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

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4 **Title**

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6 how landscape management could modify ectotherm thermal tolerance.

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8 **Running title**

9 Landscape influences insects' thermal tolerance

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Abstract

25

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.

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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

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

In the context of conservation biology, and accepting the realities of climate change and landscape simplification, there is a need to understand whether microclimatic variation due to landscape composition affect ectotherm thermal tolerance. The existence of microclimates is 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 studies (*e.g.* Suggitt *et al.*, 2011; Pincebourde & Woods, 2012; Lawson *et al.*, 2014; Maclean *et al.*, 2015) have demonstrated the importance of focusing on microclimatic variation when assessing organisms' susceptibility to climate change. These papers, along with the present 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 landscape composition could allow them to exploit more favorable temperature conditions.

There is a particular need to identify the relative roles of evolution versus behavioral and physiological plasticity in moderating the impacts of global environmental changes (Deutsch *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

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).

Material and methods

Landscape gradient determination

Parasitic wasps were monitored in winter wheat fields in northern Brittany (France). Sampling took place in the vicinity of the LTER '*Zone Atelier Armorique*' (osur.univ-rennes1.fr/za-armorique, 48°36'N, 1°32'W) along a hedgerow network landscape gradient, ranging from closed landscapes to more open landscapes. To select the fields, 16 areas (300 meter radius) containing at least one wheat field were characterized with respect to the following three 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 characterized, and only plots with annual cultures (maize or cereals) during the last 5 years 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, and each area was then identified as belonging to one of three landscape units: (1) Closed landscape (five fields) – high grassland density (>45%), many hedgerows (>3200m) and small field sizes (<0.93ha); (2) Intermediate landscape (six fields); (3) Open landscape (five 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 $\pm0.3°C$ and $\pm2\%$ respectively) and wind speed

156 (using Wind Sentry anemometer accurate to $\pm0.5m.s^{-1}$) 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.

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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 ±

187 1°C, 70 ± 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°C) connected to a digital display. A cooling rate of $0.75°C.min^{-1}$ 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 ±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).

Statistical analyses

Statistical analyses were performed using R software (R Development Core Team, 2013). Statistical analyses were carried out with a statistical error risk of 5%. Statistical differences in meteorological data among landscape types were tested using a standard repeated measures ANOVA. The meteorological variable in question was considered as a fixed effect, and recording time included as a random effect. Tukey HSD post-hoc tests were used to detect pairwise differences among landscape types for each season. Homogeneity of species 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_{min} data in both seasons, 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. The effect of these explanatory variables was tested with the "Anova" function from the "car" package, which performs an analysis of variance (Type II) with likelihood ratio tests, using a chi-square statistic (Fox & Weisberg, 2011). Tukey HSD post-hoc tests for linear models were then performed to test for differences within categories. CT_{min} differences between seasons were tested using a Mann-Whitney test. Kruskal-Wallis tests were used to test if the landscape had an effect on the size of parasitoid individuals in each season. For *A. rhopalosiphi*, two GLMs were fitted and tested as described above. The CT_{min} response of parasitoids to season x sex and season x size interactions, and of their size to a season x sex interaction were tested. Differences within seasons where then evaluated with Mann-Whitney tests.

Results

Meteorological records

For both seasons, closed landscapes were on average significantly colder than open 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 intermediate landscapes had a higher relative humidity than open landscapes. Closed 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 landscape types. There was more variation among landscapes in winter than in spring for all of the meteorological variables measured. The occurrence of days which contained at least one temperature event under zero were for closed, intermediate and open landscapes respectively, 1, 2, and 4 in winter and 0, 0, and 4 in spring.

Please insert Table 1 here

Parasitoid communities

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

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276 <center>Please insert Figure 1 here.</center>

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 ±0.07°C in

283 winter and -0.44 ±0.05°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 ±0.06°C n=98 and -0.20

291 ±0.09°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, χ^2=3.03, df=2, p=0.22) or spring (χ^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.

