

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

Kévin Tougeron, Joan van Baaren, Françoise Burel, Lucy Alford

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4	Title
5	Comparing thermal tolerance across contrasting landscapes: first steps towards understanding
6	how landscape management could modify ectotherm thermal tolerance.
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8	Running title
9	Landscape influences insects' thermal tolerance
10	
11	Authors
12	Kévin Tougeron <sup>1, 2, 3</sup> , Joan van Baaren <sup>1, 2</sup> , Françoise Burel <sup>1, 2</sup> & Lucy Alford <sup>1, 2</sup>
13	
14	Adresses
15	<sup>1</sup> UMR 6553 Ecobio, Université de Rennes 1, 263 avenue du Général Leclerc, 35042 Rennes,
16	France.
17	<sup>2</sup> Université Européenne de Bretagne (UEB), 5 boulevard Laënnec, 35000, Rennes, France.
18	<sup>3</sup> Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université
19	de Montréal, 4101 Sherbrooke Est Montréal, Québec, Canada H1X 2B2.
20	
21	Corresponding author:
22	Kévin Tougeron
23	E-mail: tougeron.kevin@gmail.com

24 Phone: (+33) 2 23 23 41 32

#### 25 Abstract

Insects are highly dependent on ambient temperatures to ensure their biological functions.
 Their persistence in the environment and their resistance to unfavorable temperatures are governed by their physiological thermal tolerance.

Global change extends beyond climatic conditions to encompass modifications to the
 landscape. However, studies of climate change and landscape composition effects on
 ecosystem services, such as biological control, are commonly performed independently.
 Moreover, coarse scales are not always relevant when assessing climate change's impacts
 on ectotherms. We aimed to better understand the ecological relationships that may exist
 between microclimatic variation and insect thermal tolerance across a landscape
 composition gradient.

36 3. To determine how landscape composition may impact insect thermal tolerance, parasitic
wasps (Hymenoptera: Braconidae: Aphidiinae) of aphids were sampled along a landscape
gradient from "closed" to "open" habitats. Sampling was performed during the winter
2013/14 and spring 2014 in cereal fields of Brittany, France. Meteorological data were
recorded along this gradient.

4. First, our results show an influence of landscape composition on local microclimate.
Additionally, parasitoids from open landscapes had a higher tolerance to low temperatures,
leading to higher physiological costs, compared to parasitoids from closed landscapes. This
trend was stronger in winter than in spring.

5. These results have numerous implications in the context of climate change, suggesting that
 targeted landscape management practices could create sheltered microclimatic areas that

47 reduce the physiological costs of thermal tolerance, and promote the persistence of48 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*

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### 54 Introduction

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

Despite numerous reports of climate change impacts, the gap between the coarse scale at which climate data are collected and the climatic conditions the organisms actually experience at a finer scale is problematic (Bennie *et al.*, 2008; Potter *et al.*, 2013; Woods *et al.*, 2015). Downscaling from global to microclimatic variations is needed to better investigate how ectotherms will respond to changes in their immediate environment. It is well known that

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

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

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

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

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

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

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

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#### 123 Material and methods

### 124 Landscape gradient determination

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

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

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#### 148 Sampling sessions and meteorological records

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

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#### 162 **Study species**

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

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#### **Sampling methods**

Parasitoids were sampled by collecting aphid mummies (*i.e.*, parasitized aphids containing a 175 176 parasitoid pupa). To assess the diversity and abundance of mummies, fields were visited twice a week during each sampling period. Sampling occurred during a 1 hour period over a surface 177 of at least 1000m<sup>2</sup>, with field borders excluded to avoid margin effects. Mummies of the three 178 major species of cereal aphids described above were sampled (every mummy found was 179 collected). The largest living aphids (larval stages L4 and above) were also sampled and kept 180 in microcages to wait for possible mummification. Each microcage consisted of a plastic tube 181 (5 cm diameter) in which wheat (Triticum aestivum L. v. Saturnus (Poaceae)) grew on a 182 vermiculite substrate. The microcages were checked daily for 10 days and newly-formed 183 mummies were conserved. Each mummy was placed individually in a gelatin capsule 184

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

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#### 197 Cold tolerance measurement

Non-lethal thermal thresholds are states of narcosis, offering a means of estimating the cold 198 tolerance of individuals, which can have important effects on insect fitness (Le Lann et al., 199 2011a; Alford et al., 2012a). Insect physiological thermal tolerance is defined as the 200 temperature range between the two thresholds of minimum critical temperature (CT<sub>min</sub>) and 201 maximum temperature ( $CT_{max}$ ) beyond which survival is impossible (Bale, 1996). Indeed, 202 survival is of little benefit if locomotion is compromised, leading to death through an inability 203 to feed or to escape predators or parasites (Alford et al., 2012a; b). Here, we only focus on the 204 CT<sub>min</sub> threshold. Indeed, in temperate climates insect fitness is expected to increase as 205 206 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 late208 winter and early spring.

