Déterminants du parasitisme larvaire du carpocapse du pommier au Sud Est de la France

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Déterminants du parasitisme larvaire du carpocapse du pommier au Sud Est de la France

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Résumé :

Dans un contexte de réduction de la pression phytosanitaire sur les cultures il est important de recourir à des méthodes permettant de réduire l'usage des pesticides pour lutter contre les ravageurs. Un des moyens consiste à réguler les ravageurs par leurs ennemis naturels (Lutte biologique par conservation). Le carpocapse du pommier, *Cydia pomonella,* est un ravageur majeur des vergers de pommiers au Sud Est de la France. L'objectif de cette thèse était de définir les déterminants du parasitisme larvaire de ce ravageur. Nous avons caractérisé la composition de la communauté des parasitoïdes sur les larves diapausantes et de saison du carpocapse. Cette communauté est majoritairement représentée par trois espèces d'hyménoptères : *Ascogaster quadridentata*, *Pristomerus vulnerator* (parasitoïdes primaires) et *Perilampus trisits* (parasitoïde secondaire d'*A. quadridentata* et de *P. vulnerator*) dans les zones étudiées. Nous avons déterminé les caractéristiques des pratiques agricoles et des éléments semi-naturels au niveau du verger et du paysage qui affectent le taux de parasitisme et la composition de la communauté de parasitoïdes pour des larves diapausantes du carpocapse. Les haies brise-vent et les haies composites autour du verger semblent jouer sur la composition de la communauté en favorisant par leur présence les parasitoïdes primaires *A. quadridentata* et *P. vulnerator* par rapport au parasitoïde secondaire *P. tristis*. La protection phytosanitaire au niveau du verger et du paysage environnant a un effet sur le taux de parasitisme. Le taux de parasitisme est plus élevé dans les vergers en agriculture biologique que dans les vergers conventionnels ainsi que dans les vergers entourés d'une faible proportion de vergers conventionnels dans un voisinage de 250 m. Nous avons étudié les dynamiques temporelles de la communauté des parasitoïdes du carpocapse. Le taux de parasitisme a globalement augmenté au cours de la saison de développement du carpocapse et il est plus élevé lorsque l'on piège de jeunes larves dans les fruits que des larves âgées dans les bandes cartonnées enroulées autour des troncs. La composition de la communauté varie au cours du temps. La proportion relative du parasitoïde secondaire *P. tristis* augmente au cours de la saison en parallèle d'une diminution de la proportion relative *d'A. quadridentata*. Les émergences des adultes *A. quadridentata* sont de plus synchronisées avec celles des adultes carpocapses. Enfin, nous avons développé et testé une méthode de PCR-RFLP et des marqueurs ADN spécifiques pour détecter et identifier les parasitoïdes du carpocapse. La PCR-RFLP permet d'identifier les parasitoïdes adultes et leurs hôtes. Les marqueurs spécifiques permettent, en outre, la détection de parasitoïdes dans les œufs et les jeunes larves de carpocapse. Ces approches moléculaires ont également permis d'évaluer le parasitisme dans des populations naturelles de carpocapse et d'estimer les interactions trophiques au sein de la communauté des parasitoïdes.

Mots-clés : Ecologie du paysage, *Cydia pomonella*, *Ascogaster quadridentata*, communauté de parasitoïdes, synchronisation, détection moléculaire, lutte biologique par conservation.

Abstract:

In the context of a more environment-friendly agriculture, it is important to design methods that enable us to reduce the use of pesticides to fight pests. One possible way consists in increasing pest regulation by their natural enemies (Conservation biological control). The codling moth, *Cydia pomonella*, is a major insect pest of apple orchards in Southeastern France. The aim of this thesis was to identify determinants of the larval parasitism of this pest. We characterized the composition of the parasitoid community on diapausing and non diapausing codling moth larvae. This community is mainly represented by three Hymenoptera species: *Ascogaster quadridentata*, *Pristomerus vulnerator* (two primary parasitoids) and *Perilampus trisits* (a secondary parasitoid of *A. quadridentata* and *P. vulnerator*) in the study sites. We determined the characteristics of agricultural practices and semi- natural habitats at the orchard and landscape level that affect the parasitism rate and the composition of the parasitoid community of diapausing codling moth larvae. The windbreak and spontaneous hedgerows around the orchard seemed to impact the parasitoid community composition by promoting, when present, the primary parasitoids *A. quadridentata* and *P. vulnerator* versus the secondary parasitoid *P. tristis*. Crop protection practices at the orchard and surrounding landscape levels affected the parasitism rate. Parasitism rate was higher in organic orchards than in conventional orchards as well as in orchards surrounded by a low proportion of conventional orchards in a 250 m vicinity. We further studied the within-season temporal dynamics of the codling moth parasitoid community. The parasitism rates globally increased along the season among cohorts of mature codling moth larvae and were higher in young larvae trapped in fruits than in mature larvae trapped in band traps around the tree trunks. The community composition varied along the season. The relative proportion of the secondary parasitoid *P. tristis* increased among the codling moth cohorts whereas the proportion of *A. quadridentata* decreased. Furthermore, the emergences of adult *A. quadridentata* were synchronized with the emergences of the adult codling moths. Finally, we developed and tested a PCR -RFLP method and specific DNA markers to detect and identify parasitoids of the codling moth. The PCR -RFLP method was powerful to identify adult parasitoids and their hosts. Specific primers allowed detection of parasitoids in the eggs and young larvae of codling moth. These DNA-based techniques allowed molecular evaluation of parasitism in *C. pomonella* natural population and reconstructing quantitative food web of the parasitoid community.

