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Comparing thermal tolerance across contrasting landscapes: first steps towards understanding how landscape management could modify ectotherm thermal tolerance

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3

4 **Title**

5 Comparing thermal tolerance across contrasting landscapes: first steps towards understanding
6 how landscape management could modify ectotherm thermal tolerance.

7

8 **Running title**

9 Landscape influences insects' thermal tolerance

10

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25 **Abstract**

- 26 1. Insects are highly dependent on ambient temperatures to ensure their biological functions.
27 Their persistence in the environment and their resistance to unfavorable temperatures are
28 governed by their physiological thermal tolerance.
- 29 2. Global change extends beyond climatic conditions to encompass modifications to the
30 landscape. However, studies of climate change and landscape composition effects on
31 ecosystem services, such as biological control, are commonly performed independently.
32 Moreover, coarse scales are not always relevant when assessing climate change's impacts
33 on ectotherms. We aimed to better understand the ecological relationships that may exist
34 between microclimatic variation and insect thermal tolerance across a landscape
35 composition gradient.
- 36 3. To determine how landscape composition may impact insect thermal tolerance, parasitic
37 wasps (Hymenoptera: Braconidae: Aphidiinae) of aphids were sampled along a landscape
38 gradient from "closed" to "open" habitats. Sampling was performed during the winter
39 2013/14 and spring 2014 in cereal fields of Brittany, France. Meteorological data were
40 recorded along this gradient.
- 41 4. First, our results show an influence of landscape composition on local microclimate.
42 Additionally, parasitoids from open landscapes had a higher tolerance to low temperatures,
43 leading to higher physiological costs, compared to parasitoids from closed landscapes. This
44 trend was stronger in winter than in spring.
- 45 5. These results have numerous implications in the context of climate change, suggesting that
46 targeted landscape management practices could create sheltered microclimatic areas that

47 reduce the physiological costs of thermal tolerance, and promote the persistence of
48 biological control agents.

49

50 **Keywords:** Climate change; Thermal biology; Critical temperatures; Insect ecophysiology;
51 Landscape ecology; Conservation ecology; Biological pest control; Hymenoptera;
52 Braconidae; Aphidiinae; *Aphidius*

53

54 **Introduction**

55 Climate models predict a global average temperature increase of between 1.7 and 6°C by the
56 year 2100 (IPCC, 2013). In addition, an increase in the frequency of extreme events (heat or
57 cold spells) is expected, especially in temperate regions (Hance *et al.*, 2007). Meanwhile,
58 European landscapes have undergone increasing homogenization since the 1950s due to
59 agricultural intensification, leading to the removal of hedges, groves, woods, and natural
60 grasslands (MEA, 2005). This homogenization has reduced the effectiveness of ecosystem
61 services, such as pest control (Thies *et al.*, 2011). Although the combined influences of
62 landscape properties and climate change (warming winters, increased incidence of cold spells)
63 on ecosystems are still poorly understood, there is increasing evidence that working on both
64 of these aspects could yield more important insights than considering them separately.

65 Despite numerous reports of climate change impacts, the gap between the coarse scale at
66 which climate data are collected and the climatic conditions the organisms actually experience
67 at a finer scale is problematic (Bennie *et al.*, 2008; Potter *et al.*, 2013; Woods *et al.*, 2015).

68 Downscaling from global to microclimatic variations is needed to better investigate how
69 ectotherms will respond to changes in their immediate environment. It is well known that

70 local microclimate can be influenced by landscape properties (*e.g.* Chen *et al.*, 1999). For
71 instance, the windbreak and antifreeze role of hedges, especially those with an embankment,
72 has been widely studied and confirmed in agricultural landscapes. Wooded and closed areas
73 generally have lower temperature amplitudes than open areas over a daily scale (Argent,
74 1992; Suggitt *et al.*, 2011), but are also colder, on average, than open areas due to the
75 retention of cold air layers by hedges (Quénol & Beltrando, 2006). The microclimatic
76 variation that occurs between open and closed landscapes is a very complex phenomenon that
77 depends on many elements such as slope, hedge density, topography, and solar radiation
78 (Quénol & Beltrando, 2006; Bennie *et al.*, 2008; Suggitt *et al.*, 2011).

79 In the context of conservation biology, and accepting the realities of climate change and
80 landscape simplification, there is a need to understand whether microclimatic variation due to
81 landscape composition affect ectotherm thermal tolerance. The existence of microclimates is
82 widely confirmed, but the roles they play with regard to small organism ecology remains
83 understudied (Potter *et al.*, 2013; Sunday *et al.*, 2014; Woods *et al.*, 2015). Recent field
84 studies (*e.g.* Suggitt *et al.*, 2011; Pincebourde & Woods, 2012; Lawson *et al.*, 2014; Maclean
85 *et al.*, 2015) have demonstrated the importance of focusing on microclimatic variation when
86 assessing organisms' susceptibility to climate change. These papers, along with the present
87 study, represent the first steps towards understanding how insects will be affected by climate
88 change at the scale of their local environment, and whether microclimatic variations due to
89 landscape composition could allow them to exploit more favorable temperature conditions.

90 There is a particular need to identify the relative roles of evolution versus behavioral and
91 physiological plasticity in moderating the impacts of global environmental changes (Deutsch
92 *et al.*, 2008; Sunday *et al.*, 2014). Acclimatization (*i.e.*, the capacity to better resist previously

93 experienced temperatures) is an important physiological trait utilized by insects to cope with
94 thermal stress. This is a well-known phenomenon that, along with behaviour, could be a
95 plastic trait that commonly allows ectotherms to cope with climate change (Andrew &
96 Terblanche, 2013; Colinet *et al.*, 2015). Body size, which is known to influence many life
97 history traits in insects (Chown & Gaston, 2010), could also influence their thermal tolerance.
98 Indeed, the Absolute Energy Demand (AED) hypothesis predicts that larger individuals
99 should be disadvantaged in the face of stressful temperature conditions, since they expend
100 energy proportionally faster than smaller individuals (Reim *et al.*, 2006).

