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1 Early life nutritional quality effects on adult memory retention in a parasitic wasp

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20 *Running Title: capital resources effects on memory of Trichogramma brassicae*

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22

23 Abstract:

24 Nutritional quality during early life can affect learning ability and memory retention of animals.  
25 Here we studied the effect of resource quality gained during larval development on the learning  
26 ability and memory retention of two sympatric strains of similar genetic background of the  
27 parasitoid *Trichogramma brassicae*: one uninfected and one infected by *Wolbachia*. *Wolbachia*  
28 is a common arthropod parasite/mutualistic symbiont with a range of known effects on host  
29 fitness. Here we studied, for the first time, the interaction between resource quality and  
30 *Wolbachia* infection on memory retention and resource acquisition. Memory retention of  
31 uninfected wasps was significantly longer when reared on high quality hosts when compared to  
32 low quality hosts. Furthermore, uninfected wasps emerging from high quality hosts showed  
33 higher values of protein and triglyceride than those emerging from low quality hosts. In contrast,  
34 the memory retention for infected wasps was the same irrespective of host quality, although  
35 retention was significantly lower than uninfected wasps. No significant effect of host quality on  
36 capital resource amount of infected wasps was observed, and infected wasps displayed a lower  
37 amount of protein and triglyceride than uninfected wasps when reared on high quality hosts.  
38 This study suggests that the nutritional quality of the embryonic period can affect memory  
39 retention of adult wasps not infected by *Wolbachia*. However, by manipulating the host's  
40 obtained capital resource amount, *Wolbachia* could enable exploitation of the maximum  
41 available resources from a range of hosts to acquire suitable performance in complex  
42 environments

43 Keywords:

44 Learning, memory, capital resources, *Trichogramma brassicae*, *Wolbachia*

45

46 **Introduction:**

47 Nutrient availability and quality during development can greatly affect adult phenotype, such as  
48 body mass, fecundity and longevity, directly affecting the fitness of the individual (Metcalf and  
49 Monaghan 2003, Schwartz and Morrison 2005). In addition, it has been reported that nutrient  
50 availability may also impact adult behavior such as dominance status, food preference or  
51 defensive behaviors, (Mevi-Schütz and Erhardt 2003, Lindstedt et al. 2009, Araújo et al. 2012,).  
52 For example, in *Nicrophorus vespilloides* burying beetles, adult size is influenced by the size of  
53 the carcass on which the individual develops; beetles reared on small carcasses are smaller than  
54 beetles reared on larger carcasses giving them less success in contest success Hopwood et al.  
55 2014). Recently, the possible connections between quality of nutrition in early development and  
56 learning ability and memory capacity in adults has received considerable research attention in  
57 vertebrates (Fisher et al. 2006, Stangl and Thuret 2009). To address this question in  
58 invertebrates, parasitic wasps represent an ideal model since nutritional resources available  
59 during development are constrained by the host, and by the efficiency of the parasitic wasps to  
60 convert host body mass to their own body mass (Jervis and Kidd 1986, Kant et al. 2012 Sanders  
61 et al. 2016). Since the resources gained by parasitoid larvae (Capital resources) are a primary  
62 factor in determining the future fitness of the adult life stage (Jervis and Kidd 1986), host quality  
63 subsequently plays an important role in adult fitness and has been shown to affect development,  
64 mortality, longevity, fecundity and adult size (Godfray 1994, Sampaio et al. 2008).  
65 In insects, alteration in diet quality during development has various effects on adults' fitness,  
66 generally called as the silver spoon effect (Chapman et al. 2012, Hopwood et al. 2014). For  
67 example, Runagall-McNaull et al. (2015) found that male and female lifespan of *Telostylinus*  
68 *angusticollis* was shortest when larvae were fed with a protein restricted diet. Restriction of

69 nutrients in insect larval instars is generally considered to negatively influence adult fitness by  
70 increasing development time and reducing adult size, male secondary sexual trait expression, and  
71 both male and female fecundity (Zwaan et al. 1991, Tatar and Tatar 2003, Boggs and Freeman  
72 2005, Bonduriansky 2007). The major nutrients involved in development are proteins,  
73 carbohydrates and lipids. Proteins play essential roles in viability, vitelogenesis, neural  
74 differentiation and mating behavior. Thus imbalances in dietary amino acids can significantly  
75 affect survival, development and fitness of adults (Joachim-Bravo et al. 2009, Even et al. 2012,  
76 Bjorum et al. 2013). Carbohydrates act as body fuel and provide energy to development and  
77 represent the mechanism by which energy is stored for future use (Rivero and Casas 1999,  
78 Tenhumberg et al. 2006).

79 *Wolbachia* is an obligate intracellular endosymbiont, belonging to a-proteobacteria group which  
80 includes a number of important arthropod-transmitted bacterial agents such as *Rickettsia*  
81 *rickettsii*, *R. felis*, *Anaplasma platys*, *Ehrlichia canis*, *E. chaffeensis*, and *E. ewingii*. (Taylor and  
82 Hoerauf 1999, Bowman 2011). It is estimated to infect 66% of arthropod species, although often  
83 at a low prevalence within a species (Hilgenboecker et al. 2008). Recently, *Wolbachia* infections  
84 have been shown to influence the fitness of their hosts in various ways, positively and  
85 negatively, via altering patterns of reproduction and resistance to microbial infections (Hedges et  
86 al. 2008, Teixeira et al. 2008). The spread of *Wolbachia* in arthropod populations depends on the  
87 continuity of *Wolbachia* transmission across generations. *Wolbachia* causes parthenogenesis to  
88 spread, thus providing an advantage to their hosts in terms of female offspring production  
89 (Stouthamer 1997). Species belonging to the *Trichogramma* genus are gregarious  
90 endoparasitoids and are potential hosts to *Wolbachia* (Kishani Farahani et al. 2015 ). Most  
91 *Trichogramma* species are associated with the eggs of Lepidoptera, although some can attack

92 dipteran, coleopteran, and symphytan eggs (Hoffmann et al. 1995, Pinto 1998, Mansfield and  
93 Mills 2002). One particular species, *Trichogramma brassicae*, is widely used as a biological  
94 control agent and has been used in biological control of some key pests (Smith 1996, Ebrahimi et  
95 al. 1998, Lundgren et al. 2002). In nature, one uninfected and one infected strain of *T. brassicae*  
96 coexists, with the infected strain only producing female offspring (Farrokhi et al. 2010,  
97 Poorjavad et al. 2012). It has been shown that both strains are genetically similar and that their  
98 differences are due only to *Wolbachia* infection (Kishani Farahani et al. 2015). However, a  
99 recent study revealed that *Wolbachia* infected *T. brassicae* possess a lower ability to evaluate  
100 host quality compared to uninfected wasps, leading authors to conclude that such behavior was  
101 the result of host manipulation (Kishani Farahani et al., 2015).

