

Are aphid parasitoids from mild winter climates losing their winter diapause?

Kévin Tougeron^{1,2,3}  · Cécile Le Lann^{1,2} · Jacques Brodeur³ · Joan van Baaren^{1,2}

Received: 29 April 2016 / Accepted: 4 November 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Temperature is both a selective pressure and a modulator of the diapause expression in insects from temperate regions. Thus, with climate warming, an alteration of the response to seasonal changes is expected, either through genetic adaptations to novel climatic conditions or phenotypic plasticity. Since the 1980s in western France, the winter guild of aphid parasitoids (Hymenoptera: Braconidae) in cereal fields has been made up of two species: *Aphidius rhopalosiphi* and *Aphidius matricariae*. The recent activity of two other species, *Aphidius avenae* and *Aphidius ervi*, during the winter months suggests that a modification of aphid parasitoid overwintering strategies has taken place within the guild. In this study, we first performed a field survey in the winter of 2014/15 to assess levels of parasitoid diapause incidence in agrosystems. Then,

we compared the capacity of the four parasitoid species to enter winter diapause under nine different photoperiods and temperature conditions in the laboratory. As predicted, historically winter-active species (*A. rhopalosiphi* and *A. matricariae*) never entered diapause, whereas the species more recently active during winter (*A. avenae* and *A. ervi*) did enter diapause but at a low proportion (maximum of 13.4 and 11.2%, respectively). These results suggest rapid shifts over the last three decades in the overwintering strategies of aphid parasitoids in Western France, probably due to climate warming. This implies that diapause can be replaced by active adult overwintering, with potential consequences for species interactions, insect community composition, ecosystem functioning, and natural pest control.

Keywords Climate change · Phenotypic plasticity · Phenology · Overwintering · Seasonal adaptations · Loss of diapause

Communicated by Roland A. Brandl.

Using fresh data and both field and laboratory experiments, we present new evidence of climate change impacts on overwintering strategies in parasitoid wasp species from mild winter areas.

Electronic supplementary material The online version of this article (doi:[10.1007/s00442-016-3770-7](https://doi.org/10.1007/s00442-016-3770-7)) contains supplementary material, which is available to authorized users.

✉ Kévin Tougeron
tougeron.kevin@gmail.com

¹ UMR 6553 Ecobio, Centre National de la Recherche Scientifique, Université de Rennes 1, 263 Avenue du Général Leclerc, 35042 Rennes, France

² Université Bretagne Loire, Cité Internationale, 1 place Paul Ricœur, 35044 Rennes, France

³ Département de Sciences Biologiques, Institut de Recherche en Biologie Végétale, Université de Montréal, 4101 rue Sherbrooke Est, Montréal, Quebec H1X 2B2, Canada

Introduction

There is extensive evidence that climate change can impact various organisms' functional traits and can lead to rapid eco-evolutionary changes, including trait loss through relaxed selection (Carroll et al. 2007; Lahti et al. 2009). Similarly, an increasing number of studies on both animals and plants indicate that modifications in mean temperatures have considerable impacts on species distribution, abundance, physiology, and phenology (Hughes 2000; Walther et al. 2002; Parmesan 2006). There is, however, limited evidences suggesting that climate change may also induce drastic shifts in overwintering strategies, with species delaying migration (e.g., Gordo et al. 2005) or remaining active throughout the winter (Andrade et al. 2016).

In temperate areas, an increase in mean temperatures in winter is expected, leading to a milder, later, and shorter winter period (IPCC 2013). This change in seasonality is likely to have widespread impacts on ectothermic organisms, such as insects, whose fitness is largely determined by how well their phenology tracks seasonal patterns (alternation between favorable and adverse conditions). Insects typically enter diapause as the photoperiod declines in fall which is used as cue of upcoming deleterious conditions (Tauber et al. 1986; Saunders 2002). This photoperiodic response is modulated and selected by winter temperatures, and consequently, diapause incidence varies between populations along winter severity gradients (Danks 1987). For example, in the parasitoid *Nasonia vitripennis* Ashmead, the incidence of diapause is higher and occurs sooner, in northern populations (which experience earlier and harsher winters) compared with southern populations (Paolucci et al. 2013). Climate warming may impose a selective pressure on insects' diapause, which may be altered in the same way as if northern populations were moving southward. As a consequence, for a given population in a given area, diapause level may currently be lower than some decades ago.

Changes in diapause expression can occur either through adaptations to novel climatic conditions or through phenotypic plasticity when facing higher temperatures (Bradshaw and Holzapfel 2001; Vázquez et al. 2015). Insect populations experiencing warmer winters could show (1) a delay in the onset of diapause, (2) a decrease in the proportion of individuals entering diapause and, ultimately (3) a loss of the capacity to enter diapause if there is a selective advantage to individuals maintaining their activities during winter (e.g., increased resource availability and increased cost of diapause; Hance et al. 2007; Bale and Hayward 2010). These potential shifts in overwintering strategies could have cascading consequences on community structures, species interactions and ecosystem functioning (Parmesan and Yohe 2003; Andrade et al. 2016) but are, to date, scantily documented in host/parasitoid systems (Hance et al. 2007; Chaianunporn and Hovestadt 2015).

The Brittany region, in western France, is characterized by an oceanic climate. Since the early 1980s, studies in this area have reported the winter guild of cereal aphid parasitoids to be dominated by two species which remained active and showed low levels or the absence of winter diapause in the field: *Aphidius rhopalosiphii* De Stefani-Perez (Hymenoptera: Braconidae) and *Aphidius matricariae* Haliday, with a proportion of diapausing parasitoids in each population of around 25% (Rabasse and Dedryver 1982; Krespi 1990; Krespi et al. 1997, Table 1). In contrast, two other parasitoid species, *Aphidius avenae* Haliday and *Aphidius ervi* Haliday, as well as all aphid hyperparasitoid species, were presumed to enter diapause in winter, because they were only encountered as active adults (or as non-diapausing prepupae

inside a mummy) during late spring, summer and early fall (Rabasse and Dedryver 1982; Krespi et al. 1997, Table 1). This situation has changed in the past few years (since winter 2011/12), with *A. avenae* (and to a lower extent *A. ervi*) now being active in winter in cereal fields, and representing, respectively, up to 52 and 3% of the total parasitoid guild each winter (Andrade et al. 2016; Tougeron et al. 2016).

Andrade et al. (2016) suggested that the change in the aphid parasitoid guild composition was related to climate warming and to increasing availability of anholocyclic cereal aphids during winter, leading to a shift in overwintering strategies in some parasitoid species. Indeed, under mild climates of Western Europe, anholocyclic cereal aphids have been active throughout the year (Dean 1974; Leather 1992; Langer et al. 1997; Dedryver et al. 2001), and have been serving as reservoirs allowing some parasitoids to overwinter as active adults, whereas others entered diapause (e.g., Langer and Hance 2000; Legrand et al. 2004). This pattern is often considered as a “spread-the-risks” strategy (polyphenism), as a local adaptation to both winter temperature conditions and to host availability (Hance et al. 2007). Overwintering as an active adult rather than as diapausing prepupae may consequently become a more common strategy in insects experiencing a warmer climate. For the above reasons, aphid parasitoids from Brittany are excellent models to study diapause expression in the context of climate change.