Keywords: Landscape ecology, *Cydia pomonella*, *Ascogaster quadridentata,* parasitoid community, phenology, molecular detection, conservation biological control.

Sommaire

Introduction

1. Intensification agricole

Les changements des systèmes de production agricole au niveau mondial durant la seconde moitié du XXème siècle poussés par les besoins grandissants de la population mondiale et les objectifs d'indépendance alimentaire ont conduit à la simplification des paysages agricoles. Ainsi le regroupement des parcelles a diminué et fragmenté un grand nombre d'éléments semi-naturels comme les haies, les prairies et les forêts (Meeus, 1993; Burel and Baudry, 1999; Tscharntke et al., 2005). Cette intensification a réduit la diversité des cultures. Au niveau des parcelles, l'intensification agricole est associée à une forte augmentation des intrants (fertilisants et pesticides), une mécanisation des exploitations et une simplification des rotations des cultures (Robinson and Sutherland, 2002; Benton et al., 2003). Bien que ces changements ont augmenté les rendements ils ont par contre négativement impacté la biodiversité des organismes (Matson et al., 1997; Tilman et al., 2001).

2. Perte de biodiversité et diminution des services écosystémiques

Les ennemis naturels des ravageurs sont l'un des bénéfices de la biodiversité. Leur abondance et diversité ainsi que les services écosystémiques qu'ils offrent aux agriculteurs sont affectés par l'intensification agricole (Roschewitz *et al.*, 2005; Winqvist *et al.*, 2011; Monteiro *et al.*, 2013; Veres *et al.*, 2013). L'utilisation des pesticides à spectre large et la simplification du paysage affecte les communautés des ennemis naturels. Cette simplification des paysages est associée à une réduction des proportions des éléments semi-naturels tel les haies qui fournissent les ressources trophiques et constituent un refuge pour un grand nombre d'ennemis naturels notamment les guêpes parasitoïdes (Forman and Baudry, 1984; Landis *et al.*, 2008). L'utilisation des pesticides a un effet négatif sur les organismes non ciblés comme les pollinisateurs et les prédateurs (Horrigan *et al.*, 2002). Les effets de la simplification des paysages et l'utilisation massive des pesticides sur la biodiversité et les services écosystémiques sont difficiles à séparer vu que ces deux pratiques

sont souvent corrélées. De plus, la diversité des éléments semi-naturels et la diversité des cultures sont corrélées dans la plupart des paysages agricoles (Fahrig *et al.*, 2011).

3. Régulation naturelle des ravageurs des cultures

L'intensification agricole a rendu les agro-écosystèmes plus vulnérables aux bioagresseurs. Les monocultures facilitent la localisation des cultures cibles par les ravageurs, ainsi que leur dispersion. La diminution des éléments semi-naturels a réduit l'abondance et la diversité des ennemis naturels. Ainsi, les monocultures subissent une forte pression des ravageurs par rapport aux systèmes plus diversifiés (Russell, 1989; Andow, 1991). L'utilisation massive des pesticides comme moyen de lutte principal contre les ravageurs a conduit à la sélection de génotypes résistants aux molécules utilisées (Van Leeuwen *et al.*, 2008). Et l'exposition récurrente aux pesticides impacte la santé des travailleurs en agriculture (Lee *et al.*, 2004).

Il est ainsi nécessaire de trouver et de développer des moyens de lutte alternatifs contre les ravageurs. La protection intégrée contre les ravageurs - *Integrated pest management* (IPM) - propose la mise en œuvre d'un ensemble de pratiques culturales qui satisfait des exigences écologiques, économiques et toxicologiques afin d'obtenir une récolte qualitativement optimale (OILB, 2002).

La lutte biologique est l'une des méthodes de lutte qui peut être employée en IPM. Il s'agit de l'utilisation d'organismes vivants pour supprimer les populations d'un ravageur ou ses impacts dans le but de le rendre moins abondant ou moins nocif (Eilenberg *et al.*, 2001). Il existe trois formes de lutte biologique : la lutte biologique par introduction (classique), la lutte biologique par augmentation et la lutte biologique par conservation. La lutte biologique par introduction consiste à introduire un agent biologique exotique (provenant de l'aire d'origine du ravageur) dans un nouvel environnement pour contrôler à long terme un ravageur non-natif. Dans la lutte biologique par augmentation il s'agit de relâcher des auxiliaires dans des environnements qui n'en contiennent pas ou pas suffisamment pour un contrôle optimal des ravageurs. L'approche de la thèse se situant dans le cadre de la lutte biologique par conservation nous allons développer ce moyen de lutte.

