



HAL
open science

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

Lucy Alford, Kévin Tougeron, Jean-sébastien Pierre, Françoise Burel, Joan van Baaren

► **To cite this version:**

Lucy Alford, Kévin Tougeron, Jean-sébastien Pierre, Françoise Burel, Joan van Baaren. The effect of landscape complexity and microclimate on the thermal tolerance of a pest insect. *Insect Science*, 2018, 25 (5), pp.905-915. 10.1111/1744-7917.12460 . hal-01810255

HAL Id: hal-01810255

<https://univ-rennes.hal.science/hal-01810255>

Submitted on 7 Jun 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Running title: Landscape influences insect thermal tolerance

Title for authors: L. Alford et al.

Correspondence: Lucy Alford, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Davidson Building, Glasgow G12 8QQ, UK. email: l.alford.02@cantab.net

*Current address: Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Davidson Building, Glasgow G12 8QQ, UK

Original article

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

Lucy Alford^{1,3*}, Kévin Tougeron^{1,2,3}, Jean-Sébastien Pierre^{1,3}, Françoise Burel^{1,3}, and Joan van Baaren^{1,3}

¹UMR 6553 ECOBIO, Centre National de la Recherche Scientifique, Université de Rennes I, 263 Avenue du Général Leclerc, 35042 Rennes Cedex, France, ²Institut de Recherche en Biologie Végétale, Université de Montréal, 4101 Sherbrooke Est Montréal, Québec H1X 2B2, Canada, and ³Université Européenne de Bretagne (UEB), 5 boulevard Laënnec, 35000, Rennes, France.

Abstract

Landscape changes are known to exacerbate the impacts of climate change. As such, understanding the combined effect of climate and landscape on agro-ecosystems is vital if we are to maintain the function of agro-ecosystems. The present study aimed to elucidate the

effects of agricultural landscape complexity on the microclimate and thermal tolerance of an aphid pest to better understand how landscape and climate may interact to affect the thermal tolerance of pest species within the context of global climate change. Meteorological data were measured at the landscape level, and cereal aphids (*Sitobion avenae*, *Metopolophium dirhodum* and *Rhopalosiphum padi*) sampled, from contrasting landscapes (simple and complex) in winter 2013/14 and spring 2014 in cereal fields of Brittany, France. Aphids were returned to the laboratory and the effect of landscape of origin on aphid cold tolerance (as determined by CT_{min}) was investigated. Results revealed that local landscape complexity significantly affected microclimate, with simple homogenous landscapes being on average warmer, but with greater temperature variation. Landscape complexity was shown to impact aphid cold tolerance, with aphids from complex landscapes being more cold tolerant than those from simple landscapes in both winter and spring, but with differences among species. This study highlights that future changes to land use could have implications for the thermal tolerance and adaptability of insects. Furthermore, not all insect species respond in a similar way to microhabitat and microclimate, which could disrupt important predator-prey relationships and the ecosystem service they provide.

Keywords Agro-ecosystems; biological control; cereal aphids; climate change; cold tolerance; landscape ecology.

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 al.*, 2013, Dong *et al.*, 2013) the effect of landscape complexity on insect thermal tolerance has been under studied (Tougeron *et al.*, 2016). If agro-ecosystem functioning is to be maintained into the future, it is imperative that the impacts of landscape complexity and climate on agro-ecosystems are no longer studied in isolation. This is especially true if we are to understand, develop, and successfully implement landscape management techniques to reduce the impacts of climate change and associated extreme weather events on crucially important organisms (e.g. organisms that provide a valuable ecosystem service such as natural pest control). Furthermore, through understanding the effect of landscape complexity on

local microclimate and insect thermal tolerance, we may elucidate which components of the microclimate (e.g. mean temperature, temperature extremes, humidity etc.) are most important in determining insect thermal tolerance.

Using cereal fields of North-West France as a study system, the present study investigates variation in microclimate and insect thermal tolerance from contrasting simple and complex landscapes.

Although landscape composition can impact the local microclimate, the relationship is a complex one involving many factors (vegetation type, cover, topography etc.), often leading to confusing and sometimes contradictory results with some studies suggesting a warming effect of landscape intensification (Chen *et al.*, 1999, Quénoel & Beltrando, 2006, Suggitt *et al.*, 2011), whilst another suggest a cooling effect (Argent, 1992). As such, meteorological data will be recorded directly from the study area to elucidate the exact relationship between landscape intensification and microclimate.

The focus species of the current study are the cereal aphids (Hemiptera: Aphididae) *Sitobion avenae* (Fabricius) common name the English grain aphid, *Metopolophium dirhodum* (Walker) common name the rose grain aphid, and *Rhopalosiphum padi* (L.) common name the bird cherry oat aphid. All three species are major pests of commercially important cereal crops in temperate climates (Fereses & Moreno, 2009, Dedryver *et al.*, 2010). These cereal aphids possess a complex lifecycle with a series of asexual and sexual generations produced over the course of a year (Moran, 1992). At the onset of winter, the production of sexual morphs is triggered and sexual reproduction occurs. This holocyclic (sexual) lifecycle results in the production of very cold hardy eggs that overwinter (Strathdee *et al.*, 1995). An alternative anholocyclic (asexual) lifecycle also exists which resulted from a stable genetic mutation affecting the photoperiodic switch, resulting in a failure to produce sexual forms in autumn (Moran, 1992; Dixon, 1998). As such, asexual, parthenogenetic reproduction may occur all year

round, resulting in the existence of genetically distinct anholocyclic clones. These clones have been shown to vary in insecticide resistance and thermal tolerance, with implications for the clonal types that come to dominate within a population (Fenton *et al.*, 2010; Alford *et al.*, 2012b).

