

Diapause expression in a Québec, Canada population of the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae)

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Abstract—*Aphidius ervi* Haliday (Hymenoptera: Braconidae) is a major natural enemy of several agricultural pests in North America. Yet little is known about its overwintering strategy, especially concerning the plastic response to photoperiod and temperature that induce diapause. Information on parasitoid overwintering patterns is of great importance if we aim to predict their phenology and better inform pest outbreak control. Moreover, there is increasing evidence of plastic and genetic changes in overwintering strategies in insect from temperate areas following climate change. We set up a laboratory approach to better understand the factors acting on diapause induction in *A. ervi*. We studied the diapause incidence in a population from Québec, Canada, using the combination of two temperatures (14 °C and 20 °C) and three photoperiod treatments (10:14, 12:12, 14:10 [light:dark] hours). We found an effect of both factors on diapause incidence; *A. ervi* expressed close to 95% of diapause at the most fall-like conditions (14 °C, 10:14 [light:dark] hours) and almost no diapause (3.5%) at the most summer-like conditions tested (20 °C, 14:10 [light:dark] hours). This parasitoid species does have the potential to enter diapause in Québec before lethal frosts, despite a recent introduction from France (1960s), where mild winter occurs compared with Québec.

Résumé—*Aphidius ervi* Haliday (Hymenoptera: Braconidae) est un des principaux ennemis naturels de plusieurs ravageurs agricoles en Amérique du Nord. Pour autant, on en sait peu sur ses stratégies d'hivernation, en particulier concernant les réponses plastiques à la photopériode et à la température qui induisent la diapause. Obtenir des informations sur les stratégies d'hivernation des parasitoïdes est d'une importance capitale si nous voulons prédire leur phenologie et mieux contrôler les pullulations de ravageurs. De plus, il y a de plus en plus de preuves de changements plastiques ou évolutifs dans les stratégies d'hivernation des insectes de milieux tempérés à cause du changement climatique. Nous avons mis en place une approche de laboratoire visant à mieux comprendre les facteurs agissant sur l'induction de la diapause chez *A. ervi*. Nous avons étudié les niveaux de diapause chez une population du Québec, Canada, en utilisant la combinaison de deux traitements de températures (14 °C et 20 °C) et trois traitements de photopériode (10:14, 12:12, 14:10 [jour:nuit] heures). Nous avons trouvé un effet des deux facteurs sur les niveaux de diapause; *A. ervi* a exprimé environ 95% de diapause à la condition la plus automnale testée (14 °C, 10:14 [jour:nuit] heures) et presque aucune diapause (3.5%) à la condition la plus estivale testée (20 °C, 14:10 [jour:nuit] heures). Cette espèce de parasitoïde semble donc avoir la capacité à entrer en diapause au Québec avant que le gel n'apparaisse, malgré une récente introduction depuis la France (années 1960), où des hivers plus doux qu'au Québec ont lieu.

Diapause allows arthropods to survive environmental conditions that are temporarily – and up to a few months – unfit for survival. In rough winter climates of North America, insects enter diapause long before the onset of unfavourable conditions to

ensure their survival. The main cues inducing insect winter diapause under temperate climates are photoperiod and temperature decrease at the end of the summer (Tauber *et al.* 1986). Timing of diapause induction is crucial because an early

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diapause may impede the insect from exploiting its environment and reproduce, and a late diapause may be lethal if the environment gets rapidly colder. For parasitoid insects, another constraint is the need to maintain synchronisation with the phenology of the host throughout the year (Godfray 1994). In parasitic Hymenoptera, photoperiod and temperature are predominant cues for diapause induction and allow synchronisation between parasitoids, their host, and the abiotic environment (Saunders *et al.* 1970; Brodeur and McNeil 1989, 1994; Tougeron *et al.* 2017).

Aphidius ervi Haliday (Hymenoptera: Braconidae) is a major natural enemy of several Aphidae (Hemiptera) species in North America, including its main aphid host the pea aphid *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae) and cereal aphids (Stary 1974), and is used worldwide for mass releases in a biological control context. *Aphidius ervi* was introduced from western France to the United States of America in the mid-1960s (Halfhill *et al.* 1972). In Canada, this species first spread in wet and mild climatic areas but rapidly invaded more northern areas of British Columbia, Ontario, and Québec (Campbell and Mackauer 1973; Stary 1974). Pike *et al.* (1997) mentioned that *A. ervi* was active from April to October in northwestern United States of America, but so far little information is available on the winter ecology of *A. ervi* in Canada. Recently, we demonstrated that a Québec population of *A. ervi* exposed to complex outdoor conditions in Québec gradually increased their level of diapause from early September to early October up to 100%, while a population from western France exposed to the same conditions expressed significantly lower diapause levels, only up to 35%, demonstrating local adaptations to winter conditions in their area of origin (Tougeron *et al.* 2018).