102 The current study was carried out to investigate whether nutritional quantity during immature  
103 development affects memory retention of adult wasps of both the infected and uninfected strain  
104 of *T. brassicae*. We hypothesized that (1) uninfected wasps, which need high quality hosts to  
105 develop (Kishani Farahani et al., 2015) will present less proteins, triglycerides and lipids when  
106 developing in low quality hosts (2) uninfected wasps with less nutrients will be affected in their  
107 learning capacities (3) infected wasps, which oviposit equally in high and low quality hosts  
108 (Kishani Farahani et al., 2015) will not present differences in nutrients and (4) they will not be  
109 affected by host quality for their learning ability.

## 110 **Material and Methods:**

### 111 **Parasitoids: choice of the strains, collection and rearing**

112 We compared two naturally infected and uninfected strains for which we have shown similar  
113 genetic background, which proved that all behavioral differences between these two strains are  
114 linked to *Wolbachia* prevalence (Kishani Farahani et al. 2015). Another possibility to conduct

115 this experiment would have been to use antibiotic treatment to obtain an uninfected strain, but  
116 some studies have shown the negative effects of antibiotics on *Wolbachia* treated arthropods  
117 (Dedeine et al. 2001, Timmermans and Ellers 2009). Removing *Wolbachia* by antibiotic  
118 treatment may affect physiology and behavior of *Wolbachia* infected arthropods and thus the  
119 observed difference between *Wolbachia* infected and antibiotic treated individuals may be due to  
120 negative effects of antibiotic treatment. In conclusion, comparing natural strains may show  
121 natural differences in all biological aspects of infected and uninfected individuals and may  
122 represent a better method by which to observe the effects of *Wolbachia*.

123 Parasitoid strains were derived from cultures maintained by the Biological Control Research  
124 Department (BCRD) of the Iranian Research Institute of Plant Protection (IRIPP). The original  
125 source of the cultures were parasitoids obtained from parasitized eggs of *Ostrinia nubilalis*  
126 Hübner (Lep.: Pyralidae), collected from northern Iran (Baboulsar Region, South of the Caspian  
127 Sea) in 2012. Parasitoids were reared at  $25\pm 1^{\circ}\text{C}$ ,  $50\pm 5\%$  RH, and 16:8 L: D on eggs of *Ephestia*  
128 *kuehniella* Zeller (Lepidoptera: Pyralidae). Eggs were obtained from a culture, reared at  $25\pm 1^{\circ}\text{C}$   
129 on wheat flour and yeast (5%), maintained at the Insectary and Quarantine Facility, University  
130 of Tehran. Approximately 20 mated female moths were kept in glass containers (500 ml) to  
131 provide eggs for experiments involving uninfected and infected wasps. Wasps were reared for 15  
132 generations on *E. kuehniella* prior to use in experiments.

133 To rear wasps on different host qualities, one hundred fresh eggs (less than one day old) (high  
134 quality eggs) and old eggs (more than 45 days old) (low quality eggs) were exposed to one day  
135 old wasp females for 24 hours. Old eggs were smaller, had a scarious chorion and a lower  
136 weight, as low-quality reward. This was carried out for both strains. After 24 hours, the eggs

137 were removed and kept under controlled conditions of  $25\pm 1^{\circ}\text{C}$ , 16L: 8 D, and  $50\pm 5\%$  RH in a  
138 growth chamber and checked until adult wasp emergence.

### 139 **Experimental design:**

140 In the current research, effects of *Wolbachia* presence and host quality during immature  
141 developmental time on adult wasps learning ability and memory retention was studied in a  
142 factorial design for four total treatments: T1: uninfected wasps emerging from high quality hosts,  
143 T2: uninfected wasps emerging from low quality hosts, T3 infected wasps emerging from high  
144 quality hosts, T4: infected wasps emerging from low quality hosts).

### 145 **Determination of Glycogen, Triglyceride and Protein amounts**

146 For the four treatments T1 to T4, the amount of glycogen, triglycerides and proteins was  
147 determined from x newly emerging females.

### 148 **Glycogen determination**

149 Fat bodies of 30 adults, from each line, were removed and immersed in 1 ml of 30%  
150 KOH w/ $\text{Na}_2\text{SO}_4$ . Tubes containing the samples were covered with foil (to avoid evaporation) and  
151 boiled for 20-30 min. Tubes were subsequently shaken and cooled in ice. 2 ml of 95% EtOH was  
152 added to precipitate glycogen from the digested solution. Samples were again shaken and  
153 incubated on ice for 30 min. Following the incubation on ice, tubes were centrifuged at 13000  
154 rpm for 30 min. Supernatant was removed and pellets (glycogen) were re-dissolved in 1 ml of  
155 distilled water and shaken. Standard Glycogen (0, 25, 50, 75 and 100 mg/ml) was prepared  
156 before adding phenol 5%. Incubation was performed on an ice bath for 30 min. Standards and  
157 samples were read at 490 nm and distilled water was used as a blank (Chun and Yin 1998).

### 158 **Triglyceride Determination**



159 A diagnostic kit from PARS-AZMOON<sup>®</sup> Co. was used to measure the amount of  
160 triglyceride in the adult parasitoid. Reagent solution contained phosphate buffer (50 mM, pH  
161 7.2), 4-chlorophenol (4 mM), Adenosine Triphosphate (2 mM), Mg<sup>2+</sup> (15 mM), glycerokinase 0.4  
162 kU/L), peroxidase (2 kU/L), lipoprotein lipase (2 kU/L), 4-aminoantipyrine (0.5 mM) and  
163 glycerol-3-phosphate-oxidase (0.5 kU/L). Samples (10 μL) were incubated with 10 μL distilled  
164 water and 70 μL of reagent for 20 min at 25 °C (Fossati and Prencipe 1982). The optic density  
165 (ODs) of samples and reagent as standard were read at 546 nm. The following equation was used  
166 to calculate the amount of triglyceride:

$$mg/dl = \frac{OD \text{ of sample}}{OD \text{ of Standard}} \times 0.01126$$

167

168

## 169 **Protein determination**

170 Protein concentrations were assayed according to the method described by Lowry et al.  
171 (1951). The method recruits reaction of Cu<sup>2+</sup>, produced by the oxidation of peptide bonds with  
172 Folin–Ciocalteu reagent. In the assay, 20 μL of the sample was added to 100 μL of reagent, and  
173 incubated for 30 min prior to reading the absorbance at 545 nm (Recommended by Ziest Chem.  
174 Co., Tehran-Iran).

