1   **The effect of landscape complexity and microclimate on the thermal tolerance of a pest**

2   **insect**

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18   **Running title:** Landscape influences insect thermal tolerance

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27 **Abstract**

28 Landscape changes are known to exacerbate the impacts of climate change. As such,

29 understanding the combined effect of climate and landscape on agro-ecosystems is vital if we

30 are to maintain the function of agro-ecosystems. The present study aimed to elucidate the

31 effects of agricultural landscape complexity on the microclimate and thermal tolerance of an

32 aphid pest to better understand how landscape and climate may interact to affect the thermal

33 tolerance of pest species within the context of global climate change. Meteorological data

34 were measured at the landscape level, and cereal aphids (*Sitobion avenae*, *Metopolophium*

35 *dirhodum* and *Rhopalosiphum padi*) sampled, from contrasting landscapes (simple and

36 complex) in winter 2013/14 and spring 2014 in cereal fields of Brittany, France. Aphids were

37 returned to the laboratory and the effect of landscape of origin on aphid cold tolerance (as

38 determined by CT_{min}) was investigated. Results revealed that local landscape complexity

39 significantly affected microclimate, with simple homogenous landscapes being on average

40 warmer, but with greater temperature variation. Landscape complexity was shown to impact

41 aphid cold tolerance, with aphids from complex landscapes being more cold tolerant than

42 those from simple landscapes in both winter and spring, but with differences among species.

43 This study highlights that future changes to land use could have implications for the thermal

44 tolerance and adaptability of insects. Furthermore, not all insect species respond in a similar

45 way to microhabitat and microclimate, which could disrupt important predator-prey

46 relationships and the ecosystem service they provide.

47

Introduction

The earth is presently experiencing rapid changes in both climatic conditions and landscape structure. Over the past century the climate has warmed by approximately 0.6°C and an increased incidence of extreme climatic events such as droughts and heat and cold waves is predicted (Easterling *et al*. 1997, 2000, Karl *et al*. 2000, Walther *et al*. 2002; IPCC 2013). Since many species can tolerate only a restricted range of environmental conditions, climate change is expected to have significant consequences for the phenology, life history and distribution of species, which will ultimately impact the severity and timing of pest outbreaks and ecosystem functioning (Hance *et al*. 2007). In addition to climatic changes, much of Europe has experienced large scale agricultural intensification over the past 50 years, resulting in the alteration of landscape patterns such as an increase in mean field size, the disappearance of semi-natural habitats and a change in farming practices combined with a dramatic increase in chemical inputs (Tscharntke *et al*. 2005). This intensification has led to a considerable increase in agricultural productivity but also to a simplification of agricultural landscapes (Roschewitz *et al*. 2005), a drastic loss of biodiversity (Krebs *et al*. 1999, Tilman *et al*. 2002), and degradation of ecosystem services (Tscharntke *et al*. 2005).

As human population density continues to rise, agricultural productivity must increase to meet demand (Tilman 1999) which necessitates an improvement or at least maintenance in the efficacy of crop protection against agricultural pests. Much research has focused on the effect of climatic conditions on the thermal tolerance and physiological plasticity of agriculturally important insects so that we may better understand and predict the potential impacts of climate change on agro-ecosystems (e.g. Collier *et al*. 1991, Chen & Kang 2004, Le Lann *et al*. 2011, Alford *et al*. 2012b, Piyaphongkul *et al*. 2012). However, given that landscape changes are known to exacerbate the impact of global climate change (Delattre *et*

77   *al.* 2013, Dong *et al.* 2013) the effect of landscape complexity on insect thermal tolerance has

78   been under studied (Tougeron *et al.* 2016). If agro-ecosystem functioning is to be maintained

79   into the future, it is imperative that the impacts of landscape complexity and climate on agro-

80   ecosystems are no longer studied in isolation. This is especially true if we are to understand,

81   develop, and successfully implement landscape management techniques to reduce the

82   impacts of climate change and associated extreme weather events on crucially important

83   organisms (e.g. organisms that provide a valuable ecosystem service such as natural pest

84   control). Furthermore, through understanding the effect of landscape complexity on local

85   microclimate and insect thermal tolerance, we may elucidate which components of the

86   microclimate (e.g. mean temperature, temperature extremes, humidity etc.) are most

87   important in determining insect thermal tolerance.