13

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 ±0.08°C and -0.45 ±0.04°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 ±0.01mm and 0.58 ±0.01mm,

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 ±0.01mm and 0.47 ±0.01mm respectively, Mann-Whitney test, W=1067, p<0.01)

315 and spring (respectively 0.58 ±0.01mm and 0.55 ±0.01mm, 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

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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

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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

Size effects

In our analysis of *A. rhopalosiphi*, female parasitoids were larger than males. This trend is almost ubiquitous among parasitoid wasps (Hurlbutt, 1987) and insects in general (Chown & Gaston, 2010). There was no direct effect of size on the individuals' thermal tolerance between landscapes, although *A. rhopalosiphi* individuals were smaller in winter than in spring. If we consider that mean winter temperatures recorded are really those experienced by the parasitoids in the fields (*i.e.*, there may have been specific locations with higher temperatures), this result does not follow the predictions of the temperature-size rule (TSR). The TSR states that insects developing at higher temperatures are generally smaller upon 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 impose a selective pressure that favors smaller parasitoids. For instance, Ismail *et al.* (2012) 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 fitness-related traits (*e.g.* fecundity, longevity; Harvey *et al.*, 1994), there could be a size induced trade-off between female fertility (large individuals) and survival (reduced size) under stressful temperature conditions. These results showing an effect of temperature variations at the seasonal scale (but not at the landscape scale) confirm our fifth hypothesis but refutes our fourth hypothesis concerning parasitoid size.

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

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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

Acknowledgments

experiments comply with the current laws of the country in which the experiments were

performed.

References

Alford, L., Blackburn, T.M. & Bale, J.S. (2012a) Effects of acclimation and latitude on the activity thresholds of the aphid *Myzus persicae* in Europe: Variation in aphid activity thresholds. *Journal of Applied Entomology*, **136**, 332–346.

Alford, L., Hughes, G.E., Blackburn, T.M. & Bale, J.S. (2012b) Walking speed adaptation ability of *Myzus persicae* to different temperature conditions. *Bulletin of Entomological Research*, **102**, 303–313.

Andrade, T.O., Hervé, M., Outreman, Y., Krespi, L. & van Baaren, J. (2013) Winter host exploitation influences fitness traits in a parasitoid. *Entomologia Experimentalis et Applicata*, **147**, 167–174.

Andrew, N.R. & Terblanche, J.S. (2013) The response of insects to climate change. (Salinger, J. ed.). *Climate of Change: Living in a Warmer World*, pp. 38–50, CSIRO Publishing, Auckland, New-Zealand.

Argent, R.M. (1992) The influence of a plant canopy on shelter effect. *Journal of Wind Engineering & Industrial Aerodynamics*, **41**, 2643–2653.

Bahrndorff, S., Holmstrup, M., Petersen, H. & Loeschcke, V. (2006) Geographic variation for climatic stress resistance traits in the springtail *Orchesella cincta*. *Journal of Insect Physiology*, **52**, 951–959.

Bale, J.S. (1996) Insect cold hardiness: A matter of life and death. *European Journal of Entomology*, **93**, 369–382.

Bauerfeind, S.S. & Fischer, K. (2014) Simulating climate change: temperature extremes but not means diminish performance in a widespread butterfly. *Population Ecology*, **56**, 239–250.

Bennie, J., Huntley, B., Wiltshire, A., Hill, M.O. & Baxter, R. (2008) Slope, aspect and climate: Spatially explicit and implicit models of topographic microclimate in chalk grassland. *Ecological Modelling*, **216**, 47–59.

Chen, J., Saunders, S.C., Crow, T.R., Naiman, R.J., Brosofske, K.D., Mroz, G.D., Brookshire, B.L. & Franklin, J.F. (1999) Microclimate in Forest Ecosystem and Landscape

468   Ecology : Variations in local climate can be used to monitor and compare the effects
469   of different management regimes. *BioScience*, **49**, 288–297.

