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Variabilité de la diapause chez les parasitoïdes de pucerons dans le cadre des changements climatiques

Implications en lutte biologique

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Résumé – Variabilité de la diapause chez les parasitoïdes de pucerons dans le cadre des changements climatiques ; implications en lutte biologique

Les changements climatiques altèrent la phénologie des organismes, c'est-à-dire la succession dans le temps des éléments de leur cycle de vie. L'expression de la diapause chez les insectes, soit l'arrêt de développement permettant de survivre aux dégradations saisonnières de l'environnement biotique et abiotique, est en particulier affectée. Cette thèse aborde différents facteurs environnementaux agissant sur les stratégies saisonnières des parasitoïdes de pucerons du genre *Aphidius*. Dans les agro-systèmes céréaliers de l'ouest de la France, les communautés d'hôtes et de parasitoïdes ont changé rapidement au cours de la dernière décennie. Ainsi, plusieurs espèces de parasitoïdes n'entrent plus qu'en faible proportion en diapause, dû à l'augmentation des températures, à la baisse du nombre de jours de gel et à la présence accrue de leurs hôtes pendant l'hiver ; ces pressions de sélection agissent sur les seuils d'entrée diapause. Les parasitoïdes présentent des ajustements plastiques de leur réponse aux stimuli environnementaux qui induisent la diapause. Ils y entrent en plus forte proportion s'ils se développent dans des morphes de pucerons sexués (annonciateurs de ressources limitées) que dans des hôtes asexués, ce qui souligne la co-évolution de leurs cycles de vie. Cette plasticité s'avère également transgénérationnelle puisque la compétition entre femelles induit la diapause estivale chez leurs descendants. De plus, l'incidence de la diapause hivernale augmente après plusieurs générations de parasitoïdes soumises aux mêmes conditions. Les adaptations locales des parasitoïdes aux nouvelles conditions environnementales mènent à la perte d'expression de la stratégie de diapause – qui comporte des coûts écologiques et physiologiques – au profit de stratégies hivernales d'activité à l'état adulte. La modification de l'activité-densité des espèces au sein des communautés de pucerons-(hyper)parasitoïdes, due aux changements de stratégie d'hivernation, pourrait bouleverser l'équilibre des réseaux trophiques et altérer positivement ou négativement l'efficacité du service écosystémique de lutte biologique.

Mots-clés : Plasticité phénotypique, Polyphénisme, Adaptations locales, Stratégies d'hivernation, Interactions spécifiques, Communauté, Température, Photopériode.

Abstract – Diapause variability in aphid parasitoids in the context of climate changes; implications for biological control

Climate changes alter the phenology of organisms, *i.e.* the succession over time of the elements of their life-cycle. Diapause expression in insects – that is, the developmental arrest allowing survival to seasonal degradations in the biotic and abiotic environment – is particularly affected. This thesis addresses the existence of different environmental factors acting on the seasonal strategies of *Aphidius* aphid parasitoids. In cereal agro-systems of western France, host and parasitoid communities have changed rapidly over the past decade. Hence, some parasitoid species enter diapause at low levels due to temperature increase, decrease in frost events and the presence of their hosts during winter, which impose selection on diapause induction thresholds. Parasitoids show plastic adjustments of their response to environmental stimuli inducing diapause. They enter diapause at higher proportion if they develop in sexual morphs of aphids (which inform for upcoming limited resources) than in asexual hosts, which underlines the co-evolution of their life-cycles. Transgenerational plasticity is also involved in these responses since maternal competition induces summer diapause in their offspring. Moreover, winter diapause incidence increases after some parasitoid generations experience the same environmental conditions. Local adaptations of parasitoids to new environmental conditions lead to a loss of diapause expression – which involves ecological and physiological costs – to the benefit of adult overwintering strategies. Modifications in species activity-density within aphid-(hyper)parasitoid communities, due to changes in overwintering strategies, could affect food-webs' stability and alter positively or negatively the efficiency of biological pest control.

Keywords: Phenotypic plasticity, Polyphenism, Local adaptations, Overwintering strategies, Species interactions, Community, Temperature, Photoperiod.

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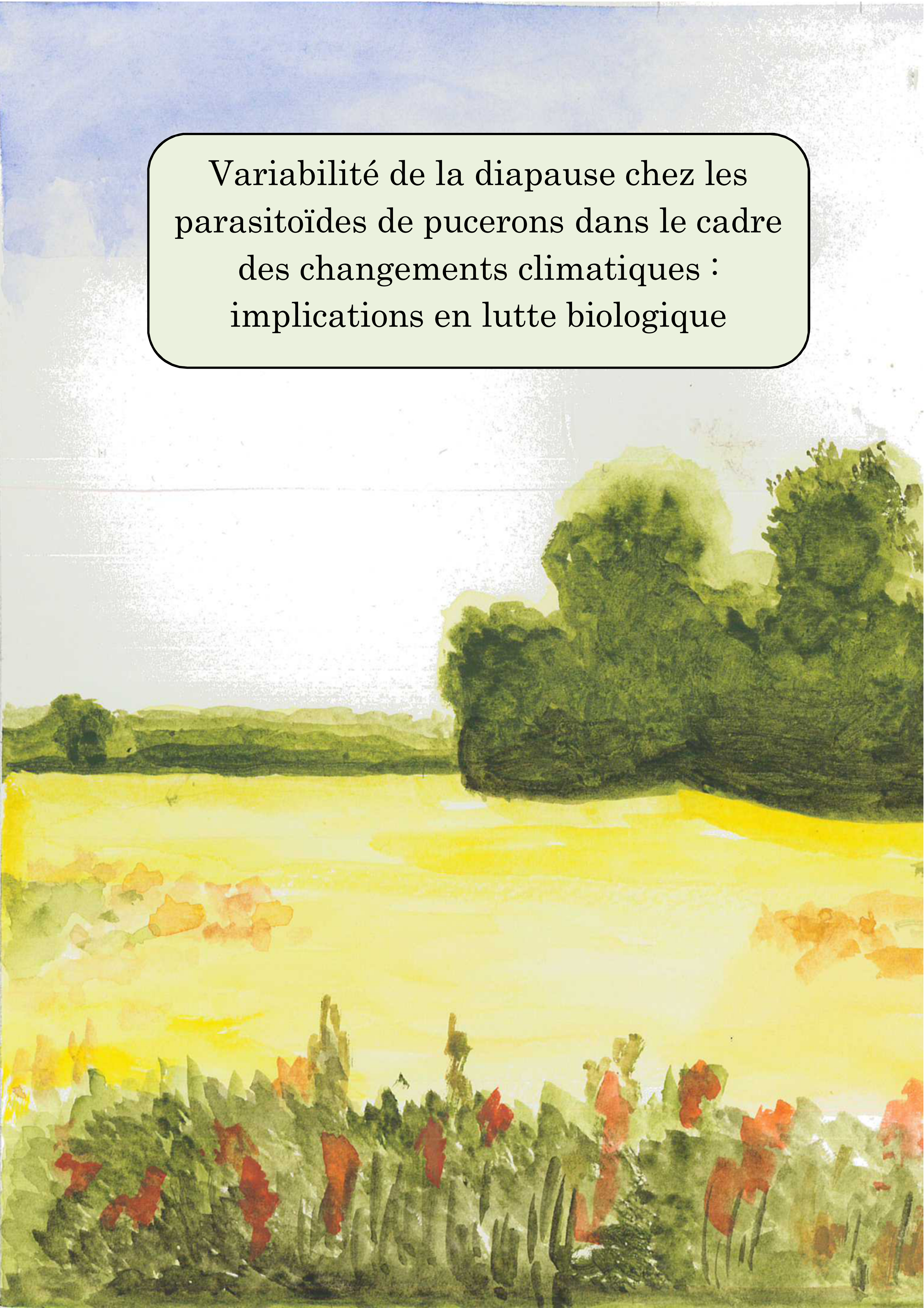

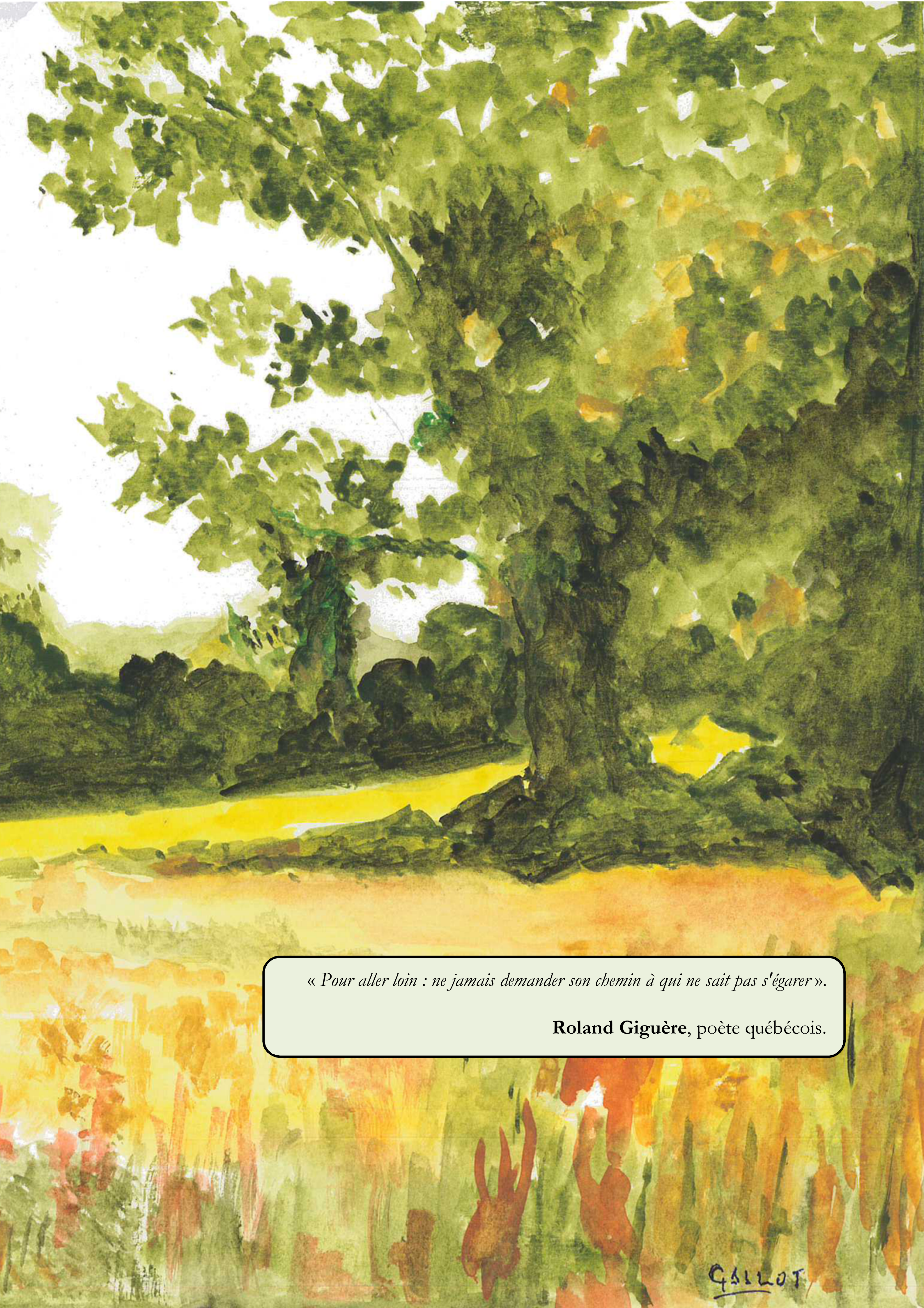

« *Pour aller loin : ne jamais demander son chemin à qui ne sait pas s'égarer* ».

Roland Giguère, poète québécois.

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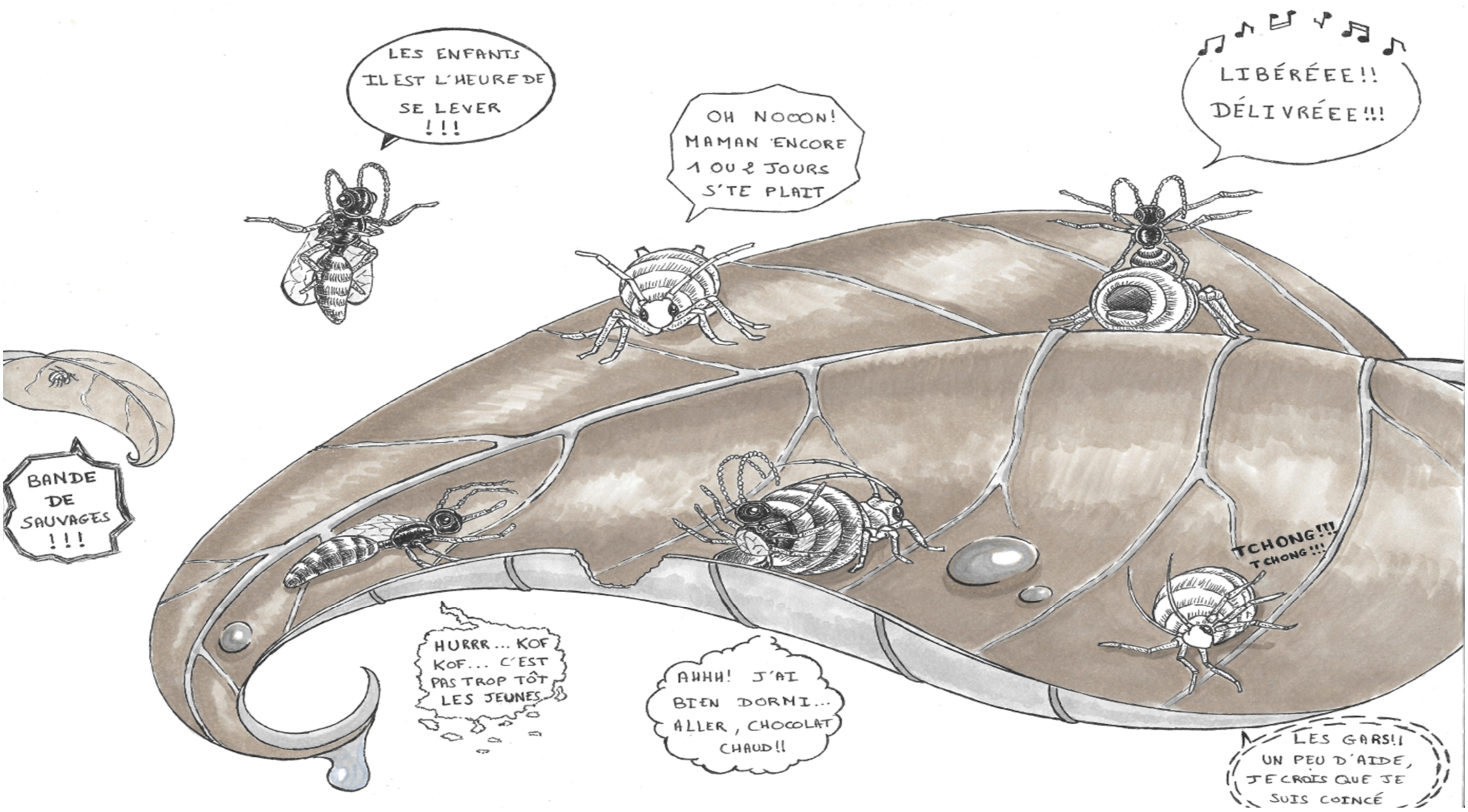

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**“Diapause variability in aphid parasitoids in the context of climate changes;
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Partie I

Introduction

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Chapitre 2 : Revue de la littérature

"Winter is coming."

George R.R. Martin, A Game of Thrones

Chapitre 1

Introduction générale

Les changements climatiques

Généralités

Les changements climatiques sont en majeure partie issus de l'intensification des activités humaines au cours des deux derniers siècles (Karl & Trenberth, 2003). Les températures moyennes du globe ont augmenté rapidement au cours des dernières décennies (Figure 1.1), en particulier en hiver, et selon les scénarios les plus optimistes du GIEC[1], les températures pourraient s'accroître de +2,4°C à la fin du siècle, les scénarios les plus pessimistes faisant état de +5,5°C (IPCC, 2014). De plus, la fréquence des évènements climatiques extrêmes, comme des épisodes de sécheresse, de vagues de froid ou de chaud, ne cesse d'augmenter (Easterling *et al.*, 2000).

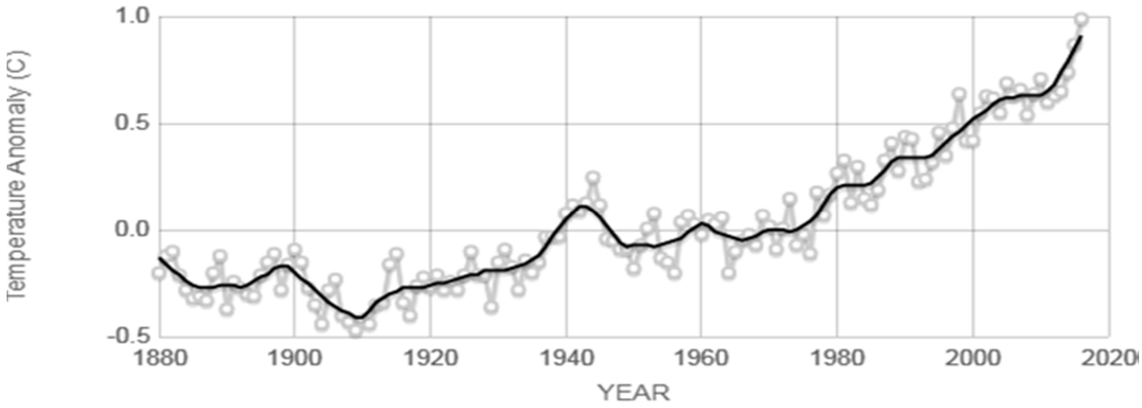

Figure 1.1 : Changement de températures (°C) à l'échelle du globe par rapport à la moyenne de 1951 à 1980. Plus de 95% des années les plus chaudes ont été enregistrées depuis 2001. L'année 2016 a été la plus chaude jamais enregistrée. Source *NASA's Goddard Institute for Space*.

Pour les organismes, les changements climatiques ont pour conséquence directe de les exposer à des conditions sub-optimales voire stressantes à un rythme sans précédent à l'échelle évolutive, mais aussi de diminuer la prévisibilité de leur environnement (Vasseur *et al.*, 2014). Les effets des changements climatiques se superposent à ceux des autres éléments des changements globaux comme les modifications d'usage des sols et l'exploitation des ressources naturelles (Oliver & Morecroft, 2014), menaçant le maintien de la biodiversité à l'échelle mondiale, et accroissant les pressions environnementales qui agissent sur les organismes (Travis, 2003, Hughes, 2012). Prédire les réponses des espèces aux changements globaux et leurs conséquences sur le fonctionnement des écosystèmes est donc un enjeu majeur de l'écologie évolutive (Walther *et al.*, 2002).

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[1] Groupe d'experts intergouvernemental sur l'évolution du climat

Principales réponses des organismes

Les effets des changements climatiques sont perceptibles à plusieurs échelles d'organisation écologique, des individus aux communautés et écosystèmes. Ils affectent les traits comportementaux, morphologiques et physiologiques des individus en plus de contraindre leur écologie comme leur tolérance au stress ou leur capacité de dispersion. Les changements climatiques affectent à la fois la dynamique (mortalité, natalité) et la structure des populations (démographie). Enfin, à l'échelle des communautés, ils modifient la diversité et l'abondance des espèces et la qualité des interactions spécifiques (Walther *et al.*, 2002, Parmesan, 2006, Ovaskainen *et al.*, 2013). Ces effets peuvent se répercuter d'un niveau écologique à un autre, menant à des réponses très complexes et très dépendantes de l'environnement local considéré (Hughes, 2012).

En réponse aux changements climatiques, certaines espèces modifient leur aire de distribution, généralement vers les pôles ou vers de plus hautes altitudes afin de suivre le changement de températures (Davis & Shaw, 2001, Lenoir *et al.*, 2008, Sánchez-Guillén *et al.*, 2016). Les espèces qui ne changent pas de distribution géographique doivent répondre localement aux nouvelles conditions environnementales. Ces réponses peuvent impliquer des adaptations génétiques locales et/ou l'expression de la plasticité phénotypique (Parmesan, 2006, Hoffmann & Sgrò, 2011). Déterminer la part relative qu'occupent ces deux éléments dans l'adaptation des organismes fait l'objet d'une attention particulière dans le cadre des changements climatiques (Berteaux *et al.*, 2004, Anderson *et al.*, 2012, Merilä & Hendry, 2014, Duputié *et al.*, 2015).

Les réponses génétiques et plastiques des organismes face aux changements climatiques concernent de nombreux traits et processus écologiques comme par exemple les changements de phénologie[2], qui nous intéressent particulièrement dans cette thèse. La phénologie ayant une base génétique importante chez tous les organismes, elle est sensible aux pressions évolutives exercées par les changements climatiques (Parmesan & Yohe, 2003, Bradshaw & Holzapfel, 2006) (Encadré 1).

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[2] La phénologie d'un organisme se définit comme la succession de ses évènements écologiques périodiques au cours de l'année, par exemple la ponte, l'éclosion, la migration, etc.

Encadré 1. Phénologie et synchronisation des cycles de vie

Un point important à considérer en ce qui concerne la phénologie des organismes est la synchronisation de leur cycle de vie avec les espèces avec lesquelles ils interagissent (Johansson *et al.*, 2015, Visser, 2016). Ce point sera particulièrement discuté dans le **Chapitre 2** de la thèse.

Un exemple bien connu est l'avancement de la période de ponte chez la mésange *Parus major* (Passeriformes : Paridae) suite à une désynchronisation avec la période de disponibilité des insectes dont elle se nourrit, due au réchauffement des températures printanières (Visser *et al.*, 1998). La mésange met à la fois en jeu des réponses plastiques et génétiques pour se resynchroniser avec la phénologie de sa proie (Nussey *et al.*, 2005) (Figure 1.2).

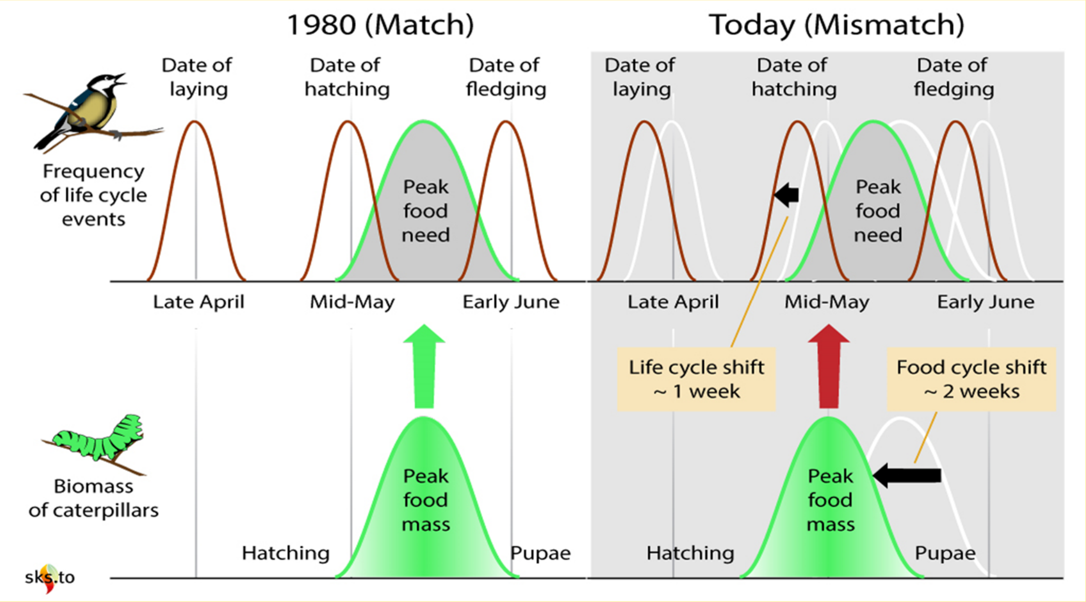

Figure 1.2 : Dans les années 1980 la disponibilité en nourriture (les chenilles du chêne) était parfaitement synchronisée avec le besoin de la mésange. Les chenilles ont avancé leur phénologie de plus de deux semaines en moins de trois décennies, en réponse au réchauffement climatique, tandis que la mésange n'a avancé sa date de ponte que d'une semaine, mettant en péril la synchronie de leurs cycles de vie. Source du graphique, *Skeptical Science Graphics*.

En bref, les changements de phénologie se traduisent principalement par une saison de reproduction décalée dans le temps (van Asch *et al.*, 2012), une avancée de la date de migration ou un allongement de la période d'activité (Hill *et al.*, 2002, Both *et al.*, 2006, Bell *et al.*, 2015) et une augmentation du nombre de générations produites chaque année (Altermatt, 2010). Il existe des exemples parmi de nombreux groupes d'êtres vivants, incluant les plantes (Fitter, 2002, Menzel *et al.*, 2006, Cleland *et al.*, 2007), les mammifères (Humphries *et al.*, 2002), les oiseaux (Charmantier *et al.*, 2008, Dunn *et al.*, 2010) et les amphibiens (Beebee, 1995) et, ce qui fera l'objet de cette thèse, les insectes (Forrest, 2016). Au sein de ce dernier groupe, les changements phénologiques se manifestent de différentes manières, notamment au niveau des stratégies d'hivernation[3]. Toutefois, la manière dont ces stratégies sont influencées reste méconnue chez beaucoup d'espèces d'insectes (voir **Chapitre 2**).

Les adaptations locales à l'environnement thermique

Les adaptations des organismes à leur environnement climatique jouent évidemment un rôle fondamental dans leur survie, dans le maintien de la biodiversité, voire également dans la préservation des interactions spécifiques (Blanquart *et al.*, 2013). Une diversité importante de processus sont impliqués dans les adaptations locales, allant des flux de gènes à la plasticité phénotypique, en passant par l'héritabilité des traits et la selection naturelle (synthèse dans Kawecki & Ebert (2004)). Ces multiples facteurs contraignent également la réponse évolutive des espèces face aux changements climatiques (Merilä & Hendry, 2014). L'adaptation à des changements d'environnements dépend de la variabilité génétique au sein d'une population et de la force de la sélection opérée sur ces organismes (Hedrick, 2011). L'évolution peut se faire rapidement, sur quelques générations, en particulier si les pressions environnementales sont importantes, comme dans le cas des changements climatiques (Thompson, 1998, Bradshaw & Holzapfel, 2006).

En général, on dit qu'un individu s'adapte localement à son milieu lorsqu'il présente une meilleure valeur adaptative (*fitness*) dans cet environnement que des individus venant d'autres environnements (*Local-Foreign*), et qu'il a une meilleure *fitness* dans son environnement d'origine que lorsqu'il est déplacé ailleurs (*Home-Away*) (Kawecki & Ebert, 2004). Il existe différents moyens de mettre en évidence les adaptations locales et de quantifier l'adaptation d'un organisme (ou d'une population) à un environnement donné (voir Blanquart *et al.* (2013) pour une synthèse). La majorité des études visant à démontrer l'existence d'adaptations locales a consisté en des comparaisons de Qst/Fst[4] (e.g. Leinonen *et al.*, 2013), en des corrélations entre des variables environnementales et des traits phénotypiques (ou génétiques) (Charmantier *et al.*, 2016), en des études le long de clines géographiques (Hut *et al.*, 2013), en des expériences de jardin commun dans lesquels différentes espèces ou populations sont soumises aux mêmes conditions (Gibert *et al.*, 2001), ou encore en des expériences de transplantations (translocations) réciproques (Hereford, 2009) (voir **Chapitre 5**).

La plasticité phénotypique face aux changements climatiques

L'hétérogénéité environnementale favorise l'apparition de la plasticité phénotypique, qui joue un rôle majeur dans l'écologie et l'évolution des organismes (Encadré 2).

La plasticité permet aux organismes de persister dans des environnements fluctuants et hétérogènes (Stearns, 1989) mais elle peut aussi permettre aux organismes de survivre à des changements directionnels de l'environnement (Chevin *et al.*, 2013). Par exemple, le rôle de la plasticité comportementale et physiologique dans les adaptations thermiques a été largement

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[3] Le terme hivernation utilisé dans ce manuscrit fait référence au fait de passer l'hiver. En ce sens, il correspond à une traduction directe de l'anglais *overwintering*. Il englobe donc toutes les stratégies adoptées par les animaux pour traverser la période défavorable des milieux tempérés.

[4] Méthode permettant de quantifier le degré de différenciation génétique entre populations. Permet de distinguer l'effet de la sélection naturelle d'autres forces évolutives, comme par exemple la dérive génétique. Peut suggérer l'existence d'adaptations locales.

reconnu (Angilletta, 2009, Abram *et al.*, 2016). En ajustant leur phénotype aux changements de température par voie plastique, certains organismes parviennent à réduire le risque d'extinction posé par les changements climatiques (Parmesan, 2006, Sgrò *et al.*, 2016).

Encadré 2. La plasticité phénotypique

La plasticité phénotypique est la capacité d'un génotype donné à exprimer des phénotypes variés (comportements, physiologie) en réponse à différents environnements (Stearns, 1989, Nylin & Gotthard, 1998, Agrawal, 2001). La relation produite par le génotype (G) et son environnement (E) se définit comme une norme de réaction, qui peut être continue ou discrète (Pigliucci, 2001, David *et al.*, 2004). Cette norme n'est pas nécessairement linéaire mais peut être quadratique, logistique, etc. (DeWitt & Scheiner, 2004). Divers moyens peuvent être utilisés pour décrire l'évolution des différentes normes de réaction et identifier l'action de la sélection sur la plasticité (voir Gibert *et al.*, 1998, David *et al.*, 2004).

Le niveau de la norme de réaction (valeur moyenne du trait considéré) définit l'adaptation d'un organisme à un environnement local, tandis que la pente de la norme de réaction définit le degré de plasticité (Via & Lande, 1985) (Figure 1.3). Les normes de réaction sont largement utilisées pour étudier l'effet des changements climatiques sur les phénotypes des organismes (Charmantier *et al.*, 2008, Baumann & Conover, 2011, van Asch *et al.*, 2012).

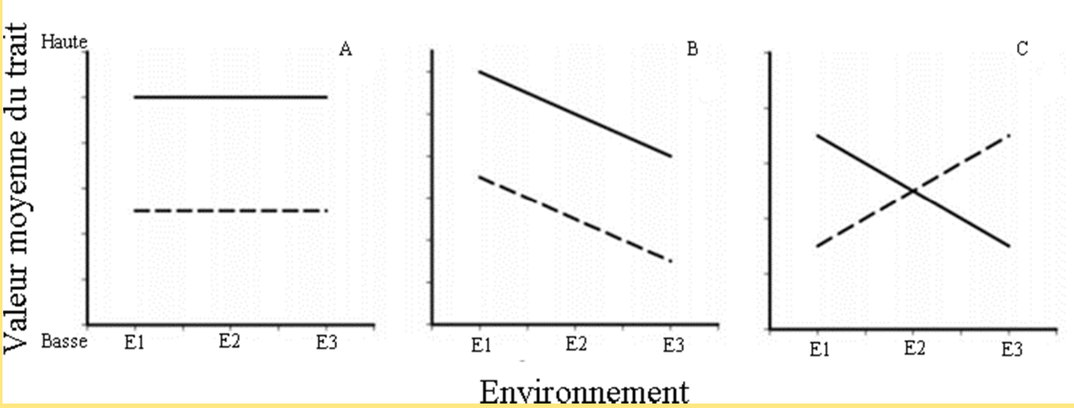

Figure 1.3 : Normes de réaction théoriques représentant la valeur d'un trait hypothétique au sein de deux types de populations (deux types de lignes) le long d'un gradient environnemental. **A.** Les génotypes diffèrent sans variation entre les environnements. **B.** L'effet de l'environnement est équivalent chez les deux populations. **C.** Interaction GxE, la plasticité phénotypique diffère entre les populations. D'après Chown & Terblanche (2006).

Chez les ectothermes, la règle taille-température (*Temperature Size-Rule*, TSR; Atkinson (1994)) est un exemple particulier de norme de réaction. En raison d'un taux métabolique plus faible, les individus se développant à basse température ont un taux de croissance ralenti et sont donc de plus grande taille une fois adultes. Cet effet est fortement conservé chez les espèces d'arthropodes et entre les sexes (Hirst *et al.*, 2015). L'étude des normes de réaction impliquant la température comme variable environnementale (e.g. la TSR ou les gammes de tolérance thermique) s'avère d'une importance capitale dans le cadre des changements climatiques. Par exemple, la taille influence de nombreux traits de vie et processus écologiques chez les insectes, tels que la fécondité ou la capacité de dispersion (Blanckenhorn, 2000). Les ectothermes adoptent également des stratégies comportementales différentes pour exploiter leurs ressources en fonction des températures (Abram *et al.*, 2016, Moiroux *et al.*, 2015).

La plasticité comporte des limites et pourrait ne pas suffire aux organismes pour répondre efficacement aux changements climatiques (Auld *et al.*, 2010, Sgrò *et al.*, 2016). Par exemple, beaucoup d'espèces d'ectothermes, en particulier dans les tropiques, vivent déjà à des températures proches de leur limites de tolérance au chaud, et ces limites sont très peu plastiques (Addo-Bediako *et al.*, 2000, Hoffmann *et al.*, 2013). Van Heerwaarden *et al.* (2016) ont montré que *Drosophila melanogaster* (Diptera : Drosophilidae) avait une faible capacité d'acclimatation développementale aux hautes températures, suggérant que la plasticité phénotypique minimise très peu les risques d'être exposé à des températures non optimales.

L'aspect adaptatif de la plasticité a fait l'objet de nombreux débats. La question est en particulier de savoir si la plasticité peut être sélectionnée et évoluer indépendamment comme n'importe quel autre trait, ou si la plasticité est seulement un sous-produit de la sélection agissant sur d'autres traits (Via, 1993, Via *et al.*, 1995, Pigliucci, 2006). Nussey *et al.* (2005) ont par exemple démontré que la plasticité phénotypique des mésanges *P. major* évoluait sous l'action de la sélection naturelle face aux changements climatiques. La plasticité n'est pas toujours adaptative car elle peut être le produit de contraintes environnementales auxquelles le génotype répond (Stearns, 1989, Whitman & Agrawal, 2009), et elle peut comporter des coûts à son maintien et à son expression, en particulier dans les environnements qui changent de façon très prévisible (DeWitt & Scheiner, 2004, Sgrò *et al.*, 2016). De plus, si la plasticité peut permettre à la sélection naturelle d'agir sur le génotype en produisant des phénotypes environnements-dépendants (Price *et al.*, 2003, Sgrò *et al.*, 2016), elle peut aussi limiter l'évolution génétique en maintenant les phénotypes hors de portée des pressions de sélection environnementales (Thompson, 1991, Hoffmann & Sgrò, 2011, Tufto, 2015).

En particulier, la plasticité développementale joue un rôle central dans les adaptations des organismes à des environnements variables (West-Eberhard, 2003). Le cours de l'ontogenèse peut être altéré par des stimuli environnementaux qui influencent de façon irréversible le phénotype individuel (Whitman & Agrawal, 2009, Gotthard & Berger, 2010). On parle de polyphénisme comme un cas particulier de la plasticité quand un stimulus environnemental modifie brutalement la pente de la norme de réaction et produit des phénotypes discrets (Nijhout, 2003, Kivelä *et al.*, 2013). La diapause des insectes est un cas classique de plasticité développementale et de polyphénisme lié aux changements environnementaux saisonniers.

Des environnements variables et imprévisibles peuvent également sélectionner des stratégies de *bet-hedging* (c.-à-d. des stratégies de minimisation des risques). Ces stratégies permettent d'accroître la variabilité phénotypique produite au sein d'une génération ou entre générations (*bet-hedging* diversifié), ou de produire constamment le même phénotype (*bet-hedging* conservatif) (Ripa *et al.*, 2010). Le *bet-hedging* est une stratégie diminuant la *fitness* moyenne au profit d'une variance de *fitness* moins importante (Simons, 2011). Il existe également une stratégie conditionnelle (*adaptive coin-flipping*) où la probabilité de réaliser un phénotype est basée sur la probabilité de rencontrer un environnement donné (Cooper & Kaplan, 1982). Ces stratégies de *bet-hedging* sont fortement liées à la plasticité transgénérationnelle qui s'exprime quand les conditions rencontrées par les générations précédentes influencent le phénotype des descendants (DeWitt & Scheiner, 2004, Galloway & Etterson, 2007).

Les trois formes d'adaptation décrites ci-dessus (évolution génétique, plasticité phénotypique et *bet-hedging*) ne sont pas mutuellement exclusives, s'inter-influencent, et s'expriment différemment en fonction de la prévisibilité de l'environnement, de sa variabilité et de la vitesse à laquelle il change (DeWitt & Scheiner, 2004, Tufto, 2015).

Compromis évolutifs et perte de traits

Les ressources étant limitées dans l'environnement, les organismes peuvent les allouer à différents traits, devant ainsi faire des compromis entre ceux-ci (Roff & Fairbairn, 2007). Par exemple, Ellers *et al.* (2000) ont démontré qu'il existe un compromis entre le nombre d'œufs produits par la femelle parasitoïde *Asobara tabida* (Hymenoptera : Braconidae) et sa longévité. Quand exprimer un trait phénotypique devient coûteux en termes de *fitness* sous certaines conditions environnementales, ce trait peut être éliminé (ou la valeur de ce trait modifiée) par la sélection naturelle (Lahti *et al.*, 2009). Un trait peut également être perdu s'il devient inutile dans un contexte environnemental particulier, si la fonction assurée est redondante avec celle produite par d'autres traits, ou encore si la fonction est déjà assurée par une autre espèce avec laquelle il y a une forte interaction (Ellers *et al.*, 2012). Par exemple, chez de nombreuses espèces d'hyménoptères parasitoïdes, la capacité à synthétiser des lipides est absente chez les adultes. La lipogenèse a probablement été perdue par compensation environnementale, les hôtes assurant la synthèse de lipides pour les parasitoïdes (Visser *et al.*, 2010).

Par extension, la théorie sur les compromis évolutifs et sur la perte de traits peut aussi s'appliquer aux stratégies d'histoire de vie puisque plusieurs traits sont souvent simultanément impliqués dans un compromis d'allocation de ressources (Stearns, 1992, Nylin & Gotthard, 1998). Cela concerne également les stratégies exprimées dans les cycles saisonniers où le *temps* plus que la *ressource* est limité (discussions dans Danks, 1994, 2007). Ces *trade-offs* temporels contraignent la phénologie des organismes qui doivent par exemple « décider » quand produire une nouvelle génération en fonction de l'environnement rencontré (Danks, 2006). Certaines stratégies d'histoire de vie sont ainsi optimisées par rapport à d'autres dans des environnements donnés, et donc sélectionnées (Stearns, 1976, Stearns, 1992). Par exemple si les températures varient peu dans l'environnement, les spécialistes de climat qui disposent d'une gamme restreinte de tolérance thermique ont une meilleure *fitness* que les généralistes de climat, chez qui être capable de tolérer une large gamme de températures implique des coûts (Nilsson-Örtman *et al.*, 2012).

Les adaptations saisonnières des insectes

Tous les environnements présentent, à divers degrés, des variations des conditions biotiques et abiotiques au cours du temps. Ces variations journalières, saisonnières ou inter-annuelles ont engendré des rythmes biologiques comme les phases de sommeil ou les rythmes saisonniers (phénologie) (Danks, 2007). Les régions tropicales sont typiquement peu variables, même si des saisons sèches et humides se succèdent. Au contraire, les changements saisonniers sont extrêmes en régions polaires, ce qui implique des adaptations très particulières des organismes qui y vivent (Everatt *et al.*, 2015). En milieu tempéré, la variation la plus remarquable est indubitablement l'alternance entre des saisons favorables et défavorables, et la nécessité de survivre à l'hiver a un impact singulièrement important sur les cycles de vie des organismes (Danks, 2007). Les conditions estivales peuvent également être stressantes (e.g. chaleur, risque de déshydratation), bien qu'elles soient généralement moins contraignantes pour l'activité des insectes (abordé plus loins dans cette introduction et au **Chapitre 6**).

Les insectes, en tant qu'ectothermes poïkilothermes[5] de petite taille, sont particulièrement sensibles à l'augmentation ou à la diminution des températures au-delà ou en-deçà de leur optimum, aux conditions de gel et de sécheresse, ainsi qu'à la baisse de disponibilité en ressources (Speight *et al.*, 2008). Les conséquences de l'exposition à des températures sub-optimales sont variées, tant sur le plan écologique, physiologique, morphologique que comportemental (Bale, 2002, Angilletta, 2009, Denlinger & Lee, 2010, Colinet *et al.*, 2015, Abram *et al.*, 2016). Les adaptations saisonnières des insectes, ont fait l'objet de nombreuses synthèses (e.g. Tauber *et al.*, 1986, Danks, 1987, 2007) et je mets dans cette thèse l'accent sur la diapause hivernale (voir sections suivantes).

Les insectes peuvent adopter différentes stratégies pour passer l'hiver ; ils peuvent migrer sur de longues distances, ce qui est limité à un ensemble assez restreint d'espèces telles que le papillon monarque *Danaus plexippus* (Lepidoptera : Nymphalidae) ; ou ils peuvent rester sur place en hiver et entrer en diapause. S'ils se maintiennent *in situ*, d'importantes adaptations physiologiques et biochimiques contribuent à accroître leur résistance au froid pendant l'hiver. Deux stratégies principales peuvent être discernées chez les insectes, soit tolérer la formation interne de gel, soit l'éviter (Bale, 1996, 2002). Dans le premier cas, les insectes ont une température de cristallisation des fluides proche de zéro et tolèrent la formation du gel en le contenant à l'extérieur de leurs cellules. Dans le second cas, les insectes utilisent la surfusion, c'est-à-dire qu'ils diminuent significativement la température à laquelle les fluides gèlent en accumulant des polyols et des sucres (synthèse du fonctionnement de ces stratégies dans Sinclair *et al.* (2003) & Everatt *et al.* (2015)).

Les capacités de tolérance au froid ou au chaud des insectes peuvent varier entre les populations et les origines géographiques, en raison de différentes adaptations aux climats locaux et de différentes capacité d'acclimatation (plasticité) aux températures rencontrées (Saunders & Hayward, 1998, Ayrinhac *et al.*, 2004, Sinclair *et al.*, 2012). A plus fine échelle, la tolérance thermique des insectes est également affectée par leurs capacité de thermorégulation comportementale et physiologique et l'utilisation de microclimats (Abram *et al.*, 2016, Pincebourde *et al.*, 2016, Tougeron *et al.*, 2016).

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[5] Animaux ectothermes dont la température corporelle est directement corrélée à celle du milieu extérieur.

La diapause chez les insectes

Contexte historique de la définition de la diapause

Dans ce bref historique, je m'intéresse à l'évolution des concepts et de la sémantique qui ont mené à la définition actuelle de la diapause. En me basant sur des études de la discipline, réalisées depuis le 18e siècle, j'expose ici l'évolution des idées et l'intérêt des scientifiques sur la diapause au cours des siècles afin de mieux comprendre ce que cela implique au regard de la recherche moderne et du contexte scientifique actuel.

L'étude des rythmes biologiques chez les êtres vivants est fascinante, à plus forte raison que l'être humain est aussi fortement influencés par les cycles circadiens, ainsi que par l'alternance des saisons. Dès le néolithique, avec l'apparition de l'agriculture et de l'élevage, l'Homme doit comprendre et maîtriser les cycles naturels des végétaux et des animaux pour les exploiter au mieux au cours de l'année. L'étude des effets du photopériodisme journalier a commencé chez les plantes avec pour modèle la sensitive *Mimosa pudica* (Fabales : Mimosaceae) qui, en ouvrant et fermant ses feuilles sur un cycle d'environ 24 h, exprime clairement une rythmicité liée à l'alternance des périodes de jour et de nuit (De Mairan, 1729). Bien avant que le terme 'diapause' fut introduit, des naturalistes s'étaient déjà intéressés à comprendre comment les animaux passaient l'hiver, sous quelles formes, quels étaient les comportements adoptés, et combien de temps durait leur inactivité pendant la saison défavorable.

C'est le naturaliste français René-Antoine Ferchault de Réaumur, membre de la *Royal Society of London* et directeur de l'Académie Royale des Sciences, qui commente pour la première fois des études détaillées sur le comportement et la physiologie des insectes pendant l'hiver, dans un ouvrage de plusieurs volumes (De Réaumur, 1734). Il évoque notamment ses recherches sur l'hivernation des abeilles et, sans le mentionner comme tel, De Réaumur fait ici la première description de la diapause chez les insectes :

> « *Je ne connais aucun insecte à qui la chaleur soit aussi nécessaire. Elles périssent de froid dans un air dont la température parait assez douce à tous les insectes de notre climat. Le froid qui arrête la végétation des plantes, qui fait perdre à nos prairies & à nos champs leurs fleurs, met les abeilles dans un état où la nourriture cesse de leur être nécessaire; il les tient dans une espèce d'engourdissement pendant lequel il ne se fait chez elles aucune transpiration, ou au moins, pendant lequel la quantité de ce qu'elles transpirent est peu considérable, qu'elle peut n'être pas réparée par des aliments, sans que leur vie courre risque. En hiver pendant qu'il gèle, on peut considérer sans crainte l'intérieur des ruches qui n'ont pas des parois transparentes; car on peut les coucher sur le côté & même les renverser sens dessus dessous, sans mettre aucune abeille en mouvement. On les voit entassées & très-pressées les unes contre les autres; peu de place aussi leur suffit alors : elles sont ordinairement entre les gâteaux vers leur partie inférieure, ou au plus, vers le milieu de la hauteur de la ruche. Si le dégel revient, si l'air se radoucit & surtout si les rayons du Soleil tombent sur la ruche & réchauffent, les mouches à miel sortent de leur espèce de léthargie ; elles agitent leurs ailes, elles se mettent en mouvement, l'activité leur est rendue.* »

La diapause a d'abord été définie par analogie à l'hibernation des mammifères, chez qui ce phénomène est bien connu depuis l'antiquité (e.g. les écrits naturalistes de Pline l'Ancien ou d'Aristote ; Bostock & Riley, 1855). Le terme « hibernation » est d'ailleurs utilisé au 19e siècle pour décrire la torpeur hivernale chez les insectes (Rennie, 1857). En étudiant le ver à soie, Duclaux (1869) différencie pour la première fois la simple torpeur due aux basses températures de la dormance[6] prolongée. Le terme « résurrection » apparait à plusieurs reprises dans la littérature ancienne pour désigner l'anabiose, un retour à la vie après une période de cryptobiose, c'est-à-dire de complète inactivité métabolique, développementale et comportementale (Preyer, 1891). Le terme « diapause » fut introduit pour la première fois par Wheeler (1893) pour décrire le stade d'arrêt de développement des œufs de criquets pendant l'hiver. Le terme « quiescence » ou « pseudo-diapause » est quant à lui introduit quelques années plus tard par Shelford (1929) et Roubaud (1930).

A partir des années 1910 et jusqu'au milieu du 20e siècle, entraîné par le développement des sciences de l'écologie et de l'évolution, il y a un renouveau de l'intérêt scientifique pour l'hivernation des insectes et leur capacité à résister aux basses températures (Sanderson, 1908, Bodine, 1923, Holmquist, 1928, Salt, 1936), notamment via l'étude des composés cryoprotecteurs. Pour une revue de l'histoire de la recherche sur la tolérance au froid des insectes, voir Sømme (2000). Au cours des années 1920 et 1930, les premiers laboratoires de chronobiologie s'installent. C'est également à cette époque que Bünning (1936) propose qu'il existe un lien entre le système photopériodique induisant la diapause et le système des rythmes circadiens. Andrewartha (1952) fait la première synthèse de ce que la diapause des insectes implique au regard de leur écologie ; une thématique qui est encore aujourd'hui loin d'être épuisée. Lees (1955) décrit dans son livre la diapause d'un point de vue écophysiologique et met en avant l'importance de l'étude des cycles saisonniers des insectes ce qui ouvrira la voie aux premières recherches sur le contrôle endocrinien et sur les bases génétiques de la diapause (Lees, 1956, Salt, 1961). A l'issue de ces travaux, les écologues et entomologistes ressentent le besoin de produire une définition et surtout une classification claire de ce qu'est la diapause. En effet, il existe alors beaucoup de confusion sur la nature des différents types de dormance chez les insectes. Un nouveau système de classification des types de dormance est alors produit dans la seconde moitié du 20e siècle (Danilevskii, 1965, Müller, 1970, Mansingh, 1971, Thiele, 1973, Beck, 1980, Tauber *et al.*, 1986) ; en particulier, une description des principaux facteurs induisant, maintenant et terminant la diapause est réalisée, les termes diapause et quiescence sont clairement définis et dissociés, les notions de diapause obligatoire ou facultative apparaissent (voir sections suivantes).

Ce rappel historique permet de discerner les paradigmes actuels de la recherche sur la diapause et en quoi il est aujourd'hui primordial de s'intéresser à ce processus écologique, dans le cadre des changements globaux et des services écosystémiques, ce qui sera largement discuté dans cette thèse. Bien que les mécanismes neuroendocriniens gouvernant la diapause sont aujourd'hui bien connus (voir plus bas), les causes et les conséquences écologiques précises accompagnant ce phénomène sont encore peu explorées. Les questions suivantes restent en suspens : (i) existe-t-il un syndrome de diapause commun à toutes les espèces d'insectes,

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[6] Dormance : Période récurrente dans la vie d'un organisme pendant laquelle le développement et la reproduction sont ralentis. Chez les insectes, elle peut se traduire par un simple ralentissement de la croissance, par de la quiescence ou de la diapause en fonction de la prévisibilité de l'environnement (Danks, 1987).

(ii) quels sont les stimuli impliqués dans l'induction, le maintien et la fin de la diapause chez les différentes espèces d'insectes, (iii) quelle est l'importance quantitative relative de ces stimuli et leurs effets combinés sur la diapause, et (iv) quel est le degré de plasticité des insectes en réponse à ces facteurs et cette réponse est-elle sujette à modification au travers de la sélection naturelle ? Je tenterai en partie de répondre à ces questions dans cette thèse.

Définition et rôle de la diapause

Le consensus scientifique établi dans les années 1980 a mené à l'obtention d'une définition précise de la diapause, qui est aujourd'hui (et dans ce manuscrit) utilisée comme référence. **La diapause est un état dynamique de faible activité métabolique, génétiquement déterminé et ayant le système neuro-hormonal pour médiateur. Elle est caractérisée par une inactivité comportementale, un arrêt de la morphogenèse et des fonctions reproductrices et un ralentissement de la croissance. La diapause a lieu à un stage spéci-spécifique de l'ontogenèse et son expression est régulée par différents signaux environnementaux qui précèdent l'arrivée des conditions défavorables (*token-stimuli*). Une fois induite, la diapause ne peut être immédiatement levée même si des conditions favorables au développement apparaissent (Tauber *et al.*, 1986).**

La diapause représente un important aspect de l'adaptation des insectes aux variations environnementales et leur procure un gain de *fitness* d'autant plus grand que l'alternance entre les saisons est marquée et prévisible (Danks, 1987). Le rôle principal de la diapause est de synchroniser le cycle de vie de l'insecte avec son environnement biotique et abiotique. En milieu tempéré, cette suspension du développement et de la croissance permet à l'insecte de survivre à la période hivernale et parfois estivale alors que les ressources sont rares, voire indisponibles, et les conditions abiotiques, comme la température, défavorables (Danks, 1987).

La diapause s'accompagne d'un syndrome physiologique, comportemental et morphologique (c.-à-d. un ensemble de traits) très caractéristique, et pouvant être propre à chaque espèce (Tauber *et al.*, 1986). En prévision de l'hiver, les insectes accumulent des réserves énergétiques et gagnent généralement en masse (Hahn & Denlinger, 2007). La dépression métabolique évoquée plus haut est le phénomène le plus communément observé chez les insectes en diapause. Par exemple chez le dendroctone du pin *Dendroctonus ponderosae* (Coleoptera : Curculionidae), le métabolisme ralentit progressivement jusqu'en décembre et remonte graduellement jusqu'en été (Lester & Irwin, 2012). L'état de diapause est généralement accompagné d'une augmentation de la résistance aux extrêmes climatiques et au gel (Tauber *et al.*, 1986), même si cet aspect reste en débat chez certaines espèces car il est difficile de dissocier les effets propres à la diapause de ceux de l'acclimatation au froid (Denlinger & Lee, 1991, Hodkova & Hodek, 2004). Pour éviter le gel de ses tissus, l'insecte en diapause élimine les agents nucléants en arrêtant de s'alimenter et en réduisant le contenu en eau de ses fluides corporels (Koštál, 2006). Le choix d'un site de diapause adéquat (par la mère ou par l'insecte qui diapause) fait partie de l'aspect comportemental de la préparation à la diapause chez beaucoup d'insectes (Brodeur & McNeil, 1989b, Boivin, 1994, Hance *et al.*, 2007). Enfin, il existe du polyphénisme morphologique ; par exemple des individus en diapause ayant des couleurs plus susceptibles de capter la radiation solaire ou qui forment des cocons plus épais que les individus non-diapausants pour résister au froid (Krespi *et al.*, 1994, Danks, 2007).

Au sein des différents types de dormance chez les ectothermes, il existe également le phénomène de quiescence qui, par opposition à la diapause, est une réponse physiologique ou comportementale immédiate et directe à un évènement stressant, le plus souvent un stress thermique ou hydrique (Tauber *et al.*, 1986). La quiescence est levée rapidement dès que l'environnement devient à nouveau favorable. Ce phénomène est, par définition, fortement lié à la plasticité phénotypique des individus. Comme la diapause, la quiescence s'accompagne ou non d'une meilleure résistance aux températures extrêmes et à la dessiccation (Leather *et al.*, 1993, Danks, 2007). Bien que la quiescence soit probablement le moyen le plus souvent utilisé par les insectes pour répondre à des périodes de stress qui ne sont pas liées à une récurrence saisonnière (Tauber *et al.*, 1986), peu de choses sont connues quant à la diversité des stratégies de quiescence à travers les différents groupes d'insectes.

L'origine évolutive de la diapause hivernale reste assez floue, mais pour mieux la comprendre il faut considérer qu'il existe un important gradient de stratégies de dormance allant du ralentissement temporaire de la croissance à la diapause profonde, en passant par la stratégie de quiescence. En effet, certains auteurs considèrent que la diapause hivernale est une acquisition récente chez les insectes, qui ne serait apparue qu'après la colonisation de milieux tempérés après la dernière glaciation (Levins, 1969). Cette hypothèse semble peu solide puisque la capacité à entrer en diapause existe également chez les arthropodes tropicaux, bien qu'elle soit souvent plus difficile à détecter qu'en milieux tempérés (Tauber & Tauber, 1981). Plus probablement, la diapause aurait une origine tropicale et les insectes n'auraient pas acquis *de novo* la capacité à entrer en diapause après la dernière glaciation. En particulier, à cause de l'aspect « anticipatoire » de la diapause, son origine serait liée à une évolution des capacités à mesurer les stimuli indiquant l'approche des changements saisonniers (Tauber *et al.*, 1986, Saunders, 2002). Comme les insectes ont colonisé des latitudes plus hautes, ils auraient développé des capacités à mesurer les changements saisonniers de photopériode à partir des horloges circadiennes existantes (Meuti & Denlinger, 2013). La sélection naturelle aurait alors agi sur ces caractéristiques du photopériodisme et de la dormance préexistantes chez les espèces tropicales jusqu'à la mise en place d'adaptations aux variations environnementales des milieux tempérés (Tauber & Tauber, 1981). On peut donc en conclure que la diapause est un phénomène phylogénétiquement très ancien chez les arthropodes.

La diapause estivale a été répertoriée chez beaucoup d'insectes et apparait dans de nombreux milieux, des déserts jusqu'aux hautes latitudes (Masaki, 1980). La diapause estivale peut être considérée comme une image miroir de la diapause hivernale, puisqu'elle se traduit par un syndrome physiologique similaire mais n'est induite que lorsque la durée de jour et les températures sont élevées (Spieth *et al.*, 2011). En revanche, les facteurs ayant sélectionné la diapause estivale sont probablement différents de ceux de la diapause hivernale puisque son rôle est de permettre la résistance aux périodes de sécheresse (Ridsdill-Smith *et al.*, 2005) et de répondre aux épisodes de baisse de qualité ou de disponibilité en ressources fréquents à la fin de l'été (Hunter & McNeil, 1997, Tsukada, 1999). Par ailleurs, la diapause estivale doit être distinguée du phénomène d'estivation (Navas & Carvalho, 2010), qui est une forme de dormance principalement exprimée chez les ectothermes de milieux tropicaux pour survivre à la saison sèche (Denlinger & Armbruster, 2014).

Finalement, la diapause hivernale ne s'exprime pas chez certains insectes, soit parce qu'ils ont développé d'autres stratégies d'hivernation, soit parce que le milieu dans lequel ils évoluent ne comporte pas de cycles saisonniers assez marqués (Tauber *et al.*, 1986). Par exemple, de nombreuses espèces de pucerons ont des cycles de vie anholocycliques, c'est-à-dire qu'ils ne produisent pas d'individus sexués et donc pas d'œufs qui pourraient entrer en diapause. Ces pucerons, principalement associés aux milieux tempérés aux hivers doux, passent l'hiver sous forme larvaire ou adulte et continuent à s'alimenter et à se reproduire (Leather, 1992). Ces phénotypes non-diapausants pendant la période hivernale sont particulièrement intéressants, car ils prennent une importance grandissante dans le cadre des changements climatiques (**Chapitres 3** et **9**).

L'expression de la diapause

Les insectes ont développé des mécanismes complexes afin mesurer la durée relative du jour et de la nuit, et ainsi réguler à la fois leurs rythmes journaliers et saisonniers (Saunders, 2002, Bradshaw & Holzapfel, 2010, Saunders, 2012). La diapause hivernale est principalement induite par la détection de la baisse de photopériode après le solstice d'été, car c'est un stimulus environnemental stable au cours des ans et donc fiable pour prédire l'arrivée de l'hiver à une latitude donnée (Tauber *et al.*, 1986). Pour chaque population d'insectes, il existe donc une durée de jour critique à laquelle 50% des individus entrent en diapause (*Critical Day Length* – CDL) (Tauber *et al.*, 1986, Bradshaw & Holzapfel, 2010) (Figure 1.4). Cette mesure permet notamment de connaitre les seuils d'induction de diapause associés à chaque population.

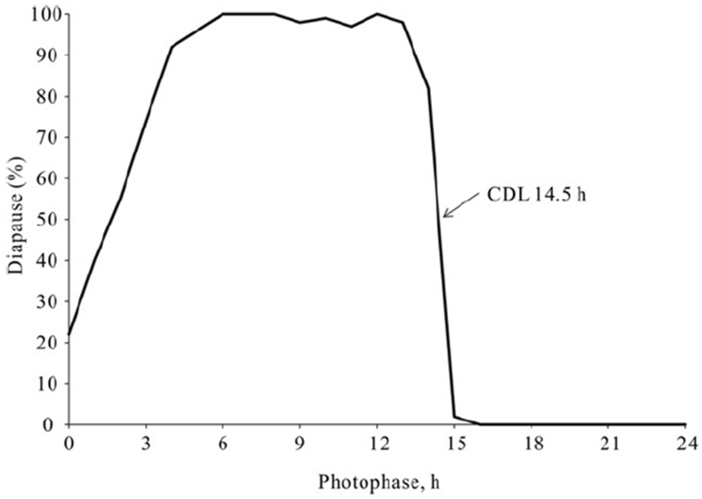

Figure 1.4 : Illustration de la durée critique de jour nécessaire à l'induction de la diapause larvaire par effet maternel chez *Calliphora vicina* (Diptera : Calliphoridae). A 20°C, la diapause est induite chez 50% des larves quand la génération maternelle perçoit une durée relative de jour de 14,5 h. D'après Saunders (2014).

L'utilisation d'un signal anticipatoire comme la photopériode permet à l'insecte de se préparer physiologiquement et comportementalement à l'arrivée des conditions hivernales (Denlinger, 2002). Néanmoins, d'autres stimuli environnementaux peuvent moduler la réponse photopériodique des insectes comme la température, l'humidité ou la disponibilité et la qualité des ressources (Danks, 1987, Tauber *et al.*, 1998, Saunders, 2014). Les interactions intra et interspécifiques entrent également en jeu dans l'expression de la diapause. Par exemple, la présence de prédateurs induit la diapause chez les femelles du tétranyque tisserand *Tetranychus urticae* (Trombidiformes : Tetranychidae) (Kroon *et al.*, 2008). La compétition ainsi que les relations hôtes-parasitoïdes peuvent également influencer la diapause (**Chapitres 6** et **7**).

Il n'y a pas d'unicité dans le fonctionnement des horloges biologiques chez les insectes, mais deux grands modèles de mesure de la photopériode ont été identifiés : (1) L'oscillateur circadien, où la diapause est induite dès qu'il y a coïncidence entre un point de l'oscillateur propre à chaque espèce avec une phase du cycle photopériodique. (2) Le sablier, où une durée critique de jour ou de nuit est nécessaire pour induire la diapause (Saunders, 2012). De plus, certains insectes répondent à la durée relative photophase/scotophase alors que d'autres se basent uniquement sur la direction (augmentation ou diminution) des changements photopériodiques (Tauber & Tauber, 1970).

a. Les phases de la diapause

Le syndrome de diapause se met en place à travers plusieurs phases distinctes. J'évoque rapidement ici les phases d'initiation, de maintien, de fin et de post-diapause (Koštál, 2006) (Figure 1.5).

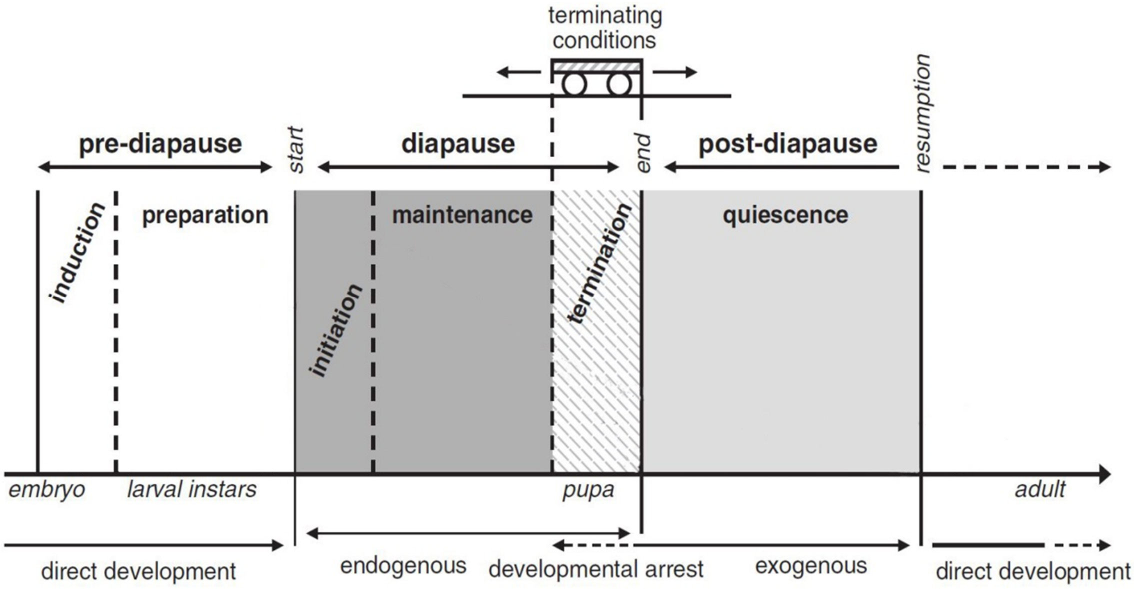

Figure 1.5 : Illustration des différentes phases de la diapause larvaire chez les insectes au cours des différents stages ontogéniques, de l'embryon à l'adulte. Ces phases sont décrites en détail dans le texte ci-dessous. Modifié d'après Koštál (2006).

La phase d'initiation est typiquement atteinte lorsque l'individu cesse son développement direct. Le(s) stade(s) sensible(s) aux signaux environnementaux peuvent varier selon les espèces (Koštál, 2006). La diapause est initiée chez la génération qui passe l'hiver mais elle peut être induite par les signaux perçus par cette génération ou la précédente (Saunders, 1965). Ces cas d'induction maternelle de diapause sont très fréquents chez les insectes (Mousseau & Dingle, 1991).