Dans la lutte biologique par conservation il s'agit de préserver les ennemis naturels présents dans les agro-écosystèmes en modifiant les pratiques agricoles et en aménageant le paysage pour les favoriser (Barbosa, 1998; Landis *et al.*, 2000). Il est alors possible de créer des habitats non cultivés (haies, bandes enherbées, buissons, fossés, …) qui serviront de refuge, de sites d'hibernation et qui fourniront les ressources trophiques nécessaires aux ennemis naturels tel le nectar, pollen, les proies et les hôtes alternatifs. Pour assurer l'efficacité de la lutte biologique par conservation il est important de diminuer l'utilisation des pesticides dans les parcelles. Ainsi, il est important de bien comprendre quels sont les facteurs agronomiques et écologiques qui affectent les populations des ennemis naturels pour mieux adapter les pratiques agricoles et les aménagements du paysage qui les favoriseront.

4. Gestion des ravageurs de la parcelle au paysage

La lutte biologique par conservation requiert d'être abordée dans une perspective paysagère. La dynamique des populations et les interactions trophiques dépendent de processus qui s'opèrent à des niveaux plus larges que ceux de la parcelle (Ricklefs and Schluter, 1993; Kareiva and Wennergren, 1995). Les ravageurs et leurs ennemis naturels traversent souvent l'interface entre les zones cultivées et celles non cultivées. D'où le besoin de mener des études combinées des systèmes gérés et naturels (Bianchi *et al.*, 2006; Rand *et al.*, 2006; Bommarco *et al.*, 2007).

La lutte biologique par conservation se basant sur la modification des pratiques agricoles et l'aménagement du paysage pour favoriser les ennemis naturels, il est important de connaître l'effet des pratiques agricoles et des éléments paysagers sur les ravageurs et leurs ennemis naturels associés.

Les pratiques agricoles et les ressources locales et paysagères affectent souvent l'abondance et la composition des communautés de parasitoïdes dans un agro-écosystème donné (Jonsson *et al.*, 2012; Mates *et al.*, 2012). Les pratiques agricoles intensives affectent négativement les parasitoïdes hyménoptères qui sont particulièrement sensibles aux pesticides (Thomson and Hoffmann, 2006). Les éléments semi-naturels peuvent fournir des proies ou des hôtes alternatifs pour les prédateurs et les parasitoïdes (Landis *et al.*, 2000).

Les habitats floraux fournissent aux parasitoïdes des ressources complémentaires notamment le pollen et le nectar. Cela augmente la longévité et la fécondité des femelles parasitoïdes (Schmale *et al.*, 2001). Les haies peuvent servir de refuge pour les populations de parasitoïdes durant les traitements pesticides. Les bois sont caractérisés par un microclimat plus modéré que le centre de la parcelle qui protège les ennemis naturels contre les fortes variations de température (Rahim *et al.*, 1991; Landis *et al.*, 2000). Les éléments semi-naturels peuvent aussi favoriser les ravageurs en abritant leur diapause hivernale ou estivale (Leather, 1993; Pywell *et al.*, 2005).

L'échelle de réponse des espèces ciblées est un élément important à prendre en considération dans l'aménagement du paysage. L'échelle à laquelle les parasitoïdes répondent aux facteurs locaux et du paysage dépend de leur biologie, particulièrement de leur capacité de dispersion ainsi que la structure de l'hétérogénéité du paysage (Ricci *et al.*, 2013). De plus, les espèces spécialistes répondent à la composition du paysage à une plus petite échelle que les espèces généralistes (Chaplin-Kramer *et al.*, 2011).

5. Les parasitoïdes : Ennemis naturels des ravageurs des cultures

Les ennemis naturels des ravageurs sont diversifiés. Ils sont des prédateurs, des parasitoïdes, des champignons ou des virus entomopathogènes.

Les parasitoïdes représentent entre 8 % et 20 % des insectes décrits. La majorité des parasitoïdes appartiennent soit à l'ordre des Hyménoptères soit à l'ordre des Diptères (Feener and Brown, 1997). Les parasitoïdes Hyménoptères sont des insectes dont les larves parasitent différents stades de développement d'autres arthropodes (insectes, acariens). Les femelles parasitoïdes piquent, injectent du venin et pondent leurs œufs dans ou sur leur hôte. La femelle peut tuer directement l'hôte au moment de la ponte ; on parle alors de parasitoïde idiobionte**.** La larve du parasitoïde peut aussi retarder son développement et/ou consommer progressivement son hôte avant de le tuer ; le parasitoïde est alors koinobionte (Quicke, 1997). Les parasitoïdes influencent ou régulent la densité des populations de leurs hôtes dans les écosystèmes naturels et agricoles (Godfray, 1994). Ils sont largement utilisés dans le contrôle biologique des ravageurs des cultures (Quicke, 1997).

Les larves des parasitoïdes se développent aux dépens d'un seul hôte (Godfray, 1994). Suivant les espèces, les parasitoïdes peuvent infester différents stades de développement de leurs hôtes. Ainsi des parasitoïdes d'œufs, de larves, de pupes et parfois même d'adultes ont été identifiés. Le parasitisme peut aussi se prolonger sur plusieurs stades de leur hôte. On distingue ainsi des parasitoïdes ovo-larvaires ou des parasitoïdes ovo-pupaux en fonction du stade auquel l'hôte est attaqué puis tué (Stireman *et al*., 2006).