Insects inhabiting higher latitudes generally exist in climates cooler than their thermal optima (Deutsch *et al.*, 2008) and this is true of the cereal aphids of temperate climates. As such, global warming could act to increase the fitness of temperate insects (Deutsch *et al.*, 2008). Instead, it is the unpredictable occurrence of extreme environmental events (e.g. cold waves) that could prove a greater challenge to insects (Godfray *et al.*, 1994, Hance *et al.*, 2007) and thus be more important in dictating insect survival and persistence. The importance of increasing extreme weather events places increased importance on the study of insect thermal tolerance. Here we report on laboratory experiments designed to investigate the cold tolerance of these three aphid species, as determined by the critical thermal minima (CT_{min}), originating from wheat fields varying in agricultural intensification regime. The following hypotheses were tested: i) Local climate will differ between simple and complex landscapes. ii) Aphid cold tolerance will vary with landscape complexity in accordance with the local microclimate. iii) Differences in aphid thermal tolerance between landscapes will be less marked in spring than winter because exposure to stressful low temperatures will be less frequent in spring months.

Materials and methods

Meteorological data

Meteorological data in the study area was recorded using established BWS200 weather stations (Campbell Scientific France) during two sampling sessions; one in winter (13th January to 10th March 2014) and one in spring (17th March to 12th May 2014). One weather station per type of landscape was used to record air temperature and relative humidity (using CS215 probes accurate at 0.3°C and 2% respectively), and wind speed (using Wind Sentry anemometer accurate at 0.5m.s⁻¹) at a sampling rate of once per hour for the duration of the sampling period. Weather stations were placed on flat ground at a height of 1 meter and at a minimum distance of 15 meters from the hedge boundary.

Aphid sampling and rearing

Laboratory cultures of *S. avenae*, *M. dirhodum* and *R. padi* were established using aphids collected in the field between the 13th January to 10th March 2014 (representing a winter population) and the 17th March to 12th May 2014 (representing a spring population). In both sampling seasons, aphids were collected on winter wheat and triticale fields in the Long Term Ecological Research (LTER) site Armorique (48 ° 36 'N, 1 ° 32' W) (<http://osur.univ-rennes1.fr/zaarmorique/>) located in Brittany, north-western France. Aphids were sampled from healthy plants to minimize any potential effect of host plant quality on aphid fitness. Sampling fields were selected to represent two contrasting landscape types: complex and simple. The landscape types were selected from a landscape intensification gradient previously established as part of the long-running project Farmland (farmland-biodiversity.org). Complex landscapes are characterized by high diversity, high hedgerow density (>3200m), small field sizes (<0.93ha) and the presence of grassland areas (>45%) and, due to these characters, are considered as fine grain heterogeneous landscapes. In contrast, simple landscapes represent intensively farmed homogenous landscapes

characterized by low diversity, large field sizes (>2.70ha) and few semi-natural elements (grassland density <20% and low hedgerow density <550m).

In winter 2014, a total of 27 fields within these contrasting landscape types were prospected for the presence of aphids. A total of 5 fields were found to contain aphids: 3 fields in the simple landscape and 2 fields in the complex landscape. In spring 2014, a total of 6 fields were sampled, with 3 fields in the simple landscape and 3 fields in the complex landscape for consistency with winter sampling. Due to sampling in agricultural landscapes, the selected fields were dependent upon the crop regime of the farmer and the presence of aphids. As a consequence, the fields sampled in winter and spring were not the same. Sampling was performed by 2 people during a 30 minute period and as many plants as possible were examined for living aphids. All living aphid adults and nymphs were collected and returned to the laboratory. Sampling occurred approximately three times per week, weather permitting, over the sampling period to produce a laboratory culture representative of the sampled fields.

All collected aphids were returned to the laboratory and identified as one of three species: *S. avenae*, *M. dirhodum* and *R. padi*. Aphids were subsequently separated by species and field and a culture established for each species x field combination. During the winter sampling period, *R. padi* and *M. dirhodum* were successfully collected in all 5 sample fields. *Sitobion avenae* was collected in only 2 of the fields: 1 in each landscape type. During the spring sampling period, *S. avenae* was successfully collected in all 6 sampled fields. *Metopolophium dirhodum* was collected in only 2 of the fields: 1 in the complex and 1 in the simple landscape. *Rhopalosiphum padi* was not found in any of the sampled fields in spring. This resulted in a total of 20 species x field combinations. The lack of certain species in some

fields represents a source of confounding effects which will be discussed under the heading of statistical analysis.

Each culture was reared on winter wheat, *Triticum aestivum*, 'Renan' cultivar grown in vermiculite within Plexiglas cages (50 × 50 × 50 cm) and housed within a controlled environment room at 20±1°C and LD 16 : 8 h photoperiod. Fresh wheat was added to the cultures on a twice weekly basis. Before field collected aphids were added to the cultures, an initial quarantine period was carried out to ensure that aphids were not host to parasitoid wasps. For this, field collected aphids were housed in microcages (L = 16 cm, Ø = 4 cm) comprising *T. aestivum* grown in vermiculite for a period of 10 days. Following this quarantine period, all aphids within the microcages which had not turned into an 'aphid mummy' (a dead aphid containing an immature parasitoid pupa) were transferred to the relevant culture. Winter field populations of aphids are markedly reduced in comparison to spring populations. However, at least 30 founding field-collected aphids were used to establish cultures for each species x field combination, with cultures subsequently allowed to build up to large enough numbers for use in experiments. Lab rearing duration was approximately 3 - 4 weeks before use in experiments.