Current rapid climate change can lead to shifts in overwintering strategy among parasitoids either by acting through the high plasticity of diapause or through evolutionary processes (Tougeron *et al.* 2017). Change in diapause expression in parasitoids can potentially destabilise food-webs and impair biological pest control (Björkman and Niemelä 2015). It is thus crucial to gather recent data on diapause expression in such insects of agronomic interest and document their overwintering capacity to more accurately

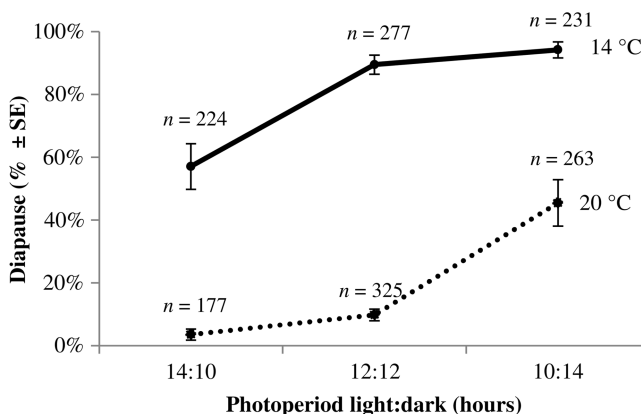
predict their phenology for pest control purposes, but also to serve as a basis (*i.e.*, reference state) for future comparative studies. The objective of this study was to better understand the plasticity of diapause induction in a population of the parasitoid *A. ervi* collected in cereal fields in Québec and exposed to different laboratory conditions of temperature and photoperiod.

We collected parasitoid individuals from about 300 aphid mummies found in different cereal fields in Québec in the south of the Saint Lawrence River (45.584° N, 73.243° W) in summer 2015. Parasitoid culture was maintained on grain aphids *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) on winter wheat (*Triticum aestivum* Linnaeus; Poaceae; Mégantic variety). Cultures were maintained under climate-controlled chambers (Convion, Winnipeg, Manitoba, Canada) at 20 °C, 75% relative humidity, and 16:8 (light: dark) hours. Aphid cohorts of homogenous size and life stage were produced for our experiments to control for potential effects on diapause (Brodeur and McNeil 1989).

The day they emerged from the aphid mummies (*i.e.*, dead aphid containing a developing parasitoid pupa), parasitoids were mated (ratio: six females per three males) and fed with a solution of honey and water for 48 hours. Six parasitoid females were then exposed together and for 24 hours to 210 (± 10) aphids on a wheat pot in a cage, under standard rearing conditions. Parasitoid females were removed from the cages, and aphids (potentially parasitised) were equally distributed on six wheat pots (≈ 35 aphids per pot). Parasitism rate was around 70%. Each pot was placed under one of the temperature and photoperiod treatment combinations: 14 °C/20 °C and 10:14/12:12/14:10 (light:dark) hours, resulting in a total of six treatments. These conditions correspond to mean temperatures and photoperiods potentially encountered by the parasitoids in their natural environment in south Québec from August to October (*i.e.*, during the typical period of diapause induction; Brodeur and McNeil 1994; Tougeron *et al.* 2018).

This procedure was repeated 10 times for each temperature and photoperiod treatment. Aphid mummy formation was verified daily, and mummies were kept individually in gelatin capsules under their respective treatment. Mummies from which a parasitoid did not emerge were

Fig. 1. Prepupal diapause incidence in a Québec population of the parasitoid *Aphidius ervi* parasitising the grain aphid *Sitobion avenae* under two conditions of temperature (14 °C, *plain line*; 20 °C, *dotted line*) and three conditions of photoperiods (14:10, 12:12, and 10:14 [light:dark] hours). *n* indicates the total number of mummies formed at each treatment (*i.e.*, sum of mummies containing diapausing, nondiapausing, and dead parasitoids).



dissected 15 days after the last adult emergence in the same cohort, and classified as dead parasitoids or diapausing prepupae following the protocol described by Tougeron *et al.* (2017). Comparisons between different treatments (photoperiod and temperature) and their interaction on diapause incidence were performed by fitting a generalised linear model (GLM) with a quasi-binomial error distribution to the data. Statistical analyses were carried out using the R software (R Core Team 2017).