101 This study examined the ecological relationships between the thermal tolerance of insects,
102 microclimatic variation, and agricultural landscape composition (crop size, hedge length, type
103 of soil cover). In particular, this work aimed to investigate how insects could adjust their
104 phenotypic responses to temperature variation among landscapes. To address these issues, the
105 cold tolerance and the size of aphid parasitoids, important biological control agents, were
106 measured across a landscape composition gradient in Brittany, France. Many studies have
107 been devoted to these species (*e.g.*, Thies *et al.*, 2005; Le Lann *et al.*, 2008, 2014) including
108 investigations of their thermal tolerance (Giri *et al.*, 1982; Legrand *et al.*, 2004; Le Lann *et*
109 *al.*, 2011a; b; Ismail *et al.*, 2012). Aphid parasitoids are interesting biological models for
110 landscape microclimate studies because they closely track the distribution of their hosts,
111 especially during the larval stage when they are immobilized inside an aphid mummy and
112 have to withstand the climatic conditions imposed upon them.

113 The following hypotheses were tested: (i) In the study area, microclimate varies along the
114 landscape gradient, with temperatures in the closed landscape being on average colder but less
115 variable than in the open landscape; (ii) If the first hypothesis is confirmed, the parasitoid

116 thermal tolerance should be landscape-dependent, with parasitoids from open, more stressful
117 areas being more cold tolerant; (iii) Due to their seasonal acclimatization capacities, and
118 because exposure to unfavorable cold temperatures are less frequent in spring months,
119 parasitoids should be more cold resistant in winter than in spring; (iv) Parasitoids are smaller
120 in open areas than in closed areas; and (v) parasitoids are smaller in winter than in spring due
121 to temperature differences between landscapes and seasons (following the AED hypothesis).

122

123 **Material and methods**

124 **Landscape gradient determination**

125 Parasitic wasps were monitored in winter wheat fields in northern Brittany (France). Sampling
126 took place in the vicinity of the LTER '*Zone Atelier Armorique*' ([osur.univ-rennes1.fr/za-](http://osur.univ-rennes1.fr/za-armorique)
127 [armorique](http://osur.univ-rennes1.fr/za-armorique), 48°36'N, 1°32'W) along a hedgerow network landscape gradient, ranging from
128 closed landscapes to more open landscapes. To select the fields, 16 areas (300 meter radius)
129 containing at least one wheat field were characterized with respect to the following three
130 parameters: total hedge length (meters), average field size (hectares), and percentage
131 grassland in the buffer zone. The land-cover over the past 5 years (2009-2013) was
132 characterized, and only plots with annual cultures (maize or cereals) during the last 5 years
133 were considered. This restriction was imposed in order to limit any potential effects of
134 grasslands on arthropod communities. One wheat field was randomly selected from each area,
135 and each area was then identified as belonging to one of three landscape units: (1) Closed
136 landscape (five fields) – high grassland density (>45%), many hedgerows (>3200m) and
137 small field sizes (<0.93ha); (2) Intermediate landscape (six fields); (3) Open landscape (five
138 fields)– low grassland density (<20%), few hedgerows (<550m) and larger fields (>2.70ha).

139 See supplementary material for a map of the study area (Appendix S1), precise locations of
140 the fields (Appendix S2), correlations between landscape variables (Appendix S3) and
141 extreme values for the landscape gradient (Appendix S4). All of the selected fields were
142 separated from one another by at least 600 meters. Although all fields were prospected,
143 parasitoids were only found in 12 fields (4 per landscape type). All of the selected fields were
144 managed with similar conventional agriculture practices. Preliminary results revealed no
145 significant differences in insect thermal tolerance within landscape types. Accordingly, data
146 from plots belonging to the same type of landscape were pooled in subsequent analyses.

147

148 **Sampling sessions and meteorological records**

149 To evaluate potential differences in insect thermal tolerance between seasons, two rounds of
150 sampling were conducted: one in late winter (13 January to 7 March 2014) and one in early
151 spring (24 March to 15 May 2014). Over the course of both sampling periods, local
152 meteorological data were recorded using BWS200 weather stations (Campbell Scientific
153 France) to characterize climate differences between seasons and among landscapes. One
154 weather station per landscape type was used, with each recording air temperature, relative
155 humidity (using CS215 probes accurate to $\pm 0.3^{\circ}\text{C}$ and $\pm 2\%$ respectively) and wind speed
156 (using Wind Sentry anemometer accurate to $\pm 0.5\text{m}\cdot\text{s}^{-1}$) once an hour during the entire
157 sampling period. Each station had a roof and was placed 1 meter above the ground. Each
158 station was placed at least 15 meters from the hedge on flat ground.

159

160

161

162 **Study species**

163 *Aphidius* parasitic wasps (Hymenoptera: Braconidae: Aphidiinae) are endoparasitoids of
164 cereal aphids. These aphids (*Sitobion avenae* Fabricius, *Rhopalosiphum padi* L. and
165 *Metopolophium dirhodum* Walker) are present during the winter in Brittany, resulting in
166 increased crop damage at the onset of spring (Le Ralec *et al.*, 2010). Parasitoids are present
167 throughout the year in Brittany with a succession of species between seasons (Krespi *et al.*,
168 1994). In this study, *Aphidius* parasitoids were included in analyses, while secondary
169 parasitoids were excluded due to their rarity in the fields early in the season. A total of 4
170 species were found during winter (*Aphidius rhopalosiphi* De Stefani-Perez, *Aphidius*
171 *matricariae* Haliday, *Aphidius avenae* Haliday and *Aphidius ervi* Haliday) and 3 species in
172 spring (*A. rhopalosiphi*, *A. avenae* and *A. ervi*).