La phase de maintien permet de verrouiller le développement de l'insecte sur une phase d'arrêt, même si l'environnement reste favorable à son activité (Koštál, 2006). La diapause est ainsi maintenue jusqu'à ce que des signaux endogènes ou exogènes permettent sa levée. La séquence de processus endogènes ou environnementaux responsable de la baisse graduelle de l'intensité de la diapause au cours de cette phase (reprise progressive de l'activité métabolique) reste encore inconnue (Koštál, 2006).

La phase de fin de diapause demeure très mal comprise et semble différer selon les espèces (Tauber *et al.*, 1986). Chez certaines espèces, la diapause est levée au bout d'une période qui semble génétiquement déterminée (horloge interne), peu importe les conditions extérieures perçues par l'insecte (Hodek, 1996, Koštál, 2006). Chez d'autres espèces, ce sont les mêmes stimuli qui induisent et mettent fin à la diapause (photopériode et température) (Tauber & Tauber, 1970), bien que les seuils requis pour induire et lever la diapause soient différents (Eizaguirre *et al.*, 2008). Chez beaucoup d'autres insectes, une période de froid précédant le retour des conditions printanières semble nécessaire à la levée de la diapause (Hodek, 1996, 2002). Au terme de la levée de diapause, le développement est réactivé et l'insecte reprend sa croissance et sa morphogenèse (Denlinger, 2002).

Enfin, la phase de levée est souvent suivie d'une phase de post-diapause (Koštál, 2006). Durant cette phase, l'insecte peut rester en quiescence pour résister aux potentielles vagues de froid printanières, tout en reprenant graduellement son activité métabolique et comportementale (Régnière, 1990).

b. Diapause obligatoire ou facultative

La diapause peut être obligatoire ou facultative. La diapause obligatoire survient à un stade spécifique et génétiquement déterminé du cycle de vie de l'insecte, elle n'est donc pas directement contrôlée par des changements environnementaux. Dans ce cas, la diapause a lieu à chaque génération et est généralement associée aux espèces univoltines (une génération par an). A l'inverse, la diapause facultative est induite en réponse à des signaux environnementaux (*token stimuli*) prédisant des conditions défavorables à venir. Elle est donc généralement associée aux espèces multivoltines (plusieurs générations par an) et induite seulement à la génération qui précède l'arrivée de l'hiver (Denlinger, 2002).

Ces termes sont parfois improprement utilisés car les patrons de voltinisme observés ne sont pas forcément liés à la diapause mais peuvent être le résultat de contraintes développementales liées à la température (Kivelä *et al.*, 2015). Par exemple, aux limites nordiques de leur aire de répartition, certains insectes peuvent ne produire qu'une seule génération par année, contraints par les faibles températures, mais ils pourraient en produire d'avantage dans des régions aux climats plus doux, où la diapause ne serait induite que pour une de ces générations (voir aussi l'**Annexe 2**).

c. Contrôle endocrinien et génétique

Le contrôle hormonal de la diapause a été très bien étudié chez les insectes. En bref, la cessation de développement résulte de la réception de signaux environnementaux par le système neuro-endocrinien. Ces signaux empêchent la production de l'hormone PTTH (prothoracotrope) par les cellules du cerveau ce qui entraine l'inactivation de la glande prothoracique et l'absence de sécrétion d'hormones de mue (ou écdystéroïdes) (Saunders, 2002). Les corps allates, impliqués dans la production des hormones juvéniles, sont aussi connus pour jouer un rôle dans la diapause des insectes. Ainsi, les titres d'hormone juvénile restent élevés chez les individus en diapause. Le rôle de cette hormone est de maintenir les caractères juvéniles et d'empêcher la reprise de la croissance et des mues (Chippendale, 1977, Tauber *et al.*, 1986). Par exemple, l'hormone juvénile régule le polyphénisme saisonnier des pucerons et la formation de morphes ailés ou sexués précédant la ponte d'œufs diapausants (Hardie, 1980, Saunders, 2002). D'autres hormones comme l'insuline, la dopamine ou l'hormone de diapause ont été identifiées comme jouant un rôle dans la diapause de certains insectes (Denlinger, 2002).

La littérature concernant les gènes impliqués dans les rythmes biologiques s'est fortement étoffée au cours des dernières années. Notamment, les gènes *period*, *timeless* et *cycle* seraient les principaux responsables du photopériodisme chez les insectes (Denlinger, 2002, Pavelka *et al.*, 2003, Saunders, 2016). Il apparait que le système génétique du contrôle des rythmes circadiens est au moins partiellement commun avec le système de contrôle des rythmes saisonniers (Ikeno *et al.*, 2010, Koštál, 2011, Meuti & Denlinger, 2013, Dolezel, 2015). Un ensemble d'autres gènes s'expriment pendant le processus de diapause, par exemple ceux responsables de la formation de molécules antigel ou de la protection contre les chocs thermiques (Hayward *et al.*, 2005). Le contrôle hormonal et moléculaire de la diapause chez les insectes sont également finement détaillés dans les synthèses de Denlinger (2002, 2013).

d. Les gradients géographiques et climatiques

Les patrons d'expression de diapause varient considérablement en fonction des latitudes et des conditions climatiques locales. Les insectes de hautes latitudes ou de climats plus froids entrent en diapause hivernale plus tôt dans l'année, en plus grande proportion et à des photopériodes plus longues que les populations du sud (Hut *et al.*, 2013). Par exemple chez le parasitoïde *Nasonia vitripennis* (Hymenoptera : Pteromalidae) la diapause est induite par les conditions rencontrées à la génération maternelle ; les femelles du nord de la Finlande produisent des descendants diapausants en plus grande quantité (jusqu'à 100% de leur portée) et significativement plus tôt que les femelles prélevées en Corse (Paolucci *et al.*, 2013). Ces patrons de diapause sont aussi vérifiés sur les gradients altitudinaux (Hodkinson, 2005).

Il est important de noter que, si l'initiation de diapause hivernale est une réponse aux changements de photopériode, le facteur environnemental principal sélectionnant pour son expression est bien la température (Tauber *et al.*, 1986). Le fait que les populations de latitudes ou climats distincts répondent différemment à différents régimes jour/nuit est une preuve de l'origine génétique de l'induction de la diapause et donc de son héritabilité (Lehmann, 2016), ainsi que d'adaptations locales à la rigueur du climat et à la succession des saisons (Saunders & Hayward, 1998, Danks, 2002, Bradshaw *et al.*, 2004).

Scénarios dans le cadre des changements climatiques

Les adaptations des insectes observées le long de gradients climatiques comme évoqué précédemment permettent de prédire que la réponse photopériodique (e.g. la CDL) pourrait être sujette à modifications par les changements climatiques. Un analogue peut en effet être établi ; les populations qui subissent localement des températures plus élevées devraient avoir une réponse similaire à des populations qui migreraient plus au sud ou vers des climats plus doux. Les insectes peuvent s'adapter aux nouvelles températures et à leur variation en changeant d'aires de répartition, en s'adaptant *in situ* en modifiant leurs capacité de tolérance thermique ou en mettant en place des stratégies de *bet-hedging*, en utilisant des processus d'acclimatation comportementale ou physiologique, ou finalement en modifiant leur phénologie, bien que les effets des changements climatiques sur la diapause restent mal connus (Bale *et al.*, 2002, Bale & Hayward, 2010, Andrew & Terblanche, 2013, Maino *et al.*, 2016)

La photopériode ne va pas être modifiée par les changements climatiques, à une latitude donnée, mais la température oui. De ce fait, la durée de jour à laquelle les insectes sont actuellement adaptés pour entrer en diapause pourrait ne plus être un signal fiable de l'arrivée de l'hiver. Cela pourrait engendrer des réponses plastiques ou évolutives du photopériodisme et de la phénologie des insectes afin de s'adapter aux nouvelles conditions saisonnières (Bale & Hayward, 2010). Ces éléments ont été au cœur de mon travail et sont discutés en détail dans le **Chapitre 2**, en ciblant particulièrement les insectes parasitoïdes.

Les modèles biologiques

Les insectes parasitoïdes

Plus de 40% des espèces connues sont des parasites et la majorité des réseaux trophiques impliquent au moins une espèce parasitaire (Dobson *et al.*, 2008). Les parasitoïdes représentent environ 10% des insectes connus (Heraty, 2009) et occupent une place extrêmement importante sur le plan écologique et fonctionnel au sein des écosystèmes (Godfray, 1994). La plupart des parasitoïdes sont des hyménoptères (plus de 60 000 espèces), les autres étant principalement des diptères (15 000 espèces) et des coléoptères (3 500 espèces) (Godfray, 1994). Le besoin de synchroniser leur cycle de vie avec celui de leur hôte est un aspect important de leur écologie et fait donc partie des éléments environnementaux qui sélectionnent et régulent la durée de la diapause (Tauber *et al.*, 1986). En plus d'exploiter une grande variété d'espèces, les parasitoïdes arborent une diversité très importante de modes de vie et de stratégies d'exploitation de leurs hôtes (Encadré 3).

Les parasitoïdes ont développé des mécanismes pour optimiser les comportements d'exploitation de leurs hôtes. En effet, pour maximiser leur *fitness*, les femelles parasitoïdes doivent parasiter le plus grand nombre d'hôtes de la plus grande qualité possible (van Alphen *et al.*, 2003). Pour ce faire, elles doivent d'abord localiser l'habitat de leurs hôtes et localiser le (ou les) hôte(s) à proprement parler. Cela se fait principalement à l'aide de signaux visuels ou chimiques comme les phéromones émises par les hôtes ou les composés relâchés par les plantes infectées par les hôtes (Wajnberg *et al.*, 2008). Puis, les femelles doivent évaluer la qualité de l'hôte grâce à des signaux externes ou internes, détectés par les antennes ou par les sensilles de l'ovipositeur (Van Lenteren, 1981, van Baaren & Nénon, 1996).

Lorsqu'elles estiment la qualité d'un hôte, les femelles parasitoïdes sont également capables de détecter si celui-ci est sain ou parasité (Godfray, 1994, Goubault *et al.*, 2004). Les hôtes restant dans l'environnement immédiatement après le parasitisme, il peut arriver que, même chez les parasitoïdes solitaires, plusieurs œufs soient pondus dans le même hôte (Mackauer, 1990). Dans le cas où la même femelle pond une seconde fois dans le même hôte, on parle de superparasitisme monogyne. Dans le cas où c'est une autre femelle de la même espèce, on parle de superparasitisme intraspécifique. Enfin, si l'hôte est parasité par plusieurs espèces de parasitoïdes simultanément, on parle de multiparasitisme. Ces derniers éléments sont le résultat d'une compétition indirecte qui peut être défavorable, ou non, aux parasitoïdes (van Alphen & Visser, 1990). Les parasitoïdes ont donc développé des adaptations comportementales pour mesurer et répondre à la compétition interspécifique et intraspécifique. Cela a été très bien étudié dans le cadre de l'exploitation optimale des patchs d'hôtes en situation de compétition directe ou indirecte (Haccou *et al.*, 2003, Boivin *et al.*, 2004, Goubault *et al.*, 2005, 2007, Le Lann *et al.*, 2011a, Robert *et al.*, 2016).

Encadré 3. Les insectes parasitoïdes

Les insectes parasitoïdes (Figure 1.6) sont des organismes situés à la frontière entre les parasites métazoaires et les prédateurs. Ils se développent dans (endoparasitoïdes) ou sur leur hôte (ectoparasitoïdes) et se nourrissent de celui-ci pendant leurs stades immatures (embryon, larves et nymphe). Ils émergent de leur hôte au stade adulte (ou parfois avant la nymphose) et ont alors une vie libre. Les stades d'hôtes attaqués vont des œufs aux adultes en passant par les larves et les nymphes (Godfray, 1994).

Il existe des parasitoïdes spécialistes qui ne s'attaquent qu'à un nombre limité d'espèces d'hôtes, alors que les parasitoïdes généralistes s'attaquent à de nombreuses espèces, parfois dans des taxa très distincts.

Il existe des parasitoïdes grégaires chez qui plusieurs individus se développent dans un même hôte, ou des parasitoïdes solitaires chez qui un seul individu émerge par hôte.

Enfin, on distingue les parasitoïdes koïnobiontes qui maintiennent leur hôte en vie jusqu'à la nymphose ou jusqu'à ce que l'adulte en émerge et les idiobiontes qui tuent ou paralysent leurs hôtes au moment de l'oviposition (du parasitisme).

Figure 1.6 : Chenille de *Papilio demoleus* (Lepidoptera : Papilionidae) parasitée par une guêpe Braconidae du genre *Apanteles*, un endoparasitoïde grégaire. Les amas blancs sous la chenille sont les cocons qui contiennent les nymphes parasitoïdes en développement. Les orifices par lesquels les larves sont sorties de la chenille sont visibles. Source Wikimedia Commons.

Enfin, les hyperparasitoïdes sont des parasitoïdes secondaires (c.-à-d., ils s'attaquent aux parasitoïdes primaires). Ils représentent donc le niveau trophique supérieur et sont adaptés de plusieurs façons à trouver des hôtes déjà parasités et à évaluer leur qualité (Brodeur, 2000, Frago, 2016). Par exemple, de manière identique aux parasitoïdes primaires, les hyperparasitoïdes utilisent les signaux volatiles émis par les plantes en réponse à une attaque de phytophages afin de localiser leurs hôtes (Poelman *et al.*, 2012).

Les pucerons et leurs parasitoïdes

Les pucerons au sens strict (Hemiptera : Aphididae) sont distribués dans le monde entier, bien qu'ils soient particulièrement présents en régions tempérées. Les pucerons peuvent produire jusqu'à 20 générations par an, en fonction des milieux qu'ils exploitent (Dixon, 1985).

Leur cycle de vie est très particulier puisqu'il peut alterner entre un mode de reproduction sexué et asexué (ou parthénogénétique) (Dixon, 1985). On parle d'anholocyclie quand le cycle de reproduction est « incomplet », c'est-à-dire qu'il ne comporte pas de phase sexuée. Dans ce cas, majoritairement retrouvé dans les régions aux hivers doux (Dedryver *et al.*, 2001), les pucerons se reproduisent toute l'année uniquement par parthénogenèse. Un cycle complet, ou holocycle, est associé à la production d'une génération d'individus sexués et de plusieurs générations parthénogénétiques (Encadré 4). Les deux types de cycles peuvent coexister au sein d'une même espèce voire d'une même population de pucerons, car la capacité à produire des individus sexués est génétiquement déterminée (Simon *et al.*, 2002).

Certaines espèces de puceron sont dites dioéciques, elles doivent alterner entre deux types de plantes très différentes au cours de leur cycle de vie ; la phase sexuée a lieu sur l'hôte primaire tandis que le reste du cycle se déroule sur la plante hôte secondaire. Beaucoup d'espèces sont monoéciques ; elles effectuent l'ensemble de leur cycle de vie sur le même type de plante hôte (Dixon, 1985).

Encadré 4. Biologie saisonnière des pucerons

Figure 1.7 : De gauche à droite, femelle parthénogénétique du puceron *Acyrthosiphon pisum* avec de jeunes larves, mâle et femelle en cours d'accouplement, femelle ovipare (sexuée) ayant déposé un amas d'œufs. Source Kévin Tougeron (photo de gauche), Bernard Chaubet (autres photos).

Au printemps, les femelles fondatrices engendrent une ou plusieurs générations de femelles vivipares parthénogénétiques. Les femelles parthénogénétiques donnent naissance à de jeunes larves (clones) qui commencent immédiatement à se déplacer et à s'alimenter. Au fil du temps, des individus ailés sont produits dans la population, permettant de coloniser de nouvelles plantes (Hardie, 1980). Au début de l'automne, des femelles parthénogénétiques sexupares sont formées. Elles donnent naissance à des mâles et des femelles ovipares qui s'accouplent. Avant l'hiver, des œufs sont déposés sur la plante hôte et ils y passeront la saison défavorable, en diapause (Figure 1.8).

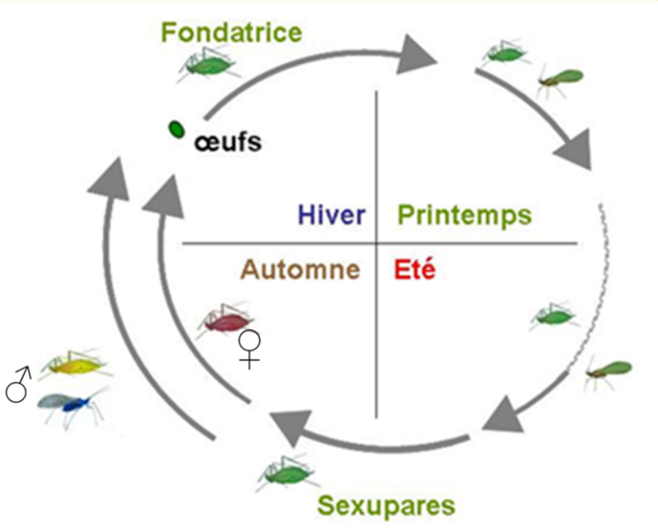

Figure 1.8 : Cycle simplifié d'une espèce de puceron holocyclique monoécique. C'est le cas notamment du puceron du pois (*Acyrthosiphon pisum*) et des épis des céréales (*Sitobion avenae*). Source : INRA Encyclop'aphid.

Les parasitoïdes de pucerons sont représentés par les Aphelinidae et les Aphidiinae, ces derniers sont des hyménoptères de la famille des Braconidae mesurant quelques millimètres (Starý, 1970). Chez les Aphidiinae il existe environ 400 espèces réparties en 50 genres (Smith & Kambhampati, 2000). La plupart des espèces d'hyménoptères parasitoïdes sont arrhénotoques; les femelles proviennent d'œufs fécondés tandis que les mâles proviennent d'œufs non fécondés (système haplo-diploïde) (Godfray, 1994). Assez cosmopolites, ils se retrouvent toutefois en majeure partie dans les zones tempérées où ils suivent les zones de distribution de leurs hôtes (Boivin *et al.*, 2012).

Les parasitoïdes de pucerons sont des endoparasitoïdes solitaires qui parasitent préférentiellement les jeunes larves de pucerons (Boivin *et al.*, 2012). La larve de parasitoïde se développe au sein du puceron et consomme ses tissus. Une fois les ressources du puceron épuisées, la prépupe du parasitoïde tisse un cocon dans lequel elle effectue sa nymphose. Le puceron mort, dont ne subsiste à ce stade que la cuticule, est appelé « momie ». Une fois métamorphosés, les mâles et les femelles parasitoïdes émergent et cherchent des partenaires pour s'accoupler avant que les femelles ne trouvent des hôtes à parasiter (Figure 1.9).

Chez les parasitoïdes de pucerons, la diapause a lieu au stade prépupe, au sein de la momie (Starý, 1970). Ces parasitoïdes doivent s'adapter au cycle de vie saisonnier complexe de leurs pucerons hôtes, ce qui peut influencer leur diapause (discuté au **Chapitre 7**).

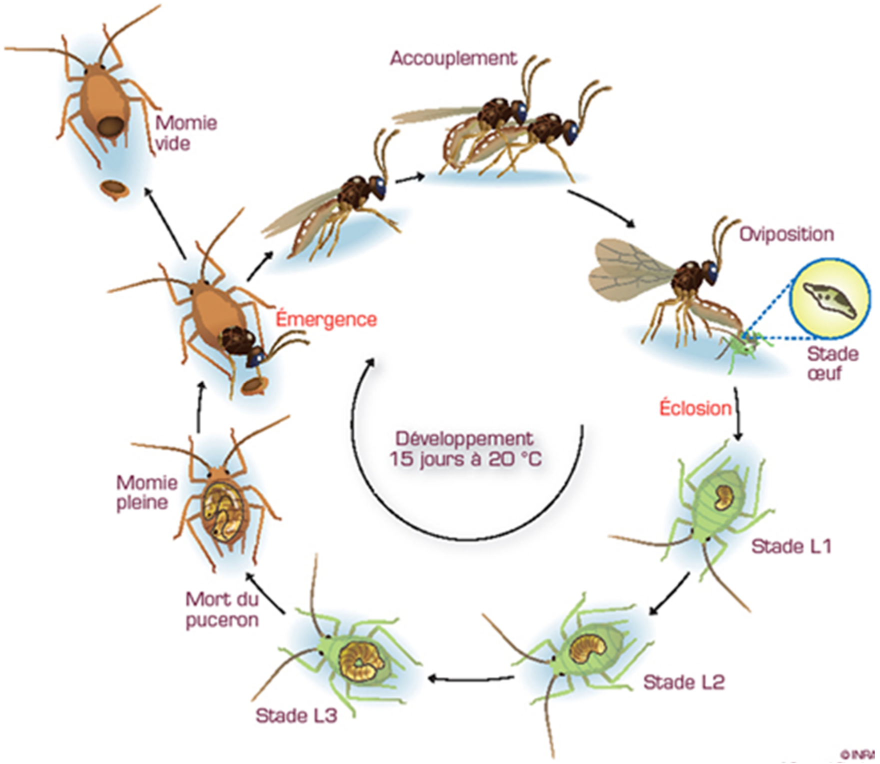

Figure 1.9 : Cycle biologique d'un hyménoptère parasitoïde de pucerons. D'après Chaubet (INRA Encyclop'aphid).

Contrôle des ravageurs

Les parasitoïdes assurent un service écosystémique clé de contrôle naturel des ravageurs en milieu agricole et forestier, au même titre que certains prédateurs et pathogènes (Naylor & Ehrlich, 1997). Les parasitoïdes sont eux-mêmes sensibles à une gamme importante d'ennemis naturels qui peuvent limiter l'efficacité du service de contrôle biologique qu'ils procurent (Boivin *et al.*, 2012). Par exemple, les hyperparasitoïdes, en attaquant les parasitoïdes primaires, réduisent grandement leur efficacité et empêchent leur maintien ou leur expansion à long-terme (Holler *et al.*, 1993, Goldson *et al.*, 2014).

La lutte biologique est le moyen de contrôler une espèce jugée nuisible pour les activités humaines à l'aide d'un ennemi naturel de cette espèce (DeBach & Rosen, 1991). La lutte biologique est un des piliers des pratiques de lutte intégrée en agriculture qui visent notamment à réduire l'utilisation de produits phytosanitaires dans les agrosystèmes (Naranjo *et al.*, 2015). Il existe trois types de lutte biologique (Bale *et al.*, 2008) ; la lutte classique par introduction (ou inoculation) qui consiste à introduire une espèce d'ennemi naturel originaire de la même zone géographique qu'un ravageur exotique, la lutte par augmentation qui consiste à effectuer des lâchers périodiques d'ennemis naturels élevés en grande quantité, et la lutte par conservation qui consiste à améliorer l'efficacité des ennemis naturels déjà présents dans le milieu à contrôler, notamment par l'aménagement de l'environnement, par exemple par le moyen de bandes fleuries qui procurent du nectar aux parasitoïdes.

En particulier, les pucerons comptent au nombre des plus importants ravageurs agricoles puisqu'ils s'attaquent à plus de 3 000 espèces cultivées et sont aussi vecteurs de très nombreux phytovirus (Van Emden & Harrington, 2017). Les parasitoïdes de pucerons sont des ennemis naturels de choix puisqu'ils sont plus spécialisés que des prédateurs, parasitent de nombreux individus et peuvent attaquer tous les stades de développement des pucerons, sauf les œufs (Boivin *et al.*, 2012). Ils sont également relativement faciles à élever en vue de lâchers massifs en serres ou en champs (van Lenteren, 2000, Boivin *et al.*, 2012).

L'élevage industriel de parasitoïdes en vue de lâchers massifs requiert des moyens de stockage efficaces des individus produits sans diminuer leur *fitness* et leur capacité à contrôler leurs hôtes. Le stockage au froid est une méthode communément utilisée dans les protocoles de production de masse de parasitoïdes (van Lenteren & Tommasini, 1999, Colinet & Boivin, 2011). Toutefois, l'exposition des parasitoïdes à des températures sub-optimales a d'importantes conséquences sur le plan comportemental et physiologique (van Baaren *et al.*, 2005, Hance *et al.*, 2007, Amice *et al.*, 2008, Colinet & Hance, 2010, Bourdais *et al.*, 2012). Etudier la diapause dans ce contexte est donc extrêmement intéressant si l'on souhaite découvrir des moyens efficaces de stocker sur le long-terme les parasitoïdes et d'effectuer des lâchers au moment opportun (Colinet & Boivin, 2011).

Le contexte écologique et climatique en Bretagne

Les systèmes pucerons-parasitoïdes ont souvent été utilisés comme modèle pour attester de l'effet des changements globaux sur la biodiversité, les réseaux trophiques et les interactions spécifiques (van Baaren *et al.*, 2010, Gagic *et al.*, 2011, Tylianakis & Binzer, 2014). C'est également ce système que j'ai étudié pour préparer cette thèse dans laquelle je me suis principalement intéressé à des espèces d'endoparasitoïdes koïnobiontes solitaires du genre *Aphidius* Nees (Hymenoptera : Braconidae) naturellement présentes en Europe de l'Ouest (Krespi, 1990).

Les espèces de parasitoïdes étudiées sont *Aphidius avenae* Haliday, *Aphidius ervi* Haliday, *Aphidius matricariae* Haliday et *Aphidius rhopalosiphi* De Stephani Perez. Ces parasitoïdes s'attaquent principalement à trois espèces de pucerons des céréales : *Metopolophium dirhodum* (Walker), *Rhopalosiphum padi* (Linnaeus) et *Sitobion avenae* (Fabricius). L'abondance saisonnière de ces trois espèces de pucerons dans le milieu agricole en zone tempérées est présentée (Figure 1.10), bien qu'elle puisse changer localement. Le puceron du pois *Acyrthosiphon pisum* (Harris), espèce modèle, a également été utilisé au cours de cette thèse. Il est principalement attaqué par *A. ervi* dans le milieu naturel.

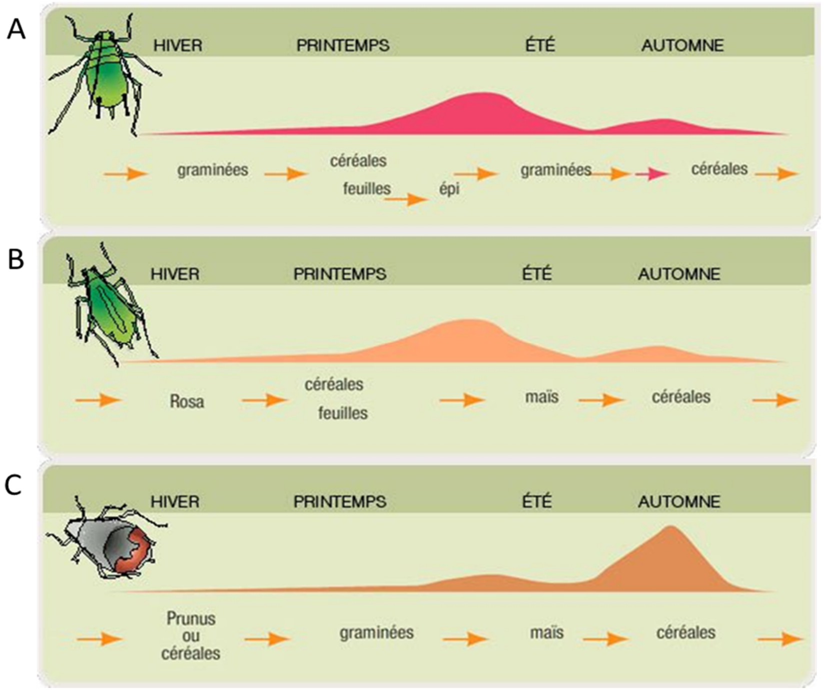

Figure 1.10 : Abondances saisonnières des pucerons des céréales en milieux tempérés. **A.** *Sitobion avenae*, **B.** *Metopolophium dirhodum* et **C.** *Rhopalosiphum padi*. Certaines espèces de pucerons restent sur la même plante hôte toute l'année (puceron monoécique comme *S. avenae*), tandis que d'autres alternent entre hôtes primaires et secondaires (pucerons dioéciques comme *M. dirhodum* et *R. padi*), seulement pour les populations holocycliques. Source du graphique Bayer France.

Les individus étudiés proviennent d'élevages établis depuis 2015 à partir d'insectes prélevés sur le terrain en Bretagne dans l'Ouest de la France. Sous des conditions hivernales peu rigoureuses, comme en Europe de l'Ouest, les parasitoïdes et leurs hôtes peuvent maintenir leur développement et leur activité toute l'année, sans entrer en diapause, au moins pour une partie de la population (Williams & Wratten, 1987, Langer *et al.*, 1997, Langer & Hance, 2000, Hance *et al.*, 2007). Cela contraint évidemment les stratégies d'exploitation des ressources des parasitoïdes, car les pucerons hôtes sont présents à faible densité en hiver (Andrade *et al.*, 2013, Eoche-Bosy *et al.*, 2016). Les parasitoïdes actifs en hiver doivent également développer des capacités de résistance au froid sans entrer en diapause. Les différences de niche thermiques au sein des communautés de parasitoïdes (Le Lann *et al.*, 2011c) et de pucerons (Alford *et al.*, 2016) expliquent en partie la répartition spécifique saisonnière particulière à la Bretagne, avec historiquement les parasitoïdes *A. matricariae* et *A. rhopalosiphi* dominants en hiver, en exploitant les pucerons *R. padi* (majoritairement) et *S. avenae* (Rabasse *et al.*, 1983, Krespi, 1990). Chaque espèce présente donc une norme de réaction particulière à la température (un optimum particulier) et la modification des températures pourrait affecter leur phénologie (voir **Chapitres 3** et **4**).

L'étude de l'écologie des pucerons et de leurs antagonistes durant leur phase de faible densité et de faible activité, c'est-à-dire pendant l'hiver, a souvent été négligée. Pourtant avec le réchauffement du climat en Bretagne (Figure 1.11), des hivers plus courts et plus chauds qu'auparavant (Météo France, 2012) et le fait que l'hiver se réchauffe plus vite que l'été (IPCC, 2014), l'intérêt d'étudier l'écologie des insectes pendant la période hivernale devient croissant.

L'activité des parasitoïdes pendant l'hiver et au début du printemps est également cruciale pour assurer un contrôle efficace des populations de pucerons (Langer *et al.*, 1997, Plantegenest *et al.*, 2001).

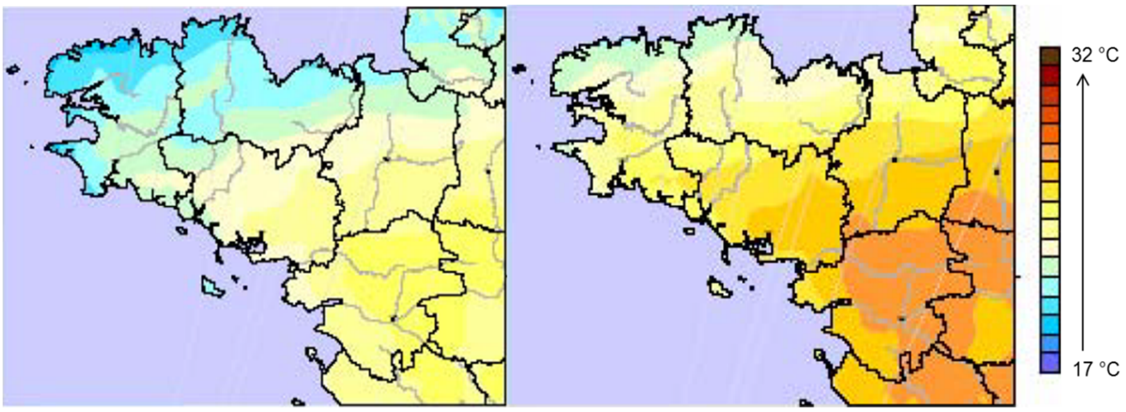

Figure 1.11 : Climatologie de référence 1971-2000 (à gauche) et projection à l'horizon 2050 (à droite) des températures maximales moyennes de juin à août pour l'Ouest de la France, selon le scénario médian A1B du GIEC (Météo France, 2012).

Au cours de cette thèse, j'ai également comparé les réponses de ces populations d'hiver doux avec des parasitoïdes des mêmes espèces, collectés au Canada, et dont les individus se sont adaptés à des hivers rudes (**Chapitre 5, 7** et **8**).

Problématique et hypothèses de travail

Dans ce contexte environnemental, la problématique est la suivante : **L'écologie saisonnière – et plus spécifiquement la diapause – des parasitoïdes de pucerons de milieux tempérés est-elle modifiée par les changements climatiques actuels ?**

L'hypothèse générale de cette thèse est que les parasitoïdes de pucerons font face à différentes pressions évolutives et variables environnementales, d'ordre abiotique ou biotique, qui altèrent l'expression de la diapause. On suppose que les parasitoïdes sont localement adaptées à la variation saisonnière de leur environnement et qu'ils peuvent répondre de manière plastique aux modifications environnementales liées aux changements climatiques via la mise en place de stratégies saisonnières particulières.

Dans ce cas, il est possible de prédire que :

- En fonction de leurs préférences thermiques, certaines espèces de parasitoïdes peuvent être actives ou non pendant l'hiver. La composition spécifique hivernale de la communauté de parasitoïdes et de pucerons diffère en fonction des conditions de température rencontrées, et change avec le réchauffement du climat.
- L'incidence de diapause est faible chez les populations de parasitoïdes adaptées aux hivers doux. Les seuils d'induction de diapause ne sont pas ou peu atteints. Les parasitoïdes provenant de régions aux d'hivers rudes ont une plus forte incidence de diapause car ils répondent fortement aux stimuli environnementaux informant de l'arrivée de l'hiver.

- La ressource hôte revêt une forte importance dans l'expression de la diapause chez les parasitoïdes et influence leur plasticité développementale. En particulier, le fait de rencontrer des hôtes de qualité différente au cours des saisons représente un signal environnemental à part entière qui module la diapause chez les parasitoïdes. La diminution de la ressource en hôtes pendant l'été, entrainant une forte compétition entre femelles parasitoïdes, influence l'induction de diapause.
- Il y a un avantage sélectif à entrer ou non en diapause selon les environnements (biotiques et abiotiques) rencontrés. Dans les régions où les hivers sont doux, la balance coûts/bénéfices penche en faveur d'une réduction, voire d'un arrêt de l'expression de la diapause au profit d'autres stratégies d'hivernation.

Ces hypothèses sont explicitées en détail dans les différents chapitres de la thèse. En résumé, je m'intéresse à comprendre la nature des facteurs environnementaux et des pressions de sélection responsables de la variabilité de la diapause observée au niveau intra-populationnel et inter-populationnel. Je décompose et analyse ensuite la diversité des réponses des parasitoïdes face aux changements climatiques. Je discute des conséquences d'un changement de stratégie d'hivernation des parasitoïdes sur les communautés d'insectes, et notamment les implications dans le cadre de la lutte biologique. Pour répondre à ces questions, j'ai utilisé des approches de terrain et de laboratoire.

Organisation de la thèse

Dans la suite de cette **Première Partie** de la thèse, après une revue de la littérature sur les stratégies saisonnières des parasitoïdes, nous détaillons par quels mécanismes les changements climatiques peuvent agir sur leur diapause et nous proposons différents scénarios quant à l'évolution de ces stratégies saisonnières (**Chapitre 2**).

Dans la **Partie II**, nous nous intéressons au cas particulier des parasitoïdes de pucerons en Bretagne.

- Le **Chapitre 3**, comporte une description des changements de communauté d'hôtes et de parasitoïdes au cours de la dernière décennie dans le but de montrer l'évolution des abondances relatives et des occurrences spécifiques en lien avec le réchauffement climatique durant l'hiver.
- Dans le **Chapitre 4** (Tougeron *et al.*, 2017a), nous déterminons en laboratoire l'incidence et le niveau d'expression de la diapause chez quatre espèces de parasitoïdes présentes en Bretagne (décrites plus haut) en les exposants à différentes conditions de photopériode et de température.
- Dans le **Chapitre 5**, via une expérience de transplantation entre des populations françaises et canadiennes de parasitoïdes de la même espèce en jardin expérimental, nous étudions l'importance des adaptations locales et de la plasticité phénotypique à déterminer les niveaux de diapause exprimés.

Dans la **Partie III**, nous explorons l'importance de la relation entre les parasitoïdes et leurs hôtes dans le cadre de la diapause.

- Dans le **Chapitre 6** (Tougeron *et al.*, 2017b) , nous testons l'influence de signaux encore méconnus chez les parasitoïdes du genre *Aphidius* telle que la densité de l'hôte et de la compétition intraspécifique sur l'induction de la diapause estivale.
- Dans le **Chapitre 7**, nous menons une analyse de l'influence de la qualité des hôtes sur la diapause des parasitoïdes. En particulier, nous testons l'influence des morphes sexués de pucerons et de leur contenu métabolique afin de mieux comprendre l'importance de la synchronisation des cycles-de vie entre espèces ayant fortement co-évolué, et des mécanismes biochimiques sous-jacents.

Dans la **Partie IV**, nous nous intéressons aux traits liés au syndrome de diapause et aux mécanismes ayant pu mener à la perte d'expression de la diapause dans certains environnements.

- Le **Chapitre 8** se focalise sur les effets de la plasticité transgénérationnelle et notamment maternelle sur la diapause et les traits de vie des parasitoïdes de pucerons. Nous souhaitons déterminer si les effets maternels peuvent inhiber la diapause des descendants si les températures sont trop élevées.
- Dans le **Chapitre 9**, nous évoquons les liens existant entre la diapause et la tolérance au froid. Le but est d'étudier les compromis existant entre le stade diapausant et le stade non-diapausant chez les parasitoïdes de milieux tempérés à hivers doux afin de mieux comprendre les coûts de la diapause et ce que cela implique au regard de l'évolution des stratégies d'hivernation.

Dans la **Partie V (discussion générale)**, nous discutons de l'ensemble de ces résultats et ouvrons des perspectives de recherche sur la diapause des insectes en général.

L'**Annexe 1** présente une étude portant sur l'importance des microrefuges climatiques liés au paysage dans la tolérance au froid et l'hivernation des parasitoïdes de pucerons dans le milieu agricole. En **Annexe 2**, nous nous intéressons au fait que, dans les milieux où les étés sont courts et les hivers rudes, comme dans les Prairies d'Amérique du Nord, le réseau trophique hôtes/parasitoïdes peut être extrêmement simple en terme de richesse spécifique et de liens trophiques, et que la stratégie de diapause *in situ* ne permet pas nécessairement le passage de l'hiver.

Chapitre 2

Revue de la littérature

How climate changes affect the seasonal ecology of insect parasitoids?

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En préparation pour *Global Change Biology*

Note: To avoid overlapping with Chapter 1, the introduction of this article has been slightly reduced.

Summary

In the context of global change, modifications in winter conditions may disrupt insect seasonal phenology patterns, including diapause expression, and thereby lead to underappreciated outcomes at different ecological scales. Parasitoids are key-components of terrestrial ecosystems in terms of functioning and species richness. Even if parasitoids share common responses to climate-change with other insects, their close relationship with their hosts and their particular life-cycle alternating between parasitic and free life-forms make them to be considered as special cases. In this article, we first examine adaptations of parasitoids to overwinter in temperate areas. We next review the mechanisms liable to plastic or evolutionary adjustments and through which climate-change may modify diapause expression. Finally, we propose different scenarios for the evolution of parasitoid seasonal ecology by exploring three foreseen outcomes of climate change: (i) decrease in winter cold severity, (ii) decrease in winter duration and (iii) increase in seasonal extreme climatic events and environmental stochasticity. We highlight that parasitoids have great capacities to adapt to new environmental conditions, either through plasticity or genetic evolution. They may reduce diapause expression, adapt new cues to initiate or terminate diapause, increase in voltinism and develop diversified bet-hedging strategies. Their response may however be constrained by those of their hosts, which may have consequences on species interactions, host-parasitoid phenology synchrony, food-web functioning and for the outcome on ecosystem services such as biological pest control.

Key-words: Insect seasonality, Climate change, Overwintering strategies, Diapause, Quiescence, Plasticity, Phenology, Scenario, Predictions.

Introduction

In temperate regions, warmer average temperatures, extended growing season, higher climatic variability and lower winter predictability are important facets of climate change (Easterling *et al.*, 2000, IPCC, 2014). Through either plasticity or local adaptations, ectotherms may respond to climate changes in three ways. They can (i) shift their geographic distribution to match their current thermal preferences, (ii) alter their phenology to cope with a new pattern of seasonal climatic conditions, and (iii) adjust their thermal tolerance capacities to match local changes in temperatures (Hughes, 2000, Walther *et al.*, 2002, Parmesan & Yohe, 2003). For insects from temperate and polar regions, modifications in phenology are the most commonly reported responses to climate change (Forrest, 2016). For instance, they have been shown to migrate and reproduce at earlier dates, increase the number of generations per year, and delay, reduce, or abandon diapause expression (e.g. Musolin, 2007, Tobin *et al.*, 2008, van Asch *et al.*, 2012, Bell *et al.*, 2015).

Parasitoid insects are key components of terrestrial ecosystems through their diversity, abundance and functions (Waage & Greathead, 1986). The life histories of parasitoids are closely linked to those of their hosts (Godfray, 1994), a primary feature being the seasonal synchrony between host availability and parasitoid activity (Danks, 1987). Asymmetric changes in seasonal species activities (*i.e.* phenology) between closely interacting (sympatric) species, such as pollinators and plants, predators and prey or parasitoids and hosts, are expected to disrupt the synchronization of their life cycles (Visser & Both, 2005, Ovaskainen *et al.*, 2013). The trophic-rank hypothesis predicts that higher trophic level organisms should be more harshly affected by environmental changes and disturbances than lower levels, because of cascading effects on the food chain (Holt *et al.*, 1999, Gilman *et al.*, 2010). Predicting effects of climate change on host-parasitoid relationships is thus challenging because it likely affects both organisms separately as well as their trophic interactions and may create timing mismatches, leading to complex outcomes in the structure and dynamics of populations, communities, and food webs.

This review examines the potential effects of climate changes on the seasonal ecology of insects, focusing on diapause and parasitoids. We first briefly summarize knowledge on diapause and quiescence in insects. Next, we examine the mechanisms through which environmental changes could influence their diapause. We finally propose different scenarios for the evolution of parasitoids seasonal ecology in the context of rapid climate changes and stress for consequences on food webs functioning and ecosystem services.

Diapause as an adaptation to temperate climates

As for other ectotherms, temperature is the primary abiotic factor affecting insects' development, survival, fitness, reproduction, foraging behavior, distribution range, and the scheduling of their activities (Angilletta, 2009, Abram *et al.*, 2016, Sánchez-Guillén *et al.*, 2016). In temperate and polar regions, insects have evolved three different strategies to survive recurring periods of unsuitable environmental conditions for growth and reproduction, and seasonal extremes of low or high temperatures. First, insects can migrate to more favorable climates, but since long-range active migration has not been reported for parasitoids, this option is not covered in this article. Second, insects can rapidly enter into quiescence when they face non-cyclic, often short periods of atypical environmental conditions (Leather *et al.*, 1993). Third, insects can enter into diapause, a hormonally mediated state of low metabolic activity characterized by arrested development and often associated with an increased resistance to environmental extremes (Tauber *et al.*, 1986, Hodkova & Hodek, 2004) (see **Chapter 1** of this thesis).

Diapause is a pivotal point in insect life cycles since it is an anticipatory mechanism responding to environmental conditions that switches off growth and reproduction (Tauber *et al.*, 1986). The "physiological decision" to enter or not diapause cannot be reversed, which is an important point to consider in the context of warmer but unpredictable winters conditions (Bale & Hayward, 2010). An early or a late entry into diapause may result in fitness reduction, respectively because the insect forgoes growth and breeding opportunities or dies from adverse conditions (Bradshaw *et al.*, 2004).

Within a population, diapause is often expressed at different environmental thresholds and its duration varies between individuals, which serves as a developmental buffer against seasonal variability and unpredictability (Tauber & Tauber, 1981). This intrapopulational variability can originate from diversified bet-hedging strategies or from genetic polymorphism of mixed pure-strategies, maintained by balancing selection (Bradshaw & Holzapfel, 2001, Soula & Menu, 2003), which results in different plastic responses to environmental cues within a population.

Diapause can also refer to aestivation (summer diapause) (Masaki, 1980), an overlooked phenomenon in insect parasitoids (He *et al.*, 2010, and references therein). In this article, we mainly focused on the evolution of overwintering strategies because continental temperatures are increasing faster in the winter than in the summer (IPCC, 2014). A specific mention will be made in the text when referring to aestivation.

Mechanisms through which environmental changes may modify overwintering strategies

Climate changes may impact environmental stimuli which act directly on parasitoids, indirectly through their host, or on both the parasitoid and the host. Hosts and parasitoids ability to persist in a changing seasonal environment is determined by both plastic adjustments in their diapause syndrome, timing, and expression thresholds and strength of directional selection on diapause-associated traits or on phenotypic plasticity itself (Bradshaw & Holzapfel, 2006, Sgrò *et al.*, 2016). A specific example is the maternal plasticity associated with summer diapause in the aphid parasitoids *Aphidius avenae* and *Aphidius rhopalosiphi*; mothers produce diapausing offspring when exposed to high intraspecific competition for hosts (Tougeron *et al.*, 2017b). A specific example of genetic changes to altered seasonality in insect is the adaptation of the pitcher-plant mosquito *Wyeomyia smithii* (Diptera : Culicidae) to longer growing seasons in North America through the modification of its critical photoperiod for larval diapause induction in the fall (*i.e.* towards shorter day lengths thresholds) (Bradshaw & Holzapfel, 2001). Disentangling the relative effects of plasticity and genetic on shifts in phenology has been identified as one of the greatest challenges in evolutionary research on organisms' responses to climate changes (Merilä & Hendry, 2014, Schilthuizen & Kellermann, 2014).

Response to temperature and photoperiod cues for diapause-induction

In insects which have wide geographic distributions, there is a genetically determined critical day length that optimizes the timing of diapause induction in each population to match local seasonal variations (Tauber *et al.*, 1986). This pattern reflects local adaptation to earlier and harsher winter at higher latitudes, and temperature-dependent clines are susceptible to be highly modified by climate changes in terms of onset, duration, and incidence of diapause in a population (Hut *et al.*, 2013). An analogy can be established between these geographic clines and the expected effects of climate-warming (Figure 2.1).

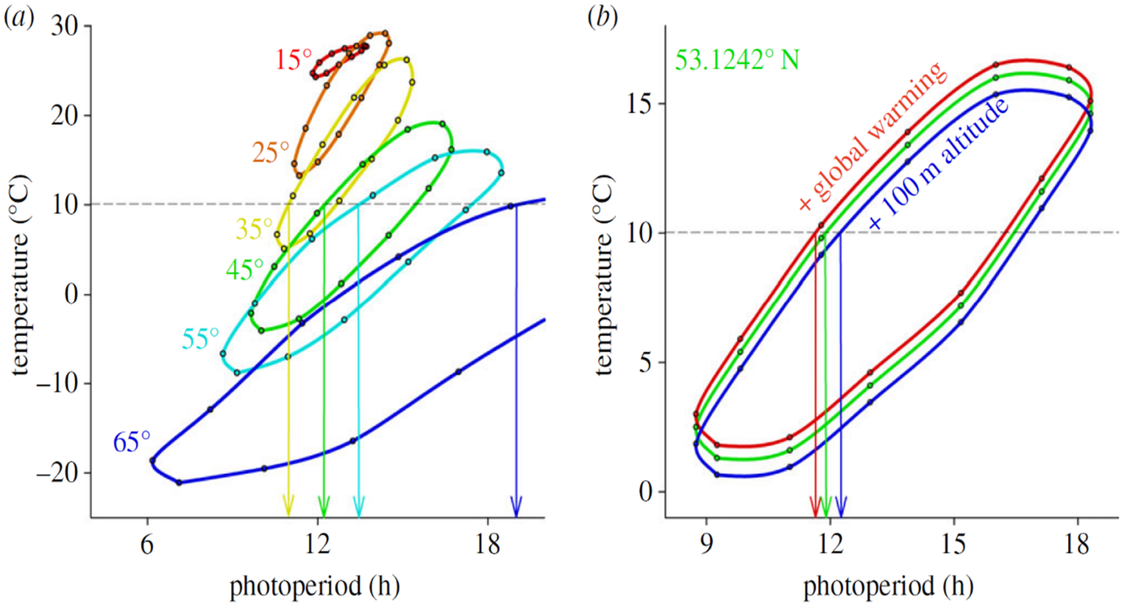

Figure 2.1: **(a)** At any given latitude, there is an ellipse-like relationship between temperature and photoperiod across the year (dots on each ellipse indicate each month conditions). The period of the year at which an insect enters diapause is thus variable across latitudes. As indicated by the dotted line, the critical photoperiodic threshold for diapause induction increases with latitude. **(b)** At a given latitude, 0.5°C warmer temperatures will shift these ellipses up - resulting in a decrease in critical photoperiod inducing diapause - whereas 100 m increase in altitude will shift them down showing that photoperiod-diapause reaction norm is liable to evolutionary or plastic changes. From Hut *et al.* (2013).

The seasonal pace of temperature changes is shifting with climate-warming, while the pace of seasonal photoperiodic changes remains unaffected. The photoperiodic response (*i.e.* diapause) of many insects may thus become mismatched with the actual growing season (mild temperatures, host availability, etc.) (Forrest, 2016). These conflicts between environmental cues may strongly impact insect fitness and drive plastic or evolutionary changes in diapause expression because insects will need to adjust their responses to diapause triggers (Bradshaw & Holzapfel, 2006, Forrest, 2016). This also raises the question of the best stimuli on which a parasitoid should rely to enter diapause in a changing climate.

Maternal effects on diapause induction

Transgenerational plasticity (TGP), including maternal effects, occurs when environmental cues experienced by the previous generation(s) modify offspring's phenotypic reaction norms (Burgess & Marshall, 2014, Sgrò *et al.*, 2016). TGP is beneficial to the insect if the maternal environment is analogous or reliably predicts the one of the offspring (Galloway & Etterson, 2007). TGP is an important mechanism to consider because it is liable to evolutionary changes and allows adaptive responses to rapid environmental changes (Sheriff & Love, 2013). Changes in diapause expression can thus arise either from responses of the diapausing individual or from TGP.

The sensitive stage can be the ovipositing female and diapause is initiated in its offspring (Saunders, 1965). If so, diapause is induced when the female detects environmental cues that inform for deleterious conditions to come (Danks, 1987, Mousseau & Dingle, 1991) and influences carbohydrates and polyols contents of their eggs, which acts on the developmental plasticity of the embryos (Denlinger, 2002). Voinovich *et al.* (2015) showed that diapause was induced by maternal effects in *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) while it was averted in the offspring when mothers were reared at high temperatures. Maternal sensitivity to environmental cues also varies among populations depending on the selective pressures on maternal perception of the environment (Mousseau & Dingle, 1991).

In some parasitoid species, there is a non-photo-thermo-sensible generation at which it is impossible to induce diapause, as a potential adaptation to avoid diapause induction in parasitoids which develop in late spring (where environmental conditions are similar as in fall). Such a maternal inhibition would prevent the induction of a maladaptive diapause phenotype in spring (Reznik & Samartsev, 2015). This phenomenon is based upon an epigenetic counter. It acts through DNA methylation induced by the parasitoids that hatch from winter diapause in order to inhibit diapause induction in offspring (Reznik & Samartsev, 2015), and could last over several generations (Uller, 2008).

Mechanisms of diapause termination

Modification in diapause termination timing could threaten the synchrony of parasitoids with their host and other resources in spring. Many insects resume development and end diapause earlier if they have first been chilled for a few days or weeks (Tauber & Tauber, 1976). For example, the parasitoid *Colpoclypeus florus* (Hymenoptera: Eulophidae) requires a chilling period >5 weeks at 4°C to terminate diapause (Milonas & Savopoulou-Soultani, 2000). Some other species simply require the presence of sufficiently high temperatures during a defined period of time; Mehrnejad & Copland (2005) noticed in the parasitoid *Psyllaephagus pistaciae* (Hymenoptera: Encyrtidae) that chilling conditions were not necessary to break diapause but the number of days required for emergence of adults decreased when the diapausing pupae experienced cold conditions. Schoonhoven (1963) demonstrated that the tachinid parasitoid *Eucarcelia rutilla* (Diptera: Tachinidae) required a hormonal stimulus from its host *Bupalus piniarius* (Lepidoptera: Geometridae) to end diapause. The plasticity for diapause termination remains to be extensively studied as well as the evolutionary potential of diapause termination systems in a warming climate.

Costs of diapause

Costs of plasticity expression in response to photoperiod are expected in a rapid-changing thermal environment, such as timing-costs (e.g. entering diapause under warm winters), production costs (e.g. producing unnecessary cryoprotectant molecules) or ecological costs (e.g. reproductive arrest under mild winters) (Sgrò *et al.*, 2016). These different costs applied on diapause could be a way through which climate changes modify parasitoid seasonal ecology. Parasitoids are limited by energetic resources they take from their hosts to survive winter, and may thus be particularly sensitive to increasing metabolic costs.

Diapause involves inherent metabolic costs during the overwintering period and following spring emergence. Fecundity and survival are often reduced in insects recovering from diapause due to physiological stress, cryoprotectant production, and energetic reserves consumption (Zhou *et al.*, 1995b, Ellers & Van Alphen, 2002, Hahn & Denlinger, 2007). Diapause costs are directly linked to diapause duration by a linear consumption of the reserves in function of time. For example, in the parasitoid *Asobara tabida* (Hymenoptera: Braconidae), a decrease in diapause duration entailed reduced costs, whereas an increased duration led to increasing mortality and decreasing fecundity (Ellers & Van Alphen, 2002).

One way for insects to save energy is to enter into a deep state of metabolic and respiratory decrease during diapause (Wadsworth *et al.*, 2013), which is less costly than a lighter diapause (Williams *et al.*, 2012), particularly during cold winters (Irwin & Lee, 2003). In species with a deep state of diapause, warmer winters may increase the cost of diapause because of increasing reserve consumption and no possibility for bringing forward diapause termination.

The host signal

For parasitoid insects, the host ecology represents an important evolutionary force acting on diapause (Tauber *et al.*, 1986). Any change in host quality or quantity across different time scales can be a selective pressure that may modify diapause timing and expression in parasitoids. In idiobiont parasitoids, which kill or paralyze their host at oviposition, the host may not be an important signal for diapause induction. In koinobiont parasitoids, where the host continues to develop during parasitism, the host represents the whole physiological environment and can thus be a signal for diapause induction (Polgár *et al.*, 1991).

The onset of parasitoid diapause can be triggered by the onset of host diapause and there is an endogenous physiological synchronization (Polgár & Hardie, 2000, Gerling *et al.*, 2009). For example, the Pteromalid hyperparasitoid *Catolaccus aeneoviridis* enters diapause at high proportions when attacking diapausing primary parasitoids (McNeil & Rabb, 1973). On the other hand, parasitoid diapause can be decoupled from its host and be induced by different or similar environmental cues. The tachinid parasitoid *Myiopharus doryphorae* is not affected by the diapause status of its host, the Colorado potato beetle, whereas diapause of *M. aberrans*, another parasitoid of the same host, is strongly influenced by the physiological status of the host as it follows perfectly the host life cycle (Gollands *et al.*, 1991).

Summer diapause in parasitoids is induced by a decrease in resources in the environment, mainly host and adult food, and competition among females (Masaki, 1980, Tsukada, 1999, Tougeron *et al.*, 2017b). These stimuli may act either directly or indirectly (*i.e.* mediated by the photoperiod and temperature) on summer diapause induction. Patterns of summer diapause expression may thus be particularly sensitive to any modification in seasonal host densities due to climate changes.

Few is known about the influence plants may have on parasitoid diapause, but one can suspect that plant quality can act on parasitoid diapause directly, or indirectly through its impact on the host (Polgár *et al.*, 1995, Hunter & McNeil, 1997). Host plant is known to play a crucial role in aphid seasonal polymorphism since most aphid populations shift from a primary host plant before winter, which induces the production of sexual morphs (Dixon, 1985). In turn, sexual aphids can be a signal for diapause induction in their parasitoids (Polgár *et al.*, 1991).

Scenarios for the evolution of overwintering strategies under climate changes

In this section, we present different scenarios for the evolution of diapause and quiescence if climate changes act through the mechanisms detailed in the previous section. Scenarios are based upon the predictions that (i) severity of winter cold will decrease, (ii) seasonal suitability for reproduction will start earlier and/or will be longer, and (iii) extreme and/or unexpected climatic events such as heat waves, cold spells, or drought episodes will be more frequent (Easterling *et al.*, 2000, Hughes, 2000). The three different facets of climate changes can produce the same ecological outcomes but we highlight which of them could be the most impactful on parasitoids ecology (Figure 2.2).

Decrease in winter cold severity

a. Decrease of diapause expression

In a climatic scenario where relatively warm winter conditions persevere, diapause costs could be diminished through a reduction in diapause duration (Ellers & Van Alphen, 2002, Ito, 2007). However, as metabolic rates increase with temperature (Gillooly *et al.*, 2001), there will possibly be costs at maintaining high metabolic rates during diapause or extended quiescence under a warmer climate (Perez & Noriega, 2013), because of higher energy consumption before the return of favorable conditions (Williams *et al.*, 2012, Xiao *et al.*, 2016). If so, the capacity to delay or avert diapause or reduce its duration may provide a selective advantage that could be maintained in insect populations (Bale & Hayward, 2010), especially when winter environmental conditions allow winter activities, including reproduction (Forrest, 2016).

The best strategy under warmer climates would be to enter in a deep state of diapause early in winter and to be able to go out easily and early. Studies are required to test how the degree of metabolic suppression during diapause, as a proxy of diapause deepness, may lead to differences in diapause expression shift in response to global climate changes (Wadsworth *et al.*, 2013). Additionally, species for which a period of chilling is required to end diapause will suffer more from warmer winters than the others. For instance, Stuhldreher *et al.* (2014) showed that the cold-adapted species *Erebia medusa* (Lepidoptera: Nymphalidae) had an earlier termination of diapause under simulated warmer winter, which led to lower adult survival rates. For species in which diapause termination is exclusively under photoperiodic control, the effect of rising temperatures on diapause termination should be low.

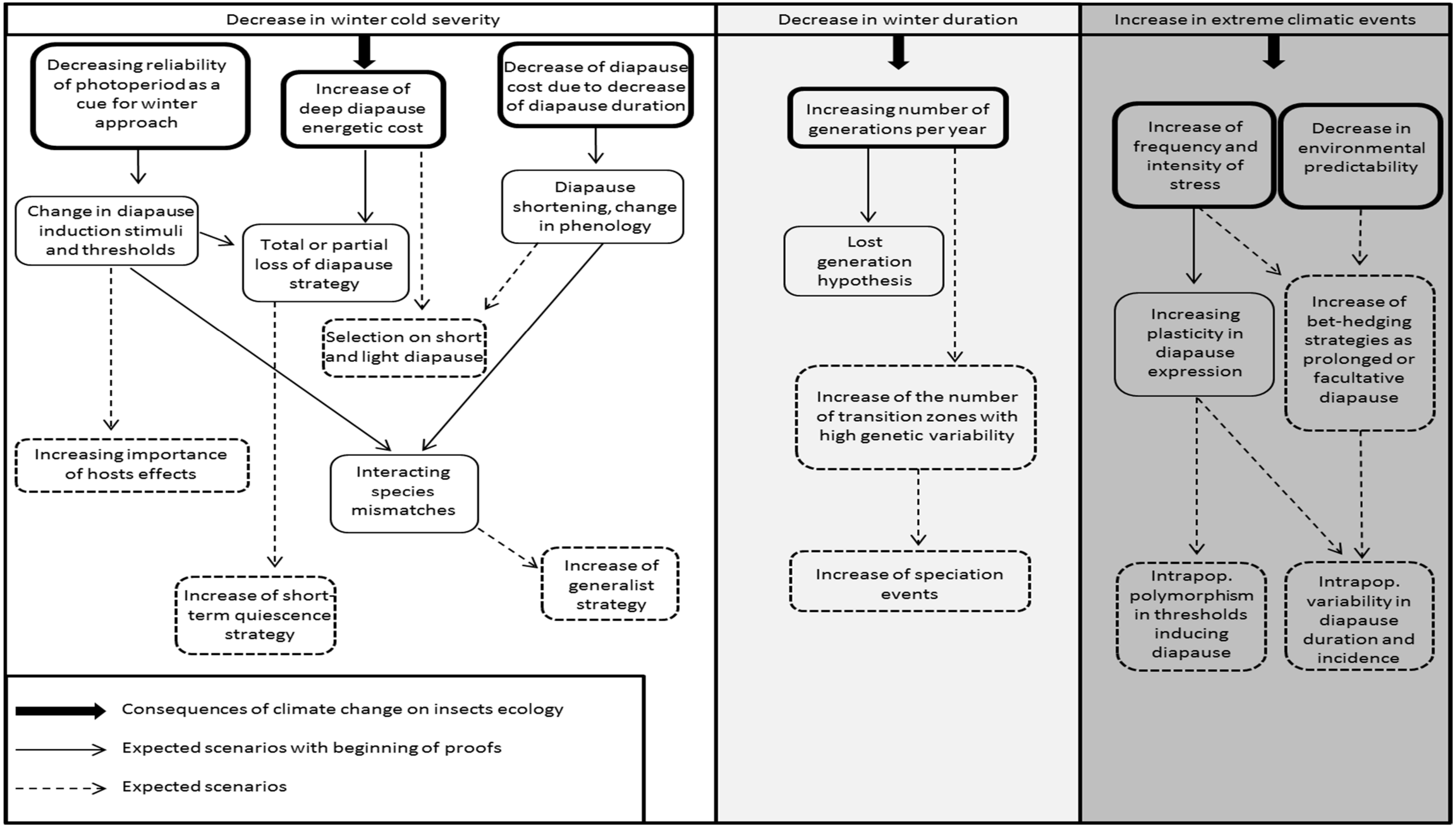

Figure 2.2: Schematic of the consequences on parasitoids seasonal ecology of decrease in winter cold severity, winter duration and increase in extreme climatic events. We present consequences of climate-change that are already well established in the literature, consequences with beginning of empirical evidence and expected scenarios.

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A likely scenario would be the reduction of diapause expression by directional natural selection for lower diapause induction thresholds. This could result in an increase in frequency of quiescence events, which represents a more plastic strategy, as the time needed to react to changes in climatic conditions is shorter than for diapause (Perez & Noriega, 2013). A quiescent strategy would allow parasitoids to track more accurately temperature changes (e.g. Rundle & Hoffmann, 2003). Quiescence should also become less costly, as the production of cryoprotective molecules will become less often necessary and for shorter periods if there are not too many extreme events. Quiescence may thus allow the insect to deal with warmer average, but more variable, winter temperatures if they can sporadically increase their cold tolerance, for instance through rapid cold hardening (Owen *et al.*, 2013). Knowledge is reduced about the propensity of species that shift from diapause strategy to quiescence as well as on the plasticity of such shifts depending on the thermal and photic environment. Development of metabolic tools and yardsticks to disentangle diapause syndrome from quiescence syndrome will greatly help to identify what type of dormancy is expressed in a given population.

The scenario of a complete loss of diapause is highly credible as different clues already underline this eventuality. When the expression of a functional trait under some environmental conditions involves costs, this trait can be lost through the process of selection (Lahti *et al.*, 2009), or its expression reduced through plastic adjustments. Strong shifts in diapause-level expression have been detected when populations were reared in the laboratory for a long time at constant temperature and photoperiod conditions, such as parasitoids cultures destined to mass-releases for biological control. For example, the aphid parasitoid *Binodoxys communis* lost its capacity to enter diapause in less than 300 generations when reared in the laboratory, leading to 100% mortality when the parasitoid was next exposed to overwintering conditions in Canada (Gariepy *et al.*, 2015). Andrade *et al.* (2016) & Tougeron *et al.* (2017a) showed than since 2010, because of warmer winters in western France, the Braconid parasitoids *Aphidius avenae* and *A. ervi,* which were known to diapause in winter the last 30 years, abandoned their diapause strategy by remaining active adults reproducing on aphids in cereal fields. Also, in mild-winter areas of north-western Spain, the parasitoid *Anaphes nitens* does not express a true diapause but rather a stage of winter quiescence that allows them to fit their dormancy duration to temperatures experienced during winter (Santolamazza-Carbone *et al.*, 2009).