88

89   Using cereal fields of North-West France as a study system, the present study investigates

90   variation in microclimate and insect thermal tolerance from contrasting simple and complex

91   landscapes. Although landscape composition can impact the local microclimate, the

92   relationship is a complex one involving many factors (vegetation type, cover, topography

93   etc.), often leading to confusing and sometimes contradictory results with some studies

94   suggesting a warming effect of landscape intensification (Chen *et al.* 1999, Quénol &

95   Beltrando 2006, Suggitt *et al.* 2011), whilst another suggest a cooling effect (Argent 1992).

96   As such, meteorological data will be recorded directly from the study area to elucidate the

97   exact relationship between landscape intensification and microclimate.

98

99   The focus species of the current study are the cereal aphids (Hemiptera: Aphididae) *Sitobion*

100   *avenae* (Fabricius) common name the English grain aphid, *Metopolophium dirhodum*

101   (Walker) common name the rose grain aphid, and *Rhopalosiphum padi* (L.) common name

102   the bird cherry oat aphid. All three species are major pests of commercially important cereal

103   crops in temperate climates (Fereres & Moreno 2009, Dedryver *et al*. 2010). These cereal

104   aphids possess a complex lifecycle with a series of asexual and sexual generations produced

105   over the course of a year (Moran, 1992). At the onset of winter, the production of sexual

106   morphs is triggered and sexual reproduction occurs. This holocyclic (sexual) lifecycle results

107   in the production of very cold hardy eggs that overwinter (Strathdee *et al*. 1995). An

108   alternative anholocyclic (asexual) lifecycle also exists which resulted from a stable genetic

109   mutation affecting the photoperiodic switch, resulting in a failure to produce sexual forms in

110   autumn (Moran, 1992; Dixon, 1998). As such, asexual, parthenogenetic reproduction may

111   occur all year round, resulting in the existence of genetically distinct anholocyclic clones.

112   These clones have been shown to vary in insecticide resistance and thermal tolerance, with

113   implications for the clonal types that come to dominate within a population (Fenton *et al*.

114   2010; Alford *et al*. 2012b).

115

116   Insects inhabiting higher latitudes generally exist in climates cooler than their thermal optima

117   (Deutsch *et al*. 2008) and this is true of the cereal aphids of temperate climates. As such,

118   global warming could act to increase the fitness of temperate insects (Deutsch *et al*. 2008).

119   Instead, it is the unpredictable occurrence of extreme environmental events (e.g. cold waves)

120   that could prove a greater challenge to insects (Godfray *et al*. 1994, Hance *et al*. 2007) and

121   thus be more important in dictating insect survival and persistence. The importance of

122   increasing extreme weather events places increased importance on the study of insect thermal

123   tolerance. Here we report on laboratory experiments designed to investigate the cold

124   tolerance of these three aphid species, as determined by the critical thermal minima (CT_{min}),

125   originating from wheat fields varying in agricultural intensification regime. The following

126   hypotheses were tested: 1) Local climate will differ between simple and complex landscapes.

127   2) Aphid cold tolerance will vary with landscape complexity in accordance with the local

128   microclimate. 3) Differences in aphid thermal tolerance between landscapes will be less

129   marked in spring than winter because exposure to stressful low temperatures will be less

130   frequent in spring months.

131

132   **Materials and Methods**

133

134   *Meteorological data*

135   Meteorological data in the study area was recorded using established BWS200 weather

136   stations (Campbell Scientific France) during two sampling sessions; one in winter (13th

137   January to 10th March 2014) and one in spring (17th March to 12th May 2014). One weather

138   station per type of landscape was used to record air temperature and relative humidity (using

139   CS215 probes accurate at 0.3°C and 2% respectively), and wind speed (using Wind Sentry

140   anemometer accurate at $0.5m.s^{-1}$) at a sampling rate of once per hour for the duration of the

141   sampling period. Weather stations were placed on flat ground at a height of 1 meter and at a

142   minimum distance of 15 meters from the hedge boundary.

143

144   *Aphid sampling and rearing*

145   Laboratory cultures of S. *avenae*, *M. dirhodum* and *R. padi* were established using aphids

146   collected in the field between the 13th January to 10th March 2014 (representing a winter

147   population) and the 17th March to 12th May 2014 (representing a spring population). In both

148   sampling seasons, aphids were collected on winter wheat and triticale fields in the Long Term

149   Ecological Research (LTER) site Armorique (48 ° 36 'N, 1 ° 32' W) (http://osur.univ-

150   rennes1.fr/zaarmorique/) located in Brittany, north-western France. Aphids were sampled

151   from healthy plants to minimize any potential effect of host plant quality on aphid fitness.