173

174 **Sampling methods**

175 Parasitoids were sampled by collecting aphid mummies (*i.e.*, parasitized aphids containing a
176 parasitoid pupa). To assess the diversity and abundance of mummies, fields were visited twice
177 a week during each sampling period. Sampling occurred during a 1 hour period over a surface
178 of at least 1000m², with field borders excluded to avoid margin effects. Mummies of the three
179 major species of cereal aphids described above were sampled (every mummy found was
180 collected). The largest living aphids (larval stages L4 and above) were also sampled and kept
181 in microcages to wait for possible mummification. Each microcage consisted of a plastic tube
182 (5 cm diameter) in which wheat (*Triticum aestivum* L. v. *Saturnus* (Poaceae)) grew on a
183 vermiculite substrate. The microcages were checked daily for 10 days and newly-formed
184 mummies were conserved. Each mummy was placed individually in a gelatin capsule

185 (Capsule T1 - Capsugel®) and subsequent parasitoid emergence was verified twice per day.
186 Aphids, mummies and parasitoids were maintained in the laboratory after sampling at $20 \pm$
187 1°C , $70 \pm 10\%$ relative humidity, and a photoperiod of L16:D8. The sex of parasitoids was
188 determined, with individuals identified according to their collection site. Following thermal
189 tolerance experiments (see below), parasitoids were freeze-killed at -20°C and identified
190 using an optical microscope and the identification key developed by Hullé *et al.* (2006). Host
191 aphid species were also identified for each parasitoid using a binocular microscope. This
192 enabled the determination of the composition of the parasitoid guild and the creation of
193 quantitative food webs for both seasons. Comparisons of thermal tolerance and size between
194 winter and spring-collected parasitoids were only made for *A. rhopalosiphi*, because it was the
195 only species present in large numbers during both seasons.

196

197 **Cold tolerance measurement**

198 Non-lethal thermal thresholds are states of narcosis, offering a means of estimating the cold
199 tolerance of individuals, which can have important effects on insect fitness (Le Lann *et al.*,
200 2011a; Alford *et al.*, 2012a). Insect physiological thermal tolerance is defined as the
201 temperature range between the two thresholds of minimum critical temperature (CT_{min}) and
202 maximum temperature (CT_{max}) beyond which survival is impossible (Bale, 1996). Indeed,
203 survival is of little benefit if locomotion is compromised, leading to death through an inability
204 to feed or to escape predators or parasites (Alford *et al.*, 2012a; b). Here, we only focus on the
205 CT_{min} threshold. Indeed, in temperate climates insect fitness is expected to increase as
206 temperature rises (Deutsch *et al.*, 2008) while sudden cold spells in winter could have a major

207 impact on parasitoid populations and could decrease the effectiveness of pest control in late
208 winter and early spring.

209 To measure parasitoid cold tolerance (CT_{min}), a double-walled glass column based on the
210 model of Huey *et al.* (1992), and improved by Powell & Bale (2006), was used. A cryostat
211 (VWR-CryoBath®) was used to cool the tube. There was no temperature difference between
212 the top and the bottom of the tube. Temperature was controlled using a thermocouple probe
213 (accurate to $0.01^{\circ}C$) connected to a digital display. A cooling rate of $0.75^{\circ}C.min^{-1}$ was chosen
214 to avoid inducing a rapid cold hardening response in the test insects (Powell & Bale, 2006).
215 Relative humidity in the tube, which could not be directly controlled, was $35 \pm 10\%$. Each of
216 the 272 parasitoids tested was individually placed in a tube. An individual's CT_{min} was
217 considered to have been reached when the parasitoid could no longer cling to the wall of the
218 vertical column, and dropped from the tube wall (Le Lann *et al.*, 2011a). After CT_{min}
219 measurements, individuals were placed individually in Eppendorf® tubes and stored in the
220 freezer. In order to avoid any confounding factors (temperature, atmospheric pressure, solar
221 radiation in the laboratory), individuals originating from different field types were tested on
222 each day of the experiments.

223

224 **Size measurements**

225 The size of parasitoids was measured after thermal tolerance experiments. The average length
226 of each parasitoid's two hind tibia (a good proxy for parasitoid size) was measured and
227 averaged. Images were captured with a Zeiss AxioCam ERc5s® HD camera mounted on a x9
228 binocular magnifier, and measurements (precise to 0.01 mm) were performed with the
229 Image J® software (v. 1.48).

230 **Statistical analyses**

231 Statistical analyses were performed using R software (R Development Core Team, 2013).
232 Statistical analyses were carried out with a statistical error risk of 5%. Statistical differences
233 in meteorological data among landscape types were tested using a standard repeated measures
234 ANOVA. The meteorological variable in question was considered as a fixed effect, and
235 recording time included as a random effect. Tukey HSD post-hoc tests were used to detect
236 pairwise differences among landscape types for each season. Homogeneity of species
237 distributions among landscapes was tested using a Chi-square test of homogeneity for each
238 season. A generalized linear model (GLM) was fitted to parasitoid CT_{min} data in both seasons,
239 with landscape type, parasitoid species, sex, size, aphid host, parasitoid species x landscape
240 interaction, and parasitoid species x aphid host species interaction as explanatory variables.
241 The effect of these explanatory variables was tested with the “Anova” function from the “car”
242 package, which performs an analysis of variance (Type II) with likelihood ratio tests, using a
243 chi-square statistic (Fox & Weisberg, 2011). Tukey HSD post-hoc tests for linear models
244 were then performed to test for differences within categories. CT_{min} differences between
245 seasons were tested using a Mann-Whitney test. Kruskal-Wallis tests were used to test if the
246 landscape had an effect on the size of parasitoid individuals in each season. For *A.*
247 *rhopalosiphi*, two GLMs were fitted and tested as described above. The CT_{min} response of
248 parasitoids to season x sex and season x size interactions, and of their size to a season x sex
249 interaction were tested. Differences within seasons were then evaluated with Mann-Whitney
250 tests.