To obtain apterous adults synchronized in age for use in experiments, reproductive adults were taken from the stock culture and were allowed to reproduce for 24 h in microcages. Resultant nymphs were allowed to develop into pre-reproductive adults for use in experiments.

Determination of low temperature activity thresholds (CT_{min})

The low temperature at which an insect is rendered immobile, known as the critical thermal minima (CT_{min}), was determined for pre-reproductive adults of the three aphid species.

CT_{min} was chosen as the measure of cold tolerance since such non-lethal thresholds are considered to be of more ecological relevance than lethal thresholds (Macdonald *et al.*, 2004), because survival at unfavorable temperatures is of little importance if the insect is rendered inactive and thus unable to find food, mate or escape predation (Mellanby, 1939, Bale, 1987).

CT_{min} was measured using a glass column, as described by Powell and Bale (2006); a modified design from apparatus previously used by Weber and Diggins (1990) and Huey *et al.* (1992). The glass column (350 × 50 mm) was connected to a programmable alcohol bath (Haake F3, Thermo Electron Corp., Karlsruhe, Baden-Württemberg, Germany), enabling the circulation of alcohol fluid around the outer chamber and thus fine control over the air temperature experienced within the inner column. Previous work has shown that air temperature is consistent along the length of the column (Powell & Bale, 2006). In addition, due to the relatively small body size of the test insects, it is concluded that the air temperature of the column approximates the body temperature of the insects (Huey *et al.*, 1992).

Consequently, a single thermocouple was placed against the glass surface of the inner column to monitor air temperature during experimentation.

In all experiments, approximately 10–15 adult aphids of one species from one field were inserted into the bottom of the column pre-set to the culture temperature of 20°C. The column was subsequently closed with a sponge stopper to reduce air flow and maintain a stable thermal environment within the inner column. Following a 10 min acclimatization period, the

programmable alcohol bath was set to decrease the temperature of the column from 20°C to -10°C at a rate of 0.5°C min⁻¹.

During the cooling phase, the CT_{min} was determined as the temperature at which an individual aphid was no longer able to cling to the vertical column and subsequently fell to the bottom of the column. The CT_{min} temperature was recorded manually from the thermocouple display reading to an accuracy of 0.1°C. The procedure was repeated to obtain CT_{min} values for 50 individuals of each species × field combination.

Statistical analysis

Meteorological data differences between landscapes were tested using an ANOVA for repeated measures and a Tukey HSD post-hoc test to compare data among landscapes for both seasons using the R software (R Core Team, 2013). The meteorological variable in question was considered as a fixed effect, and recording time was included as a random effect to control for correlation between meteorological data at recording times t and $t-1$.

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

CT_{min} data were analyzed using a Cox regression / proportional hazard model which belongs to the class of generalized linear models. It applies to survival data which are typically not normally distributed (exponential, Weibull, Gamma, etc.). The model has the advantage of being "semi parametric" in the sense that it does not require any knowledge of the underlying distribution. The only hypothesis is that the factors and covariates act multiplicatively on the

death rate $\lambda(t)$. The original data (death time) are then transformed to a death rate, and subsequently regressed on the factors and covariates by the equation

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

where $\lambda_0(t)$ is the so called "baseline hazard", X_i is the i^{th} covariate or factor among p and β_i regression coefficients. Here, we take advantage of the confusion between time and temperature to treat the temperature of fall as a death time. Cox models give access to all classic models of variance (deviance) analysis, covariance and regression. When positive, the coefficients β_i indicate an increase of the death rate and thus a negative effect on survival, and the opposite when they are negative (Cox, 1972, Fox, 2002, Collett, 2003). The replication of fields in each environment was treated as a frailty factor (random factor) (Therneau & Grambsch, 2000). The program used was *coxph* in the package *survival* of R version 3.03 (R Core Team, 2013). Analysis of deviance tests are issued from the χ^2 approximation of the deviance likelihood ratio. The overall model tested was the following:

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

Here, Surv (Survival) indicates the death rate by degrees Celsius, intercept is checked, Species indicates the aphid species, Landscape indicates the type of landscape, and exp represents the replication of fields within the landscape type. In Cox model analysis, the Frailty function defines a factor as random. Where there is no replication, the model is only estimable when this random effect is assumed to be additive. The within error, associated to

the cross levels Species x Landscape x exp does not need any distribution assumption as the Cox model is distribution free. This lack of replication is especially true in winter, and for *S. avenae* originating from the simple landscape, where there is only one replicate. Thus, the additive frailty term is estimated necessarily as equal to what is observed in the complex landscape. A confounding effect *Sitobion* x replication A x complex landscape and *Sitobion* x replication C x simple landscape is theoretically possible; a fact we have to keep in mind when discussing the results.

Results

Meteorological data

For both seasons, complex landscapes were on average colder than simple landscapes. Temperature amplitude and standard deviations revealed complex landscapes to be less variable in temperature than simple landscapes. In winter, complex landscapes showed higher relative humidity than simple landscapes, and a lower wind speed. Results further revealed greater variation between complex and simple landscapes in winter than in spring for the meteorological factors measured (Table 1). The number of days in which temperatures dropped below zero were 1 and 4 for complex and simple landscapes respectively in winter, and 0 and 4 respectively in spring.