Trait convergence can be expected when environmental conditions and consequent filtering are particularly harsh (Cornwell *et al.*, 2006). In this case, diapause is expected to be maintained in parasitoid community experiencing both harsh winters and host scarcity. In contrast, traits are expected to diverge in communities with high levels of competition and higher resource availability (MacArthur & Levins, 1967). In this case, we expect the formation of communities (or populations) in which both diapausing and non-diapausing species (or individuals) coexist, as a temporal avoidance of competition. With climate changes, it could be expected that traits linked to winter survival strategies will become more convergent among species, because environmental filtering will increase at the community level (*i.e.* warmer temperatures leading to diapause avoidance) (Le Lann *et al.*, 2014b).

b. Modification in host-parasitoid interactions

Climate changes could result in asymmetrical shifts in overwintering strategies between hosts and parasitoids because phenology is shifting at different rates between trophic levels and species (Thackeray *et al.*, 2016). For instance, a parasitoid may rely on temperature-related cues to enter diapause whereas its host may use non-climatic cues such as day length, leading to potential desynchronization between interacting species (Walther, 2010). However, decoupling phenology may also appear when both species respond differently to the same climatic cues. Desynchronization in phenology can in turn induce shifts in diapause expression because a parasitoid must remain synchronized with its host.

Parasitoids which enter diapause under the control of their host (e.g. McNeil & Rabb, 1973) will conform to the host's diapause period or duration and they will likely remain synchronized. If the host stops entering diapause, the parasitoid will probably conform to it and also avoid diapause. Predictions are harder to make for species that rely on multiple factors and only partially rely on their host status to enter diapause. For example, the Braconid parasitoids *Aphidius matricariae* and *A. ervi* enter diapause at high levels when parasitizing oviparous aphids, probably in response to aphid metabolic or hormonal content, whereas diapause is induced by photoperiod and temperature when the parasitoid attacks viviparous aphids (Polgár *et al.*, 1991, Tougeron *et al.*, in prep). Yet, aphids produce less oviparous morphs in warmer environments (Dedryver *et al.*, 2001). We can thus predict that climate warming will modify parasitoid diapause expression, through modifications in host phenology, first by suppressing one of the stimuli involved in diapause induction, and then by producing contradictory environmental signals for the parasitoid if sexual morphs induce parasitoids' diapause even if winter temperatures are warm enough for them to survive. However, host diapause expression is often selected by the same cues as parasitoids, so it is unlikely that such conflicting cues appear between hosts and abiotic signals. Aphid parasitoids would more likely stop using sexual morphs of their hosts as a diapause-inducing signal (Tougeron *et al.*, in prep).

The physiological or behavioral thermal envelope is likely to be different between a parasitoid and its host(s) (van Baaren *et al.*, 2010, Le Lann *et al.*, 2014b, Moiroux *et al.*, 2016, Furlong & Zalucki, 2017). This is likely to impact parasitoids' capacity of adaptation to new climates because of mismatches in thermal preferences among trophic levels (Berg *et al.*, 2010, Visser, 2016). Differences in thermal sensitivities between parasitoids and their hosts may be a crucial point to consider in the context of climate changes (Gilman *et al.*, 2010). A potential scenario would be that a host is more resistant to climatic variability than its parasitoid, which can decrease the parasitoid success at finding and attacking the host (Le Lann *et al.*, 2014b). Moreover, the speed of adaptation to the new conditions could be quicker for the hosts than for the parasitoids due to higher reproductive and growth rates that could contribute to the loss of synchronization (Hance *et al.*, 2007). Mismatches in phenology could thus occur through asymmetric thermal preferences between interacting species. For instance, it is known that parasitoids and their hosts differ in thermal acclimation responses when facing microclimatic variations across a landscape (Tougeron *et al.*, 2016, Alford *et al.*, 2017).

As well as plants cannot spread their spatio-temporal ranges beyond their pollinators' one (Klein *et al.*, 2008), specialized parasitoids will not be able to spread beyond the spatial and temporal range of their hosts (Schweiger *et al.*, 2008), which will constrain shifts in parasitoids' phenology. At parasitoid community level, generalist species - for any trait, such as thermal tolerance or degree of host specialization - should be able to better adapt to climate changes than more specialist species, and the latest should consequently be under stronger selection for phenological adjustments (Rand & Tscharntke, 2007, Tylianakis *et al.*, 2008, Vázquez *et al.*, 2015). Generalist parasitoids that change their phenology could thus be able to find an at least suboptimal host species available at the time they need to oviposit. On the opposite, parasitoids with higher host specialization would not change their phenology if their hosts poorly tracks climate change with their seasonal response.

In host-parasitoid systems, host manipulation by the parasitoid has often been shown (e.g. Khudr *et al.*, 2013), even in the context of diapause (Brodeur & McNeil, 1990). We can thus expect that if host manipulation occurs as a form of behavioral plasticity, the parasitoid will be less affected by climate changes. For example, Josso *et al.* (2011) showed that parasitized and unparasitized larvae of Drosophilidae by *A. tabida* pupated at different depths in experimental humid substrate, and this result could be linked to differences in development time between parasitized and unparasitized pupae. This implies that manipulative parasitoids are probably able to drive their host to a favorable overwintering location, which will limit negative impacts of climate changes. However, the extent to which climate changes may modify host-parasitoid interactions by acting on behavioral manipulation remains an unresolved question which still needs to be explored.

In a recent review, Johansson *et al.* (2015) proposed different scenarios on the evolution of matches and mismatches of interacting-species synchrony under a climate-change perspective. They suggested that several aspects of biological binary interactions might be affected by phenological asynchrony (e.g. when both entities undergo and leave diapause asynchronously), such as demographic effects or changes in life-history traits. It is often unclear what will the effects of asynchrony be, mainly because of the lack of empirical evidences, especially considering potential coextinctions (Dunn *et al.*, 2009). Klapwijk *et al.* (2010) demonstrated that the synchrony between *Euphydryas aurinia* (Lepidoptera: Nymphalidae) and its parasitoid *Cotesia bignellii* (Hymenoptera: Braconidae) was not altered under realistic scenarios of climate warming, as the variance in developmental duration in the parasitoid species is large enough to ensure an overlap between host availability and parasitoid emergence under different climatic contexts.

Decrease in winter duration

a. Shifts in voltinism

By being a product of multiple environmental constraints (Tobin *et al.*, 2008), changes in voltinism are tricky to predict, especially in the case of interacting species such as parasitoids and their hosts. In species with facultative diapause, changes in voltinism are expected to be the simplest response of insects to climate changes (Sgrò *et al.*, 2016). For a few decades, several species have added one or more generations to their life cycle (Altermatt, 2010), either through an increase of developmental rate or a reduction of diapause duration (Tobin *et al.*, 2008, Bentz & Powell, 2014). For example, Tobin *et al.* (2003) observed that in the grape-berry moth *Paralobesia viteana* (Lepidoptera: Tortricidae), there could be a partial fifth generation in the southern United States, particularly when temperatures exceed normal values. Small modifications in temperatures can result in large shifts in voltinism (Parmesan & Yohe, 2003), as long as hosts are available for parasitoids, allowing them to take advantage of suitable winter conditions and longer growing season. When parasitoids and their hosts shift synchronously and symmetrically their voltinism patterns, this may not lead to interaction disruption.

A decrease in voltinism could also be expected if some insects fail to prevent diapause or enter in diapause early because of mismatches between photoperiod and thermal conditions, as discussed in Forrest (2016).

Kivelä *et al.* (2013) noted that for many insect species presenting a facultative diapause, if populations were compared among latitudes, a saw-tooth pattern in life histories aroused because the expected gradual change of these traits in relation to season length is interrupted by abrupt changes in voltinism. Geographic gradients studies (e.g. Bradshaw & Holzapfel, 2001, Winterhalter & Mousseau, 2007, Paolucci *et al.*, 2013) may help to forecast where such populations with mixed strategies may appear and predict the proportion of diapausing individuals at a given location under different climate-warming scenarios, using spatial and thermal analogues (Figure 2.3). In transition areas between populations with a different number of generations (e.g. Winterhalter & Mousseau, 2007, Aalberg Haugen & Gotthard, 2015, van Dyck *et al.*, 2015), the genetic variability is expected to be high, which results in higher life-history variability (Nylin *et al.*, 1994, Blanckenhorn & Fairbairn, 1995) and possibly hybridization between different voltinism patterns (Wadsworth *et al.*, 2013) and references therein). In addition, differential dormancy patterns between different populations of a given species may ultimately lead to speciation when the life cycles of these populations become too different and reproductively isolate them (Wadsworth *et al.*, 2013).

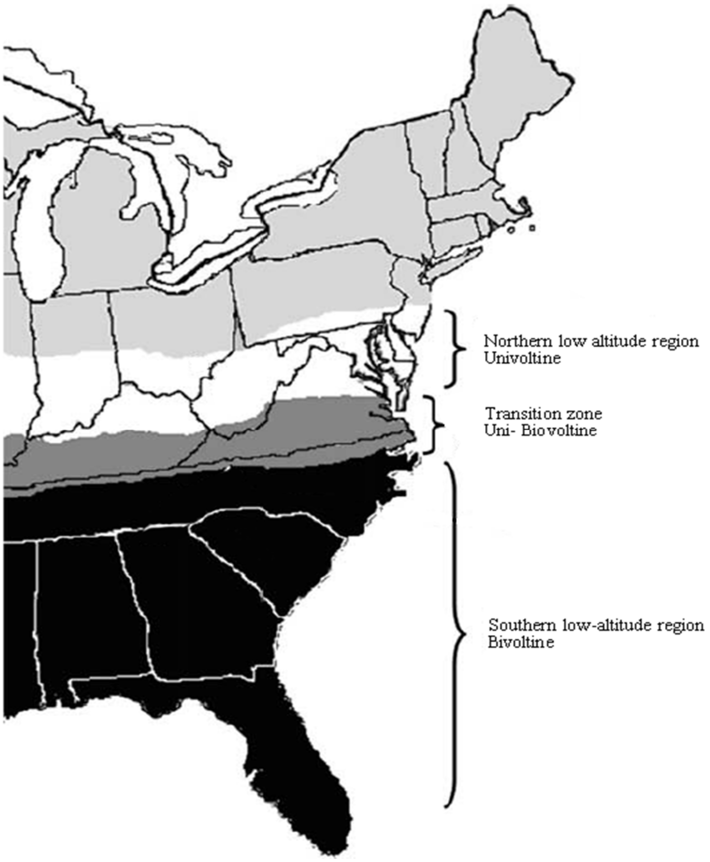

Figure 2.3: Voltinism repartition of populations of the cricket *Allonemobius socius* across the USA. Univoltinism is expressed at northern ranges whereas polyvoltinism is expressed in southern distribution range. There is a large transition zone where the two voltinism patterns coexist and genetic hybridization can occur. Modified after Winterhalter & Mousseau (2007).

Species with an obligatory diapause occurring at a genetically determined stage may be even more influenced by the rapidly rising global temperatures than species with facultative diapause, in terms of mismatches between diapause timing and seasonal events. Although not yet reported, these species may produce another generation each year due to temperature constraints on developmental rate, but diapause could still be expressed at each of these generations, leading to strong physiological and ecological costs for the insect. Indeed, as obligatory diapause is strongly genetically conserved in a given species (Košťál, 2006), plasticity is, in this case, unlikely to be involved in any kind of resynchronization with seasonality. We expect high mortality rates as well as potential local extinctions if adaptations of univoltine species to new climatic environments cannot occur, which could in turn force the selection toward increasing voltinism. Moreover, univoltine species that would theoretically be able to increase their voltinism may fail because of conflicting signals between temperature, hosts, and photoperiod, as discussed before. At this point, we can only predict that species harboring an obligatory diapause may be subject to strong selective pressures on diapause duration (Forrest, 2016).

Whether or not shifts in voltinism in response to climate change are adaptive is often unknown (Duputié *et al.*, 2015) because physiological and ecological costs may arise from such developmental responses (Sgrò *et al.*, 2016). In some cases, the delay preceding diapause induction is a developmental trap which results in the production of a latter generation before winter unable to survive or enter diapause (e.g. Musolin, 2007, van Dyck *et al.*, 2015; lost-generation hypothesis (Figure 2.4)). In this case, this delay could be a product of constraints (*i.e.* higher temperatures acting on plasticity expression) and not be adaptive for the insect. For example, Musolin (2007) states that the increase in temperature by 1-2°C in Osaka (Japan) region from the 1950s to the 1990s allowed the stink bug *Nezara viridula* (Hemiptera: Pentatomidae) to enter diapause later, leading to the production of a late generation in fall unable to survive winter. In all the regions where insects cannot overwinter out of diapause, delaying diapause is a developmental trap and a maladaptative phenotypic plasticity response. On the contrary, if insect populations from mild winter areas can remain active throughout winter (*i.e.* they can feed and reproduce), their capacity to delay or avert diapause may provide a selective advantage that could be maintained in the populations (Bale & Hayward, 2010).

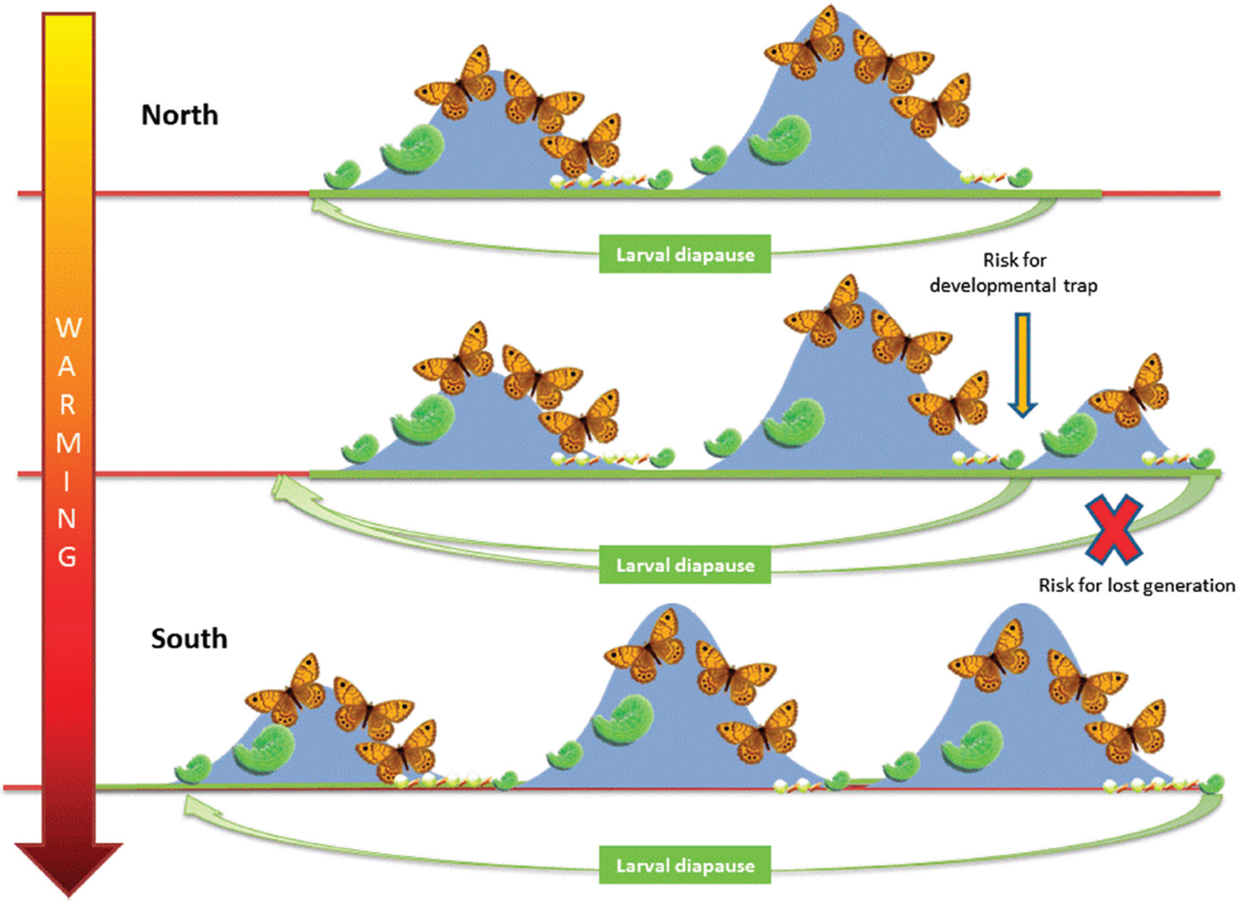

Figure 2.4: Illustration of the lost-generation hypothesis. The wall-brown butterfly *Lasiommata megera* has a larval diapause induction. At northern latitudes the species is bivoltine whereas it is trivoltine in southern and warmer parts of its distribution area. At mid-latitudes, it produces a partial third generation which is unable to enter diapause before winter, resulting in a developmental trap and important fitness costs for this generation. This example illustrates processes appearing through a product of constraints that could cause maladaptive responses in the context of climate change. From van Dyck *et al.* (2015).

b. Increasing maternal effects

Transgenerational plasticity (TGP) may become increasingly important for diapause induction in the context of longer growing season. Parasitoids need to fit more accurately seasonal changes by getting better proxies of upcoming winter or of the return of favorable conditions. Patterns of photoperiodic variations will remain unaffected by climate change but its reliability as an indicator of upcoming seasonal changes in temperature may be highly diminished. Then, in the context of climate changes, maternal perception of current temperatures or host availability could become a more adaptive way to induce and/or terminate diapause at the right period of the year (Mousseau & Dingle, 1991). Van Asch *et al.* (2010) showed that the winter moth *Operophtera brumata* (Lepidoptera: Geometridae) fitted its phenology to spatial changes in oak bud opening thanks to maternal effects on offspring developmental time, without the need for genetic changes.

However, in some areas, adults could be exposed to higher late-fall temperatures and may not induce diapause in their progeny whereas their offspring may still need to enter diapause to survive winter cold, leading to potential mismatches in the transgenerational transfer of environmental information and impact insects winter survival (Coleman *et al.*, 2014). There might thus be mother-offspring conflicts in overwintering strategies, which can trigger a loss of cold resistance during winter. Depending on environmental correlations between generations, maternal effects may either decrease or increase the rate of response to selection and thus accelerate or slow down diapause evolutionary changes to climate warming (Kirkpatrick & Lande, 1989). When there is environmental autocorrelation, TGP may be beneficial for the offspring whereas when the environment is unpredictable, the range of offspring phenotypes (e.g. diapause) should increase (Marshall & Uller, 2007, Sgrò *et al.*, 2016). In extremely disconnected mother-offspring environments, biotic (e.g. host availability) and abiotic constraints (e.g. warmer temperatures) may trigger transgenerational conflicts in overwintering strategies, potentially leading to the offspring death, as also proposed by the lost generation hypothesis.

Increase in extreme events frequency and environmental stochasticity

It has been first argued that yearly variation in thermal environment was too small to select for risk-spreading (Hopper, 1999). Thus, evolution of bet-hedging strategies has been overlooked in the context of climate changes (Sgrò *et al.*, 2016). To cope with a new selection pressure led by increasing environmental stochasticity (variability and unpredictability), mixed strategies such as bet-hedging strategies should become more frequent (Simons, 2011, Kivelä *et al.*, 2013). Bet-hedging strategies are likely to be widespread among univoltine insects with true obligatory diapause (e.g. Bradford & Roff, 1993, Menu *et al.*, 2000). Among these species, conservative risk-spreading strategies may arise with early obligatory diapause as well as delayed post-diapause emergence (Hopper, 1999). For species with facultative diapause, diversifying risk-spreading strategies may lead to variable diapause induction and termination thresholds within a population; individuals of the same genotype may or may not diapause under identical environmental cues (Hopper, 1999).

Increased climatic variability could select for increased phenotypic plasticity within a population (Vázquez *et al.*, 2015), with only a part of the population undergoing diapause whereas the other part is remaining active in the environment or entering adult quiescence, as observed in aphid parasitoids (Starý, 1970, Andrade *et al.*, 2016). This pattern is likely to occur in transition zones between a voltinism pattern and another where the evolution of phenotypic plasticity will be fostered and diapause induction may become a flexible conditional strategy (Blanckenhorn & Fairbairn, 1995). The proportion of parasitoids that enter diapause each winter should match the probability for a parasitoid to undergo harsh winters and experience host shortage, in an adaptive coin-flipping strategy (Hopper, 1999, Rajon *et al.*, 2014). This raises the question of the role of environmental predictability in the establishment of such mixed strategies (Burgess & Marshall, 2014), and bet-hedging strategies are thus strongly linked to maternal effects discussed in the precedent paragraphs.

Climate warming leads to a decrease in snow-cover, reducing its shelter effect on organisms (Zhang, 2005). Yet, most parasitoid species cannot use behavioral thermoregulation because they overwinter inside their immobile hosts or in the soil, but they can select overwintering sites to deal with microclimatic variability (Hance *et al.*, 2007). It is known that some insects such as the mymarid parasitoid *Anaphes victus* (Hymenoptera: Mymaridae) choose different sites to lay their diapausing eggs depending on the probability to be snow-covered in winter (Boivin, 1994). An increase in both mean winter temperatures and variability will decrease the proportion of sites which will remain protected all winter-long and may increase parasitoid mortality during diapause (Hance *et al.*, 2007), especially if reduced snow-cover leads to increasing exposure to freeze-thaw cycles and cold spells (Roland & Matter, 2016). In both obligatory and facultative-diapause species, unpredictable winter conditions such as the timing and location of snow-cover appearance should increase the occurrence of behavioral bet-hedging strategies concerning the choice of oviposition sites (e.g. Boivin, 1994).

Among the seasonal bet-hedging strategies, the prolonged diapause is perhaps the most well-known for many arthropods (Menu & Debouzie, 1993, Soula & Menu, 2003) and references therein), but may have been overlooked among parasitoids (Valera *et al.*, 2006). This diversified bet-hedging strategy (Menu *et al.*, 2000), with individuals of the population ending their diapause over successive years, insures the survival of at least a few individuals in case of a particularly catastrophic year (Ringel *et al.*, 1998). However, in favorable years, such individuals face ecological costs because they miss breeding opportunities and expose themselves to predators, parasites, and pathogens (Denlinger, 1981, Menu & Desouhant, 2002). Parasitoids enter and leave prolonged diapause when their hosts do so, showing that both the host and the parasitoid are often timed by the same external cues, potentially due to strong coevolution (Hanski, 1988, Corley *et al.*, 2004). We can expect that prolonged diapause occurrence and duration variability will increase in several insect species with increasing climatic variability and unpredictability, especially in winter (Hanski, 1988, Lalonde, 2004).

Foray *et al.* (2014) highlighted that a thermal specialist strain (stenotherm) of the parasitoid *Venturia canescens* (Hymenoptera: Ichneumonidae) was more harshly affected by temperature fluctuations than a generalist strain (eurytherm) for a set of life-history traits, highlighting that jack-of-all-trades is master of none. Evolution often tends to select for climate-specialist populations in high or low latitudes (Hoffmann, 2010, Nilsson-Örtman *et al.*, 2012), because adaptation to a wide range of temperatures is energetically costly, especially when temperature variations are low or only occur between generations (Hoffmann *et al.*, 2013). In eurytherm species, the diapause strategy could be lost more quickly because they will be able to survive warmer winters but harsher cold spells at the same time, without the need of entering diapause. More, they could track their host phenology more accurately than stenotherms which will need to adapt to new hosts thermal range preferences.

Consequences of changes in diapause expression

Predictive models on the consequences of global changes on insects often ignore trophic-level interactions within a food web, in which changes in phenology (such as changes in diapause expression), can ultimately translate into modifications in strength, occurrence, and frequency of the multitrophic interactions (Davis *et al.*, 1998, Thomson *et al.*, 2010, Gilbert *et al.*, 2014). Outstanding questions currently concern potential outcomes of shifts in species interactions on food-web functioning as well as on the efficiency of ecosystem services such as biological pest control (Gilman *et al.*, 2010, Chaianunporn & Hovestadt, 2015, Valiente-Banuet *et al.*, 2015).

Changes in community structure and food-web functioning

In a meta-analysis, Tylianakis *et al.* (2008) showed that every type of species interactions can be influenced by climate changes. Consequently, the effects of climate changes should be considered not only at the individual level (e.g. physiological acclimation and temperature stress) but also for the entire community (e.g. shifts in interactions involving more than two species) from the plant to the hyperparasitoid levels (van der Putten *et al.*, 2010, Thackeray *et al.*, 2016, Visser, 2016). What can be expected at the community level in response to rising temperatures is an increase in competition through trait convergence (Le Lann *et al.*, 2014a). It is also possible that interaction extinctions lead to functional loss through loss of functional redundancy in an ecosystem (Valiente-Banuet *et al.*, 2015). Novel interactions will appear, altering the abundances, distributions, and functions of species in a food web (Walther *et al.*, 2002, Gilman *et al.*, 2010), potentially leading to increased antagonism (predation, parasitism, or competition), and affecting partners' fitness because of the lack of co-evolutionary history (Gilman *et al.*, 2010). Moreover, when some species track changing temperatures better than their enemies or competitors, because of better adaptation for thermal tolerance or acclimation capacities, the occurrence and intensity of species interactions in a community may be modified. Such "ecological releases" are likely to occur in the case of range shifts (e.g. biological invasions) but also have to be considered regarding changes in phenology.

Novel interactions appearing through climate warming could involve trade-offs on some life-history traits (e.g. Andrade *et al.*, 2013), impossibility to search for microhabitats where the novel host live, or even an evolutionary trap if the novel host is behaviorally but not physiologically accepted by the parasitoid due to lack of adaptations to defend against host's immune system (Abram *et al.*, 2014). Moreover, because 'jack-of-all-trades is master of none', generalists may be disadvantaged if a new specialist species settles in the community (e.g. through changes in phenology) and better exploit the same resource. In insect communities, some species will stop entering diapause which could completely modify the food-web functioning if these species are key species in the community. For example, in the parasitoid community of cereal aphids in Western France, the generalist aphid parasitoid *Aphidius avenae* (Hymenoptera: Braconidae) has greatly reduced its winter diapause incidence because of warmer winters (Tougeron *et al.*, 2017a), and it became the dominant species in winter inducing the rarefaction of previously most abundant species *A. rhopalosiphi* and *A. matricariae* (Andrade *et al.*, 2016). If parasitoids become active in a period of the year at which they never appeared before, trophic niche could overlap with other parasitoids, creating higher intraguild competition.

There is no doubt that thermal tolerance capacities can shift asynchronously between different species between trophic levels (Hance *et al.*, 2007, van Baaren *et al.*, 2010). We argue that differences in thermal sensitivity will play a major role in determining the strength of species interactions by acting on species seasonal (or even daily) occurrences. For example, Dong *et al.* (2013) demonstrated that increasing temperatures led to cascading effects from plant phenology to aphid and parasitoid abundance in a natural ecosystem, and to asymmetric responses across trophic levels. It is also still unclear how parasitoids or hosts experiencing novel thermal and photoperiodic combinations may be affected in term of life-history traits and fitness and in what extent it could modify their interactions (Joschinski *et al.*, 2017, Tougeron *et al.*, in prep).

Finally, the role that bacterial endosymbionts may play in structuring communities (e.g. Sanders *et al.*, 2016) or shaping host-parasitoid interactions in a climate change context remains poorly understood. Endosymbionts effects on insect life-history traits and species interactions are very diversified (Oliver *et al.*, 2014). It appears that both obligatory and facultative bacterial endosymbionts are heat-sensitive and are commonly lost in insects that experience a heat shock (Hance *et al.*, 2007, Xin *et al.*, 2016). Moreover, their action on host-parasitoid interactions seem to be temperature and symbiont-dependent with some endosymbionts becoming less effective when temperature rises (Bensadia *et al.*, 2006, Cayetano & Vorburger, 2013). They influence host-parasitoid coevolution and may be consequently involved in their responses to climate change. However, it remains unknown if host and/or parasitoid endosymbionts could act on diapause expression (Pintureau *et al.*, 2003), even if symbionts may be involved in insect survival during overwintering for instance by improving thermal tolerance of their hosts (Kashima *et al.*, 2006, Dunbar *et al.*, 2007, Koehler *et al.*, 2013).

Impacts on biological control

Climate change is expected to increase damages caused by agricultural pests through increasing population growth, accumulative pest generations per year, facilitation of pest dispersion, earlier pest outbreaks, and both decreasing natural enemies efficiency and plant resistance (Thomson *et al.*, 2010, Klapwijk *et al.*, 2012, Björkman & Niemelä, 2015, Eigenbrode *et al.*, 2015, Pincebourde *et al.*, 2016). Climate warming might also be beneficial for biological control agents that will be able to find more hosts to parasitize and for a longer time window. For example, Van Nouhuys & Lei (2004) showed that warmer springs improved the synchrony of the specialist parasitoid *Cotesia melitaearum* with its host, the butterfly *Melitaea cinxia*, leading to increased parasitoid colonization at the metapopulation scale. An increase in parasitoids abundance and richness following increasing temperatures can lead to an overall enhancement of parasitism rate and a better biological control service (Péré *et al.*, 2013).

Absence of accurate temporal host-tracking by a specialized parasitoid may result in strong yield losses (Eigenbrode *et al.*, 2015). For example, Grabenweger *et al.* (2007) showed that parasitoids of the invasive horse-chestnut leaf-miner (*Cameraria ohridella*) broke diapause too early in spring, when no host available, resulting in poor synchronization between both species. Desynchronization may not be problematic for biological control when there is enough functional redundancy within a guild of parasitoid species to control a host (Hance *et al.*, 2007). It is thus crucial to (i) determine diapause levels, diapause induction thresholds and main mechanisms of diapause initiation in candidate species for introduction to control a local pest (Gariepy *et al.*, 2015) and (ii) include diapause (and diapause changes) in population dynamic models destined to inform pest control decisions (Lalonde, 2004). Simulation models on insect seasonality (e.g. Powell & Logan, 2005) and host-parasitoid interactions (e.g. Kalinkat & Rall, 2015) in response to temperature changes may provide great insight into pest and natural enemies phenological responses to climate change and help forecasting predictions on biological pest control.

At first sight, the loss or decrease of diapause expression in natural population of parasitoids could be advantageous because early suppression of pests is crucial for efficient biological control (Neuville *et al.*, 2015). However, change in food-web composition (see previous section) may decrease biological control efficiency through increasing intraguild competition or appearance of new antagonist species. For example, hyperparasitism levels tend to rise, even at seasons in which no secondary parasitoids were reported before (Tougeron *et al.*, 2017a), suggesting that the loss of diapause expression can occur across different trophic levels and that hyperparasitoid pressure may increase in winter, leading to poorer pest control by primary parasitoids (Gómez-Marco *et al.*, 2015).

Finally, manipulation of parasitoid diapause for inundative releases of biological control agents in the industrial context is an exciting field of research, and has been extensively discussed elsewhere (Denlinger, 2008, Colinet & Boivin, 2011). We expect parasitoid populations used for mass releases in biological control to suffer more from increasing climatic variability once released in the field because they are often reared under constant temperature conditions which may suppress their diapause and reduces their range of thermal tolerance.

Conclusion

Forecasting scenario on the evolution of insect seasonality is thorny because dormancy is not a simple binary active versus inactive state but rather a syndrome; *i.e.* a physiologically complex and dynamic alternative developmental pathway, part of a continued process within the insect life cycle, with varying characteristic throughout diapause induction, maintenance, termination and insect ontogenesis (Koštál, 2006, Wadsworth *et al.*, 2013). Further investigations on the cost-benefit balance of diapause are required as it will determine how insects will adapt their diapause timing and incidence under future environmental conditions. Parasitoids will obviously share common responses to climate-change with other insects but we highlighted that some of these responses are unique to parasitoids because of their particular lifestyle. We detailed throughout this review that different parasitoid "functional" groups may be more or less subject to shifts in overwintering strategies along with climate change. This framework includes univoltine *vs.* multivoltine species, generalist *vs.* specialist species for hosts and temperature, koinobionts *vs.* idiobionts parasitoids, host-diapause-conformer *vs.* independent cues received by the parasitoid, and differences in photoperiodic and thermal responses for diapause induction as well as different requirements for diapause termination.

Finally, future research will have to assess the relative importance and limits of local adaptation by genetic changes and both behavioral and physiological phenotypic plasticity in mediating the responses of parasitoid species and their interacting partners to climatic changes, focusing on their capacity to adopt new seasonal strategies and to change their phenology (Bradshaw & Holzapfel, 2006, Auld *et al.*, 2010, Merilä & Hendry, 2014, Duputié *et al.*, 2015). This could be achieved by experimental evolution set-ups, common garden or transplantation experiments, and exploring long-term datasets. Models can also help at determining whether a shift in phenology is heritable or not in a population (e.g. van Asch *et al.*, 2007).

In the light of the discussion, it appears that parasitoid species are affected in several aspects by current climate changes but we also highlighted different ways through which parasitoids could cope well with these changes. As a plausible scenario, we suggest that parasitoids will locally modify their phenology to fit their hosts' and new thermal environment and seasonal pace rather than moving towards more northern areas, as also predicted for other species (Parmesan & Yohe, 2003). We expect successful adaptation to rapid climate change to involve both rapid genetic shifts in diapause expression and plastic responses, including maternal effects, allowing them to successfully track their hosts and adapt to novel biotic and abiotic conditions. A better appreciation of the processes triggering insect phenology is needed to forecast the consequences of such changes on species interactions and synchrony across multiple trophic levels, community functioning and ecosystem services such as biological pest control (Forrest, 2016, Visser, 2016), especially because these responses might be taxonomically constrained (Thackeray *et al.*, 2016).

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Author contribution statement

JVB & JB initiated the review project. KT, JVB, JB, CLL wrote the manuscript. All authors disclose any potential sources of conflict of interest.

Partie II

La diapause chez les parasitoïdes du genre *Aphidius*

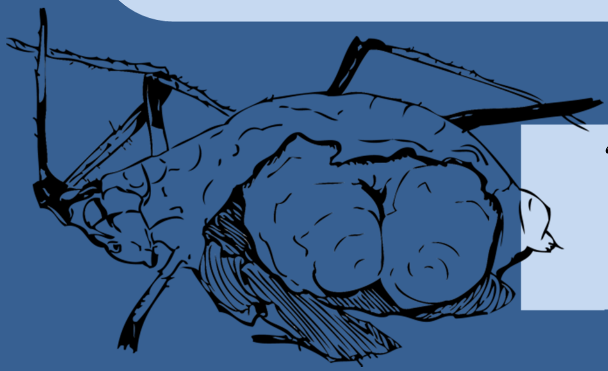
Chapitre 3 : Evolution des communautés hivernales
Chapitre 4 : Etude de l'incidence de la diapause en Bretagne
Chapitre 5 : Expérience de translocation

En bref :

- Les communautés d'hôtes et de parasitoïdes ont changé au cours de la dernière décennie ; de nouvelles espèces de parasitoïdes sont apparues (*Aphidius avenae* et *Aphidius ervi*) et les abondances relatives des espèces de parasitoïdes et de pucerons au sein du réseau trophique varient entre années. Aucune tendance claire liée à la température n'est toutefois décelée.

- Les deux espèces historiquement présentes en Bretagne en hiver (*Aphidius rhopalosiphi* et *Aphidius matricariae*) n'expriment plus de diapause lorsqu'elles sont soumises aux conditions de températures et de photopériode automnales qui prévalent dans cette région. Les deux espèces récemment apparues expriment de faibles niveaux de diapause. Les réponses de ces quatre espèces de parasitoïdes de Bretagne pourraient être dues au réchauffement climatique et à des adaptations locales au climat hivernal doux.

- Les parasitoïdes de Bretagne soumis au climat rude du Canada entrent en diapause en plus forte proportion qu'en Bretagne, mais en moins forte proportion que les populations canadiennes, démontrant que l'expression de leur plasticité est adaptée aux conditions climatiques de leur région d'origine respective. La capacité à exprimer la diapause n'est donc pas perdue, mais les seuils d'induction ne sont pas ou peu atteints pour les populations de Bretagne.



> *"When winter first begins to bite and stones crack in the frosty night, when pools are black and trees are bare, 'tis evil in the Wild to fare."*
>
> **J.R.R. Tolkien**, The Lord of the Rings

Chapitre 3

Evolution des communautés hivernales

Changes in host-parasitoid communities over the years in cereal crops of Western France: Does climate warming matters?

Kévin Tougeron, Maxime Damien, Cécile Le Lann, Jacques Brodeur & Joan van Baaren
En préparation

Note: This thesis chapter consists of preliminary analyses of host-parasitoid associations over recent years. More detailed analyses will be performed subsequently in order to link community data to climatic variations.

Summary

Consequences of climate changes can have a knock-on effect from individual to community scale. In particular, changes in species phenology are becoming frequent and modify the structure and composition of communities, with potential consequences on their functioning and the provision of ecosystem services. In mild climate areas, aphids can be present in cereal fields throughout the winter, which allows aphid parasitoids to remain active. Using a seven-year dataset of aphid-parasitoid winter trophic webs in cereal fields of western France, we first describe changes in community structure and composition across the years. We then explore the potential role of an increase in mean winter temperatures in observed community changes. We report that the community structure and composition that prevails before 2011 has recently shifted toward a more diversified community, with the presence of at least two new parasitoid species (*Aphidius ervi* and *Aphidius avenae*) and one aphid species (*Metopolophium dirhodum*). Strong bottom-up effects potentially determined the relative importance of parasitoid species because important compartmentalization with their hosts is reported. While changes in species occurrences are have been detected and probably resulted from milder climatic conditions, shifts in relative abundances across years could not be statistically linked to inter-annual changes in winter temperature. We suggest the recent changes in community composition to be linked to shifts in diapause expression (reduction or arrest of the use of winter diapause), and highlight the implications for natural biological control in cereal fields.

Key-words: Species diversity, Occurrence, Abundance, Aphids, Competition, Biological control, Diapause.

Introduction

There is large evidence of climate change impacts on the geographic distribution, diversity, abundance of organisms (Walther *et al.*, 2002, Parmesan, 2006). In particular, climate warming strongly influence their phenology and some species now migrate at different earlier in spring or later in fall, increase the number of generations per year, while some other reduce the duration or abandon diapause (Roy & Sparks, 2000, Altermatt, 2010, Bale & Hayward, 2010). Overwintering has been particularly neglected in the study of ecological responses to climate warming and few data are available, mostly due to the difficulty of winter field sampling. Yet, temperatures are increasing faster in winter than in summer, leading to overall milder, shorter and later winter periods in temperate areas (IPCC, 2014). Plastic or adaptive responses of organisms to new thermal environments could modify species interactions such as competition, predation and parasitism and impact the structure and stability of communities (Hughes, 2000).

In the context of global diversity crisis, studies increasingly focus on how trophic networks respond to global changes (Parmesan, 2006, Chaianunporn & Hovestadt, 2015). Indeed, species interactions within and between communities support the majority of ecosystem services and must be considered as study system *per se* (Montoya *et al.*, 2003). Food-webs structure and composition are quite fragile and are likely to change in the context of climate warming (Montoya *et al.*, 2006). Understanding how and why these food-webs vary in space and time is a central objective in understanding ecosystem responses to global environmental changes (Facey *et al.*, 2014). New-species appear, others disappear from the food-web and changes in species interactions between trophic levels occur (Chaianunporn & Hovestadt, 2015, Tylianakis *et al.*, 2008). However, our knowledge on the liability of ecological networks to change in time and space under climate-warming pressure still remains incomplete.

Parasites are very diverse and abundant in almost every food-webs (Dobson *et al.*, 2008) and their interactions with hosts greatly contribute to ecosystems functioning (Lafferty *et al.*, 2008). Tightly interacting species such as parasitoid insects and their hosts are likely to be influenced by recent climate change, threatening the provision of ecosystem services such as natural biological pest control (Hance *et al.*, 2007, Jeffs & Lewis, 2013). The impacts of climate-change or inter-annual variations in weather conditions on host-parasitoid communities remain little explored compared to other types of food-webs (e.g. plant-herbivores networks; Singer & Parmesan, 2010) and there has been few attempts at predicting their future structure and composition under different scenarios of climate change (Jeffs & Lewis, 2013).

In regions characterized by mild winter temperature, the absence of lethal frosts allows aphids and their parasitoids to overwinter as active adults. In cereal crops of Western France, aphid-parasitoid communities in winter were historically composed of two aphid species *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabricius) and two parasitoid species; *Aphidius rhopalosiphi* De Stefani-Perez and *Aphidius matricariae* Haliday. From late-spring to fall, additional species were present, including the aphid *Metopolophium dirhodum* (Walker) and the parasitoids *Aphidius ervi* Haliday and *Aphidius avenae* Haliday and (Rabasse *et al.*, 1983, Krespi, 1990, Krespi *et al.*, 1997). These seasonal variations in aphid and parasitoid species' occurrence seem to be consistent across Western Europe in cereal crops (Lumbierres *et al.*, 2007) and reflect thermal niche separation (Le Lann *et al.*, 2011c, Andrade *et al.*, 2016). *Aphidius avenae* has been shown to have less cold resistance and more heat resistance than *A. rhopalosiphi* (Le Lann *et al.*, 2011c).

Sitobion avenae prefers warm and dry microclimates as opposed to *R. padi* which prefers cool conditions (Jarošík *et al.*, 2003, van Baaren *et al.*, 2010, Alford *et al.*, 2016).

Recently, Andrade *et al.* (2016) showed that host-parasitoid communities of western France have undergone shifts in species composition and relative abundance, with parasitoid species usually not encountered during winter now being active throughout the season and exploiting anholocyclic aphids. They demonstrated that fine-scale intra-seasonal temperature variations (*i.e.* temperature experienced by the insect during its development) significantly influenced the relative abundance of aphids and parasitoids within the community. *Aphidius avenae* was more abundant at warm developmental temperatures and was suggested to supplant its competitor *A. rhopalosiphi*. Higher temperatures were associated with increasing abundances of *S. avenae* and a decrease in *R. padi* (Andrade *et al.*, 2016). In the present study, we extended the work of Andrade *et al.* (2016) to adopt a community-wide approach and analyze the effects of long-term (inter-annual) variations in temperature on aphid and parasitoid species occurrence and relative abundance. Using a 7-years dataset, we first describe temporal changes in winter aphid-parasitoid associations, and attempt to link these changes to modifications in mean winter temperatures.

Material & Methods

Data collection

Data consisted in aphid-parasitoid pairs of species gathered from different studies conducted in the Long Term Ecological Research ZA Armorique, France (48,29 °N - 1,35 °W), each winter from 2009/10 to 2016/17, at variable dates between 28th November and 14th March of each year (excepted in 2009/10; conducted between January and February). Data from winter 2009/10 to winter 2012/13 were obtained from Eoche-Bosy *et al.* (2016) and Andrade *et al.* (2016), data of 2013/14 from Tougeron *et al.* (2016), data from 2014/15 from Tougeron *et al.* (2017a), data of 2015/16 from (Damien *et al.* (2017) and data from 2016/17 from unpublished field results. Winter 2010/2011 was excluded from our analyses because no parasitoids nor aphids were found in the fields due to frost conditions during 15 consecutive days (Andrade *et al.*, 2016).

Sampling and quantitative food-webs

In each of these studies, sampling was performed following the protocol of Andrade *et al.* (2016), with differences in the location of sampled fields due to inter-annual culture rotations. In brief, sampling was conducted on a 10-days basis in at least six cereal fields each year; mainly winter wheat, but also barley and triticale. All aphids and aphid mummies (*i.e.* dead aphid containing a developing parasitoid) were collected during a one hour period over an approximate area of 1000 m². As the aphid-parasitoid network is stable over the sampling period (Andrade *et al.*, 2016, Damien *et al.*, 2017), data were pooled for the entire winter season. Live aphids were brought back to the laboratory and reared until mummification or death. All mummies were maintained at ambient temperature (17-20°C) until parasitoid emergence. Emerging adult primary parasitoids, as well as aphid hosts were then identified to the species based on morphological characters (Hullé *et al.*, 2006). Hyperparasitoids (secondary parasitoids) were identified to the genera level. Parasitoids that emerged more than 25 days after sampling, representing each year less than 25% of the total number of sampled mummies, were excluded from the food-web analysis to avoid accounting for diapausing individuals when characterizing winter-active communities. Important variations in sampling sizes are due to differences in sampling effort among years and to differences in climatic conditions. To examine trophic interactions between host and parasitoid species, quantitative food webs using the relative abundance (%) of each species were determined for each winter (Memmott *et al.*, 1994). Hyperparasitoids were put on the same trophic level than primary parasitoids because it was impossible to distinguish from which parasitoid host they came. Mean daily temperature data per sampling period were obtained from Météo France (2017).

Analyses

Food webs were compared among years using different quantitative and qualitative metrics calculated for each year (Dormann *et al.*, 2009): Connectance - the overall complexity of the food web (realized proportion of possible links); Web Asymmetry - the balance between numbers of parasitoid and aphid species (negative values indicate more higher than lower trophic-level species); H2 - the level of specialization within a network, from 0 (no specialization) to 1 (perfect specialization); Generality - the weighted mean number of aphid species exploited by each parasitoid species; Vulnerability - the weighted mean number of parasitoid species attacking a given aphid species.

A Principal Component Analysis (PCA) was performed to separate each year of sampling based on species relative abundance. Values of the first axis of the PCA were extracted and their correlation with mean temperatures was assessed using a Linear Model to determine if the separation between years on the graph was due to temperature variations. A coinertia analysis with a Monte-Carlo test was performed to link species relative abundance variations between years to mean temperature variations.

All analyses were performed using R software (R Core Team, 2017), the *ade4* (Dray & Dufour, 2007), the *bipartite* (Dormann *et al.*, 2009) and the *codyn* packages (Hallett *et al.*, 2016).

Results

Changes in species richness and relative abundances were observed from winter 2009/10 to winter 2016/17 in the host-parasitoid food-webs, with important inter-annual variations (Figure 1).

The year 2009/10 was similar to the past three decades (as presented in introduction) with *A. rhopalosiphi* and *A. matricariae* being the only two parasitoid species active in winter and exploiting *S. avenae* and *R. padi*. *Aphidius avenae* was observed for the first time in winter 2011/12 with 52% relative abundance. *Aphidius ervi* appeared in the network in 2013/14 with 5% relative abundance. Both species remained present in the network until now, although at variable relative abundances among years. *Aphidius rhopalosiphi* is present every winter while *A. matricariae* occurrence and relative abundance tend to decrease over the years. *Ephedrus plagiator* (Nees) and *Diaeretiella rapae* (M'intosh), two generalist species, were anecdotally reported in winter 2011/12 and 2012/13. Hyperparasitoid species were also present in two out of seven winters. The aphid *M. dirhodum* is also present every winter starting 2011/12 at 12%, and it represented up to 68% of the aphid species relative abundance in winter 2015/16 (Figure 3.1). Only parasitoids from the *Aphidius* genera were considered for further analyses.

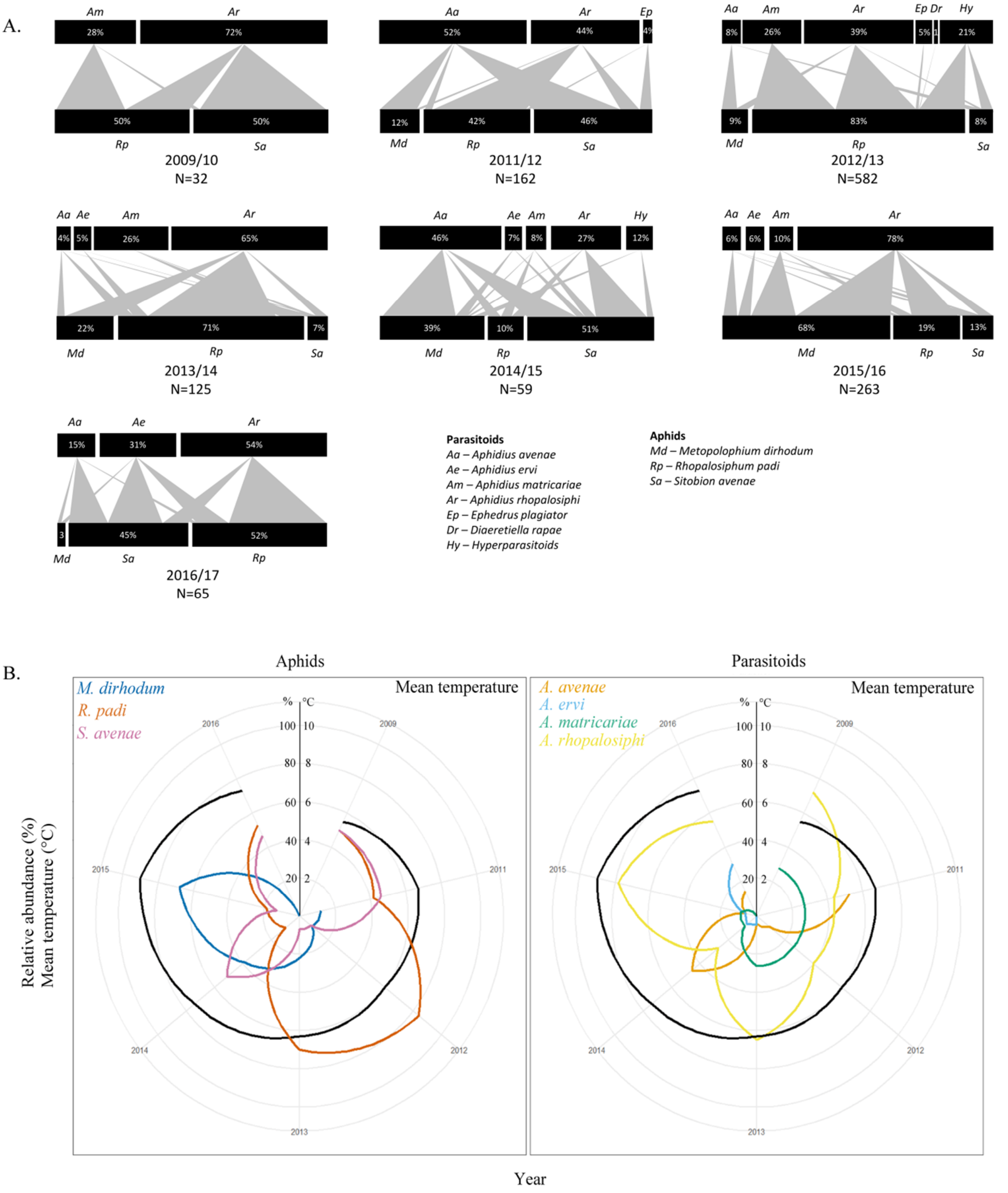


Figure 3.1: **A.** Quantitative food-webs of parasitoid and aphid community composition in winters 2009/10 to 2016/17 (there were no parasitoids in 2010/11). Upper and lower bars represent the relative abundance (%) of parasitoid and aphid species, respectively. The thickness of the arrows between bars is proportional to the interaction strength between a pair of species. Total number of individuals (N) used to construct each food-web is shown below each year. **B.** Rankplots showing aphid (left panel) and *Aphidius* parasitoid (right panel) relative abundances (%) each winter from 2009/10 to 2016/17. Mean winter temperatures (°C) are in black.

The food-web connectance was >0.6 every year; almost all possible links between each parasitoid and aphid species were realized. From 2012/13 to 2015/16, the food-web was asymmetric, with more parasitoid species than aphid species. The degree of specialization within the food-web (H2 index) tends to decrease over the years. Accordingly, the generality and vulnerability indexes tend to increase over time; each parasitoid species attacks more aphids and each aphid species is exploited by more parasitoid species, in general (Table 3.1).

Table 3.1: Number of species in each trophic level (primary parasitoids and aphids) and food-web metrics for each sampled winter.

Year	2009/10	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17
Number of parasitoid species	2	3	5	4	4	4	3
Number of aphid species	2	3	3	3	3	3	3
Connectance	1.00	0.89	0.60	0.83	0.92	0.92	0.89
Web Asymmetry	0.00	0.00	-0.25	-0.14	-0.14	-0.14	0.00
H2	0.53	0.71	0.41	0.36	0.24	0.22	0.34
Generality	1.63	1.43	2.18	2.41	2.67	2.26	2.25
Vulnerability	1.77	2.00	1.96	2.47	2.41	2.73	2.26

Preferential associations between hosts and primary parasitoids species occurred, as shown by the correlation circle of pooled data across years (Figure 2). The aphid *S. avenae* was mostly associated with the parasitoids *A. avenae* and *A. ervi* while the aphid *R. padi* was mostly associated with the parasitoids *A. rhopalosiphi* and *A. matricariae*. *Metopolophium dirhodum* was not preferentially associated with any parasitoid in the food-web.

Graphically, coldest winters seem to overall be associated with the co-occurrence and higher relative abundance of both *A. rhopalosiphi* and *A. matricariae* for parasitoids, and *R. padi* for aphids while warmest winters seem to be associated with co-occurrence and higher abundance of both *A. avenae* and *A. ervi* for parasitoids, and *S. avenae* for aphids. *Metopolophium dirhodum* was highly abundant in the warmest winter (2015/16) and mainly associated with *A. rhopalosiphi* (Figures 1, 2). However, the PCA is only supported by 41.8% inertia on the PC1, indicating that species separation on this figure is partially explained by the sampling year. Statistically, the repartition of the years across the PC1 was not correlated with mean winter temperatures (LM, t=0.20, R^2=-0.1, p=0.86) (Figure 3.2). We found no significant influence of mean temperatures on global aphid-parasitoid relative abundances across years (Monte-Carlo permutation test, p=0.38).

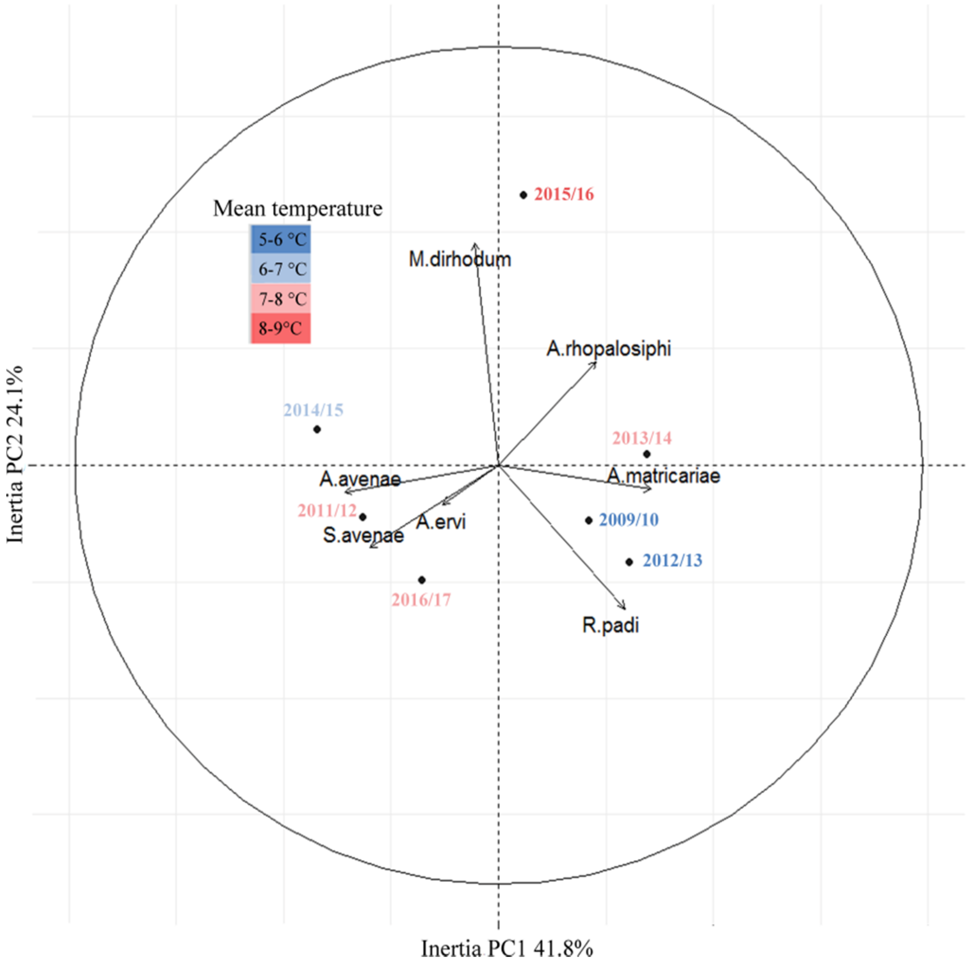


Figure 3.2: PCA separating aphid and parasitoid species relative abundances by sampling years. Colors indicate mean winter temperature over the sampling season for each year; from 5 to 9°C (refer to the color chart).

Discussion

Our results illustrate how winter climate warming could have rapidly, over a few years, translated into a community-level change in species composition. Two parasitoid species *A. avenae* and to a minor extent *A. ervi,* and one aphid species *M. dirhodum* are now active during winter along with other species of the community. The winter trophic network composition in cereal fields in Western France is getting similar to what is usually described in spring. The food-web is becoming more diversified in aphid and parasitoid species and, while the connectance (realized links) remains stable over time, the degree of specialization tends to decrease, suggesting that parasitoids exploit aphids in function of their relative abundance, as reported in spring (Andrade *et al.*, 2016). These changes in occurrence cannot arise from modifications in distribution range of the species, since *A. avenae*, *A. ervi* and *M. dirhodum* are commonly observed in spring at the same location (Krespi, 1990, Andrade *et al.*, 2016). Our results suggest a recent shift in overwintering strategy in *A. avenae* and *A. ervi* populations with at least some individuals remaining active throughout the winter rather than entering diapause. This hypothesis is supported by results from a laboratory experiment showing that diapause incidence in both parasitoid species was low (<15%), even when parasitoids were reared under fall-like temperature conditions (**Chapter 4**, Tougeron *et al.*, 2017a).

Changes in species composition in the food-web can be due to differences in thermal niche; the most cold-resistant species usually remained active during winter (e.g. *A. rhopalosiphi*, *A. matricariae* and *R. padi*) whereas less cold-resistant species (e.g. *A. avenae*, *A. ervi*, *M. dirhodum* and hyperparasitoids) were mostly active from spring to fall (Krespi, 1990, Le Lann *et al.*, 2011c, Alford *et al.*, 2016, Andrade *et al.*, 2016). Overwintering temperature may now be warm enough to allow niche overlapping of these species during winter. However, our data did not reveal a trend in species relative abundances change over the years; fine-scale temperature variations (Andrade *et al.*, 2016) rather than long-term variations may thus better explain species relative abundances. Other environmental variables may shape community dynamics across years, including species competition and host availability.

For instance, changes in species abundance could be due to interaction strength and to high host-parasitoid compartmentalization within the food-web. Indeed, there is a strong co-variation in abundances of host-parasitoid pairs over time, which could contribute to shape the structure of the community. We showed that the variation in relative abundance of some species is highly correlated with abundance of other species. Andrade *et al.* (2016) also demonstrated a strong compartmentalization of the species in the food-web, and that abundances of parasitoids were explained by both maximum temperatures experienced during their development and abundance of their hosts, suggesting bottom-up effects on parasitoid abundance, at short time-scales.

Modifications in parasitoid species relative abundances could also be due to shifts in competition for hosts after occurrence of new species in the food-web. For example, the recent addition of *A. avenae* in the overwintering food-web, which is a better competitor than other parasitoids at exploiting the aphid *S. avenae*, may have reduced the relative abundance of *A. matricariae* and *A. rhopalosiphi* (Eoche-Bosy *et al.*, 2016, Andrade *et al.*, 2016). Female parasitoids show seasonal variations in foraging behavior (Roitberg *et al.*, 1992) and can adapt their foraging strategies to competition or host-patch quality (Goubault *et al.*, 2004, Outreman *et al.*, 2005, Barrette *et al.*, 2010). This highlights the importance of host species in shaping parasitoid response to global change, which must be more thoroughly investigated (Barton & Ives, 2014, Chaianunporn & Hovestadt, 2015).

It has been shown that temperatures played a more important role than landscape features in shaping local aphid-parasitoid communities (Andrade *et al.*, 2015). However, numerous other factors than mean temperature may participate to inter-annual variations in species abundances, occurrences and species interactions during winter. Parasitoids and their hosts may for instance be influenced by microclimatic refuges in the landscape (Tougeron *et al.*, 2016, Alford *et al.*, 2017), by surrounding plant covers (Gagic *et al.*, 2012, Damien *et al.*, 2017), or by fine temporal scale variations in climate. Winter 2016/17 was on average warmer than other winters but important cold spells occurred in December and January, which may have conducted to higher abundances of *R. padi* and *A. rhopalosiphi*, the more cold tolerant species in the food-web (Le Lann *et al.*, 2011c, Alford *et al.*, 2016), and quasi-disappearance of *M. dirhodum* from the system, through environmental filtering. Such thermal extremes may reduce or eliminate any advantages of global warming for some species (Ma *et al.*, 2015, Sgrò *et* *al.*, 2016), and may impede proper evaluation of climate change effects on community dynamics.

Climate warming challenges the coexistence between ecologically related species and their interaction, which may in turn impact community stability and ecosystem functioning (van der Putten *et al.*, 2004, Tylianakis *et al.*, 2008). For instance, overwintering reproduction in parasitoids plays an important role in suppressing early cereal aphid populations (Langer *et al.*, 1997). Increasing number of parasitoid species during winter due to climate warming can enhance aphid natural biological control, but niche overlapping between parasitoid species may reduce its efficiency (Andrade *et al.*, 2016). The presence of non-diapausing hyperparasitoids, reported for the first time in winter in Western France in 2012/13 (Tougeron *et al.*, 2017a), may reduce the efficiency of biological control in the fields. For example, in Spain, characterized by relatively warm winters, hyperparasitism remains high throughout the year which disrupts biological control by primary parasitoids in orchards (Gómez-Marco *et al.*, 2015). With an expected temperature increase from 0.5 to 2°C in the next decades (Karl & Trenberth, 2003), occurrences of new species in food-webs can be more common, either through shifts in geographic distribution (e.g. biological invasions) or shifts in phenology (e.g. reduction of diapause expression).

Predictive analyses on the community structures should now integrate changes in overwintering strategies of one or more species along with climate change prediction at local scales to identify the potential effect of climate change in the ecosystem service provided by parasitoids. Next steps of this work will involve a more precise identification of the main biotic and climatic factors that shape community changes across and among the year as well as a predictive work on future changes of aphid-parasitoid community, using recent modelization and predictive tools (van de Pol *et al.*, 2016, Blonder *et al.*, 2017).

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Chapitre 4

Etude de l'incidence de la diapause en Bretagne

Are parasitoids from mild winter areas losing their winter diapause?

Kévin Tougeron, Cécile Le Lann, Jacques Brodeur & Joan van Baaren

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Summary

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 photoperiod 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.

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, 2014). 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 autumn 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 to 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 & Holzapfel, 2001, Vázquez *et al.*, 2015). Insect populations experiencing warmer winters could show (i) a delay in the onset of diapause, (ii) a decrease in the proportion of individuals entering diapause and, ultimately (iii), 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, 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 & Yohe, 2003, Andrade *et al.*, 2016) but are, to date, scantily documented in host/parasitoid systems (Hance *et al.*, 2007, Chaianunporn & 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 absence of winter diapause in the field: *Aphidius rhopalosiphi* 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 4.1). 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 4.1). 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 a 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: (i) are these populations expressing winter diapause in both the field and laboratory; (ii) if yes, under what condition of photoperiod and temperature; and (iii) in what proportion for each species and conditions? 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 thirty years ago in the same area and on the same species (details in Table 4.1). According to previous studies on these insect populations (e.g. Krespi *et al.* 1997; Andrade *et al.* 2016), the following hypotheses were tested: (i) the incidence of parasitoid diapause is higher at lower temperatures and photoperiods; (ii) the incidence of parasitoid diapause varies together with the seasonal occurrence of each species in natural environments, with *A. matricariae* and *A. rhopalosiphi* expressing no or little diapause, and *A. avenae* and *A. ervi* having the highest diapause incidence; and (iii) as a response to mild winters and the availability of hosts in winter, overall parasitoid diapause incidence is low in Brittany populations.

Table 4.1: Summary of results on overwintering parasitoids in Brittany, Western France in the 1970-1980s. 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 six 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.

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)		
Aphidius avenae[1]	4	none	Unspecified[a]	High: Adults rarely or never encountered in winter but present in late fall and early spring[ab]
Aphidius ervi	2	none	Unspecified[a]	High: Adults rarely or never encountered in winter but present in late fall and early spring[ab]
Aphidius rhopalosiphi[2]	29	427	Throughout winter (<1 month after sampling for most individuals)[ab] 29% required between 54 and 224 days to emerge[b]	Some individuals in diapause[ab] The rest overwinters as active adult on anholocyclic populations of aphids[a]
Aphidius matricariae	21	224	Throughout winter (<1 month after sampling for most individuals)[ab] 24% required between 124 and 427 days to emerge[b]	Some individuals in diapause[ab] The rest overwinters as active adult on anholocyclic populations of aphids[a]
Hyperparasitoids: *Asaphes sp.*, *Alloxysta sp.*, *Dendrocerus sp.*, *Phaenoglyphis sp.*	68	Very scarce, no abundance available	April to June[ab] Long delay of emergence (>100 days in most cases) Only 4 out of 68 emerged 14 to 50 days after sampling[b]	Almost all individuals enter diapause and emerge in spring or summer[ab]

[1]Formerly named *Aphidius picipes*. [2]Formerly named *Aphidius uzbekistaniscus*.
[a]Rabasse and Dedryver (1982); [b] Krespi *et al.* (1997).