251

252

253 **Results**

254 **Meteorological records**

255 For both seasons, closed landscapes were on average significantly colder than open
256 landscapes (Table 1). With respect to its amplitude and standard deviation, temperature varied
257 less in closed landscapes than in intermediate and open landscapes. In winter, closed and
258 intermediate landscapes had a higher relative humidity than open landscapes. Closed
259 landscapes had a lower wind speed than intermediate and open landscapes. The weather in
260 winter was on average colder, with greater wind exposure and humidity than in spring for all
261 landscape types. There was more variation among landscapes in winter than in spring for all
262 of the meteorological variables measured. The occurrence of days which contained at least
263 one temperature event under zero were for closed, intermediate and open landscapes
264 respectively, 1, 2, and 4 in winter and 0, 0, and 4 in spring.

265 Please insert Table 1 here

266

267 **Parasitoid communities**

268 The parasitoid community (Fig. 1) was dominated by *A. rhopalosiphi* in winter and spring.
269 The second most abundant species was *A. matricariae* in winter, although this species was
270 completely absent in spring. *A. avenae* was the second most abundant species in spring. The
271 aphid community was dominated by *R. padi* in winter and *M. dirhodum* in spring. In winter,
272 *A. matricariae* and *A. ervi* were never reared from *M. dirhodum*. In spring, parasitoids were
273 reared from different host species in accordance with the hosts' relative abundance. The
274 species were homogeneously distributed across all landscape types in both winter (Chi-
275 squared test, $\chi^2=7.88$, $df=6$, $p=0.25$) and spring ($\chi^2=1.32$, $df=4$, $p=0.86$).

276

Please insert Figure 1 here.

277

278 **Thermal tolerance**

279 **All parasitoid species**

280 Figure 2 shows the data for all species (pooled) for both seasons. Statistical information is
281 provided in Table 2. For all species and across all landscapes, the winter parasitoid
282 community was significantly more cold resistant than the spring community ($-0.64 \pm 0.07^\circ\text{C}$ in
283 winter and $-0.44 \pm 0.05^\circ\text{C}$ in spring, Mann-Whitney test, $W=10273$, $p<0.05$).

284 In winter, there was a significant effect of landscape type on CT_{\min} . Individuals from open
285 environments were significantly more cold resistant than those from intermediate (Tukey
286 HSD, $z=-2.48$, $p<0.05$) and closed ($z=-3.53$, $p<0.01$) environments. There was a marginally
287 non-significant difference between individuals from intermediate and closed environments
288 ($z=-2.22$, $p=0.06$). A similar but non-significant trend was observed for spring populations. In
289 spring, a significant effect of host species was found; parasitoids reared from *M. dirhodum*
290 had significantly lower CT_{\min} than those reared from *S. avenae* ($-0.51 \pm 0.06^\circ\text{C}$ $n=98$ and -0.20
291 $\pm 0.09^\circ\text{C}$ $n=39$ respectively, Tukey HSD, $z=2.76$, $p<0.05$). There were no differences between
292 the CT_{\min} of parasitoids reared from *R. padi* compared to those reared from other species.
293 There were no significant differences in CT_{\min} among species or between sexes, neither in
294 winter or spring. There was no effect of body size on CT_{\min} for either winter or spring-
295 collected parasitoids, and parasitoid size was not influenced by the landscape gradient in
296 winter (Kruskal-Wallis test, $\chi^2=3.03$, $df=2$, $p=0.22$) or spring ($\chi^2=0.13$, $df=2$, $p=0.94$). There
297 were no interaction effects among landscape gradient, parasitoid species, or aphid host species
298 affecting the CT_{\min} of parasitoids in winter or spring.

299 Please insert Table 2 here

300 Please insert Figure 2 here

301

302 **For *A. rhopalosiphi* only**

303 Winter sampled individuals from all types of landscape were more resistant to cold than those
304 sampled in the spring (respectively $-0.66 \pm 0.08^{\circ}\text{C}$ and $-0.45 \pm 0.04^{\circ}\text{C}$, Anova, LR=3.32, df=1,
305 $p=0.05$). There was no difference in CT_{min} between sexes in either season (LR=0.001, df=1,
306 $p=0.93$). There was no influence of parasitoids' body size on their thermal tolerance for either
307 sex (LR=0.63, $p=0.42$). Furthermore, there was no effect of the interaction between season
308 and sex (LR=0.56, df=1, $p=0.46$) or between season and size (LR=0.75, df=1, $p=0.39$) on
309 parasitoids' CT_{min} (Fig. 3a). Winter-collected *A. rhopalosiphi* individuals were smaller than
310 spring individuals (respectively and for both sexes, $0.50 \pm 0.01\text{mm}$ and $0.58 \pm 0.01\text{mm}$,
311 LR=57.84, df=1, $p<0.001$). There was no effect of the interaction between season and sex on
312 parasitoid body size (LR=0.65, df=1, $p=0.42$). In both seasons, size differed significantly
313 between sexes (LR=13.03, df=1, $p<0.001$). Females were significantly larger than males in
314 winter ($0.52 \pm 0.01\text{mm}$ and $0.47 \pm 0.01\text{mm}$ respectively, Mann-Whitney test, $W=1067$, $p<0.01$)
315 and spring (respectively $0.58 \pm 0.01\text{mm}$ and $0.55 \pm 0.01\text{mm}$, $W=1098$, $p<0.01$) (Fig. 3b).