In this study, we asked to what extent the diapause could be affected by rapid climate changes in aphid parasitoid populations from a mild winter region, in Western Europe. The following questions were addressed: (1) are these populations expressing winter diapause in both the field and laboratory; (2) if yes, under what condition of photoperiod and temperature; and (3) in what proportion for each species and condition? To answer these questions, we first conducted a field survey in winter 2014/15 to assess levels of diapause incidence in natural populations of aphid parasitoids in cereal fields and to determine if *A. avenae* and *A. ervi* remained constituent of the host-parasitoid food web in Brittany as observed since 2011/12 (Andrade et al. 2016; Tougeron et al. 2016). Then, we quantified diapause induction in the four cereal aphid parasitoids species under different combinations of photoperiod and temperature in the laboratory. We aimed at highlighting changes in diapause expression by comparing current diapause expression to those recorded about 30 years ago in the same area and on the same species (details in Table 1). According to the previous studies on these insect populations (e.g. Krespi et al. 1997; Andrade et al. 2016), the following hypotheses were tested: (1) the incidence of parasitoid diapause is higher at lower temperatures and photoperiods; (2) the incidence of parasitoid diapause varies together with the seasonal occurrence of each species in natural environments, with

Table 1 Summary of results on overwintering parasitoids in Brittany, Western France in the 1970–1980s

Parasitoid species	Total number of mummies collected in winter		Emergence period and time to emergence of winter-collected mummies	Conclusions of authors on winter diapause
	Rabasse and Dedryver (1982)	Krespi et al. (1997)		
<i>Aphidius avenae</i> ^a	4	None	Unspecified ^c	High: adults rarely or never encountered in winter but present in late fall and early spring ^{ab}
<i>Aphidius ervi</i>	2	None	Unspecified ^c	High: adults rarely or never encountered in winter but present in late fall and early spring ^{cd}
<i>Aphidius rhopalosiphi</i> ^b	29	427	Throughout winter (<1 month after sampling for most individuals) ^{cd} 29% required between 54 and 224 days to emerge ^d	Some individuals in diapause ^{cd} The rest overwinters as active adult on anholocyclic populations of aphids ^c
<i>Aphidius matricariae</i>	21	224	Throughout winter (<1 month after sampling for most individuals) ^{cd} 24% required between 124 and 427 days to emerge ^d	Some individuals in diapause ^{cd} The rest overwinters as active adult on anholocyclic populations of aphids ^c
Hyperparasitoids	68	Very scarce, no abundance available	April to June ^{cd} Long delay of emergence (>100 days in most cases)	Almost all individuals enter diapause and emerge in spring or summer ^{cd}
<i>Asaphes</i> sp.				
<i>Alloxysta</i> sp.				
<i>Dendrocerus</i> sp.				
<i>Phaenoglyphis</i> sp.			Only 4 out of 68 emerged 14 to 50 days after sampling ^d	

For both studies, aphid mummies that may contain either diapausing or non-diapausing parasitoids were collected and the time from mummy collection to emergence of adult parasitoids and hyperparasitoids was determined under outdoors conditions. This provides information on the proportion of diapause among these species. Rabasse and Dedryver (1982) sampled in wheat fields mainly at Le Rheu in Brittany, and in other locations in Western France. They conducted surveys from late October to early April for 6 years, starting in 1975. They reported that the aphid community was dominated by *Sitobion avenae* and *Rhopalosiphum padi*. Krespi et al. (1997) collected mummies on wheat from October to March in 1984 and 1985. Sampling periods will be roughly named the “winter period” (i.e., the period during which diapause can occur). In this table, we report pooled data for all sampling years and the main conclusions of the authors concerning diapause expression in aphid parasitoids that emerge from winter-collected mummies. We only show data from species of interest in the present study

^a Formerly named *Aphidius picipes*

^b Formerly named *Aphidius uzbekistanicus*

^c Rabasse and Dedryver (1982)

^d Krespi et al. (1997)

A. matricariae and *A. rhopalosiphi* expressing no or little diapause, and *A. avenae* and *A. ervi* having the highest diapause incidence; and (3) as a response to mild winters and the availability of hosts in winter, overall parasitoid diapause incidence is low in Brittany populations.

Materials and methods

Meteorological data

We analyzed minimum, maximum, and average daily temperature between (1) 1976–1985 and (2) 2010–2015. These

periods correspond to the range of sampling dates in (1) Rabasse and Dedryver (1982) and Krespi et al. (1997) and (2) Andrade et al. (2016) and Tougeron et al. (2016) and this study in Brittany. In our analysis, we chose to only account for the evolution of the temperature conditions between the two time periods and not for inter-annual variations within a given time period. Data were analyzed for seasonal periods of parasitoid diapause induction and maintenance: the fall (from September 22 to December 21) and the winter (from December 22 to March 20). We also analyzed for each season, across the two time periods, the variation in the average number of days with cold spell events (minimum temperature ≤ 0 °C, close to potential freezing

temperature and above activity and developmental thresholds of most *Aphidius* parasitoids (Sigsgaard 2000; Alford et al. 2014)), and heat spell events (maximum temperature ≥ 22 °C, stressful for *Aphidius* parasitoids (Le Lann et al. 2011b)). Data were recorded by a Météo France weather station situated next to the sampled fields (48°04'06"N, 1°44'00"W, elevation: 36 m) with a classic resistance temperature detector probe (1976–1985) and a Pt100 probe (2010–2015, Pyrocontrole, Meyzieu, France).

Winter field sampling

To assess for potential diapausing primary and secondary parasitoids in the field in winter and to build an aphid parasitoid quantitative food web, we sampled aphids and aphid mummies in different cereal crops from the 5th to the 29th of January, 2015, in Brittany, Western France. A preliminary prospection on several crops allowed us to assess the presence of aphids and to select a total of seven different fields in which we performed the sampling. Fields were separated one from another by at least 500 m. Precise locations of the fields were as follows (°North; °West): 48.09458; 1.81386, 48.11095; 1.81403, 48.10777; 1.78203, 48.48282; 1.60453, 48.10046; 1.81459, 48.10636; 1.81395 and 48.12238; 1.53869. We visited each field twice per week. Field borders were excluded to avoid potential margin effects. A standardized surface of 1000 m² was prospected in each field and every aphid and mummy found were collected and stored in the lab at 20 °C, with a Light:Dark (LD, h) 16:8 photoregime. Living aphids were kept on winter wheat 10 days to wait for potential mummification and were identified thereafter. Following emergence, parasitoids and hyperparasitoids were freeze-killed at -20 °C and identified (along with the species of aphid they emerged from), using an optical microscope and the identification key developed by Hullé et al. (2006). Hyperparasitoids were only identified to the genus level. Aphid mummies from which no parasitoid emerged were kept for 70 days in gelatine capsules ($L = 2$ cm, $\varnothing = 0.5$ cm) at 20 °C, with any emergence scored daily. Remaining mummies were then dissected and their content identified as a dead individual or a diapausing parasitoid prepupae.