Material & Methods

Meteorological data

We analyzed minimum, maximum and average daily temperature between (i) 1976-1985, and (ii) 2010-2015. These periods correspond to the range of sampling dates in (i) Rabasse and Dedryver (1982) and Krespi *et al.* (1997) and (ii) (Andrade *et al.*, 2016, Tougeron *et al.*, 2016) and the present 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

In order 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 follow (°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 m^2 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, Ø = 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 10 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 (50x50x50 cm) at 20 ±1°C, 70 ±20 % RH and 2 000 lx under a 16:8 LD photoregime in climate-controlled chambers.

Conditions for diapause induction

In order 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, 14:10 LD) with three temperature regimes (14°C, 17°C, 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 & McNeil, 1989a, Langer & Hance, 2000, Polgár & Hardie, 2000). Only five climate chambers were available at the same time. Thus, we first tested 5 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 4 treatments (20°C 12:12 LD / 17°C 10:14 LD / 17°C 12:12 LD / 17°C 14:10 LD) + 1 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 & 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 5 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, Ø = 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 ±10 %. Starting one 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 4.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 in order 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 dataset, 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 Core Team, 2017). The significance of each explanatory variable was tested with the 'Anova' function from the package *car*, using a F test for diapause quasibinomial data (Fox & 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 (Figure 4.1, Table 4.2). 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 4.2).

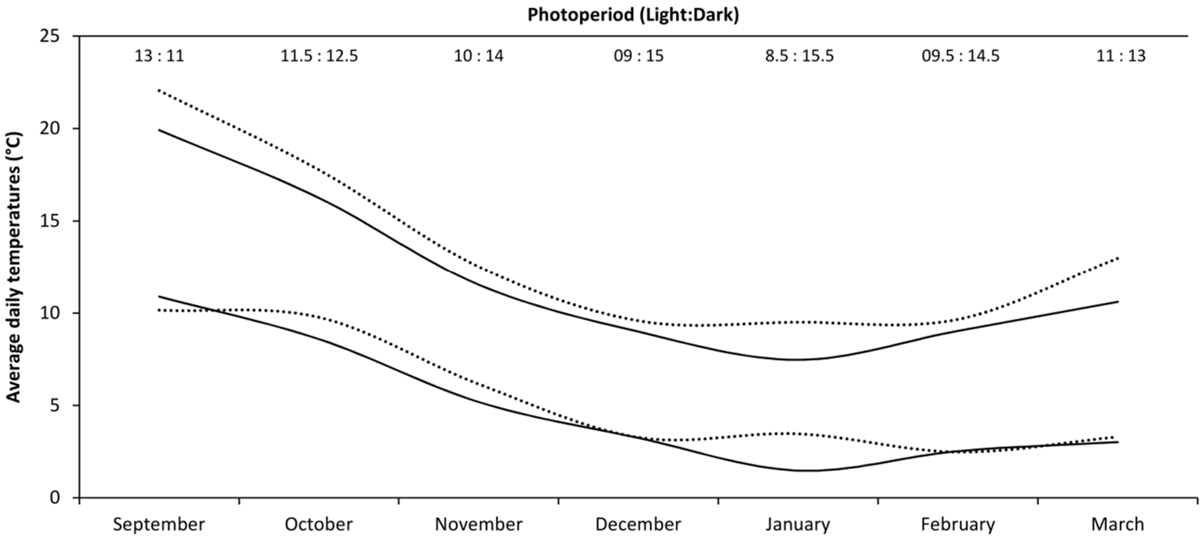

Figure 4.1: Average minimum and maximum daily temperatures from September 22 to March 20 calculated across two periods: 1975-1985 (solid lines) and 2010-2015 (dotted lines). The corresponding photoperiod (at 48 °N) is indicated for the 1st day of each month.

Table 4.2: Minimum, average and maximum daily temperatures and mean frequency of days with cold (≤0°C) and heat spells (≥22°C) (± 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		1976-1985		2010-2015		Average change	
Season		Fall	Winter	Fall	Winter	Fall	Winter
Temperature (°C)	Min average	6.55 ±0.16	2.44 ±0.15	7.02 ±0.23	3.26 ±0.19	**+0.47**	**+0.82**
	Average	9.73 ±0.15	5.45 ±0.13	10.50 ±0.22	6.58 ±0.16	**+0.77**	**+1.13**
	Max average	13.53 ±0.17	8.98 ±0.14	14.63 ±0.25	10.37 ±0.17	**+1.10**	**+1.39**
Mean frequency	Cold spells (≤0°C)	9.00 ±0.69	24.10 ±0.72	7.67 ±0.85	16.00 ±0.98	**-15%**	**-34%**
	Heat spells (≥22°C)	2.90 ±0.74	0.00	6.50 ±0.67	0.00	**+124%**	**0%**

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 to 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 than 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 2 *A. avenae*, 7 *A. ervi* and 9 hyperparasitoids from three genera (*Alloxysta* sp., *Asaphes* sp., *Phaenoglyphis* sp.*)* emerging more than two 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 (Figure 4.2). The two remaining mummies contained unidentifiable dead individuals.

The aphid-primary parasitoid food web in winter 2014-2015 (Figure 4.2) was dominated by *A. avenae* and *A. rhopalosiphi*. *Aphidius ervi* and *A. matricariae* represented 15 and 5% of the total parasitoid abundance, respectively. The three hyperparasitoid genera mentioned above were also detected at high abundances (21% of the food-web total abundance). *Sitobion avenae* and *Metopolophium dirhodum* (Walker) dominated the aphid community and *Rhopalosiphum padi* (L.) was present at a lower abundance.

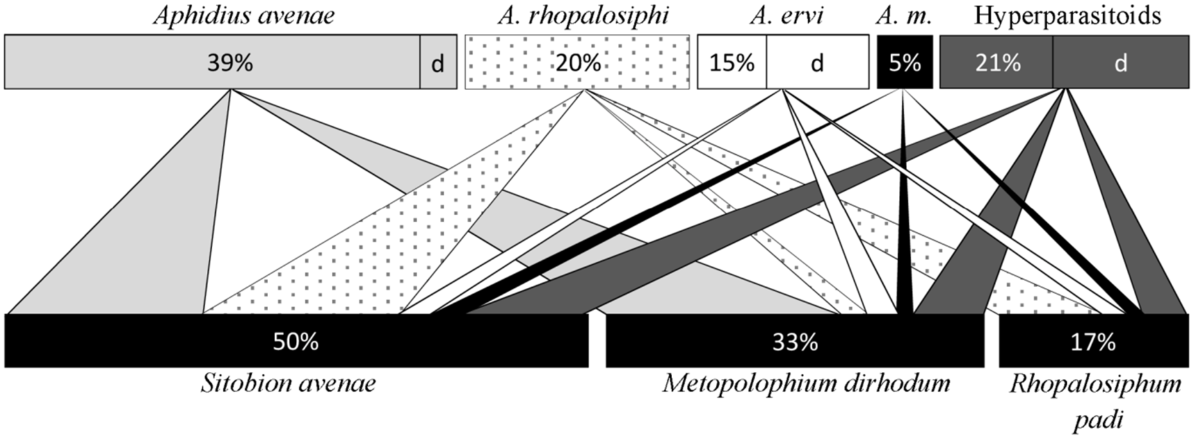

Figure 4.2: Quantitative food web showing the parasitoid and aphid community composition in winter 2014-2015 in cereal fields. Upper bars represent parasitoid of the genus *Aphidius* relative abundance. *A. m.* abbreviation stands for *Aphidius matricariae*. Percentages include diapausing and non-diapausing parasitoids, with lowerscript 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.

Diapause expression in the laboratory

A total of 1,482 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. rhopalosiphi* (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 ±1.6 %) (Figure 4.3a) and *A. ervi* (11.2 ±4.9 %) (Figure 4.3b).

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" conditions. 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 4.3), 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.

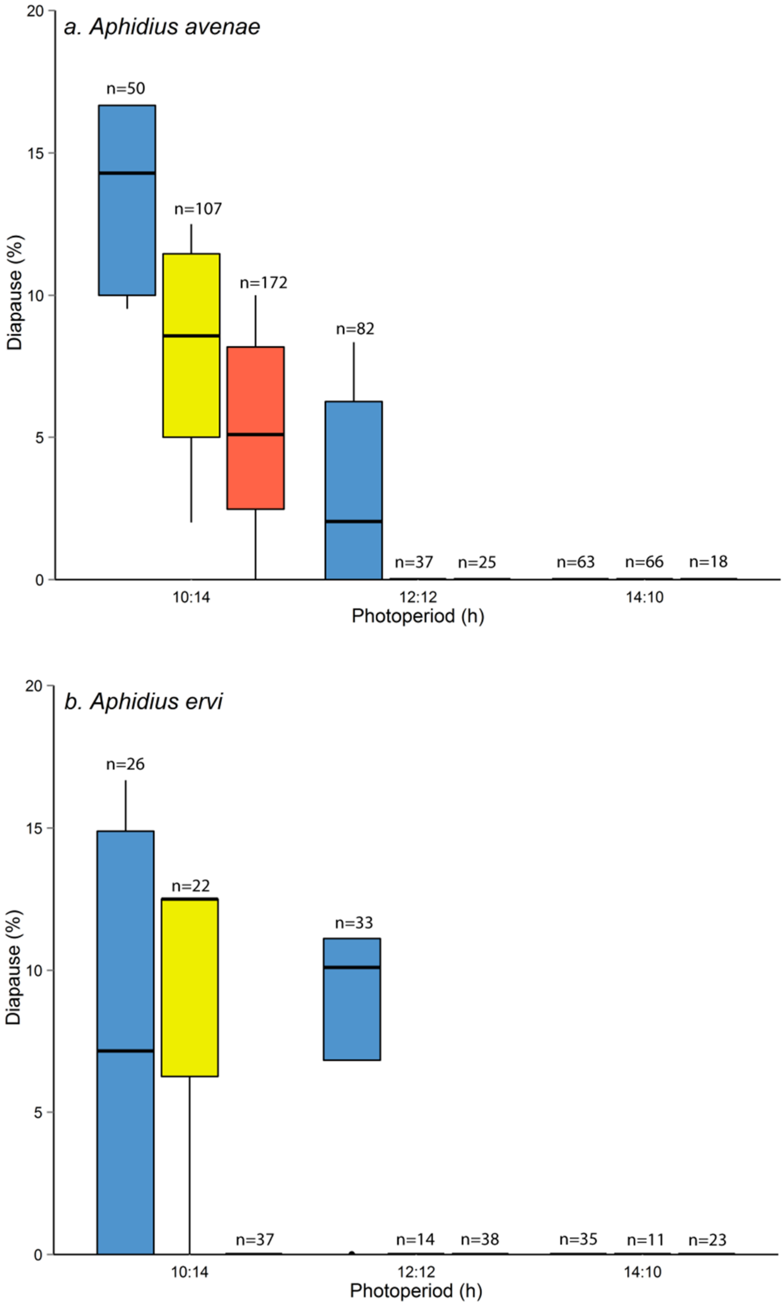

Figure 4.3: Prepupal diapause incidence (%) in Brittany populations of **(a)** *Aphidius avenae* (n=620, 85 dead, 513 emerged, 22 in diapause) and **(b)** *Aphidius ervi*, (n=239, 31 dead, 199 emerged, 9 in diapause) when parasitized aphids were reared under different photoperiodic (Light:Dark (hours): 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.

Table 4.3: Factors influencing diapause incidence of Brittany populations of *Aphidius avenae* and *Aphidius ervi*.

Factor \ Species	*Aphidius avenae*				*Aphidius ervi*			
	F	df	p > F		F	df	p > F	
Photoperiod	52.15	2	p<0.001	***	42.22	2	p<0.001	***
Temperature	13.62	2	p<0.001	***	61.29	2	p<0.001	***
Photoperiod x Temperature	1.87	4	p=0.14	NS	4.49	4	p<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.

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 little diapause expression. By comparing current diapause levels to those recorded thirty years ago in Western France (Table 4.1, Krespi 1990) and by analyzing current diapause levels in other aphid parasitoid species in from more northern or southern areas (Langer & 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. We provide 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 population 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 & 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, Tougeron *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, 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 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.*, 2011c). 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*: (i) 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. (ii) 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, Gariepy *et al.* (2015) showed that a strain of the parasitoid *Binodoxys communis* reared for 350 generations (≈ 7 years) in such conditions genetically lost 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 & 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 4.1). In the 1980s, *A. rhopalosiphi* 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 & Dedryver, 1982, Krespi, 1990, Krespi *et al.*, 1997). In contrast, we found 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 & 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.*, 1995a). 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 to 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 & Hardie, 1999). In *A. rhopalosiphi* 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 & 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 & Van Alphen, 2002, Ito, 2007). Alternatively, the cost of diapause may increase with climate warming (i) 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 & Copland, 2005), and (ii) 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 & 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 analyzes 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.

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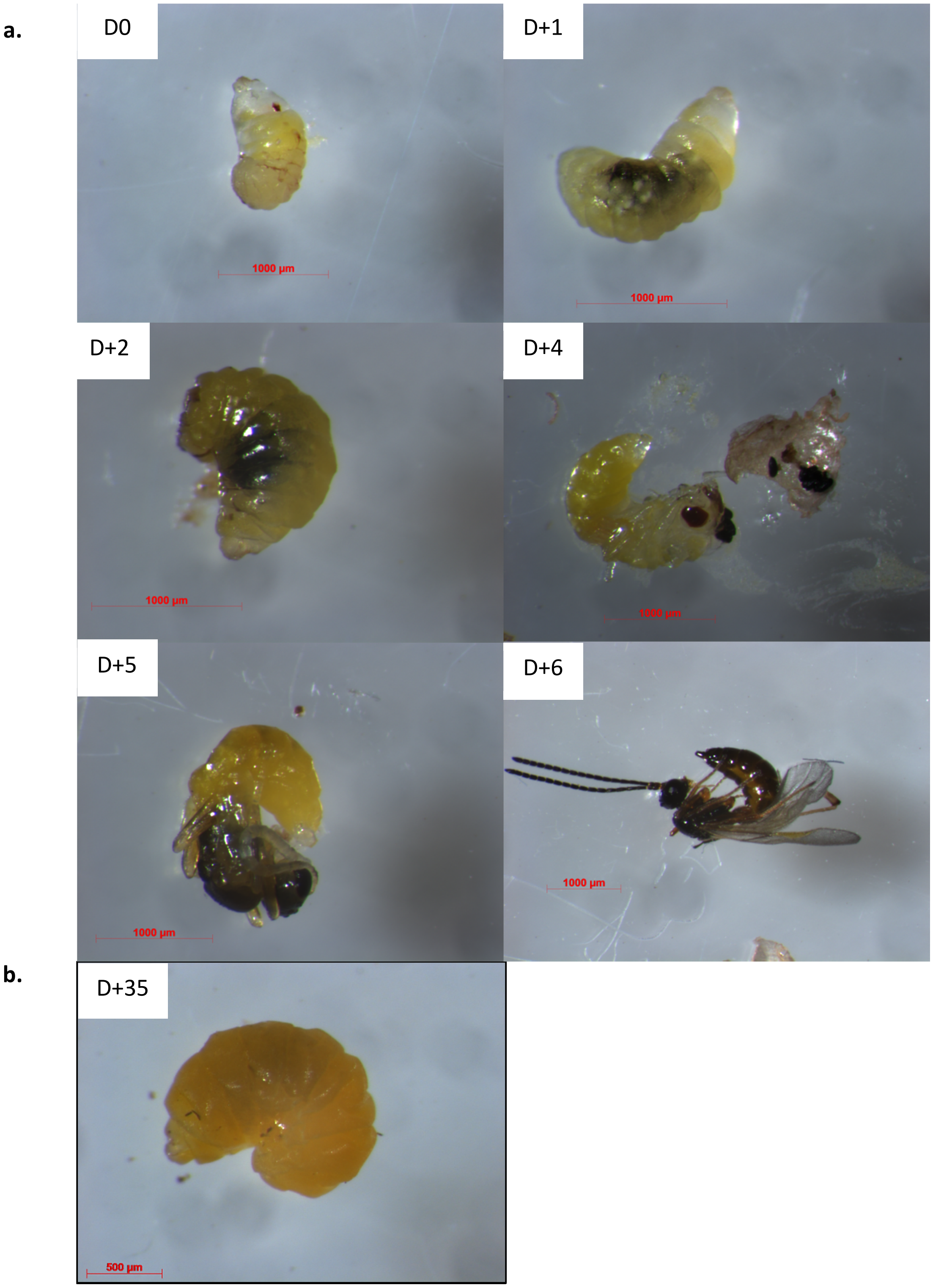
Appendix 4.S1: a. Typical life forms during development of non-diapausing parasitoids within the mummy. *Aphidius ervi* mummies were taken from the colony from Day 0 to Day 6 after mummification at 20°C, 16:8 h LD. All individuals were alive when the mummy cuticle was opened. Larvae are light-yellow. Egg to mummy development usually takes 9 days and mummy to adult emergence takes 5 more days ±2 days at 20°C in *Aphidius ervi.* **b.** Diapausing prepupae of *A. ervi* 35 days following mummification; the body is fat and golden-yellow and the meconium had been rejected inside the mummy. For a complete description and pictures of larval morphology and development of *Aphidius sp.*, see Muratori *et al.* (2004).



Chapitre 5

Expérience de translocation

Disentangling plasticity from local adaptations in diapause expression of parasitoids from and within contrasted thermal environments

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En préparation

Summary

An important question in evolutionary ecology is to understand the drivers of phenotypic variations in contrasted environments. Disentangling plasticity from evolutionary responses in such contexts allows a better knowledge of how organisms adapt their phenotypes to changing climates. Various aspects of the seasonal ecology of insect populations are essential for their persistence in the environment, including their capacity to overwinter. Phenotypic plasticity should allow insects to adjust their locally adapted diapause levels to actually experienced environmental conditions. Using an outdoor reciprocal translocation experimental design, we compared diapause expression of Canadian and French populations of two aphid parasitoid species of the genus *Aphidius* in both their respective and foreign bioclimatic regions, thus varying temperature conditions under a similar latitude (*i.e.* same photoperiod). From June to December 2016, diapause and mortality levels were recorded every two weeks. We found different Genotype × Environment reaction norms between parasitoid populations and experimental locations. For both parasitoid species, the incidence of diapause was higher in Canadian populations (up to 90%) than French populations (<20%) at either location, suggesting local adaptations to harsh (Canadian populations) or mild (French populations) winter climatic conditions of their area of origin. Phenotypic plasticity played an important role in mediating diapause incidence at different temperatures but similar photoperiod, as diapause was induced at higher levels in Canada than in France, independently of parasitoid's origin. There were important mortality levels (up to 90%) from October to December in populations of both species, highlighting timing costs, probably due to lethal frosts in Canada and high energy expenditure in France. We conclude that both plastic and evolutionary responses may be involved in the adaptation of parasitoids' overwintering strategies to ongoing climate warming.

Keywords: Climate change, Translocation Experiment, Transplant, Adaptation, Reaction Norms, Parasitoids.

Introduction

Understanding the basis of phenotypic variations across varying environments is an important question in evolutionary ecology. Reaction norms (RN) describe the degree to which a genotype (G) responds, in plastic way, to environmental changes (E), in a manner that G x E interactions can be measured (Via & Lande, 1985, Scheiner, 1993). This implies that organisms' trait expression, and *in fine* their fitness, can change with the environmental conditions at which they were selected (level of the RN) and which they actually experience (slope of the RN) (Ayrinhac *et al.*, 2004, Nussey *et al.*, 2007). Organisms can respond to environmental changes through genetic evolution or plasticity (Parmesan, 2006, Sgrò *et al.*, 2016). Disentangling the relative effects of plastic and evolutionary responses to environmental changes is crucial if we aim at predicting how organisms will face ongoing global climate changes (Gienapp *et al.*, 2008, Merilä & Hendry, 2014). Transplant (*i.e.* translocation) experiments between contrasted environments provide interesting ways to study RN and to infer organisms' responses to climate changes (Kawecki & Ebert, 2004, Hoffmann & Sgrò, 2011).

Diapause is a well-documented example of developmental plasticity, allowing insects to cope with variable environments (Nylin & Gotthard, 1998). Insects from temperate regions typically enter diapause in response to decreasing photoperiod that reliably indicates unfavorable conditions to come (Tauber *et al.*, 1986). However, the photoperiodic response is often modulated by temperature and other environmental factors (Danks, 1987) and climate warming would diminish the reliability of photoperiod as a predictor of winter onset, leading to potential phenological mismatches (Bale & Hayward, 2010, van Dyck *et al.*, 2015, Forrest, 2016). It is thus necessary to separate the respective effects of photoperiod and temperature when studying diapause reaction norms across environmental gradients. Few studies have examined the consequences of photoperiod-based adaptations, such as diapause, when organisms face new climates (Grevstad & Coop, 2015).

Timing is of the essence since too early or too late diapause induction can lead to impair insect fitness and induce mortality (Sgrò *et al.*, 2016). Insect populations minimize such timing costs by adapting diapause incidence and initiation to local environmental seasonal variations (Danks, 1987). Therefore, for a given insect species, there are geographic clines in diapause expression; diapause being induced earlier and at lower environmental threshold in northern populations (Winterhalter & Mousseau, 2007, Hut *et al.*, 2013, Paolucci *et al.*, 2013). A few transplant experiments in the context of diapause study (e.g. Chen *et al.*, 2014) have been conducted but, to our knowledge, no previous study on diapause RN has held the photoperiodic environment constant while varying the thermal environment. The experimental design described below allows disentangling local adaptations and plasticity in diapause expression without confounding the effects of temperature and photoperiod.

This study focused on parasitoids species of the genus *Aphidius* (Hymenoptera: Braconidae); *Aphidius ervi* (Haliday) and the sister species *Aphidius rhopalosiphi* (Haliday) / *Aphidius avenaphis* (Fitch). Photoperiod and temperature interact to induce diapause in these species (Brodeur & McNeil, 1989a, Tougeron *et al.*, 2017a). The aim of this study was to investigate and compare the RNs of diapause expression in multivoltine populations of aphid parasitoids from contrasted winter climate areas (Canada *vs.* western France) in outdoor semi-experimental conditions across the season for diapause induction. In this longitudinal translocation experiment, we tested parasitoids' species and population responses to fall and winter climatic conditions to which they were not adapted, in order to measure the degrees of local adaptation and plasticity in diapause expression. In their native environment, Canadian parasitoids start to enter winter diapause in mid-July and at 100% incidence before lethal frosts occur (Brodeur & McNeil, 1994). In contrast, in western France parasitoids of some species can remain active throughout winter, at least for a part of the population, due to mild temperatures and host availability (Krespi *et al.*, 1997, Andrade *et al.*, 2016).

We hypothesized that; (i) Canadian populations enter diapause at higher levels and earlier in the season than French populations, due to adaptations to conditions of their area of origin; (ii) Diapause levels are higher and parasitoids enter diapause earlier in Canada than in France, regardless of the origin of the parasitoid population, due to phenotypic plasticity to local temperatures; (iii) Mortality levels are higher for parasitoid populations tested in the foreign location.

Material & Methods

Meterological data

Minimum, mean and maximum temperatures were recorded every hour throughout the experiment using LOG32 data logger (Dostmann, Germany) in Brittany and HOBO data logger (Onset, MA, USA) in Canada directly placed in one of the outdoor experimental cages. Cages did not produce any greenhouse effect since temperature was similar to outdoor weather stations close to the experimental sites. Data for each date was averaged using data of the following 14 days to have reliable information on climatic conditions experienced by developing parasitoids.

Biological material

Four aphid parasitoid populations of two different species were collected from cereal fields in 2015. One population of *Aphidius ervi* was collected in Québec, Canada (45.58°N, 73.24°W) and one population was collected in Brittany, France (48.11°N, 1.67°W). *Aphidius rhopalosiphi* (Eurasian species) and *Aphidius avenaphis* (North America species) are phylogenetically, ecologically and morphologically closely related species (Kos *et al.*, 2011, Tomanović *et al.*, 2013) and were considered as sister species in this study. *Aphidius rhopalosiphi* parasitoids were collected in Brittany, France, and *A. avenaphis* parasitoids were collected in Saskatchewan, Canada (52.21°N, 106.66°W). Parasitoid colonies were maintained in climatic chambers at 20°C, 16:8 h LD photoperiod and 75% Relative Humidity (RH) on cereal aphids colonies (*Sitobion avenae* (Fabricius)) collected either in Brittany (for the maintenance of and experiments on French parasitoid populations) or in Québec (for Canadian parasitoid populations).

Experimental design

Experiments were conducted every 14 days from 20th June to 5th December, 2016 in outdoor mesocosms in Montréal 45.55 °N, 73.55 °W (QC, Canada) and Rennes 48.11 °N, 1.65 °W (Brittany, France), thus providing approximately the same photoperiodic regime (similar latitude) in both locations across the season. In France, experiments were not performed from 4th July to 29th August (4 tested periods) because no diapause was expected prior to this period and to avoid measuring summer diapause (Krespi *et al.*, 1997). The first observation (20th June) was done in France to obtain a common starting point with Canada. The Canadian population of *A. ervi* could not be tested on the 24/10 in France.

Four days before each date of outdoor releases, fifteen parasitoid females and five to ten males from each population, less than 48 h old, were taken from the colony and placed during 24 h in three different plastic tubes (h=10 cm, ⌀=3 cm) at a ratio of 5 females for 3 males for mating and with a dilution of honey for feeding. Parasitoid females were then placed in three fine netting cages (30x30x30 cm) in presence of 200 to 300 second and third instar larvae *S. avenae* aphids on a wheat pot for 60 to 72 h, with a dilution of honey. Female parasitoids were then removed from the cages and the three wheat pots with parasitized aphids were put together in a new fine netting cage. The four cages (one per population), protected from direct rain by a plastic roof, were put outside in a semi-shaded place, and this procedure was repeated every two weeks in both locations.

Twice a week, the formation of mummies (*i.e.* dead aphid containing a parasitoid prepupa or pupa) was checked in each cage. Mummies were collected from the plants and were individually put in 1.5 mL plastic microtubes with a tiny hole on the top for venting and were let in their respective cages. Adult emergence from the mummies was checked three times a week and mummies from which no parasitoid emerged within 15 days after the last adult emergence were dissected (or 45 days after mummy formation when no emergence occurred in a cage). Contents from the mummies' dissections were categorized as dead parasitoid or gold-yellow diapausing prepupa (Tougeron *et al.*, 2017a).

Statistical analyses

The origin of parasitoid population and the area where experiments had been conducted were combined to create a variable representing each parasitoid population at each location (Canada/Canada, Canada/France, France/France and France/Canada). For each species separately (*A. ervi* and the sister species *A. rhopalosiphi* / *A. avenaphis*), a Generalized Linear Model was fitted to the data to test for differences in diapause incidence on the combined variable (parasitoid origin and tested location), the photoperiod, the temperature and the interaction effects of these factors. For each species, data was split between population origin (French or Canadian) and experimental location (France or Canada) and GLMs were fitted to this data. The same procedure was used to analyze mortality levels. Minimal temperatures appeared to better explain diapause and mortality levels across the season than mean and maximum temperatures; this factor was thus kept in the final models. The significance of each explanatory variable was tested with the 'Anova' function from the package *car* in R (R Core Team, 2017).

Results

Mean temperatures were almost equal in both areas until early October, but minimal temperatures were on average 6.5°C lower in Canada than in France across the experimental season. Temperatures in France were more buffered (lower min-max amplitude) than temperatures in Canada which also dropped faster in fall. Negative temperatures were only encountered a few days in late November in France (down to -2.5°C) while several frost events (down to -23.5°C) occurred almost every day in Canada from early October (Figure 5.1). First snow fall occurred on 20th November in Canada. There was no snow in France during the time of this experiment. Relative humidity was on average higher in France (88.16 ±1.69%) than in Canada (70.54 ±2.26%). Daylength at 46.50°N (representative of both experimental locations) decreased from 15.7 h to 8.8 h between the 20th June and the 5th December.

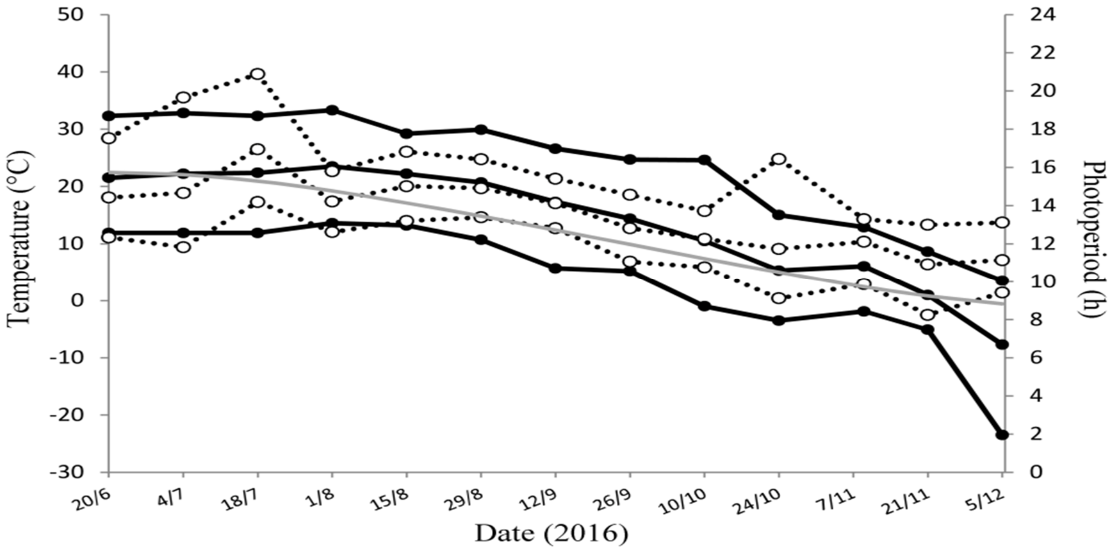

Figure 5.1: Minimum, mean and maximum temperatures (°C) in Montréal, QC, Canada (solid lines) and Rennes, Brittany, France (dotted lines) from 20th June to 5th December, 2016. Data shown at each date is the average of temperature data for the 14 days following this date. Photoperiod at 46.50°N is shown for each date (grey line).

The total incidence of diapause was significantly different among population origins and experimental locations, for both species (*i.e.* among each curve on Figure 5.2) (GLM, LR=43.2, df=3, p<0.001 and LR=45.0, df=3, p<0.001, for *A. ervi* and *A. rhopalosiphi* / *A. avenaphis*, respectively), as described below. Figure 5.3 shows mean reaction norms of each population in the origin or foreign climatic area, for each parasitoid species. Basal levels of Canadian parasitoids' reaction norms were higher than French populations. The slope of the reaction norm was higher for *A. ervi* than for other species, for both French and Canadian populations (Figure 5.3).

Canadian populations of both species had overall higher diapause levels than French populations when tested in Canada (GLM, LR=20.0, df=1, p<0.001 and LR=19.0, df=1, p<0.001, for *A. ervi* and *A. rhopalosiphi* / *A. avenaphis*, respectively) and in France (GLM, LR=10.1, df=1, p<0.005 and LR=36.4, df=1, p<0.001, for *A. ervi* and *A. rhopalosiphi* / *A. avenaphis*, respectively) (Figures 5.2 and 5.3).

Diapause levels were higher for *A. ervi* tested in Canada than in France for both the Canadian-origin populations (GLM, LR=8.25, df=1, p<0.005) and the French-origin population (GLM, LR=13.4, df=1, p<0.001). There were however no significant differences in diapause levels between experimental locations (France *vs.* Canada) for *A. avenaphis* (Canadian-origin population, GLM, LR=0.05, df=1, p=0.83) and *A. rhopalosiphi* (French-origin population, GLM, LR=2.9, df=1, p=0.09).

Canadian populations entered diapause up to a maximum of 75.5% and 27.8% for *A. ervi*, and 91.8% and 61.8% for *A. avenaphis*, in Canada and in France, respectively. French populations entered up to a maximum of 17.1% and 3.7% for *A. ervi*, and 7.5% and 12.0% for *A. rhopalosiphi*, in Canada and in France, respectively. In Canada, diapause peaks (*i.e.* mean maximum) occurred on the 24th October for Canadian populations while it occurred one month later for French populations (21st November). In both Canada and France experimental locations, first entrance in winter diapause was observed earlier for Canadian-origin parasitoids (as early as 20th June) than for French-origin parasitoids (not earlier than 26th September, except for *A. rhopalosiphi* on 20th June, see discussion).

Diapause levels increased with the photoperiod decrease over the season (GLM, LR=17.1, df=1, p<0.001 and LR=7.2, df=1, p<0.005 for *A. ervi* and *A. rhopalosiphi* / *A. avenaphis*, respectively). There was however no effect of photoperiod on diapause incidence for the French *A. ervi* population tested in France (GLM, LR=19.1, df=3, p<0.001) because almost no diapause was expressed over the season. The effect of photoperiod was not different between populations and locations for *A. rhopalosiphi* / *A. avenaphis* (GLM, LR=7.1, df=3, p=0.07).

The decrease in minimal temperatures had a small effect on increasing *A. ervi* diapause (GLM, LR=4.4, df=1, p=0.046). This effect was the same for parasitoid origin and experimental location (GLM, LR=2.54, DF=3, p=0.47). Minimal temperatures had no significant effect on *A. rhopalosiphi* / *A. avenaphis* diapause (GLM, LR=1.3, df=1, p=0.26) for parasitoid origin and experimental location (GLM, LR=1.2, DF=3, p=0.75).

Diapause levels decreased at the end of the season (late October to December) while mortality levels increased in both Canadian and French populations, especially when reared in Canada (between 59 and 95% mortality in early December). Mortality levels were overall significantly different among parasitoid population origins and location of the experiment (GLM, LR=12.7, df=1, p<0.01 and LR=39.0, df=1, p<0.001 for *A. ervi* and *A. rhopalosiphi*/*avenaphis*, respectively). Mortality levels were influenced by minimal temperatures (GLM, LR=36.1, df=1, p<0.001 and LR=17.0, df=1, p<0.001). Notably, in France, end-season mortality levels were higher for Canadian parasitoid populations than for French populations. Reversely in Canada, end-season mortality levels were higher for French populations than for Canadian populations, for both species.

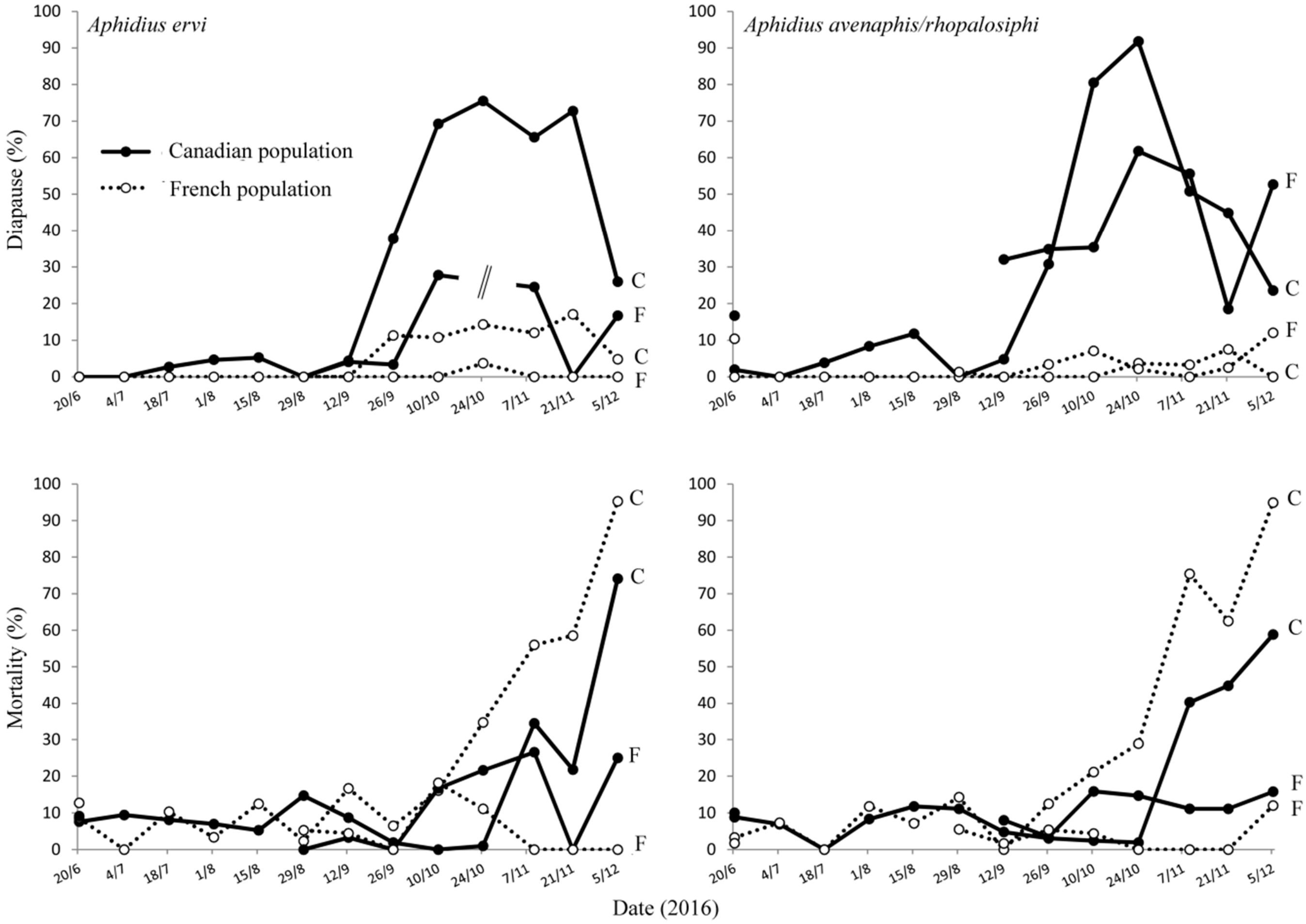

Figure 5.2: Seasonal levels of diapause and mortality recorded every two weeks from 20th July to 5th December (*i.e.* representing changes in photoperiod over the season), 2016 in the Canadian (solid lines) and France (dotted lines) originated parasitoid populations of *Aphidius ervi* and *Aphidius avenaphis / Aphidius rhopalosiphi*. Letters on the right of each panel indicate the location of the tested population: Canada (C) and France (F) (*i.e.* representing different thermal environment). Experiments in France started on the 20th June to have an initial common date with experiments conducted in Canada, but were interrupted until the 29th August. Percentages were calculated on a minimum of 25 individuals for each data point (excepted on the 5th December in Canada where 7 to 12 mummies were sampled).

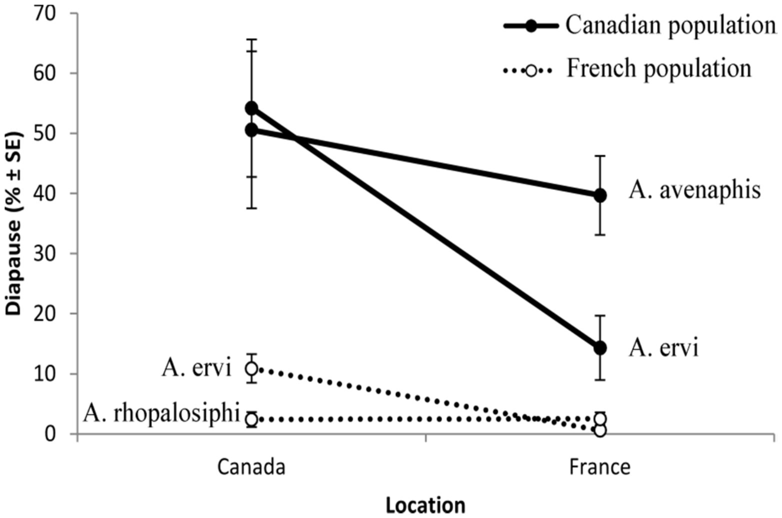

Figure 5.3: Reaction norms of diapause incidence (mean % ± SE) of Canadian (full line) and French (dotted line) populations of *A. ervi* and *A. avenaphis* / *A. rhopalosiphi* reared in outdoor location either in Canada or in France. Mean diapause levels were calculated from 12th September to 21st November (*i.e.* over the experimental dates in common between France and Canada). Data from the 5th December was excluded due to too important mortality levels which were artificially dropping diapause levels. N>600 mummies collected for each population in Canada, and N>350 in France.

Discussion

In the context of climate changes, animal populations will increasingly be exposed to environmental conditions to which they are not currently adapted. We provide evidence, using parasitoid insects, that both plasticity and local adaptations can be involved in insect response to varying climates, suggesting that might also be the case in response to climate change. This translocation experiment allowed separating the effects of local adaptations from those of plasticity, as well as the effects of photoperiod and temperature on diapause incidence. As predicted, aphid parasitoid populations originating from the harsh winter area (Canada) expressed higher levels of diapause than populations from the mild winter area (Brittany), when tested either in France or in Canada (as seen by levels of the RN). This first pattern reflects adaptations to climatic conditions of their respective area of origin. Diapause incidence was overall higher for parasitoids tested in Canada than in Brittany (as seen by slopes of the RN), especially for *A. ervi*. This second pattern highlights plastic responses of diapause expression under different thermal environments.

The relative importance of photoperiod and temperature at inducing diapause can differ between populations of a same species because the reliability of these stimuli may vary among regions (Piercey & Maly, 2000, Pruisscher *et al.*, 2017). Northern populations normally enter diapause under strict photoperiodic control and earlier than southern populations where diapause may be more sensitive to modulation by temperature (Hut *et al.*, 2013, Paolucci *et al.*, 2013). We observed that, at a given date (*i.e.* a specific photoperiod) and for a given location (*i.e.* a specific temperature regime), Canadian parasitoid populations entered diapause at higher levels and earlier than French populations. In France, Canadian populations entered diapause at important levels (up to 27.8% for *A. ervi* and 61.8% for *A. avenaphis*) even if temperatures were warm enough for continuous development and adult survival (developmental thresholds are around +3°C in this genera; Sigsgaard, 2000, Zamani *et al.*, 2007), suggesting a strong photoperiodic effect in harsh winter populations. On the other hand, French parasitoids expressed overall low diapause levels in both Canada and France, suggesting a weak photoperiodic response. Their diapause incidence was higher in Canada than in France, indicating a temperature effect. These results suggest that parasitoids from mild winter climates could be relatively more sensitive to temperature than to photoperiod when entering diapause. It would allow them to match more accurately their overwintering strategy to actual winter temperatures. Nevertheless, both temperature and photoperiod stimuli interact to induce diapause in *Aphidius* species (Brodeur & McNeil, 1989a, Tougeron *et al.*, 2017a) and the direction of these stimuli (e.g. increasing or decreasing photoperiod) could also be involved in diapause induction (Saunders, 2014).

In the laboratory, Tougeron *et al.* (2017a) observed very little levels in diapause incidence in species of aphid parasitoids from mild winter climate. Under the most winter-like condition tested (14°C, 10:14 h LD), no diapause was expressed in *A. rhopalosiphi* and an average level of 11.2 ± 4.9% was observed in *A. ervi*. Here we demonstrate that, although mild winter populations of these two species express overall low diapause levels in both Canada and France, they have the capacity to enter diapause at higher levels through developmental plasticity when exposed to conditions of colder temperature and lower photoperiod than what was tested in the laboratory. Our results confirm the hypothesis concerning the selection for low diapause induction thresholds (low temperature and day-length) and little plastic responses in populations from mild winter areas, associated with adaptations to overwinter as active adults (Andrade *et al.*, 2016).

Several studies have used transplant experiments to move populations to warmer climates and study organisms' evolutionary and plastic responses in the context of climate warming (e.g. Andrew and Hughes, 2007, Logan *et al.*, 2014, Nooten *et al.*, 2014). For diapause, Chen *et al.*, (2014) demonstrated that the moth *Hyphantria cunea* (Lepidoptera: Arctiinae), transferred from its temperate origin area to a subtropical location, has the capacity to enter summer diapause to cope with warmer summers, thus highlighting a plastic developmental response. The moth however faced environmental conditions to which it was not adapted, and higher mortality levels were observed (Chen *et al.*, 2014). Our results strengthen the idea that, while photoperiod is the main cue inducing diapause (Tauber *et al.*, 1986), temperature is one of the main environmental factor selecting for diapause expression in a population because it directly acts on mortality levels (Danks, 1987).

When parasitoids were put outside in Canada at the end of the experimental season, it was too late for them to enter diapause and/or to acclimate to cold before dying from low temperatures. Higher mortality levels appeared for French populations held in Canada, which may be poorly resistant to cold temperatures (Le Lann *et al.*, 2011c, Tougeron *et al.*, 2016). Although high mortality levels observed at this period highlight timing costs of being active too late in the season, they are not ecologically relevant to interpret, precisely because parasitoids are adapted to enter in diapause before the onset of lethal frosts in their respective environments. Canadian populations reared in France also had important mortality levels despite the relatively warm conditions across the experimental season. It is possible that, for

Canadian populations in France, developmental costs occurred; temperatures were too hot to be sustainable in a diapause state, leading to important mortality levels. Indeed, mild winter temperatures lead to higher metabolic activity during the process of diapause, potentially increasing mortality in insects (Bosch *et al.*, 2010, Williams *et al.*, 2012, Xiao *et al.*, 2016). These results strengthen the idea that there is a strong evolutionary pressure to enter early diapause in harsh winter climates, but not necessarily in mild winter climates in which diapause can involve physiological (*i.e.* energy consumption) and ecological costs (*i.e.* not exploiting available hosts in the environment).

When diapause levels are calculated based on the number of diapausing parasitoids divided by the number of emerged parasitoids (*i.e.* by excluding dead individuals), *A. ervi* population from France showed up to 100% diapause in December in Canada (see figure 5.S1). Thus, the decrease in diapause incidence observed in November and December in Canada is an artifact due to high mortality levels and cannot be directly interpreted. It is also interesting to notice that in the French population of *A. rhopalosiphi* held in France outdoor conditions, around 10% diapause levels have been recorded in June, when it is unexpected to obtain winter diapause. This could be the result of summer diapause expression which can be initiated in the offspring when female parasitoids encounter competitors for host resources, under summer like conditions (Krespi *et al.*, 1997, Tougeron *et al.*, 2017b).

We showed that *A. ervi* had higher diapause reaction norms slopes than the other species, which may suggest that it is more plastic to environmental changes. Moreover, minimal temperatures only influenced diapause in *A. ervi* while *A. avenaphis* and *A. rhopalosiphi* were only influenced by photoperiodic changes, and no difference in diapause incidence was found between experimental sites. This suggests that *A. avenaphis* and *A. rhopalosiphi* may rely more on photoperiod than on temperature cues to initiate their diapause. The response to climate change in *Aphidius* parasitoids' diapause could thus be different between species and could depend on their respective responses to environmental cues (temperature or photoperiod).

To conclude, parasitoid populations showed diapause levels adapted to their respective climates, but some degree of plasticity in diapause expression was expressed in response to thermal conditions. When a population face new climates, the genetic-based photoperiod response is likely to be mal-adapted because at a given location, photoperiod will not change whereas temperatures will (Grevstad & Coop, 2015). Increasing costs of diapause expression under an inappropriate thermal environment are thus to be expected (Bale & Hayward, 2010, Sgrò *et al.*, 2016), for instance in case of unexpected cold spells in typically mild winter areas. If species have to adapt to new environments, one could expect the reaction norm (*i.e.* the phenotypic response to the environment) to change genetically (Winterhalter & Mousseau, 2007, van Asch *et al.*, 2012). Examples of genotypic changes when facing warmer climates (e.g. Bradshaw and Holzapfel, 2001, Logan *et al.*, 2014) remain however less common than reports on phenotypic change (Hill *et al.*, 2002, Berteaux *et al.*, 2004).

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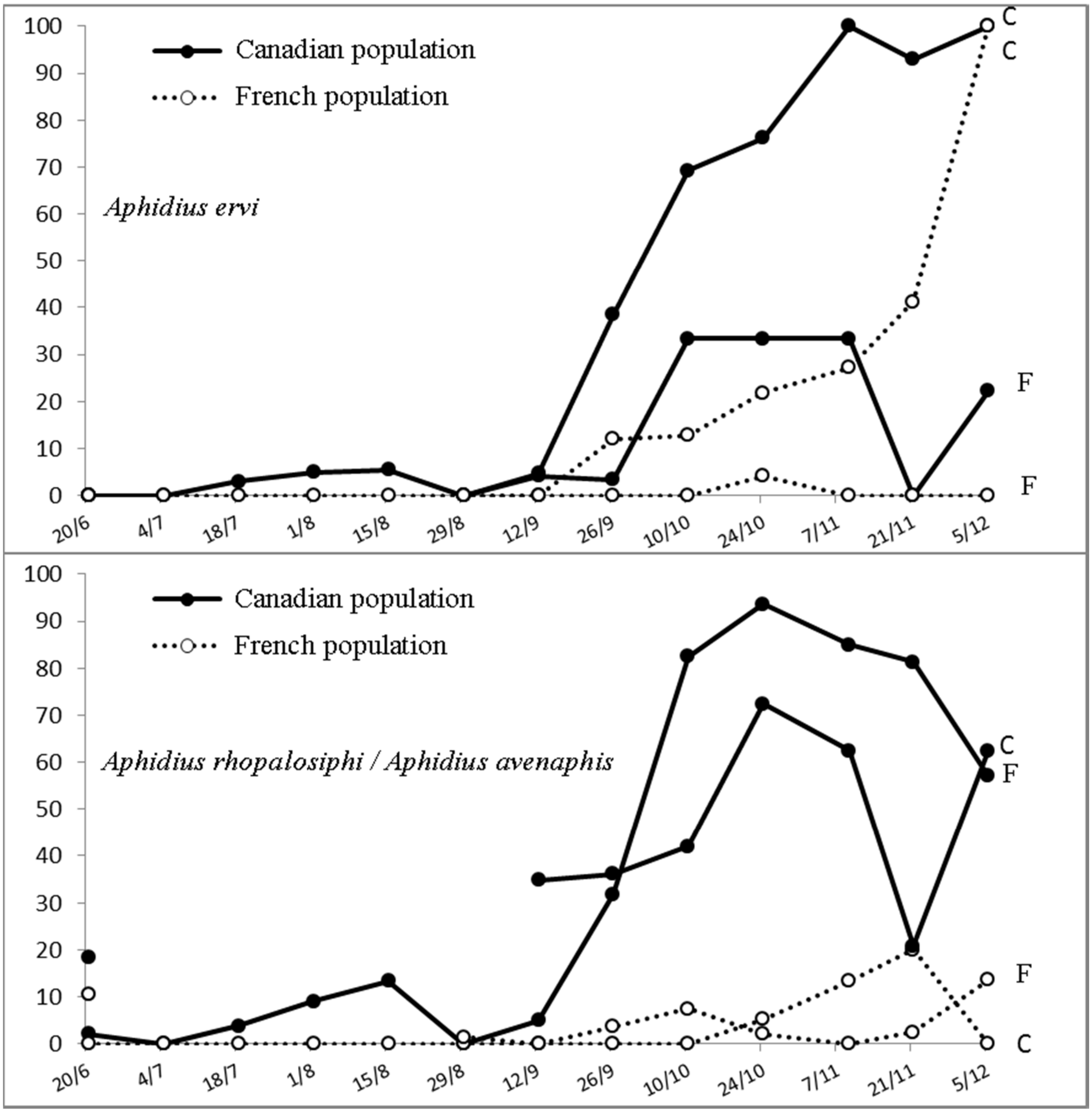

Appendix 5.S1: Diapause levels recorded every two weeks from 20th July to 5th December (*i.e.* representing changes in photoperiod over the season), 2016 in the Canadian (solid lines) and France (dotted lines) originated parasitoid populations of *Aphidius ervi* and *Aphidius avenaphis / Aphidius rhopalosiphi*. Diapause levels are here calculated by removing dead individuals from the dataset (*i.e.* diapause (%) = number of diapausing individuals / number of emerged parasitoids x 100). Letters on the right of each panel indicate the location of the tested population, either in Canada (C) or France (F) (*i.e.* representing different thermal environments). Note that experiments in France were started on the 20th June to have a starting point in common with experiments conducted in Canada, but were interrupted until the 29th August. Percentages were calculated on a minimum of 25 individuals for each data point (excepted on the 5th December in Canada where 7 to 12 mummies were sampled).

Partie III

Importance des hôtes pour la diapause des parasitoïdes

Chapitre 6 : Diapause estivale et compétition intraspécifique

Chapitre 7 : Effet des hôtes sexués sur la diapause

En bref :

- La synchronisation du cycle de vie des parasitoïdes avec celui de leur hôte est primordiale à leur survie. Les variations quantitatives et qualitatives des hôtes au cours de l'année pourraient donc être un signal à part entière indiquant un changement saisonnier et induisant la diapause.

- Les femelles parasitoïdes *A. rhopalosiphi* et *A. avenae* induisent une diapause estivale chez une partie de leurs descendants lorsqu'elles rencontrent de forts niveaux de compétition intra-spécifique. La densité d'hôtes rencontrée par les femelles n'influence pas l'entrée en diapause estivale. Cela représente un cas de plasticité transgénérationnelle adaptative ; les individus de la génération suivante souffrent moins de la compétition et du manque d'hôtes associé.

- Les parasitoïdes *A. ervi* entrent en plus forte proportion en diapause quand ils se développent dans des hôtes *A. pisum* sexués que dans des hôtes asexués. Les morphes sexués sont métaboliquement différents des morphes asexués, et représentent les derniers hôtes disponibles avant l'hiver. L'effet du morphe hôte est indépendant de l'effet de la température et de la photopériode sur la diapause.

- Les *A. ervi* provenant du Canada répondent de cette manière aux hôtes sexués, mais pas la population de Bretagne. Cela peut être dû à la faible présence d'hôtes sexués en Bretagne et pourrait en partie expliquer les faibles niveaux de diapause rencontrés dans cette région.

"What good is the warmth of summer,
without the cold of winter to give it sweetness."

J. Steinbeck, Travels with Charley: In Search of America

Chapitre 6

Diapause estivale et compétition intraspécifique

Intraspecific maternal competition induces summer diapause in insect parasitoids

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Summary

Organisms often live in unpredictable environments and have to adopt life history strategies that optimize their fitness under these conditions. According to bet-hedging theory, individuals can reduce variation in fitness outcomes by investing in different strategies at the same time. For arthropods, facultative summer diapause enables survival during dry and hot periods of the year, and can be triggered by a decrease in resource abundance. However, the effect of resource depletion on diapause induction has never been disentangled from the effect of the perception of the presence of competitors. Using two solitary parasitoid species of cereal aphids as a model system, *Aphidius avenae* Haliday and *Aphidius rhopalosiphi* De Stefani-Perez (Hymenoptera: Braconidae), we tested whether (i) low absolute host density and/or (ii) high levels of parasitoid females' competition lead to maternal-induced summer diapause in parasitoid offspring. Under summer-like climatic conditions, emerging parasitoid females were (i) reared alone and exposed to different host densities (from 5 to 130 aphids), or (ii) reared together with competing females (from 2 to 20 females) and then exposed individually to 50 aphids. For both parasitoid species, low aphid densities did not induce summer diapause. However, the incidence of summer diapause increased up to a maximum of 11% with increasing levels of competition experienced by female parasitoids. More than 60% of the females produced both diapausing and non-diapausing offspring after being kept at the two highest competition densities. Such a "spreading-the-risk" strategy has likely evolved to optimize parasitoid fitness by preventing the following generation from exposure to low populations of suitable hosts and high mortality from superparasitism. These results provide the first experimental evidence of direct maternal competition-induced diapause in insects, and may change the way we apprehend the evolution of arthropod seasonal ecology, by considering intraspecific competition.

Key-words: Aestivation, Bet-hedging, Host density, Intraspecific competition, Superparasitism

Introduction

Phenotypic plasticity allows a given genotype to produce a range of phenotypes in response to variable environmental conditions (Auld *et al.*, 2010). For example, alternative life-histories can arise from maternal effects (*i.e.* transgenerational plasticity), whereby the proportion of individuals expressing a given phenotype depends on the conditions experienced by the preceding generation (Mousseau & Dingle, 1991). In insects, facultative diapause, a period of deferred development, can be induced by environmental cues perceived either by the diapausing individual or by its mother (Saunders, 1965, Tauber *et al.*, 1986, Brodeur & McNeil, 1989a). In the latter case, the female can change carbohydrates and polyols contents of the egg through hormonal regulation which acts on the egg's development (Yamashita *et al.*, 2001, Denlinger, 2002). Such 'anticipatory' maternal effects allow the mother to alter the phenotype of her offspring to increase their survival in the future environment by buffering against anticipated environmental stressors (Burgess & Marshall, 2014). However, when a female cannot exactly assess which phenotype would be favored in the future environment, she may produce a range of offspring phenotypes to optimize her fitness (*i.e.* she "hedges her bets"). The proportion of each offspring phenotype may be adjusted to the probability of encountering a given environment, when biotic or abiotic cues make it partially predictable (Hopper, 1999, Menu *et al.*, 2000, Marshall & Uller, 2007).

Summer diapause is a phase of dormancy that is followed by an active phase in fall and occurs either at the same developmental stage as winter diapause, or at a different one (Masaki, 1980). Summer diapause should be distinguished from aestivation which is a form of dormancy mostly expressed in tropical insects during the dry season (Navas & Carvalho, 2010, Denlinger & Armbruster, 2014). Although overlooked, summer diapause is likely to be as widespread as winter diapause among insects (Masaki, 1980), and has been observed in few hymenopterous parasitoid species from temperate regions (He *et al.* 2010, and references therein). Whereas the developmental, physiological and hormonal syndrome associated with summer diapause is quite similar to winter diapause syndrome (*i.e.* metabolic depression, developmental arrest and increasing resistance to adverse conditions) (Masaki, 1980, Denlinger & Armbruster, 2014), there is still a black-box in insect ecology and physiology concerning diapause induction, maintenance and termination stimuli (Koštál, 2006). It has been shown that summer diapause is predominantly induced by overcrowding on a resource patch, by a decrease in food resource availability and/or by dry and warm environmental conditions (Masaki, 1980, Tauber *et al.*, 1998). In the leaf-curling midge parasitoid *Platygaster demades* Walker (Hymenoptera: Platygastridae), there is a bet-hedging strategy on embryonic summer diapause levels and duration to match expected periods of host-shortage, thus avoiding fitness impairment and asynchronism with hosts (He *et al.*, 2010). In addition to limited food resources, competition avoidance has been proposed as a factor selecting for diapause evolution in insects because competition may also lead to a reduction in resource availability (Danks, 1987, Lalonde, 2004). Induction of diapause through competition (*i.e.* due to overcrowding on a resource patch) has been reported in rotifers (Gilbert, 2004) and some insects among the Lepidoptera, Hemiptera, Coleoptera and Hymenoptera (Brown *et al.*, 1979, Hagstrum & Silhacek, 1980, Harada & Spence, 2000, He *et al.*, 2010, Togashi, 2016). However, to our knowledge, the influence of resource scarcity due to competition was never disentangled from the influence of competition perception by itself on diapause induction, and increasing maternal competition was never shown to induce diapause in their offspring. Separating these effects would bring new evolutionary insights on intraspecific interactions theories, on arthropods' seasonal ecology in response to varying environments and in the face of climate change, as well as on still overlooked token stimuli that control dormancy in insects.

For insect parasitoids, the quality of their environment depends on both the suitability and abundance of hosts which vary throughout the seasons (Vercken *et al.*, 2015). Parasitoids have evolved behavioral responses to cope with both intra- and interspecific competition for hosts which can be used as a proxy for measuring resource availability and the risk of superparasitism (Boivin & Brodeur, 2006, Cusumano *et al.*, 2016). Superparasitism is repeated parasitism of a host by female parasitoids of the same species. It is typically deleterious for solitary parasitoids (van Alphen & Visser, 1990, Harvey *et al.*, 2013) and increases with the level of competition between females in a population. Competition should thus be avoided in order to reduce the probability of both resource (host) scarcity and superparasitism. In aphid parasitoids, competing females have the capacity to modify their foraging behaviour in response to the presence of competitors, mostly detected through visual and chemical cues (Mackauer, 1990, Barrette *et al.*, 2009, van Baaren *et al.*, 2009). For instance, *Aphidius ervi* Haliday (Hymenoptera: Braconidae) increase their patch residence time when exploiting an aphid colony in presence of competitors (direct perception of competition) or foraging on a patch previously exploited by competitors (indirect perception of competition) (Le Lann *et al.*, 2011a).

We aimed to disentangle the effect of low host availability from the effect of maternal perceived competition (*i.e.* low relative host availability) on the incidence of summer diapause. We also measured the duration of summer diapause, which remains un-documented in most parasitoid species. We conducted experiments under laboratory conditions using two sympatric parasitoid species of the grain aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae); *Aphidius avenae* Haliday and *Aphidius rhopalosiphi* De Stefani-Perez (Hymenoptera: Braconidae). In cereal fields, these parasitoid species experience rapid decrease in host availability in summer (Rabasse *et al.*, 1983), especially because host-plants are drying and aphids are subject to deleterious heat shocks (Alford *et al.*, 2012a). Previously observed levels of summer diapause were relatively low in *A. avenae* and *A. rhopalosiphi* under natural conditions (<5% of the population), when aphid abundance was low in the field (Starý, 1966, Krespi *et al.*, 1997). The cues responsible for triggering summer diapause in these species were not identified. In cereal fields of Western France, *A. avenae* is most abundant from spring to fall while *A. rhopalosiphi* presents higher abundance from autumn to spring (Krespi, 1990, Andrade *et al.*, 2016, Tougeron *et al.*, 2016). The two species differ in their capacity to enter winter diapause; *A. rhopalosiphi* does not produce diapausing individuals whereas *A. avenae* enters winter diapause at low levels (Tougeron *et al.*, 2017a). *Aphidius avenae* is about 1°C more resistant to high temperatures than *A. rhopalosiphi* (Le Lann *et al.*, 2011c) which may imply lower summer diapause incidence in the former species. Hence, the following hypotheses were tested: the incidence of summer diapause in parasitoid offspring is higher (i) when females encounter low aphid densities, (ii) when females experience high levels of conspecific competition, and (iii) in *A. rhopalosiphi* than in *A. avenae*, considering differences in their heat tolerance and seasonal occurrence.

Material & Methods

Biological material

Aphidius avenae and *A. rhopalosiphi* were collected from cereal fields in Western France (Long Term Ecological Research area *ZA Armorique*, 48°08' N, 1°80' W) in 2014 and 2015 (details in Tougeron *et al.*, 2017a) and have since been reared on a colony of the grain aphid *S. avenae* established from a single female collected at Le Rheu, France, in 1990. Parasitoids were reared at 20°C, 70% relative humidity (RH), and under 16:8h light:dark (LD) photoperiod while aphids were reared at 24°C, 55% RH, and 16:8h LD on winter wheat (*Triticum aestivum* var. Mégantic).

General experimental conditions

Experiments were conducted at 24°C, 55% RH, and 16:8 LD. These conditions simulate those that are typically encountered in the study area when aphid parasitoid summer diapause is assumed to occur, between 20th June and 31st August (Météo France, 2015; Krespi *et al.* 1997). Ten days prior to a test, apterous parthenogenetic aphids were placed on sprouted wheat (about 2 cm high) in experimental arenas (plastic pots ⌀ 10 cm) to produce aphid cohorts of 2nd or 3rd instar larvae. When necessary, aphids of the same age from the aphid colony were added to the infested wheat plants to reach the required density.

Last larval instars of Aphidiinae parasitoids make a cocoon and pupate inside the dead aphid host; the resulting structure is called a 'mummy.' Aphid mummies were taken from the parasitoid colonies, isolated in small gelatin capsules (⌀ 5 mm), and acclimated to the experimental abiotic conditions until adult emergence (3 to 5 days). Following emergence, each parasitoid female was placed in the presence of 2 males for mating and provided a honey solution (70% dilution) for 36 h. This duration allows females to reach their maximum egg-load (Le Lann *et al.*, 2012). Parasitoid females were then held along with aphids and honey in the experimental arenas for 48 h, following the two protocols described below. Mummy formation in the arenas was checked every day, beginning one week after oviposition. Mummies were kept individually in gelatin capsules at 20°C and adult emergence was checked daily. Twenty days after the first adult emergence within a single female offspring, the remaining mummies in the cohort were dissected, except for the diapause duration experiment (see below), and the content was recorded as being a dead individual or a diapausing golden-yellow prepupa (Tougeron *et al.*, 2017a).