152   Sampling fields were selected to represent two contrasting landscape types: complex and

153   simple. The landscape types were selected from a landscape intensification gradient

154   previously established as part of the long-running project Farmland (farmland-

155   biodiversity.org). Complex landscapes are characterized by high diversity, high hedgerow

156   density (>3200m), small field sizes (<0.93ha) and the presence of grassland areas (>45%)

157   and, due to these characters, are considered as fine grain heterogeneous landscapes. In

158   contrast, simple landscapes represent intensively farmed homogenous landscapes

159   characterized by low diversity, large field sizes (>2.70ha) and few semi-natural elements

160   (grassland density <20% and low hedgerow density <550m).

161

162   In winter 2014, a total of 27 fields within these contrasting landscape types were prospected

163   for the presence of aphids. A total of 5 fields were found to contain aphids: 3 fields in the

164   simple landscape and 2 fields in the complex landscape. In spring 2014, a total of 6 fields

165   were sampled, with 3 fields in the simple landscape and 3 fields in the complex landscape for

166   consistency with winter sampling. Due to sampling in agricultural landscapes, the selected

167   fields were dependent upon the crop regime of the farmer and the presence of aphids. As a

168   consequence, the fields sampled in winter and spring were not the same. Sampling was

169   performed by 2 people during a 30 minute period and as many plants as possible were

170   examined for living aphids. All living aphid adults and nymphs were collected and returned

171   to the laboratory. Sampling occurred approximately three times per week, weather permitting,

172   over the sampling period to produce a laboratory culture representative of the sampled fields.

173

174   All collected aphids were returned to the laboratory and identified as one of three species: *S.*

175   *avenae*, *M. dirhodum* and *R. padi*. Aphids were subsequently separated by species and field

176   and a culture established for each species x field combination. During the winter sampling

177 period, *R. padi* and *M. dirhodum* were successfully collected in all 5 sample fields. *Sitobion*

178 *avenae* was collected in only 2 of the fields: 1 in each landscape type. During the spring

179 sampling period, *S. avenae* was successfully collected in all 6 sampled fields. *Metopolophium*

180 *dirhodum* was collected in only 2 of the fields: 1 in the complex and 1 in the simple

181 landscape. *Rhopalosiphum padi* was not found in any of the sampled fields in spring. This

182 resulted in a total of 20 species x field combinations. The lack of certain species in some

183 fields represents a source of confounding effects which will be discussed under the heading

184 of statistical analysis.

185

186 Each culture was reared on winter wheat, *Triticum aestivum*, 'Renan' cultivar grown in

187 vermiculite within Plexiglas cages (50 x 50 x 50 cm) and housed within a controlled

188 environment room at $20\pm1°C$ and LD 16 : 8 h photoperiod. Fresh wheat was added to the

189 cultures on a twice weekly basis. Before field collected aphids were added to the cultures, an

190 initial quarantine period was carried out to ensure that aphids were not host to parasitoid

191 wasps. For this, field collected aphids were housed in microcages (L = 16 cm, Ø = 4 cm)

192 comprising *T. aestivum* grown in vermiculite for a period of 10 days. Following this

193 quarantine period, all aphids within the microcages which had not turned into an 'aphid

194 mummy' (a dead aphid containing an immature parasitoid pupa) were transferred to the

195 relevant culture. Winter field populations of aphids are markedly reduced in comparison to

196 spring populations. However, at least 30 founding field-collected aphids were used to

197 establish cultures for each species x field combination, with cultures subsequently allowed to

198 build up to large enough numbers for use in experiments. Lab rearing duration was

199 approximately 3-4 weeks before use in experiments.

200

201 To obtain apterous adults synchronized in age for use in experiments, reproductive adults

202 were taken from the stock culture and were allowed to reproduce for 24 h in microcages.

203 Resultant nymphs were allowed to develop into pre-reproductive adults for use in

204 experiments.

205

206 ***Determination of low temperature activity thresholds (CTmin)***

207 The low temperature at which an insect is rendered immobile, known as the critical thermal

208 minima (CT_{min}), was determined for pre-reproductive adults of the three aphid species.

209 CT_{min} was chosen as the measure of cold tolerance since such non-lethal thresholds are

210 considered to be of more ecological relevance than lethal thresholds (Macdonald *et al*. 2004),

211 because survival at unfavorable temperatures is of little importance if the insect is rendered

212 inactive and thus unable to find food, mate or escape predation (Mellanby 1939, Bale 1987).