Biological material

Laboratory cultures of *Aphidius* species were established from individuals collected in Brittany during different sampling sessions in fall 2014. From 84 to 125 females and from 31 to 78 males (depending on the species) were sampled in five cereal fields in a 30 km area around the city of Rennes (48.113°N, 1.674°W) in 2014. The genetic pool was refreshed in January 2015 by adding 57 non-diapausing parasitoids from the seven fields that we used in this

study, and mentioned above. This was done to ensure a sufficient genetic diversity in the laboratory parasitoid culture. They were reared for less than ten generations before being tested. To control for host effect on parasitoid diapause incidence, we used a single aphid clone of *Sitobion avenae* (Fabricius) that was established from one single parthenogenetic female collected in 1990 at Le Rheu, Brittany. Parasitoids were maintained on parthenogenetic *S. avenae* aphids, reared on winter wheat (*Triticum aestivum* L. v. *Saturnus*). Insects and plants were maintained in Plexiglas cages (50 × 50 × 50 cm) at 20 ± 1 °C, $70 \pm 20\%$ RH and 2000 lx under a 16:8 LD photoregime in climate-controlled chambers.

Conditions for diapause induction

To test the effects of photoperiod and temperature on parasitoid diapause incidence during larval and pupal development, nine constant conditions were used, representing the combinations of three photoregimes (10:14, 12:12, and 14:10 LD) with three temperature regimes (14, 17, and 20 °C). These values correspond to temperatures and photoperiods encountered by the parasitoids from August 15 to November 30, 2014, in natural conditions in Brittany (Météo France). These temperature and photoperiod treatments are known to be diapause-inducing in aphid parasitoids from temperate regions at equivalent latitude and represent standard treatments when assessing for diapause expression in insects, which helps at better comparing our results to other studies' (Brodeur and McNeil 1989a; Langer and Hance 2000; Polgár and Hardie 2000). Only five climate chambers were available at the same time. Thus, we first tested five treatments on the four species (20 °C 10:14 LD/20 °C 14:10 LD/14 °C 10:14 LD/14 °C 12:12 LD/14 °C 14:10 LD), and next, we tested the last four treatments (20 °C 12:12 LD/17 °C 10:14 LD/17 °C 12:12 LD/17 °C 14:10 LD) + one repetition of one previous treatment (20 °C 10:14 LD) to control for any bias in the experiment, such as artificial selection in the cultures, cohort, or generational effects. There were no differences in diapause incidence between these two batches of experiments at 20 °C, 10:14 LD, for any of the four species (GLM, $df = 1$, $F = 0.08$, $p = 0.79$).

Insect production for the experiments

Because host size and life stage have been shown to influence parasitoid diapause incidence (Brodeur and McNeil 1989a), aphid cohorts of homogenous size were produced for our experiments. For each parasitoid species, mummies were collected from the culture, isolated in gelatin capsules, and then maintained in climate chambers until emergence. On the day of emergence, five females were placed

in a plastic tube for 12–24 h with access to two males for mating and honey and water for feeding. The next day, under standard rearing conditions, the five females were exposed together for 24 h to 150 (± 10) *S. avenae* second instar aphids on a wheat pot in a cage, also with access to honey and water.

Diapause induction

After 24 h, parasitoid females were removed from the cage and the 150 potentially parasitized aphids were randomly and equally distributed on 5 wheat pots (≈ 30 aphids per pot). Pots consisted of plastic tubes ($L = 16$ cm, $\varnothing = 5$ cm) filled with vermiculite substrate and closed with a fine mesh netting. Each of these “microcages” was placed under one of the experimental photoperiod/temperature conditions described above. Temperature was set using MIR-153 climate chambers (Sanyo, Osaka, Japan) and controlled daily using the incorporated probe. Light was provided by 20 W fluorescent tubes with an intensity of 2000 lx (4000 K cool white color) in each climate chamber. Relative humidity was maintained at $80 \pm 10\%$. Starting 1 week after the experiments began, microcages were checked daily. Once aphid mummies were formed, they were isolated individually in gelatin capsules and kept under their respective treatments. Parasitoid emergence was verified twice daily. This whole procedure was repeated from 5 to 11 times for each treatment and species. Mummies from which a parasitoid did not emerge were dissected 15 days after the last adult emergence in the same cohort, and classified as dead parasitoids or diapausing prepupae (see Appendix S1 for more details and pictures of the dissections).

Statistical analyses

We considered both the number of diapausing individuals and the number of non-diapausing individuals, including dead ones, for each temporal replicate (i.e., each wheat microcage with 30 parasitized aphids at any given condition). First, a Generalized Linear Model (GLM) with a quasi-binomial error distribution (to account for overdispersion) was fitted to the data to assess differences in the proportion of individuals in diapause between the four species and treatments (i.e., photoperiod and temperature), including the interactions between these explanatory variables. Second, for each of the two species that had at least some individuals entering diapause, another GLM with quasi-binomial errors was run to assess diapause incidence differences among treatments (photoperiod, temperature, and their interaction as explanatory variables). To assess for a potential effect of differential mortality rates among conditions, we re-ran the model after removing dead individuals in the data set, thus only considering diapausing versus

emerged parasitoids. We attempted to include the identity of the wheat pot as a random factor in our analysis, but this procedure consistently generated convergence errors in our generalized linear mixed models. Thus, our final analyses rest on the assumption that there was no temporal effect on diapause induction, which is probably justified, because the experiments were all conducted within a few weeks (i.e., over the course of a maximum of three parasitoid generations). All statistical analyses were carried out using the R software (R Development Core Team 2015). The significance of each explanatory variable was tested with the ‘Anova’ function from the package *car*, using an *F*-test for diapause quasi-binomial data (Fox and Weisberg 2011).

Results

Meteorological data

From 1976 to 2015, the daily average temperature during the potential season of diapause induction increased by an average of 0.77 °C in fall and 1.13 °C in winter (Fig. 1; Table 2). Furthermore, days with cold spell events during both fall and winter decreased in frequency since the 1970s, while the frequency of days with heat spell events more than doubled in the fall (Table 2).

Winter field sampling

As observed by Andrade et al. (2016), aphids and their parasitoids are generally encountered at very low abundances in cereal fields in winter compared with spring. A total of 93 aphids and aphid mummies were collected. Sixteen aphids were unparasitized and a total of 77 mummies were formed, i.e., a parasitism rate of 83%, as defined by the total number of mummies (sampled mummies + aphids that turned into mummies) divided by the total number of individuals (mummies + unparasitized aphids). Among these mummies, 57 hatched within the few days after sampling (< 7 days). Adult emergence was greatly delayed in 23% of the aphid mummies with two *A. avenae*, seven *A. ervi*, and nine hyperparasitoids from three genera (*Alloxyta* sp., *Asaphes* sp., and *Phaenoglyphis* sp.) emerging more than 2 months after sampling. These individuals were considered to be in diapause and represented 7, 64, and 56% of the total number of mummies of their species or group of species, respectively. The 75 individuals were included in the quantitative food web (Fig. 2). The two remaining mummies contained unidentifiable dead individuals.