316 Please insert Figure 3a and 3b here

317

318 **Discussion**

319 The results confirmed our first and second hypotheses, highlighting a landscape composition
320 effect on different climatic factors and on the cold tolerance of parasitoids. This effect was
321 more pronounced in winter. This study also revealed that parasitoids were smaller and more

322 cold resistant in the winter than in spring, in accordance with our third and fifth hypotheses.
323 Parasitoid size was not affected by the landscape gradient, which refutes our fourth
324 hypothesis.

325

326 **Landscape effects on microclimate**

327 Between an open area and a hedged zone or forest remnant, there is generally a decrease in
328 daytime temperatures but an increase in humidity (Argent, 1992; Quénol & Beltrando, 2006).
329 These tendencies were confirmed by our measurements in winter and, to a lesser extent, in
330 spring. Indeed, hedgerows usually limit wind speed, which acts to increase relative humidity.
331 Additionally, cooling typically occurs along the borders of hedges and in the fields behind
332 them. These important shelter effects limit the mixing of air layers and thus accentuate the
333 cooling of fields surrounded by hedgerows (Quénol & Beltrando, 2006). Another
334 consequence of sheltered environments is that temporal variations in temperature and
335 humidity are less pronounced than in open landscapes (Suggitt *et al.*, 2011; also confirmed by
336 our results). In spring, temperature is less variable among landscapes than in winter, since
337 vegetation begins to grow in the fields, and hedgerow foliage increases in density. The present
338 study showed that open landscapes, especially in winter, present more stressful climatic
339 conditions for ectotherms such as wind exposure, frost, more extreme temperatures, and
340 greater temperature variation.

341

342 **Landscape effects on the cold tolerance**

343 We demonstrated the importance of considering microhabitat variation when assessing
344 organisms' susceptibility to climate change. Winter sampled parasitoids from open landscapes

345 were approximately 1°C more resistant to cold than those from closed landscapes, and 0.6°C
346 more resistant than individuals from intermediate landscape. The magnitude of these effects
347 greatly decreased in spring, although a similar trend was observed. Differences in parasitoid
348 CT_{min} along the landscape gradient may have resulted from phenotypic plasticity
349 (acclimatization), which is a particularly effective way for insects to cope with temperature
350 stresses at different temporal scales (Lee & Denlinger, 2010; Colinet *et al.*, 2015). The
351 thermal conditions encountered during the larval stage greatly influences the thermal
352 tolerance of adults in most insects (Hoffmann *et al.*, 2013), and in open landscapes,
353 parasitoids are less protected against cold spells. Thus, parasitoids from open landscapes
354 could have acclimatized to sudden colder temperatures during their development, resulting in
355 greater adulthood cold resistance. The results of Bahrndorff *et al.* (2006) on springtails and
356 Bauerfeind & Fischer (2014) on butterflies also support the contention that thermal extremes
357 explain variation in thermal resistance traits better than average temperatures. Separating the
358 effects of mean and extreme temperatures on ectotherms – a recently developing field of
359 research in thermal biology (Easterling *et al.*, 2000; Thompson *et al.*, 2013; Colinet *et al.*,
360 2015) – is one of the novelties of this study. In sheltered environments, parasitoids might be
361 better able to resist cold due to higher relative humidity, and therefore might have no need to
362 develop the strong physiological resistance required of open landscape parasitoids. Indeed, as
363 suggested by De Bach (1943), desiccation is one of the main reasons for death under cold
364 stress conditions in Aphidiinae. Higher humidity facilitates cold resistance by diminishing the
365 risks of desiccation (Bahrndorff *et al.*, 2006; Hoffmann *et al.*, 2013).

366 Winter temperatures are stressful for parasitoids, since they are often below the insects'
367 developmental thresholds. The thermal threshold for mummy-to-adult development has been

368 estimated at 7.2°C for *A. rhopalosiphi*, 7.9°C for *A. matricariae*, and 6.6°C for *A. ervi*
369 (Sigsgaard, 2000; Colinet & Hance, 2010). In spring, however, temperatures become warmer
370 on average and less extreme. Thus, even if microclimatic differences along the landscape
371 gradient persist (as it has been shown in this study), the temperatures reached are rarely or
372 never stressful enough to make parasitoid acclimatization necessary. For this reason, it may
373 not be possible to reveal differences between landscapes with respect to the cold tolerance of
374 spring-collected parasitoids. In spring, the microclimatic gradient effect could also be
375 compensated by a smaller scale impact of vegetation within the plots. For example, it has
376 previously been shown that vegetation cover in crops provides a stable and beneficial
377 microhabitat for insects both in terms of temperature and wind exposure (Waterhouse, 1955).
378 Nevertheless, as shown in this study for *A. rhopalosiphi*, parasitoid cold tolerance differs
379 between seasons. Winter-collected individuals acclimatized to cold temperatures are therefore
380 more resistant to cold and vice versa in spring, supporting our third hypothesis.

381 There were no effects of sex or parasitoid species on cold tolerance in winter or spring.
382 Parasitoids of all species emerging from *S. avenae* in spring were less cold tolerant than those
383 emerging from *M. dirhodum*. We can thus infer bottom-up effects of host quality on
384 parasitoid thermal tolerance. It is already known that parasitoids emerging from *S. avenae* are
385 larger than parasitoids emerging from other aphid species (which increases their fecundity),
386 and that parasitoids emerging from *R. padi* have a higher emergence rate (Andrade *et al.*,
387 2013). A trade-off may then exist between cold resistance and other life history traits that
388 determine host choice and availability.