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

For aphids sampled in winter, there was a significant effect of aphid species ($\chi^2_2 = 139.29$, $P < 0.001$) and landscape complexity ($\chi^2_1 = 19.56$, $P < 0.001$) on the rate of drop-off (CT_{min}) from a vertical column (Fig. 1). No interaction effect was observed between aphid species

and landscape complexity ($\chi^2_2 = 3.62$, $P = 0.164$). The β coefficients were negative for *R. padi* and positive for *S. avenae*, and both proved significantly different from *M. dirhodum* considered as the baseline ($\beta = 0$, default option in *coxph*). Thus, for the species effect, the greatest cold tolerance, when measured as CT_{\min} induced drop-off from the vertical column, was observed for *R. padi*. *Sitobion avenae* displayed the lowest cold tolerance and dropped from the column at warmer temperatures, with *M. dirhodum* proving intermediate of the two species. A significant effect of landscape complexity was observed for *R. padi* ($\chi^2_1 = 3.91$, $P = 0.048$), *M. dirhodum* ($\chi^2_1 = 11.54$, $P < 0.001$) and *S. avenae* ($\chi^2_1 = 4.41$, $P = 0.036$). The β coefficients were negative for the complex landscape, as compared to the simple landscape considered as the baseline, meaning that aphids remain attached for longer and accordingly at lower temperatures when originating from complex landscapes. Thus, for all three species, aphids from complex landscapes displayed a reduced rate of CT_{\min} induced drop-off from the vertical column indicative of a greater cold tolerance than aphids from simple landscapes. The frailty effect caused by replications within the same environment was always very low and non-significant. It is pointed out that this effect is poorly known for *Sitobion avenae* due to the lack of replicates within landscapes. Although this effect is coherent with that observed for the two other species, we cannot exclude a sampling bias in this case.

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

For aphids sampled in spring, there was a significant effect of aphid species ($\chi^2_1 = 16.60$, $P < 0.001$) and a weak effect of landscape complexity ($\chi^2_1 = 4.14$, $P = 0.042$) on the rate of CT_{\min} induced drop-off from a vertical column (Fig. 2). No interaction effect was observed between aphid species and landscape complexity ($\chi^2_1 = 1.757$, $P = 0.185$). The greatest cold tolerance

was displayed by *M. dirhodum* and the lowest cold tolerance by *S. avenae*. When analyzing the data separately for each species, a significant effect of landscape complexity was found for *S. avenae* ($\chi^2_1 = 10.32$, $P = 0.001$) with CT_{\min} induced drop-off from a vertical column occurring at a faster rate for individuals originating from simple landscapes. The effect of landscape complexity proved non-significant for *M. dirhodum* ($\chi^2_1 = 0.003$, $P = 0.954$).

Discussion

Effects of landscape complexity on microclimate

Different components of the landscape such as topography, vegetation type and cover directly impact the microclimate (Chen *et al.*, 1999). Although landscape composition is known to affect the microclimate, the relationship is complex, often leading to confusing and sometimes contradictory results (Argent, 1992; Chen *et al.*, 1999; Quénol & Beltrando, 2006; Suggitt *et al.*, 2011). In the current study, simple landscapes (homogenous landscapes characterized by low diversity, large field sizes and few semi-natural elements) were, on average, warmer than more complex landscapes (fine grain heterogeneous landscapes characterized by high species diversity, high hedgerow density, small field sizes and the presence of grassland areas) in both winter and spring. This supports hypothesis 1 that local climate will differ between simple and complex landscapes. However, whilst warmer with regard to mean temperature, simple landscapes displayed an increased range of temperature extremes.

As a landscape becomes progressively more open, it is exposed to increasing amounts of daytime short radiation, acting to raise local temperatures (Chen *et al.*, 1999; Suggitt *et al.*,

2011). Furthermore, the increased hedgerow density characteristic of complex landscapes provides a windbreak function, causing a reduction in local wind speed and retention of denser, cooler air (Quénol & Beltrando, 2006), acting to lower mean local temperatures. This windbreak effect and reduction in wind speed further functions to raise relative humidity, as observed in the current study during winter sampling. As the season changes from winter to spring, increased vegetation growth across all landscape types results in a buffering of temperatures. Such buffering of temperature leads to a reduction in variation between simple and complex landscapes, although the complex landscape still remains significantly colder, and the simple landscape with greater extremes. Meteorological data collected in the current study therefore support the idea that landscape intensification and homogenization could act to raise local temperatures, whilst increasing temperature variations and extremes, and reducing relative humidity.

Seasonal aphid population composition

The general decline in dominance of *R. padi* from winter to spring, with a concurrent increase to dominance of *S. avenae* by spring is a commonly observed pattern in the population composition of cereal aphids (Andrade *et al.*, 2013, 2015; Alford *et al.*, 2014). In the current study, during winter sampling, *R. padi* and *M. dirhodum* were present in all sampled fields and *S. avenae* in 2 of the 5 sampled fields. However, by spring, *R. padi* was no longer present. *Sitobion avenae* had increased to dominance and was found in all spring sampled fields, whilst *M. dirhodum* was present in only 2 of the 6 sampled fields. This observation is further supported by the significant inter-species variation in thermal tolerance observed in the current study, with *R. padi* displaying the lowest temperatures of CT_{min} and *S. avenae* the highest. It is known that *R. padi* prefers cool, humid conditions, whilst *S. avenae* has a

preference for warm and dry conditions (Honek, 1985; Leather *et al.*, 1989; Jarošík *et al.*, 2003). The ability of *R. padi* to remain active and attached to the substrate at colder temperatures than its counterparts could therefore provide the species with a fitness advantage in winter. This theory is supported by recent research which revealed behavioral variations that could contribute to the dominance of *R. padi* in winter (Alford *et al.*, 2014), offering an explanation to the observed variation in aphid sampling between seasons.