Diapause incidence of *A. ervi* increased when photoperiod and temperature decreased, up to $94.2 \pm 2.5\%$ at 14 °C and 10:14 (light:dark) hours (Fig. 1). Both photoperiod (GLM, $F = 35.7$, $df = 2$, $P < 0.001$) and temperature (GLM, $F = 237.7$, $df = 1$, $P < 0.001$) had a significant influence on diapause incidence, but the interaction between photoperiod and temperature did not significantly influence diapause incidence (GLM, $F = 1.3$, $df = 2$, $P = 0.3$), meaning that the photoperiod effect was similar for any tested temperature, and reversely.

Emerging individuals represented the majority of nondiapausing parasitoids, while dead parasitoids only represented a marginal proportion of it; 0.8, 2.5, and 5.8% of the total number of mummies at 14 °C and 4.9, 9.6, and 12.4% at 20 °C, for 10:14, 12:12, and 14:10 (light:dark) hours, respectively.

We showed that *A. ervi* entered diapause at higher incidence when exposed to short photoperiods and relatively low temperatures, which

fits the common pattern observed in insects in response to these major cues for diapause induction (Tauber *et al.* 1986). This particular population of *A. ervi* from Québec is highly sensitive to these diapause-initiating cues, as shown by the high thresholds at which at least some diapausing individuals were observed. Some of the tested parasitoids expressed diapause even at temperature and photoperiod treatments corresponding to late August conditions in south Québec (20 °C, 14:10 [light:dark] hours), which is typical of populations from harsh winter areas. For example, the braconid parasitoid *Aphidius nigripes* Ashmead from Québec expresses 100% of diapause as early as 1 September (Brodeur and McNeil 1994). At the opposite, a population of *A. ervi* from western France where mild winters occur do not express diapause at all under similar conditions in the laboratory (Tougeron *et al.* 2017). Both laboratory and outdoor mesocosms results (Tougeron *et al.* 2018) indicate that diapause incidence in *A. ervi* is very plastic to temperature, so any change in late summer or fall temperatures due to climate warming could reduce diapause incidence, as long as hosts and other resources are available. In addition to a direct plastic response of diapause expression by parasitoids, long-term adaptation of overwintering strategies to new photoperiod–temperature conditions is to be expected as reported for many insect species (Bale and Hayward 2010).

In the area where parasitoids were sampled, the average first frost date occurs on 10 October over the period 2010–2015 (Environment Canada data from <http://climate.weather.gc.ca>). Almost 90% of the parasitoids were in diapause at 14 °C and 12:12 (light:dark) hours, which are conditions corresponding to mid-September. Thus, under laboratory conditions, the tested *A. ervi* population seems to respond to cues triggering diapause in a way that would allow them to avoid lethal frosts in natural conditions of south Québec. Since the introduction of *A. ervi* to eastern Canada in the 1960s (Halfhill *et al.* 1972), its overwintering strategy seems to be adapted to winter conditions occurring in Québec. Insect activity records in a given area and their capacity to enter diapause under field-realistic photoperiod and temperature conditions do not presume for their capacity to overwinter onsite. For instance, most aphids do not overwinter in Canada and migrate each spring from southern areas, carried by winds (Irwin and Thresh 1988; Butts 1992; Zhu *et al.* 2006; Gavolski and Meers 2011). For *A. ervi* to overwinter in Québec, it would require that (i) hosts are available before winter, and that these hosts are suitable for the parasitoid to enter diapause (species, physiological status, developmental stage, size) and (ii) that *A. ervi* has sufficient cold-tolerance capacities as prepupae within aphid mummies to survive the winter. As the overwintering sites of *A. ervi* in Québec remain unknown, one could speculate that this species recolonises each year from southern areas as immature larvae within parasitised aphids carried by airstreams, as it has been demonstrated for other parasitoid species (Vorley and Wratten 1987). Testing this hypothesis would require identifying potential overwintering sites and hosts to observe whether or not *A. ervi* overwinters in Québec region, as related species such as *A. nigripes* do.

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