389

390

391 **Size effects**

392 In our analysis of *A. rhopalosiphi*, female parasitoids were larger than males. This trend is
393 almost ubiquitous among parasitoid wasps (Hurlbutt, 1987) and insects in general (Chown &
394 Gaston, 2010). There was no direct effect of size on the individuals' thermal tolerance
395 between landscapes, although *A. rhopalosiphi* individuals were smaller in winter than in
396 spring. If we consider that mean winter temperatures recorded are really those experienced by
397 the parasitoids in the fields (*i.e.*, there may have been specific locations with higher
398 temperatures), this result does not follow the predictions of the temperature-size rule (TSR).
399 The TSR states that insects developing at higher temperatures are generally smaller upon
400 emergence as a result of shorter development time (Van der Have & De Jong, 1996). Our
401 results are, however, consistent with the AED hypothesis which states that a winter should
402 impose a selective pressure that favors smaller parasitoids. For instance, Ismail *et al.* (2012)
403 showed that smaller individuals of the parasitoid *A. ervi* that smaller individuals had have
404 better resistance to cold stress than larger wasps. Since parasitoid size is intimately linked to
405 fitness-related traits (*e.g.* fecundity, longevity; Harvey *et al.*, 1994), there could be a size
406 induced trade-off between female fertility (large individuals) and survival (reduced size)
407 under stressful temperature conditions. These results showing an effect of temperature
408 variations at the seasonal scale (but not at the landscape scale) confirm our fifth hypothesis
409 but refutes our fourth hypothesis concerning parasitoid size.

410

411 **Conclusion**

412 A clear impact of the landscape on the thermal tolerance of parasitoids in winter, and to a
413 lesser extent in spring, was observed in this study. The physiological thermal tolerance of

414 parasitoids seemed to respond to the varying weather conditions (especially extreme
415 temperatures and relative humidity) encountered among landscapes and also among seasons.
416 As stated in the introduction, broad scale temperature variations and predictions can be very
417 different from those directly experienced by insects. These data highlight the need to better
418 understand the relationship between insect physiology and landscape structure, especially
419 under stressful conditions. We must use fine scale studies combined with information on the
420 microhabitat if we are to understand climate change effects on insect populations and
421 individual physiology. Climate change effects could be exacerbated by agricultural
422 intensification leading to more open landscapes, subjecting insects to increasing physiological
423 stress in the future. From a biological control perspective, our results imply that it may be
424 possible to manipulate parasitoid cold resistance using protective elements in the landscape
425 that reduce microclimatic variations.

426

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438 performed.

439

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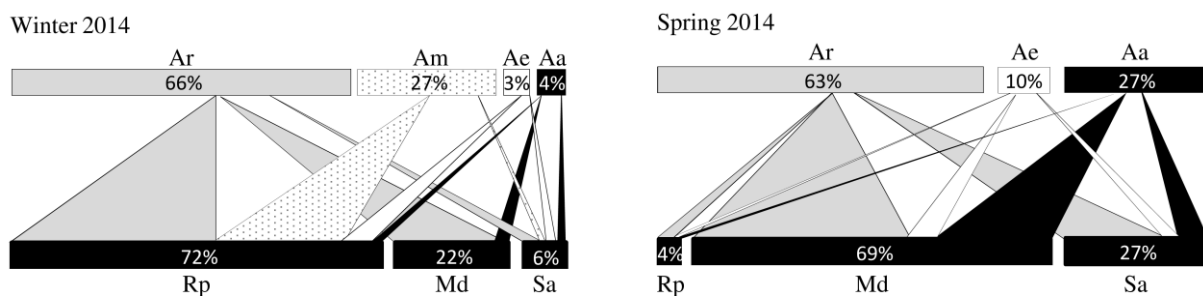
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586 **Figures**

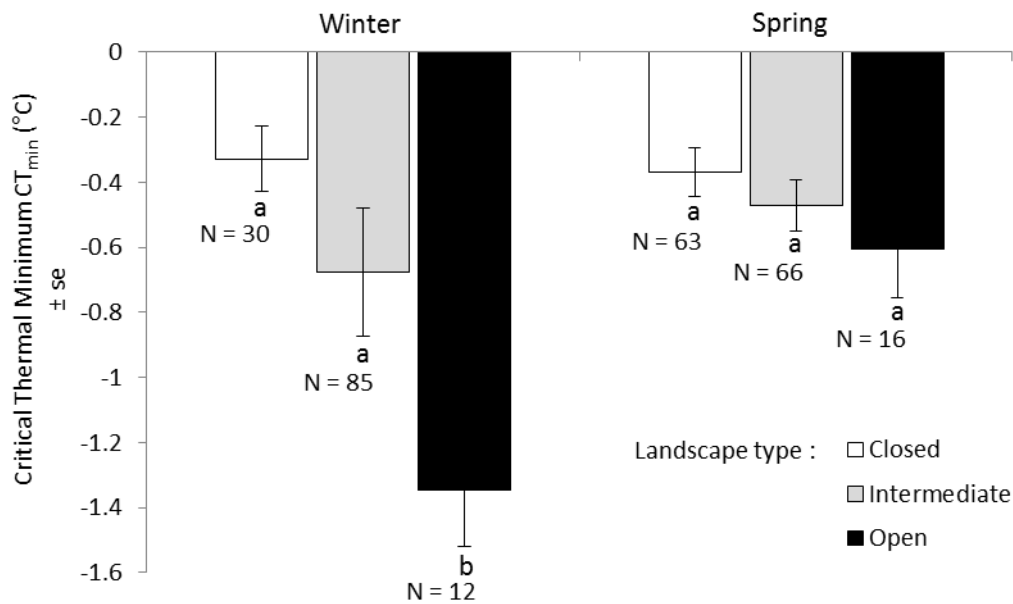


587 **Figure 1:** Quantitative food webs of the parasitoid and aphid community composition in winter and spring 2014.
 588 **Ar** (*Aphidius rhopalosiphī*), **Am** (*Aphidius matricariae*), **Ae** (*Aphidius ervi*), **Aa** (*Aphidius avenae*), **Rp**
 589 (*Rhopalosiphum padi*), **Md** (*Metopolophium dirhodum*), **Sa** (*Sitobion avenae*). Upper bars represent parasitoid
 590 relative abundance and lower bars represent aphid relative abundance for each species. The thickness of the
 591 arrows between two bars is proportional to the relative number of trophic interactions between species (e.g. in
 592 winter 56% of the parasitoids emerged from *R. padi* mummies were *A. rhopalosiphī*). The figures do not include
 593 individuals that could not be identified. The total number of individuals are n=127 and n=141 for parasitoids and
 594 n=141 and n=142 for aphids in winter and spring, respectively.