Effects of landscape complexity on aphid thermal tolerance

Hypothesis 2 states that aphid thermal tolerance will vary with landscape complexity in accordance with the local climate. With the microclimate data collected in the current study, two opposing sub-hypotheses are proposed. Firstly, aphids from simple landscapes could possess an enhanced thermal tolerance in response to greater temperature variations (representing a more thermally stressful environment); a pattern recently observed for the parasitoid wasps of cereal aphids (Tougeron *et al.*, 2016). Secondly, aphids from complex landscapes could possess an enhanced thermal tolerance in response to colder mean temperatures. Interestingly, the current study supported the latter sub-hypothesis, with the aphid species from complex landscapes being more cold tolerant. Since the response to the landscape is observed at the guild level, it suggests that the strength of external filtering (Violle *et al.*, 2012) constrains traits to a narrow range within a guild exposed to the same environmental conditions (Diaz & Cabido, 2001; White, 2008; Aparicio *et al.*, 2012). When exposed to unfavorable low temperatures, survival is dependent upon a number of factors including the extremes of temperature, the rate of cooling, the duration of exposure and the extent to which the temperature fluctuates around freezing (Sinclair *et al.*, 2003). For many insects, and arthropods more generally, temperature extremes are considered to be of

greater importance in determining thermal resistance than temperature means (Paaijmans *et al.*, 2003; Bahrndorff *et al.*, 2006; Estay *et al.*, 2014; Lawson *et al.*, 2015). However, whilst research evidence highlights the importance of temperature extremes in determining thermal tolerance, the relationship is perhaps not that simple, with any environmental unpredictability acting to undermine the strength of the environmental cue received by the insect (Deere *et al.*, 2006). For this reason, plasticity is predicted to be more common in environments that are predictably variable (Deere *et al.*, 2006). As such, the increased temperature variation and enhanced extremes around a warmer mean recorded in simple landscapes may provide an unpredictable cue to the aphids. In contrast, the colder mean temperatures with reduced temperature extremes recorded in complex landscapes could provide a more reliable cue, thus eliciting a greater physiological response and acting to increase aphid cold tolerance.

The relationship between temperature variation and thermal tolerance is therefore a complex one, with low temperature exposure being far more multifaceted than simply a mean temperature. However, whilst researchers are increasingly factoring in temperature extremes and fluctuations into models in pursuit of enhancing our understanding of insect thermal tolerance, particularly in the face of global climate change (Estay *et al.*, 2014, Vasseur *et al.*, 2014), we cannot rule out the possibility that insects may respond to different aspects of the temperature exposure. A recent study investigating the thermal tolerance of *Aphidius* (Hymenoptera: Braconidae) parasitoids along a landscape intensification gradient found that parasitoids originating from intensive 'simple' landscapes were significantly more cold tolerant than those originating from more natural 'complex' landscapes (Tougeron *et al.*, 2016); the reverse pattern to what was observed for their aphid host in the current study. As temperature exposure is multifaceted, so too are the mechanisms involved in conferring cold

tolerance, ranging from behavioral to molecular (Bale, 2002; Sulmon *et al.*, 2015), and strategies concerned with withstanding freezing or avoiding freezing altogether (Bale, 1991). As such, insects may utilize different mechanisms and respond to different environmental triggers. For example, whilst increased temperature variation depressed the lower limits of the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae) and the moth *Helicoverpa armigera* (Lepidoptera: Noctuidae), the same pattern was not observed for the aphid *Acyrtosiphon pisum* (Hemiptera: Aphididae) (Estay *et al.*, 2014). Instead, the lower limit of *A. pisum* was raised following increased temperature fluctuations (variance of temperature around the mean), therefore acting to reduce cold tolerance, with more importance placed on a constant mean temperature (Estay *et al.*, 2014).

The work of Estay *et al.* (2014), in conjunction with the current study, suggests that, although temperature extremes are important in determining thermal resistance for many insects, temperature means could be of greater importance in determining aphid thermal resistance.

Aphids are phloem feeding insects, dependent upon a host plant. Subsequently, any engagement in behavioral thermoregulation e.g. seeking out more thermally suitable microhabitat could result in lost feeding opportunity and be detrimental to the individual. Furthermore, the aphid phloem sap diet is high in cryoprotectant sugars and thus continuation of feeding at unfavorable low temperatures acts to enhance cold tolerance (Danks, 1978; Sømme & Zachariassen, 1981). As such, an aphid may withstand rather than evade unfavorable cold temperatures, remaining fixed to the host plant. For this reason, aphids may be more sensitive to mean temperatures, displaying enhanced thermal tolerance in response to smaller temperature variations around a lower mean temperature, as recorded in complex landscapes.

Seasonal variation in aphid thermal tolerance

The effect of landscape intensification on aphid thermal tolerance was observed in both winter and spring, although the relationship was more pronounced in winter. This offers support to hypothesis 3 that differences in aphid thermal tolerance between landscapes will be less marked in spring than winter because exposure to stressful low temperatures will be less frequent in spring months. This finding further suggests that seasonal thermal tolerance of aphids is conferred via phenotypic plasticity as opposed to genetic adaptation. Aphids, as with all organisms, can respond to variation in environmental temperature via phenotypic plasticity (Addo-Bediako *et al.*, 2000; Ayrinhac *et al.*, 2004) and display a high degree of plasticity in their inherent thermal tolerance (Powell & Bale, 2008; Alford *et al.*, 2012a,b). Natural selection is unlikely to favor unnecessary thermal tolerance and, as such, any acquired cold tolerance should be lost on cessation of winter temperatures. In the study system, mean temperatures increased from winter to spring. In conjunction with this temperature increase, variation in microclimate with landscape complexity became less pronounced. As such, variation in aphid thermal tolerance with landscape complexity should also become less pronounced in spring, as was observed in the current study.