470   Chown, S.L. & Gaston, K.J. (2010) Body size variation in insects: a macroecological
471   perspective. *Biological Reviews*, **85**, 139–169.

472   Colinet, H., Sinclair, B.J., Vernon, P. & Renault, D. (2015) Insects in Fluctuating Thermal
473   Environments. *Annual Review of Entomology*, **60**, 123–140.

474   Colinet, H. & Hance, T. (2010) Interspecific variation in the response to low temperature
475   storage in different aphid parasitoids. *Annals of Applied Biology*, **156**, 147–156.

476   De Bach, P. (1943) The effect of low storage temperature on reproduction in certain parasitic
477   Hymenoptera. *Pan-Pacific Entomologist*, **19**, 112–119.

478   Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. &
479   Martin, P.R. (2008) Impacts of climate warming on terrestrial ectotherms across
480   latitude. *Proceedings of the National Academy of Sciences*, **105**, 6668–6672.

481   Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R. & Mearns, L.O.
482   (2000) Climate Extremes: Observations, Modeling, and Impacts. *Science*, **289**, 2068–
483   2074.

484   Fox, J. & Weisberg, H.S. (2011) *An R Companion to Applied Regression*, 2nd ed. Sage.

485   Giri, M.K., Pass, B.C., Yeargan, K.V. & Parr, J.C. (1982) Behavior, net reproduction,
486   longevity, and mummy-stage survival of *Aphidius matricariae* [Hym. Aphidiidae].
487   *Entomophaga*, **27**, 147–153.

488   Hance, T., van Baaren, J., Vernon, P. & Boivin, G. (2007) Impact of Extreme Temperatures
489   on Parasitoids in a Climate Change Perspective. *Annual Review of Entomology*, **52**,
490   107–126.

491   Harvey, J.A., Harvey, I.F. & Thompson, D.J. (1994) Flexible Larval Growth Allows Use of a
492   Range of Host Sizes by a Parasitoid Wasp. *Ecology*, **75**, 1420–1428.

493   Hoffmann, A.A., Chown, S.L. & Clusella-Trullas, S. (2013) Upper thermal limits in terrestrial
494   ectotherms: how constrained are they? *Functional Ecology*, **27**, 934–949.

495   Huey, R.B., Crill, W.D., Kingsolver, J.G. & Weber, K.E. (1992) A method for rapid
496   measurement of heat or cold resistance of small insects. *Functional Ecology*, **6**, 489–
497   494.

498   Hullé, M., Turpeau, E. & Chaubet, B. (2006) Encyclop'aphid, a key for aphids and their
499   parasitoids

500 Hurlbutt, B. (1987) Sexual size dimorphism in parasitoid wasps. *Biological Journal of the*
501   *Linnean Society*, **30**, 63–89.

502 IPCC. (2013) *Climate Change 2013 the Physical Science Basis: Final Draft Underlying*
503   *Scientific-Technical Assessment : Working Group I Contribution to the IPCC Fifth*
504   *Assessment Report.* (Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J.
505   Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley eds.). 1535p., Cambridge
506   University Press, Cambridge, United Kingdom and New York, NY, USA.

507 Ismail, M., Vernon, P., Hance, T., Pierre, J.-S. & van Baaren, J. (2012) What are the possible
508   benefits of small size for energy-constrained ectotherms in cold stress conditions?
509   *Oikos*, **121**, 2072–2080.

510 Krespi, L., Dedryver, C.A., Rabasse, J.-M. & Nénon, J.P. (1994) A morphometric comparison
511   of aphid mummies containing diapausing vs. non-diapausing larvae of *Aphidius*
512   *rhopalosiphi* (Hymenoptera: Braconidae). *Bulletin of Entomological Research*, **84**,
513   45–50.

514 Lawson, C.R., Bennie, J., Hodgson, J.A., Thomas, C.D. & Wilson, R.J. (2014) Topographic
515   microclimates drive microhabitat associations at the range margin of a butterfly.
516   *Ecography*, **37**, 732–740.