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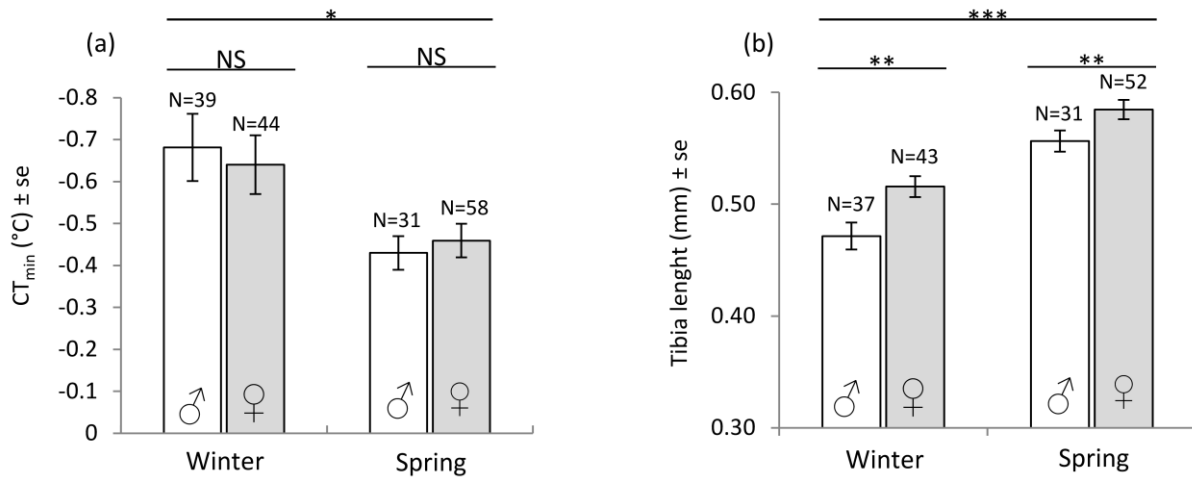
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598 **Figure 2:** Average critical thermal minimum of parasitoids (all species) in winter and spring depending on the type
 599 of landscape. N=127 and 145 in winter and spring, respectively. Lower-case letters show significant differences
 600 between landscapes for each season. For inter-seasonal comparisons refer to the text.



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603 **Figure 3:** Average critical minimal temperature (a) and average tibia length (b) for *A. rhopalosiphi* in winter and
 604 spring. N=172 for CT_{min} measurements and 163 for size measurements. Symbols indicate parasitoids sexes
 605 males (♂) and females (♀). *** p<0.001 ** p<0.01 * p<0.05 n.s.=not significant.



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609 **Table 1:** Daily average meteorological data (\pm standard error) in the study area (Brittany, France) in winter (13th
610 January to 7th March) and spring (24th March to 15th May) for different types of landscape. Results of repeated
611 measures ANOVA comparing meteorological factors among landscapes are shown. n.s.=not significant.
612 Superscript letters (a, b, c) denote significant differences among landscapes for each meteorological factor within
613 each season.

Season	Landscape type	Air temperature (°C)	Min Max temperature (°C)	Relative humidity (%)	Wind speed (m.s ⁻¹)
Landscape comparison					
Winter	Closed	6.58 \pm 0.07 ^(a)	-0.32 14.61	90.82 \pm 0.25 ^(a)	2.18 \pm 0.04 ^(a)
	Intermediate	7.23 \pm 0.08 ^(b)	-0.53 23.37	90.18 \pm 0.25 ^(a)	3.39 \pm 0.06 ^(b)
	Open	8.39 \pm 0.16 ^(c)	-2.07 24.91	85.82 \pm 0.54 ^(b)	2.67 \pm 0.07 ^(c)
	ANOVA (df=2)	F=67.01, p<0.001		F=67.8, p<0.001	F=148.2, p<0.001
Spring	Closed	10.78 \pm 0.10 ^(a)	0.51 20.10	83.63 \pm 0.40	1.84 \pm 0.03 ^(a)
	Intermediate	11.12 \pm 0.11 ^(a,b)	0.08 20.89	84.46 \pm 0.38	1.91 \pm 0.04 ^(a)
	Open	11.49 \pm 0.12 ^(b)	-1.02 21.78	83.66 \pm 0.43	2.71 \pm 0.05 ^(b)
	ANOVA (df=2)	F=10.1, p<0.001		n.s.	F=130.4, p<0.001
Seasonal comparison					
Winter	All	7.12 \pm 0.05	-2.07 24.91	89.70 \pm 0.18	2.77 \pm 0.03
Spring	All	11.13 \pm 0.06	-1.02 21.78	83.91 \pm 0.23	2.16 \pm 0.03

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621 **Table 2:** Factors influencing parasitoid CT_{min} . Test statistics from Generalized Linear Models (GLMs) are shown
 622 for winter (N=127 individuals, 72 females and 45 males) and spring (N=142 individuals, 80 females and 62
 623 males). There were large differences in community assembly between seasons so they had to be tested
 624 separately; see text of Results. The Likelihood-Ratio chi-square is calculated for each model using the “car”
 625 package. *** p<0.001. ** p<0.01. * p<0.05.