## 175 **Learning ability:**

### 176 **Conditioning**

177 To study the innate preference of the wasps towards one odor (peppermint and lemon odors were  
178 tested) against a filter paper without odor, 50 naïve wasps of each strain were exposed to either  
179 the odor of peppermint or the odor of lemon (98% purity, by Adonis Gol Darou Group, Iran) in

180 the wind tunnel as previously described by Yong et al. (2007) and their responses were recorded.  
181 Wasps were also exposed to odor versus clean air. To do this, single naïve female wasps were  
182 introduced into the flight chamber. The tested odor was presented on strips of filter paper (1× 2  
183 cm) on which 1 µl of one or other solution (peppermint or lemon) had been placed on one filter  
184 paper, and no odor on the second filter paper. Each filter paper was attached to a glass pipette  
185 placed vertically on a stand and spaced 10 cm apart. Twenty-five of the 50 wasps underwent this  
186 procedure using the peppermint odor (at least 97% pure) and the other 25 underwent the  
187 procedure using the lemon odor (at least 97% pure). The responses of the wasps to the odors  
188 were observed in the flight tunnel during a flight time of 15 min. Any individual that landed or  
189 hovered on an odor site for more than 2 minutes was recorded as a responder wasp. Females that  
190 did not complete a flight or did not fly over the start area in the flight chamber were scored as  
191 displaying no response.

192 The ability of wasps to learn was determined using a Pavlovian conditioning procedure whereby  
193 an odor stimulus was associated with the reward of ovipositing (Bleeker et al. 2006). Several  
194 studies have shown that oviposition is a reward for parasitoids (for example van Baaren et al.  
195 2005, Bleeker et al. 2006). A flight chamber was subsequently used to observe the behavior of  
196 conditioned wasps. Sixty five one-day old naïve females, reared on high quality hosts and 65  
197 reared on low quality hosts, were exposed individually to host eggs for 15 minutes to gain  
198 oviposition experience. This was to avoid the variability in sequence and the retention of  
199 behavioral events associated with learning from the first host encountered (Mills and Kuhlmann  
200 2004). Since some wasps died, were lost or did not oviposit during the manipulation,  
201 approximately sixty wasps per treatment were used. Half of the test individuals (n=30) were  
202 conditioned using peppermint odor and the remaining half using lemon odor. For conditioning,

203 one adult wasp was introduced to a vial (2×10 cm) containing 100 host eggs (one day old eggs,  
204 glued on a cardboard) and was transferred into the conditioning tank (25×25×25 cm).  
205 Subsequently, peppermint or lemon odor (98% purity, by Adonis Gol Darou Group, Iran) were  
206 used to condition the wasps. During experiments, the conditioning odor (either peppermint or  
207 lemon) was pumped into the tanks with a speed of 1 m/s speed. The conditioning process lasted a  
208 total of 2 hours and was repeated for both uninfected and infected females reared on high and  
209 low quality hosts (60 females of each of the treatments T1 to T4). The conditioning time of 2  
210 hours was set based on the average time of patch leaving of 100 adult wasps exposed to 100  
211 eggs. This time was recorded by direct observation.

#### 212 **Test of odor preference after conditioning**

213 Fifteen minutes after conditioning, infected and uninfected female wasps reared on high and low  
214 quality hosts were placed individually in the flight chamber, The responses of 50 female wasps  
215 (randomly selected from the surviving wasps of the 60 conditioned), 25 conditioned on  
216 peppermint and 25 conditioned on lemon, were tested for the four treatment groups (totaling 4 x  
217 50 females). The responses of the wasps to the conditioned odors were observed in the flight  
218 tunnel during a flight time of 15 min. If females displayed a preference towards the conditioned  
219 odor (i.e. the individual landed or hovered on the conditioned odor site for more than 2 minutes),  
220 it was assumed that associative learning between the odor and the reward of oviposition had  
221 occurred. The number of rejection responses and non-responding wasps were recorded to  
222 determine behavioral response variation by both strains. Females that did not complete a flight or  
223 did not fly after 5 min were scored as displaying no response. All flight responses were tested at  
224 25°C, 50% RH, and a light intensity of 2000 lux.

225           **Test of memory retention duration:**

226   Memory retention was assumed when wasps showed a significant preference for the conditioned  
227   odor (peppermint/lemon). To determine the duration of memory retention, experimental wasps of  
228   both strains, reared on low and high quality hosts, were observed 2, 4, 6, 8, 10, 12, 14, 16, 18, 20,  
229   24, and 30h after training in a choice test experiment between the conditioned and unconditioned  
230   odor in the flight tunnel. For each time interval, 50 new wasps of each strain (25 conditioned  
231   with each odor) reared on high quality hosts and 50 reared on low quality hosts were observed in  
232   the wind tunnel as described, totaling 2400 tested wasps.

233           **Statistical analysis:**

234   **Comparison of Glycogen, Triglyceride and Protein amounts**

235           Amount of protein, triglyceride and glycogen were analyzed in a two way ANOVA in  
236   GLM Proc. by SAS ver. (9.1). In all cases, the factors were the strain and the host quality.  
237   When a significant effect of treatment was found, Tukey *post hoc* test was performed. Data are  
238   presented as means  $\pm$  SE and statistical analyses were performed using SAS software (SAS  
239   Institute Inc. 2003).

240           **Learning and memory**

241   The innate responses of both strains were compared by Chi-Square tests using SAS software  
242   (SAS Institute 2003). To compare the responses of the two strains before and after conditioning,  
243   a Generalized Linear model implemented in the procedure GENMOD of SAS software (ver. 9.1)  
244   was used with the binomial family error and logit link. The least square estimates of the  
245   proportions in each level were then compared by the Chi-square approximation (an option  
246   offered by GENMOD).