213

214 CT_{min} was measured using a glass column, as described by Powell and Bale (2006); a

215 modified design from apparatus previously used by Weber and Diggins (1990) and Huey *et*

216 *al*. (1992). The glass column (35 x 5cm) was connected to a programmable alcohol bath

217 (Haake F3, Thermo Electron Corp., Karlsruhe, Baden-Württemberg, Germany), enabling the

218 circulation of alcohol fluid around the outer chamber and thus fine control over the air

219 temperature experienced within the inner column. Previous work has shown that air

220 temperature is consistent along the length of the column (Powell & Bale 2006). In addition,

221 due to the relatively small body size of the test insects, it is concluded that the air temperature

222 of the column approximates the body temperature of the insects (Huey *et al*. 1992).

223 Consequently, a single thermocouple was placed against the glass surface of the inner column

224 to monitor air temperature during experimentation.

225

226   In all experiments, approximately 10-15 adult aphids of one species from one field were

227   inserted into the bottom of the column pre-set to the culture temperature of 20°C. The column

228   was subsequently closed with a sponge stopper to reduce air flow and maintain a stable

229   thermal environment within the inner column. Following a 10 min acclimatization period, the

230   programmable alcohol bath was set to decrease the temperature of the column from 20°C to -

231   10°C at a rate of 0.5°C min^{-1}.

232

233   During the cooling phase, the CT_{min} was determined as the temperature at which an

234   individual aphid was no longer able to cling to the vertical column and subsequently fell to

235   the bottom of the column. The CT_{min} temperature was recorded manually from the

236   thermocouple display reading to an accuracy of 0.1°C. The procedure was repeated to obtain

237   CT_{min} values for 50 individuals of each species x field combination.

238

239   ***Statistical analysis***

240   Meteorological data differences between landscapes were tested using an ANOVA for

241   repeated measures and a Tukey HSD post-hoc test to compare data among landscapes for

242   both seasons using the R software (R Core Team 2013). The meteorological variable in

243   question was considered as a fixed effect, and recording time was included as a random effect

244   to control for correlation between meteorological data at recording times t and t-1.

245   Meteorological data differences between seasons were tested using a Welch's two sample t-

246   test.

247

248   CT_{min} data were analyzed using a Cox regression / proportional hazard model which belongs

249   to the class of generalized linear models. It applies to survival data which are typically not

250   normally distributed (exponential, Weibull, Gamma, etc.). The model has the advantage of

251   being "semi parametric" in the sense that it does not require any knowledge of the underlying

252   distribution. The only hypothesis is that the factors and covariates act multiplicatively on the

253   death rate $\lambda(t)$. The original data (death time) are then transformed to a death rate, and

254   subsequently regressed on the factors and covariates by the equation

255
$$\lambda(t) = \lambda_0(t) \exp\left(\sum_{i=1}^{p} \beta_i X_i\right)$$

256   where $\lambda_0(t)$ is the so called "baseline hazard", X_i is the ith covariate or factor among p and

257   β_i regression coefficients. Here, we take advantage of the confusion between time and

258   temperature to treat the temperature of fall as a death time. Cox models give access to all

259   classic models of variance (deviance) analysis, covariance and regression. When positive, the

260   coefficients β_i indicate an increase of the death rate and thus a negative effect on survival,

261   and the opposite when they are negative (Cox 1972, Fox 2002, Collett 2003). The replication

262   of fields in each environment was treated as a frailty factor (random factor) (Therneau and

263   Grambsch 2000). The program used was *coxph* in the package *survival* of R version 3.03 (R

264   Core Team 2013). Analysis of deviance tests are issued from the χ^2 approximation of the

265   deviance likelihood ratio. The overall model tested was the following:

266

267     $\text{Surv}(CT_{\min}) = \text{intercept} + \text{Species} * \text{Landscape} + \text{frailty(exp)} + \text{error}$

268

269   Here, Surv (Survival) indicates the death rate by degrees Celsius, intercept is checked,

270   Species indicates the aphid species, Landscape indicates the type of landscape, and exp

271   represents the replication of fields within the landscape type. In Cox model analysis, the

272   Frailty function defines a factor as random. Where there is no replication, the model is only

273   estimable when this random effect is assumed to be additive. The within error, associated to

274   the cross levels Species x Landscape x exp does not need any distribution assumption as the

275   Cox model is distribution free. This lack of replication is especially true in winter, and for *S.*

276   *avenae* originating from the simple landscape, where there is only one replicate. Thus, the

277   additive frailty term is estimated necessarily as equal to what is observed in the complex

278   landscape. A confounding effect *Sitobion* x replication A x complex landscape and *Sitobion* x

279   replication C x simple landscape is theoretically possible; a fact we have to keep in mind

280   when discussing the results.