Conclusion

The current study suggests that landscape intensification could act to raise local temperatures, whilst increasing temperature variations and extremes. Indeed, future land use changes could prove as important in dictating local microclimate as global climate change (Pyke, 2004, Oliver & Morecroft, 2014). In the current study, the thermal tolerance of cereal aphids was shown to vary with landscape complexity, with aphids from more natural ‘complex’

landscapes proving significantly more cold tolerant than those originating from intensively farmed, homogenous ‘simple’ landscapes. Future land use changes could therefore have profound implications for the thermal tolerance and adaptability of insects. Furthermore, the study highlights that all insect species may not respond in a similar manner to thermal conditions and changes to microhabitat and microclimate. If true, this could prove particularly problematic if the response of pest insects and their biological control agents are in anyway decoupled, as suggested in the current study and the study by Tougeron *et al.* (2016) on aphid parasitoids. This is an important consideration when faced with land use decisions or landscape manipulation strategies, particularly with the aim of maintaining agro-ecosystem function into the future.

Acknowledgments

The authors gratefully acknowledge Mathilde Méheut and Charlotte Alford for assistance in the field, to Stephanie Llopis, Herve Amat, Jean-Luc Foulon, Thierry Fontaine and Fouad Nassur for technical assistance, and to Alban Thomas, Valérie Bonnardot and Hervé Quénol from COSTEL lab for their help in collecting meteorological data. This work was funded by a Marie Curie Intra-European Fellowship for the project ‘Climland’ (FP7-PEOPLE-2012-IEF-326943) awarded to L. Alford, F. Burel and J. van Baaren.

Disclosure

The authors have no conflict of interesting to declare.

References

- Addo-Bediako, A., Chown, S.L. and Gaston, K.J. (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 267, 739–745.
- Alford, L., Andrade, T.O., Georges, R., Burel, F. and van Baaren, J. (2014) Could behaviour and not physiological thermal tolerance determine winter survival of aphids in cereal fields? *PLoS ONE*, 9, e114982.
- Alford, L., Blackburn, T.M. and Bale, J.S. (2012a) Effects of acclimation and latitude on the activity thresholds of the aphid *Myzus persicae* in Europe. *Journal of Applied Entomology*, 135, 332–346.
- Alford, L., Blackburn, T.M. and Bale, J.S. (2012b) Effect of latitude and acclimation on the lethal temperatures of the peach-potato aphid *Myzus persicae*. *Agricultural and Forest Entomology*, 14, 69–79.
- Andrade, T.O., Hervé, M., Outreman, Y., Krepsi, L. and van Baaren, J. (2013) Winter host exploitation influences fitness traits in a parasitoid. *Entomologia Experimentalis et Applicata*, 147, 167–174.

Andrade, T.O., Outreman, Y., Krespi, L., Plantegenest, M., Vialatte, A., Gauffre, B. and van Baaren, J. (2015) Spatiotemporal variations in aphid-parasitoid relative abundance patterns and food webs in agricultural ecosystems. *Ecosphere*, 6, 113–113.

Aparicio, A., Hampe, A., Fernandez-Carrillo, L. and Albaladejo, R.G. (2012) Fragmentation and comparative genetic structure of four Mediterranean woody species: complex interactions between life history traits and the landscape context. *Diversity and Distributions*, 18, 226–235.

Argent, R.M. (1992) The influence of a plant canopy on shelter effect. *Journal of Wind Engineering and Industrial Aerodynamics*, 44, 2643–2653.

Ayrinhac, A., Debat, V., Gibert, P., Kister, A.G., Legout, H., Moreteau, B., Vergilino, R. and David, J.R. (2004) Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology*, 18, 700–706.

Bahrndorff, S., Holmstrup, M., Petersen, H. and 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. (1987) Insect cold hardiness – freezing and supercooling – an ecophysiological perspective. *Journal of Insect Physiology*, 33, 899–908.

Bale, J.S. (1991) Insects at low-temperature – a predictable relationship. *Functional Ecology*, 5, 291–298.

Bale, J.S. (2002) Insects and low temperatures: from molecular biology to distributions and abundance. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 357, 849–861.

Chen, B. and Kang, L. (2004) Variation in cold hardiness of *Liriomyza huidobrensis* (Diptera: Agromyzidae) along latitudinal gradients. *Environmental Entomology*, 33, 155–164.

Chen, J.Q., Saunders, S.C., Crow, T.R., Naiman, R.J., Brosfoske, K.D., Mroz, G.D., Brookshire, B.L. and Franklin, J.F. (1999) Microclimate in forest ecosystem and landscape ecology – Variations in local climate can be used to monitor and compare the effects of different management regimes. *Bioscience*, 49, 288–297.

Collett, D. (2003) *Modelling Survival Data in Medical Research*. Second Edition. Chapman and Hall, London, UK.

Collier, R.H., Finch, S., Phelps, K. and Thompson, A.R. (1991) Possible impact of global warming on cabbage root fly (*Delia radicum*) activity in the UK. *Annals of Applied Biology*, 118, 261–271.

Cox, D.R. (1972) Regression models and life tables. *Journal of the Royal Statistical Society, Series B*, 34,187–220.

Danks, H.V. (1978) Modes of seasonal adaptation in insects .1. Winter survival. *Canadian Entomologist*, 110, 1167–1205.

Dedryver, C.A., Le Ralec, A. and Fabre, F. (2010) The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *Comptes Rendus Biologies*, 333, 539–553.