Le nombre d'hôtes pouvant être parasités varie en fonction des espèces. Certains parasitoïdes sont hautement généralistes. D'autres espèces sont spécialisées sur une ou quelques espèces d'hôtes uniquement. Les Diptères parasitoïdes sont généralement plus généralistes que les Hyménoptères (Wajnberg and Ris, 2007). Les parasitoïdes spécialistes d'une espèce donnée d'hôte contribuent globalement à plus de mortalité de cet hôte que les parasitoïdes généralistes (Elzinga et al, 2007; Godfray et al, 1995; Stefanescu et al, 2012).

Certaines femelles parasitoïdes disposent de presque la totalité de leurs œufs matures à leur émergence. Elles sont qualifiées de proovogéniques. Alors que pour d'autres, la maturation des œufs s'effectue après l'émergence au cours de leur vie reproductive. Elles sont qualifiées de synovogéniques (Flanders, 1950). Il existe dans la nature un continuum entre ces 2 stratégies. Les parasitoïdes synovogéniques ont une durée de vie supérieure à celle des parasitoïdes proovigéniques (Jervis *et al.*, 2001). Les ressources florales comme le pollen et le nectar augmentent la longévité et la fécondité des femelles parasitoïdes en particulier celle des espèces synovogéniques (Schmale *et al.*, 2001).

Les stades immatures des parasitoïdes peuvent se développer à l'intérieur (endoparasitoïdes) ou à l'extérieur de leur hôte (ectoparasitoïdes). Certains parasitoïdes peuvent paralyser leur hôte et interrompre son développement. Ils sont dits idiobiontes. Les parasitoïdes koïnobiontes se nourrissent de leur hôte tout en lui permettant de poursuivre son développement (Askew and Shaw, 1986). Il existe des covariations entre les traits d'histoire de vie des parasitoïdes. Il y a une dichotomie entre les endoparasitoïdes koïnobiontes qui sont plutôt proovogéniques et spécialistes sur un nombre restreint d'hôtes et les ectoparasitoïdes idiobiontes synovogéniques et plutôt généralistes (Godfray, 1994 ; Jervis *et al.*, 2001 ; Wajnberg and Ris, 2007).

Il existe plusieurs systèmes de déterminisme du sexe chez les parasitoïdes : la diplo-diploïdie, l'haplo-diploïdie et la parthénogenèse thélytoque (Normak, 2003). Chez les Diptères le

système principal est la diplo-diploïdie. Chaque individu est issu d'un ovocyte fécondé. Le sexe est déterminé par la combinaison des chromosomes sexuels. L'haplo-diploïdie est répandue chez la majorité des Hyménoptères. Les mâles sont haploïdes se développant à partir d'ovocytes non fécondés (parthénogenèse arrhénotoque) alors que les femelles sont diploïdes se développant à partir d'ovocytes fécondés. Par conséquence, la femelle fécondée peut déterminer la proportion de mâles et de femelles dans sa descendance (sexe ratio) en contrôlant le nombre d'œufs à féconder. Dans le cas de thélytoquie, les femelles sont seulement présentes.Elles se reproduisent de façon aséxuée sans s'accoupler. La connaissance du déterminisme du sexe est importante pour comprendre l'évolution et l'écologie des parasitoïdes et ainsi optimiser la lutte biologique contre les ravageurs des cultures (Wajnberg and Ris, 2007).

Les parasitoïdes peuvent rentrer en compétition en parasitant des hôtes déjà parasités. Il s'agit de superparasitisme quand la femelle parasitoïde parasite un hôte déjà parasité par un parasitoïde de la même espèce (Vanalphen and Visser, 1990). Le multiparasitisme correspond au parasitisme d'un hôte par différentes espèces de parasitoïdes (Fisher, 1961). Les hyperparasitoïdes sont des parasitoïdes secondaires qui se développent aux dépens d'un parasitoïde primaire. Ainsi, ils représentent un quatrième niveau trophique (Sullivan and Volkl, 1999). L'effet des hyperparasitoïdes sur l'efficacité des parasitoïdes primaires est un sujet important en lutte biologique (Rosenheim, 1998 ; Sullivan and Volkl, 1999). Le cleptoparasitisme a lieu quand une espèce de parasitoïdes se développe aux dépens d'une autre espèce en lui volant son hôte pour le développement de ses propres descendants (Eggleton and Belshaw, 1992).

Pour détourner la réponse immunitaire de leur hôte, les parasitoïdes ont développé un ensemble de facteurs de virulence présents dans leur venin et/ou produits par des gènes de polyDNAvirus symbiotiques (PDV) (Bezier *et al.*, 2013). Le venin a pour rôle de paraliser l'hôte soit de façon temporaire pour faciliter l'oviposition soit de façon permanente. Il peut empêcher la mue de l'hôte et peut chez certaines espèces de parasitoïdes faciliter le rôle d'autres facteurs de virulence comme les PolyDNAvirus. Les PolyDNAvirus sont des symbiotes de parasitoïdes Hyménotère chez les Braconidae et les Ichnmonidae (Wajnberg and Ris, 2007) dont l'ADN est intégré au génome de l'hôte. Les particules virales sont injectées à l'oviposition de l'œuf du parasitoïde dans l'hôte. L'expression des gènes de

virulence entraîne des changements physiologiques de l'hôte comme l'inhibition de l'encapsulation de l'œuf du parasitoïde et des manipulations développementales permettant le développement et l'émergence du parasitoïde (Moreau, 2003; Beckage and Gelman, 2004; Moreau *et al.*, 2009; Beckage, 2012). Chez d'autres espèces de parasitoïdes des particules proches des virus mais dépourvues d'ADN jouent un rôle semblable aux PDV (Wajnberg and Ris, 2007).