281

282   **Results**

283

284   *Meteorological data*

285   For both seasons, complex landscapes were on average colder than simple landscapes.

286   Temperature amplitude and standard deviations revealed complex landscapes to be less

287   variable in temperature than simple landscapes. In winter, complex landscapes showed higher

288   relative humidity than simple landscapes, and a lower wind speed. Results further revealed

289   greater variation between complex and simple landscapes in winter than in spring for the

290   meteorological factors measured (Table 1). The number of days in which temperatures

291   dropped below zero were 1 and 4 for complex and simple landscapes respectively in winter,

292   and 0 and 4 respectively in spring.

293

294   *The effect of landscape complexity on the CT_{min} of winter aphids*

295   For aphids sampled in winter, there was a significant effect of aphid species ($\chi^2_2 = 139.29$,

296   p<0.001) and landscape complexity ($\chi^2_1 = 19.56$, p<0.001) on the rate of drop-off (CT_{min})

297   from a vertical column (Fig. 1). No interaction effect was observed between aphid species

298   and landscape complexity ($\chi^2_2 = 3.62$, p=0.164). The β coefficients were negative for *R. padi*

299   and positive for *S. avenae*, and both proved significantly different from *M. dirhodum*

300   considered as the baseline ($\beta = 0$, default option in *coxph*). Thus, for the species effect, the

301   greatest cold tolerance, when measured as CT_{min} induced drop-off from the vertical column,

302   was observed for *R. padi*. *Sitobion avenae* displayed the lowest cold tolerance and dropped

303   from the column at warmer temperatures, with *M. dirhodum* proving intermediate of the two

304   species. A significant effect of landscape complexity was observed for *R. padi* ($\chi^2_1 = 3.91$,

305   p=0.048), *M. dirhodum* ($\chi^2_1 = 11.54$, p<0.001) and *S. avenae* ($\chi^2_1 = 4.41$, p=0.036). The β

306   coefficients were negative for the complex landscape, as compared to the simple landscape

307   considered as the baseline, meaning that aphids remain attached for longer and accordingly at

308   lower temperatures when originating from complex landscapes. Thus, for all three species,

309   aphids from complex landscapes displayed a reduced rate of CT_{min} induced drop-off from the

310   vertical column indicative of a greater cold tolerance than aphids from simple landscapes.

311   The frailty effect caused by replications within the same environment was always very low

312   and non-significant. It is pointed out that this effect is poorly known for *Sitobion avenae* due

313   to the lack of replicates within landscapes. Although this effect is coherent with that observed

314   for the two other species, we cannot exclude a sampling bias in this case.

315

316   ***The effect of landscape complexity on the CT_{min} of spring aphids***

317   For aphids sampled in spring, there was a significant effect of aphid species ($\chi^2_1 = 16.60$,

318   p<0.001) and a weak effect of landscape complexity ($\chi^2_1 = 4.14$, p=0.042) on the rate of

319   CT_{min} induced drop-off from a vertical column (Fig. 2). No interaction effect was observed

320   between aphid species and landscape complexity ($\chi^2_1 = 1.757$, p=0.185). The greatest cold

321   tolerance was displayed by *M. dirhodum* and the lowest cold tolerance by *S. avenae*. When

322   analyzing the data separately for each species, a significant effect of landscape complexity

323   was found for *S. avenae* ($\chi^2_1 = 10.32$, p=0.001) with CT_{min} induced drop-off from a vertical

324   column occurring at a faster rate for individuals originating from simple landscapes. The

325   effect of landscape complexity proved non-significant for *M. dirhodum* ($\chi^2_1 = 0.003$,

326   p=0.954).

327

328   **Discussion**

329

330   *Effects of landscape complexity on microclimate*

331   Different components of the landscape such as topography, vegetation type and cover directly

332   impact the microclimate (Chen *et al*. 1999). Although landscape composition is known to

333   affect the microclimate, the relationship is complex, often leading to confusing and

334   sometimes contradictory results (Argent 1992, Chen *et al*. 1999, Quénol & Beltrando 2006,

335   Suggitt *et al*. 2011). In the current study, simple landscapes (homogenous landscapes

336   characterized by low diversity, large field sizes and few semi-natural elements) were, on

337   average, warmer than more complex landscapes (fine grain heterogeneous landscapes

338   characterized by high species diversity, high hedgerow density, small field sizes and the

339   presence of grassland areas) in both winter and spring. This supports hypothesis 1 that local

340   climate will differ between simple and complex landscapes. However, whilst warmer with

341   regard to mean temperature, simple landscapes displayed an increased range of temperature

342   extremes.

343

344   As a landscape becomes progressively more open, it is exposed to increasing amounts of

345   daytime short radiation, acting to raise local temperatures (Chen *et al*. 1999, Suggitt *et al*.