The aphid-primary parasitoid food web in winter 2014–2015 (Fig. 2) was dominated by *A. avenae* and *A. rhopalosiphii*. *Aphidius ervi* and *A. matricariae* represented 15 and 5% of the total parasitoid abundance, respectively.

Fig. 1 Average, minimum, and maximum daily temperatures from September 22 to March 20 calculated across two periods: 1975–1985 (solid lines) and 2010–2015 (dotted lines). The corresponding photoperiod (at 48°N) is indicated for the 1st day of each month

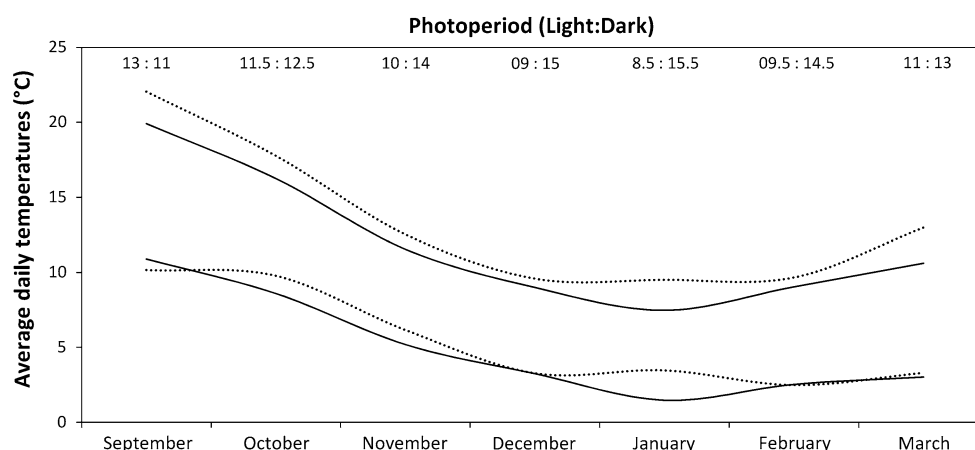


Table 2 Minimum, average, and maximum daily temperatures and mean frequency of days with cold (≤ 0 °C) and heat spells (≥ 22 °C) ($\pm$ standard error of the mean) for fall (from 22nd September to 21st

December) and winter (from 22nd December to 20th March) calculated across two periods: 1975–1985 and 2010–2015 Data were provided by Météo France

Years Season	1976–1985		2010–2015		Average change	
	Fall	Winter	Fall	Winter	Fall	Winter
Temperature (°C)						
Min average	6.55 $\pm$ 0.16	2.44 $\pm$ 0.15	7.02 $\pm$ 0.23	3.26 $\pm$ 0.19	+0.47	+0.82
Average	9.73 $\pm$ 0.15	5.45 $\pm$ 0.13	10.50 $\pm$ 0.22	6.58 $\pm$ 0.16	+0.77	+1.13
Max average	13.53 $\pm$ 0.17	8.98 $\pm$ 0.14	14.63 $\pm$ 0.25	10.37 $\pm$ 0.17	+1.10	+1.39
Mean frequency						
Cold spells (≤ 0 °C)	9.00 $\pm$ 0.69	24.10 $\pm$ 0.72	7.67 $\pm$ 0.85	16.00 $\pm$ 0.98	–15%	–34%
Heat spells (≥ 22 °C)	2.90 $\pm$ 0.74	0.00 $\pm$ 0.00	6.50 $\pm$ 0.67	0.00 $\pm$ 0.00	+124%	0%

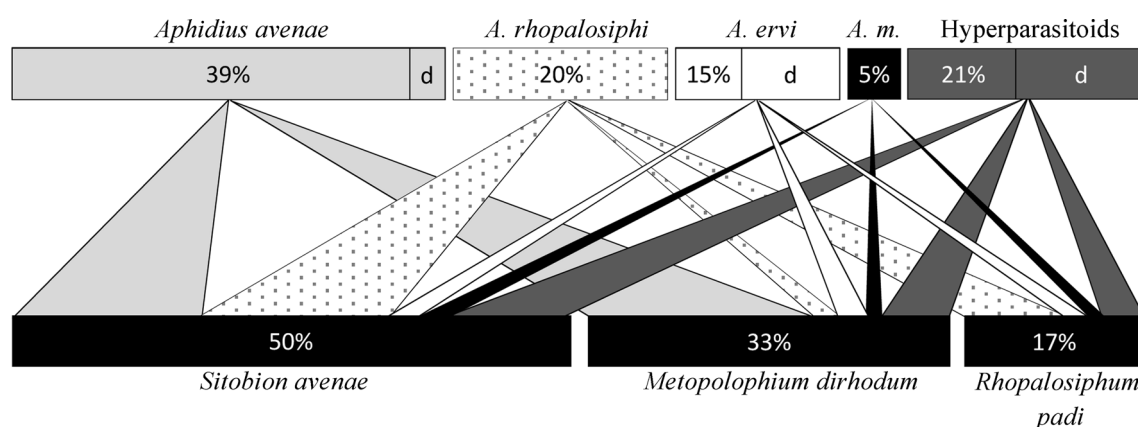


Fig. 2 Quantitative food web showing the parasitoid and aphid community composition in winter 2014–2015 in cereal fields. Upper bars represent parasitoid of the genus *Aphidius* relative abundance. *A. m.* abbreviation stands for *Aphidius matricariae*. Percentages include diapausing and non-diapausing parasitoids, with lower script letter “d” indicating the proportion of individuals in diapause. Lower bars

represent aphid relative abundance for each species. The thickness of the arrows between two bars is proportional to the relative number of trophic interactions between species (e.g., 64% of the parasitoids emerging from *M. dirhodum* mummies were *A. avenae*). The total number of aphid parasitoids pairs represented is $n = 75$

The three hyperparasitoid genera mentioned above were also detected at high abundances (21% of the food web total abundance), and for the first time in winter. *Sitobion*

avenae and *Metopolophium dirhodum* (Walker) dominated the aphid community and *Rhopalosiphum padi* (L.) was present at a lower abundance.