6. Questions abordées dans la thèse et plan du manuscrit

Durant ma thèse, je me suis intéressée en particulier aux parasitoïdes du carpocapse du pommier. Le but de mon travail était de définir les déterminants du parasitisme larvaire du carpocapse du pommier dans le Sud Est de la France, dans une perspective de lutte biologique par conservation.

Les questions qui ont guidées mon travail étaient :

1- **Quelles sont les variations temporelles du parasitisme du carpocapse ?**
La composition de la communauté de parasitoïdes change-t-elle sur les larves de carpocapse au cours de la saison? Ces parasitoïdes sont-ils synchrones avec le carpocapse ? La phénologie des parasitoïdes varie-t-elle au cours du temps ? Comment le taux de parasitisme du carpocapse varie-t-il au cours du temps ?

2- **Quelles sont les variations spatiales du parasitisme du carpocapse ?**
Quels sont les effets des pratiques agricoles et des caractéristiques des éléments semi-naturels au niveau du verger et du paysage sur la composition de la communauté des parasitoïdes et le taux de parasitisme du carpocapse ?

3- **Quelles sont les interactions hôte – parasitoïde et parasitoïde – parasitoïde au sein de la communauté de parasitoïdes du carpocapse ?**
Quelles sont les interactions entre le carpocapse et ses parasitoïdes ? Quelles

sont les interactions intra - et inter - spécifiques dans la communauté de parasitoïdes du carpocapse ?

Ce manuscrit s'organise autour de ces questions de la façon suivante :

Cette introduction générale est suivie d'une introduction de la biologie du carpocapse du pommier et ses parasitoïdes. Cette section comprend des planches de photos des parasitoïdes qui sont jointes au manuscrit de thèse. Une partie « Matériels et méthodes » est présentée pour décrire les sites d'étude, les jeux de données et les méthodes d'échantillonnage. La suite du manuscrit se présente en 3 articles.

Le **chapitre 1** aborde la première question dans un article soumis au journal *Biological Control* : « **Temporal dynamics of parasitoid assemblages on the codling moth** ».

Le **chapitre 2** traite la question 2 dans un article publié dans le journal *Agriculture, Ecosystems and Environment* : « **Codling moth parasitism is affected by semi-natural habitats and agricultural practices at orchard and landscape levels** ».

Le **chapitre 3** traite la question 3 par une série d'expérimentations notamment par le développement d'outils moléculaires pour la détection et l'identification des parasitoïdes du carpocapse. Cela est développé dans un article en préparation pour *Molecular Ecology Resources*: « **Molecular tools for the detection and the identification of *Cydia pomonella* parasitoids**».

Le manuscrit se termine par une discussion générale des résultats de la thèse et les perspectives ainsi que trois annexes.

Modèle biologique

1. Le carpocapse du pommier : *Cydia pomonella*

Biologie du carpocapse

Le carpocapse du pommier, *Cydia pomonella* Linnaeus, est un Lepidoptère de la famille des Tortricidae dont les adultes mesurent entre 15 et 22 mm d'envergure. Il a une coloration spécifique qui le distingue des autres Tortricidae. Ses ailes antérieures sont gris-cendré et présentent à leur extrémité distale un speculum à fond brun (Figures 1 et 2) (Bovey, 1966). C'est un ravageur majeur des pommes, son hôte préféré, mais il se développe aussi sur d'autres plante-hôtes comme les poiriers, les pêchers, les abricotiers et les noyers. Ce papillon a suivi la dispersion des pommiers à travers le monde en adaptant son cycle de vie aux conditions climatiques et trophiques (Audemard, 1991). Il est originaire d'Asie centrale, zone d'origine du pommier domestique (Geibel *et al.*, 2000; Harris *et al.*, 2002; Mills, 2005). Les adultes peuvent voler jusqu'à 50 m dans leur verger d'origine (Audemard, 1991). La durée moyenne de vie des adultes varie de 9 à 13 jours. Ce sont des papillons à mœurs crépusculaires (Bovey, 1966). Les œufs sont pondus séparément sur les feuilles, les rameaux proches des fruits et sur les fruits eux-mêmes. Une seule larve se développe par fruit vu la distribution des œufs et le comportement de cannibalisme des larves sur et dans le fruit (Audemard, 1991). L'incubation des œufs dure de 1 à 3 semaines suivant la température. Les chenilles néonates cherchent à pénétrer les fruits durant une phase dite « stade baladeur » de 2 à 4 jours. Les larves creusent une galerie en spirale sous la surface du fruit puis la prolongent en direction de la zone des pépins qui sont complètement consommés (Figures 3 et 4). Le développement larvaire dure de 20 à 30 jours et comporte 5 stades successifs. Par la suite, la larve de 5ème stade quitte le fruit et descend le long du tronc de l'arbre pour chercher un abri pour former son cocon. Les larves de stade L5 atteignent 18 à 20 mm de long (Bovey, 1966).