Effect of host density on offspring diapause incidence

To test the effects of host density on summer diapause incidence, we exposed parasitoids to the following host densities: 5, 20, 50, 65 and 130 aphids. These densities are encountered in the fields on wheat shoots (Roschewitz *et al.*, 2005) and encompass the realized fecundities for 48 h of both parasitoid species. *Aphidius avenae* and *A. rhopalosiphi* are able to produce about 62 and 31 mummies in two days, respectively (Le Lann *et al.*, 2012). Naive mated females (*i.e.* without oviposition experience or contact with other females) were individually introduced into experimental arenas to parasitize aphids. This experiment was replicated using four different females for each parasitoid species and aphid density modality (for a total of 20 females per species).

Effect of competition between females on offspring diapause incidence

To test the effect of competition among parasitoid females on summer diapause incidence, mated females were placed in plastic tubes (⌀=4 cm, L=9 cm) for 48 h at the following densities: 2, 5, 10 and 20 females. These densities were chosen to represent the range of direct competition a female might experience in either natural (as indicated by natural parasitism rates (Roschewitz *et al.*, 2005)) or laboratory rearing settings; with two and twenty females competing being highly credible in the field and in mass-rearing condition, respectively. Females were individually introduced in an experimental arena containing 50 aphids. This experiment was replicated using fifteen different females for each parasitoid species and competition treatment (for a total of 60 females per species).

Diapause duration in the offspring after mothers' competition experience

To measure the duration of summer diapause, four additional females of each parasitoid species from the 20-females-competition treatment were allowed to parasitize 50 aphids each. Following parasitoid development, mummies were placed in gelatin capsules and parasitoid emergence was checked every day. Data are accurate to ± 2 days since parasitoids that emerged during the weekend were pooled with those emerging on Monday. Parasitoids with a delay of emergence >20 days after first adult emergence were considered to be in diapause. This 20-days threshold was determined *a priori* since egg-to-adult development in non-diapausing *Aphidius sp.* usually lasts from 12 to 16 days at 20°C (Zamani *et al.*, 2007), and confirmed *a posteriori* following the distribution of emergence data. The remaining mummies were dissected 30 days after no emergence had been recorded in a given cohort to determine the status (dead or in diapause) of the parasitoid.

Statistical analyses

No diapausing individuals were produced following the host density experiment (see results section) and therefore no statistical analysis was conducted on this dataset. To test for the effects of female competition (categorical effect), Generalized Linear Models (GLMs) with a quasi-binomial error distribution (to account for overdispersion) and a logit-link function were fitted to the data separately for each parasitoid species. The response variable was the number of diapausing and non-diapausing individuals, including dead ones, for each replicate (ovipositing female). Another GLM with a quasi-binomial error distribution was fitted to the data to test for differences in diapause induction between parasitoid species, competition treatment as well as their interaction. The significance of each factor was tested by implementing an iterative procedure with the 'Anova' function from the package *car*, using F-tests for quasi-binomial data (Fox & Weisberg, 2011). Differences between competition levels were compared with Tukey Contrasts (Hothorn *et al.*, 2008). Data for diapause duration within a species was pooled and thus was not compared among the offspring of different females. Statistical analyses were carried out with *R* software (R Core Team, 2017).

Results

Effect of host density on offspring diapause incidence

Aphid density (5, 20, 50, 65 and 130 individuals) had no effect on summer diapause expression; none of the mummies contained diapausing individuals for either *A. avenae* (n=219 total mummies formed) or *A. rhopalosiphi* (n=205). Parasitism rates (*i.e.* number of mummies formed / total number of aphids proposed to parasitoids) varied between aphid density treatments but were constant within a given density. They ranged between 80 and 100% depending on the female parasitoid at the density of 5 aphids and between 10 and 30% at the density of 130 aphids.

Effect of competition between females on offspring diapause incidence

Increasing competition between females led to increasing summer diapause induction in their offspring for both *A. avenae* (GLM, F=76.8, df=3, p<0.001, n=1327) and *A. rhopalosiphi* (F=89.2, df=3, p<0.001, n=1174) (Figure 6.1). In the competition treatment of 20 females, diapause incidence reached maximums of 11.22 ± 1.5% and 9.92 ± 1.3% (mean ± SE) for *A. avenae* and *A. rhopalosiphi*, respectively. The 20-female competition treatment showed significantly higher proportions of parasitoids in summer diapause than the competition treatment of 10 females in *A. avenae* (Tukey Contrasts; z=3.73, p<0.005) but not in *A. rhopalosiphi* (z=1.10, p=0.69). There was no summer diapause in the other two competition treatments (2 and 5 females) for either parasitoid species. There was no significant difference in summer diapause incidence between species (GLM, F=0.75, df=1, p=0.39) and no significant interaction between species and competition level (F=2.28, df=3, p=0.08).

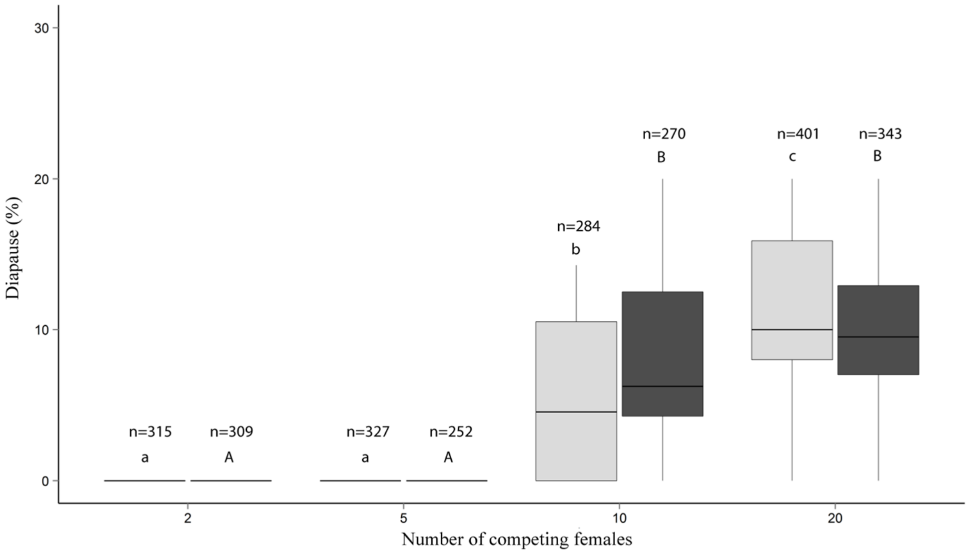

Figure 6.1: Percentages (±CI 95%) of aphid mummies containing parasitoid prepupae in summer diapause in relation to the number of females in competition for *Aphidius avenae* (grey) and *Aphidius rhopalosiphi* (black). Different lower case letters (for *A. avenae*) and upper case letters (for *A. rhopalosiphi*) indicate significant differences between competition treatments (Tukey Contrasts). The total number of mummies is provided for each experimental condition.

Not all females produced diapausing offspring; in the competition treatment with 20 females, for both parasitoid species, 93.3% of the tested females (14 out of 15) produced a minimum of one offspring in diapause. In the competition treatment with 10 females, the proportion of females inducing summer diapause in their offspring decreased to 60% (9 out of 15) and 86.7% (13 out of 15) for *A. avenae* and *A. rhopalosiphi*, respectively. Moreover, there was high variability in the diapause incidence within each female offspring for females that produced diapausing individuals; from 2.9% to 20% in *A. avenae* and from 4.8% to 20% in *A. rhopalosiphi* (in the 20-female competition treatment for both species). In the 10-female competition treatment this proportion ranged from 2.6% to 14.3% in *A. avenae* and from 3.0% to 20% in *A. rhopalosiphi*.

Diapause duration in the offspring after mothers' competition experience

Most parasitoids did not enter diapause, emerging from 12 to 27 days after oviposition (80.5%, n=62 for *A. avenae;* 75%, n=54 for *A. rhopalosiphi*). Nine (11.7%) *A. avenae* and seven (9.7%) *A. rhopalosiphi* individuals emerged more than 32 days after oviposition (*i.e.* more than 20 days after the first emergence in their cohort) and were considered to be in summer diapause. The mean summer diapause duration was similar for both parasitoid species (*A. avenae,* 55.6 ±5.2 days; *A. rhopalosiphi,* 54.7 ±4.3 days), although emergences spread over a longer period in *A. avenae* (Figure 6.2). All remaining mummies dissected 113 days after oviposition (last adult emergence occurred at day 83 after oviposition + 30 days without any emergence) contained dead, non-diapausing individuals (15 adults and 2 pupae).

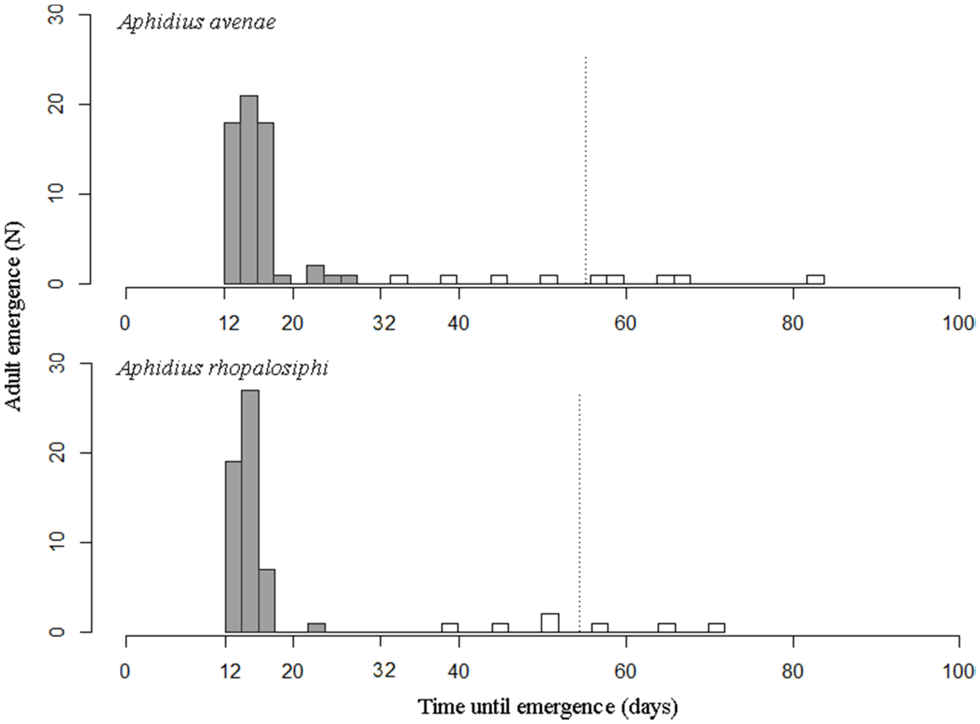

Figure 6.2: Distribution over time of parasitoid (*Aphidius avenae* and *Aphidius rhopalosiphi*) emergence at 20°C for non-diapausing (grey bars) and diapausing (open bars) offspring developing in *Sitobion avenae*. For each parasitoid species, distributions represent pooled offspring data from four females. Vertical dashed lines represent the mean emergence time of parasitoids in summer diapause. Day zero is the day of oviposition.

Discussion

In addition to its other functions, diapause allows species exploiting the same ecological niche to avoid competition (Danks, 1987, Tauber *et al.*, 1998, Alekseev & Starobogatov, 1996, Lalonde, 2004). Although competition avoidance has been invoked as a potential biotic factor selecting for the evolution of diapause (Danks, 1987), perception of competition has not been identified as a cue *per se* for summer or winter diapause induction in insects – probably because it has never been decoupled from the influence of resource scarcity due to overcrowding. We report no effect of resource scarcity (host density), but we did observe a clear effect of increasing maternal competition on summer diapause induction in the progeny of two *Aphidius* parasitoids. This is the first evidence, to our knowledge, of maternal perception of competition for resources being identified as a biotic cue for summer diapause induction in insects. Summer diapause was triggered by the same cues and expressed in a similar way (*i.e.* incidence and duration) in both *A. avenae* and *A. rhopalosiphi.* Summer diapause led to a developmental arrest occurring at the same developmental stage (prepupa) as for winter diapause (Brodeur & McNeil, 1989a, Tougeron *et al.*, 2017a). Although only expressed in a part of the population, summer diapause can have important consequences for parasitoids' life-cycle, population dynamics and interactions with their hosts.

Although the syndrome associated with summer and winter diapause is similar (Masaki, 1980, Denlinger & Armbruster, 2014), their inducing environmental cues differ as suggested by Masaki (1980), Tauber *et al.* (1986) and Košťál (2006), mostly because summer diapause serves different ecological functions from winter diapause. Maternal effects on winter diapause expression in *Aphidius* sp. can be significant but remains marginal compared to other environmental stimuli, mainly photoperiod and temperature (Brodeur & McNeil, 1989a, Langer & Hance, 2000). In the genus *Aphidius*, conditions directly encountered by the early sensitive stage (egg, young larva) represent the main cues initiating diapause (Brodeur & McNeil, 1989a, Tougeron *et al.*, 2017a). In contrast, for other parasitoid species such as *Nasonia vitripennis*, winter diapause is triggered only by maternal environmental perception (Saunders, 1965) and is linked to seasonal patterns of host availability (Saunders *et al.*, 1970). In contrast to winter diapause, our results suggest that summer diapause has a strong maternal component in *Aphidius* species.

Summer diapause induction in *Aphidius* offspring appears to arise from the perception of direct conspecific competition among females, when no hosts are available. This perception may be a proxy for measuring the risk of both host shortage and superparasitism in the future offspring environment. Our results also suggest that solitary parasitoids may respond differently to the risk of self-superparasitism (*i.e.* by a single female; host-density experiment) which can be neutral or beneficial (van Alphen & Visser, 1990), than to the risk of conspecific superparasitism (competition experiment), which is typically detrimental. Indeed, in the experiment on host density, transgenerational diapause induction was not observed, even when only 5 aphids were offered for 48 h, which strengthens the idea that increasing competition alone induces summer diapause.

Not all parasitoid females produced diapausing offspring, and individual mothers that did lay diapausing offspring did so in only a subset of their progeny. This variability could be explained either by differing sensitivity to competition among mothers (*i.e.* high genetic polymorphism in the population) or high degrees of plasticity in parasitoid females. Because the offspring's environment cannot be fully predicted from the conditions experienced by the mother, evolution can select for maternal genotypes that produce both diapausing and non-diapausing offspring, and modify the frequency of each phenotype regarding relevant environmental cues (Hopper, 1999), such as the level of competition for host resources. In this context, summer diapause expression induced by parasitoid mothers could be viewed as a type of 'conditional diversified bet-hedging' strategy (*sensu* de Jong *et al.* 2011). In the light of the above, we hypothesize that risks may be spread both among mothers at the population scale (not all mothers produced diapausing offspring) and at the progeny scale (individual mothers induced diapause in only a part of their offspring).

The highest female density we tested (n=20) is likely to occur in laboratory or industrial mass-rearing but are less credible in field conditions. In cereal fields, *Aphidius* sp. abundances per aphid patch are relatively low, greatly vary between years (Roschewitz *et al.*, 2005), and high levels of direct intraspecific competition may rarely occur among solitary parasitoids (Vollhardt *et al.*, 2008, Wajnberg *et al.*, 2008). It may however occur for interspecific competition (e.g. host-patches exploited by different parasitoid females at the same time leading to high multiparasitism levels) or in other parasitoid species (e.g. gregarious parasitoids). The incidence of summer diapause induced by maternal competition under natural conditions nevertheless remains unknown. Our results are likely to translate to other parasitoids, including those released in inundative biological control programs. In the context of biological control, improving knowledge of factors inducing diapause is also of great importance if we aim at improving conditions of mass-rearing (Boivin *et al.*, 2012).

The emergence of *A. avenae* and *A. rhopalosiphi* individuals following summer diapause was spread over a long period of time (up to 11 weeks after oviposition). This pattern could represent a form of temporal risk-spreading to avoid parasitoid overcrowding on a resource patch and would also be beneficial when extreme climatic events could impair parasitoid survival. However, summer diapause duration, maintenance and termination mechanisms remain to be measured in field conditions. This pattern contrasts with other aphid parasitoid species, such as *Aphidius nigripes* (Ashmead), in which spring emergence after winter diapause is highly synchronized and occurs within three weeks (Brodeur & McNeil, 1994). We also showed that development from egg to adult emergence extended to a maximum of 83 days for parasitoids entering summer diapause. This is substantially less than winter diapause, which lasts for several months in overwintering aphid parasitoids from temperate areas (Brodeur & McNeil, 1994). These findings strengthen the idea that summer diapause has evolved in insects to cope with short-term deleterious conditions such as high competition for hosts - which are ephemeral resources - rather than predictive, long-term environmental fluctuations.

Parasitoid pupae developing inside the aphid mummy are highly vulnerable to natural enemies and adverse climatic conditions (Brodeur & Rosenheim, 2000). The optimal proportion of offspring entering summer diapause might be selected to balance risks of competition for hosts and mortality from, disease, extreme climatic events, predation or hyperparasitism (which levels can be high at the end of the summer (Holler *et al.*, 1993)). There are, for instance, a few examples of predator-induced diapause in arthropods (Slusarczyk, 1995, Kroon *et al.*, 2008). Rausher (1986) also argued that a female butterfly's decision to induce diapause or not in a given proportion of its offspring may also depend on the 'decision' taken by its competitors. In this regard, intra- and interspecific interactions between insects as well as predation and hyperparasitism levels must be considered in future research when studying summer diapause as a density-dependent process. However, the underlying ecological processes behind summer diapause induction and duration are poorly understood for insects from temperate regions and substantially more knowledge is needed concerning the multiple biotic and abiotic factors other than competition, and their interactions, that modulate summer diapause.

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Chapitre 7

Effet des hôtes sexués sur la diapause

Sex makes them “sleepy”: sexual morphs of aphids induce diapause in parasitoids

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Summary

When organisms coevolve, any change in traits and ecology in one interacting species can produce phenotypic changes in the other species. To overwinter successfully, parasitoids have to synchronize their life-cycle to both abiotic conditions and their hosts’ phenology. Although winter diapause in parasitoids was shown to be mostly induced by photoperiod and temperature, host phenotypic changes along the year may also constitute independent induction cues. To test the effect of host reproduction strategy on parasitoid diapause induction, we used a single holocyclic clone of the pea aphid *Acyrthosiphon pisum* producing both sexual and asexual morphs. *Aphidius ervi* parasitoids from contrasted climatic origin (harsh *vs.* mild winter areas) were allowed to parasitize each morph and developing parasitoids were placed under either fall-like or summer-like temperature-photoperiod conditions. We provide for the first time measurement of forty-seven metabolites using gas chromatography and lipid reserves in sexual and asexual aphid morphs. We found out that sexual aphids were cues *per se* for parasitoid diapause induction; parasitoids entered diapause at higher levels (19.4 ± 3.0 %) when developing in oviparous aphids than in viviparous aphids (3.6 ± 1.3 %), only under summer-like conditions. This pattern was only observed in parasitoids from the harsh winter area since overall low diapause levels and no effect of the aphid morph were observed in the other population, underlying local adaptations to overwintering cues. Our analyses suggest parasitoids’ response to be mainly influenced by sexual aphids’ physiology, with higher proportion of polyols and sugars, and more fat reserves being found in oviparous aphids. Our results underline strong coevolutionary processes between hosts and parasitoids in their area of origin, leading to phenology synchronization.

Key-words: Coevolution, Phenotypic plasticity, Phenology, Synchronization, Environmental cue, Sugar, Lipid, Metabolism, Physiology

Introduction

Interacting individuals from two species can adjust their phenotypes in response to cues from each other, even when these cues vary across time (Agrawal, 2001). Beneficial or antagonistic interactions, from mutualism to parasitism, predation and competition may lead to adaptive phenotypic responses. When tight interactions persist over time, coevolution can occur and species adapt to the interacting species' life history traits, phenology and ecology (Agrawal, 2001, Ellers *et al.*, 2012). Interaction-norms (Thompson, 1988) arise from ecological responses of interacting organisms in varying environments, as any phenotypic change occurring in one "partner" species can cascade to the other species' phenotype (Fordyce, 2006, Hughes, 2012). Cues produced by the interacting species may indirectly inform a species of environmental changes. For example, plant senescence in fall can inform herbivorous insects of upcoming detrimental winter conditions and induces phenotypic changes (e.g. diapause induction) or migration behaviour (Archetti *et al.*, 2009).

Parasitoids are excellent models to study phenotypic expression in interacting species because they are strongly influenced by phenotypic variation of their hosts, which represent the whole nutritional and physiological environment during immature stages (Godfray, 1994). Diapause is an important ecological process in insects allowing them to survive recurrent unfavorable environmental conditions (Tauber *et al.*, 1986). For parasitoids, dormancy also contributes to maintain synchronization with their host's seasonal reproductive-cycle; it is induced before suitable hosts vanish from the environment (Lalonde, 2004). As in most of insect species, diapause in parasitoids is induced by abiotic cues perceived either by the generation that will enter diapause or by the maternal generation (Tauber *et al.*, 1986). In addition to abiotic cues, there have been few reports that the onset of parasitoids diapause can be triggered by the onset of host diapause (Polgár & Hardie, 2000, Gerling *et al.*, 2009), or through intraspecific competition for hosts (Tougeron *et al.*, 2017b). However, whether the phenotype of a non-diapausing host can influence diapause in the parasitoid remains poorly studied.

Aphids serve as hosts for Aphidiinae parasitoids and have a very complex cycle showing an alternation between parthenogenetic and sexual morphs across the year (Dixon, 1985). Sexual morphs of aphids are present at higher proportion in harsh than in mild winter climates (Dedryver *et al.*, 2001), and they represent the last hosts available for aphid parasitoids before winter as they produce overwintering eggs triggered by environmental cues in late-fall (Leather, 1992). Sexual morphs of aphids have been suggested to promote diapause in parasitoids, indicating possible effect of host's physiology (Polgár *et al.*, 1991, Polgár *et al.*, 1995, Christiansen-Weniger & Hardie, 1997). No mechanistic understanding of this phenomenon has been proposed and the effects of the host morph *per se* have not been detangled from confounding factors such as host genotype, host size, host geographic origin, host sampling season or abiotic conditions. Hosts and parasitoids share common evolutionary history, they respond to similar seasonal cues and the physiological syndrome associated with overwintering is highly conserved among insects (Tauber *et al.*, 1986, Denlinger, 2002). As a result, the host's physiological state, and related metabolic content, may be used as a reliable signal for parasitoids, informing them for upcoming seasonal changes.

Hormones and carbohydrates are involved in the control of diapause expression in insects (Chippendale, 1977, Christiansen-Weniger & Hardie, 1999, Denlinger, 2002). For aphid parasitoids, metabolic and proteomic profiles differ between diapausing and non-diapausing individuals, with higher amounts of sugar and polyols being found in diapausing parasitoids (Colinet *et al.*, 2012). In aphids, morphs have different morphological and physiological phenotypes. Sexual aphid females accumulate reserves to produce energetically costly diapausing eggs (Le Trionnaire *et al.*, 2008) with cryoprotectant compounds such as mannitol and glycerol (Sømme, 1969), whereas parthenogenetic females metabolize resources rapidly to produce embryos. Aphids' triglyceride reserves change quantitatively and qualitatively across the seasons with alternation between morphs (Greenway *et al.*, 1974), and aphid parasitoids are known to consume sugars and lipid reserves of their hosts (Jervis *et al.*, 2008), so they may be influenced by the host's reserves.

We questioned the extent to which sexual and asexual morphs of a single clone of the pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) influences winter diapause expression in the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) under summer and fall conditions. Under laboratory conditions, we compared the response to aphid morphs of two populations of parasitoids from mild and harsh winter areas that differed in their level of diapause expression. In *Aphidius* species, winter diapause is initiated at the prepupal stage within the aphid mummy (*i.e.* dead aphid containing a developing parasitoid) following stimuli perceived by early developmental stages or by the mother (Brodeur & McNeil, 1989a, Tougeron *et al.*, 2017a). We hypothesized that parasitoids of both populations developing in sexual aphids enter diapause at higher proportion than those developing in asexual aphids, independently of photoperiod and temperature. We predicted this pattern to originate from differences in aphids' physiological contents. We thus performed metabolomic analyzes to measure lipid content and quantify aphid morphs energetic reserves. We also hypothesized parasitoids from mild winter area to be less responsive to diapause inducing cues from the host and the environment, because parasitoid populations should be adapted to climatic conditions and relative occurrence of sexual hosts in their respective area of origin.

Material & Methods

Biological materials

Two populations from contrasted climatic origins of the parasitoid *A. ervi* were established in 2015 from parasitoids collected at the mummy stage in pea fields near Montréal, QC, Canada (45.584°N, 73.243°W; harsh winter area) and Rennes, France (48.113°N, 1.674°W; mild winter area). Parasitoids were reared using a cyclically parthenogenetic clone (clone F2-X9-47) of the pea aphid *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) provided by INRA Le Rheu, France, known to produce both sexual and asexual aphid morphs (Jaquiéry *et al.* 2014). All insects were maintained on fava bean *Vicia faba* (Fabaceae) at 20°C, 70% relative humidity (RH) and 16:08 h Light:Dark (LD) photoregime.

Three aphid morph treatments were used for the experiments; oviparous females (O), viviparous females (V) and a control treatment for viviparous females (C).

Production of sexual and asexual aphids

Three parthenogenetic *A. pisum* adult females from the aphid cultures were put on fava bean plants (N=15) and allowed to lay larvae during four days. Females were then removed and plants infested by aphid larvae were put in a growing chamber at 17°C, 70% RH, 12:12 h LD, and under 36W, IRC 85, 6500 K light-day type fluorescent tubes to induce the production of sexual aphids (Le Trionnaire *et al.*, 2009). At each generation, fava plants were renewed and less than five aphids were maintained per plant to prevent formation of alate individuals and overcrowding (Hardie, 1980). As the embryos directly detect photoperiodic cue through the cuticle of the grand-mother (Le Trionnaire *et al.*, 2008), the first sexual males (~20%) and oviparous females (30 to 60%) were formed, along with viviparous females (20 to 50%), after three generations maintained under these conditions. A control group of parthenogenetic females was produced by maintaining aphids at non-sexual-inductive conditions, 20°C, 70% RH, 16:08 h LD. This was done to control for any stress effects of the sexual-inductive conditions on the aphid, and because a mix of morphologically undistinguishable sexuparous aphids (parthenogenetic aphids producing sexual morphs) and viviparous aphids (parthenogenetic aphids producing parthenogenetic morphs) can be produced at 17°C, 12:12 h LD. Sexual and asexual aphid morphs were differentiated under a stereo microscope (x10) by observing the shape of their legs: sexual female aphids have rhinaria on the tibia, oviparous females have a femur of the same width as the tibia, viviparous females have a wider tibia than the femur and do not possess rhinaria (Lamb & Pointing, 1972, Hullé *et al.*, 2006). Aphid males were not used in our experiment since parasitoids did not parasitize them (Tougeron *et al.*, unpublished data).

Diapause induction

Aphid mummies from the colonies were isolated in a small gelatin capsule until parasitoid emergence. Newly emerged parasitoids were put during 24 h in a 5 cm plastic tube for mating (5 females with 2 males) and were fed with a dilution of honey (70 %). In order to test potential maternal genotype effects and egg-laying order in different host phenotypes on diapause induction, and to control for parasitoids' age or host preference, twelve *A. ervi* females were individually allowed to parasitize 16 aphids of the same size and of each of the three morphs (oviparous female, viviparous female, control viviparous females) for 12 h over three consecutive days by alternating the order of presentation of aphid morphs among females. Parasitoids rested at night, with an access to diluted honey. Aphids were introduced in a plastic tube (10 x 3 cm) and were given a few minutes to settle on a fava bean cut plant, after which a parasitoid was introduced in the tube. Four parasitoid females were first individually put in presence of oviparous aphids, then moved to a second tube with control viviparous aphids and next moved to a third tube containing viviparous aphids. Four other females were first offered viviparous aphids, and the last four females were first offered control viviparous aphids (Figure 7.1).

After each oviposition period, the 16 potentially parasitized aphids of each morph type were transferred by group of 8 on two fava bean plants. Plants were next enclosed in microperfored plastic bags and placed at either 20°C, 16:8 h LD (summer-like conditions not inducing diapause in *A. ervi*) or 17°C, 10:14 h LD (autumn-like conditions known to induce diapause) (Brodeur & McNeil, 1989a, Tougeron *et al.*, 2017a). Precautions were taken to uniformize light-exposure in rearing chambers. When the plants began to dry, aphids were gently transferred to another plant with a small paintbrush. Mummy formation was checked daily and newly formed mummies were placed individually in gelatin capsules and remained under their respective temperature and photoperiod treatment until adult emergence. Mummies from which no parasitoid had emerged 15 days after mummification were dissected and the content was recorded as dead parasitoids or diapausing individuals (golden-yellow prepupae) (Tougeron *et al.*, 2017a). This experiment was repeated twice per parasitoid populations.

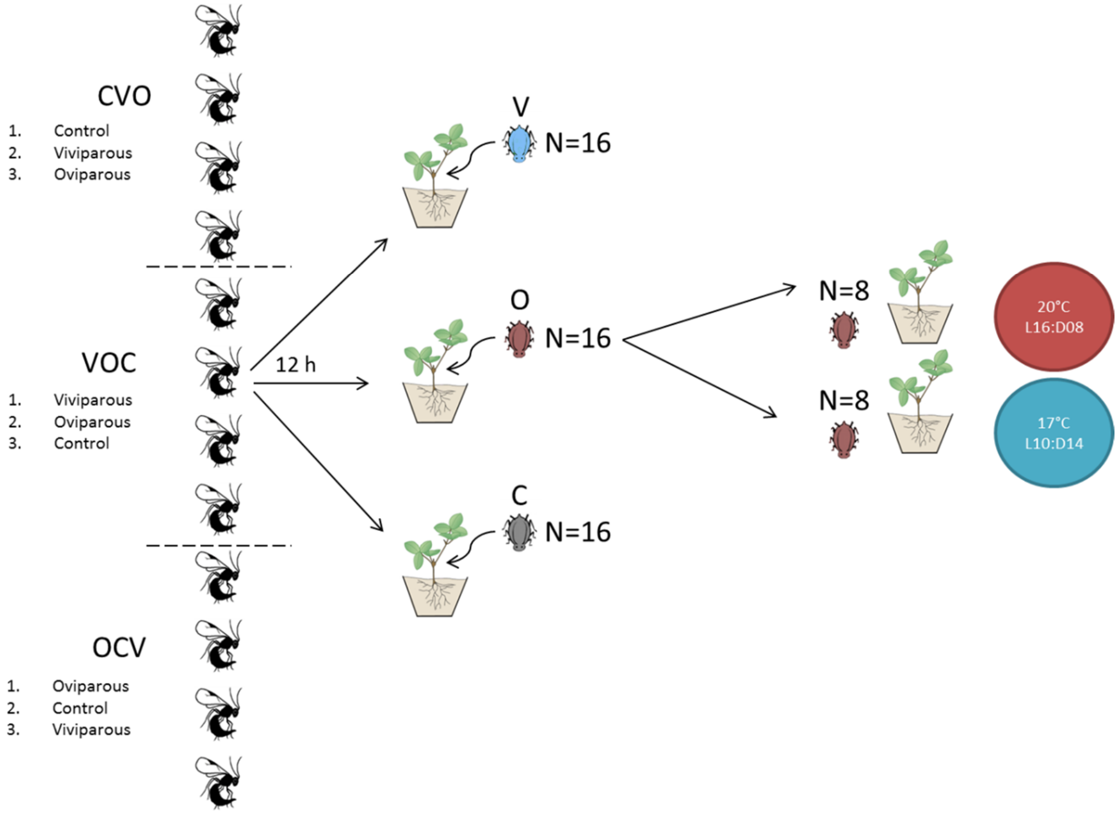

Figure 7.1: Experimental design for diapause induction in the parasitoid *Aphidius ervi*. Twelve parasitoid females were individually allowed to parasitize 16 *Acyrthosiphon pisum* from each of the three aphid morphs for 12 h: viviparous (V), oviparous (O) and control viviparous (C). First contact (parasitism sequence) with an aphid was alternated between the three morphs (VOC, CVO, OCV). Following parasitism, the aphid cohort was split in two and individuals were reared under a diapause-inductive condition (17°C 10:14 h LD) or a non-diapause-inductive condition (20°C 16:8 h LD). This protocol was repeated twice for each parasitoid population (originating from mild or harsh winter).

To disentangle an effect of the aphid morph (inter-individual differences within a population due to developmental plasticity) from an effect of the aphid clone (differences in reproduction modes genetically determined between populations) on parasitoid diapause, we compared the influence of the cyclically parthenogenetic clone (holocyclic) described above with an obligate parthenogenetic clone, producing only viviparous females (anholocyclic clone F2-X9-19; Jaquiéry *et al.* 2014). Five *A. ervi* females were individually allowed to sequentially parasitize 35 viviparous aphids of each clone during 12 h. Parasitized aphids were next placed at 17°C 10:14 h LD and diapause induction was measured as described above. Any clone effect was excluded; Diapause incidence was similar for parasitoids developing in viviparous aphids of either the holocyclic (59.9 ± 10.1 %, n=132 mummies) or the anholocyclic (66.0 ± 7.7 %, n=112 mummies) clone (GLM, p=0.97). The cyclically parthenogenetic clone was thus used for the experiments.

Metabolomic analyses and lipid reserves

Non-parasitized apterous adult aphids of each morph and of the same age (between 24 and 48 h after imago moult) were kept at -20°C for metabolomic and lipid analyses. Samples were dried at 60°C for 2 days in a freeze-dryer and then weighed to measure dry mass using a Mettler-toledo precision balance (accurate to 0.01 mg).

For metabolic analyses, 18 aphids of each morph (viviparous and oviparous females) were used. Since dry mass of a single aphid was low (mean dry mass 0.92 mg), nine replicates were analysed for each morph condition, each consisting of a pool of two aphid females. The samples were put in 600 µL of chlorophorm-methanol (1:2) solution and homogeneized using a tungsten-bead beating apparatus at 30 Hz for 1 min. Then, 400 µL of MilliQwater was added to each tube and samples were centrifuged at 5°C, 4,000 g for 5 min. Finally, 90 µL of the upper aqueous phase containing metabolites were transferred to chromatographic vials. Injection order of the samples was randomized prior mass spectrometry detection. Metabolomic fingerprinting process was performed following the protocol of Colinet *et al.* (2012). Chromatograms were analyzed using XCalibur software (Thermo Fischer Scientific, Waltham, MA, USA). We obtained measures of 47 metabolites: 14 amino acids, 7 polyols, 11 sugars, 11 acidic metabolic intermediate and 4 other metabolites and amines (Table 7.1). Details of metabolite amounts measured for each morph are provided in Figure 7.S1.

Table 7.1: Metabolites detected in two morphs (viviparous and oviparous females) of the pea aphid, *Acyrthosiphon pisum*. Abbreviations used on Figure 1 are in brackets.

Amino acids

Alanine (Ala)
Citrulline (Citr)
Dopamine (Dop)
Glutamic acid (Ac_Glu)
Glycine (Gly)
Isoleucine (Ile)
Leucine (Leu)
Lysine (Lys)
Ornithine (Orn)
Proline (Pro)
Serine (Ser)
Valine (Val)
Threonine (Thr)
Phenylananine (Phe)

Polyols

Adonitol
Arabitol
Galacticol
Glycerol
Inositol
Mannitol
Xylitol (Xyl)

Other metabolites

Glycerol-phosphate (Gly_Ph)

Metabolic intermediates

Aspartic acid (Ac_Asp)
Citric acid (Ac_cit)
Galacturonic acid (Ac_Gal)
Gamma aminobutyric acid (GABA)
Gluconic lactone acid (Ac_GL)
Glyceric acid (Ac_Glyc)
Lactic acid (Ac_Lact)
Malic acid (Ac_Mal)
Phosphoric acid (Ac_Phos)
Pipecolic acid (Ac_Pipe)
Quinic acid (Ac_quin)

Sugars

Arabinose
Fructose
Fructose-6-phosphate (F6P)
Galactose
Glucose
Glucose-6-phosphate (G6P)
Maltose
Mannose
Ribose
Saccharose (Sac)
Trehalose

Amines

Putrescine (Put)
Cadaverine (Cad)
Triethanolamine (TEA)

Lipid reserves were measured on 52 oviparous females and 23 viviparous females. Each dry aphid was left for two weeks in an Eppendorf tube containing 1 mL of chloroform-methanol solution (1:2) to extract lipids (see Terblanche *et al.*, 2004). Aphids were then rinsed with the same solution, and placed back in the freeze-dryer for 24 h to eliminate the residues of the extracting solution and next weighted again to measure fat content (= fat mass (mg) / lean dry mass (mg)) (Colinet *et al.*, 2007).

Statistical analyses

Data of the two parasitoid populations were analyzed separately. Generalized linear mixed-effects models (GLMM) with binomial distributions were fit to the data using the *lme4* package. The response variable was the proportion of diapausing parasitoids. The aphid morph (three modalities), the temperature/photoperiod conditions and their interaction were considered as fixed factors, the parasitoid female identity and the egg-laying (parasitism) order were considered as random effect factors in the models. Significance of each term in the model was analyzed using the package *car*. A Principal Component Analysis (PCA) was performed to detect which metabolites (expressed in nmol.mg^{-1}) differed the most between aphid morphs. An ANOVA was performed to test differences in fat content between aphid morphs. All statistical analyses were performed using the R software (R Core Team, 2017).

Results

Diapause incidence

Aphid morphs influenced diapause incidence only in the Canadian population at 20°C 16:8 h LD (GLMM, $\chi^2=16.9$, df=2, $p<0.001$; Figure 2). Parasitoid diapause incidence was higher when they developed in oviparous aphids (19.4 ± 3.0 %) than in viviparous aphids (3.6 ± 1.3 %) or viviparous control aphids (3.8 ± 1.4 %). The aphid morph did not have an effect on diapause at 17°C 10:14 h LD (Figure 2).

Diapause incidence was higher at 17°C 10:14 h LD than at 20°C 16:8 h LD, for both the Canadian (76.9 ± 2.5 % *vs.* 9.0 ± 1.5 %, GLMM, $\chi^2=250$, df=1, $p<0.001$) and the French population (27.9 ± 2.1 % *vs.* 0 %, GLMM, $\chi^2=238$, df=1, $p<0.001$). At 20°C 16:8 h LD, low levels of diapause were observed for the Canadian population, excepted in presence of oviparous aphids (Figure 7.2), whereas no diapause was expressed for the French population.

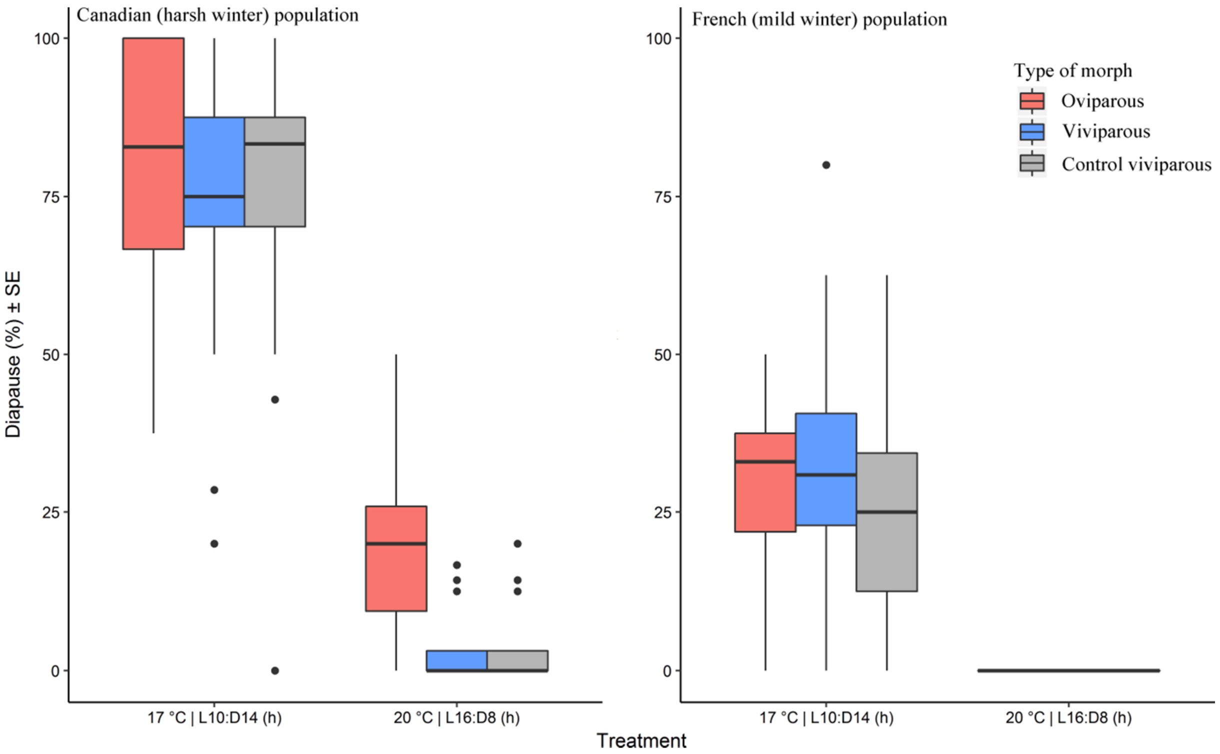

Figure 7.2: Diapause incidence (% ± SE) of two *Aphidius ervi* parasitoid populations (Canadian (harsh winter, left panel) and French (mild winter, right panel)) when parasitoids developed in different morphs of the pea aphid *Acyrthosiphon pisum* (oviparous sexual females, viviparous parthenogenetic females, control viviparous females) and under two abiotic conditions (17°C 10:14 h LD or 20°C 16:8 h LD).

Metabolomic analyses and lipid reserves

Differences ion metabolites between aphid morphs were mainly observed along the first principal component (PC1) of the PCA, which represented 35.6% of the total inertia. The second component (PC2) accounted for 22.5% of the inertia and mostly represented variation within each group of aphid morph (Figure 7.3). Metabolites that mostly contributed to the discrimination along PC1 are amino acids and glycerol (positively) and trehalose and ribose (negatively).

Polyols and sugars were mostly represented in oviparous females, at the exception of glucose and glycerol which were more concentrated in viviparous morphs. Noticeably, oviparous aphids had higher concentrations of fructose, trehalose, galactose, ribose, mannose, maltose, glucose-6-phosphate, fructose-6-phosphate, galacticol, adonitol, arabitol and mannitol than viviparous aphids (Figure 7.S1).

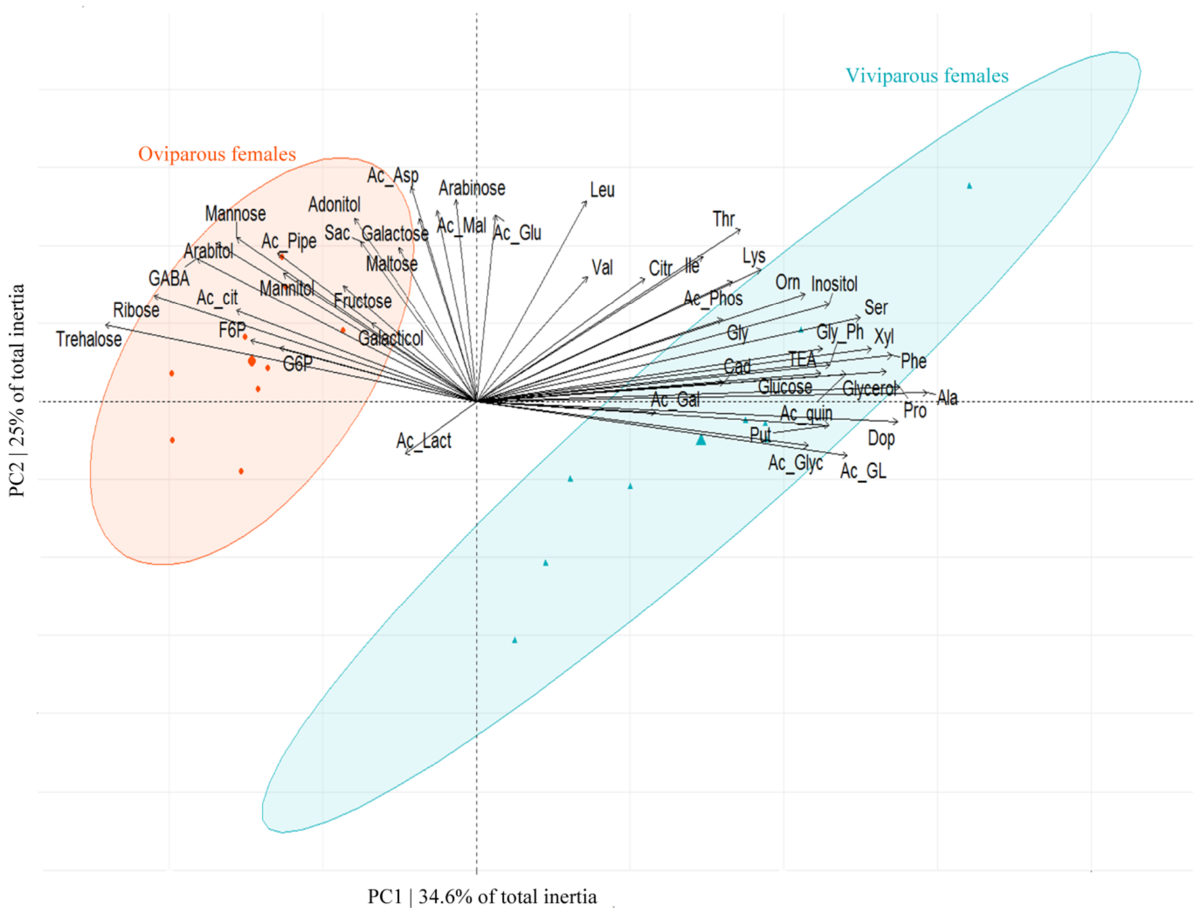

Figure 7.3: Multivariate analysis (PCA) on the first two principal components (PC) representing links between metabolic compounds (47 variables, nmol.mg^{-1}) and two aphid morphs (oviparous vs. viviparous females) of *Acyrthosiphon pisum*. Confidence ellipses are constructed around each aphid group centroid (n=9 replicates by morph). Abbreviations are listed in Table 1.

Oviparous females had a higher fat content ratio (mg fat/mg dry mass) than viviparous females (0.63 ±0.02 and 0.51 ±0.03, n= 52 and n= 23, respectively) (ANOVA, LR=8.0, df=1, $p<0.005$).

Discussion

Species have to adapt to environmental changes and to related phenotypic variations in species with whom they interact. The host plays a major role in parasitoid ecology and it was demonstrated that, in addition to abiotic factors such as photoperiod and temperature, the host species, size or life-stage can modulate parasitoid diapause (Tauber *et al.*, 1986, Danks, 1987). We report that aphid parasitoids can use aphid sexual morph as a cue for diapause induction, with higher diapause incidence (up to 20%) expressed in *A. ervi* developing in oviparous *A. pisum* aphids than in parthenogenetic conspecifics, likely due to differences in aphid's physiology. Of significance, only parasitoids from the harsh winter area and exposed to summer-like conditions relied on aphid morph as a cue for diapause induction.

Parasitoid populations of *A. ervi* from contrasted climatic environments (Canada and France) do not respond the same way to both abiotic (photoperiod and temperature) and host cues. French populations of *Aphidius* sp. have recently adapted to climate warming by remaining active under mild winter conditions, with none or small proportions of individuals entering diapause (Tougeron *et al.*, 2017a). The opposite pattern is observed in Canada where all aphid parasitoids overwinter in diapause (Brodeur & McNeil, 1994). In mild winter areas, non-diapausing parasitoids exploit anholocyclic aphids and their populations are maintained on parthenogenetic hosts during winter (Langer & Hance, 2000, Andrade *et al.*, 2015, 2016). These mild-winter parasitoid populations may not have evolved response to sexual aphids, or may have lost the capacity to respond to it, because sexual morphs are rare in warmer climatic areas (Dedryver *et al.*, 2001).

In cold temperate regions, sexual morphs of aphids are produced at the end of the growing season and represent the last hosts available for parasitoids before the onset of unfavorable conditions. In addition, parasitism of aphid sexual morphs on primary host plants allows parasitoids to overwinter nearby their hosts, thereby favoring host availability in spring for newly emerged parasitoids and improving reproductive-cycles synchronization (Höller, 1990, Christiansen-Weniger & Hardie, 1997). In harsh winter climates, parasitoids have coevolved with the seasonal occurrence of aphid morphs. Parasitoids may have adapted to it as cues for changing environments (*i.e.*, as a convergent signal with temperature and photoperiod decrease in fall). Parasitoids may also directly respond to the sexual hosts' metabolites that also induce their own diapause. Indeed, the overwintering metabolic and physiological syndrome is highly conserved among insects (Tauber *et al.*, 1986) and both aphids and parasitoids may respond to the same molecules involved in diapause.

Parasitoid diapause has evolved following two selective pressures; lethal winter temperatures and host availability that may have led to two distinct signals inducing diapause (photoperiod/temperature decrease and presence of sexual hosts, respectively). Parasitoids should remain active as long as hosts are available and temperatures are favorable. They should however enter diapause as soon as aphids modify their phenotype, regardless of abiotic conditions. In natural settings, alternative host species are present and both anholocyclic and holocyclic aphid populations can coexist (Dedryver *et al.*, 2001), which may limit the proportion of parasitoids entering diapausing following the host-cue. Canadian Aphidiinae parasitoids enter diapause for a part of the population as early as July (Brodeur & McNeil 1994; **Chapter 5**, this thesis) as a potential adaptation to avoid early lethal frosts, and we showed that sexual morphs only influenced diapause under summer-like conditions, suggesting that encountering sexual aphids informs the parasitoids for upcoming deleterious conditions and modulates diapause expression. In fall-like conditions, the morph effect was overridden by the temperature/photoperiod effect, which thus remains the main signal for diapause induction.

We found that oviparous females had higher fat reserves than viviparous females. Oviparous aphids contain eggs with yolk (vitellus) mostly composed of fat and proteins (Brough & Dixon, 1990). Egg maturation represents an important mobilization from the fat-body to the ovaries in insects (Arrese & Soulages, 2010). Fatty acids serve as a main source of energy for physiological or ecological processes, including flight, gametes production and hormones production, and can represent up to 30% of aphids' fresh mass (Dillwith *et al.*, 1993, Sayah, 2008). Reserves from the fat-body such as glycogen and tryglycerides play major roles in overwintering insects since they are metabolized to produce sugar-based cryoprotectant molecules (Storey & Storey, 1991, Hahn & Denlinger, 2011). Aphidiinae parasitoids diapause as prepupae and accumulation of reserves prior overwintering is critically important (Colinet *et al.*, 2010). Diapause entails important energetic costs for insects (Ellers & Van Alphen, 2002, Hahn & Denlinger, 2011) and parasitoids may enter diapause only if a critical body-mass or amount of energetic reserves is reached (Colinet *et al.,* 2010); developing in an oviparous aphid could contribute to it.

Antifreeze molecules play important roles in insect cold hardiness, allowing them to survive overwintering (Storey & Storey, 1991, Bale, 2002). Metabolic analyses showed sugars and polyols to be mostly concentrated in oviparous females containing eggs intended to overwinter. Glucose-6-phosphate and fructose were found at high concentrations in oviparous morphs of *A. pisum* and are precursors of sorbitol, a cryoprotective compound that was also observed in diapausing mummies of the aphid parasitoid *Praon volucre* (Colinet *et al.*, 2012). Fructose-6-phosphate is a precursor of mannitol, and both are cryoprotectant molecules (Storey & Storey, 1991) highly concentrated in oviparous females. Prior entering diapause, parasitoids could mobilize sugars from their host to synthetize different polyols. Overwintering eggs of the aphid *Hyalopterus pruni* are characterized by high values of mannitol and trehalose (Sømme 1969), which we also observed in *A. pisum* oviparous morphs. In our study, high concentrations of glucose and glycerol (other cryoprotectants) were found in viviparous females. Glycerol is as a cryoprotective substance usually associated with the diapause syndrome (Hayward *et al.*, 2005) but parthenogenetic females can also overwinter and may use it as well (Leather, 1992), while glucose may be used as a rapidly assimilable source of energy by viviparous morphs and their offspring (Dixon, 1985). Cryoprotectant molecules are part of the diapause syndrome (Hodkova & Hodek, 2004) but are not known to initiate diapause. We suggest that the presence of some polyols and sugar metabolites in the aphid, as well as accumulation of fat reserves associated with the overwintering process, may either directly induce diapause or may trigger the endocrine cascade responsible for diapause induction in the parasitoid. Diapausing *P. volucre* prepupae show overall similar metabolites than oviparous morphs of aphids (Colinet *et al.*, 2012), which concur to the hypothesis that parasitoids enter diapause when exposed to these metabolites while developing inside and/or consuming their hosts.

In addition to metabolites, the role of aphid hormones needs to be thoroughly explored in such a bottom-up interaction since diapause in endoparasitoids is likely dependent of the host's endocrine system. Application of ecdysteroids and juvenile hormones at particular insect life stages induce various phenotypic changes and can also initiate diapause (Hardie, 1980, Hodkova & Hodek, 2004, Zhang *et al.*, 2011). In aphids, the endocrine system regulates sexual reproduction and the production of sexual morphs, by transmitting environmental signals from the brain to the ovaries (Hardie *et al.*, 1985, Polgár *et al.*, 1996, Ishikawa *et al.*, 2012).

Finally, in the context of global warming, host-parasitoid interactions may play a crucial role in shaping ecological responses to environmental changes (Jeffs & Lewis, 2013, Delava *et al.*, 2016). Bottom-up effects on diapause, such as reported in our study, should be considered as a potential factor explaining the low levels of diapause expression recorded in parasitoids from mild winter areas (Andrade *et al.*, 2016, Tougeron *et al.*, 2017a). The absence of sexual hosts in the environment may be a missing signal for parasitoids acting together with increasing temperatures to reduce diapause expression. Studying the expression of sex in aphids in different climatic areas will help at forecasting predictions on parasitoids' diapause expression.

Acknowledgments

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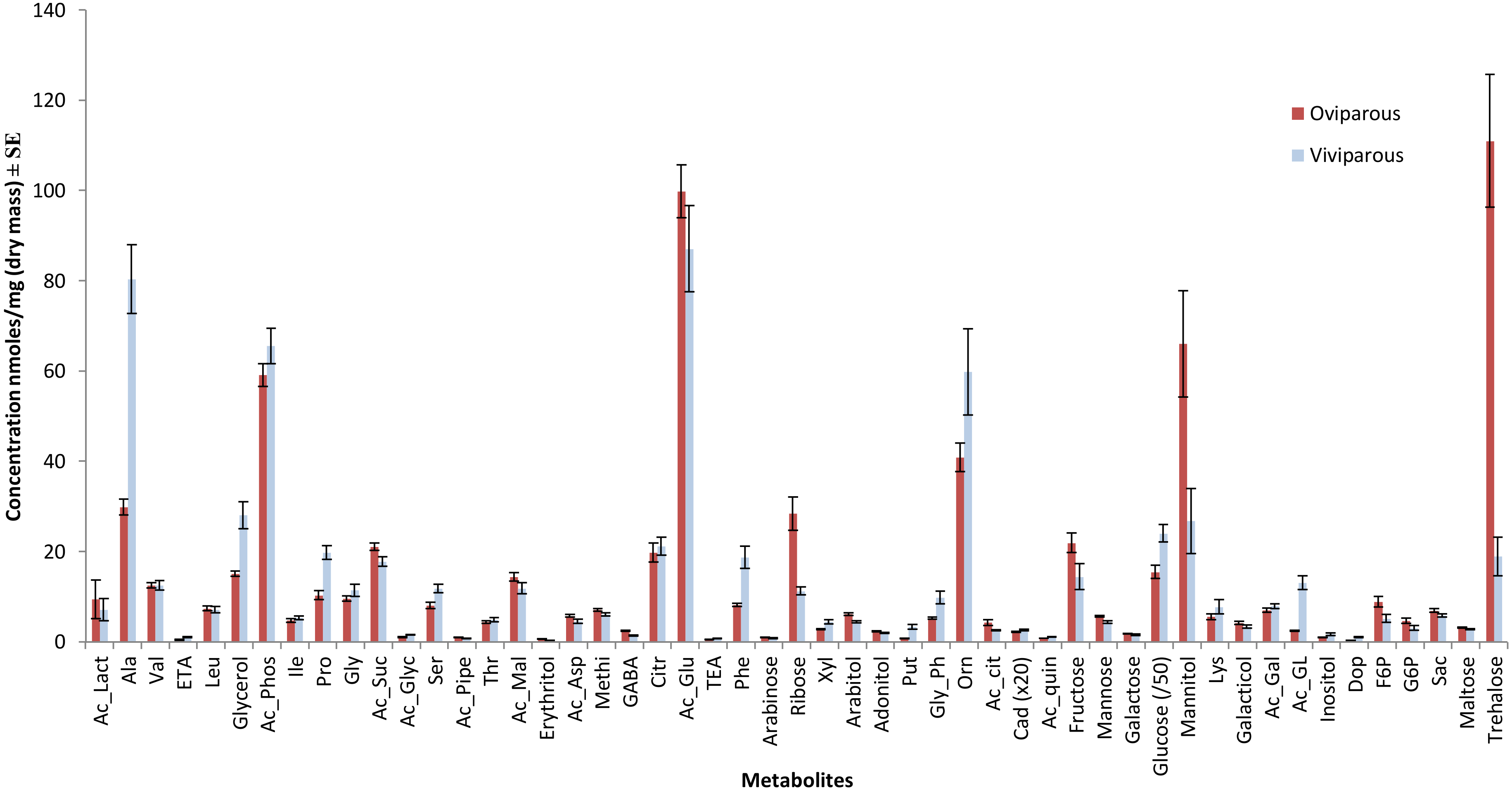

Appendix 7.S1: Mean concentration (nmoles.mg^{-1}) of each of the 47 metabolite observed by gas-chromatography spectrometry in oviparous females (red) and viviparous females (blue) of the pea aphid *Acyrthosiphon pisum*. Note that cadaverine levels (Cad) have been multiplied by 20 and glucose levels have been divided by 50 on this graph to improve scaling. Alanine (Ala), Aspartic acid (Ac_Asp), Cadaverine (Cad), Citric acid (Ac_cit), Citrulline (Citr), Dopamine (Dop), Fructose-6-phosphate (F6P), Galacturonic acid (Ac_Gal), Gamma aminobutyric acid (GABA), Gluconic lactone acid (Ac_GL), Glucose-6-phosphate (G6P), Glutamic acid (Ac_Glu), Glyceric acid (Ac_Glyc), Glycerol-phosphate (Gly_Ph), Glycine (Gly), Isoleucine (Ile), Lactic acid (Ac_Lact), Leucine (Leu), Lysine (Lys), Malic acid (Ac_Mal), Ornithine (Orn), Phenylananine (Phe), Phosphoric acid (Ac_Phos), Pipecolic acid (Ac_Pipe), Proline (Pro), Putrescine (Put), Quinic acid (Ac_quin), Saccharose (Sac), Serine (Ser), Threonine (Thr), Triethanolamine (TEA), Valine (Val), Xylitol (Xyl). N=9 replicates/morph.

Partie IV

Mécanismes liés à la perte d'expression de la diapause

Chapitre 8 : Effets transgénérationnels sur la diapause

Chapitre 9 : Diapause et tolérance au froid

En bref :

- Certains mécanismes liés à la baisse d'expression de la diapause chez les parasitoïdes sont explorés. Les parasitoïdes *A. ervi* entrent en plus forte proportion en diapause après deux générations passées sous les mêmes conditions « automnales ». Cela suggère que si la génération maternelle perçoit de trop hautes températures, elle pourrait inhiber la diapause chez ses descendants. Des effets transgénérationnels sont également visibles sur le temps de développement ainsi que sur plusieurs traits de vie.

- Il existe un compromis dans les capacités de résistance au froid entre le stade qui diapause (prépupe) et le stade adulte chez les parasitoïdes. Les adultes qui proviennent de prepupes qui ne sont pas entrées en diapause ont une meilleure résistance au froid et une masse plus importante que les adultes formés à partir d'individus en diapause.

- Dans les milieux où les hivers sont doux, la stratégie de diapause pourrait donc être éliminée au profit d'autres stratégies d'hivernation car elle est à la fois coûteuse et n'apporte pas un avantage pour survivre aux températures douces présentes l'hiver en Bretagne.

> *"Only model organisms live in a world of endless summer."*
>
> **W. E. Bradshaw *et al.***, (2004)

Chapitre 8

Effets transgénérationnels sur la diapause

Maternal and trans-generational effects on *Aphidius ervi* diapause and life history traits

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En préparation

Summary

Transgenerational effects, including maternal effects, act on a wide range of insects' life history traits and can be involved in the control of developmental plasticity, such as winter diapause expression. Yet, recent studies indicate a decrease in diapause expression in some insect populations, in both natural and laboratory conditions. Two hypotheses could explain a loss of diapause expression: (1) some individuals have no longer the genetic capacity to express it or (2) individuals do not encounter the environmental cues required to trigger diapause. For instance, insect mothers could inhibit diapause expression in their offspring and adjust the proportion of diapausing offspring produced under different environments, as a bet-hedging strategy. In this study, we explored transgenerational effects in one industrial and one wild strain originating from Canada of the parasitoid *Aphidius ervi*. These strains were reared under short photoperiod (8:16 h LD) and low temperature (14°C) over two generations, and came from mothers reared at either 14°C or 20°C. Diapause levels, developmental times, physiological and morphological traits were measured. Diapause levels increased after one generation at 14°C in the wild but not in the industrial strain. This suggests transgenerational plasticity effects on diapause for the wild strain and the presence of low diapause-induction thresholds for the industrial strain. We also observed transgenerational modifications due to maternal effects in developmental times and in life-history traits in both strains, after maternal exposure to different thermal regimes. These results highlight that it may be possible to recover higher diapause levels by acting on transgenerational plasticity in response to diapause-induction cues, provided that the environmental conditions used are reaching the induction-thresholds specific to each population.

Keywords: Bet-hedging, Phenotypic plasticity, Parasitoids, Temperature, Maternal effect, Biological control.

Introduction

Transgenerational plasticity (TGP) represents a particular type of phenotypic plasticity in that environments experienced by the grand-maternal or maternal generation generate phenotypic variations in the offspring generations (Mousseau & Dingle, 1991). TGP effects on insect ecology and life history traits are quite common and have been proved to be adaptive when the parental environment accurately predicts or is similar to the offspring's environment (Sgrò *et al.*, 2016). TGP can involve DNA methylation (*i.e.* epigenetics) (Youngson & Whitelaw, 2008), which is known to act on phenotypic expression and reaction norms, such as diapause (Zhou *et al.*, 2013) and methylation of genes can persist over several generations (Uller, 2008, Sgrò *et al.*, 2016).

Maternal effects have been particularly well studied in the context of developmental plasticity in insects (Mousseau & Fox, 1998, Uller, 2008). In the majority of insects, facultative diapause, a phase of developmental arrest and low metabolic activity, can be induced directly by environmental conditions encountered by the overwintering generation (Tauber *et al.*, 1986). However, in some species, after experiencing environmental cues that inform for deleterious conditions to come, the female may influence carbohydrates and polyols contents of the egg which acts on the developmental plasticity of the embryo (Denlinger, 2002). In this case the sensitive stage is the ovipositing female and diapause is initiated in its offspring (Saunders, 1965, Lacour *et al.*, 2015, Tougeron *et al.*, 2017b). In most cases, both maternal and diapausing generation plasticity are involved in determining diapause incidence (Tauber *et al.*, 1986).