517 Lee, R.E.J. & Denlinger, D.L. (2010) Rapid cold-hardening: Ecological significance and
518   underpinning mechanisms. (Lee, R.E.J. & Denlinger, D.L. eds.). *Low Temperature*
519   *Biology of Insect*, pp. 35–58, Cambridge University Press, NY, USA.

520 Legrand, M.A., Salin, C., Langer, A. & Hance, T. (2004) Are mummy characteristics reliable
521   indicators of diapause and cold tolerance in the parasitoid wasp Aphidius rhopalosiphi
522   (Braconidae, Aphidiinae)? *CryoLetters*, **25**, 161–166.

523 Le Lann, C., Outreman, Y., Van Alphen, J.J.M., Krespi, L., Pierre, J.-S. & Van Baaren, J.
524   (2008) Do past experience and competitive ability influence foraging strategies of
525   parasitoids under interspecific competition? *Ecological Entomology*, **33**, 691–700.

526 Le Lann, C., Roux, O., Serain, N., Van Alphen, J.J.M., Vernon, P. & Van Baaren, J. (2011a)
527   Thermal tolerance of sympatric hymenopteran parasitoid species: does it match
528   seasonal activity? *Physiological Entomology*, **36**, 21–28.

529 Le Lann, C., Wardziak, T., van Baaren, J. & van Alphen, J.J.M. (2011b) Thermal plasticity of
530   metabolic rates linked to life-history traits and foraging behaviour in a parasitic wasp:
531   Temperature affects physiology and behaviour of a parasitoid. *Functional Ecology*, **25**,
532   641–651.

533 Le Lann, C., Visser, B., Mériaux, M., Moiroux, J., van Baaren, J., van Alphen, J.J.M. &
534   Ellers, J. (2014) Rising temperature reduces divergence in resource use strategies in
535   coexisting parasitoid species. *Oecologia*, **174**, 967–977.

536 Le Ralec, A., Anselme, C., Outreman, Y., Poirié, M., Van Baaren, J., Le Lann, C. & Van
537   Alphen, J.J.-M. (2010) Evolutionary ecology of the interactions between aphids and
538   their parasitoids. *Comptes rendus biologies*, **333**, 554–565.

539 Maclean, I.M.D., Hopkins, J.J., Bennie, J., Lawson, C.R. & Wilson, R.J. (2015)
540   Microclimates buffer the responses of plant communities to climate change. *Global
541   Ecology and Biogeography*, **24**, 1203–1362.

542 Millennium Ecosystem Assessment. (2005) *Ecosystems and Human Well-Being: Biodiversity
543   Synthesis*, World Resources Institute. Washington, DC.

544 Pincebourde, S. & Woods, H.A. (2012) Climate uncertainty on leaf surfaces: the biophysics
545   of leaf microclimates and their consequences for leaf-dwelling organisms. *Functional
546   Ecology*, **26**, 844–853.

547 Potter, K.A., Arthur Woods, H. & Pincebourde, S. (2013) Microclimatic challenges in global
548   change biology. *Global Change Biology*, **19**, 2932–2939.

549 Powell, S.J. & Bale, J.S. (2006) Effect of long-term and rapid cold hardening on the cold
550   torpor temperature of an aphid. *Physiological Entomology*, **31**, 348–352.

551 Quénol, H. & Beltrando, G. (2006) Microclimate in forest ecosystem and landscape ecology.
552   *Climatologie*, **3**, 9–23.

553 R Development Core Team. (2013) *R: A Language and Environment for Statistical
554   Computing. R Foundation for Statistical Computing, Vienna, Austria.*

555 Reim, C., Teuschl, Y. & Blanckenhorn, W.U. (2006) Size-dependent effects of larval and
556   adult food availability on reproductive energy allocation in the Yellow Dung Fly.
557   *Functional Ecology*, **20**, 1012–1021.