346   2011). Furthermore, the increased hedgerow density characteristic of complex landscapes

347   provides a windbreak function, causing a reduction in local wind speed and retention of

348   denser, cooler air (Quénol & Beltrando 2006), acting to lower mean local temperatures. This

349   windbreak effect and reduction in wind speed further functions to raise relative humidity, as

350   observed in the current study during winter sampling. As the season changes from winter to

351   spring, increased vegetation growth across all landscape types results in a buffering of

352   temperatures. Such buffering of temperature leads to a reduction in variation between simple

353   and complex landscapes, although the complex landscape still remains significantly colder,

354   and the simple landscape with greater extremes. Meteorological data collected in the current

355   study therefore support the idea that landscape intensification and homogenization could act

356   to raise local temperatures, whilst increasing temperature variations and extremes, and

357   reducing relative humidity.

358

359   *Seasonal aphid population composition*

360   The general decline in dominance of *R. padi* from winter to spring, with a concurrent increase

361   to dominance of *S. avenae* by spring is a commonly observed pattern in the population

362   composition of cereal aphids (Andrade *et al*. 2013, 2015, Alford *et al*. 2014). In the current

363   study, during winter sampling, *R. padi* and *M. dirhodum* were present in all sampled fields

364   and *S. avenae* in 2 of the 5 sampled fields. However, by spring, *R. padi* was no longer

365   present. *Sitobion avenae* had increased to dominance and was found in all spring sampled

366   fields, whilst *M. dirhodum* was present in only 2 of the 6 sampled fields. This observation is

367   further supported by the significant inter-species variation in thermal tolerance observed in

368   the current study, with *R. padi* displaying the lowest temperatures of CT_{min} and *S. avenae* the

369   highest. It is known that *R. padi* prefers cool, humid conditions, whilst *S. avenae* has a

370   preference for warm and dry conditions (Honek 1985, Leather *et al*. 1989, Jarošík *et al*.

371   2003). The ability of *R. padi* to remain active and attached to the substrate at colder

372   temperatures than its counterparts could therefore provide the species with a fitness

373   advantage in winter. This theory is supported by recent research which revealed behavioral

374 variations that could contribute to the dominance of *R. padi* in winter (Alford *et al*. 2014),

375 offering an explanation to the observed variation in aphid sampling between seasons.

376

377 ***Effects of landscape complexity on aphid thermal tolerance***

378 Hypothesis 2 states that aphid thermal tolerance will vary with landscape complexity in

379 accordance with the local climate. With the microclimate data collected in the current study,

380 two opposing sub-hypotheses are proposed. Firstly, aphids from simple landscapes could

381 possess an enhanced thermal tolerance in response to greater temperature variations

382 (representing a more thermally stressful environment); a pattern recently observed for the

383 parasitoid wasps of cereal aphids (Tougeron *et al*. 2016). Secondly, aphids from complex

384 landscapes could possess an enhanced thermal tolerance in response to colder mean

385 temperatures. Interestingly, the current study supported the latter sub-hypothesis, with the

386 aphid species from complex landscapes being more cold tolerant. Since the response to the

387 landscape is observed at the guild level, it suggests that the strength of external filtering

388 (Violle *et al*. 2012) constrains traits to a narrow range within a guild exposed to the same

389 environmental conditions (Diaz & Cabido, 2001; White, 2008; Aparicio *et al*. 2012).

390 When exposed to unfavorable low temperatures, survival is dependent upon a number of

391 factors including the extremes of temperature, the rate of cooling, the duration of exposure

392 and the extent to which the temperature fluctuates around freezing (Sinclair *et al*. 2003). For

393 many insects, and arthropods more generally, temperature extremes are considered to be of

394 greater importance in determining thermal resistance than temperature means (Paaijmans *et*

395 *al*. 2003, Bahrndorff *et al*. 2006, Estay *et al*. 2014; Lawson *et al*. 2015). However, whilst

396 research evidence highlights the importance of temperature extremes in determining thermal

397 tolerance, the relationship is perhaps not that simple, with any environmental unpredictability

398 acting to undermine the strength of the environmental cue received by the insect (Deere *et al*.

399   2006). For this reason, plasticity is predicted to be more common in environments that are

400   predictably variable (Deere *et al*. 2006). As such, the increased temperature variation and

401   enhanced extremes around a warmer mean recorded in simple landscapes may provide an

402   unpredictable cue to the aphids. In contrast, the colder mean temperatures with reduced

403   temperature extremes recorded in complex landscapes could provide a more reliable cue, thus

404   eliciting a greater physiological response and acting to increase aphid cold tolerance.