Powell & Bale (2008) and Coleman *et al.* (2014) demonstrated the existence of adaptive maternal effects on cold hardening in overwintering insects, respectively in the grain aphid *Sitobion avenae* (Hemiptera: Aphididae) and the blow fly *Calliphora vicina* (Diptera: Calliphoridae). Among parasitoid insects, Voinovich *et al.*, (2015) showed that diapause was induced by maternal effects in *Trichogramma* (Hymenoptera: Trichogrammatidae) while it was averted in the offspring when mothers were reared at high temperatures. In some parasitoid species, there is a non-photo-thermo-sensible generation at which it is impossible to induce diapause, as a potential adaptation to avoid diapause induction in spring during which environmental conditions are similar to fall) (Reznik & Samartsev, 2015). This phenomenon is based upon a TGP "timer" as parasitoids hatching from winter diapause inhibit diapause induction in their offspring over several generations (Reznik & Samartsev, 2015).

There is growing evidences that, in response to climate changes, or when reared in the laboratory for a long time at constant favorable temperature conditions, insects reduce or abort winter diapause (Musolin, 2007, Tougeron *et al.*, 2017a). For instance, the parasitoid *Binodoxys communis* (Hymenoptera: Braconidae) lost its capacity to enter diapause in less than 300 generations maintained in the laboratory (Gariepy *et al.*, 2015). These shifts in overwintering strategies can be triggered either by (i) evolutionary (genetic) changes (e.g. Bradshaw & Holzapfel 2001) on diapause induction thresholds or (ii) by plastic responses, when parasitoids receive an improper environmental signal to enter diapause, which could include developmental TGP and bet-hedging (Mousseau & Dingle, 1991, Hopper, 1999). If diapause expression in parasitoids is silenced by environmental conditions encountered by the mother, it should be possible to re-induce higher level of diapause by acting on TGP.

The first aim of this study was to measure maternal effects on diapause incidence, developmental time and life-history traits, as parasitoid mothers could induce diapause in their offspring and prepare them for overwintering when they encounter diapause-inductive environmental signals. In *Aphidius* parasitoid wasps, little maternal effects on diapause have been reported, confirming that both parental and direct diapause induction can occur (Brodeur & McNeil, 1989a, Langer & Hance, 2000, Tougeron *et al.*, 2017b) but the effects on development time and morphological traits remain unknown. The second aim was to investigate the propensity of parasitoids to increase their diapause levels and modify trait expression over several generations exposed to these diapause-inductive conditions. We questioned whether low proportion of diapausing individuals in a population could be due to genetic loss of diapause or to the inhibition of plasticity expression by the maternal environment.

To achieve these goals, we exposed over two generations two strains of the parasitic wasp *Aphidius ervi* Haliday (Hymenoptera: Braconidae) to known diapause-inducing conditions, and we manipulated thermal conditions of the maternal generation. If the low levels of diapause are due to a genetic lost, then all of part of the female parasitoids should not produce any individual that enter diapause. If the diapause plasticity is conserved, each female could be able to adjust the proportion of diapausing offspring in response to the perceived environment, as a bet-hedging strategy. We used an industrial strain, kept in non-diapausing rearing conditions for decades and known to express barely no diapause (<5%) (Mubashir-Saeed *et al.* in prep), and a Canadian wild strain known to express higher diapause levels (>90%) (Tougeron *et al.* in prep).

Material & Methods

Insect production

For the present study, rearing cultures were started with two strains of *A. ervi*: an industrial one provided by the Viridaxis S.A. company (Charleroi, Belgium), and a Canadian strain collected in the fields in summer 2015 (Québec, 45.584 °N, 73.243 °W). Both strains were maintained on parthenogenetic pea aphids *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) reared on faba bean (*Vicia faba*, Fabaceae) in pots of potting soil treated with diflubenzuron 25%, an insecticide for shore flies. Insects and plants were maintained at 20°C, 75 ± 5% Relative Humidity (RH) and with a long day photoregime of 16:8 h Light:Dark (LD).

To produce the first generation, mummies (*i.e.* dead aphid containing a parasitoid prepupa) were collected from the rearing culture and isolated in Eppendorf tubes (1.5 mL) until parasitoid emergence. Within the first 48 h after emergence, batches of five female and five male parasitoids were placed in Petri dishes (15 cm diameter) for 24 h for mating, with access to honey and water for feeding. Female parasitoids of each batch were then allowed for 48 h to parasitize a cohort of 100 (± 10) parthenogenetic *A. pisum* second instar aphids on a pot of *V. faba* bean, closed by a piece of fine netting, and with an access to honey and water. The pots of parasitized aphids were then placed in climate chambers for the experiments (see experimental design) and, once formed, mummies were isolated in Eppendorf tubes (1.5 mL) with honey until adult emergence. A part of these newly emerged adults was freeze-killed and used for trait measurements (see below), the rest (n>70) was used to produce the next generation. Two successive generations were produced this way. To obtain the first generation, mating and parasitism were both conducted under rearing conditions. To obtain the next generation, manipulations were conducted in the climate chambers (see below).

Experimental design

a. Transgenerational effects on diapause and life-history traits

Individuals of the two strains were reared for two successive generations at 14°C and 8:16 h LD photo-regime (Figure 8.1A). Note that a third generation will be produced to complete this data, but results are not yet available. The conditions were selected as they are known to induce diapause in aphid parasitoids from temperate areas (Christiansen-Weniger & Hardie, 1999, Tougeron *et al.*, 2017a) while allowing a shorter development time than colder temperatures (Sigsgaard, 2000). This experiment was replicated in three climate chambers to which pots of parasitized aphids were randomly assigned at each generation.

b. Maternal effect

In parallel, maternal effect was tested on two generations, using the industrial strain only. For this purpose, mothers from the rearing culture and their offspring were put in different thermal conditions (14°C or 20°C, at the same photoperiod regime of 8:16 h LD), to have lineages of different temperature history (Figure 8.1B). The temperature of 20°C is not known to induce diapause when associated with short photoperiod regime (Tougeron *et al.*, 2017a) and is different enough from 14°C to expect differences of development (Malina & Praslicka, 2008). Three additional climate chambers at 20°C were used to randomly assign the aphid pots.

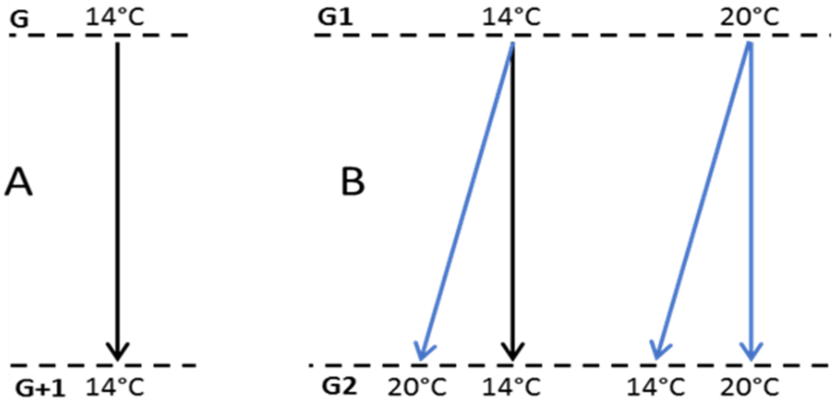

Figure 8.1: Design of the experiments conducted on successive generations of parasitoids. In the first experiment (**A**) 3 generations of individuals from the 2 strains were maintained in the same conditions (14°C, 8:16 h LD) during their entire development. The second experiment (**B**) was conducted only with the industrial strain over two generations. Individuals of the first generation were randomly distributed between 14°C and 20°C, and their offspring (within the parasitized aphids) were also randomly distributed between 14°C and 20°C.

Measures

a. Emergence patterns and diapause

For each replicate of a same cohort (*i.e.* individuals of a same generation, strain and temperature treatment), we measured time from oviposition to first emergence and time from first emergence to half of the total emergence (median date). We also measured the sex ratio and the proportions of emerged, dead and diapausing individuals. Emergences were checked once a day at the same hour and 24 h to 48 h after emergence adult parasitoids were frozen in liquid nitrogen and stored at -80°C for further analyses. Mummies from which no parasitoid emerged 10 days after the last adult emergence in the same cohort were dissected to verify if they were in diapause (moving gold-yellow plump parasitoid larva) or dead (Tougeron *et al.*, 2017a).

b. Fitness-related traits

Morphological traits, related to fitness in parasitoids species, were measured on individuals of each cohort and treatment (generation, strain, or maternal origin). The length of the front right tibia, an estimation of adult size (Ellers, 1995) and the surface of the right wing were measured by image analysis method. Digital pictures of tibia and wing were captured with a camera (Sony N50) mounted on a stereomicroscope and the pictures were analyzed using the numeric image analysis software ImageJ (Wayne Rasband, USA). Individuals were first weighted with a Mettler-electrobalance (Me22; sensitivity: 1µg) to obtain their fresh mass. The dry mass was measured after individuals were dried at 60°C during 3 days in oven. Each dried individual was placed in an Eppendorf tube (1.5 mL) containing 1 mL of a chloroform:methanol solution (1:2) and left for 2 weeks on agitator. They were dried again for 12 h in oven at 60°C to eliminate the residues of the solution before being weighted a last time to obtain the lean dry mass (LDM). The fat contents (FC), a good measure of survival (Ellers, 1995), were then obtained with the dry mass (DM) and the LDM, using the following formula : Fat mass = DM– LDM and FC= FM/LDM (Colinet *et al.*, 2007).

Statistical analyses

All statistical analyses were carried out using the R software (R Development Core Team 2015). Differences in emergence patterns and morphological traits between cohorts were assessed by testing the effect of the variables "strain", "generation", their interaction and the variable "sex" in the experiment on transgenerational effects, or the unique variable "temperature history" in the maternal effect experiment. The significance of each explanatory variable and their interactions were tested using the 'Anova' function (package *car*). Analyses were followed by Tukey multiple comparison post-hoc tests (using package *multcomp* and *lsmeans*), in order to assess differences among modalities of significant variables.

Emergence patterns were fitted to a survival logistic-rank model (using package *survival*) with adding a censoring factor for non-emerged living mummies and models were compared using a likelihood-ratio chisquare method. The proportions of emerged, dead and diapausing individuals were also compared between cohorts by fitting generalized linear models based on a quasibinomial distribution and a logit link function for each parameter, and models were compared by using a Fisher statistic. Data of each morphological trait (fresh mass, tibia length, wing surface and fat contents) was fitted to linear mixed models (using package *nlme*), and the climate chambers identity was considered as a random factor in these models.

Results

Transgenerational effects on diapause and life-history traits

a. Emergence patterns and diapause

Patterns of emergence (at 14°C) were significantly different between generations, with the second generation taking longer to develop than the first one (Survival, LR=52.97, p<0.001) but were not different between strains (Survival, LR=2.58, p=0.108), which both exhibited the same variation between generations (Survival, LR=0.26, p=0.608) (Figure 8.2). In the Canadian strain, there was however no difference to get the first emergences, as it took an average of 27.33 ± 1.85 (mean ± SE) days in the first generation and 27.00 ± 1.15 in the second. For the industrial strain, it took an average of 22.67 ± 0.67 days before the first emergences in the first generation and 27.33 ± 1.33 days in the second. After the first emergence, in the Canadian strain, half of the individuals emerged within 5.33 ± 0.88 days (median date) in the first generation and 7.33 ± 2.60 days in the second one. In the industrial strain, it took 5.00 ± 0.1 days and 6.33 ± 1.86 days, respectively for the first and second generations (Figure 8.2).

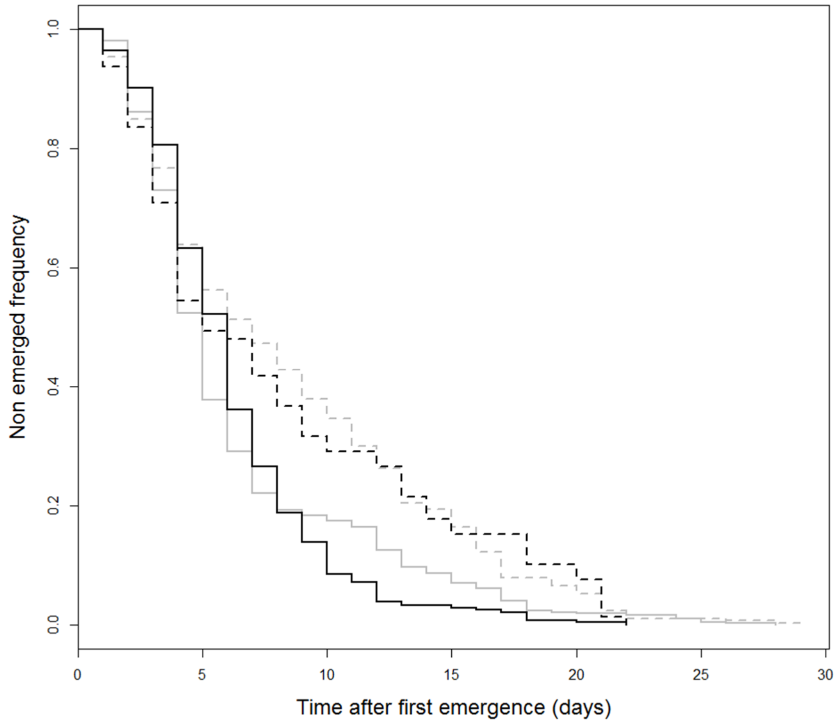

Figure 8.2: Emergence patterns after first parasitoid emergence in each strain, Canadian (black, N=362) and industrial ones (grey, N=730), in the 1st (full line) and 2nd (dashed line) generations.

The proportion of individuals entering diapause was significantly different between strains (GLM, F=6.16, p<0.05) and generations (GLM, F=9.59, p<0.05). Levels of diapause were overall lower in the industrial strain (Tukey, z=-1.96, p<0.05), and in the first generation (Tukey, z=2.96, p<0.005). There was an interaction effect between strain and generation (GLM, F=6.95, p<0.05); between first and second generations, the levels of diapausing individuals statistically increased in the Canadian strain (Tukey, z=2.96, p<0.05), but were not different in the industrial strain (Tukey, p=0.99, z=0.26) (Table 8.1). Every female batch produced diapausing offspring at the first and second generation in the wild strain.

The same proportions of individuals emerged in both strains (GLM, F=4.11, p=0.07) and generations (GLM, F=0.93, p=0.36), and the same proportions died in both strains (GLM, F=0.67, p=0.44) and generations (GLM, F=0.14, p=0.72) (Table 8.1). There were significant differences of sex ratio between strains (GLM, F=14.6, p<0.01) and a significant interaction of strain and generation (GLM, F=7.62, p<0.05). There was a higher proportion of males in the Canadian strain (Tukey, z=4.49, p<0.001) at the first generation, and both strains presented a balanced sex-ratio at the second generation (Tukey, z=0.20, p=0.99) (Table 8.1).

Table 8.1: means ± SE of sex ratio and percentage of emerged, dead and diapausing individuals for each generation of both strains (C: Canadian strain and V: industrial strain from Viridaxis).

Generation	Strain	N	Sex ratio	% Emerged	% Dead	% Diapause
1	C	476	0.65 ± 0.04	52.73 ± 21.33	38.30 ± 17.20	8.97 ± 4.45
	V	615	0.14 ± 0.02	69.21 ± 5.42	30.00 ± 5.88	0.79 ± 0.46
2	C	291	0.57 ± 0.27	22.47 ± 4.47	44.74 ± 5.50	21.66 ± 9.97
	V	422	0.52 ± 0.09	69.35 ± 6.77	30.05 ± 6.43	0.60 ± 0.60

b. Fitness-related traits

There were significant morphological differences between sexes; females being, compared to males (mean ± SE), heavier (LMM, χ^2=19.8, p<0.001, 0.43 ± 0.01 mg *vs.* 0.33 ± 0.01 mg), having longer tibias (LMM, χ^2=52.5, p<0.001, 0.53 ± 0.01 mm *vs.* 0.49 ± 0.01 mm) and had higher fat content ratio (χ^2=15.5, p<0.001, 0.36 ± 0.02 *vs.* 0.24 ± 0.02). There was no difference between wing surfaces (LMM, χ^2=0.22, p=0.64, 1.14 ± 0.01 mm^2 *vs.* 1.16 ± 0.02 mm^2).

Canadian parasitoids had overall lower weights than industrial ones (LMM, χ^2=73.29, p<0.001, 0.32 ± 0.007 mg *vs.* 0.43 ± 0.008 mg, respectively), but it did not change between generations (LMM, χ^2=0.02, p=0.88) (Figure 8.3A). There was no significant difference of tibia length between the two strains (LMM, χ^2=0.33, p=0.56), but parasitoids had overall smaller tibias at the second generation (LMM, χ^2=4.71, p<0.05, 0.54 ± 0.003 mm *vs.* 0.49 ± 0.004 mm, for G1 and G2, respectively) (Figure 8.3B).

There were significant differences of wing surface between both strains (LMM, χ^2=35.9, p<0.001) and generations (LMM, χ^2=31.3, p<0.001), with a significant interaction effect (LMM, χ^2=9.84, p<0.001). Canadian individuals had overall wider wings than industrial ones (1.23 ± 0.02 mm^2 *vs.* 1.10 ± 0.01 mm^2, respectively). Wing surfaces of Canadian individuals decreased at the second generation (Tukey, t.ratio=5.60, p<0.001) while those of the industrial strain remained the same (Tukey, t.ratio=2.44, p=0.07) (Figure 8.3C).

Parasitoids from the Canadian strain had a lower fat contents ratio than parasitoids from the industrial strain (LMM, $\chi^2=8.12$, $p<0.001$, ratio of 0.22 ± 0.02 vs. 0.37 ± 0.01, for Canadian and industrial strain, respectively). There was no difference between generations (LMM, $\chi^2=2.0$, $p=0.15$) (Figure 8.3D).

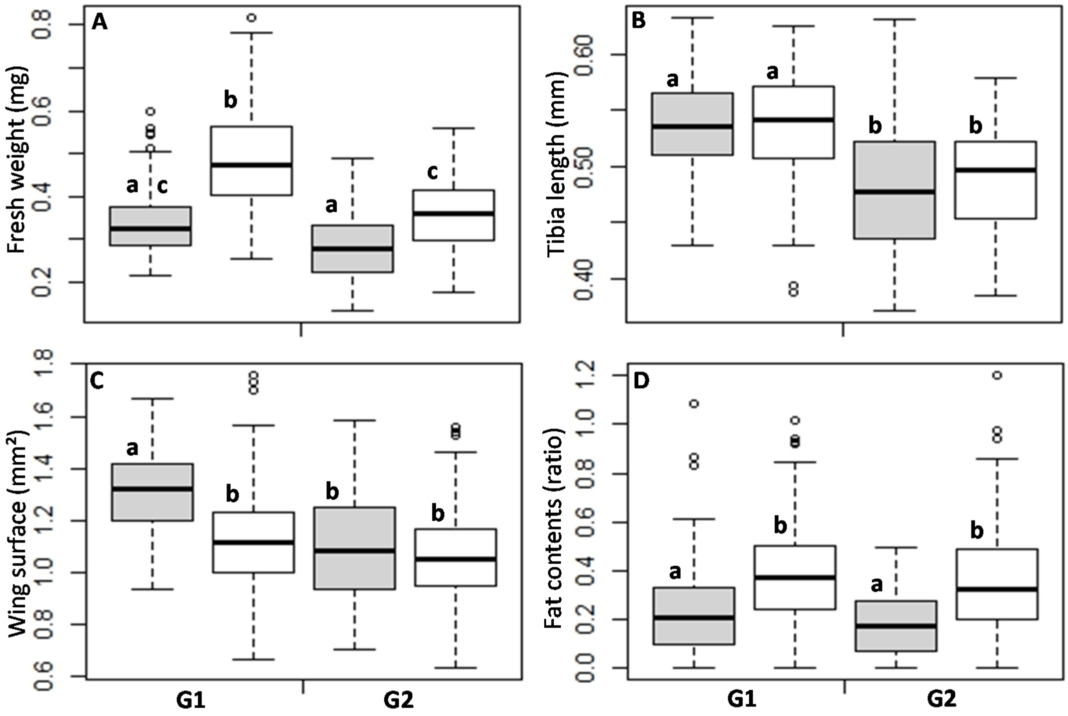

Figure 8.3: Mean trait values of individuals (± CI 95 %). **A**: fresh weight (mg), **B**: tibia length (mm), **C**: wing surface (mm²) and **D**: fat contents (ratio fat mass/lean dry mass) for the two strains of *A. ervi*, Canadian (grey) and industrial one (white), reared at 14°C for the 1st generation (G1, left) and 2nd generation (G2, right). N=40, 120, 32, 50, from left to right, respectively. Lowercase letters indicate significant differences (Tukey post-hoc tests).

Maternal effect

Here we only present data concerning the offspring generation reared at 14°C (from mothers reared either at 14°C or 20°C). Data from offspring generation reared at 20°C is not avalaible at this time.

a. Emergence patterns and diapause

This experiment was done on the industrial strain only. Patterns of emergence were significantly different between temperature conditions and generations (Survival, LR=495.34, $p<0.001$) (Figure 8.4). For the maternal generation, it took an average of 15.7 ± 1.8 (mean ± SE) days after eggs were laid to get the first emergences at 20°C, while it took 22.67 ± 0.67 days at 14°C. In the offspring generation, first emergence was observed later in individuals from mothers reared at 14°C than from mothers reared at 20°C (27.33 ± 1.3 days and 26 ± 1.1 days respectively) (Figure 8.4).

Half of the mothers reared at 20°C emerged 2.3 ± 0.33 days after the first emergences while half of their offspring emerged 3.9 ± 0.62 days after the first emergences. Half of the mothers reared at 14°C emerged 5.0 ± 0.18 days after the first emergences while half of their offspring emerged 6.9 ± 0.65 days after the first emergences) (Figure 8.4).

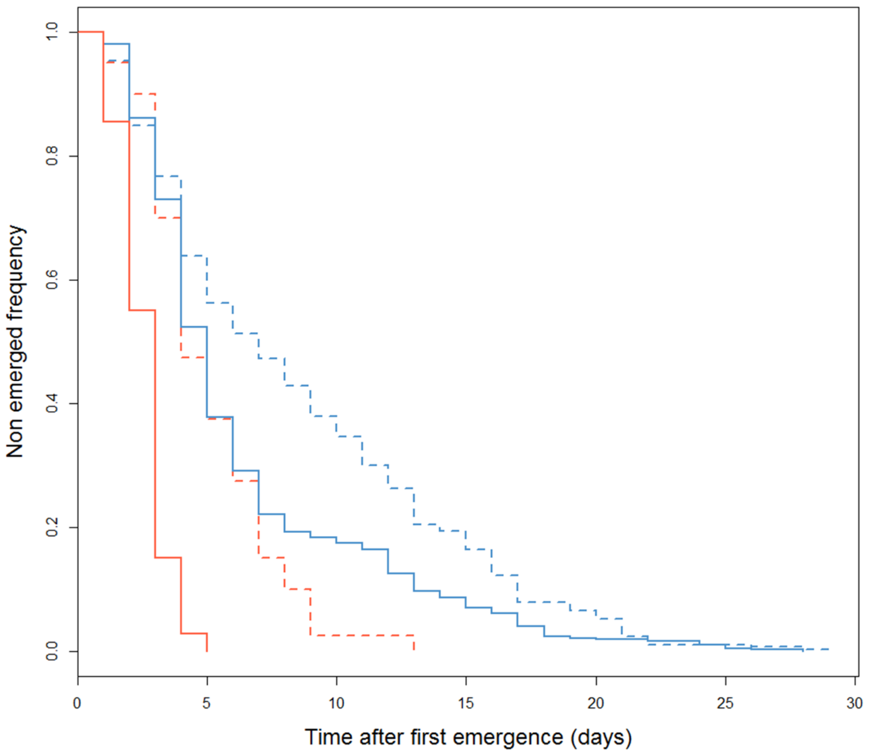

Figure 8.4: Emergence patterns of maternal generations reared at 14°C (full blue line, N=426) or 20°C (full red line, N=180) and offspring reared at 14°C from mothers reared at 14°C (dashed blue line, N=304) or 20°C (dashed red line, N=40).

Among the different cohorts, similar proportions of individuals emerged (GLM, F=1.67, p=0.26), died (GLM, F=0.47, p=0.30) and entered in diapause (GLM, F=0.97, p=0.46) (Table 8.2). Three out of 6 female batches (*i.e.* parasitized aphid pots) never produced diapausing individuals. Sex ratios differed between conditions (GLM, F=10.53, p<0.01), as mothers reared at 14°C produced more males in their offspring than mothers reared at 20°C (Table 8.2).

Table 8.2: Means ± SE of sex ratio and percentage of emerged, dead and diapausing individuals for each generation of both strains. Data show trait measurements on parasitoids of the first (maternal) generation reared either at 14°C or 20°C and of the second (offspring) generation reared at 14°C but produced by mothers from 14°C or 20°C. The photoregime was 8:16 h LD for each temperature condition.

Mothers' rearing temperature	Rearing temperature	N	% Emerged	% Dead	% Diapause	Sex-ratio
Maternal generation	14°C	615	69.21 ± 5.42	30.00 ± 5.88	0.79 ± 0.46	0.14 ± 0.02
Maternal generation	20°C	212	83.97 ± 3.92	16.03 ± 3.92	0	0.21 ± 0.08
14°C	14°C	422	69.35 ± 6.77	30.05 ± 6.43	0.60 ± 0.60	0.52 ± 0.09
20°C	14°C	71	69.66 ± 9.93	49.43 ± 10.67	0.91 ± 0.74	0.23 ± 0.05

b. Fitness-related traits

There were highly significant differences of fresh weight (LMM, F=27.03, p<0.001) (Figure 8.5A), length tibia (LMM, F=19.39, p<0.001) (Figure 8.5B) and wing surface (LMM, F=25.31, p<0.001) (Figure 8.5C), and marginally not significant difference in fat content (LMM, p=0.07, F=2.34) (Figure 8.5D) between maternal and offspring female cohorts.

Mothers reared at 14°C were significantly heavier than the mothers reared at 20°C (Tukey, z=3.16, p<0.01, 0.49 ± 0.01 mg *vs.* 0.35 ± 0.01 mg, respectively). In parallel, their offspring were heavier than those of the mothers reared at 20°C (Tukey, z=3.02, p<0.05, 0.36 ± 0.008 mg *vs.* 0.29 ± 0.02 mg) (Figure 8.5A).

Mothers reared at 14°C had longer tibia than mothers reared at 20°C (Tukey, z=3.13, p<0.01, 0.54 ± 0.004 mm *vs.* 0.49 ± 0.005 mm, respectively), and their offspring were bigger than those from mothers reared at 20°C (Tukey, z=3.14, p<0.05, 0.50 ± 0.005 *vs.* 0.46 ± 0.007, respectively) (Figure 8.5B).

Finally, mothers reared at 14°C had significantly bigger wing surface than mothers reared at 20°C (Tukey, z=6.52, p<0.001, 1.13 ± 0.02 mm² *vs.* 0.89 ± 0.02 mm², respectively), and their offspring had wider wing surface than those from mothers reared at 20°C (Tukey, z=4.81, p<0.001, 1.07 ± 0.02 mm² *vs.* 0.83 ± 0.03 mm², respectively) (Figure 8.5C).

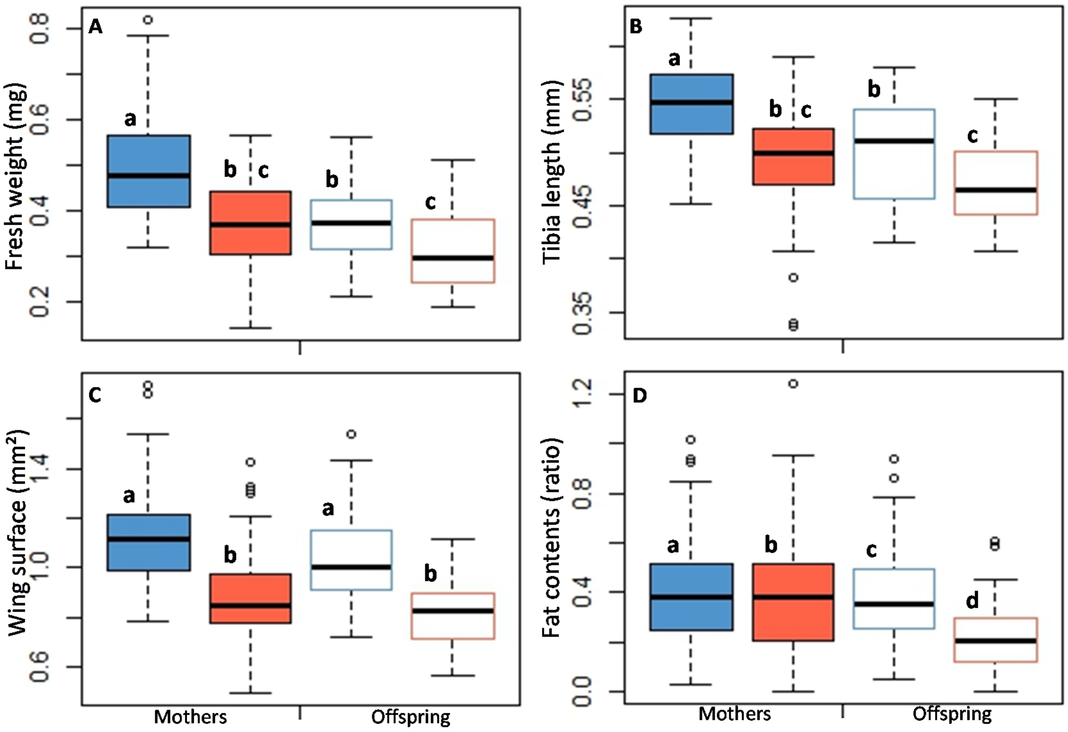

Figure 8.5: Mean trait values of individuals (± CI 95 %). **A**: fresh weight (mg), **B**: tibia length (mm), **C**: wing surface (mm²) and **D**: fat contents (ratio fat mass/lean dry mass) for the different thermal conditions and generations. Left, the maternal generation reared at 14°C (filled in blue) (N=120) and 20°C (filled in red) (N=75). Right, the second generation (offspring) reared at 14°C but from mothers reared at 14°C (blue line) (N=50) or 20°C (red line) (N=22). Lowercase letters indicate significant differences (Tukey post-hoc tests).

Discussion

In the Canadian wild parasitoid strain, but not in the industrial strain, we succeeded in increasing diapause levels over two generations. This result suggest that transgenerational plasticity is involved in diapause induction in *A. ervi* and that higher diapause levels can be retrieved after only one generation, provided that induction thresholds are sensitive enough to environmental cues. In the wild population, parasitoid females could adjust the proportion of diapausing offspring to environmental conditions perceived (bet-hedging). Both strains exhibited a lengthening of the delays of emergence and changes among fitness-related traits over the two generations. Our results could not directly highlight implication of the maternal environment on diapause induction, as too few individuals entered diapause in the industrial strain tested. Yet, maternal thermal conditions influenced fitness-related traits of their non-diapausing offspring, highlighting that phenotypes of non-diapausing parasitoids should be more thoroughly investigated (Tougeron *et al.*, in prep).

Transgenerational effects on diapause and life-history traits

Working on the same industrial strain of *A. ervi* that we used, Mubashir-Saeed *et al.* (in prep.) found 4.7% diapause at the first generation, and an increase to 23.75% at the second, for parasitoids maintained at 8°C (*vs.* 14°C for our study) and 8:16 h LD photoperiod. While we made sure that individuals encountered diapause inducing conditions according to previous studies on *A. ervi* (Christiansen-Weniger & Hardie, 1999, Langer & Hance, 2000), our results may highlight insufficiently low temperatures to reach the thresholds inducing diapause in this industrial strain. Indeed, at the opposite of natural strains, industrial strains are closed genetic pools, maintained under non-diapause inductive rearing conditions over decades and parasitoids are not subject to evolutionary pressures maintaining diapause, which may have led to selection on low diapause induction thresholds, as also suggested in wild parasitoid populations from mild winter climates after climate change (Tougeron *et al.*, 2017a). As we found that some females never produced diapausing offspring, we cannot completely exclude the hypothesis of a genetic loss of diapause in this strain. Testing lower temperatures and photoperiod would be required to conclude on this particular point.

It could also require more than two generations for recovering diapause in this strain at this temperature, as shown in two *Trichogramma* species exposed to non-inducing conditions, in which diapause was re-expressed normally after five successive generations under inducing conditions (Reznik & Samartsev, 2015). Lengthening of development time at the second generation for both strains may thus eventually lead to a gradual return of diapause, as in natural settings, plurivoltine insects set up the overwintering syndrome over several generations (Danks, 2002). This lengthening may also be due to maternal effects (see below), as the first generation had mothers reared at 20°C while the second generation had mothers reared at 14°C.

We obtained surprisingly low levels of diapause in the Canadian strain compared to what was expected for population from harsh winter climates (Tougeron *et al.*, in prep.; Brodeur and McNeil, 1989), with a maximum of 8.97 % ± 4.45 in the second generation, while Tougeron *et al.* (in prep.) found 94.0 ± 2.5 % in similar conditions (14°C, 10:14 h LD). However, they tested individuals shortly after they were collected in the field in summer of the same year

(October 2015). The Canadian strain has then been maintained in constant non-inducing diapause conditions for several generations. A decrease in diapause induction has already been observed after only one (Reznik & Samartsev, 2015) or few (Gariepy *et al.*, 2015, Lauga-Reyrel, 1990) insect generations exposed to non-inducing conditions. However, it is unlikely that diapause has been genetically lost in the Canadian population (*i.e.* that the low diapause levels are due to strains of females never producing diapausing offspring). Indeed, almost all females produced at least one offspring in diapause, suggesting that in this population, the diapause levels we observed were the result of a maternal bet-hedging response to rearing conditions.

Differences in fitness-related traits of non-diapausing individuals were found between strains and generations. Differences between strains may be explained by artificial selection carried out on the industrial strain to increase parasitoids' survival, fecundity and foraging efficiency (Boivin *et al.*, 2012). We found that they were heavier and contained more fat reserves than the wild strain. Differences between generations may be due to physiological costs at developing and living at 14°C, which is a sub-optimal temperature for *Aphidius* parasitoids (Sigsgaard, 2000, Le Lann *et al.*, 2011b). Decrease in size at the second generation exposed to 14°C for both strains does not follow the temperature-size rule in insects, which states that exposure to low-temperature increases adult size (Van der Have & De Jong, 1996). This pattern seems however to be consistent with the Absolute Energy Demand hypothesis which predicts that larger individuals are disadvantaged under stressful conditions (Blanckenhorn, 2000). As a matter of fact, reduction in adult size has been observed several times in *Aphidius* species experiencing cold stress (Ismail *et al.*, 2012; Tougeron *et al.*, 2016; **Chapter 9**, this thesis).

Maternal effect

In our investigation, we could not provide evidence for maternal effect on diapause induction. As discussed before, the industrial strain may have low diapause-induction thresholds that were not reached in this study. In addition, there may be missing signals for maternal effects to be expressed, such as food or humidity (Mousseau & Dingle, 1991, Tauber *et al.*, 1998). However, our results indicated that offspring's traits and development time were influenced by temperatures experienced by their mothers.

Influence of maternal age or of photoperiod experienced by the parental generation on offspring development time have been shown in insects (Mousseau & Dingle, 1991, Fox, 1994). Heritability of development times has also been well studied in insects (Sequeira & Mackauer, 1992, Bradshaw *et al.*, 1997, Roff, 2000), but we are not aware of any example on change in offspring development time after maternal exposure to different temperatures. We demonstrated here that the delay in development time (half-emergence) between female parasitoids reared at 14°C and 20°C (*i.e.* 2.7 days) was comparable to the delay in development time between their respective offspring, both reared at 14°C (*i.e.* 3 days), suggesting an effect of transgenerational plasticity.

Morphological and physiological traits were also concerned by these maternal effects, as differences in mean trait value between both types of females (14°C and 20°C) were passed on their respective offspring. We believe we can exclude any effect of trait genetic heritability on the differences we observe since we started the experiments with a sufficient number of parasitoids and since the industrial parasitoid strain is very homogeneous in mean traits. In any case, we propose that such "predictive" maternal effect at 14°C would prepare the offspring to better face their incoming environment, as we observed them to be heavier, taller, with wider wings and with more fat reserves.

To conclude, diapause levels expressed in a population are modulated by TGP, but selection may act on lowering diapause induction thresholds and on modifying the proportion of diapausing offspring produced by each mother (bet-hedging). If the maternal environment is analogous to the offspring's environment, one can expect that silencing diapause and modifying traits through TGP will result in an adaptive phenotype for the overwintering generation. If not, fitness costs can occur for the offspring, caused by mismatches in the transgenerational transfer of environmental information (mother-offspring conflict, Coleman *et al.*, 2014). In this sense, adaptive significance of maternal effects is still controverted (Marshall & Uller, 2007) because mothers do not always know the best (Henry *et al.*, 2005) and cannot necessarily anticipate incoming environments (Uller *et al.*, 2013). For instance, Coleman *et al.*, (2014) demonstrated that *C. vicina* adults reared at 20°C reduced of 3.5°C their diapausing offspring's cold resistance compared to adults reared at 15°C, suggesting limits of transgenerational plasticity in the context of climate warming. Predicting insect responses to global climate change thus implies a better understanding of maternal and transgenerational effects, in particular on fitness-related traits and on phenotypes that are extremely plastic to seasonal variability, such as diapause. Knowledge is also particularly lacking on the genetic basis of diapause expression and diapause induction thresholds in parasitoids (but see Paolucci *et al.*, 2016 for a study on clock genes polymorphism across a latitudinal gradient).

Acknowledgments

MD performed the experiments. MD and KT wrote the manuscript. All co-authors participated at elaborating the protocol and revising the manuscript. KT was supported by the French Region Bretagne and the Canada Research Chair in biological control. The authors thank technical support provided at the UCL and at the IRBV, Montréal.

Chapitre 9

Diapause et tolérance au froid

Trade-off between diapause and cold-resistance in aphid parasitoids

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En préparation pour *Journal of Insect Physiology*

Summary

To overwinter, insects from mild temperate areas can either enter diapause or remain active. Both strategies involve costs and benefits depending on the environment. In the first case, the emerging individuals will resist winter but have a reduced fitness because diapause entails physiological and ecological costs. In the second case, individuals need to be cold-resistant enough to withstand winter temperatures during their immature and adult stages, but could avoid diapause-associated costs. In mild temperate areas, the cost-benefit balance between the diapause and the non-diapause strategy would likely change in response to climate warming. A trade-off between these two strategies should lead to reduction of diapause expression in some populations. We explored the importance of such trade-off through the comparison of cold resistance capacities among different life stages in diapausing and non-diapausing individuals in a population of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) originating from western France where a decrease of diapause incidence was recently observed. As a measure of insect cold resistance, the Super Cooling Point (SCP) was determined for non-diapausing and diapausing prepupae and adults that went through prepupal diapause or not. Diapausing and non-diapausing prepupae were equally cold-resistant (-24.20 ±0.30°C *vs.* -24.74 ±0.36°C, respectively), and overall more resistant than adults. Adults that went through diapause as prepupae were less cold resistant (-17.85 ±1.10°C) than adults that have not undergone through diapause (-21.10 ±0.54°C). We also found that diapausing prepupae and adults that have undergone diapause were lighter than their non-diapausing counterparts, at a comparable size, suggesting higher energetic expenses during diapause. These results suggest a trade-off between diapause expression in prepupae and cold resistance and life-history-traits in adults. We conclude that selection could favor insects that do not enter diapause and thus avoid its associated costs while taking advantage at exploiting the mild winter environment.

Key-words: Overwintering, Super Cooling Point, Cold resistance, Mass, Size, Plasticity, Life-history-traits.

Introduction

In temperate areas, insects have evolved various strategies to spatially or temporally cope with recurrent unfavorable periods (Danks, 2006). They can migrate, resist freezing conditions or, as for most insects, avoid lethal freezing of body fluids (Bale, 1987). These freeze-intolerant insects have the capacity to lower the temperature at which their cell fluids crystalize (*i.e.* the Super Cooling Point (SCP)) by producing cryoprotectant molecules (e.g. polyols, sugars), eliminating ice nucleators in gut contents and partially dehydrating their tissues (Bale, 1996, Danks, 2007).

Increasing cold-resistance can be triggered by environmental stimuli that also induce diapause (*i.e.* developmental arrest), such as the photoperiodic decrease, and in this case entering diapause also provides higher cold-resistance (Tauber *et al.*, 1986, Koštál, 2006). In some species, increased cold resistance is accomplished through an acclimation process (*i.e.* response to stress) when insects experience low temperatures (Lagerspetz, 2006). In such a case, acclimation is not necessarily associated with diapause (Hayward *et al.*, 2014). The cause-effect relationship between diapause and cold-resistance in insects is still debated, and both phenomenon may not be directly related depending on species or populations (Denlinger & Lee, 1991, Xie *et al.*, 2015) (for a review on these links, see Hodkova and Hodek, 2004).

Diapause is generally expressed at one specific stage of the insect's development (Tauber *et al.*, 1986). Diapause during an immature stage has inherent metabolic costs because of the use of energetic reserves to withstand winter temperatures and survive throughout winter in an inactive stage (Schmidt & Conde, 2006, Hahn & Denlinger, 2011), and therefore involves a trade-off with some life-history traits at the adult stage (Kroon & Veenendaal, 1998, Fordyce *et al.*, 2006). For instance, in the parasitoid *Asobara tabida*, the duration of larval diapause negatively impacts emerging females' egg load after diapause (Ellers & Van Alphen, 2002). Larval diapause was also shown to be associated with a decrease in adult longevity in the parasitoid *Praon volucre* (Colinet *et al.*, 2010).

Cold resistance itself, with or without diapause, can negatively affect different life-history traits such as longevity or heat-resistance in insects (Zera & Harshman, 2001, Hayward *et al.*, 2005, Basson *et al.*, 2012, Sulmon *et al.*, 2015). Within insect populations, different thermal performance and overwintering strategies (diapause *vs.* non-diapause) can be observed, as a result of local adaptations or plastic responses to environmental variability, such as bet-hedging (Hopper, 1999). Depending on the strategy used by an individual insect, trade-off in cold resistance could appear between life-stages when resources allocated at the diapausing immature stage become less abundant for the adult non-diapausing stage.

Aphidiinae aphid parasitoids (Hymenoptera: Braconidae) enter diapause as prepupae which allows measurement of potential trade-offs with the adults and to study costs and benefits of the diapause strategy. In populations from mild winter areas (e.g. Western Europe), diapause incidence has been decreasing for a few years and parasitoids of different species now mostly remain active throughout the year (Lumbierres *et al.*, 2007, Gómez-Marco *et al.*, 2015, Andrade *et al.*, 2016, Tougeron *et al.*, 2017a). Exploring these trade-offs will help at understanding the rationale of changes in overwintering strategies in the context of climate warming. Parasitoids that do not enter diapause should be cold-resistant enough to withstand low temperatures during both their immature and adult stages. Parasitoids that remain active may have a fitness advantage because they avoid diapause associated costs while taking advantage at exploiting anholocyclic aphid populations present throughout the year. If so, selection should favor them over diapausing parasitoids, which could lead to a reduction in diapause expression in mild winter areas populations.

This study aimed at exploring the relationship between diapause and cold-resistance in a parasitoid population of *Aphidius ervi* from a mild winter area where both diapause and non-diapause strategies are expressed during winter (Andrade *et al.*, 2016, Tougeron *et al.*, 2017a). We first hypothesized that energetic costs of diapause engender a trade-off between diapause at the prepupal stage and cold-resistance at the adult stage; adults that have been in prepupae diapause should be less cold-resistant than diapausing prepupae. We also expected adults that have not undergone diapause (*i.e.* adults that are active during winter) to have a higher fitness; they should be heavier and bigger than adults that come from diapausing prepupae. We then examined cold resistance in diapausing *vs.* non-diapausing individuals, as well as associated fitness consequences. We hypothesized that diapausing prepupae are more cold-resistant than non-diapausing ones, but due to trade-offs, adults that remain active in the winter should be more cold-resistant than adults that have undergone prepupal diapause.

Material & Methods

Biological material

A colony of the aphid *Sitobion avenae* F. (Hemiptera: Aphididae) was established from a single female collected at Le Rheu (France) in 1990. Aphids were reared on winter wheat sprouts (*Triticum aestivum*). Aphids served as hosts for the parasitoid *A. ervi*, whose colony was established from individuals collected in 2015 around Rennes, France (48.1°N; 1.7°W). Both insects were reared at 20 ±1°C, 16:8 h Light:Dark (LD) photoregime and 70 ±10 % relative humidity (RH).

Diapause induction

Thirty < 48h-old, mated female parasitoids were split in two groups and introduced in two cages (50x50x50cm) with 350 to 400 *S. avenae* aphids of second or third instar for 48 h. Parasitoids were then removed from the cages and the cages with parasitized aphids were placed at 17°C, 10:14 h LD, 70% RH, conditions known to induce 8.3 ±4.2% diapause in our *A. ervi* population (Tougeron *et al.* 2017a). Aphid mummification (*i.e.* dead aphids containing a parasitoid prepupa) was checked daily - starting 5 days after oviposition - and mummies were isolated in eppendorf tubes. This protocol was repeated three times over the course of the experiment (4 months) to obtain sufficient number of parasitoids to be tested.

Five different modalities were tested and produced as follow (Figure 9.1); Less than 24 h after mummification, 27 mummies were taken to measure the SCP of the one-day-old prepupae (P1). Early parasitoid instars are the sensitive stage for diapause initiation in Aphidiinae (Brodeur & McNeil, 1989a) but it was impossible to visually separate mummies containing diapausing or non-diapausing prepupae in the P1 treatment. We therefore tested the SCP of 21 one-day-old non-diapausing parasitoid prepupae formed at 20°C, 16:8h LD (P1C), a condition that does not induce diapause (Tougeron *et al.*, 2017a). Twenty-four adult parasitoids emerged up to 10 days after mummification were tested for their SCP within 12 h after their emergence (adults that have not undergone diapause, AND). Sixteen mummies from which no adults had emerged 15 days after mummification were considered to contain a 15-days-old diapausing prepupa (P15; Tougeron *et al.*, 2017) and their SCP was measured. The remaining mummies were transferred at 20°C, 16:8 h LD to terminate diapause. The SCP of 25 adults emerging from this treatment was measured within 12 h after adult emergence (adults that went through diapause at their prepupae stage, AD). This latter treatment allowed us to measure the number of days required to break diapause in *A. ervi* under the experimental conditions tested (we stopped recording emergence 100 days after the mummies were placed at 20°C). To make sure parasitoids stayed in diapause long enough to see an effect on their SCP, we only tested individuals that emerged at least 40 days after being placed at 20°C.

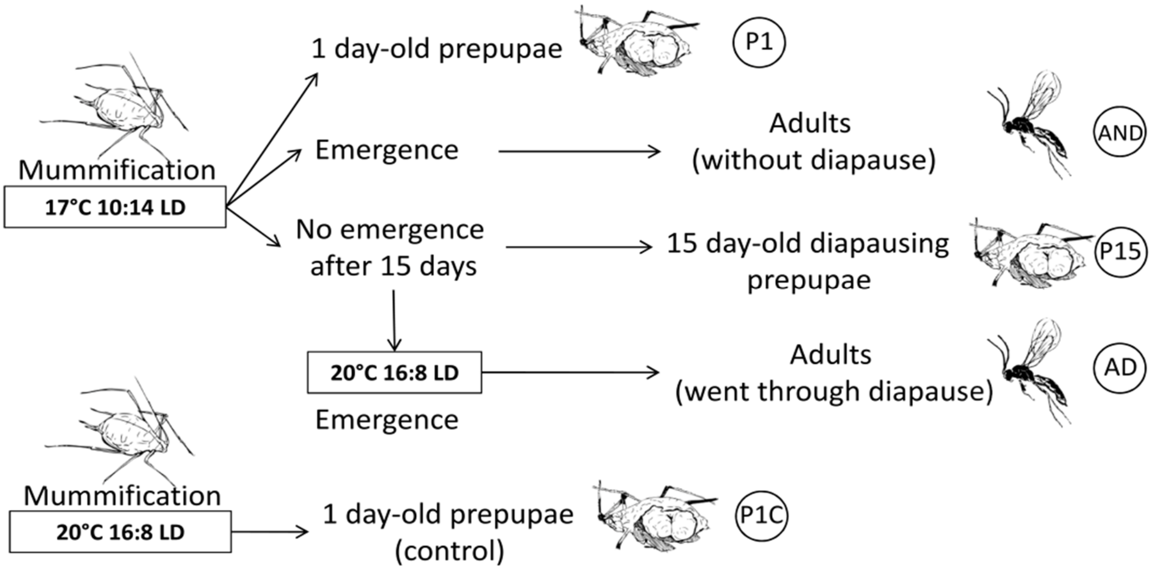

Figure 9.1: Schematic of the experimental design used to produce the five parasitoid treatments on which Super Cooling Points were measured. One-day-old prepupae in the mummy (P1), adults that did not enter prepupal diapause (AND), fifteen-days-old diapausing prepupae (P15), adults that went through prepupal diapause (AD), and one-day-old prepupae (Control; P1C).

SCP measurements

Super Cooling Points of prepupae (within the mummy) and emerged adults were measured using metallic thermocouples plugged in a high resolution temperature data-logger (Testo 176-T4, ±0.3°C, Germany). Parasitoids were placed in a 7mm diameter gelatin capsule (Capsugel Coni-snap 1EL, NJ, USA) in direct contact with the thermocouple (Hanson & Venette, 2013). Capsules were then placed in glass test tubes on a rack that was immerged in the cryostatic bath. Temperature was set to cool at a rate of 0.2°C per minute, from +5°C to -30°C. Data was extracted from the data-logger using Testo Comfort Software 5.0. SCP corresponds to the onset of the exotherm produced by the latent heat of freezing, just before the death of the insect (Colinet *et al.*, 2007, Renault *et al.*, 2002).

Mass, volume and size measurements

We determined if the SCP was influenced by the mass of the parasitoids and the size of the adults or the volume of the mummies. Mass was measured using a ± 0.1 µg precision balance (Mettler-Toledo XP2U). Aphid mummies were weighted before and after each SCP experiment to test for potential effect of freezing on the mass. No difference was found (before SCP measurements: 0.36 ±0.04 mg, and after: 0.35 ±0.04 mg, Student paired t-test t=1.42, p=0.19, n=17), thus only the mass obtained after SCP measurements was kept for analyzes. Size of the adults and volume of the mummies were measured using a camera AxioCam ERc 5s (Zeiss, Germany) and the ImageJ software (v1.51). For adults, the length of the left hind tibia was measured. For mummies, length (L) and width (W) were measured and the volume (V) was obtained using the relation: $V = \pi L W^2/6$ (Colinet *et al.*, 2007). The mass/volume and mass/size ratio were then calculated and used in the analyses to determine if they had an influence on SCP. The sex of the adults was also determined as sexual dimorphism in size exists in *A. ervi* (Hurlbutt, 1987) and could influence the SCP (Renault *et al.*, 2002).

Statistical analyses

A generalized linear model (GLM) was fitted to the data to test for differences in SCP among the five modalities (diapausing prepupae, non-diapausing prepupae, control prepupae, adult which went through immature diapause and adult which emerged without diapause) and using the sex and the ratio mass/size or mass/volume as covariables. Tukey post hoc tests for linear models (package '*multcomp*') were then performed to test for differences among treatments. Data was then split between adults and prepupae and GLMs were fitted to the data in order to assess for differences in mass/size or mass/volume ratio between diapausing and non-diapausing parasitoids (sex was used as a covariable for adults). Differences between explanatory variables were assessed using a type-II Anova from the package '*car*'. All statistical analyses were performed using R software (R Core Team, 2017). The SCP distribution among the three prepupae treatment was plotted in order to make sure that no subgroup of prepupae (e.g. diapausing *vs.* non-diapausing) was observed within each treatment.

Results

Super Cooling Points differed significantly among parasitoid treatments (GLM, LR=75.6, df=4, p<0.001) (Figure 9.2). The lowest SCP (*i.e.* the highest cold resistance) was found in the prepupae; both types of prepupae had similar SCPs (-24.2 ±0.3°C and -24.7 ±0.4°C for one-day, fifteen-days old diapausing prepupae, respectively, Tukey Contrasts, p=0.96). Their SCPs did not differ from the control group of one-day-old prepupae reared at 20°C 16:8 h LD (-24.1 ±0.6°C, Tukey Contrasts, p=0.97). The three types of prepupae had a lower SCP than adults that have not undergone diapause (-21.1 ±0.5°C, Tukey Contrasts, p<0.05) and adults that have undergone diapause (-17.8 ±1.1°C, Tukey Contrasts, p<0.05). Adults that have not undergone diapause had a higher cold-resistance than adults that went out of diapause (Tukey Contrasts, p<0.05).

From the prepupae placed at 20°C 16:8 h LD to break diapause, parasitoid adults took from 22 to 82 days to emerge (AD treatment) with a mean (±SE) emergence time of 61.4 ±3.3 days.

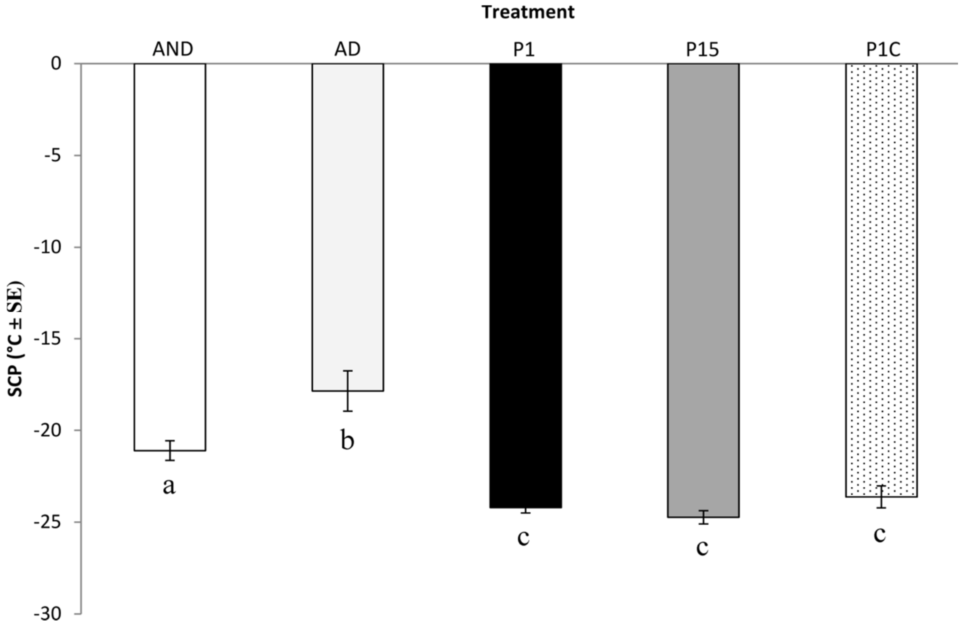

Figure 9.2: Differences in SCP (±SE) among parasitoid treatments; Adults that did not emerge from a diapausing prepupae (AND, n=24), adults that went through diapause at the immature instar (AD, n=25), one-day-old prepupae in the mummy (P1, n=27), fifteen-days-old diapausing prepupae in the mummy (P15, n=16), and control one-day-old prepupae in the mummy (P1C, n=21). Lowerscript letters indicate significant differences (p<0.05) between factors according to Tukey post-hoc tests for linear models following a GLM.

The distribution of SCP among prepupae treatments was homogeneous and unimodal (Figure 9.3). Even if both diapausing and non-diapausing prepupae could have been present in the P1 treatment, no SCP subgroup was observed.

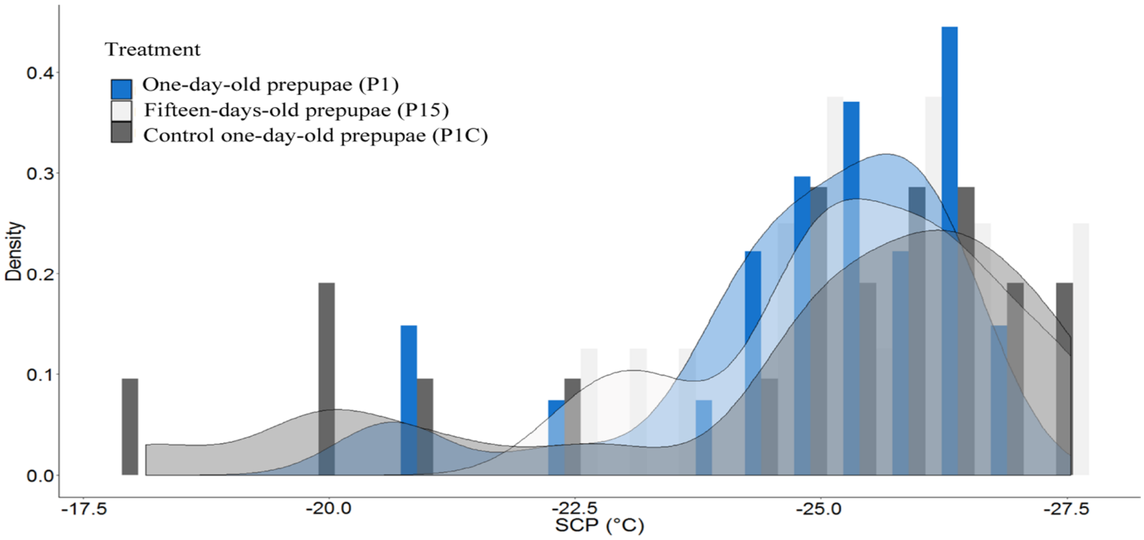

Figure 9.3: SCP distribution and density curves among the three different prepupae treatment; one-day-old prepupae (P1, blue), fifteen-days-old prepupae (P15, light gray) and control one-day-old prepupae (P1C, dark gray).

Mass/size ratios were smaller in AD (0.13 ±0.02) than in AND (0.22 ±0.02) and in P15 (0.26 ±0.06) than in P1 (0.47 ±0.06) (Figure 9.4). This means that for a given body size, adults that have undergone diapause (AD) had a mass 1.6x lower than their non-diapausing counterparts (AND) (GLM, LR=7.0, df=1, $p<0.01$). Prepupae in diapause (P15) had a mass 1.8x lower than non-diapausing prepupae (P1) (GLM, LR=5.7, df=1, $p<0.05$) (Figure 9.4).

The mass/size ratio did not influence the SCP of adults (GLM, LR=2.6, df=1, p=0.10), for both AD and AND treatments (GLM interaction, LR=0.3, df=1, p=0.57), neither did the mass/volume ratio for prepupae (GLM, LR=0.005, df=1, p=0.98), for both P1 and P15 treatments (GLM interaction, LR=0.9, df=1, p=0.34) (Figure 9.4). Sex had no effect on the SCP of the adults (GLM, LR=0.6, df=1, p=0.43).

There was SCP variability within treatments that could be explained by mass and size differences. When analyzed separately from the mass, small adults had a lower SCP than big ones (GLM, LR=6.2, df=1, $p<0.05$, $R^2=0.14$) for both adults that have undergone diapause and adults that have not undergone diapause (interaction, GLM, LR=0.03, df=1, p=0.84). Moreover, when analyzed separately from the size, light adults had a lower SCP than heavy ones (GLM, LR=4.4, df=1, $p<0.05$, $R^2=0.05$), for both adult treatments (interaction, GLM, LR=0.58, df=1, p=0.44). This relationship was not true for the volume (GLM, LR=1.8, df=1, p=0.18) and the mass (GLM, LR=0.96, df=1, p=0.33) of the prepupa treatments.

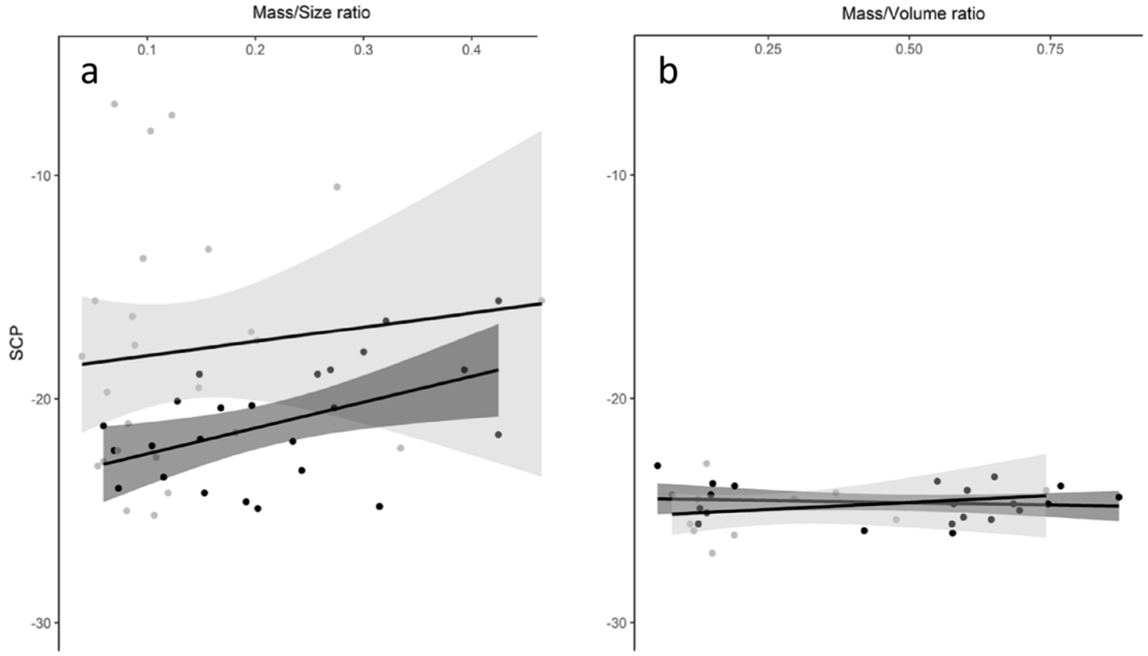

Figure 9.4: SCP (°C) variation depending on the (a) mass/size ratio of adults and (b) the mass/volume ratio of mummies, for diapausing prepupae (P15, n=16) or adults that come from diapause (AD, n=25) (grey) and non-diapausing prepupae (P1, n=27) or adults that did not undergo diapause (AND, n=24) (black). Lines and shaded areas show GLM predictions ± SE.

Discussion

Aphidius ervi adults that emerged from individuals that went through diapause had a lower cold resistance (*i.e.* a higher SCP) than any parasitoids from the other treatments suggesting a trade-off in cold-resistance within and between life-stages, depending on the diapause *vs.* non-diapause strategy used by the parasitoid. Our results also suggest that the non-diapause strategy could be advantageous in mild winter areas because parasitoids are able to withstand winter temperatures while avoiding diapause costs. Unexpectedly, diapausing prepupae did not have a higher cold resistance than non-diapausing prepupae, since no SCP differences were observed between 1-day-old and 15-days-old (diapausing) prepupae.

Our results suggest a trade-off between immature (prepupa) and adult cold-resistance when parasitoids enter diapause. This phenomenon might be the result of energetic expenses during diapause, leading to less energetic reserves to sustain low temperatures for the emerging individual. We also found that adult parasitoids that have undergone diapause and diapausing prepupae were lighter than their non-diapausing counterparts, highlighting a physiological cost to diapause. It is known in some parasitoid species that the fitness of adults emerging following a diapause episode decreases with diapause duration (Ellers & Van Alphen, 2002). We also highlighted costs in term of physiological functions as shown by the lower SCP of adults that went through larval diapause. The observed trade-off in cold-resistance is consistent to the fact that parasitoids terminate diapause when favorable conditions return. In this case, energy could be allocated to sustain other functions than thermal resistance.

Aphidius ervi exhibited SCP values in the range of values observed in closely related species (Langer & Hance, 2000, Colinet *et al.*, 2007) but no SCP difference was found between the three types of prepupae in the present study. In aphid parasitoids, the relation between SCP and diapause is not yet clear, as the SCP of immature instars of *A. ervi* and *Aphidius rhopalosiphi* was found to be low during diapause (*i.e.* high cold-resistance) (Langer & Hance, 2000) while no link was found in *Praon volucre* (Colinet *et al.*, 2010). First, excretion of nucleating gut content (*i.e.* meconium) after the nymphal metamorphosis may have limited action of cold on both types of prepupae in a similar fashion. Second, cold-hardening may happen later in the process of diapause (after 15 days), as parasitoids can remain in diapause over several months in natural conditions (Koštál, 2006). In addition, higher cold-resistance may appear only if insects are exposed to cold temperatures, following acclimation in winter, either during diapause or not (Denlinger & Lee, 1991, Hodkova & Hodek, 2004, Lester & Irwin, 2012). In our study, parasitoids from each treatment developed and acclimated at the same temperature.