247 The estimation of forgetting relies on a series of observations recorded at different times  $t_1; t_2;$   
 248  $\dots t_n$  after conditioning. At each time, a set of  $n_t$  subjects was subjected to a choice test with three  
 249 possible responses:  $a; b;$  and  $c$ , which correspond respectively to a preference for the odor side of  
 250 the flight chamber, a rejection (choice of the opposite side of the chamber), and a null choice, i.e.  
 251 no preference of chamber side and a null response. The forgetting of conditioning results in a  
 252 switch from a high level to a lower level of positive responses, a simultaneous switch from a low  
 253 level to a high level of null responses, and a switch from a very low to a moderate level of  
 254 negative responses. A constraint links the three responses as  $n_a + n_b + n_c = n_t$  or  $n_c = n_t - n_a - n_b$ .  
 255 The course of these three responses over time can be described by two logistic functions written  
 256 here as probabilities,

257  $p_a, p_b, p_c$ , constrained by  $p_a + p_b + p_c = 1$ :

258

$$(1) \quad p_a = k_a - \frac{k_a - a_a}{1 + e^{(-b_a(t-t_0))}} + a_a$$

259  
260

$$(2) \quad p_c = \frac{k_c - a_c}{1 + e^{(-b_c(t-t_0))}} + a_c$$

261  
262

$$(3) \quad p_b = 1 - p_a - p_c$$

263  
264

265  $k_a$ , respectively  $k_c$ , and  $a_a$ , respectively  $a_c$  define the sill and baselines of the logistic models (1)  
 266 and (2): the baselines are  $a_a$  and  $a_c$ , and the seals are  $k_a + a_a$  in model(1),  $k_c + a_c$  in model (2).  $k_a$   
 267  $+ a_a$  estimates the initial state in model (1), and  $a_c$  the final state. It is the inverse in model (2),  
 268 where  $a_c$  is the initial state and  $k_c+a_c$  the final state.

269 A supplementary restriction lies in the fact that, as  $t_0$  represents the mean time to forgetting, *i.e.*  
 270 the inflection time point of the logistics functions; it has to be the same in all three equations.

271 The data consist of a vector of three counts:  $V_t = (n_{at}, n_{bt}, n_{ct})$  the respective number of subjects

272 responding  $a$ ;  $b$  or  $c$  at time  $t$ . An R script was written to enable this. The experimental design  
273 was a balanced factorial design with two factors: the type of strain, with two levels (uninfected  
274 and infected), and the number of conditioning experiments, with five levels. This design resulted  
275 in ten crossed levels. The model, defined by equations 1 to 3, was fitted individually on each set  
276 of ten data. The maximization of the likelihood cannot be fully automatic, and requires an initial  
277 guess of the seven parameters  $k_a$ ;  $a_a$ ;  $b_a$ ;  $k_c$ ;  $a_c$ ;  $b_c$ ;  $t_0$ . This was done by a visual evaluation of  
278 each graphic representation of the crossed levels. After maximization, we obtained seven  
279 estimates of each parameter, along with an estimate of their standard error through the hessian  
280 matrix, which is provided on demand by the *optim* function of R (R core team 2013). All  
281 correlations between pairs of coefficients were lower than 0.9, a threshold commonly accepted as  
282 critical for the correct inversion of the hessian matrix (Bouvier 1996 by Bouvier and Huet 1994).  
283 As our main focus was  $t_0$ , we will give only the results for this parameter, which was accurately  
284 estimated with a standard errors ranging from 0.3 to 1.8 hours. Recorded forgetting times  
285 between high and low quality reared uninfected and infected wasps were compared by Cox  
286 proportional hazard models.

## 287 **Results:**

### 288 **Amounts of Glycogen, Triglyceride and Protein**

289 For all physiological measurements, there was a difference between wasps reared on high quality  
290 food vs. low quality food only when they were uninfected with *Wolbachia*.

291 Effects of strain, host quality and their interaction on amount of acquisitioned resource by  
292 uninfected and infected wasps are shown in Table 1. Uninfected wasp protein amount did not  
293 show significant difference by infected wasps in high and low quality host reared wasps  
294 (Uninfected wasps:  $p = <.0001$ ; Infected wasps:  $p = 0.211$ ). Triglyceride amount in uninfected

295 wasps was significantly higher in wasps reared on high quality hosts ( $p=0.006$ ) while infected  
296 wasps showed similar amount ( $p= 0.587$ ) (Figure 1). Glycogen amount of uninfected wasps  
297 reared on high quality hosts differed significantly ( $p=0.009$ ) (Figure 1) whereas infected wasps  
298 showed no significant difference ( $p= 0.837$ ).

### 299 **Test of odor preference after conditioning**

300 Naïve females of both the uninfected ( $\chi^2=0.13, p=0.93, df= 2, N=50$ ) and the infected  
301 ( $\chi^2=0.69, p=0.70, df= 2, N=50$ ) strains displayed no significant innate preference for the odor of  
302 lemon or that of peppermint against the filter paper with no odor or for no response wasps  
303 ( $\chi^2=0.95, p=0.62, df= 4, N=100$ ). Furthermore, when uninfected (peppermint vs air:  $\chi^2=2.31,$   
304  $p=0.12, df= 2, N=50$ ; lemon vs air:  $\chi^2=0.63, p=0.42, df= 2, N=50$ ) and infected wasps  
305 (peppermint vs air:  $\chi^2=1.44, p=0.22, df= 2, N=50$ ; lemon vs air:  $\chi^2=0.02, p=0.86, df= 2, N=50$ )  
306 were tested with odor and clean air showed no significant difference in their behavior.

307 Effects of strain, conditioning and their interaction on the response of uninfected and infected  
308 wasps are shown in table 2. The rate of no response of uninfected wasps reared on high quality  
309 hosts decreased significantly after conditioning for both odors (GENMOD of SAS, lemon odor:  
310  $\chi^2=6.36, p=0.0117, df=1, N=25$ ; peppermint odor:  $\chi^2=5.67, p=0.0173, df=1, N=25$ ) (Fig. 2a). The  
311 rate of no response of uninfected wasps reared on low quality hosts was not significantly  
312 different before and after conditioning (lemon odor:  $\chi^2=2.18, p=0.14, df=1, N=25$ ; peppermint  
313 odor:  $\chi^2=1.37, p=0.241, df=1, N=25$ ) (Fig. 2a). For individuals of the uninfected strain emerging  
314 from high and low quality hosts, the rate of positive response towards the odor increased  
315 significantly after conditioning (Proc GENMOD of SAS: lemon odor:  $\chi^2=10.59, p=0.0011, df=1,$   
316  $N=25$ ; peppermint odor:  $\chi^2=9.08, p=0.0026, df=1, N=25$  for high quality host reared wasps

317 (Figure 3a); lemon odor:  $\chi^2=4.94$ ,  $p=0.026$ ,  $df=1$ ,  $N=25$ ; peppermint odor:  $\chi^2=4.94$ ,  $p=0.026$ ,  
318  $df=1$ ,  $N=25$  for low quality host reared wasps, (Figure 3a).