Figure 1: Carpocapse du pommier adulte (source: internet)

Figure 2: Carpocapse adulte à l'émergence (source : Equipe EPI, INRA)

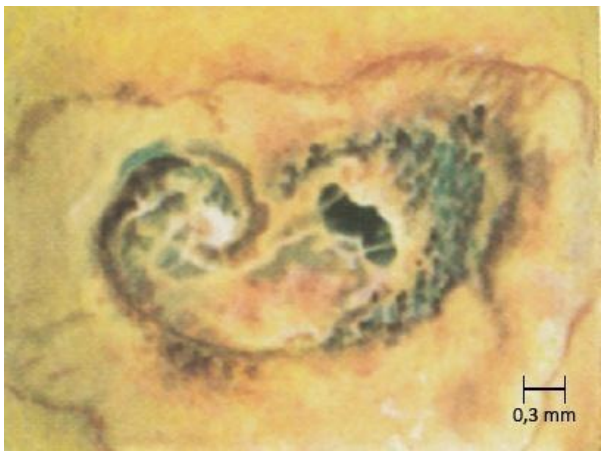

Figure 3 : Galerie en spirale creusée sous l'épiderme par la jeune larve de carpocapse (source: Association de coordination technique agricole, 1986, fiche 82)

Figure 4 : Galerie creusée par le carpocapse dans une pomme attaquée (source: Association de coordination technique agricole, 1986, fiche 82)

Phénologie du carpocapse

Le cycle de vie du carpocapse est composé de générations annuelles variant entre une à quatre en fonction du climat (latitude et altitude), l'année et la plante-hôte. A la fin de la saison de développement les larves matures entrent en diapause dans un cocon sur l'arbre ou dans le sol. Une proportion des larves entre en diapause durant la saison de développement à chaque génération (Audemard, 1991). Le carpocapse ajuste son cycle de

vie au climat et à la plante-hôte en adaptant le temps de diapause (Audemard, 1991). Les larves peuvent rester exceptionnellement en diapause pendant deux ans (Yothers and Carlson, 1941). Dans le sud est de la France, le carpocapse a entre 2 et 3 générations annuelles. De plus en plus de larves de troisième génération sont observées (Boivin *et al.*, 2005). Cela peut être du au rallongement des conditions climatiques favorables à l'émergence des adultes en conséquence du réchauffement climatique et/ou de la plantation de nouvelles variétés de pommes récoltées plus tardivement comme les Pink Lady.

Dégâts du carpocapse et méthodes de lutte

Le carpocapse du pommier peut engendrer jusqu'à 90% de pertes de la production fruitière dans les vergers non traités (Audemard, 1991). Les fruits attaqués par le carpocapse au début de leur croissance tombent généralement au sol. Dans le cas contraire, le fruit est dans tous les cas non commercialisable. Les fruits même endommagés superficiellement sont retirés du marché des fruits à croquer (Figure 5).

Figure 5 : Attaque de carpocapse sur pomme (source: Equipe EPI, INRA)

Il existe plusieurs stratégies de lutte contre le carpocapse du pommier. Le seuil de tolérance de la présence du carpocapse dans un verger est de 1 à 2%. Par conséquent, le carpocapse est la cible d'environ 90% des traitements insecticides dans les vergers de pommiers (10 à 20 traitements par année en Basse vallée de la Durance, (Monteiro *et al.*, 2013)). Des moyens de lutte biologiques comme la pulvérisation de virus de la granulose et la confusion sexuelle

sont utilisés à la fois dans les vergers en agriculture biologique et conventionnelle. Le virus de la granulose est spécifique du carpocapse. Il se multiplie rapidement dans la larve et cause sa mort. La confusion sexuelle consiste à placer des diffuseurs d'un analogue synthétique de phéromone sexuelle dans le verger pour désorienter les mâles et réduire ainsi le taux de reproduction des papillons adultes. Une autre technique de lutte qui se développe depuis 2007 consiste à protéger les arbres avec des filets de protection qui limitent à la fois la reproduction et l'oviposition des carpocapses (Sauphanor *et al.*, 2012).

L'utilisation massive des insecticides a conduit à l'apparition de résistances à la plupart des molécules utilisées (Reyes *et al.*, 2007; Franck *et al.*, 2012) y compris le virus de la granulose (Asser-Kaiser *et al.*, 2007). D'où la nécessité de trouver des méthodes alternatives et durables pour la lutte contre le carpocapse.

Méthodes de capture

L'évaluation des effectifs de la population de carpocapse dans un verger peut se faire par un échantillonnage périodique des fruits qui seront disséqués. Tous les fruits d'un certain nombre d'arbres y compris les fruits tombés doivent être examinés. Ceci permet d'évaluer la population de carpocapse au niveau du verger par extrapolation. Une autre méthode consiste à utiliser des pièges en carton ondulé enroulés autour des troncs des arbres pour échantillonner les larves diapausantes et les larves matures des générations successives (Audemard, 1991). Il existe aussi des piégeages attractifs des mâles en utilisant un analogue synthétique de la codlémone, phéromone sexuelle femelle (Riedl and Croft, 1974; Riedl *et al.*, 1976).