Deere, J.A., Sinclair, B.J., Marshall, D.J. and Chown, S.L. (2006) Phenotypic plasticity of thermal tolerances in five oribatid mite species from sub-Antarctic Marion Island. *Journal of Insect Physiology*, 52, 693–700.

Delattre, T., Baguette, M., Burel, F., Stevens, V.M., Quénol, H. and Vernon, P. (2013) Interactive effects of landscape and weather on dispersal. *Oikos*, 122, 1576–1585.

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

Diaz, S. and Cabido, M. (2001) Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution*, 16, 646–655.

Dixon, A.F.G. (1998) *Aphid Ecology: An Optimization Approach*, Second Edition, Chapman and Hall, U.K.

Dong, Z.K., Hou, R.X., Ouyang, Z. and Zhang, R.Z. (2013) Tritrophic interaction influenced by warming and tillage: A field study on winter wheat, aphids and parasitoids. *Agriculture, Ecosystems and Environment*, 181, 144–148.

Easterling, D.R., Evans, J.L., Groisman, P.Y., Karl, T.R., Kunkel, K.E. and Ambenje, P. (2000) Observed variability and trends in extreme climate events: A brief review. *Bulletin of the American Meteorological Society*, 81, 417–425.

Easterling, D.R., Horton, B., Jones, P.D., Peterson, T.C., Karl, T.R., Parker, D.E., Salinger, M.J., Razuvayev, V., Plummer, N., Jamason, P. and Folland, C.K. (1997) Maximum and minimum temperature trends for the globe. *Science*, 277, 364–367.

Estay, S.A., Lima M. and Bozinovic, F. (2014) The role of temperature variability on insect performance and population dynamics in a warming world. *Oikos*, 123, 131–140.

Fenton, B., Margaritopoulos, J.T., Malloch, G.L. and Foster, S.P. (2010) Micro-evolutionary change in relation to insecticide resistance in the peach–potato aphid, *Myzus persicae*. *Ecological Entomology*, 35, 131–146.

Fereres, A. and Moreno, A. (2009) Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus Research*, 141, 158–168.

Fox, J. (2002) Cox proportional-hazards regression for survival data: appendix to an R and S-PLUS companion to applied regression. Volume, 1–18.

Godfray, H.C.J., Hassell, M.P. and Holt, R.D. (1994) The population dynamic consequences of phenological asynchrony between parasitoids and their hosts. *Journal of Animal Ecology*, 63, 1–10.

Hance, T., van Baaren, J., Vernon, P. and Boivin, G. (2007) Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology*, 52, 107–126.

Honek, A. (1985) Plant density and abundance of cereal aphids (Hom., Aphidina). *Journal of Applied Entomology*, 100, 309–315.

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

IPCC (2013) *Climate Change 2013 the Physical Science Basis: Final Draft Underlying Scientific-Technical Assessment: Working Group I Contribution to the IPCC Fifth Assessment Report* (eds. T.F. Stocker, Qin, D., Plattner, G-K., Tignor, M., Allen, S. K.,

Boschung, J., Nauels, A., Xia, Y., Bex V. and Midgley, P. M.) Cambridge University Press, Cambridge, UK and New York.

Jarošík, V., Honěk, A. and Tichopád, A. (2003) Comparison of field population growths of three cereal aphid species on winter wheat. *Plant Protection Science*, 39, 61–64.

Karl, T.R., Knight, R.W. and Baker, B. (2000) The record breaking global temperatures of 1997 and 1998: Evidence for an increase in the rate of global warming? *Geophysical Research Letters*, 27, 719–722.

Krebs, J.R., Wilson, J.D., Bradbury, R.B. and Siriwardena, G.M. (1999) The second silent spring? *Nature*, 400, 611–612.

Lawson, C. R., Vindenes, Y., Bailey, Y. L. and Van De Pol. M. (2015) Environmental variation and population responses to global change. *Ecology Letters*, 18, 724-736.

Leather, S.R., Walters, K.F.A. and Dixon, A.F.G. (1989) Factors determining the pest status of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), in Europe: a study and review. *Bulletin of Entomological Research*, 78, 345–360.

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

Macdonald, S.S., Rako, L., Batterham, P. and Hoffmann, A.A. (2004) Dissecting chill coma recovery as a measure of cold resistance: evidence for a biphasic response in *Drosophila melanogaster*. *Journal of Insect Physiology*, 50, 695–700.

Mellanby, K. (1939) Low temperature and insect activity. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 127, 473–487.

Moran, N.A. (1992) The evolution of aphid life cycles. *Annual Review of Entomology*, 37, 321–348.

Oliver, T.H. and Morecroft, M.D. (2014) Interactions between climate change and land use change on biodiversity: attribution problems, risks, and opportunities. *Wiley Interdisciplinary Reviews - Climate Change*, 5, 317–335.

Paaajmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C. and Thomas, M.B. (2013) Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19, 2373–2380.

Piyaphongkul, J., Pritchard, J. and Bale, J.S. (2012) Can tropical insects stand the heat? A case study with the brown planthopper *Nilaparvata lugens* (Stal). *PLoS ONE*, 7, e29409.

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

Powell, S.J. and Bale, J.S. (2008). Intergenerational acclimation in aphid overwintering. *Ecological Entomology*, 33, 95–100.

Pyke, C.R. (2004) Habitat loss confounds climate change impacts. *Frontiers in Ecology and the Environment*, 2, 178–182.

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

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

Roschewitz, I., Gabriel, D., Tschamtker, T. and Thies, C. (2005) The effects of landscape complexity on arable weed species diversity in organic and conventional farming. *Journal of Applied Ecology*, 42, 873–882.