319 The number of null responses of infected wasps reared on high and low quality hosts did not  
320 decrease significantly after conditioning (lemon odor:  $\chi^2=0.37$ ,  $p=0.5451$ ,  $df=1$ ,  $N=25$ ;  
321 peppermint odor:  $\chi^2=0.1$ ,  $p=0.7578$ ,  $df=1$ ,  $N=25$  for high quality host reared wasps (Figure 2b);  
322 and lemon odor:  $\chi^2=0.39$ ,  $p=0.529$ ,  $df=1$ ,  $N=25$ ; peppermint odor:  $\chi^2=0.4$ ,  $p=0.53$ ,  $df=1$ ,  $N=25$   
323 for low quality host reared wasps) (Figure 2b). In both cases (wasps reared on high and low  
324 quality hosts) approximately 30% of individuals did not respond to the odor (Figure 2b). Infected  
325 wasps reared on high and low quality hosts significantly associated the presence of a conditioned  
326 stimuli with oviposition (Lemon odor:  $\chi^2=6.18$ ,  $p=0.0129$ ,  $df=1$ ,  $N=25$ ; peppermint odor:  
327  $\chi^2=7.55$ ,  $p=0.006$ ,  $df=1$ ,  $N=25$  for high quality host reared wasps; lemon odor:  $\chi^2=7.56$ ,  $p=0.007$ ,  
328  $df=1$ ,  $N=25$ ; peppermint odor:  $\chi^2=7.55$ ,  $p=0.006$ ,  $df=1$ ,  $N=25$  for low quality host reared wasps)  
329 (Figure 3b).

### 330 **Memory retention**

331 To reveal significant differences in memory retention of both uninfected and infected wasps  
332 reared on high and low quality hosts, we carried out a three-factor analysis of variance to verify  
333 the conclusions. Differences between the strains were highly significant ( $F= 0.23$ ,  $p= 0.006$ ), as  
334 were the host quality ( $F = 49.05$ ,  $p= 0.0003$ ), with odor type ( $F =0.015$ ,  $p =0.09$ ) and their  
335 interaction ( $F=7.63$ ,  $p=0.02$ ) being significant. For the uninfected strain, the memory duration  
336 was longer for wasps reared on high quality hosts than for wasps reared on low quality hosts ( $p=$   
337  $0.029$  for lemon odor;  $p= 0.030$  for peppermint odor) (Fig. 4). For the infected strain, the  
338 memory duration did not vary significantly with host quality ( $p= 0.84$  for lemon odor;  $p= 0.42$   
339 for peppermint odor) (Fig. 4). The memory retention of infected wasps was lower than



340 uninfected wasps when reared on high quality hosts ( $p=0.009$  for lemon odor;  $p=0.009$  for  
341 peppermint odor). However, memory retention did not significantly differ between infected and  
342 uninfected wasps when reared on low quality hosts ( $p=0.52$  for lemon odor;  $p=0.84$  for  
343 peppermint odor).

#### 344 **Discussion:**

345 In accordance with our main hypotheses, host quality affected learning ability and memory  
346 retention of uninfected wasps, while memory retention of infected wasps was not affected by  
347 host quality. Host quality significantly affected memory retention of uninfected wasps, with  
348 uninfected wasps reared on high quality hosts displaying greater memory retention than those  
349 reared on low quality hosts. For the amount of resources, uninfected wasps reared on high  
350 quality hosts obtained significantly more proteins, triglycerides and glycogen from their host  
351 during their development whereas infected wasps obtained the same amount of resources on both  
352 high and low quality hosts. We will first discuss the case of the uninfected wasps and then the  
353 case of the infected wasps.

354 Recent studies have shown the effects of nutrition during the early life stages on adult cognition  
355 and learning capacity in different vertebrates such as rats, pigs, primates and humans (Tveden-  
356 Nyborg et al. 2009, Anwar Zainuddin and Thuret 2012, Nyaradi et al. 2013). In most cases, it  
357 was shown that nutritional quality strongly affects neurogenesis in organs which is involved in  
358 learning and memory formation during adulthood due to lack of minerals, vitamins, amino acids  
359 and fatty acids (Lucassen et al. 2013, Nyaradi et al. 2013, Hoeijmakers et al. 2015). Our results  
360 showed that uninfected wasps reared on low quality hosts displayed shorter memory retention  
361 than wasps reared on high quality hosts and this may be due to a lack of the requirements in low  
362 quality hosts. For insect parasitoids, the host represents the sole nutritional and physiological

363 environment during immature development (Jervis and Kidd 1986). Many endoparasitic  
364 Hymenopterans like *Trichogramma* spp. have no yolk in their eggs and so lay their eggs inside  
365 the body of hosts, which subsequently provides the nutrients for both embryonic and larval  
366 development (Chapman 2012). Consequently, host quality is important for overall parasitoid  
367 growth and development and may influence the developmental time, mortality rate, longevity,  
368 fecundity and behavior (Harvey and Strand 2002, Sampaio et al. 2008). In adult wasps, it was  
369 shown that storage macromolecules have critical roles in searching behavior, flying, and  
370 reproduction. Since efficient parasitoids must have appropriate searching behavior, these  
371 macromolecules provide required energy by processing carbohydrates, proteins and lipids (Lee  
372 and Park 2004, Bauerfeind and Fischer 2005, Plácido-Silva et al. 2006, Jordão et al. 2010).  
373 Moreover, most parasitic species (and those of this study) are incapable of lipogenesis as adults  
374 (Visser et al. 2010), and, as such, their lipid resource consists solely of lipids obtained from the  
375 host during development. Thus acquisitioned energetic and structural resources during immature  
376 development of parasitoid wasps play an important role in adult behavioral and physiological  
377 fitness. Glycogen, triglycerides and proteins are the three main storage macromolecules in the  
378 body of insects responsible for several energetic demanding processes. Phosphorylation of  
379 glycogen and triglycerides, as well as transamination of protein molecules, provides intermediate  
380 components for electron transport system providing energy, oxygen and water (Nation 2008). In  
381 eggs, presence of these components is essential for development of the embryo. Learning and  
382 storing memory is a costly process which affects animal fitness (Niven et al. 2003, Jones and  
383 MacLarnon 2004, Isler and van Schaik 2006 a b, Niven et al. 2007, Niven and Laugiun 2008,).  
384 The brain is a specialized tissue in which functionality depends upon the generation of electrical  
385 potentials and their conduction through long axonal components of cell-bodies and through the

386 synaptic gaps between these cell-bodies (Rosales et al. 2009). These special functions of brain  
387 are reflected in a higher need for nutrients such as fats and glycogen, and nutrition plays a critical  
388 role at the cross-roads of the biological and nurturing factors that mediate brain growth and  
389 development. As our results showed that uninfected wasps reared on high quality hosts contained  
390 more glycogen, which is the most readily available energy source, this enables thee wasps to  
391 spend more energy resources on daily activities such as movement or memory formation.  
392 Triglycerides are a major form of lipids stored into the fat body, and when triglycerides are  
393 transformed into diglycerides, they are transported by a specific lipoprotein to various  
394 organs/tissues, including the brain to provide the required energy. Octopamine, as an involved  
395 neurotransmitter in learning (Unoki et al. 2006), does play a role in metabolism because  
396 activating octopaminergic cells increases triglycerides (Erion et al. 2012). Thus extra resources  
397 of triglycerides in uninfected wasps reared on high quality hosts can be used as energy resources  
398 needed to consolidate longer memory. In conclusion, the lack of appropriate nutrients seemed to  
399 be the cause of the decrease of memory retention, although the precise physiological mechanisms  
400 remain to be elucidated.