Ennemis naturels du carpocapse

Les ennemis naturels du carpocapse sont nombreux et la plupart ne sont pas spécifiques de ce ravageur. Certaines espèces de Trichrogrammes (*Trichogramma platneri, Trichogramma embryophagum, Trichogramma cacoesiae*), le nematode *Neoaplectana* et le virus de la granulose ont fait l'objet d'essais de lutte biologique. Le champignon *Beauveria bassiana* Vuillemin provoque la mortalité du carpocapse dans les vergers durant les hivers froids et

humides (Hagley, 1971). Le carpocapse est aussi attaqué par un complexe de parasitoïdes des larves et des nymphes (Mills, 2005). La prédation par les oiseaux et d'autres arthropodes agit comme un important facteur limitant des populations de carpocapse dans certaines situations (Audemard, 1991; Mols and Visser, 2002, 2007).

Dans cette thèse nous avons étudié les parasitoïdes larvaires du carpocapse du pommier dans le Sud Est de la France. Les photos des insectes pour la suite de ce chapitre sont présentées dans une planche de photos jointe au manuscrit.

2. Les parasitoïdes du carpocapse des pommes

Les parasitoïdes du carpocapse ont été identifiés dans plusieurs régions dans le monde. Le complexe de parasitoïdes diffère entre l'Amérique du nord et l'Eurasie (Mills, 2005). Mills (2005) a synthétisé l'ensemble des espèces attaquant le carpocapse dans la région Paléarctique (Figure 6). En Europe, plusieurs parasitoïdes primaires (Braconidae et Ichneumonidae) et un hyperparasitoïde (Perilampidae) ont été collectés de larves diapausantes de carpocapse (Rosenberg, 1934; Athanassov *et al.*, 1997; Diaconu *et al.*, 2000; Mills, 2005). Les espèces dominantes dans cette communauté est le braconide *Ascogaster quadridentata* Wesmael et deux ichneumonides *Pristomerus vulnerator* Panzer et *Trichomma enecator* Rossi (Athanassov *et al.*, 1997; Mills, 2005).

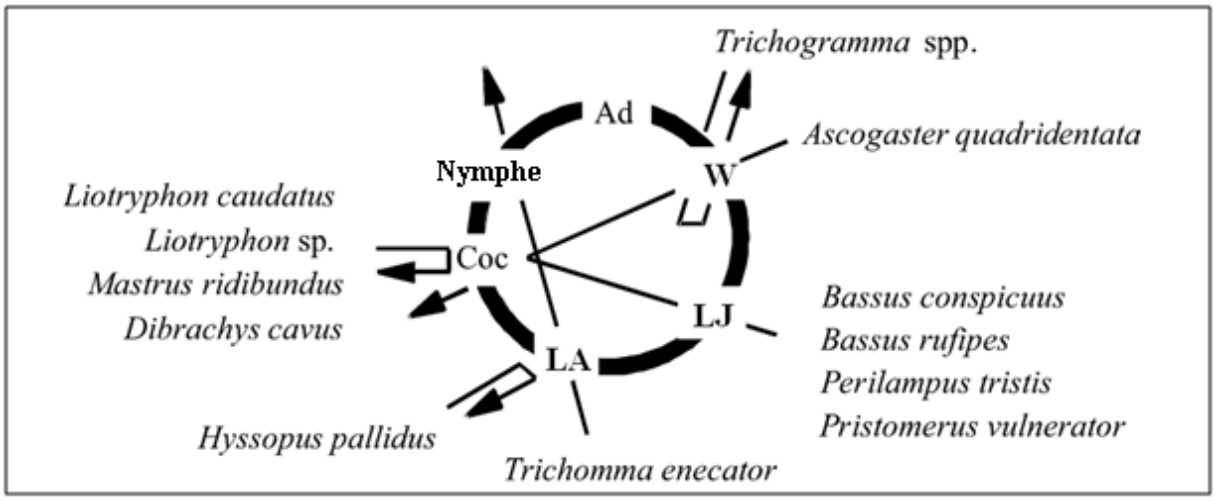

Figure 6 : Le complexe des parasitoïdes associés au carpocapse des pommes en Eurasie. Le cercle représente le cycle de développement du carpocapse (W : œuf, LJ : larve jeune, LA : larve âgée, Coc. : cocon). Les flèches représentent le stade attaqué et tué par les parasitoïdes associés. Les parasitoïdes dont les flèches restent en dehors du cercle sont des ectoparasitoïdes et ceux dont les flèches le traversent sont des endoparasitoïdes (Mills, 2005).

Les parasitoïdes de larves de carpocapse décrits ci-dessous sont ordonnés selon leurs abondances relatives dans les vergers de pommiers en Europe d'après la littérature (Rosenberg, 1934; Athanassov *et al.*, 1997; Diaconu *et al.*, 2000; Mills, 2005).