Diapause expression in the laboratory

A total of 1482 mummies were formed in laboratory tests. Overall, there was a significant difference in diapause incidence among species (GLM, $df = 3$, $F = 149.1$, $p < 0.001$); there was no diapause observed in *A. rhopalosiph* ($n = 312$ mummies, 18 dead, 294 emerged) nor in *A. matricariae* ($n = 311$ mummies, 40 dead, 271 emerged), regardless of temperature/photoperiod treatments, whereas diapause was observed in the two other parasitoid species, *A. avenae* and *A. ervi*, but at overall low levels of expression. The maximum diapause incidence was observed at 14 °C and 10:14 LD for both *A. avenae* ($13.4 \pm 1.6\%$) (Fig. 3a)

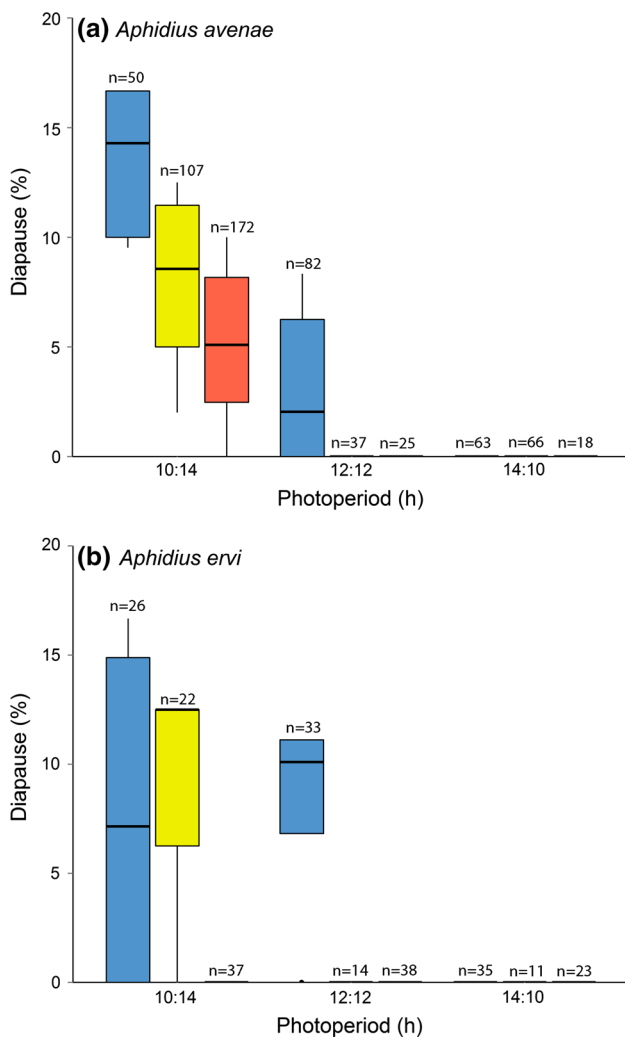


Fig. 3 Preupal diapause incidence (%) in Brittany populations of **a** *Aphidius avenae* ($n = 620$, 85 dead, 513 emerged, 22 in diapause) and **b** *Aphidius ervi* ($n = 239$, 31 dead, 199 emerged, 9 in diapause) when parasitized aphids were reared under different photoperiodics [Light:Dark (h): 10:14; 12:12; 14:10] and temperature conditions (14, 17, and 20 °C, blue, yellow, and red), from left to right, respectively. The number of parasitoids tested is provided on the figure for each condition (color figure online)

and *A. ervi* ($11.2 \pm 4.9\%$) (Fig. 3b). There was no diapause observed at 14:10 LD for either species. *Aphidius ervi* diapause was never observed at 20 °C, i.e., under the more “summer-like” temperature condition. However, there was an interaction effect between species and temperature treatments (GLM, $df = 6$, $F = 14.5$, $p < 0.001$), a marginally non-significant interaction effect between species and photoperiod treatments (GLM, $df = 6$, $F = 2.1$, $p = 0.06$) and a significant three-way interaction effect between species, photoperiod, and temperature (GLM, $df = 12$, $F = 4.3$, $p < 0.001$), showing that parasitoid response to temperature and photoperiod treatments was species-specific. Responses of species showing diapausing were thus analyzed separately in different models. Photoperiod and temperature significantly influenced diapause induction in *A. avenae* and *A. ervi* (Table 3), with diapause levels increasing with a decrease in photoperiod and temperature. The interaction of these two factors, however, only influenced diapause incidence for *A. ervi*, suggesting that the influence of temperature on diapause varies with the photoperiod treatment (and reversely). When removing dead individuals from the analysis, we found the same results, showing that they were not biased by parasitoid mortality.

Discussion

This study aimed to understand how recent species-specific changes in the overwintering strategies within a parasitoid guild were linked to the phenotypic expression of diapause. Results obtained for *A. avenae* and *A. ervi* conform to the general pattern of insects from temperate areas relying on photoperiod and temperature stimuli to enter diapause, confirming our first hypothesis on diapause induction cues. However, we also revealed that only individuals from two out of four parasitoid species entered diapause under the conditions tested, and that diapause incidence was very low for both species that did enter diapause (<15%). This result confirms our second hypothesis regarding interspecific differences, as well as our third hypothesis about a little diapause expression. By comparing current diapause levels to those recorded 30 years ago in Western France (Table 1, Krespi 1990) and by analyzing current diapause levels in other aphid parasitoid species from more northern or southern areas (Langer and Hance 2000; Sigsgaard 2002; Lumbierres et al. 2007; Gómez-Marco et al. 2015), we argue that climate warming can trigger shifts in parasitoid diapause expression.

Winter field sampling

Field data show that most individuals within populations of the four primary parasitoid species were active in January.

Table 3 Factors influencing diapause incidence of Brittany populations of *Aphidius avenae* and *Aphidius ervi*

Factor/species	<i>Aphidius avenae</i>				<i>Aphidius ervi</i>			
	<i>F</i>	<i>df</i>	<i>p</i> > <i>F</i>		<i>F</i>	<i>df</i>	<i>p</i> > <i>F</i>	
Photoperiod	52.15	2	<i>p</i> < 0.001	***	42.22	2	<i>p</i> < 0.001	***
Temperature	13.62	2	<i>p</i> < 0.001	***	61.29	2	<i>p</i> < 0.001	***
Photoperiod × temperature	1.87	4	<i>p</i> = 0.14	NS	4.49	4	<i>p</i> < 0.01	**

Results from Generalized Linear Models (GLMs) with quasi-binomial errors are shown for both species. Significance of each term was assessed with iterative *F* tests

*** *p* < 0.001, ** *p* < 0.01, NS not significant

We provide first evidence that aphid hyperparasitoids have become active during the winter in cereal fields in Brittany and at a relatively high abundance (>20% of total parasitoid abundance). We also found some diapausing *A. avenae* and *A. ervi*, as well as some diapausing hyperparasitoids in the field, but no diapausing *A. rhopalosiphi* or *A. matricariae*, in line with our laboratory results on diapause incidence. In warmer climates in Spain (39°N), Gómez-Marco et al. (2016) showed that *Aphis spiraecola* was parasitized during winter by Aphididae parasitoids. Lumbierres et al. (2007) also showed that winter-active aphids on cereal crops in Catalonia (41°N) were parasitized by a diversity of aphid parasitoids, including *A. rhopalosiphi*, *A. matricariae*, and *A. ervi*, suggesting that a part of the parasitoid populations overwintered as active adults. At the opposite in colder climates, such as Zealand (56°N, Denmark), *Aphidius* spp. are absent of the fields before May, since they cannot overwinter as active adults on their aphid hosts which are purely holocyclic (i.e., they produce diapausing eggs in winter) (Sigsgaard 2002). However, diapause incidence in the field may have been under-estimated by the sampling method we used (Brodeur and McNeil 1990). Indeed, some aphid parasitoids have the capacity to modify host behaviour prior to mummification and Brodeur and McNeil (1989b) showed that aphids containing parasitoid larvae destined to enter diapause frequently leave the host plant and mummify in concealed microhabitats where they become better protected against adverse climatic conditions. Moreover, we cannot exclude that *A. ervi* or *A. avenae*, the more generalist species in our study, did enter diapause at higher levels in populations exploiting different plant–aphid associations.