401         For the infected strain, there was no effect of host quality, either for the learning ability or  
402 for the amount of nutrients at the end of the development. There is evidence in the literature that  
403 *Wolbachia* may utilize the host's recourses like a parasite. For example, Caragata et al. (2014)  
404 determined competition for nutrients between *Wolbachia* and *Aedes aegypti*. Infection of  
405 mosquitoes with the virulent *Wolbachia* strain wMelPop caused a significant reduction of  
406 fecundity and egg viability. The authors explained the finding by competition between *A. aegypti*  
407 and the symbiont for essential blood meal nutrients. Moreover, they found cholesterol and amino  
408 acids had critical roles in egg structural development, with *Wolbachia* infection reduceing total

409 cholesterol levels in mosquitoes by 15-25% (Caragata et al. 2014). As such, both *Wolbachia*  
410 and host may be involved in competition over nutritional resources, which will strongly affect  
411 the amount of available capital resources in adults. Interestingly, results of our study suggest that  
412 this is not the case, with infected wasps reared on low quality hosts displaying the same amount  
413 of nutrients as infected wasps reared on high quality hosts.

414 In our study, *Wolbachia* may manipulate and maximize resource acquisition in its host,  
415 resulting in a similar amount of nutritional resources of *Wolbachia* infected wasps reared on high  
416 and low quality hosts. *Wolbachia* infected wasp energy resources contained more triglycerides,  
417 meaning that these wasps save their energy under the form of triglycerides instead of glycogen as  
418 with uninfected wasps. Kishani Farahani et al. (2015) stated that infected wasps spend more time  
419 in host feeding during their adult life compared to uninfected wasps. This host-feeding behavior  
420 provides them with more proteins and glycogen. As shown by Kishani Farahani et al. (2015),  
421 superparasitism rate in infected wasps was higher than in uninfected wasps; a behavior which  
422 helps *Wolbachia* to disperse between uninfected individuals via horizontal transfer. As such, a  
423 shorter memory retention may lead to a higher superparasitism rate by forgetting host parasitized  
424 marking cues. It seems that *Wolbachia* is using an adaptive strategy to enhance dispersal  
425 between individuals by shortening memory retention and maximizing the same energy resource  
426 amount from high and low quality hosts. However, at present, it cannot be concluded whether the  
427 differences observed in the current study represent a strategy employed by *Wolbachia* or are  
428 simply a physical or physiological side effect of *Wolbachia* infection.

429 Finally, it seems that differences between the learning ability and memory retention of  
430 uninfected and infected wasps may be derived from the amount of energy which can be utilized  
431 daily for brain function, since these organs are energetically expensive. Further research is

432 therefore required to investigate the effects of *Wolbachia* infection on the Central Nervous  
433 System and other vital organs involved in lifetime information acquisition.

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639 Table 1. Effects of *Wolbachia* presence, host quality and the interaction of these two factors on  
640 the amount of protein, triglyceride and glycogen of wasps. Significant results are shown in bold.

	<b>Host quality</b>		<b><i>Wolbachia</i> presence</b>		<b>Interaction</b>	
	<b>F value</b>	<b>Pr &gt; F</b>	<b>F value</b>	<b>Pr &gt; F</b>	<b>F value</b>	<b>Pr &gt; F</b>
<b>Protein</b>	25.71	<b>0.001</b>	6.01	<b>0.039</b>	48.87	<b>0.0001</b>
<b>Triglyceride</b>	8.87	<b>0.017</b>	2.65	0.14	4.75	0.060
<b>Glycogen</b>	5.10	0.053	3.39	0.103	6.54	<b>0.033</b>

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647 **Table 2.** Effects of host quality, conditioning and the interaction of these two factors on  
 648 responses of uninfected and infected wasps. Significant results are shown in bold.

			Host quality		Conditioning		Interaction	
			$\chi^2$	P value	$\chi^2$	P value	$\chi^2$	P value
<b>Uninfected</b>	<b>Peppermint</b>	<b>No response</b>	2.51	0.1128	14.62	<b>0.0001</b>	2.51	0.1128
		<b>Positive</b>	1.03	0.3098	30.39	<b>&lt;.0001</b>	1.03	0.3098
	<b>Lemon</b>	<b>No response</b>	6.30	0.0121	21.67	<b>&lt;.0001</b>	6.30	<b>0.0121</b>
		<b>Positive</b>	0.44	0.5077	43.39	<b>&lt;.0001</b>	4.51	<b>0.0337</b>
<b>Infected</b>	<b>Peppermint</b>	<b>NO response</b>	0.11	0.7396	0.89	0.3457	0.11	0.7396
		<b>Positive</b>	0.00	1.0000	32.97	<b>&lt;.0001</b>	0.00	1.0000
	<b>Lemon</b>	<b>NO response</b>	0.11	0.7363	2.37	0.1240	0.11	0.7363
		<b>Positive</b>	0.10	0.7554	29.66	<b>&lt;.0001</b>	0.10	0.7554

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651 **Figure legends:**

652 **Figure 1.** Mean± SE of protein, triglyceride and glycogen amount in uninfected and infected  
653 wasps in low- (LQE) and high-quality (HQE) hosts. Different letters indicate significant  
654 differences between the treatments after Bonferroni correction (P=0.0125).

655 **Figure 2.** Percentage of null responses by uninfected wasps (a) and infected wasps (b) reared on  
656 high or low quality hosts after and before conditioning by peppermint and lemon odor.  
657 Responses of 25 wasps (uninfected, only females, and infected) to peppermint/lemon odor were  
658 compared. Different letters indicate significant differences. N.S.: Not Significant.