Finally, in insects, the relation between supercooling temperature and sex or morphological traits such as size or mass is still in debate. Colinet *et al.*, (2007) showed that the more important the volume of the mummy, the less cold resistant *Aphidius colemani* prepupae in the mummy was. The ability to supercool (*i.e.* to resist crystallization) decreases as mass, size and volume increase (Colinet *et al.*, 2007). Such a pattern was not observed in our *A. ervi* population. This may be due to interspecific differences or to different rearing photoperiods (16:8 h LD in Colinet *et al.* (2007) *vs.* 10:14 h LD in our study) as it was shown to influence morphological traits, development times and insects' fitness independently of the temperature (Joschinski *et al.*, 2015, Tougeron *et al.* in prep).

Sexual dimorphism in size, mass and energetic reserves have to be carefully considered when exploring differences in thermal tolerance (Blanckenhorn, 2000, Le Lann *et al.*, 2011c, Ismail *et al.*, 2012). In adults, when taken apart from each other, size and mass influenced the SCP of the parasitoids. This is in line with previous studies on parasitoids showing that small individuals are more cold-resistant than large parasitoids (Ismail *et al.*, 2012, Tougeron *et al.*, 2016), due to more parsimonious use of energetic reserves (Reim *et al.*, 2006). Differential resistance to cold between sexes has been reported in aphid parasitoids (Le Lann *et al.*, 2011c), with adult females being less resistant to cold than males, probably due to size differences between sexes. We observed no such difference in our study, suggesting that Aphidiinae parasitoids' SCP is influenced by the size and not by sex *per se.*

The population of *A. ervi* used in this study does not express high levels of diapause (mean 11.2 ± 4.9% at 14°C, 10:14 h LD; Tougeron *et al.*, 2017), and parasitoids can remain active as non-diapausing individuals throughout winter (Andrade *et al.*, 2016), suggesting a risk-spreading strategy. The relative proportions of diapausing and non-diapausing parasitoids is likely to be determined by the risk of encountering cold spells or harsh winters at a given location (Hopper, 1999). Parasitoids can either (i) overwinter as diapausing prepupae, and then resist winter cold but miss opportunities to exploit a favorable winter environment and have a lower fitness as emerging adults, or (ii) abort diapause and be better cold-resistant at the adult stage to withstand possible cold spells and exploit the winter environment. This latter strategy may be advantageous and thus selected if diapausing prepupae does not have a better cold resistance than non-diapausing ones, and if parasitoids are cold-resistant enough to overwinter as adults, which seems to be the case in mild-winter areas such as western Europe (Lumbierres *et al.*, 2007, Andrade *et al.*, 2016, Tougeron *et al.*, 2016, 2017a).

To conclude, diapause expression could be reduced if the balance between its physiological (e.g. energetic demand) or ecological costs (e.g. no exploitation of the winter environment, less generation produced each year) and its benefits (e.g. increased cold resistance, synchronization with the host) is shifted by climate warming (Bale & Hayward, 2010, Sgrò *et al.*, 2016). In more natural setting, insects can adopt behavioural adjustments of their thermal tolerance capacities, which may participate in adjusting their response to climate warming (Sunday *et al.*, 2014). For instance, parasitoids are known to manipulate their host in the context of diapause to make them choose sheltered areas in which they can overwinter (Brodeur & McNeil, 1989b). Non-diapausing parasitoids and their hosts can also live in microclimatic habitats (thermal shelters) that can modify their thermal tolerance capacities (Tougeron *et al.*, 2016, Alford *et al.*, 2017). There is now a need to better understand the winter ecology and physiology of non-diapausing insects, as they are facing increasing winter temperatures, especially in the context of climate warming (Owen *et al.*, 2013; Stuhldreher *et al.*, 2014).

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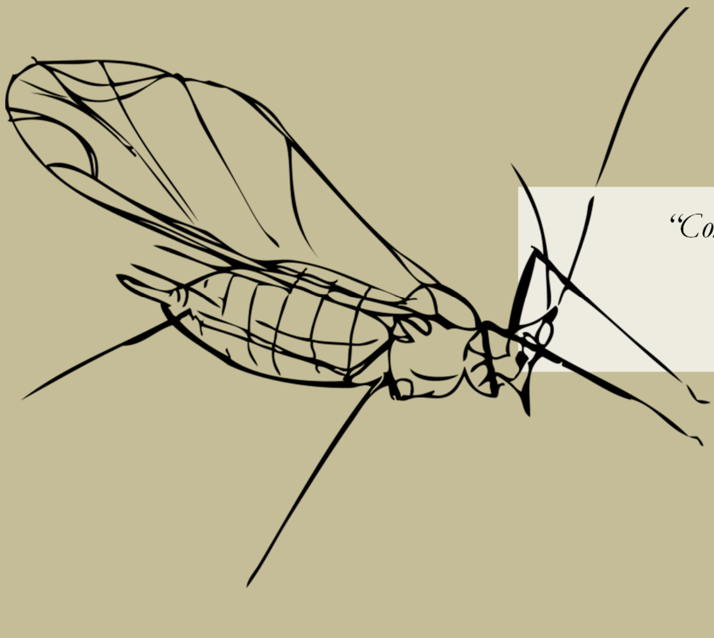
Partie V

Discussion générale

Perspectives de recherche

Conclusion



> *"Coming back to where you started is not the same as never leaving."*
>
> **Terry Pratchett**, A Hat Full of Sky

Discussion

Discussion générale des apports de la thèse

Dans cette thèse, j'ai conduit différentes expériences avec des approches de terrain et de laboratoire, faisant état de la diapause chez les parasitoïdes du genre *Aphidius* et des différents facteurs environnementaux pouvant agir sur sa variabilité, à la fois au sein d'une population, entre populations et entre espèces. Toute altération dans les conditions abiotiques mais aussi biotiques perçues par les parasitoïdes modifie les pressions de sélections qui agissent sur la diapause et influence leur phénologie et leurs stratégies d'hivernation, soit via leur plasticité phénotypique, soit par adaptation génétique (**Chapitre 2**). Les résultats présentés dans les différents chapitres ouvrent sur d'intéressantes perspectives concernant l'évolution de la plasticité développementale des insectes dans le cadre des changements climatiques.

En premier lieu, les communautés d'hôtes et de parasitoïdes du milieu agricole sont différentes entre années, à la fois en termes de richesse spécifique et d'abondance relative des espèces. Certaines espèces de parasitoïdes et de pucerons qui étaient absentes en hiver du réseau trophique il y a une quinzaine d'années y sont maintenant actives. Bien que ces changements soient difficiles à relier directement à l'augmentation des températures – car de multiples facteurs peuvent entrer en jeu (e.g. jours de gel) – ils pourraient être liés à l'évolution des stratégies d'hivernation des parasitoïdes et en particulier à la perte d'expression de la diapause chez un nombre croissant d'espèces (**Chapitre 3** et **4**).

Parmi les stimuli environnementaux qui induisent la diapause des parasitoïdes de pucerons, la température et la photopériode sont particulièrement importants. Cela reflète l'aspect adaptatif de la diapause chez les insectes, qui est une préparation à l'arrivée des conditions hivernales (Danks, 1987). Toutefois, nous montrons que les niveaux de diapause sont faibles voir nuls chez les quatre espèces de parasitoïdes étudiées, et ce, même soumises à des stimuli de température et de photopériode typiques des conditions de fin d'automne rencontrées en Bretagne. Ces niveaux semblent également plus faibles que ceux rencontrés dans les années 1980 dans la même région, mettant en évidence que les parasitoïdes ne rencontrent plus des stimuli environnementaux suffisants pour induire la diapause (**Chapitre 4**).

Les adaptations locales des parasitoïdes à des hivers doux pourraient expliquer les faibles niveaux de diapause observés chez ces différentes espèces. Pour le prouver, nous avons comparé des populations de parasitoïdes venant de France et du Canada au cours d'une expérience de translocation. Les populations françaises produisent de relativement faibles niveaux de diapause comparé aux populations canadiennes, sous les conditions environnementales du Canada, même si la diapause est un prérequis à la survie hivernale dans cette région (Brodeur & McNeil, 1994). Ces résultats suggèrent également que la capacité à produire des individus diapausants n'est pas génétiquement perdue chez les populations françaises, et que les seuils d'induction de la diapause ne sont pas atteints par les stimuli environnementaux en France (**Chapitre 5**).

La diapause estivale, une composante négligée de l'écologie saisonnière des insectes, s'exprime chez les parasitoïdes de pucerons par plasticité transgénérationnelle lorsque les mères rencontrent des compétitrices. Cela représente vraisemblablement une adaptation à la diminution des ressources, directement corrélée au degré de compétition perçu par les femelles parasitoïdes. Contrairement à ce qui était attendu, la densité en hôtes n'affecte pas l'incidence de diapause, c'est donc bien la compétition intraspécifique *in fine*, en tant qu'élément déterminant le nombre d'hôtes de qualité disponibles pour chaque femelle, qui contrôle la proportion d'individus qui entre en diapause (**Chapitre 6**).

La ressource hôte est d'une grande importance dans l'expression de la diapause des parasitoïdes puisqu'ils doivent synchroniser leur cycle de vie avec celui de leurs hôtes (Godfray, 1994). Les parasitoïdes de pucerons entrent en diapause à plus forte proportion quand ils se développent dans des hôtes ovipares, qui diffèrent significativement des vivipares en terme de métabolites et de réserves énergétiques. Cette réponse plastique (individuelle ou par effet maternel) au signal environnemental que représente l'hôte reflète une adaptation à la synchronisation des cycles de vie. La réponse à l'hôte semble toutefois être contrainte génétiquement, puisque la diapause des populations d'*A. ervi* venant de milieux à hiver doux (Bretagne) ne semble pas affectée par les morphes sexués, contrairement à la population canadienne (**Chapitre 7**).

La diapause des parasitoïdes du genre *Aphidius* est en partie dépendante des conditions rencontrées par la génération précédente. Les effets maternels semblent être importants sur de nombreux traits d'histoire de vie des parasitoïdes. Nous montrons toutefois qu'une des principales limites à l'étude de la diapause est la connaissance des seuils environnementaux auxquels elle est induite, seuils qui diffèrent selon les populations utilisées et notamment leur origine géographique et climatique (**Chapitre 8**).

Enfin, les parasitoïdes de pucerons qui évoluent en hivers doux semblent assez résistants au froid pour survivre sans entrer en diapause. De plus, le fait d'entrer en diapause pendant les stades immatures réduit considérablement la résistance au froid des parasitoïdes une fois adultes. Il existe donc un compromis entre la phase de diapause et la phase de vie active. Cela nous éclaire sur les coûts de la stratégie de diapause et sur de possibles mécanismes pouvant mener à son abandon sous certaines conditions environnementales (**Chapitre 9**). Bien que les hivers soient plus chauds en moyenne, des vagues de froid létales peuvent survenir (Andrade *et al.*, 2016). Pour minimiser les risques liés à ce type d'environnement, les parasitoïdes peuvent développer des stratégies de *bet-hedging*, et produire à la fois des individus qui entrent en diapause l'hiver et d'autres qui n'y entrent pas.

Une approche mécanistique de l'expression de la diapause chez les parasitoïdes

Chez les parasitoïdes, plusieurs pressions de sélection agissant sur la diapause sont identifiées, et peuvent expliquer les différences d'expression de diapause entre les populations et au sein des populations.

Variabilité inter-populationnelle

La diapause est un phénomène par définition plastique à des stimuli environnementaux (polyphénisme saisonnier). Cette plasticité est héritable (Roff & Bradford, 2000, Lehmann, 2016) et représente un interrupteur *ON/OFF* permettant à un individu de prendre la « décision physiologique » de poursuivre le développement ou d'entrer en diapause (Tauber *et al.*, 1986). Les différences d'incidence de diapause entre populations, par exemple le long de gradients climatiques, reflètent les adaptations locales des parasitoïdes à leur milieu. La sélection naturelle agit localement sur les seuils de réponse aux stimuli environnementaux (principalement à la photopériode et la température) via deux principaux facteurs ; la rigueur hivernale et la disponibilité en hôtes (c.-à-d. la ressource limitante) (Tauber *et al.*, 1986). Dans les milieux où les hivers sont rudes, les seuils d'induction de diapause sont facilement atteints et les parasitoïdes entrent en diapause tôt avant l'hiver et en forte incidence. Si les températures hivernales sont assez chaudes et les hôtes disponibles pour permettre l'activité des parasitoïdes pendant l'hiver, les seuils induisant la diapause ne sont pas ou peu atteints par les stimuli extérieurs, et le polyphénisme n'est donc pas ou peu exprimé. Localement, des stratégies de *bet-hedging* peuvent être sélectionnées, et la proportion de diapausants dans une population est déterminée selon la probabilité de rencontrer un hiver rude chaque année. Ce *bet-hedging* peut-être soit conservatif, en cas d'hivers constamment rudes ou constamment doux, soit diversifié, si les conditions hivernales sont variables et imprévisibles (Hopper, 1999).

Variabilité intra-populationnelle

Au sein d'une population, on observe de la variabilité dans les seuils d'induction de diapause ; à une condition environnementale donnée, tous les individus n'entrent pas en diapause. En plus du *bet-hedging* évoqué ci-dessus, cela peut être dû à du polymorphisme génétique dans les seuils d'induction menant à des différences de réponse plastiques entre individus. Ce polymorphisme peut être maintenu localement par de la sélection balancée si les températures hivernales varient d'une année à l'autre (Hopper, 1999). Les différences entre individus peuvent également être dues à des effets maternels au sens strict (plasticité transgénérationnelle) qui, selon les conditions environnementales rencontrées (e.g. le nombre de compétitrices, la qualité des hôtes, les températures, …), favorisent ou inhibent la diapause de leurs descendants (Mousseau & Dingle, 1991). Enfin, la variabilité intrapopulationnelle de diapause peut être liée à des différences locales dans les stratégies de *bet-hedging*, c'est-à-dire des mères qui produisent plus ou moins d'individus en diapause que d'autres, par exemple en fonction des conditions qu'elles rencontrent (*bet-hedging* conditionnel).

Scenario dans le cadre des changements climatiques

Les changements climatiques modifient les pressions environnementales qui maintiennent l'expression de la diapause au sein des populations de parasitoïdes. Ces derniers peuvent y répondre rapidement, de manière plastique, ou par réponse évolutive si la diversité génétique est assez grande et si la diapause implique des coûts assez importants pour que la sélection agisse contre son expression. Les considérations générales concernant la diapause des parasitoïdes dans le cadre des changements climatiques ont été exposées au **Chapitre 2**. Qu'en est-il précisément de l'expression des stratégies hivernales des parasitoïdes du genre *Aphidius* ?

Dans les années 1980, les hôtes étaient déjà présents pendant l'hiver en Bretagne, bien que probablement en plus faibles abondances qu'aujourd'hui (Rabasse *et al.*, 1983, Krespi, 1990). Les faibles températures représentaient toutefois un facteur environnemental pouvant avantager la diapause chez certaines espèces (Krespi *et al.*, 1994). Si les hôtes restent disponibles et que le climat se réchauffe, comme au cours des dernières décennies en Bretagne, les parasitoïdes ne rencontrent plus les conditions nécessaires à l'expression de leur plasticité saisonnière ; les seuils d'induction de la diapause ne sont plus atteints chez tout ou partie des individus. Donc au sein d'une population, les niveaux de diapause peuvent diminuer *via* cette réponse plastique des parasitoïdes (**Chapitre 4**).

De plus, ces seuils d'expression peuvent être sujets à sélection directionnelle dans le cadre des changements climatiques, car le fait d'exprimer de la diapause peut avoir des coûts pour les parasitoïdes si l'hiver se réchauffe (**Chapitres 2** et **9**). Ces coûts peuvent être écologiques (e.g. diapause induite alors que les températures sont convenables et les hôtes disponibles, exposition aux prédateurs et aux hyperparasitoïdes), ou de maintenance (e.g. coûts énergétiques, compromis avec certains traits). Il y aurait donc à la fois un avantage sélectif à ne pas entrer en diapause, et un désavantage sélectif à y entrer. Dans ce cas, la frange de la population qui est la moins sensible aux stimuli d'induction de diapause est sélectionnée chaque hiver. En Bretagne, comme dans les autres régions où les hivers sont doux, l'équilibre entre les coûts et les bénéfices à exprimer la diapause penche en faveur d'un abandon ou d'une réduction de cette stratégie au profit d'une activité hivernale impliquant probablement la quiescence (Langer & Hance, 2000, Legrand, 2005).

Chez des espèces tolérantes au froid, comme *A. matricariae* et *A. rhopalosiphi*, ces stratégies de non-diapause étaient déjà reportées il y a trente ans (Krespi, 1990). Le réchauffement des températures dans cette région permet dorénavant à d'autres espèces comme *A. ervi* et *A. avenae* et des hyperparasitoïdes, moins tolérantes au froid (Krespi, 1990, Le Lann *et al.*, 2011c), de s'établir dans les réseaux trophiques d'hiver. La diapause reste toutefois exprimée à plus forte incidence chez ces espèces peu tolérantes au froid, ce qui démontre de possibles stratégies de *bet-hedging*, car même si les hivers sont en moyenne plus chauds ils sont aussi plus imprévisibles. Si le climat continue de se réchauffer, l'incidence de diapause devrait continuer à diminuer. En plus de la diapause, la niche thermique des espèces est donc essentielle à considérer si l'on veut comprendre les changements de composition spécifique au sein des réseaux trophiques (**Chapitres 3** et **4**).

On peut parler de « perte » de diapause chez certaines espèces, non pas génétiquement, mais parce que la diapause n'est jamais exprimée car les seuils ne sont, de fait, jamais atteints en Bretagne. En revanche, il semble possible d'exclure tout effet d'un polymorphisme de diapause (c.-à-d. la présence de lignées génétiques diapausantes et non-diapausantes) dans les populations d'*Aphidius* de Bretagne. En effet, au moins une partie de la descendance de chaque femelle parasitoïde est capable d'entrer en diapause si les conditions abiotiques reçues le permettent. Même chez *A. rhopalosiphi* chez qui aucun individu en diapause n'avait été détecté en laboratoire (Tougeron *et al.*, 2017, **Chapitre 4**), un faible niveau de diapause a pu être produit quand la population de Bretagne était exposée aux conditions du Québec (**Chapitre 5**) (le même phénomène a été observé chez *A. matricariae*, ces données ne sont pas présentées dans cette thèse). Cela renforce l'idée que la sélection agit sur les seuils de diapause et pas sur la diapause elle-même (voir discussion sur la génétique de la diapause plus bas).

Les parasitoïdes bretons peuvent également recevoir des signaux inadéquats qui limitent leur entrée en diapause, comme l'absence de pucerons sexués dans l'environnement (dont la présence diminue avec la hausse des températures ; Dedryver *et al.*, 2001) ou encore une possible inhibition maternelle de la diapause en cas de températures trop élevées (**Chapitre 8**, Reznik & Samartsev, 2015, Voinovich *et al.*, 2015). D'une manière générale, il semble qu'à la fois les conditions rencontrées par la génération maternelle et celles rencontrées par la génération hivernante puisse influencer la diapause des parasitoïdes du genre *Aphidius* (Brodeur & McNeil, 1989a, **Chapitres 6** et **8**). La plasticité transgénérationnelle pourrait également être impliquée dans la réponse des parasitoïdes aux pucerons sexués (**Chapitre 7**), bien que cet aspect n'ait pas été exploré. Ces changements plastiques d'expression de diapause peuvent être adaptatifs pour les parasitoïdes, sauf s'ils sont le produit de contraintes environnementales (e.g. van Dyck *et al.*, 2015). Par exemple, de trop hautes températures automnales pourraient empêcher l'induction de la diapause à une photopériode qui normalement l'aurait induite (Bale & Hayward, 2010). Le fait de retarder la diapause dans le temps voire de ne pas l'exprimer en hiver pourrait menacer la survie des parasitoïdes en cas de vague de froid soudaine.

Les changements de composition spécifique dans la communauté d'hôtes pourraient aussi modifier la diapause des parasitoïdes. Par exemple chez *A. tabida*, la diapause est induite à plus forte proportion quand le parasitoïde attaque *D. melanogaster* plutôt que l'espèce sympatrique *D. subobscura* (Kraaijeveld & van Alphen, 1995). Nous avons montré que le parasitoïde *A. ervi* a une incidence de diapause d'environ 30 % quand il se développe dans le puceron du pois *A. pisum* à 17 °C et 10:14 h LD (**Chapitre 7**), alors qu'il n'exprime que 10 % de diapause quand il se développe dans *S. avenae*, dans les mêmes conditions (**Chapitre 4**). Dans les expériences menées au cours de cette thèse, nous n'avons globalement utilisé qu'une espèce hôte, mais dans le milieu agricole, les parasitoïdes s'attaquent à plusieurs espèces (**Chapitre 3**). Des associations préférentielles existent entre espèces hôtes et parasitoïdes (Andrade *et al.*, 2013, 2015), ainsi qu'en fonction des biotypes des hôtes (c.-à-d. des hôtes d'une même espèce spécialisés sur différents types de plante) (Bilodeau *et al.*, 2013). Sur plusieurs aspects, l'influence de la qualité de l'hôte pour la diapause des parasitoïdes doit encore être largement explorée. Il faut également garder à l'esprit que nous avons étudié uniquement les espèces présentes dans les cultures de céréales et que les stratégies saisonnières adoptées par les parasitoïdes peuvent être différentes dans d'autres types de cultures, ou d'autres écosystèmes, en présence d'une communauté d'hôtes différente.

Comme exposé dans le **Chapitre 1**, la diapause est un syndrome, c'est-à-dire que plusieurs pressions de sélection environnementales (e.g. températures, ressources) ont mené au cours de l'histoire évolutive des insectes, à la sélection d'un ensemble de traits exprimés pendant l'hivernation et avec une forte intégration phénotypique (Tauber *et al.*, 1986, Danks, 1987, Hodek, 1999). Les changements climatiques risquent donc d'agir sur l'ensemble de ces traits (Gotthard & Berger, 2010, Kivelä *et al.*, 2015). L'étude de l'écologie et des traits de vie des non-diapausants devient de ce fait de plus en plus importante, car la diapause ne sera pas affectée seule par le réchauffement climatique. Chez les parasitoïdes qui n'entrent pas en diapause, des adaptations comportementales ou physiologiques sont nécessaires pour survivre à l'hiver avec cette stratégie. Les parasitoïdes pourraient par exemple choisir des microrefuges thermiques pour mieux résister aux températures hivernales (e.g. **Annexe 1**).

Il existe un consensus assez clair dans la littérature récente sur le fait que le réchauffement climatique a des effets positifs sur les insectes de milieux tempérés (Bale *et al.*, 2002, Deutsch *et al.*, 2008, Tougou *et al.*, 2009, Altermatt, 2010). Dans l'absolu, la *fitness* des parasitoïdes pourrait augmenter en hiver car les températures plus élevées permettent une meilleure survie, l'activité, la reproduction et l'exploitation des ressources. En revanche, les modèles biologiques sont souvent considérés comme des entités solitaires, ce qui est incorrect écologiquement parlant, particulièrement lorsque l'on considère les parasitoïdes (Harvey, 2015). En particulier, les optimums thermiques sont différents entre espèces au sein des réseaux trophiques (Berg *et al.*, 2010) et les réponses phénologiques aux changements climatiques sont très variables entre les taxa (Thackeray *et al.*, 2016). Les interactions antagonistes comme les relations proies-prédateurs ou hôtes-parasitoïdes sont susceptibles d'être altérées à la fois par l'augmentation des températures moyennes et par de plus hautes variations de température (Sentis *et al.*, 2017a, 2017b, Uszko *et al.*, 2017). Les prédictions dans le cadre des changements climatiques doivent donc prendre en compte la disponibilité en hôtes (plantes/pucerons), leur mode de reproduction et leur stratégie d'hivernation, l'apparition de nouveaux compétiteurs ou prédateurs dans le réseau trophique et les changements de phénologie des différentes espèces au sein d'un écosystème donné (discuté dans le **Chapitre 2** et dans Andrade *et al.* (2016)).

Enfin, dans le milieu naturel, il faut dorénavant considérer les hyperparasitoïdes comme des éléments récurrents des réseaux trophiques d'hiver, comme en Bretagne. En effet, les changements climatiques ont probablement également affecté leur phénologie car les hyperparasitoïdes rencontrent des températures hivernales assez chaudes pour survivre, et leurs hôtes (les parasitoïdes primaires) sont actifs pendant l'hiver. D'avantage de connaissances sont nécessaires sur leurs stratégies d'hivernation et sur les facteurs impliqués dans l'induction, le maintien et la levée de leur diapause. Des éléments de réponse pourraient venir de l'étude des réseaux trophiques hivernaux de milieux où les hivers sont encore plus doux qu'en Bretagne, comme par exemple en Espagne où les hyperparasitoïdes sont présents à forte abondances toute l'année dans certaines cultures (Gómez-Marco *et al.*, 2015).

Un schéma bilan des facteurs agissant sur la diapause des parasitoïdes est présenté (Figure 10.1).

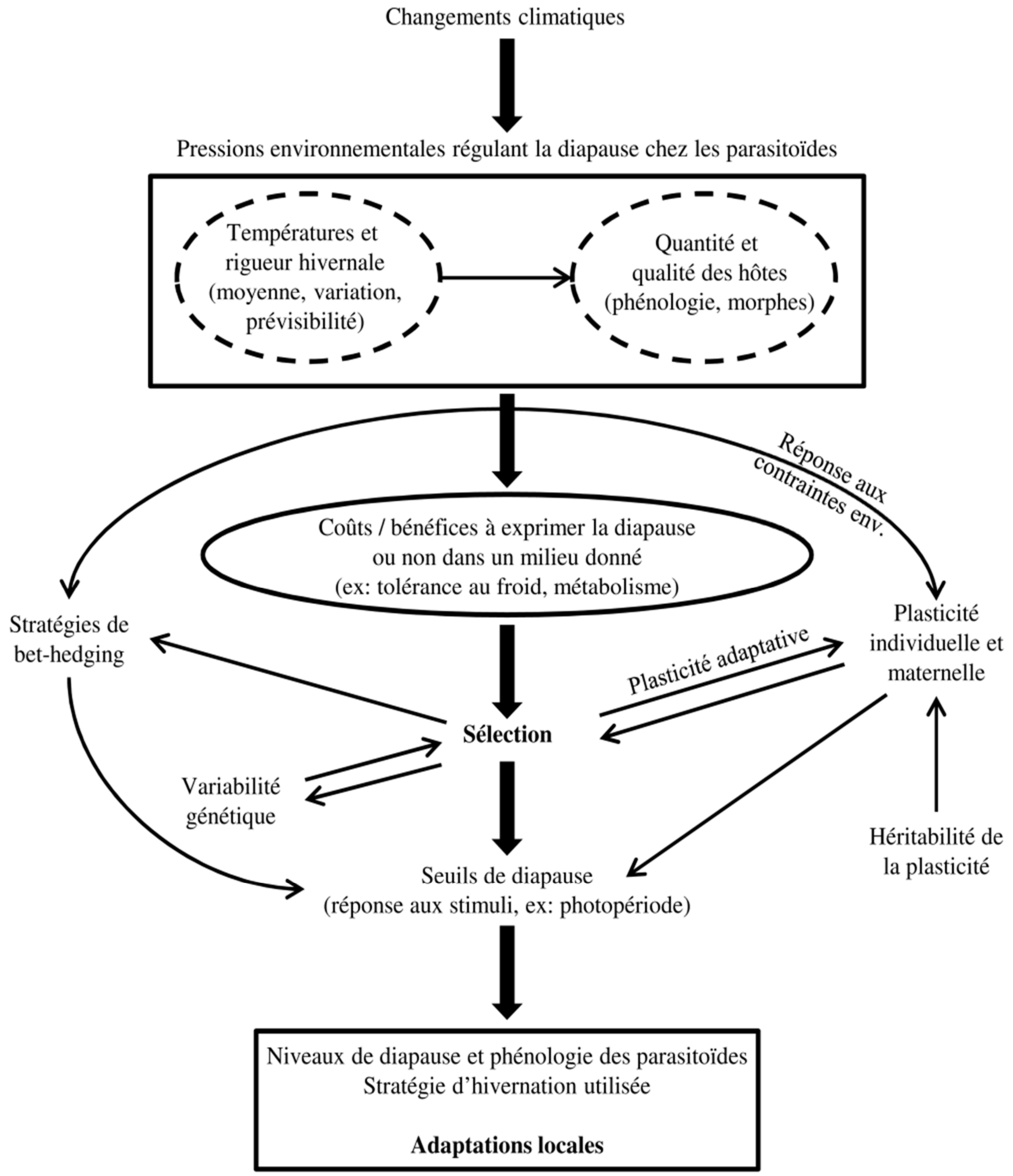


Figure 10.1 : Schéma résumant les facteurs impliqués dans la variabilité de la diapause chez les parasitoïdes et l'action des changements climatiques sur ceux-ci. Ce processus est décrit en détail dans la discussion. En résumé, la rigueur hivernale et la disponibilité en hôtes sont les deux facteurs environnementaux qui sélectionnent localement pour l'expression de la diapause des parasitoïdes (polyphénisme saisonnier), et qui agissent ultimement sur les seuils de diapause via la balance coûts/bénéfices dans un environnement donné (voie centrale, grandes flèches). La réponse des parasitoïdes aux stimuli environnementaux d'induction (et de levée) de la diapause est sensible à la plasticité maternelle et aux stratégies de *bet-hedging*, qui sont elles-mêmes sujettes à sélection, si la variabilité génétique le permet dans la population (petites flèches). Les informations présentées dans ce schéma sont issues de la revue de la littérature en introduction, et des travaux réalisés au cours de cette thèse.

Implications pour le contrôle biologique

Plusieurs éléments ont déjà été discutés sur ce point à la fin du **Chapitre 2** ; les changements de phénologie à la fois chez les hôtes (ravageurs) et chez leurs parasitoïdes (et hyperparasitoïdes) risquent de déstabiliser les réseaux trophiques et de modifier (positivement ou négativement) l'efficacité du contrôle biologique naturel (Thomson *et al.*, 2010, Furlong & Zalucki, 2017). En effet, un parallèle intéressant peut être établi entre les conséquences de changements d'aire de répartition d'espèces, par exemple dans le cadre des invasions biologiques, et celles de changements de phénologie liées aux changements climatiques ; une ou plusieurs espèces apparaissent dans le réseau trophique alors qu'elles en étaient absentes auparavant (Ghalambor *et al.*, 2007). Chez les parasitoïdes, la température a un impact important sur l'expression de la diapause (Li *et al.*, 2008, Tougeron *et al.*, 2017a), mais aussi directement sur les interactions avec l'hôte comme le taux d'attaque du parasitoïde ou sa virulence, ainsi que les comportements de défense et la résistance immunitaire de l'hôte (Cayetano & Vorburger, 2013, Le Lann *et al.*, 2014b, Delava *et al.*, 2016, Wu *et al.*, 2016). Quelques éléments nouveaux sont apportés à la lumière des travaux menés dans cette thèse.

Pouvoir contrôler la diapause des ennemis naturels ou des ravageurs de culture est un défi important pour l'avenir du contrôle biologique, en particulier dans le cadre de l'élevage de masse et du stockage au froid d'insectes (Colinet & Boivin, 2011). Il est souvent difficile de manipuler la diapause pour stocker des insectes en raison de fortes mortalités observées en milieu artificiel (van Lenteren & Tommasini, 1999), car le stockage au froid est généralement couteux en termes de traits de vie (van Baaren *et al.*, 2005, Colinet & Boivin, 2011, Ismail *et al.*, 2014), et comme nous l'avons vu, car la diapause n'augmente pas nécessairement la tolérance au froid des insectes (**Chapitre 9**, Hodkova & Hodek, 2004). Malgré ces difficultés, de nombreuses réussites ont néanmoins eu lieu en ce sens et des agents de lutte biologique peuvent être stockés au froid pendant plusieurs semaines, en diapause, sans graves conséquences sur la *fitness* (e.g. Garcia *et al.*, 2002, Hun *et al.*, 2004).

En raison d'adaptations locales aux températures, certaines espèces ou populations ont une meilleure résistance au froid que d'autres (e.g. chez la drosophile, Gibert *et al.*, 2001, Ayrinhac *et al.*, 2004) et entrent en diapause à différentes proportions (**Chapitre 5**, Hut *et al.*, 2013). Chez les parasitoïdes de pucerons, Colinet & Hance (2010) ont montré que certaines espèces toléraient mieux le stockage au froid que d'autres. Une meilleure connaissance des stratégies d'hivernation et des cycles saisonniers des populations naturelles de parasitoïdes permettrait de sélectionner des lignées appropriées pour le stockage au froid. L'étude des endosymbiontes bactériens et de leurs effets sur l'écologie des parasitoïdes et des insectes qu'ils contrôlent est également une piste de recherche intéressante et d'actualité dans le cadre de la lutte biologique (Vorburger, 2017), à plus forte raison que les interactions symbiotiques sont sensibles aux modifications de l'environnement thermique (Oliver *et al.*, 2014).

Nous avons aussi montré qu'il était difficile de ré-induire la diapause chez des souches industrielles de parasitoïdes (**Chapitre 8**), et certaines d'entre-elles semblent perdre assez rapidement leur capacité à entrer en diapause (Gariepy *et al.*, 2015). Soumettre régulièrement ces souches à des conditions automnales puis hivernales pourrait aider au maintien de l'expression de la diapause. Il est également possible de sélectionner artificiellement des lignées qui entrent ou n'entrent pas en diapause chez certaines espèces, comme *Sarcophaga bullata* (Diptera : Sarcophagidae) (Goto *et al.*, 2006). Cette possibilité reste à explorer chez les parasitoïdes de pucerons chez qui, à ma connaissance, aucune lignée génétiquement incapable de produire des individus en diapause n'a été clairement identifiée. Le fait que les pressions environnementales agissent sur les seuils de diapause laisse d'intéressantes perspectives sur la possibilité de réinduire de plus forts taux de diapause chez les populations de parasitoïdes, par sélection artificielle.

Comme la diapause est sous fort contrôle endocrinien (**Chapitre 1**, Denlinger, 2013), il est possible de l'inhiber ou de l'activer en exposant les insectes à différentes molécules de synthèse mimant l'effet des hormones naturelles comme les hormones juvéniles, les ecdystéroïdes ou d'autres hormones spécifiquement impliquées dans le syndrome de diapause (Corbitt & Hardie, 1985). Par exemple chez *A. matricariae* et *A. ervi*, une application d'ecdystéroïdes permet d'induire la diapause indépendamment des autres signaux environnementaux reçus (Christiansen-Weniger & Hardie, 1999). En utilisant ces hormones, il pourrait donc être possible de la contrôler pour les besoins du stockage au froid, mais aussi de forcer le début ou l'arrêt de la diapause au mauvais moment de l'année pour contrôler des ravageurs agricoles (discuté dans Denlinger (2008)). Il est difficile d'appliquer ces méthodes de contrôle de diapause en milieu agricole, même si certains essais ont déjà été menés (Ždárek *et al.*, 2000). Au-delà des hormones, il est envisageable de reproduire artificiellement le contenu métabolique des pucerons ovipares, puisque nous avons montré que certaines de ces molécules pourraient être liées à la diapause des parasitoïdes de pucerons (**Chapitre 7**).

Perspectives de recherche

Nous l'avons vu au **Chapitre 1**, l'étude des rythmes saisonniers des insectes occupe l'intérêt des scientifiques et des naturalistes depuis déjà plus de trois siècles. Il existe de nombreuses raisons de poursuivre ce travail, à la fois pour les sciences fondamentales et appliquées (Denlinger, 2008).

La diapause est avant tout une question fascinante en biologie du développement et nous permet de mieux appréhender les adaptations locales et la plasticité phénotypique des insectes. En particulier, la régulation génétique de la diapause (voir plus loin) est un sujet d'actualité. De plus, la régulation des rythmes photopériodiques saisonniers est fortement liée avec le système des rythmes circadiens chez les insectes (Dolezel, 2015). Il a notamment été démontré chez *A. pisum* que les gènes impliqués dans les rythmes circadiens, notamment *per*, *Clock*, *cycle* et *timeless* étaient également ceux impliqués dans l'induction de la diapause (Cortés *et al.*, 2010). Des liens intéressants peuvent de ce fait être établis entre les mécanismes qui perturbent l'expression de la diapause et ceux qui agissent sur le sommeil des insectes, incluant différents aspects des changements climatiques (Tougeron & Abram, 2017). Enfin, la diapause est un phénomène extrêmement intéressant pour l'étude de l'évolution du vieillissement chez les êtres vivants, car la sénescence est fortement ralentie pendant cette période (Tatar & Yin, 2001).

D'un point de vue appliqué, outre le fait de pouvoir manipuler la diapause pour l'efficacité du stockage au froid et la lutte biologique, il faut prendre en compte les périodes d'activité et d'inactivité des insectes dans les modèles prédisant les pullulations saisonnières de ravageurs (Samietz *et al.*, 2007). Dans un avenir proche, les modèles prédictifs de distribution et de phénologie des ravageurs et de leurs ennemis naturels (e.g. Stoeckli *et al.*, 2012) devront être ajustés en prenant en compte les effets des changements climatiques sur l'expression de la diapause, en particulier sur sa durée. Pour ce faire, une meilleure connaissance des mécanismes d'induction mais surtout de fin de diapause sont nécessaires. A l'issue de cette thèse il est possible d'identifier quelques sujets de recherche sur la diapause qui sont actuellement explorés par la communauté scientifique et/ou qui mériteraient une attention particulière dans un proche avenir.

Mécanismes de fin de diapause

Les mécanismes écologiques, hormonaux et génétiques liés à la levée de diapause nécessitent des recherches supplémentaires chez la majorité des espèces d'insectes (Koštál, 2006). Certains gènes surexprimés ou au contraire sous-exprimés pendant la phase de levée de diapause ont néanmoins été identifiés (voir discussion dans Denlinger, 2002). Etudier les causes environnementales de l'arrêt de la diapause est en particulier important dans le cadre des changements climatiques, par exemple chez les espèces qui nécessitent de rencontrer une période de gel pour entamer la phase de levée de diapause (**Chapitre 2**, Hodek, 1996).

Chez les insectes, des mécanismes cérébraux liés à la détection du photopériodisme pourraient être impliqués dans la mesure du temps écoulé pendant la diapause (Saunders, 2012). Récemment, Lehmann *et al.* (2017a) ont démontré que chez le papillon *Pieris napi* (Lepidoptera : Pieridae), les structures sensorielles liées notamment à la vision sont très bien développées chez les pupes en diapause, contrairement à d'autres structures cérébrales. Ces résultats, bien que préliminaires, suggèrent que ces structures pourraient jouer un rôle dans le maintien et la levée de la diapause (Lehmann *et al.*, 2017a).

Chez les parasitoïdes du genre *Aphidius*, nous avons observé que la diapause hivernale pouvait être levée en quelques semaines lorsque les momies de pucerons étaient laissées sous des conditions de température favorables (autour de 20 °C) (**Chapitres 4** et **9**). Cela n'exclut toutefois pas que l'exposition à des conditions froides puisse accélérer la levée de diapause, comme mis en évidence chez d'autres espèces d'insectes (e.g. Mehrnejad & Copland, 2005, Lehmann *et al.*, 2017b).

Génétique de la diapause

Récemment, des approches ARN interférence[7] ont permis de démontrer le rôle de certains gènes de l'horloge circadienne dans la diapause de *N. vitripennis* (Mukai & Goto, 2016). De plus en plus d'études s'intéressent aux variations latitudinales de l'expression de la diapause chez de nombreuses espèces d'insectes (e.g. Lehmann *et al.*, 2015, Paolucci *et al.*, 2013, Posledovich *et al.*, 2015, Pruisscher *et al.*, 2017) dont *N. vitripennis*, qui s'impose dorénavant comme modèle chez les parasitoïdes en raison de fortes variations d'incidence de diapause le long de gradients environnementaux, et parce que son génome est entièrement séquencé (Werren *et al.*, 2010). En effectuant des analyses QTL[8], Paolucci *et al.* (2016) ont trouvé que les différences d'expression de diapause entre une population de *N. vitripennis* de Corse et une autre de Finlande étaient liées à deux régions génomiques sur lesquelles sont situés les gènes

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[7] Approches permettant d'inhiber l'expression d'un gène en « neutralisant » l'ARN messager associé.

[8] *Quantitative Trait Locus*, région génomique (locus) que l'on peut corréler à l'expression d'un trait phénotypique. Cette expression peut être quantifiée.

period et *cycle*, que l'on a déjà mentionnés. Ces résultats permettent de mettre en avant les bases génétiques du contrôle des seuils d'induction de diapause et des adaptations locales du photopériodisme saisonnier au climat. Le développement des technologies d'édition de génome comme CRISPR/Cas9 ouvrent de nouvelles portes à la manipulation génétique de l'expression de la diapause. Le séquençage en cours du génome d'*Aphidius ervi* (i5K Consortium, 2013) nous permettra dans un avenir proche d'utiliser la génétique pour explorer la variabilité de la diapause chez les parasitoïdes de pucerons.

Enfin, avant d'étudier la génétique de la diapause, il serait d'abord nécessaire d'explorer les bases de son héritabilité (e.g. Bradshaw *et al.*, 1997). Par exemple, *Ostrinia furnacalis* (Lepidoptera : Crambidae) montre une dominance génétique incomplète dans le contrôle photopériodique de la diapause. Le croisement d'une souche qui diapause fortement et d'une qui n'exprime quasiment pas de diapause sous les mêmes conditions abiotiques forme une lignée hybride qui exprime la diapause de manière intermédiaire (Xia *et al.*, 2012). Ces méthodes pourraient être appliquées dans le cas des parasitoïdes de pucerons en croisant les souches canadiennes et françaises utilisées dans cette thèse et en étudiant la réponse de la génération hybride.

Certaines lignées d'insectes au sein d'une même population pourraient également être plus sensibles que d'autres aux signaux d'induction ou de fin de diapause (Roff & Bradford, 2000). Par exemple, certains individus au sein des populations de *Diabrotica barberi* (Coleoptera : Chrysomelidae), un important ravageur du maïs, expriment une diapause prolongée qui est une adaptation à la variabilité des cycles de culture. French *et al.* (2014) ont démontré que cette diapause prolongée était fortement héritable (h^2=0,70) et qu'il existait un fort polymorphisme d'expression de diapause au sein de la population. Des indications sur le sexe du parent transmettant les caractères de diapause sont également requises (e.g. Pruisscher *et al.*, 2017). Ces considérations d'héritabilité sont intimement liées aux stratégies de *bet-hedging* dont nous avons discuté précédemment car ces stratégies sont, elles-aussi, héritables (Hopper, 1999).

Au-delà de la génétique, des approches au travers de gradients géographiques peuvent permettre de mieux estimer quels vont être les impacts des changements climatiques sur les stratégies saisonnières des insectes, notamment via l'établissement d'analogues climatiques (Ramírez-Villegas *et al.*, 2011). Il serait intéressant pour les parasitoïdes de pucerons d'étudier le cline de diapause sur un gradient longitudinal de rigueur hivernale, par exemple en Europe. De tels gradients permettraient de s'affranchir d'un quelconque effet photopériodique et de ne prendre en compte que l'adaptation aux températures locales. Par analogie, si l'expression de la diapause change le long d'un gradient de températures hivernales, il est possible d'en déduire la réponse d'uneespèce au réchauffement du climat.

Mécanismes épigénétiques

Le rôle de la méthylation ADN sur la diapause a jusqu'à présent reçu peu d'attention, notamment parce que les bases génétiques de la diapause sont elles-mêmes peu connues et que les modèles biologiques sur lesquels on étudie la diapause et l'épigénétique ne sont pas les mêmes (Bewick *et al.*, 2017). Récemment chez le parasitoïde *N. vitripenni*s, chez qui la diapause est directement sous régulation maternelle, Pegoraro *et al.* (2016) ont montré que les femelles exposées à des photopériodes courtes avaient un profil de méthylation du génome très différent de celui des femelles exposées à des jours longs, ce qui influençait la diapause de leurs descendants. Il reste aujourd'hui à savoir si les insectes chez qui aucun effet maternel direct sur la diapause n'est connu montrent également des signes de méthylation ADN susceptibles, par exemple, de modifier leur tolérance thermique ou d'autres traits associés au syndrome de diapause (Powell & Bale, 2008, Reznik & Samartsev, 2015). Les avancées les plus récentes concernant l'implication de l'épigénétique dans le phénomène de diapause ont été synthétisées par Reynolds (2017).

Conclusion générale

La synthèse des connaissances sur la diapause des insectes et sur les effets des changements climatiques sur les parasitoïdes révèle l'ampleur des défis scientifiques auxquels nous devons actuellement faire face (**Partie I**). En particulier, l'expression de la diapause chez les parasitoïdes semble s'altérer rapidement face aux modifications du climat, puisque leur *fitness* est fortement liée à la synchronisation de leur phénologie avec les changements saisonniers de l'environnement (**Partie II**). Ces changements de phénologie impliquent à la fois des réponses plastiques et évolutives des organismes (Merilä & Hendry, 2014, Forrest, 2016) et menacent par la même occasion la stabilité des réseaux trophiques (Tylianakis *et al.*, 2008). Les insectes parasitoïdes sont en particulier fortement sensibles aux changements climatiques puisqu'ils dépendent de la ressource hôtes pour survivre ; ressource dont les variations tant quantitatives que qualitatives peuvent influencer leur diapause (**Partie III**). Divers mécanismes, incluant la plasticité transgénérationnelle et la balance coûts/bénéfices des stratégies d'hivernation peuvent mener à une baisse d'expression de la diapause, au profit de la stratégie de quiescence hivernale (**Partie IV**). Les parasitoïdes non-diapausants apparaissent maintenant comme le phénotype dominant dans les réseaux trophiques des régions à hivers doux. De nouvelles approches intégrant l'étude des pressions évolutives qui s'exercent sur les stratégies saisonnières des insectes, mais aussi l'utilisation de nouveaux outils comme la génétique, permettront vraisemblablement de répondre aux nouvelles questions que posent les changements climatiques (**Partie V**).

Annexes

Annexe 1 : Tolérance thermique et microclimats

Annexe 2 : Réseau hôtes-parasitoïdes en conditions extrêmes

En bref :

- Les variations microclimatiques au sein des paysages agricoles influencent la résistance au froid des parasitoïdes de pucerons. Les parasitoïdes y sont plus résistants dans les milieux ouverts exposés aux basses températures et au vent que dans les milieux boisés, plus humides et dont les températures sont plus tamponnées. Ces effets sont plus importants pendant l'hiver qu'au printemps. La plasticité phénotypique des parasitoïdes est en jeu, via le phénomène d'acclimatation aux températures rencontrées pendant leur développement. Ces différences microclimatiques pourraient être impliquées dans la survie hivernale des parasitoïdes.

- Dans les milieux où les hivers sont longs et rigoureux, le réseau trophique d'hôtes et de parasitoïdes est très pauvre en espèces. Les parasitoïdes ne complètent vraisemblablement qu'une génération chaque année avant le retour des conditions défavorables. Leur capacité à passer l'hiver en diapause dans ces milieux rudes est discuté. Les parasitoïdes pourraient recoloniser chaque année le milieu en migrant avec leurs hôtes parasités.

"Nothing burns like the cold."

George R.R. Martin, A Game of Thrones

Annexe 1

Tolérance thermique et microclimats

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

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Summary

Insects are highly dependent on ambient temperatures to ensure their biological functions. Their persistence in the environment and their resistance to unfavorable temperatures are governed by their physiological thermal tolerance. Global change extends beyond climatic conditions to encompass modifications to the landscape. However, studies of climate change and landscape composition effects on ecosystem services, such as biological control, are commonly performed independently. Moreover, coarse scales are not always relevant when assessing climate change's impacts on ectotherms. We aimed to better understand the ecological relationships that may exist between microclimatic variation and insect thermal tolerance across a landscape composition gradient. To determine how landscape composition may impact insect thermal tolerance, parasitic wasps (Hymenoptera: Braconidae: Aphidiinae) of aphids were sampled along a landscape gradient from "closed" to "open" habitats. Sampling was performed during the winter 2013/14 and spring 2014 in cereal fields of Brittany, France. Meteorological data were recorded along this gradient. First, our results show an influence of landscape composition on local microclimate. Additionally, parasitoids from open landscapes had a higher tolerance to low temperatures, leading to higher physiological costs, compared to parasitoids from closed landscapes. This trend was stronger in winter than in spring. These results have numerous implications in the context of climate change, suggesting that targeted landscape management practices could create sheltered microclimatic areas that reduce the physiological costs of thermal tolerance, and promote the persistence of biological control agents.

Keywords: Climate change, Thermal biology, Critical temperatures, Insect ecophysiology, Landscape ecology, Conservation ecology, Biological pest control, Hymenoptera, Braconidae, Aphidiinae, *Aphidius*

Introduction

Climate models predict a global average temperature increase of between 1.7 and 6°C by the year 2100 (IPCC, 2014). In addition, an increase in the frequency of extreme events (heat or cold spells) is expected, especially in temperate regions (Hance *et al.*, 2007). Meanwhile, European landscapes have undergone increasing homogenization since the 1950s due to agricultural intensification, leading to the removal of hedges, groves, woods, and natural grasslands (Millennium Ecosystem Assessment, 2005). This homogenization has reduced the effectiveness of ecosystem services, such as pest control (Thies *et al.*, 2011). Although the combined influences of landscape properties and climate change (warming winters, increased incidence of cold spells) on ecosystems are still poorly understood, there is increasing evidence that working on both of these aspects could yield more important insights than considering them separately.

Despite numerous reports of climate change impacts, the gap between the coarse scale at which climate data are collected and the climatic conditions the organisms actually experience at a finer scale is problematic (Bennie *et al.*, 2008, Potter *et al.*, 2013, Woods *et al.*, 2015). Downscaling from global to microclimatic variations is needed to better investigate how ectotherms will respond to changes in their immediate environment. It is well known that local microclimate can be influenced by landscape properties (Chen *et al.*, 1999). For instance, the windbreak and antifreeze role of hedges, especially those with an embankment, has been widely studied and confirmed in agricultural landscapes. Wooded and closed areas generally have lower temperature amplitudes than open areas over a daily scale (Argent, 1992, Suggitt *et al.*, 2011), but are also colder, on average, than open areas due to the retention of cold air layers by hedges (Quénol & Beltrando, 2006). The microclimatic variation that occurs between open and closed landscapes is a very complex phenomenon that depends on many elements such as slope, hedge density, topography, and solar radiation (Quénol & Beltrando, 2006, Bennie *et al.*, 2008, Suggitt *et al.*, 2011).

In the context of conservation biology, and accepting the realities of climate change and landscape simplification, there is a need to understand whether microclimatic variation due to landscape composition affect ectotherm thermal tolerance. The existence of microclimates is widely confirmed, but the roles they play with regard to small organism ecology remains understudied (Potter *et al.*, 2013, Sunday *et al.*, 2014, Woods *et al.*, 2015). Recent field studies (*e.g.* Suggitt *et al.*, 2011; Pincebourde & Woods, 2012; Lawson *et al.*, 2014; Maclean *et al.*, 2015) have demonstrated the importance of focusing on microclimatic variation when assessing organisms' susceptibility to climate change. These papers, along with the present study, represent the first steps towards understanding how insects will be affected by climate change at the scale of their local environment, and whether microclimatic variations due to landscape composition could allow them to exploit more favorable temperature conditions.

There is a particular need to identify the relative roles of evolution versus behavioral and physiological plasticity in moderating the impacts of global environmental changes (Deutsch *et al.*, 2008, Sunday *et al.*, 2014). Acclimatization (*i.e.,* the capacity to better resist previously experienced temperatures) is an important physiological trait utilized by insects to cope with thermal stress. This is a well-known phenomenon that, along with behaviour, could be a plastic trait that commonly allows ectotherms to cope with climate change (Andrew & Terblanche, 2013, Colinet *et al.*, 2015). Body size, which is known to influence many life history traits in insects (Chown & Gaston, 2010), could also influence their thermal tolerance. Indeed, the Absolute Energy Demand (AED) hypothesis predicts that larger individuals should be disadvantaged in the face of stressful temperature conditions, since they expend energy proportionally faster than smaller individuals (Reim *et al.*, 2006).

This study examined the ecological relationships between the thermal tolerance of insects, microclimatic variation, and agricultural landscape composition (crop size, hedge length, type of soil cover). In particular, this work aimed to investigate how insects could adjust their phenotypic responses to temperature variation among landscapes. To address these issues, the cold tolerance and the size of aphid parasitoids, important biological control agents, were measured across a landscape composition gradient in Brittany, France. Many studies have been devoted to these species (*e.g.*, Thies *et al.*, 2005; Le Lann *et al.*, 2008, 2014) including investigations of their thermal tolerance (Giri *et al.*, 1982, Legrand *et al.*, 2004, Le Lann *et al.*, 2011a, 2011b, Ismail *et al.*, 2012). Aphid parasitoids are interesting biological models for landscape microclimate studies because they closely track the distribution of their hosts, especially during the larval stage when they are immobilized inside an aphid mummy and have to withstand the climatic conditions imposed upon them.

The following hypotheses were tested: (i) In the study area, microclimate varies along the landscape gradient, with temperatures in the closed landscape being on average colder but less variable than in the open landscape; (ii) If the first hypothesis is confirmed, the parasitoid thermal tolerance should be landscape-dependent, with parasitoids from open, more stressful areas being more cold tolerant; (iii) Due to their seasonal acclimatization capacities, and because exposure to unfavorable cold temperatures are less frequent in spring months, parasitoids should be more cold resistant in winter than in spring; (iv) Parasitoids are smaller in open areas than in closed areas; and (v) parasitoids are smaller in winter than in spring due to temperature differences between landscapes and seasons (following the AED hypothesis).

Material & Methods

Landscape gradient determination

Parasitic wasps were monitored in winter wheat fields in northern Brittany (France). Sampling took place in the vicinity of the LTER '*Zone Atelier Armorique*' (osur.univ-rennes1.fr/za-armorique, 48°36'N, 1°32'W) along a hedgerow network landscape gradient, ranging from closed landscapes to more open landscapes. To select the fields, 16 areas (300 meter radius) containing at least one wheat field were characterized with respect to the following three parameters: total hedge length (meters), average field size (hectares), and percentage grassland in the buffer zone. The land-cover over the past 5 years (2009-2013) was characterized, and only plots with annual cultures (maize or cereals) during the last 5 years were considered. This restriction was imposed in order to limit any potential effects of grasslands on arthropod communities. One wheat field was randomly selected from each area, and each area was then identified as belonging to one of three landscape units: (1) Closed landscape (five fields) – high grassland density (>45%), many hedgerows (>3200m) and small field sizes (<0.93ha); (2) Intermediate landscape (six fields); (3) Open landscape (five fields)– low grassland density (<20%), few hedgerows (<550m) and larger fields (>2.70ha). See supplementary material for a map of the study area (Appendix S1), precise locations of the fields (Appendix S2), correlations between landscape variables (Appendix S3) and extreme values for the landscape gradient (Appendix S4). All of the selected fields were separated from one another by at least 600 meters. Although all fields were prospected, parasitoids were only found in 12 fields (4 per landscape type). All of the selected fields were managed with similar conventional agriculture practices. Preliminary results revealed no significant differences in insect thermal tolerance within landscape types. Accordingly, data from plots belonging to the same type of landscape were pooled in subsequent analyses.

Sampling sessions and meteorological records

To evaluate potential differences in insect thermal tolerance between seasons, two rounds of sampling were conducted: one in late winter (13 January to 7 March 2014) and one in early spring (24 March to 15 May 2014). Over the course of both sampling periods, local meteorological data were recorded using BWS200 weather stations (Campbell Scientific France) to characterize climate differences between seasons and among landscapes. One weather station per landscape type was used, with each recording air temperature, relative humidity (using CS215 probes accurate to ±0.3°C and ±2% respectively) and wind speed (using Wind Sentry anemometer accurate to ±0.5 $m.s^{-1}$) once an hour during the entire sampling period. Each station had a roof and was placed 1 meter above the ground. Each station was placed at least 15 meters from the hedge on flat ground.

Study species

Aphidius parasitic wasps (Hymenoptera: Braconidae: Aphidiinae) are endoparasitoids of cereal aphids. These aphids (*Sitobion avenae* Fabricius, *Rhopalosiphum padi* L. and *Metopolophium dirhodum* Walker) are present during the winter in Brittany, resulting in increased crop damage at the onset of spring (Le Ralec *et al.*, 2010). Parasitoids are present throughout the year in Brittany with a succession of species between seasons (Krespi *et al.*, 1994). In this study, *Aphidius* parasitoids were included in analyses, while secondary parasitoids were excluded due to their rarity in the fields early in the season. A total of 4 species were found during winter (*Aphidius rhopalosiphi* De Stefani-Perez, *Aphidius matricariae* Haliday, *Aphidius avenae* Haliday and *Aphidius ervi* Haliday) and 3 species in spring (*A. rhopalosiphi*, *A. avenae* and *A. ervi*).

Sampling methods

Parasitoids were sampled by collecting aphid mummies (*i.e.,* parasitized aphids containing a parasitoid pupa). To assess the diversity and abundance of mummies, fields were visited twice a week during each sampling period. Sampling occurred during a 1 hour period over a surface of at least $1000m^2$, with field borders excluded to avoid margin effects. Mummies of the three major species of cereal aphids described above were sampled (every mummy found was collected). The largest living aphids (larval stages L4 and above) were also sampled and kept in microcages to wait for possible mummification. Each microcage consisted of a plastic tube (5 cm diameter) in which wheat (*Triticum aestivum* L. v. *Saturnus* (Poaceae)) grew on a vermiculite substrate. The microcages were checked daily for 10 days and newly-formed mummies were conserved. Each mummy was placed individually in a gelatin capsule (Capsule T1 - Capsugel®)

and subsequent parasitoid emergence was verified twice per day. Aphids, mummies and parasitoids were maintained in the laboratory after sampling at 20 ± 1°C, 70 ± 10% relative humidity, and a photoperiod of L16:D8. The sex of parasitoids was determined, with individuals identified according to their collection site. Following thermal tolerance experiments (see below), parasitoids were freeze-killed at -20°C and identified using an optical microscope and the identification key developed by (Hullé *et al.*, 2006). Host aphid species were also identified for each parasitoid using a binocular microscope. This enabled the determination of the composition of the parasitoid guild and the creation of quantitative food webs for both seasons. Comparisons of thermal tolerance and size between winter and spring-collected parasitoids were only made for *A. rhopalosiphi*, because it was the only species present in large numbers during both seasons.

Cold tolerance measurement

Non-lethal thermal thresholds are states of narcosis, offering a means of estimating the cold tolerance of individuals, which can have important effects on insect fitness (Alford *et al.*, 2012b, Le Lann *et al.*, 2011c). Insect physiological thermal tolerance is defined as the temperature range between the two thresholds of minimum critical temperature (CT_{min}) and maximum temperature (CT_{max}) beyond which survival is impossible (Bale, 1996). Indeed, survival is of little benefit if locomotion is compromised, leading to death through an inability to feed or to escape predators or parasites (Alford *et al.*, 2012b, Alford *et al.*, 2012c). Here, we only focus on the CT_{min} threshold. Indeed, in temperate climates insect fitness is expected to increase as temperature rises (Deutsch *et al.*, 2008) while sudden cold spells in winter could have a major impact on parasitoid populations and could decrease the effectiveness of pest control in late winter and early spring.

To measure parasitoid cold tolerance (CT_{min}), a double-walled glass column based on the model of Huey *et al.* (1992), and improved by (Powell & Bale, 2006), was used. A cryostat (VWR-CryoBath®) was used to cool the tube. There was no temperature difference between the top and the bottom of the tube. Temperature was controlled using a thermocouple probe (accurate to 0.01°C) connected to a digital display. A cooling rate of $0.75°C.min^{-1}$ was chosen to avoid inducing a rapid cold hardening response in the test insects (Powell & Bale, 2006). Relative humidity in the tube, which could not be directly controlled, was 35 ±10%. Each of the 272 parasitoids tested was individually placed in a tube. An individual's CT_{min} was considered to have been reached when the parasitoid could no longer cling to the wall of the vertical column, and dropped from the tube wall (Le Lann *et al.*, 2011c). After CT_{min} measurements, individuals were placed individually in Eppendorf® tubes and stored in the freezer. In order to avoid any confounding factors (temperature, atmospheric pressure, solar radiation in the laboratory), individuals originating from different field types were tested on each day of the experiments.

Size measurements

The size of parasitoids was measured after thermal tolerance experiments. The average length of each parasitoid's two hind tibia (a good proxy for parasitoid size) was measured and averaged. Images were captured with a Zeiss AxioCam ERc5s® HD camera mounted on a x9 binocular magnifier, and measurements (precise to 0.01 mm) were performed with the Image J® software (v. 1.48).

Statistical analyses

Statistical analyses were performed using R software (R Core Team, 2014). Statistical analyses were carried out with a statistical error risk of 5%. Statistical differences in meteorological data among landscape types were tested using a standard repeated measures ANOVA. The meteorological variable in question was considered as a fixed effect, and recording time included as a random effect. Tukey HSD post-hoc tests were used to detect pairwise differences among landscape types for each season. Homogeneity of species distributions among landscapes was tested using a Chi-square test of homogeneity for each season. A generalized linear model (GLM) was fitted to parasitoid CT_{min} data in both seasons, with landscape type, parasitoid species, sex, size, aphid host, parasitoid species x landscape interaction, and parasitoid species x aphid host species interaction as explanatory variables. The effect of these explanatory variables was tested with the "Anova" function from the "car" package, which performs an analysis of variance (Type II) with likelihood ratio tests, using a chi-square statistic (Fox & Weisberg, 2011). Tukey HSD post-hoc tests for linear models were then performed to test for differences within categories. CT_{min} differences between seasons were tested using a Mann-Whitney test. Kruskal-Wallis tests were used to test if the landscape had an effect on the size of parasitoid individuals in each season. For *A. rhopalosiphi*, two GLMs were fitted and tested as described above. The CT_{min} response of parasitoids to season x sex and season x size interactions, and of their size to a season x sex interaction were tested. Differences within seasons where then evaluated with Mann-Whitney tests.

Results

Meteorological records

For both seasons, closed landscapes were on average significantly colder than open landscapes (Table 11.1). With respect to its amplitude and standard deviation, temperature varied less in closed landscapes than in intermediate and open landscapes. In winter, closed and intermediate landscapes had a higher relative humidity than open landscapes. Closed landscapes had a lower wind speed than intermediate and open landscapes. The weather in winter was on average colder, with greater wind exposure and humidity than in spring for all landscape types. There was more variation among landscapes in winter than in spring for all of the meteorological variables measured. The occurrence of days which contained at least one temperature event under zero were for closed, intermediate and open landscapes respectively, 1, 2, and 4 in winter and 0, 0, and 4 in spring.

Table 11.1: Daily average meteorological data (± standard error) in the study area (Brittany, France) in winter (13th January to 7th March) and spring (24th March to 15th May) for different types of landscape. Results of repeated measures ANOVA comparing meteorological factors among landscapes are shown. n.s.=not significant. Superscript letters (a, b, c) denote significant differences among landscapes for each meteorological factor within each season.

Season	**Landscape type**	**Air temperature (°C)**	**Min | Max temperature (°C)**	**Relative humidity (%)**	**Wind speed (m.s^{-1})**
Landscape comparison					
Winter	Closed	6.58 ±0.07$^{(a)}$	-0.32 | 14.61	90.82 ±0.25$^{(a)}$	2.18 ±0.04$^{(a)}$
	Intermediate	7.23 ±0.08$^{(b)}$	-0.53 | 23.37	90.18 ±0.25$^{(a)}$	3.39 ±0.06$^{(b)}$
	Open	8.39 ±0.16$^{(c)}$	-2.07 | 24.91	85.82 ±0.54$^{(b)}$	2.67 ±0.07$^{(c)}$
	ANOVA (df=2)	F=67.01, p<0.001		F=67.8, p<0.001	F=148.2, p<0.001
Spring	Closed	10.78 ±0.10$^{(a)}$	0.51 | 20.10	83.63 ±0.40	1.84 ±0.03$^{(a)}$
	Intermediate	11.12 ±0.11$^{(a,b)}$	0.08 | 20.89	84.46 ±0.38	1.91 ±0.04$^{(a)}$
	Open	11.49 ±0.12$^{(b)}$	-1.02 | 21.78	83.66 ±0.43	2.71 ±0.05$^{(b)}$
	ANOVA (df=2)	F=10.1, p<0.001		n.s.	F=130.4, p<0.001
Seasonal comparison					
Winter	All	7.12 ±0.05	-2.07 | 24.91	89.70 ±0.18	2.77 ±0.03
Spring	All	11.13 ±0.06	-1.02 | 21.78	83.91 ±0.23	2.16 ±0.03

Parasitoid communities

The parasitoid community (Figure 11.1) was dominated by *A. rhopalosiphi* in winter and spring. The second most abundant species was *A. matricariae* in winter, although this species was completely absent in spring. *A. avenae* was the second most abundant species in spring. The aphid community was dominated by *R. padi* in winter and *M. dirhodum* in spring. In winter, *A. matricariae* and *A. ervi* were never reared from *M. dirhodum*. In spring, parasitoids were reared from different host species in accordance with the hosts' relative abundance. The species were homogeneously distributed across all landscape types in both winter (Chi-squared test, χ^2=7.88, df=6, p=0.25) and spring (χ^2=1.32, df=4, p=0.86).