558 Sigsgaard, L. (2000) The temperature-dependent duration of development and parasitism of
559   three cereal aphid parasitoids, Aphidius ervi, A. rhopalosiphi, and Praon volucre.
560   *Entomologia Experimentalis et Applicata*, **95**, 173–184.

561 Suggitt, A.J., Gillingham, P.K., Hill, J.K., Huntley, B., Kunin, W.E., Roy, D.B. & Thomas,
562   C.D. (2011) Habitat microclimates drive fine-scale variation in extreme temperatures.
563   *Oikos*, **120**, 1–8.

564 Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T. &
565   Huey, R.B. (2014) Thermal-safety margins and the necessity of thermoregulatory
566   behavior across latitude and elevation. *Proceedings of the National Academy of
567   Sciences*, **111**, 5610–5615.

568 Thies, C., Roschewitz, I. & Tscharntke, T. (2005) The landscape context of cereal aphid-
569   parasitoid interactions. *Proceedings of the Royal Society B: Biological Sciences*, **272**,
570   203–210.

571 Thies, C., Haenke, S., Scherber, C., Bengtsson, J., Bommarco, R., Clement, L.W., Ceryngier,
572   P., Dennis, C., Emmerson, M., Gagic, V. & others. (2011) The relationship between
573   agricultural intensification and biological control: experimental tests across Europe.
574   *Ecological Applications*, **21**, 2187–2196.

575 Thompson, R.M., Beardall, J., Beringer, J., Grace, M. & Sardina, P. (2013) Means and
576   extremes: building variability into community-level climate change experiments.
577   *Ecology Letters*, **16**, 799–806.

578 Van der Have, T.M. & De Jong, G. (1996) Adult size in ectotherms: temperature effects on
579   growth and differentiation. *Journal of Theoretical Biology*, **183**, 329–340.

580 Waterhouse, F.L. (1955) Microclimatological profiles in grass cover in relation to biological
581   problems. *Quarterly Journal of the Royal Meteorological Society*, **81**, 63–71.

582 Woods, H.A., Dillon, M.E. & Pincebourde, S. (2015) The roles of microclimatic diversity and
583   of behavior in mediating the responses of ectotherms to climate change. *Journal of
584   Thermal Biology*, **218**, 1956–1967.

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586 Figures
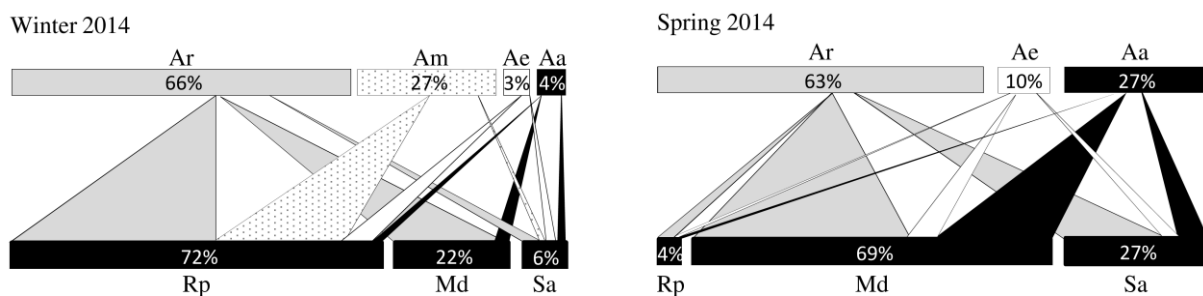
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588 **Figure 1:** Quantitative food webs of the parasitoid and aphid community composition in winter and spring 2014.