659 **Figure 3.** Percentage of recorded Positive Responses by uninfected wasps (a) and infected wasps  
660 (b) reared on high or low quality hosts after and before conditioning by peppermint and lemon  
661 odor. Responses of 25 wasps (uninfected, only females, and infected) to peppermint/lemon odor  
662 were compared. Different letters indicate significant differences. N.S.: Not Significant.

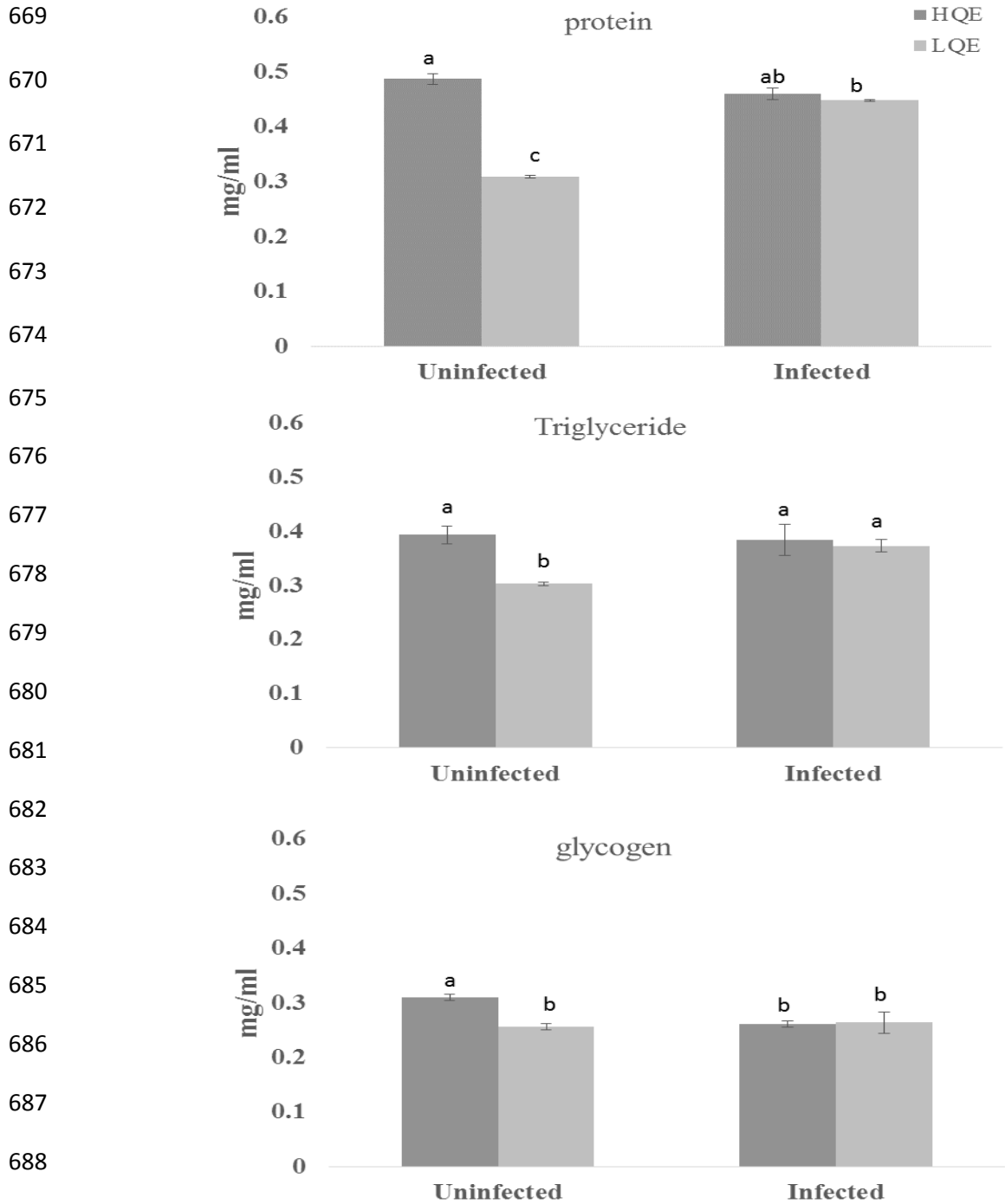
663 **Figure 4.** Differences in the forgetting mid-time depending on the host quality and the type of  
664 strain. Vertical notched bars indicate the 95% confidence interval of the estimates.

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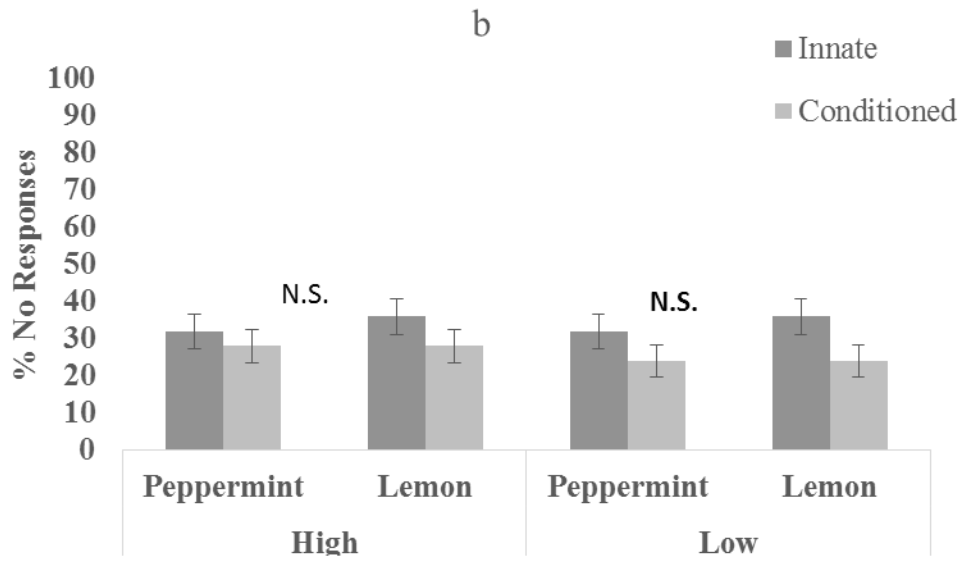
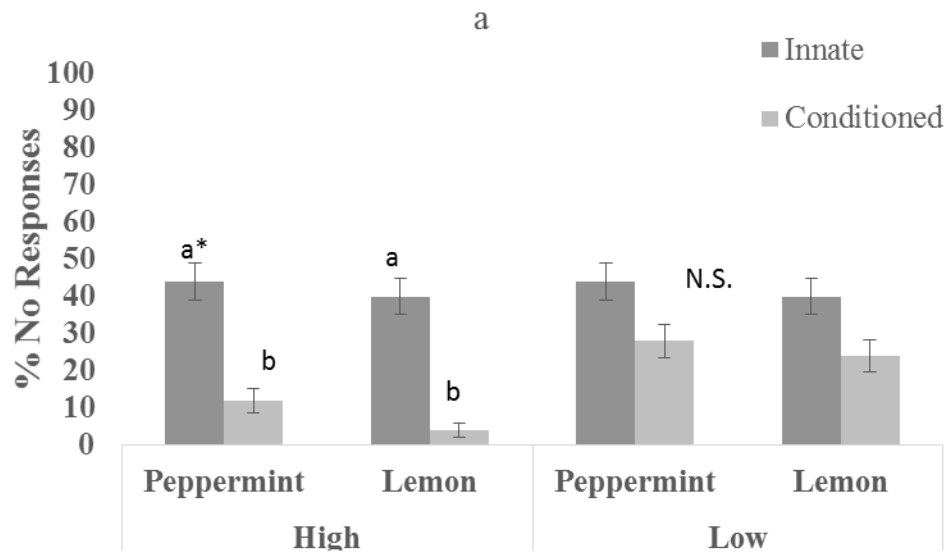
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691 Fig 1.