405

406   The relationship between temperature variation and thermal tolerance is therefore a complex

407   one, with low temperature exposure being far more multifaceted than simply a mean

408   temperature. However, whilst researchers are increasingly factoring in temperature extremes

409   and fluctuations into models in pursuit of enhancing our understanding of insect thermal

410   tolerance, particularly in the face of global climate change (Estay *et al*. 2014, Vasseur *et al*.

411   2014), we cannot rule out the possibility that insects may respond to different aspects of the

412   temperature exposure. A recent study investigating the thermal tolerance of *Aphidius*

413   (Hymenoptera: Braconidae) parasitoids along a landscape intensification gradient found that

414   parasitoids originating from intensive 'simple' landscapes were significantly more cold

415   tolerant than those originating from more natural 'complex' landscapes (Tougeron *et al*.

416   2016); the reverse pattern to what was observed for their aphid host in the current study. As

417   temperature exposure is multifaceted, so too are the mechanisms involved in conferring cold

418   tolerance, ranging from behavioral to molecular (Bale 2002, Sulmon *et al*. 2015), and

419   strategies concerned with withstanding freezing or avoiding freezing altogether (Bale 1991).

420   As such, insects may utilize different mechanisms and respond to different environmental

421   triggers. For example, whilst increased temperature variation depressed the lower limits of

422   the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae) and the moth *Helicoverpa*

423   *armigera* (Lepidoptera: Noctuidae), the same pattern was not observed for the aphid

424   *Acyrthosiphon pisum* (Hemiptera: Aphididae) (Estay *et al*. 2014). Instead, the lower limit of

425   *A. pisum* was raised following increased temperature fluctuations (variance of temperature

426   around the mean), therefore acting to reduce cold tolerance, with more importance placed on

427   a constant mean temperature (Estay *et al*. 2014).

428

429   The work of Estay *et al*. (2014), in conjunction with the current study, suggests that, although

430   temperature extremes are important in determining thermal resistance for many insects,

431   temperature means could be of greater importance in determining aphid thermal resistance.

432   Aphids are phloem feedings insects, dependent upon a host plant. Subsequently, any

433   engagement in behavioral thermoregulation e.g. seeking out more thermally suitable

434   microhabitat could result in lost feeding opportunity and be detrimental to the individual.

435   Furthermore, the aphid phloem sap diet is high in cryoprotectant sugars and thus continuation

436   of feeding at unfavorable low temperatures acts to enhance cold tolerance (Danks 1978,

437   Sømme & Zachariassen 1981). As such, an aphid may withstand rather than evade

438   unfavorable cold temperatures, remaining fixed to the host plant. For this reason, aphids may

439   be more sensitive to mean temperatures, displaying enhanced thermal tolerance in response to

440   smaller temperature variations around a lower mean temperature, as recorded in complex

441   landscapes.

442

443   ***Seasonal variation in aphid thermal tolerance***

444   The effect of landscape intensification on aphid thermal tolerance was observed in both

445   winter and spring, although the relationship was more pronounced in winter. This offers

446   support to hypothesis 3 that differences in aphid thermal tolerance between landscapes will

447   be less marked in spring than winter because exposure to stressful low temperatures will be

448   less frequent in spring months. This finding further suggests that seasonal thermal tolerance

449   of aphids is conferred via phenotypic plasticity as opposed to genetic adaptation. Aphids, as

450   with all organisms, can respond to variation in environmental temperature via phenotypic

451   plasticity (Addo-Bediako *et al*. 2000, Ayrinhac *et al*. 2004) and display a high degree of

452   plasticity in their inherent thermal tolerance (Powell & Bale 2008, Alford *et al*. 2012ab).

453   Natural selection is unlikely to favor unnecessary thermal tolerance and, as such, any

454   acquired cold tolerance should be lost on cessation of winter temperatures. In the study

455   system, mean temperatures increased from winter to spring. In conjunction with this

456   temperature increase, variation in microclimate with landscape complexity became less

457   pronounced. As such, variation in aphid thermal tolerance with landscape complexity should

458   also become less pronounced in spring, as was observed in the current study.

