methodology, Validation, Writing –  
677 Review & Editing. **Denis Thiéry:** Resources, Writing – Review & Editing. **Jérôme Moreau:**  
678 Conceptualisation, Methodology, Writing – Review & Editing, Funding acquisition.

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