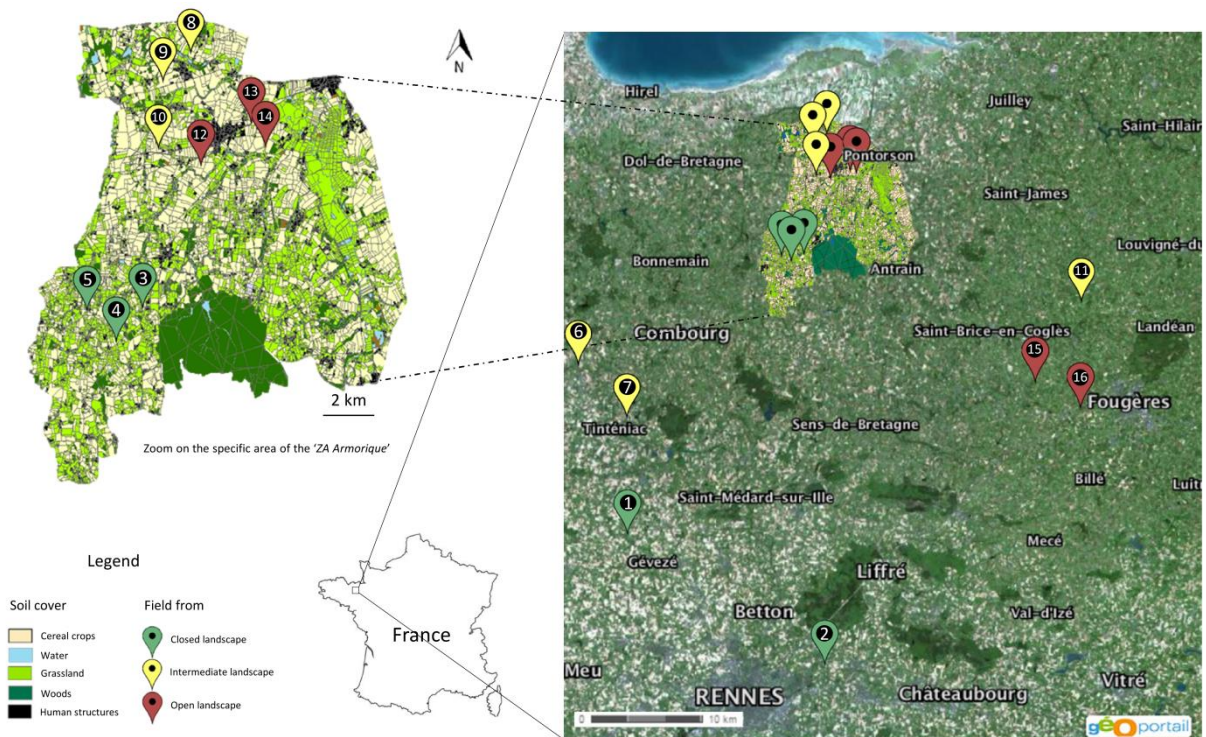
Factor / Season	Winter			Spring		
	LR (χ^2)	df	p > χ^2	LR (χ^2)	df	p > χ^2
Landscape	10.16	2	<0.01 **	3.43	2	0.18
Parasitoid species	0.99	3	0.80	0.62	2	0.73
Landscape : Parasitoid species	0.56	2	0.76	1.69	3	0.64
Sex	0.53	1	0.47	0.39	1	0.53
Host species	1.17	2	0.42	7.26	2	<0.05 *
Parasitoid species : Host species	2.16	4	0.71	0.31	4	0.98
Parasitoid size	1.96	1	0.16	0.48	1	0.49

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641 **Supplementary material:**

642 **Appendix S1:** Map of the sampling zone in northern Brittany (France) with the approximate location of the 16
643 fields sampled. The map includes a zoom on the specific area of the “ZA Armorique” where land cover is fully
644 described, in order to give the reader an idea of the landscape composition gradient. Numbers in the cursors refer
645 to corresponding numbers in Table 3 that give precise geographic locations.

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657 **Appendix S2:** geographic coordinates of the 16 fields sampled. Field numbers refer to the numbers in the cursor
 658 on the map (Appendix S1).

Landscape type	Field number	Latitude (°N)	Longitude (°W)
Closed	1	48.230	1.832
	2	48.123	1.539
	3	48.482	1.603
	4	48.479	1.612
	5	48.482	1.625
Intermediate	6	48.304	1.862
	7	48.301	1.828
	8	48.546	1.599
	9	48.557	1.575
	10	48.527	1.600
	11	48.469	1.274
Open	12	48.513	1.610
	13	48.534	1.548
	14	48.529	1.541
	15	48.383	1.349
	16	48.366	1.307

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660 **Appendix S3:** Correlations between the three variables used for landscape gradient determination. The variables
 661 were highly correlated and it was impossible to avoid this correlation for landscapes in northern Brittany. We did
 662 not consider the separate effect of each variable as it is commonly done in landscape ecology studies, but we did
 663 test the effect of the combination of the three variables on microclimatic conditions. Thus, this study deals with a
 664 “complexity gradient” or “closed to open gradient”.

Correlation between variables (R ²)	Hedge length (m)	Crop size (ha)	Proportion of grasslands (%)
Hedge length (m)	1		
Crop size (ha)	-0.81	1	
Proportion of grasslands (%)	0.45	-0.55	1

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667 **Appendix S4:** Variable values for the two 300m radius buffers (on 16 areas described) situated at the minimum
 668 and maximum extremes of the landscape gradient. High hedge length, small crop size and high proportion
 669 grassland are characteristic of the more “closed” buffer, while short hedge length, large crop size, and high
 670 proportion grassland characterize the more “open” buffer.

Extreme values	Minimum	Maximum
Hedge length (m)	200	4653
Crop size (ha)	0.93	3.36
Proportion grassland (%)	0.01	87

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