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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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.
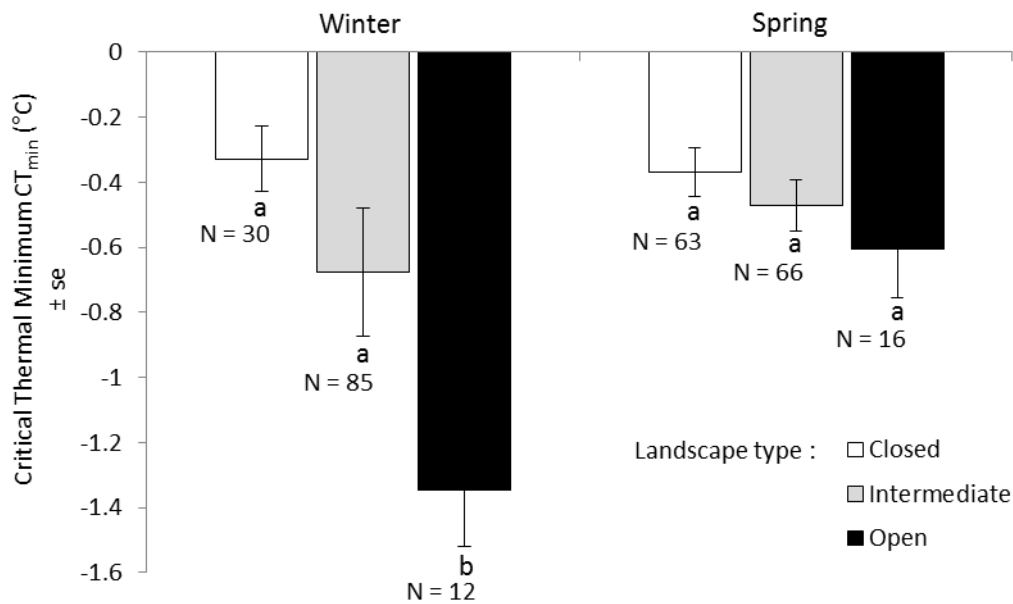
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603   **Figure 3:** Average critical minimal temperature (**a**) and average tibia length (**b**) for *A. rhopalosiphi* in winter and

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.
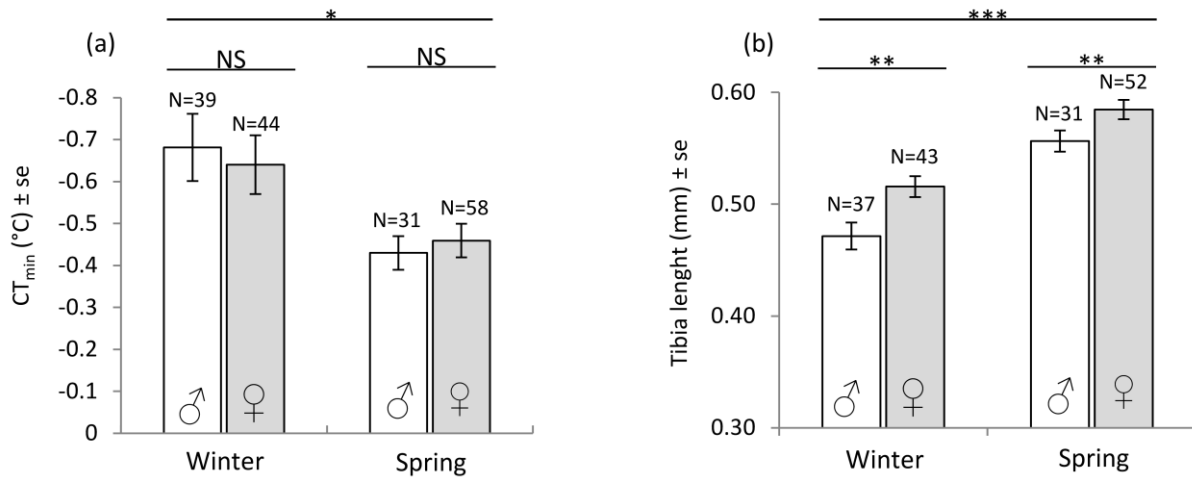
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609 **Table 1:** Daily average meteorological data (± standard error) in the study area (Brittany, France) in winter (13th