The host–parasitoid trophic web in winter 2014/15 showed a strong dominance of *A. avenae*, which represented more than 50% of the primary parasitoid relative abundance, as well as a dominance of *S. avenae* and *M. dirhodum* for the aphid community and also the presence of hyperparasitoids. In field studies conducted since 2011 in Western France, similar community compositions were encountered in spring or during mild winters, whereas cold winters were usually associated with the dominance of *A. rhopalosiphi*, *A. matricariae* parasitoids, and *R. padi*

aphids (Krespi et al. 1997; Andrade et al. 2016; Tougéron et al. 2016). This is in line with our meteorological measurements indicating that the average temperature in January 2015 was 6.1 °C. It could be considered as a mild winter, since temperatures were higher than estimated minimal developmental threshold of *Aphidius* spp., which lays between 2.2 and 4.5 °C in species we studied (Sigsgaard 2000).

Diapause expression in the laboratory

Under laboratory conditions, diapause incidence was low in *A. avenae* and *A. ervi*, and no diapause was observed in *A. rhopalosiphi* and *A. matricariae*. In the former species, a few individuals (<15%) entered in diapause, even when placed under conditions corresponding to late-November temperature and photoperiod in Brittany (14 °C, 10:14 LD), which are thresholds typically known to induce high levels of diapause in parasitoids from temperate regions with relatively cold winters. For instance, the Québec population of the parasitoid *Aphidius nigripes* (Ashmead) experiencing harsh winters started to gradually enter diapause in July and expressed 100% of diapause in the early September (Brodeur and McNeil 1994). Differential resistance to cold temperatures may partly explain the interspecific differences in diapause levels. Indeed, *A. avenae* is almost 2 °C less cold tolerant than *A. rhopalosiphi* based on walking abilities after thermal stress (Le Lann et al. 2011a). These cold tolerance differences could suggest that a “risk-spreading” strategy is under balancing selection for species that are not cold tolerant enough to survive all types of winters.

Two hypotheses can explain the absence of diapause expression in *A. matricariae* and *A. rhopalosiphi* and partial diapause expression in *A. ervi* and *A. avenae*: (1) the photoperiod and temperature conditions we tested, although encompassing the conditions potentially encountered in Western France, are not suitable to reach the threshold that induces diapause. (2) These species have genetically lost their capacity to enter diapause, whatever the conditions encountered by the parasitoids. Trait loss can occur through rapid evolutionary changes, within few years (Thompson 1998; Carroll et al. 2007) and it has previously

been demonstrated that the diapause character can be genetically lost—or its expression reduced—in parasitoids, when maintained in non-diapause rearing conditions (i.e., summer-like temperatures and hosts availability) within a small number of generations (Danks 1987). For example, Garipey et al. (2015) showed that a strain of the parasitoid *Binodoxys communis* reared for 350 generations (≈ 7 years) in such conditions genetically loses its ability to enter diapause. Another example of such changes in the pattern of seasonal activity is the genetically based shift towards shorter day-length thresholds for diapause induction in populations of the pitcher plant mosquito (*Wyeomyia smithii*) in North America over 30 years, due to climate warming (Bradshaw and Holzapfel 2001). Our results clearly highlight that if climate gets warmer, diapause might no longer be expressed in *A. avenae* and *A. ervi*, since, for any tested photoperiod, diapause incidence was significantly reduced when temperature increased.

Current diapause incidence in all four parasitoid species is now lower than the levels reported in the 1980s in the same area (Table 1). In the 1980s, *A. rhopalosiphii* and *A. matricariae* were active in the winter but between 24 and 29% of the individuals entered diapause, whereas the rest did not (Krespi et al. 1997). In contrast, we did not find any diapause induction for both species in the laboratory experiment. Moreover, *A. avenae* and *A. ervi* were never encountered as active adults or as non-diapausing prepupae within a mummy in cereal fields in winter until 2011, suggesting that they all entered in diapause before winter, even if their overwintering sites or overwintering hosts remained unspecified (Rabasse and Dedryver 1982; Krespi 1990; Krespi et al. 1997). In contrast, we found a little diapause induction in both species in the laboratory experiments. The current marginal diapause incidence could be interpreted as a response to both continuing host availability throughout the winter season and/or increasing winter temperatures that could have modified diapause induction thresholds across the years. First, on cereal crops, anholocyclic hosts have been available throughout the winter and increased in abundance in Brittany, at least since the 1970s (Rabasse and Dedryver 1982). Aphid density in winter has been shown to be higher in warmer years (Andrade et al. 2015) and to increase with climate warming (Zhou et al. 1995). Second, we showed in this study that maximum winter temperatures have increased by about 1.4 °C, along with a 34% decrease in the frequency of winter cold spell events since the 1976–1985 period. This supports the hypothesis of a decrease in the selective pressures maintaining diapause over the past 30 years, allowing the maintenance of additional parasitoid species in winter. In addition, one would expect that in more northern or cold areas, diapause incidence might currently be similar to those of western France (48°N), 30–40 years ago. Almost

all individuals from a Northern United-Kingdom *A. ervi* population (54.2°N) entered diapause when exposed to 15° C and LD 10:14 (Christiansen-Weniger and Hardie 1999). In *A. rhopalosiphii* and *A. ervi* populations from Belgium (50.3°N), diapause expression was lowered at 40% on average at 15° C and LD 09:14 (Langer and Hance 2000). Latitudinal clines of diapause expression are known in some insect species (e.g. Dedryver et al. 2001; Winterhalter and Mousseau 2007; Paolucci et al. 2013) highlighting that variation in diapause expression across a latitudinal gradient is analogous to temporal changes in diapause levels at a given location, following climate warming.

In a climatic scenario where relatively warm winter conditions persist, the capacity to enter diapause might be maintained in species able to abridge diapause as a way to reduce its fitness costs (Ellers and Van Alphen 2002; Ito 2007). Alternatively, the cost of diapause may increase with climate warming (1) if there is a lack of ability to terminate diapause, especially for insect species in which a period of chilling is required to do so (Tauber et al. 1986; Mehrnejad and Copland 2005), and (2) when mild or more variable winters induce energetic and metabolic costs in insects that are in diapause (e.g., Williams et al. 2012), especially in species that cannot shorten their diapause duration or that are in a deep stage of dormancy. In these cases, the capacity to delay or avert diapause may provide a selective advantage that could be maintained in insect populations (Bale and Hayward 2010), especially when winter environmental conditions allow winter activities, including reproduction. In any case, whether an observed change in overwintering strategies can be attributed to individual phenotypic plasticity or to local adaptation by genetic changes remains unknown and requires long-term analyses as well as further investigations on the cost and conditional expression of diapause. For instance, translocation experiments between parasitoid populations from harsh and mild winter areas and study of their diapause reaction norm may provide interesting insights on their capacity to deal with different climatic conditions.