459

460   ***Conclusion***

461   The current study suggests that landscape intensification could act to raise local temperatures,

462   whilst increasing temperature variations and extremes. Indeed, future land use changes could

463   prove as important in dictating local microclimate as global climate change (Pyke 2004,

464   Oliver & Morecroft 2014). In the current study, the thermal tolerance of cereal aphids was

465   shown to vary with landscape complexity, with aphids from more natural 'complex'

466   landscapes proving significantly more cold tolerant than those originating from intensively

467   farmed, homogenous 'simple' landscapes. Future land use changes could therefore have

468   profound implications for the thermal tolerance and adaptability of insects. Furthermore, the

469   study highlights that all insect species may not respond in a similar manner to thermal

470   conditions and changes to microhabitat and microclimate. If true, this could prove

471   particularly problematic if the response of pest insects and their biological control agents are

472   in anyway decoupled, as suggested in the current study and the study by Tougeron *et al*.

473   (2016) on aphid parasitoids. This is an important consideration when faced with land use

474   decisions or landscape manipulation strategies, particularly with the aim of maintaining agro-

475   ecosystem function into the future.

476

477   **Acknowledgments**

484

485 **References**

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753   **Table 1.** Daily average meteorological data ± standard error in the study area (Brittany,

754   France) in winter (13th January to 10th March) and spring 2014 (17th March to 12th May) for

755   complex, intermediate* and simple landscapes. The results of ANOVA and t-test analyses

756   comparing between landscape types and seasons are displayed. Letter superscripts indicate

757   significant differences between landscape types for each meteorological measure.

758

759   **Fig. 1.** Cumulative percentage drop-off of 'winter' aphids (*Rhopalosiphum padi*,

760   *Metopolophium dirhodum* and *Sitobion avenae*) from a glass column (CT_{min}) when cooled

761   from 20°C to -10°C at a rate of 0.5°C min^{-1}. Aphids were collected in winter 2013/14. Aphids

762   collected from the complex landscapes are indicated by the black line and aphids collected

763   from the simple landscapes by the grey line. Aphid drop-off is plotted from temperatures of

764   10°C and below due to the lack of aphid drop-off at temperatures of 10°C and above.

765

766   **Fig. 2.** Cumulative percentage drop-off of 'spring' aphids (*Metopolophium dirhodum* and

767   *Sitobion avenae*) from a glass column (CT_{min}) when cooled from 20°C to -10°C at a rate of

768   0.5°C min^{-1}. Aphids were collected in spring 2014. Aphids collected from the complex

769   landscapes are indicated by the black line and aphids collected from the simple landscapes by

770   the grey line. Aphid drop-off is plotted from temperatures of 10°C and below due to the lack

771   of aphid drop-off at temperatures of 10°C and above.

772

Table 1 Daily average meteorological data ± standard error in the study area (Brittany, France) in winter (13th January to 10th March) and spring 2014 (17th March to 12th May) for complex, intermediate* and simple landscapes. The results of ANOVA and t-test analyses comparing between landscape types and seasons are displayed. Letter superscripts indicate significant differences between landscape types for each meteorological measure.

Season	Landscape type	Air temperature (°C)	Min | Max temperature (°C)	Relative humidity (%)	Wind speed (m.s^{-1})
Landscape comparison					
Winter	Complex	6.86 ±0.08 [(a)]	-0.32 | 19.42	89.66 ±0.28 [(a)]	2.15 ±0.04 [(a)]
	Intermediate	7.50 ±0.08 [(b)]	-0.53 | 23.37	89.10 ±0.28 [(a)]	3.27 ±0.06 [(b)]
	Simple	8.78 ±0.17 [(c)]	-2.08 | 24.90	82.86 ±0.64 [(b)]	2.54 ±0.07 [(c)]
	ANOVA (df=2)	F=67.3, p<0.001		F=72.7, p<0.001	F=137.0, p<0.001
Spring	Complex	10.43 ±0.10 [(a)]	0.51 | 20.10	84.01 ±0.38	1.91 ±0.04 [(a)]
	Intermediate	10.81 ±0.11 [(b)]	0.08 | 20.89	84.81 ±0.36	2.00 ±0.04 [(a)]
	Simple	11.19 ±0.12 [(c)]	-1.02 | 21.78	83.88 ±0.41	2.75 ±0.05 [(b)]
	ANOVA (df=2)	F=12.42, p<0.001		n.s., p=0.20	F=124.1, p<0.001
Seasonal comparison					
Winter	All	7.43 ±0.06	-2.08 | 24.90	88.38 ±0.20	2.69 ±0.03
Spring	All	10.81 ±0.06	-1.02 | 21.78	84.26 ±0.22	2.23 ±0.03
	t -tests	t= 40.5, p<0.001		t= -13.8, p<0.001	t= -11.27, p<0.001

*Data concerning intermediate landscapes are included within the table to complete a landscape gradient from simple to complex landscapes, although aphid sampling did not occur in this landscape type.