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 ±0.07[(a)]	-0.32 | 14.61	90.82 ±0.25[(a)]	2.18 ±0.04[(a)]
	Intermediate	7.23 ±0.08[(b)]	-0.53 | 23.37	90.18 ±0.25[(a)]	3.39 ±0.06[(b)]
	Open	8.39 ±0.16[(c)]	-2.07 | 24.91	85.82 ±0.54[(b)]	2.67 ±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 ±0.10[(a)]	0.51 | 20.10	83.63 ±0.40	1.84 ±0.03[(a)]
	Intermediate	11.12 ±0.11[(a,b)]	0.08 | 20.89	84.46 ±0.38	1.91 ±0.04[(a)]
	Open	11.49 ±0.12[(b)]	-1.02 | 21.78	83.66 ±0.43	2.71 ±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 ±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

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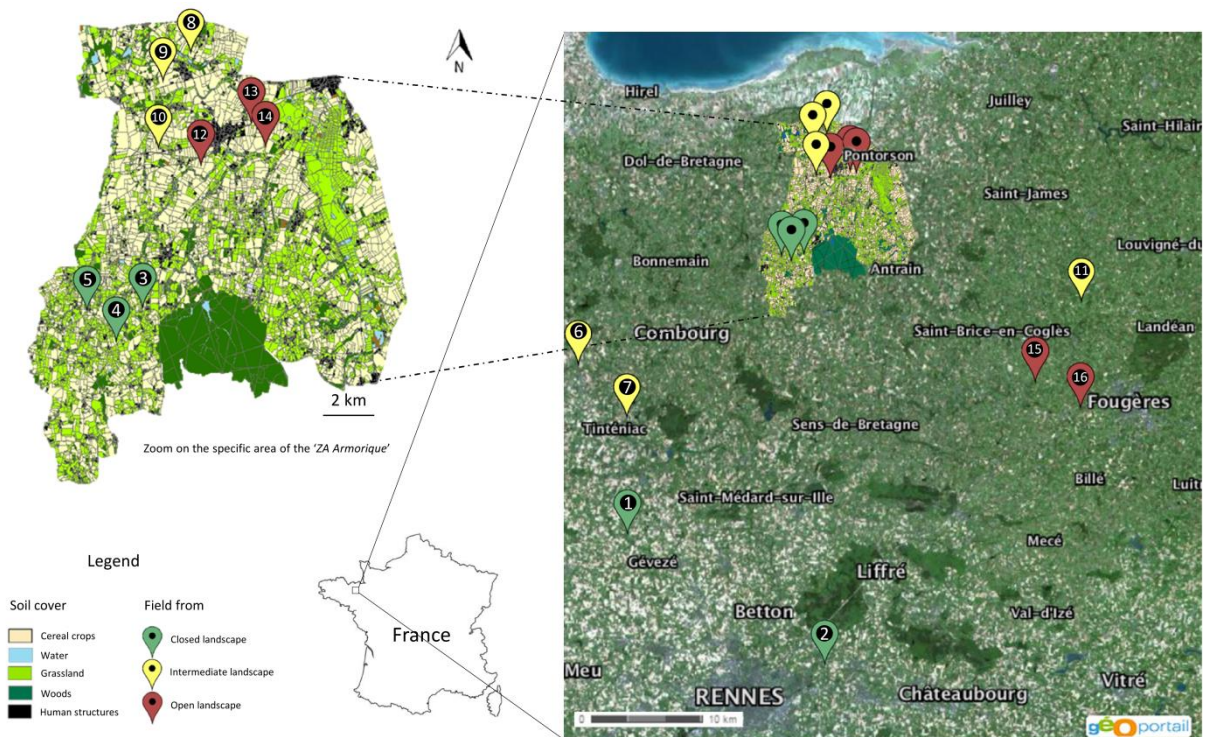
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657 **Appendix S2:** geographic coordinates of the 16 fields sampled. Field numbers refer to the numbers in the cursor

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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