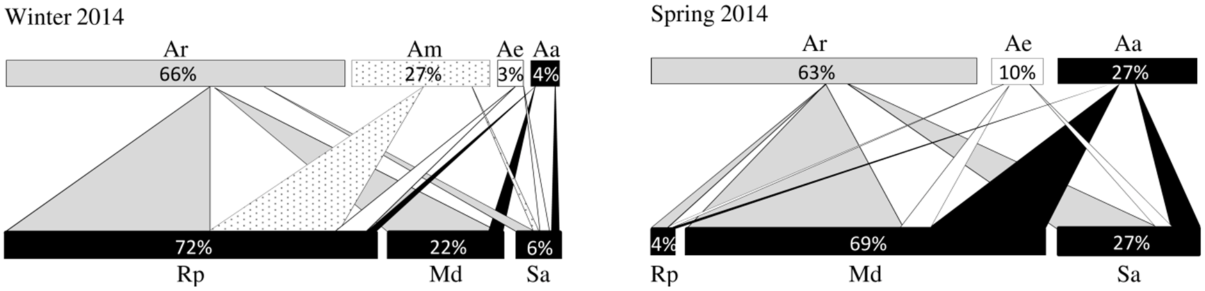

Figure 11.1: Quantitative food webs of the parasitoid and aphid community composition in winter and spring 2014. **Ar** (*Aphidius rhopalosiphi*), **Am** (*Aphidius matricariae*), **Ae** (*Aphidius ervi*), **Aa** (*Aphidius avenae*), **Rp** (*Rhopalosiphum padi*), **Md** (*Metopolophium dirhodum*), **Sa** (*Sitobion avenae*). Upper bars represent parasitoid relative abundance and 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.* in winter 56% of the parasitoids emerged from *R. padi* mummies were *A. rhopalosiphi*). The figures do not include individuals that could not be identified. The total number of individuals are n=127 and n=141 for parasitoids and n=141 and n=142 for aphids in winter and spring, respectively.

Thermal tolerance

a. All parasitoid species

Figure 11.2 shows the data for all species (pooled) for both seasons. Statistical information is provided in Table 11.2. For all species and across all landscapes, the winter parasitoid community was significantly more cold resistant than the spring community (-0.64 ±0.07°C in winter and -0.44 ±0.05°C in spring, Mann-Whitney test, W=10273, p<0.05).

In winter, there was a significant effect of landscape type on CT_{min}. Individuals from open environments were significantly more cold resistant than those from intermediate (Tukey HSD, z=-2.48, p<0.05) and closed (z=-3.53, p<0.01) environments. There was a marginally non-significant difference between individuals from intermediate and closed environments (z=-2.22, p=0.06). A similar but non-significant trend was observed for spring populations. In spring, a significant effect of host species was found; parasitoids reared from *M. dirhodum* had significantly lower CT_{min} than those reared from *S. avenae* (-0.51 ±0.06°C n=98 and -0.20 ±0.09°C n=39 respectively, Tukey HSD, z=2.76, p<0.05). There were no differences between the CT_{min} of parasitoids reared from *R. padi* compared to those reared from other species. There were no significant differences in CT_{min} among species or between sexes, neither in winter or spring. There was no effect of body size on CT_{min} for either winter or spring-collected parasitoids, and parasitoid size was not influenced by the landscape gradient in winter (Kruskal-Wallis test, χ^2=3.03, df=2, p=0.22) or spring (χ^2=0.13, df=2, p=0.94). There were no interaction effects among landscape gradient, parasitoid species, or aphid host species affecting the CT_{min} of parasitoids in winter or spring.

Table 11.2: Factors influencing parasitoid CT_{min}. Test statistics from Generalized Linear Models (GLMs) are shown for winter (N=127 individuals, 72 females and 45 males) and spring (N=142 individuals, 80 females and 62 males). There were large differences in community assembly between seasons so they had to be tested separately; see text of Results. The Likelihood-Ratio chi-square is calculated for each model using the "car" package. *** p<0.001. ** p<0.01. * p<0.05.

Factor / Season	Winter				Spring			
	LR (χ^2)	df	p > χ^2		LR (χ^2)	df	p > χ^2	
Landscape	10.16	2	**<0.01**	**	3.43	2	0.18	
Parasitoid species	0.99	3	0.80		0.62	2	0.73	
Landscape : Parasitoid species	0.56	2	0.76		1.69	3	0.64	
Sex	0.53	1	0.47		0.39	1	0.53	
Host species	1.17	2	0.42		7.26	2	**<0.05**	*
Parasitoid species : Host species	2.16	4	0.71		0.31	4	0.98	
Parasitoid size	1.96	1	0.16		0.48	1	0.49	

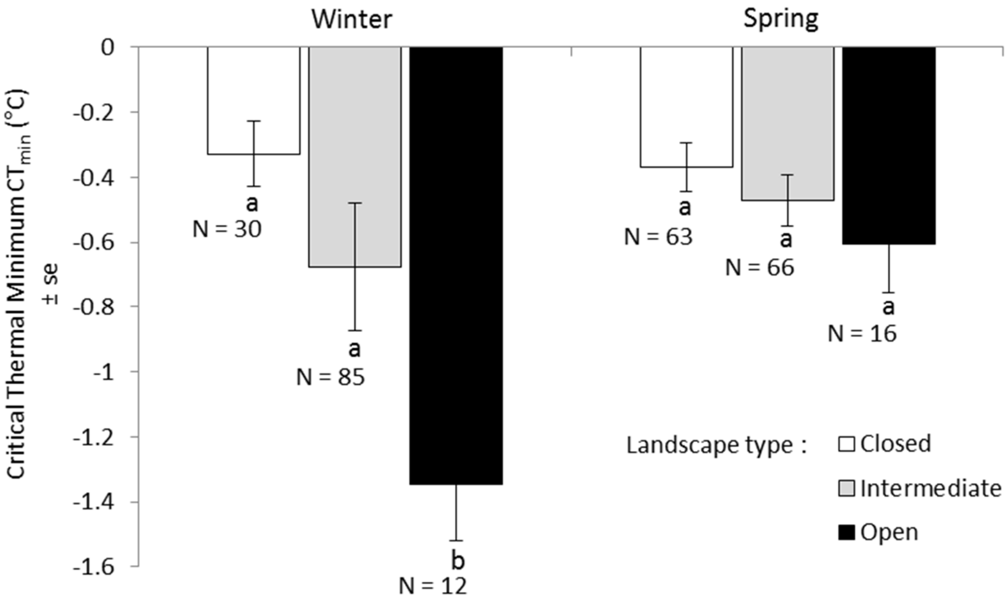

Figure 11.2: Average critical thermal minimum of parasitoids (all species) in winter and spring depending on the type of landscape. N=127 and 145 in winter and spring, respectively. Lower-case letters show significant differences between landscapes for each season. For inter-seasonal comparisons refer to the text.

b. For *A. rhopalosiphi* only

Winter sampled individuals from all types of landscape were more resistant to cold than those sampled in the spring (respectively -0.66 ±0.08°C and -0.45 ±0.04°C, Anova, LR=3.32, df=1, p=0.05). There was no difference in CT_{min} between sexes in either season (LR=0.001, df=1, p=0.93). There was no influence of parasitoids' body size on their thermal tolerance for either sex (LR=0.63, p=0.42). Furthermore, there was no effect of the interaction between season and sex (LR=0.56, df=1, p=0.46) or between season and size (LR=0.75, df=1, p=0.39) on parasitoids' CT_{min} (Figure 11.3a). Winter-collected *A. rhopalosiphi* individuals were smaller than spring individuals (respectively and for both sexes, 0.50 ±0.01mm and 0.58 ±0.01mm, LR=57.84, df=1, p<0.001). There was no effect of the interaction between season and sex on parasitoid body size (LR=0.65, df=1, p=0.42). In both seasons, size differed significantly between sexes (LR=13.03, df=1, p<0.001). Females were significantly larger than males in winter (0.52 ±0.01mm and 0.47 ±0.01mm respectively, Mann-Whitney test, W=1067, p<0.01) and spring (respectively 0.58 ±0.01mm and 0.55 ±0.01mm, W=1098, p<0.01) (Figure 11.3b).

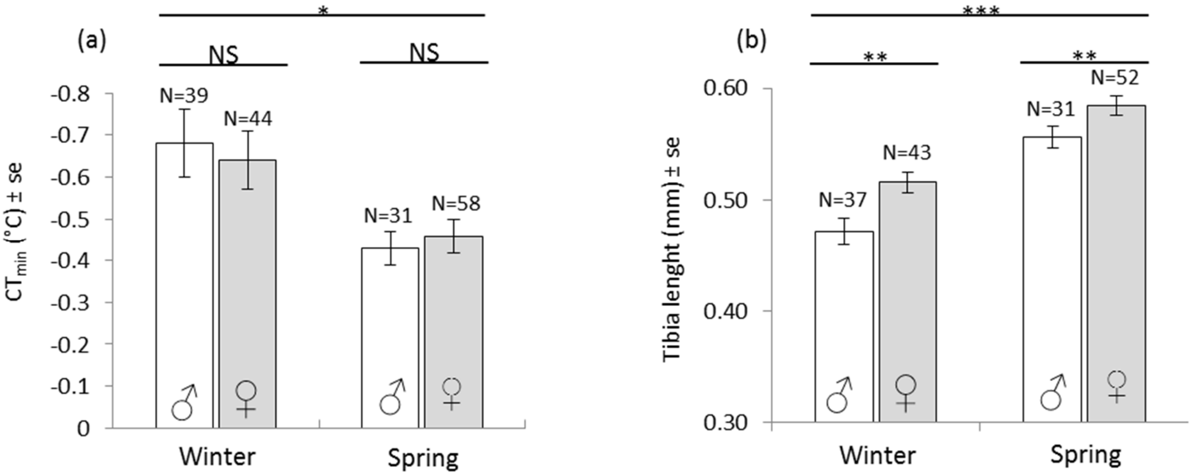

Figure 11.3: Average critical minimal temperature (**a**) and average tibia length (**b**) for *A. rhopalosiphi* in winter and spring. N=172 for CT_{min} measurements and 163 for size measurements. Symbols indicate parasitoids sexes males (♂) and females (♀). *** p<0.001 ** p<0.01 * p<0.05 n.s.=not significant.

Discussion

The results confirmed our first and second hypotheses, highlighting a landscape composition effect on different climatic factors and on the cold tolerance of parasitoids. This effect was more pronounced in winter. This study also revealed that parasitoids were smaller and more cold resistant in the winter than in spring, in accordance with our third and fifth hypotheses. Parasitoid size was not affected by the landscape gradient, which refutes our fourth hypothesis.

Landscape effects on microclimate

Between an open area and a hedged zone or forest remnant, there is generally a decrease in daytime temperatures but an increase in humidity (Argent, 1992, Quénol & Beltrando, 2006). These tendencies were confirmed by our measurements in winter and, to a lesser extent, in spring. Indeed, hedgerows usually limit wind speed, which acts to increase relative humidity. Additionally, cooling typically occurs along the borders of hedges and in the fields behind them. These important shelter effects limit the mixing of air layers and thus accentuate the cooling of fields surrounded by hedgerows (Quénol & Beltrando, 2006). Another consequence of sheltered environments is that temporal variations in temperature and humidity are less pronounced than in open landscapes (Suggitt *et al.*, 2011). In spring, temperature is less variable among landscapes than in winter, since vegetation begins to grow in the fields, and hedgerow foliage increases in density. The present study showed that open landscapes, especially in winter, present more stressful climatic conditions for ectotherms such as wind exposure, frost, more extreme temperatures, and greater temperature variation.

Landscape effects on the cold tolerance

We demonstrated the importance of considering microhabitat variation when assessing organisms' susceptibility to climate change. Winter sampled parasitoids from open landscapes were approximately 1°C more resistant to cold than those from closed landscapes, and 0.6°C more resistant than individuals from intermediate landscape. The magnitude of these effects greatly decreased in spring, although a similar trend was observed. Differences in parasitoid CT_{min} along the landscape gradient may have resulted from phenotypic plasticity (acclimatization), which is a particularly effective way for insects to cope with temperature stresses at different temporal scales (Lee & Denlinger, 2010, Colinet *et al.*, 2015). The thermal conditions encountered during the larval stage greatly influences the thermal tolerance of adults in most insects (Hoffmann *et al.*, 2013), and in open landscapes, parasitoids are less protected against cold spells. Thus, parasitoids from open landscapes could have acclimatized to sudden colder temperatures during their development, resulting in greater adulthood cold resistance. The results of Bahrndorff *et al.* (2006) on springtails and Bauerfeind & Fischer (2014) on butterflies also support the contention that thermal extremes explain variation in thermal resistance traits better than average temperatures. Separating the effects of mean and extreme temperatures on ectotherms – a recently developing field of research in thermal biology (Easterling *et al.*, 2000, Thompson *et al.*, 2013, Colinet *et al.*, 2015) – is one of the novelties of this study. In sheltered environments, parasitoids might be better able to resist cold due to higher relative humidity, and therefore might have no need to develop the strong physiological resistance required of open landscape parasitoids. Indeed, as suggested by De Bach (1943), desiccation is one of the main reasons for death under cold stress conditions in Aphidiinae. Higher humidity facilitates cold resistance by diminishing the risks of desiccation (Bahrndorff *et al.*, 2006, Hoffmann *et al.*, 2013).

Winter temperatures are stressful for parasitoids, since they are often below the insects' developmental thresholds. The thermal threshold for mummy-to-adult development has been estimated at 7.2°C for *A. rhopalosiphi*, 7.9°C for *A. matricariae*, and 6.6°C for *A. ervi* (Sigsgaard, 2000, Colinet & Hance, 2010). In spring, however, temperatures become warmer on average and less extreme. Thus, even if microclimatic differences along the landscape gradient persist (as it has been shown in this study), the temperatures reached are rarely or never stressful enough to make parasitoid acclimatization necessary. For this reason, it may not be possible to reveal differences between landscapes with respect to the cold tolerance of spring-collected parasitoids. In spring, the microclimatic gradient effect could also be compensated by a smaller scale impact of vegetation within the plots. For example, it has previously been shown that vegetation cover in crops provides a stable and beneficial microhabitat for insects both in terms of temperature and wind exposure (Waterhouse, 1955). Nevertheless, as shown in this study for *A. rhopalosiphi*, parasitoid cold tolerance differs between seasons. Winter-collected individuals acclimatized to cold temperatures are therefore more resistant to cold and vice versa in spring, supporting our third hypothesis.

There were no effects of sex or parasitoid species on cold tolerance in winter or spring. Parasitoids of all species emerging from *S. avenae* in spring were less cold tolerant than those emerging from *M. dirhodum*. We can thus infer bottom-up effects of host quality on parasitoid thermal tolerance. It is already known that parasitoids emerging from *S. avenae* are larger than parasitoids emerging from other aphid species (which increases their fecundity), and that parasitoids emerging from R. *padi* have a higher emergence rate (Andrade *et al.*, 2013). A trade-off may then exist between cold resistance and other life history traits that determine host choice and availability.

Size effects

In our analysis of *A. rhopalosiphi*, female parasitoids were larger than males. This trend is almost ubiquitous among parasitoid wasps (Hurlbutt, 1987) and insects in general (Chown & Gaston, 2010). There was no direct effect of size on the individuals' thermal tolerance between landscapes, although *A. rhopalosiphi* individuals were smaller in winter than in spring. If we consider that mean winter temperatures recorded are really those experienced by the parasitoids in the fields (*i.e.*, there may have been specific locations with higher temperatures), this result does not follow the predictions of the temperature-size rule (TSR). The TSR states that insects developing at higher temperatures are generally smaller upon emergence as a result of shorter development time (Van der Have & De Jong, 1996). Our results are, however, consistent with the AED hypothesis which states that a winter should impose a selective pressure that favors smaller parasitoids. For instance, Ismail *et al.* (2012) showed that smaller individuals of the parasitoid *A. ervi* that smaller individuals had have better resistance to cold stress than larger wasps. Since parasitoid size is intimately linked to fitness-related traits (*e.g.* fecundity, longevity; Harvey *et al.* (1994)), there could be a size induced trade-off between female fertility (large individuals) and survival (reduced size) under stressful temperature conditions. These results showing an effect of temperature variations at the seasonal scale (but not at the landscape scale) confirm our fifth hypothesis but refutes our fourth hypothesis concerning parasitoid size.

Conclusion

A clear impact of the landscape on the thermal tolerance of parasitoids in winter, and to a lesser extent in spring, was observed in this study. The physiological thermal tolerance of parasitoids seemed to respond to the varying weather conditions (especially extreme temperatures and relative humidity) encountered among landscapes and also among seasons. As stated in the introduction, broad scale temperature variations and predictions can be very different from those directly experienced by insects. These data highlight the need to better understand the relationship between insect physiology and landscape structure, especially under stressful conditions. We must use fine scale studies combined with information on the microhabitat if we are to understand climate change effects on insect populations and individual physiology. Climate change effects could be exacerbated by agricultural intensification leading to more open landscapes, subjecting insects to increasing physiological stress in the future. From a biological control perspective, our results imply that it may be possible to manipulate parasitoid cold resistance using protective elements in the landscape that reduce microclimatic variations.

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Annexe 2

Réseau hôtes-parasitoïdes en conditions extrêmes

Host-parasitoids network in extreme conditions, the case of cereal crops in Saskatchewan, Canada

Joan van Baaren, Tyler Wist, Julie Soroka & Kévin Tougeron
En préparation pour *Biodiversity and Conservation*

Summary

At the northern limit of ectotherm's distribution areas, food webs can be less complex and less persistent compared to those of more favorable areas. In agricultural areas, the conditions at the northern limit of crop cultures could impact pest regulation. In this paper, we study the aphid-parasitoid-hyperparasitoid network in cereal fields around Saskatoon (SK, Canada), one of the most rigorous climatic conditions for cereal crops in the world, inducing an extremely short period of vegetation growth. We have investigated the aphid- parasitoid-hyperparasitoid species relative abundances during two consecutive years (2014 and 2015). In 2014, a cold spring, only aphids were found. In 2015, aphids, parasitoids and hyperparasitoids arrived in the cereal fields at the same period (beginning of August, whereas the cereals are harvested at the end of the month). Three species of primary parasitoids were found, *Aphidius avenaphis* (91.9%), *Aphidius ervi* (9.54%) and *Diaeretiella rapae* (only one individual), for a parasitism rate of 0.08%, and four genera of hyperparasitoids, with a hyperparasitim rate of 20.1%. There was no specialization as the parasitoids and hyperparasitoids used the available aphid species as a function of their relative abundance. There were 87.4% apterous aphids, and the proportion of apterous and winged (alate) aphids did not differ between the 3 aphid species and between the primary and between hyperparasitoid species. The sex ratio was biased towards females for the primary parasitoids *A. avenaphis* and *A. ervi*, and for the most abundant hyperparasitoid species, *A. suspensus*. Identification criteria (number of antennomeres, characteristics of the mummies) are provided for the sampled species.

Key-words: Food-web, community, biodiversity, phenology, biological control

Introduction

The development and survival of ectotherms are intimately linked to atmospheric temperature. These organisms have evolved a diversity of morphological, physiological, and behavioral adaptations to survive within a given range of climatological conditions. Temperature governs species distribution, ecosystem function, community composition, population abundances, life history traits of individuals and synchronization of life-cycles of interacting species (Bale *et al.*, 2002, Hance *et al.*, 2007) At the extreme limits of ectotherm's distribution areas, food webs can be less complex (*i.e.* with less species richness and fewer trophic links) compared to those of more favorable areas. Therefore, in the northernmost distribution area of a species occurring in the Northern Hemisphere, the multitrophic interactions among ectotherms can be hazardous, with annual colonization from southern areas occurring more commonly than overwintering (Irwin & Thresh, 1988, Brodeur & McNeil, 1994). Hammill *et al.* (2015) showed that food web simplicity generally decreased the persistence of species.

In agricultural areas, which represent highly-disturbed and less resilient ecosystems, pest regulation at the periphery of crop cultivation could be different than in more concentrated crop growing regions. Indeed, a positive relationship between biodiversity and ecosystem functioning has been observed in many studies. For example, generalist predators often benefit from high prey diversity which is itself favored by high plant diversity (Symondson *et al.*, 2002, Miyashita *et al.*, 2012). Several studies have shown that more diverse communities are functionally less susceptible to environmental stresses (Steudel *et al.*, 2012).

Host-parasitoid systems are particularly interesting to investigate the links between biodiversity and the providing of ecosystem services, as variations in host-parasitoid community structures are subject to the expansions and regressions of the spatial distribution of their insect hosts (Folcher *et al.*, 2011). Several studies have focused on the influence of climate (Stireman *et al.*, 2005) or land cover patterns (Bennett & Gratton, 2012) on species richness, abundance and diversity of parasitoid communities (Folcher *et al.*, 2011). The fitness of parasitoids is linked to the spatial and temporal availability of the host species they exploit (Henry *et al.*, 2008). Therefore, one can expect the specific composition of the host community and their relative abundance to be major factors explaining the relative abundances of parasitoid species.

In this paper, we study the aphid-parasitoid-hyperparasitoid network in cereal fields around Saskatoon, Saskatchewan (SK), Canada, which has rigorous climatic conditions for cereal crop production, including an extremely short period of vegetation growth. Interestingly, the pest status of aphids in cereals differs across the world: they are major pests in most invaded regions (e.g. North America), but often minor pests in their native regions (e.g. Europe). This could account for the lower number of natural enemies and the simpler food webs observed in non-European countries and thus explain the higher impact of those pests outside Europe. Three aphid species largely present in cereals in North America probably came from Europe; *Rhopalosiphum padi* (Linnaeus), which has been present in North America at least since 1900 (Blackman & Eastop, 2005), *Schizaphis graminum* (Rondani) for which the first North American report was on wheat and barley in Virginia around 1882 (Webster & Phillips, 1912) and the English grain aphid, *Sitobion avenae* (Fabricius), a native of Europe, which was probably accidentally introduced everywhere in the world along with its cereal crop hosts (Asia, North and South America). In most non-European countries, biological control programs against *S. avenae* were organized and several species of parasitoids were introduced, and sometimes native parasitoid species adapted themselves to these new aphid hosts. Therefore, the host-parasitoid communities could be very similar in all the parts of the world where cereals are cultivated. For example, in North America, the parasitoid *Aphidius ervi* Haliday had been introduced from Europe to the USA in the mid-1960s (Halfhill *et al.*, 1972). This parasitoid species first spread in wet and mild climatic areas but rapidly invaded more northern areas of British Columbia and Ontario-Quebec (Campbell & Mackauer, 1973, Stary, 1974, Kos *et al.*, 2011). The native *Aphidius avenaphis* (syn. *Praon avenaphis* Fitch 1861) is a generalist North American aphid parasitoid (Marsh, 1979, Pike *et al.*, 1997) and was reported to occur in the US and Canada as early as in the 19th century (Fitch, 1856, Muesebeck *et al.*, 1951; in Kos *et al.*, 2011). *Aphidius avenaphis* specializes on the English grain aphid when this resource becomes the most abundant aphid in cereal crops.

In this study, we investigated the species relative abundances in an aphid-parasitoid system in cereal fields in Saskatchewan, during the two consecutive years 2014 and 2015. Relative abundances of parasitoid species and their aphid hosts in cereal fields were determined during the very short summer period. We aimed at investigating; (1) the species of aphids, parasitoids and hyperparasitoids present, their phenology, sex ratio and their links within the trophic web; (2) whether parasitoids and hyperparasitoids exploited different host species at distinct levels (*i.e.* resource specialization or resources equally exploited)

Our hypotheses were: (1) this host-parasitoid system harbored a limited number of species compared to the same system in Europe; (2) this system presented a high inter-annual variability due to the poor number of species; (3) this system harbored proportionally more generalist species than the same system in Europe due to few host species available and potential spatio-temporal variability in hosts' abundances; (4) the proportion of winged aphids should have been high, and parasitoids and hyperparasitoids arrived during the nymphal instar inside the aphids, due to the short season; (5) the sex ratio was female biased for parasitoids and hyperparasitoids, as it is commonly observed in cereal aphid-parasitoid systems in Europe (Andrade *et al.*, 2015); (6) the occurrence of diapause could be observed in the migrant parasitoid generation as the conditions did not allow the succession of many generations before winter.

Material & Methods

Meteorological data

Meteorological data was recorded at Environment Canada's Saskatoon weather station (Saskatoon, SK, Canada, 52.17°N; 106.72°W, elevation 504m) for the 2014 and 2015 seasons. For each sampling season, mean, lowest and highest daily temperatures were recorded, as well as the precipitation amount (mm). We only display minimal temperatures on the figure 12.1, as they seem to greatly differ between 2014 and 2015. Meteorological data were then used to explain fluctuations in aphid and parasitoid abundance at a fine temporal scale, based on aphid and parasitoid developmental temperature thresholds in the field (Sampaio *et al.*, 2007).

Field data

Sampling was conducted in cereal fields near Saskatoon and in a radius up to 200 km north east of Saskatoon. Five fields where cereal aphids appeared were monitored over time each year in 2014 and 2015. In 2014, the monitored fields were near Alvena (52.50°N, 106.02°W), the University of Saskatchewan's Kernen Research farm (52.16°N, 106.55°W), Agriculture and Agri-Food Saskatoon Research and Development Centre's (AAFC SRDC) Melfort Research farm (52.82°N, 104.60°W), AAFC SRDC's Saskatoon Research Farm (52.13°N, 106.63°W), and a grower's field near Yellow Creek (52.75483°N, 105.22063W). In 2015, they were at a grower's field near the 2014 field at Alvena *52.50°N, 106.02°W*), the U of S Kernen Research Farm (52.16°N, 106.55°W), the AAFC Melfort Research farm (52.82°N, 104.60°W), a field at the Saskatchewan Conservation Centre near Prince Albert (53.07°N, 105.83°W) and the AAFC Saskatoon Research Farm (52.13°N, 106.63°W). All fields were conventionally rather than organically managed. Living aphids were collected directly from the cereal plants and brought to the laboratory to allow for aphid mummy formation (*i.e.* parasitized aphids containing a larva of a primary parasitoid, or a hyperparasitoid when the parasitoid larva was parasitized). Mummies of parasitoids and hyperparasitoids also were collected directly from the plants and brought to the laboratory until their emergence. Cereal aphids, their primary parasitoids and their hyperparasitoids were then identified to species level and enumerated. As 2014 and 2015 were very different years in regards to weather and the target insect's presence and abundances, the sampling protocols were different for each year.

For 2014, the dates of sampling were August 28, September 2, 5, 9 and 12. Because very few aphids were found in this year, and these aphids were limited to green cereal plants growing in low, wet areas of a few fields, it was impossible to evaluate the aphid density. In each field, all the visually encountered aphids were collected and kept in the laboratory at 20°C, RH 70%, DL16:8, to observe the possible formation of mummies (which did not occur). Therefore, the available data consisted of the total number of aphids per field, and their identification to the species level.

For 2015, fields were visited at two week intervals between the end of June to the end of August. Sampling began at the time of first occurrence of aphids on August 11. Before this date, no aphids or mummies were found. The sampling was done by inspecting each leave visually one hour per field. The aphid abundance and parasitism rate were determined at the date of maximum aphid abundance, around August 25-September 2. For aphid abundance, 50 stems per field were thoroughly examined for aphid presence in the field. The living aphids observed were counted and their species were identified based on morphological traits. The number of mummies was counted and the parasitism rate calculated as the number of aphids divided by the number of mummies + aphids. Another count of relative abundance of the different species of aphids was done taking from the field 50 plants containing aphids. Each plant was carefully examined in the lab and each aphid was determined and counted. Mummies were collected randomly from the field until a minimum of 250 mummies were collected per field. Each mummy was placed in an individual gelatine capsule and kept in the lab at 20°C, RH 70%, DL16:8 until parasitoid emergence. The species of the emerged parasitoids and the species of the aphids from which those parasitoids emerged were identified based on morphology using various binomial keys (Pike *et al.*, 1997, Kavallieratos *et al.*, 2005, Tomanović *et al.*, 2012, Kavallieratos *et al.*, 2013, Kavad & Korat, 2014). The proportion of winged aphids was evaluated on both living aphids and on aphid mummies (wings remain visible after mummification). The emerged hyperparasitoids were identified based on morphology at the genus level. The sex ratio was evaluated for each species of primary parasitoid. Each unhatched mummy was dissected around one month after the peak of emergence to observe the occurrence of mortality at the mummy instar and the percentage of diapausing individuals (gold-yellow prepupae; Brodeur & McNeil, 1989a, Tougeron *et al.*, 2017a).

The morphological criteria of identification of the parasitoids from the genus *Aphidius* are not completely consistent in the literature, especially concerning the number of antennal antennomeres. The number of antennomeres was counted for each male and female, and compared with the available data in the literature. For the *Aphidius* genus, one major criteria of identification is the patterning of metasomal tergite 1. We observed some individuals with forms that had never been described.

Images

Image sequences of aphid species, mummies and metasomal tergite 1 and heads and antennae of *Aphidius* wasps were captured with a Nikon Digital Sight DS-L3 Digital Camera System mounted to a Nikon SMZ25 dissecting microscope and aligned and z-stacked with the Nikon Elements BR package software (Nikon Canada).

Statistical analyses

Quantitative food web graphical representations (Memmott *et al.*, 1994) were made based on the identified aphid mummies and their respective emerging parasitoids for 2015, in order to illustrate the degree to which relative abundances of aphids and parasitoids were linked.

We used the “bipartite” R package (Dormann *et al.*, 2009) to analyze the 2015 quantitative food-web using different metrics. The “connectance” describes the proportion of realized links in a food-web among all links possible. Co-occurrence of parasitoids on hosts describes the similarity in host-preferences. The “generality” index describes the mean number of prey species per predator. Finally, the “H2’” index measures the degree of specialization (from 0 to 1) of species in a network.

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 winged and apterous aphids between each aphid species and between each parasitoid species. All data analyses were performed using R software (R Core Team, 2017). Means and standard errors (SE) are provided.

Results

Meteorological data

We observed no differences in the snow cover or wind speed between the two years. 2014 was colder than 2015 during the wheat growing season, especially when looking at the minimal temperatures (Figure 12.1). The precipitation was higher at the beginning of the season and lowest during the summer in 2014 compared to 2015 (Figure 12.1). The minimum temperature during the growing season was -4.14 ± 0.72°C in 2014 and -0.86±0.66°C in2015. The maximum temperature was 7.4 ± 0.78°C in 2014 and 10.22 ± 0.74°C in 2015.

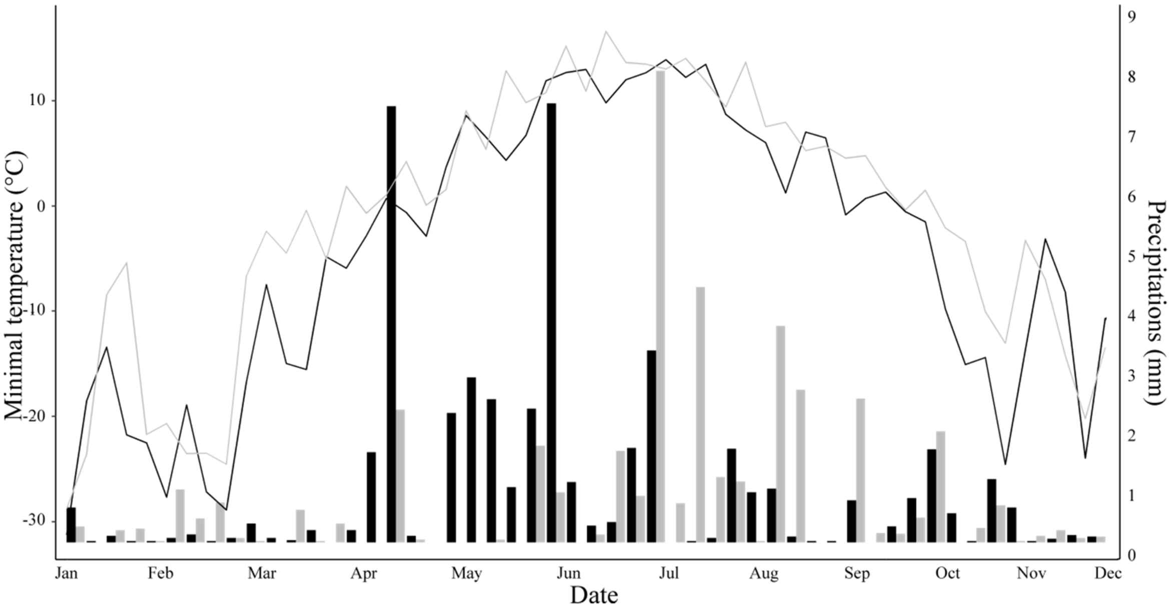

Figure 12.1: Combined graph of minimal temperatures (lines) and precipitations (bars) for 2014 (black) and 2015 (grey), from Saskatoon, Saskatchewan, Canada, data averaged weekly.

Aphids, parasitoids and hyperparasitoids

a. Year 2014

In the year 2014, only two species of aphids were found, with a total of 432 *R. padi* and 285 *S. avenae* collected in the 5 fields. *Rhopalosiphum padi* were regularly distributed among the 5 different fields, whereas for *S. avenae*, 273 of the total 285 were found in the same Alvena field, with one or two individuals in each of the other four fields. No mummies formed from any of these aphids when they were collected and held under laboratory conditions, and no mummies were collected in the fields.

b. Year 2015

Aphids

In 2015, three aphid species were found, with unequal abundances. On the 50 tillers per field that were randomly sampled, a total of 485 aphids were found, from which 397 were *S. avenae* (81.8%), 74 were *R. padi* (15.2%) and 14 were *S. graminum* (2.8%). *Sitobion avenae* had a density of 1.58±0.38, *R. padi* of 0.29±0.11 and *S. graminum* of 0.06±0.03 per plant. A total of 40 mummies were found on these 50 plants per field, for a parasitism rate of 40/525=7.62%.

On the 50 plants with aphids per field, 2034 aphids total were found; 90.1% were *S. avenae*, 9.0% were *R. padi* and 0.8% were *S. graminum*. Fifty-two plants out of 250 had at least two different species of aphids.

Parasitoids

The first mummies were observed on the 11th of August 2015, at the same time that cereal aphids were found in the fields. In total, 1190 mummies were taken from the five fields and their emergences followed in the lab. There were 98 dead parasitoids inside the mummies (8.2%), 304 empty mummies (25.5%), and 30 mummies (2.5%) containing a larva in diapause (among the dead mummies, 55 contained a dried larva that may have died during diapause due to dry storage conditions in the capsules).

From the 747 mummies from which wasps emerged, there were 597 primary parasitoids and 150 hyperparasitoids, a hyperparasitism rate of 20.1%. Three species of primary parasitoids were found, *Aphidius avenaphis* (Fitch) (549 individuals, 91.9%), *Aphidius ervi* (Haliday) (47 individuals, 9.54%), *Diaeretiella rapae* (M'intosh) (only one individual), and four genera of hyperparasitoids, *Asaphes suspensus* (Nees) (Hymenoptera: Pteromalidae) (102 individuals, 68%), *Dendrocerus bicolor* (Kieffer) (syn. *Dendrocerus aphidum* (Rondani)) (Hymenoptera: Megaspilidae) (40 individuals, 26.6%), *Alloxysta* sp. Förster (Hymenoptera: Figitidae) (4 individuals, 2.67%) and *Coruna* sp. Walker (Hymenoptera: Pteromalidae) (4 individuals, 2.67%). The quantitative food-web composed by these species is shown in Figure 12.2.

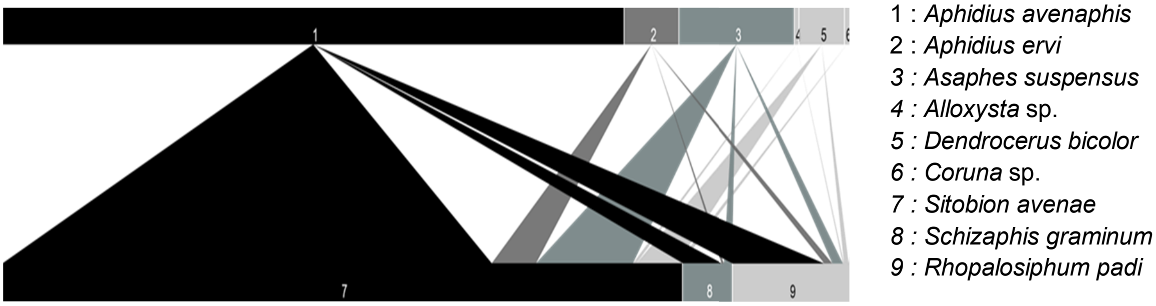

Figure 12.2: Trophic web of cereal aphids, their parasitoids and hyperparasitoids collected from cereal fields in northeastern Saskatchewan, Canada, in the summer of 2015. Upper bars represent parasitoid relative abundance and lower bars represent aphid relative abundance for each species. The thickness of the arrows between two bars is proportional to the relative number of trophic interactions between species.

Connectance was 0.77, co-occurrence of parasitoids on one given host was 1.9 out of 3 maximum and co-occurrence of hosts parasitized by the same parasitoid species was 3.7 out of 6 maximum, showing that all hosts and parasitoid species were strongly linked in the food-web. The mean number of parasitoid species attacking a particular aphid species was 2.43 and the mean number of hosts per parasitoid species was 1.85. There was no specialization as the parasitoids and hyperparasitoids used the available aphid species as a function of their relative abundance (H2'=0.01). Apterous aphids comprised 87.4% of the collection, and the proportion of apterous and alate aphids did not differ between the three aphid species (GLM, df=2, F=2.10, p=0.120) (Figure 12.3a), nor between the primary or hyperparasitoid species (GLM, df=5, F=2.20, p=0.063) (Figure 12.3b). The sex ratio was biased towards females for the primary parasitoids *A. avenaphis* and *A. ervi* (62% and 65%, respectively), and for the most abundant hyperparasitoid species, *A. suspensus* (87%). This ratio was close to 50% for *D. bicolor*. The sex ratio was not measured for *Alloxysta* sp. and *Coruna* sp. as there were only a few individuals.

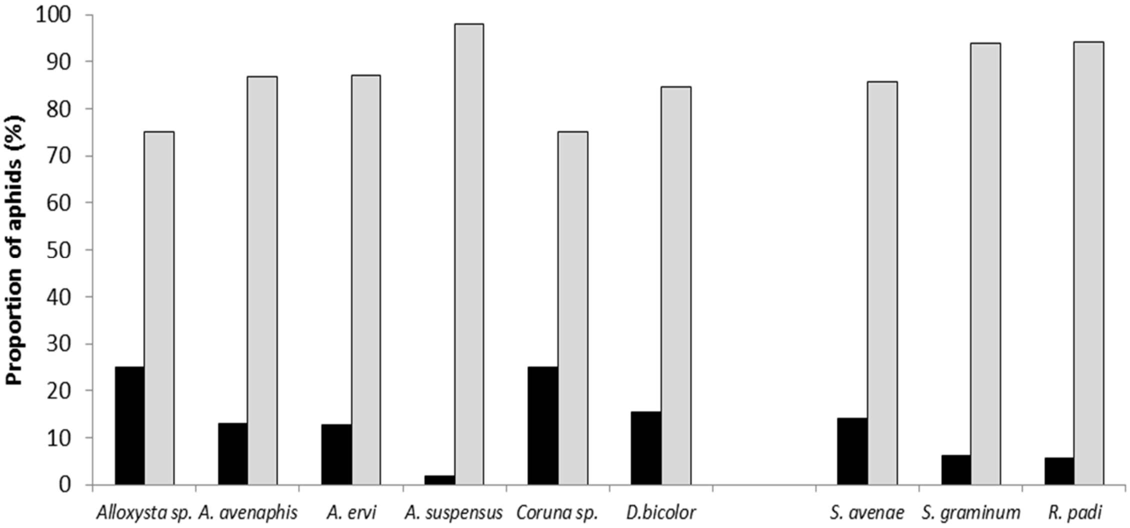

Figure 12.3: **a.** Proportion of apterous (grey) and winged (alate; black) aphid mummies for cereal aphid species, and **b.** Proportion of apterous and winged mummies where primary parasitoids and hyperparasitoids emerged from aphids collected from cereal fields in northeastern Saskatchewan, Canada, in the summer of 2015.

The development duration of the hyperparasitoids was longer than that of the primary parasitoids when kept in the laboratory at 20°C (Figure 12.4), with hyperparasitoids emerging later than primary parasitoids.

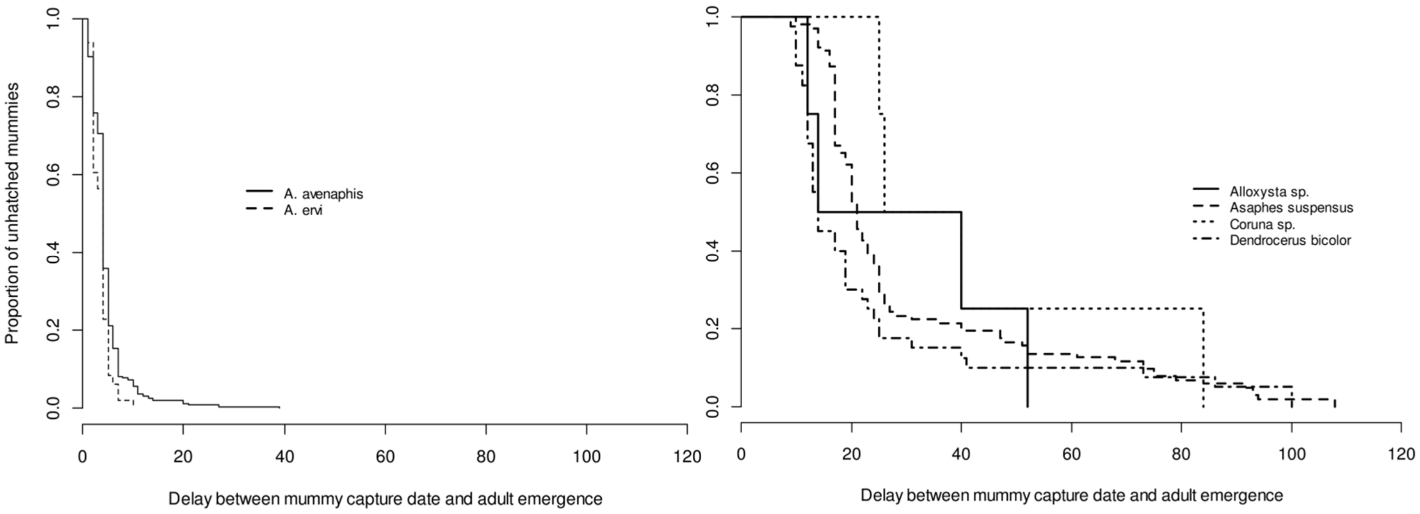

Figure 12.4: Time between mummy collection and emergence date (days) at 20°C in the laboratory for primary (left panel) and hyperparasitoid species (right panel) of cereal aphids from Saskatchewan, Canada, in 2015.

Mummies of *S. avenae* had dark and long cornicles, and long antennae which reached the cornicles (Figure 12.5a). Mummies of *S. graminum* had medium length, pale cornicles and medium length antennae (Figure 12.5b). Mummies of *R. padi* had the shortest cornicles and the shortest antennae (Figure 12.5c).

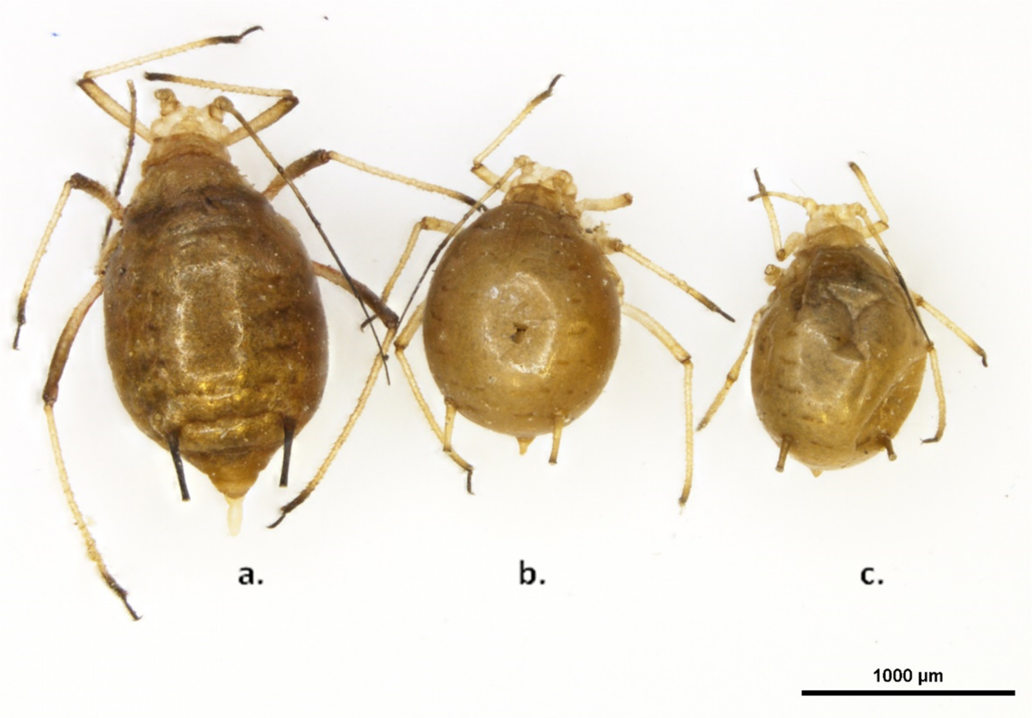

Figure 12.5: Representative mummies of a. *S. avenae*, b. *S. graminum*, and c. *R. padi.*

In several identification keys (Pike *et al.*, 1997, Kavallieratos *et al.*, 2005, Hullé *et al.*, 2006, Tomanović *et al.*, 2012, Kavallieratos *et al.*, 2013, Kavad & Korat, 2014), two morphological characters are important for the identification of the different species of the genus *Aphidius* - the patterning of metasomal tergite 1 and the number of antennomeres (Figure 12.6a). Individual *A. avenaphis* typically have a metasomal tergite 1 with thin striations (costulate), as do *A. rhopalosiphi*, *A. matricariae* and *A. uzbekistanicus* (Figure 12.6b). Most of the individuals of *A. avenaphis* we sampled had the typical metasomal tergite 1 of this group of species; however a few individuals had a metasomal tergite 1 with thicker striations (Figure 12.6c), but less thick than the pattern of striations of *A. avenae* or *A. colemani* which presented a few thick striations (Figure 6d). The *A. ervi* all had rugose metasomal tergite 1 typical of *A. ervi* (Figure 12.6e).

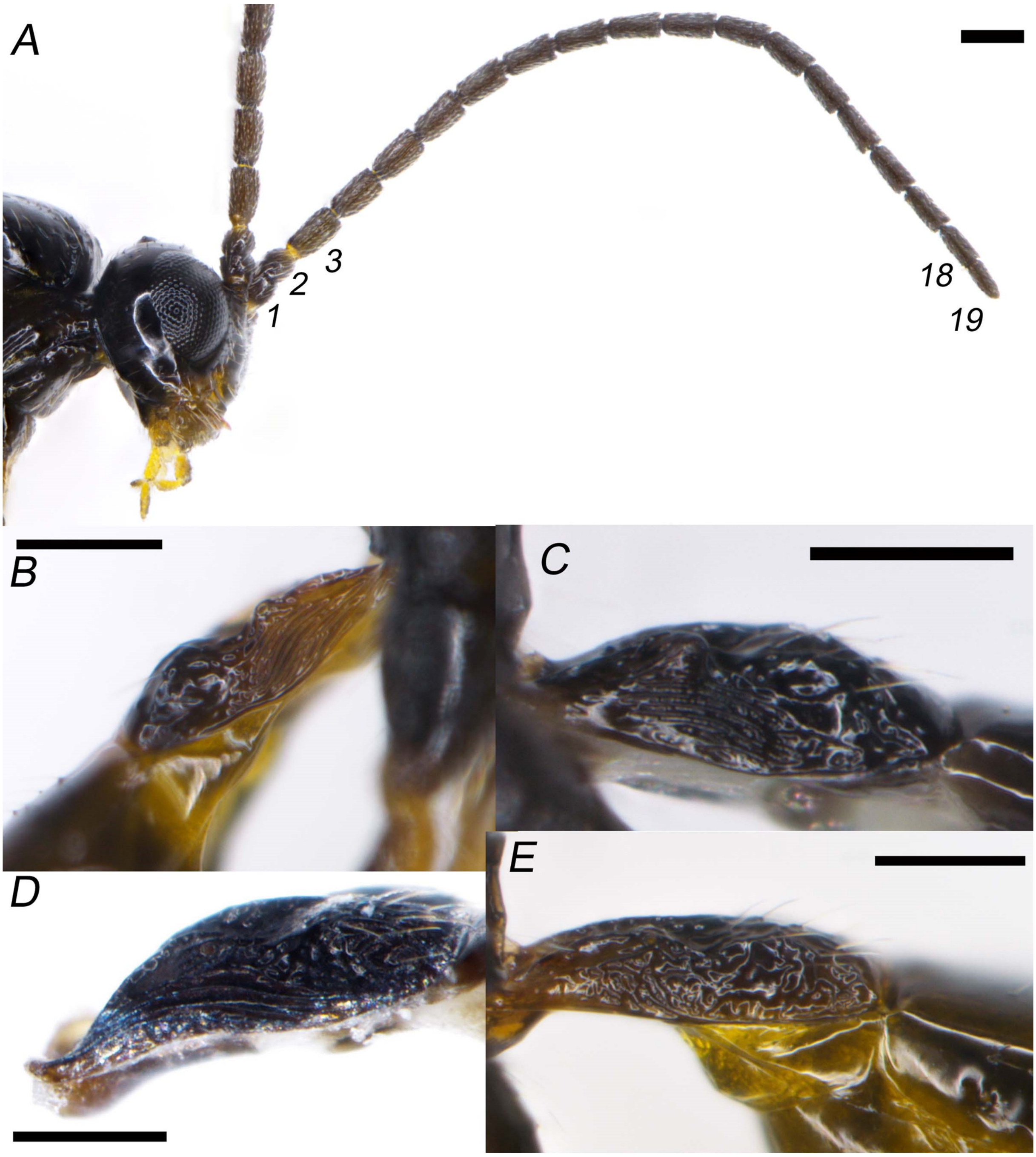

Figure 12.6: a. Head and antennae of an *Aphidius avenaphis* male showing the antennomere numbering pattern, b. Metasomal tergite 1 of typical *A. avenaphis*, c. of individuals with thicker striations, d. of typical *A. avenae*, and e. of typical *A. ervi*. All individuals were sampled around Saskatoon, Saskatchewan excepted *A. avenae* which comes from Brittany, France. Scale bars = 100µm.

Table 12.1 summarizes the numbers of antennomeres found on the individuals of *A. avenaphis* and *A. ervi* that we sampled in 2015 compared to data in the literature. For *A. avenaphis*, we observed 129 females (78%) with 16 antennomeres against four (2%) with 15 antennomeres and 33 (20%) with 17 antennomeres. For the males, 59 individuals (49%) had 19 antennomeres (Figure 12.6a), 6 (5%) presented 18 antennomeres, 41 (42%) presented 20 antennomeres and 5 (4%) presented 21 antennomeres. For *A. ervi*, the number of antennomeres varied from 17 (one individual, 6%), to 18 (nine individuals, 56%) and 19 (six individuals, 38%) for the females and from 19 (three individuals, 27%), to 20 antennomeres (four individuals, 36%) and to 21 antennomeres (four individuals, 36%) for males.

Table 12.1: Number of antenna antennomeres according to different sources from the literature for both male and female individuals of the main Aphidiinae species. Counting of antennomeres did not include the scape and the pedicel for (a) and (d), and is not specified for the other references.

Species	Males	Females
Aphidius avenae Haliday	(18-21) [a]	(16-17) [f] (17-18) [db]
Aphidius avenaphis (Fitch)	(19-21) [a]	(15-16-17) [a] (16-17) [b]
Aphidius ervi Haliday	(19-21) [a]	(17-19) [a] (17-18) [d] (18-20) [f]
Aphidius colemani Viereck	(16-17) [a]	(13-14) [d] (15-16) [bdf]
Aphidius matricariae Haliday	(16-17) [a]	(14-15) [abcdf]
Aphidius rhopalosiphi De Stephani Perez	(18-19) [a]	(16-17) [df] (17-18) [b]
Aphidius uzbekistaniscus Luzhetzki	(18-19) [a]	(16-17) [db]
Ephedrus plagiator (Nees)	(11) [ad]	(11) [acd]
Diaeretiella rapae (M'Intosh)	(16-18) [ae]	(13-15) [adef]
Praon volucre (Haliday)	(18-21) [a]	(16-18) [dc]

References: (a) Our observations, (b) Pike *et al.*, 1997, (c) Kavallieratos *et al.*, 2005, 2013, (d) Hullé *et al.* 2006, (e) Kavad and Korat 2014, (f) Tomanoviç *et al.*, 2012.

Discussion

Our results showed that the trophic web linked to cereals in Saskatchewan was very variable between the two sampling years, one year without any parasitoids and one year with the complete trophic levels (parasitoids and hyperparasitoids). These diverse results indicate that the populations are probably not established onsite and are likely limited by winter cold preceding the growing season, as none of the upper trophic levels was observed during the colder year 2014 whereas the complete trophic web was present during the warmer year 2015. If there are southerly winds early enough in the spring at the same time as high populations occur down south, they could come in early enough to establish and be parasitized. Portions of the aphids were probably already parasitized when they migrated into the cereal fields, with the first generation of parasitoids, evidenced by aphid mummies, present within four days of the appearance of the aphids in fields. The trophic web presented only a few species. All of the parasitoids and hyperparasitoids are generalists that exploited all the aphid hosts species available. The sex ratio was female biased as observed in these systems in other areas (Andrade *et al.*, 2016). The growing season in Saskatchewan is short (typically from 90 to 130 days); the first aphid individuals arrived in the middle of August, and cereals are harvested at the end of August or the beginning of September. Diapause of the parasitoids can occur as soon as the first generation of parasitoids is produced, meaning that for some species only one generation occurs during the wheat growing period, before entering winter diapause. Finally, the morphological criteria of parasitoid species identification available in the literature are not always conclusive, with the observation of some intermediate forms.

Our food web was dominated by two major species in cereals, *S. avenae* and *R. padi* as on all other continents, Andrade *et al.* (2016) for Europe, Zúniga (1990) for South America, Horrocks *et al.* (2010) for New Zealand where there is *R. padi* but not *S. avenae*. All the aphids are introduced species, probably from Eurasia and have colonized North America for more than one hundred years. In the colder year 2014, *R. padi* was the dominant aphid, whereas in warmer 2015 it was *S. avenae*. In Western Europe, *R. padi* is more cold resistant than *S. avenae* (Alford *et al.*, 2016). There is only one native primary parasitoid in Saskatchewan, *A. avenaphis*, a generalist whose original host range is unknown, and one other *Aphidius* species, *A. ervi,* which was introduced into North America in the mid-1960s (Halfhill *et al.*, 1972). At least four genera of hyperparasitoids were found, which were probably generalist native species. The total number of aphid species (three) is comparable to those observed in other continents (three to five), whereas the number of primary parasitoid species (three with *Diaeretiella rapae* occurring with only one individual) is strongly reduced, for on other continents and in the southern half of North America there can be up to 13 primary parasitoid species (Pike *et al.* (1997) for the Washington state, USA, Andrade *et al.* (2016) for Europe, Zúniga, (1990) for South America). Moreover, for primary parasitoids, *A. avenaphis* represented more than 90% of the parasitoids sampled. The number of genera of hyperparasitoids is similar to that observed elsewhere in the world, but little is known about hyperparasitoid abundance and diversity in other aphid systems.

No primary parasitoids found in 2014 and a late arrival in the middle of August 2015 coinciding with the arrival of the aphid hosts strongly suggests a colonization by parasitoids from the southern part of North America instead of a resident population. Indeed, in the case of resident populations, there are two possibilities: (1) the aphids are on the cereal fields as soon as the wheat is sown and persist all season, such as in countries with mild winters where at least a part of the aphid population is parthenogenetic all year around (eg. in the Western part of France (Andrade *et al.*, 2016, Tougeron *et al.*, 2016), or in Florida (Spencer, 1926)), or (2) come out of diapause at the beginning of the spring (Vollhardt *et al.*, 2008, Gagic *et al.*, 2012). Other populations of aphid primary parasitoids in more southerly latitudes of North America were suspected to not overwinter onsite but to arrive from southern areas by migrations in the spring (Spencer, 1926, Shands *et al.*, 1965, Brodeur & McNeil, 1994). These migrations can be in successive waves, with some of them rarely reaching the northern areas, or arriving late in the season, more in the summer than in the spring (Spencer, 1926). In the state of Washington where 13 species of primary parasitoids are found, Pike *et al.* (1997) showed that 5 primary parasitoids occur as soon as April, whereas other species arrive later, with *A. avenaphis* observed from June to November, *Ephedrus* sp. from August to October and *A. matricariae*, detected only during the summer growing season. Migrations of aphids from southern to northern USA were well documented in several crops, for example flights of *Myzus persicae* into potato fields (Zhu *et al.*, 2006), Messina (1993) for the Russian wheat aphid *Diuraphis noxia* in Utah or Irwin and Thresh (1988) for *R. maidis* and *S. graminum* in cereal fields in Illinois.

The poor trophic web of our Saskatchewan fields was composed of two generalist species as primary parasitoids, and of several species of hyperparasitoids that are all generalists. The two primary parasitoids used each species of aphid according to their relative abundances, and the same pattern is observed between hyperparasitoids and the primary parasitoid. Kaneryd *et al.* (2012) showed that highly specialized systems are particularly vulnerable to variable environments. In host-parasitoid communities, the fluctuations in the quality and quantity of host resources are expected to exert a strong influence on the resource exploitation strategy of parasitoids (Andrade *et al.*, 2013, Andrade *et al.*, 2015). A high variance in the availability and the quality of host resources would be favourable for generalist parasitoids. On the contrary, a prevalence of specialist behaviour could be expected in a stable environment (Peers *et al.*, 2012). Our trophic web was not equilibrated, with one highly dominant species (*A. avenaphis*) and few rare species. Hammill *et al.* (2015) showed that the poorest in number of species an ecosystem is, the less persistent in time it is. Kamjunke *et al.* (2004) showed that in extreme conditions, the food webs presented only a few species, with no functional redundancy, as many species are excluded due to the stressful conditions compared to more favorable habitats. In such systems, the effect of the higher trophic level is enhanced. In our study, we observed a rate of hyperparasitism of 20% in the first generation, suggesting that primary parasitoid populations and aphid populations may have difficulties to increase in abundance.

As the proportion of apterous and winged (alate) aphids did not differ between the three aphid species and were equally parasitized by the different primary and hyper parasitoid species, a large proportion of the parasitoids likely arrived as larvae inside winged aphids. As soon as the first mummies were produced, the hyperparasitoids attacked them, suggesting that hyperparasitoids, probably generalists, were already present and likely originated from aphids on other crops or uncultivated grasses around the fields. It should be noted that in 2014, the year without primary parasitoids in cereal aphids, some hyperparasitoids were sampled by sweep netting, present in fields without aphid mummies present. Parasitoid migration as larvae inside aphids was previously documented by Vorley and Wratten (1987) in southern England. The sex ratio in our study was biased towards females, a result typical of these aphid-parasitoid-hyperparasitoid communities (Andrade *et al.*, 2015, 2016).

The duration of development of our hyperparasitoids was longer than of the primary parasitoids. Because aphids and primary parasitoids arrived late in the growing season, there is little probability of a second hyperparasitoid generation in northern fields before the cereals are harvested and the temperature decreases in fall. Some of the hyperparasitoids were in diapause, as were a portion of the primary parasitoids. In areas of Quebec, Canada, diapause begins as early as August under natural conditions (Brodeur and McNeil, 1994; Tougeron *et al.* unpublished data). In northern Saskatchewan, the first generation should enter into diapause or it will not survive. However, there is no evidence that even the individuals entering diapause survive through winter until spring, which returns typically at the end of May. The duration of the winter is probably too long for survival of the primary and hyperparasitoids, for even if they survived the winter, they might die as there are no aphid hosts available until end of July (Wist *et al.*, 2012, Wist *et al.*, 2013) or in August. However, as generalist hyperparasitoids, aphid mummies on other host plants might suffice as hosts for a spring generation of hyperparasitoids but this hypothesis requires further testing.

In this study, we determined the primary parasitoids by two morphological criteria that are recognized as important for the identification of the different species of the genus *Aphidius*, the number of antennomeres and the patterning of metasomal tergite 1 (Pike *et al.*, 1997, Kavallieratos *et al.*, 2005, Hullé *et al.*, 2006, Tomanović *et al.*, 2012, Kavallieratos *et al.*, 2013, Kavad & Korat, 2014). We evaluated the variation level in the number of antennomeres, which is quite high, both in *A. avenaphis* and *A. ervi*, with around 20% of the individuals harboring a higher or a lower number of antennomeres than average. Some individuals presented asymmetric antennae with a different number of antennomeres on the two antennae (around 3% of the individuals). For *A. avenaphis* we found some males (5%) with 18 antennomeres, and this lower number has not been mentioned in other studies. It should be noted that the number of antennomeres varied between the different studies (list of references in the Table 1), showing either local variations, or a sampling with too few individuals to record all the possible variability. For example, in our study we sampled few *A. ervi*. This species represented less than 10% of our sampling and we did not observed all the range of antennomeres mentioned in the literature, whereas with our large sample of *A. avenaphis*, we found a larger variation that was mentioned in the literature. However, we cannot exclude some local differences, as a species like *A. ervi* has worldwide distribution. Concerning the second criterion, the patterning of metasomal tergite 1, individuals of *A. avenaphis* typically have a metasomal tergite 1 with thin striations (costulate), as do *A. rhopalosiphi*, *A. matricariae* and *A. uzbekistanicus,* and in our sampling we found this typical pattern in most of the individuals. However, a few individuals have a metasomal tergite 1 with thicker striations, but thinner than the pattern of striations of *A. avenae* or *A. colemani,* which have fewer but thicker striations. It is difficult to make hypotheses about this variation. As no *A. avenae* or *A. colemani* were sampled, the hypothesis of a possible hybridization is difficult to consider, although these species occur in the state of Washington to the southwest of our sampled area.

Finally, if we consider the possible impact of these parasitoid species as biological control agents against the cereal aphids, it likely would be quite limited, as the parasitoids, even with concurrent arrival with the cereal aphids, had the opportunity of only one or two generations, and their numbers are reduced by hyperparasitoid attack upon mummification of the aphids. In this system, the parasitism rate was less than 10%, considerably lower than what is observed in other areas of the world (Pike *et al.* (1997) for the Washington state, USA, Andrade *et al.* (2016) for Europe, Zúniga (1990) for South America, Horrocks *et al.* (2010) for New Zealand). The impact of predators on cereal aphid populations might be higher than that of parasitoids. Pérez-Rodríguez *et al.* (2015) showed the importance of a good synchronization between the different trophic levels, and particularly between the spring migrations of aphids and their parasitoids in England to insure efficient biocontrol. However, in areas with extreme weather conditions and short growing seasons, the limited length of the growing season does not allow effective biocontrol through parasitism, although the synchronization level of parasitoids to their aphid hosts is high. With global climate change, the shortening of the winters in Western Canada could induce an earlier arrival of aphids in the spring and a faster increase of their population and crop damage. However, their parasitoids will also arrive sooner and this earlier arrival could increase both the richness of the food web and the efficiency of the parasitoid biological control. As cereal crops are a major aspect of the Canadian prairie landscape (Dosdall *et al.*, 2011), it is important to study the evolution of these food webs in future years.

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1. **Tougeron K. & Abram P. K.,** 2017. An ecological perspective on insect sleep deprivation. *The American Naturalist* **190:(3)**, E55-E66. doi: 10.1086/692604
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