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

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#### 224 Size measurements

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

#### 230 Statistical analyses

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

251

252

# 253 **Results**

#### 254 Meteorological records

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

265

Please insert Table 1 here

266

#### 267 Parasitoid communities

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

Please insert Figure 1 here. 276 277 **Thermal tolerance** 278 All parasitoid species 279 Figure 2 shows the data for all species (pooled) for both seasons. Statistical information is 280 provided in Table 2. For all species and across all landscapes, the winter parasitoid 281 community was significantly more cold resistant than the spring community ( $-0.64 \pm 0.07$ °C in 282 winter and  $-0.44 \pm 0.05$  °C in spring, Mann-Whitney test, W=10273, p<0.05). 283 In winter, there was a significant effect of landscape type on CT<sub>min</sub>. Individuals from open 284 285 environments were significantly more cold resistant than those from intermediate (Tukey HSD, z=-2.48, p<0.05) and closed (z=-3.53, p<0.01) environments. There was a marginally 286 non-significant difference between individuals from intermediate and closed environments 287 288 (z=-2.22, p=0.06). A similar but non-significant trend was observed for spring populations. In spring, a significant effect of host species was found; parasitoids reared from M. dirhodum 289 had significantly lower CT<sub>min</sub> than those reared from S. avenae (-0.51 ±0.06°C n=98 and -0.20 290  $\pm 0.09^{\circ}$ C n=39 respectively, Tukey HSD, z=2.76, p<0.05). There were no differences between 291 the CT<sub>min</sub> of parasitoids reared from R. padi compared to those reared from other species. 292 There were no significant differences in CT<sub>min</sub> among species or between sexes, neither in 293 winter or spring. There was no effect of body size on CT<sub>min</sub> for either winter or spring-294 collected parasitoids, and parasitoid size was not influenced by the landscape gradient in 295 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 296 were no interaction effects among landscape gradient, parasitoid species, or aphid host species 297 affecting the CT<sub>min</sub> of parasitoids in winter or spring. 298

299

# Please insert Table 2 here

Please insert Figure 2 here

300 301

#### **For** *A. rhopalosiphi* **only**

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

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Please insert Figure 3a and 3b here

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#### 318 **Discussion**

The results confirmed our first and second hypotheses, highlighting a landscape composition effect on different climatic factors and on the cold tolerance of parasitoids. This effect was more pronounced in winter. This study also revealed that parasitoids were smaller and more 14 cold resistant in the winter than in spring, in accordance with our third and fifth hypotheses.
Parasitoid size was not affected by the landscape gradient, which refutes our fourth
hypothesis.

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#### 326 Landscape effects on microclimate

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

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

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

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

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

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

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#### 391 Size effects

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

410

# 411 Conclusion

A clear impact of the landscape on the thermal tolerance of parasitoids in winter, and to a
lesser extent in spring, was observed in this study. The physiological thermal tolerance of 18

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

426

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- 585
- 586 Figures



587 588 Figure 1: Quantitative food webs of the parasitoid and aphid community composition in winter and spring 2014. 589 Ar (Aphidius rhopalosiphi), Am (Aphidius matricariae), Ae (Aphidius ervi), Aa (Aphidius avenae), Rp 590 (Rhopalosiphum padi), Md (Metopolophium dirhodum), Sa (Sitobion avenae). Upper bars represent parasitoid 591 relative abundance and lower bars represent aphid relative abundance for each species. The thickness of the 592 arrows between two bars is proportional to the relative number of trophic interactions between species (e.g. in 593 winter 56% of the parasitoids emerged from R. padi mummies were A. rhopalosiphi). The figures do not include 594 individuals that could not be identified. The total number of individuals are n=127 and n=141 for parasitoids and 595 n=141 and n=142 for aphids in winter and spring, respectively.

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



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



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

Season	Landscape type	Air temperature (°C)	Min   Max temperature (°C)	Relative humidity (%)	Wind speed (m.s <sup>-1</sup> )
Landsca	pe comparison				
Winter	Closed	6.58 ±0.07 <sup>(a)</sup>	-0.32   14.61	90.82 ±0.25 <sup>(a)</sup>	2.18 ±0.04 <sup>(a)</sup>
	Intermediate	7.23 ±0.08 <sup>(b)</sup>	-0.53   23.37	90.18 ±0.25 <sup>(a)</sup>	3.39 ±0.06 <sup>(b)</sup>
	Open	8.39 ±0.16 <sup>(c)</sup>	-2.07   24.91	85.82 ±0.54 <sup>(b)</sup>	2.67 ±0.07 <sup>(c)</sup>
	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 ±0.10 <sup>(a)</sup>	0.51   20.10	83.63 ±0.40	1.84 ±0.03 <sup>(a)</sup>
	Intermediate	<sup>(a,b)</sup> 11.12 ±0.11	0.08   20.89	84.46 ±0.38	1.91 ±0.04 <sup>(a)</sup>
	Open	11.49 ±0.12 <sup>(b)</sup>	-1.02   21.78	83.66 ±0.43	2.71 ±0.05 <sup>(b)</sup>
	ANOVA (df=2)	F=10.1, p<0.001		n.s.	F=130.4, p<0.001
Seasonal comparison					
Winter	All	7.12 ±0.05	-2.07   24.91	89.70 ±0.18	2.77 ±0.03
Spring	All	11 13 +0 06	-1 02 21 78	83 91 +0 23	2 16 +0 03

- **Table 2:** Factors influencing parasitoid CT<sub>min</sub>. 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 <sup>2</sup> )	df	$p > \chi^2$		LR ( $\chi^2$ )	df	$p > \chi^2$	
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	

#### Supplementary material:

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



657 Appendix S2: geographic coordinates of the 16 fields sampled. Field numbers refer to the numbers in the cursor

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
I	13	48.534	1.548
	14	48.529	1.541
	15	48.383	1.349
	16	48.366	1.307

on the map (Appendix S1).

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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

not consider the separate effect of each variable as it is commonly done in landscape ecology studies, but we did

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 <sup>2</sup> )	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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Appendix S4: Variable values for the two 300m radius buffers (on 16 areas described) situated at the minimum and maximum extremes of the landscape gradient. High hedge length, small crop size and high proportion grassland are characteristic of the more "closed" buffer, while short hedge length, large crop size, and high proportion grassland characterize the more "open" buffer.

Extreme valuesMinimumMaximumHedge length (m)2004653Crop size (ha)0.933.36Proportion grassland (%)0.0187