Sinclair, B.J., Vernon, P., Klok, C.J. and Chown, S.L. (2003) Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution*, 18, 257–262.

Sømme, L. and Zachariassen, K.E. (1981) Adaptations to low-temperature in high-altitude insects from Mount Kenya. *Ecological Entomology*, 6, 199–204.

Strathdee, A.T., Howling, G.G. and Bale, J.S. (1995) Cold-hardiness of overwintering aphid eggs. *Journal of Insect Physiology*, 41, 653–657.

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

Sulmon, C., van Baaren, J., Cabello-Hurtado, F., Gouesbet, G., Hennion, F., Mony, C., Renault, D., Bormans, M., El Amrani, A., Wiegand, C. and Gerard, C. (2015) Abiotic stressors and stress responses: What commonalities appear between species across biological organization levels? *Environmental Pollution*, 202, 66–77.

Therneau, T.M., and Grambsch, P.M. (2000) *Modelling Survival Data, Extending the Cox Model*. First Edition. Springer, New York, USA.

Tilman, D. (1999) Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 5995–6000.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S. (2002) Agricultural sustainability and intensive production practices. *Nature*, 418, 671–677.

Tougeron, K., van Baaren, J., Burel, F. and Alford, L. (2016). Comparing thermal tolerance across contrasting landscapes: first steps towards understanding how landscape management could modify ectotherm thermal tolerance. *Insect Conservation and Diversity*, 9, 171–180.

- Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I. and Thies, C. (2005) Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecology Letters*, 8, 857–874.
- Vasseur, D.A., Delong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., Mccann, K.S., Savage, V., Tunney, T.D., and O'connor, M.I. (2014) Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 281, 20132612.
- Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V. and Messier, J. (2012) The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution*, 27, 244–252
- Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F. (2002) Ecological responses to recent climate change. *Nature*, 416, 389–395.
- Weber, K.E. and Diggins, L.T. (1990) Increased selection response in larger populations. II. Selection for ethanol vapour resistance in *Drosophila melanogaster* at two population sizes. *Genetics*, 125, 585–597.
- White, T.C.R. (2008) The role of food, weather and climate in limiting the abundance of animals. *Biological Reviews*, 83, 227–248.

Accepted Article

Manuscript received June 06, 2016

Final version received January 10, 2017

Accepted February 08, 2017

Table 1. Daily average meteorological data \pm 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 \pm 0.08 ^(a)	-0.32 19.42	89.66 \pm 0.28 ^(a)	2.15 \pm 0.04 ^(a)
	Intermediate	7.50 \pm 0.08 ^(b)	-0.53 23.37	89.10 \pm 0.28 ^(a)	3.27 \pm 0.06 ^(b)
	Simple	8.78 \pm 0.17 ^(c)	-2.08 24.90	82.86 \pm 0.64 ^(b)	2.54 \pm 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 \pm 0.10 ^(a)	0.51 20.10	84.01 \pm 0.38	1.91 \pm 0.04 ^(a)
	Intermediate	10.81 \pm 0.11 ^(b)	0.08 20.89	84.81 \pm 0.36	2.00 \pm 0.04 ^(a)
	Simple	11.19 \pm 0.12 ^(c)	-1.02 21.78	83.88 \pm 0.41	2.75 \pm 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 \pm 0.06	-2.08 24.90	88.38 \pm 0.20	2.69 \pm 0.03
Spring	All	10.81 \pm 0.06	-1.02 21.78	84.26 \pm 0.22	2.23 \pm 0.03
	<i>t</i> -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.

Fig. 1. Cumulative percentage drop-off of 'winter' aphids (*Rhopalosiphum padi*, *Metopolophium dirhodum* and *Sitobion avenae*) from a glass column (CT_{min}) when cooled from 20°C to -10°C at a rate of 0.5°C min⁻¹. Aphids were collected in winter 2013/14. Aphids collected from the complex landscapes are indicated by the black line and aphids collected from the simple landscapes by the grey line. Aphid drop-off is plotted from temperatures of 10°C and below due to the lack of aphid drop-off at temperatures of 10°C and above.

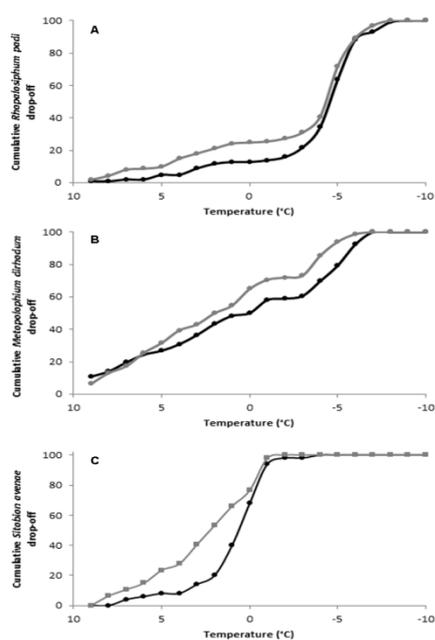


Fig. 2. Cumulative percentage drop-off of ‘spring’ aphids (*Metopolophium dirhodum* and *Sitobion avenae*) from a glass column (CT_{\min}) when cooled from 20°C to -10°C at a rate of 0.5°C min⁻¹. Aphids were collected in spring 2014. Aphids collected from the complex landscapes are indicated by the black line and aphids collected from the simple landscapes by the grey line. Aphid drop-off is plotted from temperatures of 10°C and below due to the lack of aphid drop-off at temperatures of 10°C and above.