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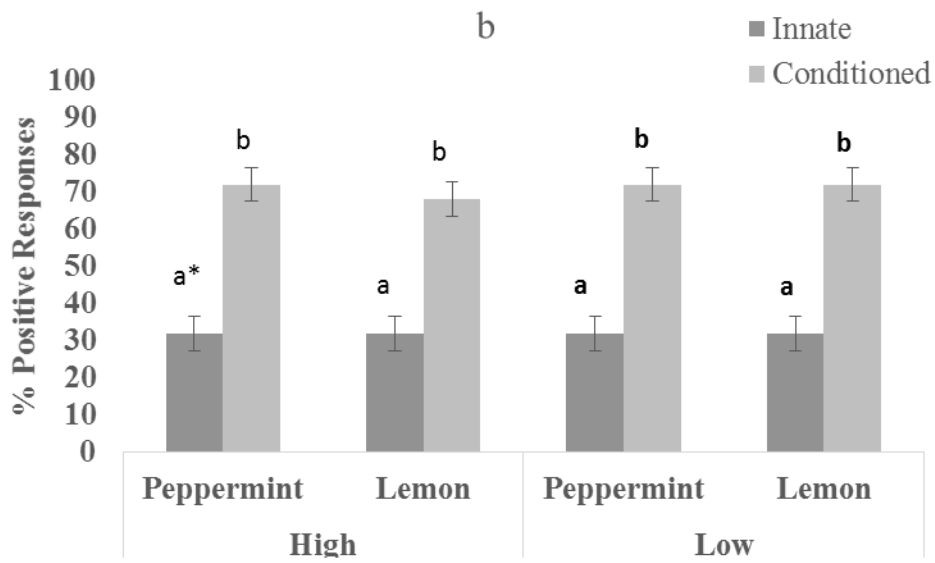
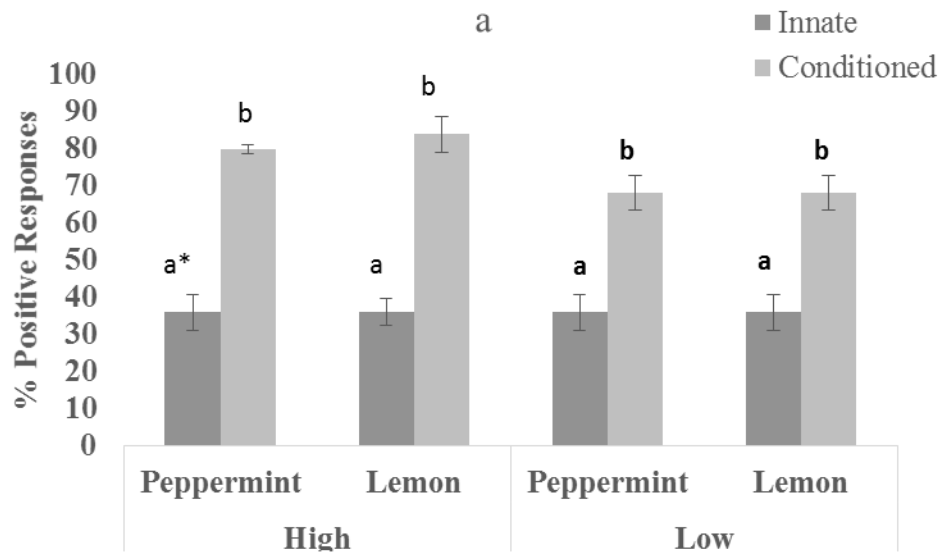
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Fig 2.

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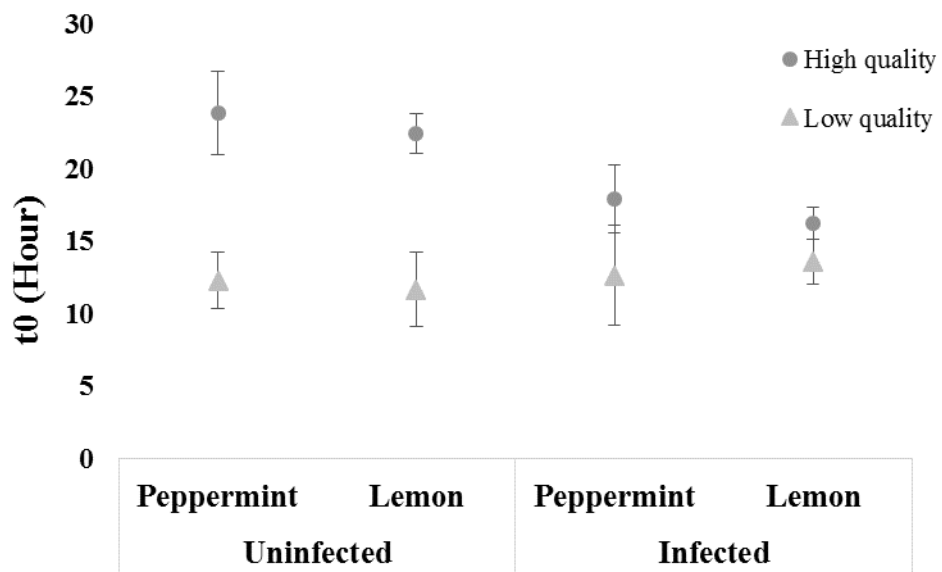
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702 Fig 3.

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Fig 4.