Perspectives and conclusions

From an ecological services perspective, a loss of diapause in parasitoids could increase levels of aphid populations' suppression, since parasitism in winter and early spring is crucial for effective natural biological control (Langer et al. 1997; Neuville et al. 2015). However, if hyperparasitoids persist in winter as we showed here, this could have undesirable consequences for natural biological control of aphids in the following spring (Gómez-Marco et al. 2015). Ultimately, modifications in insect phenology due to climate change—including diapause incidence and duration—could have important impacts on guild and community

structure, which could, in turn, change species interactions and coexistence in food webs (Andrade et al. 2016), with unpredictable consequences on ecosystem services, such as naturally present biological pest control.

To conclude, field and laboratory data suggest that in Western France, two aphid parasitoid species may have lost their capacity to enter diapause, whereas for two other species, a small fraction of their populations entered diapause. The diapause expression is marginal, even under abiotic conditions corresponding to late-November. We also provide support to the idea that photoperiodic responses are liable to evolutionary changes and that a reduction or a loss of diapause expression can occur within a few decades in short-living organisms experiencing warmer winters. We predict that the loss of diapause will become a widespread response to climate change among arthropods.

Acknowledgements This study was funded by the French Région Bretagne (Grant ARED awarded to KT) and by the Canada Research Chair in Biological Control awarded to JB. All field and laboratory works conducted in this study comply with French and Canadian laws. All applicable institutional and/or national guidelines for the care and use of animals were followed. We thank the handling editor and one anonymous reviewer for their valued comments on an earlier version of this manuscript. We are grateful to Jimmy Debuire, Stéphanie Llopis, Valérie Briand, and Josée Doyon for technical support and Paul K. Abram for English revision.

Author contribution statement This work is part of the Ph.D. thesis of the first author. All authors conceived and designed the experiments. KT performed the experiments and conducted field work. KT analyzed the data and wrote the manuscript. All co-authors substantially contributed to revisions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Alford L, Andrade TO, Georges R et al (2014) Could behaviour and not physiological thermal tolerance determine winter survival of aphids in cereal fields? *PLoS One* 9:e114982
- Andrade TO, Outreman Y, Krespi L et al (2015) Spatiotemporal variations in aphid-parasitoid relative abundance patterns and food webs in agricultural ecosystems. *Ecosphere* 6:1–14. doi:[10.1890/ES15-00010.1](https://doi.org/10.1890/ES15-00010.1)
- Andrade TO, Krespi L, Bonnardot V et al (2016) Impact of change in winter strategy of one parasitoid species on the diversity and function of a guild of parasitoids. *Oecologia* 180:877–888. doi:[10.1007/s00442-015-3502-4](https://doi.org/10.1007/s00442-015-3502-4)
- Bale JS, Hayward SAL (2010) Insect overwintering in a changing climate. *J Exp Biol* 213:980–994. doi:[10.1242/jeb.037911](https://doi.org/10.1242/jeb.037911)
- Bradshaw WE, Holzapfel CM (2001) Genetic shift in photoperiodic response correlated with global warming. *Proc Natl Acad Sci* 98:14509–14511. doi:[10.1073/pnas.241391498](https://doi.org/10.1073/pnas.241391498)
- Brodeur J, McNeil JN (1989a) Biotic and abiotic factors involved in diapause induction of the parasitoid, *Aphidius nigripes* (Hymenoptera: Aphidiidae). *J Insect Physiol* 35:969–974
- Brodeur J, McNeil JN (1989b) Seasonal microhabitat selection by an endoparasitoid through adaptive modification of host behaviour. *Science* 244:226–228
- Brodeur J, McNeil JN (1990) Overwintering microhabitat selection by an endoparasitoid (Hymenoptera: Aphidiidae): induced phototactic and thigmokinetic responses in dying hosts. *J Insect Behav* 3:751–763
- Brodeur J, McNeil JN (1994) Seasonal Ecology of *Aphidius nigripes* (Hymenoptera: Aphidiidae), a parasitoid of *Macrosiphum euphorbiae* (Homoptera: Aphididae). *Environ Entomol* 23:292–298
- Carroll SP, Hendry AP, Reznick DN, Fox CW (2007) Evolution on ecological time-scales. *Funct Ecol* 21:387–393. doi:[10.1111/j.1365-2435.2007.01289.x](https://doi.org/10.1111/j.1365-2435.2007.01289.x)
- Chaianunporn T, Hovestadt T (2015) Evolutionary responses to climate change in parasitic systems. *Glob Change Biol* 21:2905–2916. doi:[10.1111/gcb.12944](https://doi.org/10.1111/gcb.12944)
- Christiansen-Weniger P, Hardie J (1999) Environmental and physiological factors for diapause induction and termination in the aphid parasitoid, *Aphidius ervi* (Hymenoptera: Aphidiidae). *J Insect Physiol* 45:357–364
- Danks HV (1987) Insect dormancy: an ecological perspective. *Biological survey of Canada (Terrestrial Arthropods)*
- Dean GJ (1974) The overwintering and abundance of cereal aphids. *Ann Appl Biol* 76:1–7
- Dedryver C-A, Hüllé M, Le Gall J-F et al (2001) Coexistence in space and time of sexual and asexual populations of the cereal aphid *Sitobion avenae*. *Oecologia* 128:379–388. doi:[10.1007/s004420100674](https://doi.org/10.1007/s004420100674)
- Development Core Team R (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Ellers J, Van Alphen JJ (2002) A trade-off between diapause duration and fitness in female parasitoids. *Ecol Entomol* 27:279–284
- Fox J, Weisberg HS (2011) An R companion to applied regression, 2nd edn. Sage, Thousand Oaks
- Garipey V, Boivin G, Brodeur J (2015) Why two species of parasitoids showed promise in the laboratory but failed to control the soybean aphid under field conditions. *Biol Control* 80:1–7. doi:[10.1016/j.biocontrol.2014.09.006](https://doi.org/10.1016/j.biocontrol.2014.09.006)
- Gómez-Marco F, Urbaneja A, Jaques JA et al (2015) Untangling the aphid-parasitoid food web in citrus: can hyperparasitoids disrupt biological control? *Biol Control* 81:111–121. doi:[10.1016/j.biocontrol.2014.11.015](https://doi.org/10.1016/j.biocontrol.2014.11.015)
- Gómez-Marco F, Urbaneja A, Tena A (2016) A sown grass cover enriched with wild forb plants improves the biological control of aphids in citrus. *Basic Appl Ecol* 17:210–219. doi:[10.1016/j.baae.2015.10.006](https://doi.org/10.1016/j.baae.2015.10.006)
- Gordo O, Brotons L, Ferrer X, Comas P (2005) Do changes in climate patterns in wintering areas affect the timing of the spring arrival of trans-Saharan migrant birds? *Glob Change Biol* 11:12–21. doi:[10.1111/j.1365-2486.2004.00875.x](https://doi.org/10.1111/j.1365-2486.2004.00875.x)
- Hance T, van Baaren J, Vernon P, Boivin G (2007) Impact of extreme temperatures on parasitoids in a climate change perspective. *Annu Rev Entomol* 52:107–126. doi:[10.1146/annurev.ento.52.110405.091333](https://doi.org/10.1146/annurev.ento.52.110405.091333)
- Hughes GE (2000) Biological consequences of global warming: is the signal already apparent? *Trends Ecol Evol* 15:56–61
- Hüllé M, Turpeau E, Chaubet B (2006) Encyclop'aphid, a key for aphids and their parasitoids. In: INRA (French Institute for Agronomical Research)
- 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. In: Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds). Cambridge

- University Press, Cambridge, p 1535. ISBN 978-1-107-05799-1, 978-1-107-66182-0
- Ito K (2007) Negative genetic correlation between diapause duration and fecundity after diapause in a spider mite. *Ecol Entomol* 32:643–650
- Krespi L (1990) Etude de la biocénose parasitaire des pucerons des céréales dans le bassin de Rennes: cas particulier d'*Aphidius uzbekistanicus* Luz. PhD dissertation. Université de Rennes 1, France
- Krespi L, Dedryver C-A, Creach V et al (1997) Variability in the development of cereal aphid parasitoids and hyperparasitoids in oceanic regions as a response to climate and abundance of hosts. *Popul Ecol* 26:545–551
- Lahti DC, Johnson NA, Ajie BC et al (2009) Relaxed selection in the wild. *Trends Ecol Evol* 24:487–496. doi:[10.1016/j.tree.2009.03.010](https://doi.org/10.1016/j.tree.2009.03.010)
- Langer A, Hance T (2000) Overwintering strategies and cold hardiness of two aphid parasitoid species (Hymenoptera: Braconidae: Aphidiinae). *J Insect Physiol* 46:671–676
- Langer A, Stilmant D, Verbois D, Hance T (1997) Seasonal activity and distribution of cereal aphid parasitoids in Belgium. *Entomophaga* 42:185–191
- Le Lann C, Roux O, Serain N et al (2011a) Thermal tolerance of sympatric hymenopteran parasitoid species: does it match seasonal activity? *Physiol Entomol* 36:21–28. doi:[10.1111/j.1365-3032.2010.00758.x](https://doi.org/10.1111/j.1365-3032.2010.00758.x)
- Le Lann C, Wardziak T, van Baaren J, van Alphen JJM (2011b) Thermal plasticity of metabolic rates linked to life-history traits and foraging behaviour in a parasitic wasp: temperature affects physiology and behaviour of a parasitoid. *Funct Ecol* 25:641–651. doi:[10.1111/j.1365-2435.2010.01813.x](https://doi.org/10.1111/j.1365-2435.2010.01813.x)
- Leather SR (1992) Aspects of aphid overwintering (Homoptera: Aphidinea: Aphididae). *Entomol Gen* 17:101–113. doi:[10.1127/entom.gen/17/1992/101](https://doi.org/10.1127/entom.gen/17/1992/101)
- Legrand MA, Colinet H, Vernon P, Hance T (2004) Autumn, winter and spring dynamics of aphid *Sitobion avenae* and parasitoid *Aphidius rhopalosiphi* interactions. *Ann Appl Biol* 145:139–144
- Lumbierres B, Starý P, Pons X (2007) Seasonal parasitism of cereal aphids in a Mediterranean arable crop system. *J Pest Sci* 80:125–130. doi:[10.1007/s10340-006-0159-0](https://doi.org/10.1007/s10340-006-0159-0)
- Mehrnejad MR, Copland MJ (2005) Diapause strategy in the parasitoid *Psyllaephagus pistaciae*. *Entomol Exp Appl* 116:109–114
- Neuville S, Le Ralec A, Outreman Y, Jaloux B (2015) The delay in arrival of the parasitoid *Diaeretiella rapae* influences the efficiency of cabbage aphid biological control. *Biocontrol*. doi:[10.1007/s10526-015-9702-3](https://doi.org/10.1007/s10526-015-9702-3)
- Paolucci S, van de Zande L, Beukeboom LW (2013) Adaptive latitudinal cline of photoperiodic diapause induction in the parasitoid *Nasonia vitripennis* in Europe. *J Evol Biol* 26:705–718. doi:[10.1111/jeb.12113](https://doi.org/10.1111/jeb.12113)
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol Syst* 37:637–669. doi:[10.1146/annurev.ecolsys.37.091305.110100](https://doi.org/10.1146/annurev.ecolsys.37.091305.110100)
- Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 521:37–42
- Polgár LA, Hardie J (2000) Diapause induction in aphid parasitoids. *Entomol Exp Appl* 97:21–27
- Rabasse J, Dedryver C (1982) Overwintering of primary parasites and hyperparasites of cereal aphids in western France. In: Aphid antagonists. Proceedings of a meeting of the EC Experts' Group, Portici, Italy, 23–24 November 1982. AA Balkema, pp 57–64
- Saunders DS (ed) (2002) Insect clocks, 3rd edn. Elsevier, Amsterdam
- Sigsgaard L (2000) The temperature-dependent duration of development and parasitism of three cereal aphid parasitoids, *Aphidius ervi*, *A. rhopalosiphi*, and *Praon volucre*. *Entomol Exp Appl* 95:173–184
- Sigsgaard L (2002) A survey of aphids and aphid parasitoids in cereal fields in Denmark, and the parasitoids' role in biological control. *J Appl Entomol* 126:101–107
- Tauber MJ, Tauber CA, Masaki S (1986) Seasonal adaptations of insects. Oxford University Press, New York
- Thompson JN (1998) Rapid evolution as an ecological process. *Trends Ecol Evol* 13:329–332
- Tougeron K, van Baaren J, Burel F, Alford L (2016) Comparing thermal tolerance across contrasting landscapes: first steps towards understanding how landscape management could modify ectotherm thermal tolerance. *Insect Conser Diver* 9:171–180. doi:[10.1111/icad.12153](https://doi.org/10.1111/icad.12153)
- Vázquez DP, Gianoli E, Morris WF, Bozinovic F (2015) Ecological and evolutionary impacts of changing climatic variability. *Biol Rev*. doi:[10.1111/brv.12216](https://doi.org/10.1111/brv.12216)
- Walther G-R, Post E, Convey P et al (2002) Ecological responses to recent climate change. *Nature* 416:389–395
- Williams CM, Marshall KE, MacMillan HA et al (2012) Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. *PLoS One* 7:e34470. doi:[10.1371/journal.pone.0034470](https://doi.org/10.1371/journal.pone.0034470)
- Winterhalter WE, Mousseau TA (2007) Patterns of phenotypic and genetic variation for the plasticity of diapause incidence. *Evolution* 61:1520–1531. doi:[10.1111/j.1558-5646.2007.00127.x](https://doi.org/10.1111/j.1558-5646.2007.00127.x)
- Zhou X, Harrington R, Woiwod I et al (1995) Effects of temperature on aphid phenology. *Glob Change Biol* 1:303–313