2.1. *Ascogaster quadridentata*

Ascogaster quadridentata Wesmael (Figures 7-12) est un hyménoptère endoparasitoïde ovo-larvaire de la famille des Braconidae (sous-famille des Cheloninae). Il est spécialiste des larves de Tortricidae. C'est un parasitoïde primaire du carpocapse. Il se reconnaît à son abdomen dont les segment sont soudées en un seul bouclier en face dorsale (Athanassov *et al.*, 1997). Les femelles ont des antennes comptant 29 à 33 segments et présentant une légère dilatation dans la partie médiane du flagellum (Figures 7, 8 et 10) (Huddleston, 1984).

Les femelles sont prêtes à parasiter juste après l'émergence (proovigénique). Les larves parasitées du carpocapse qui ont atteint leur maximum de développement sont un quart à

un tiers de leur taille normale. Elles sont blanchâtres contrairement aux larves saines qui présentent une couleur rose (Figure 12) (Clausen, 1940). Les larves de carpocapse parasitées par *A. quadridentata* sont castrées (Reedlarsen and Brown, 1990). La femelle *A. quadridentata* pond son œuf dans le cytoplasme de l'œuf du carpocapse. La larve du parasitoïde qui vient d'émerger pénètre dans l'embryon de son hôte (Clausen, 1940). La larve L1 d'*A. quadridentata* est de 0.2 mm de longueur juste à l'éclosion. Elle augmente de taille et de volume respectivement de 10 et 250 fois avant la première mue (Rosenberg, 1934). Le parasitoïde reste au premier stade larvaire jusqu'au moment où son hôte entre dans son dernier stade de développement. Les larves de carpocapse parasitées par *A. quadridentata* commencent à se déplacer précocement et à tisser leur cocon 2 jours après la mue au stade L4. Au même moment la larve de parasitoïde mue au stade L2. Cette dernière se développe et atteint son volume maximal en 2 à 3 jours. Les fluctuations des concentrations des hormones endocriniennes du carpocapse informent le parasitoïde de l'état du développement de son hôte. Les ecdystéroïdes de l'hôte permettent au parasitoïde d'effectuer sa première mue (Lawrence, 1986). Au stade L3, la larve du parasitoïde devient ectoparasite et consomme complètement son hôte (Brown and Friedlander, 1995). *A. quadridentata* tisse un cocon fin de couleur blanche brillant à l'intérieur de celui de son hôte (Clausen, 1940; Diaconu *et al.*, 2000). Le parasitoïde se développe en nymphe puis émerge en adulte (Figure 13).

La larve du premier stade d'*A. quadridentata* entre en diapause avec son hôte. La larve L1 du parasitoïde déclenche son développement en réponse au 20-hydroxyecdysone, une hormone de mue secrétée par le carpocapse à la fin de la diapause. Dans les larves diapausantes saines cette hormone vise à renouveler la spermatogenèse dans les testicules (Friedländer, 1989; Brown *et al.*, 1990). Ainsi il est important pour *A. quadridentata* de castrer son hôte pour pouvoir bien détecter les changements hormonaux et par conséquence le développement de l'hôte afin de régler son propre développement (Brown and Friedlander, 1995).

Ascogaster quadridentata

Figure 7: *A. quadridentata* adulte mâle (12-266, cliché M. Maalouly).

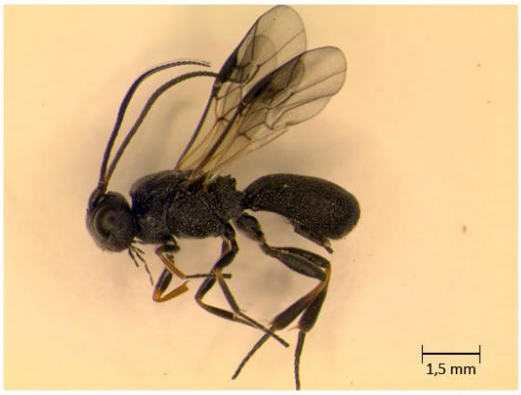

Figure 8: *A. quadridentata* adulte femelle (12-484, cliché M. Maalouly).

Figure 9: *A. quadridentata* adulte mâle avec son cocon (12-483, cliché M. Maalouly).

Figure 10: *A. quadridentata* femelle (à gauche, 12-484) et mâle (à droite, 12-266) (cliché M. Maalouly).

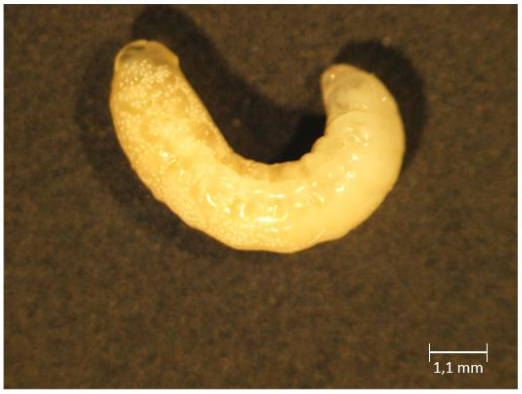

Figure 11: Larve d'*A. quadridentata* mature (cliché M. Maalouly).

Figure 12: Larve saine de carpocapse (en bas) et larve parasitée par *A. quadridentata* (en haut) (cliché M. Maalouly).

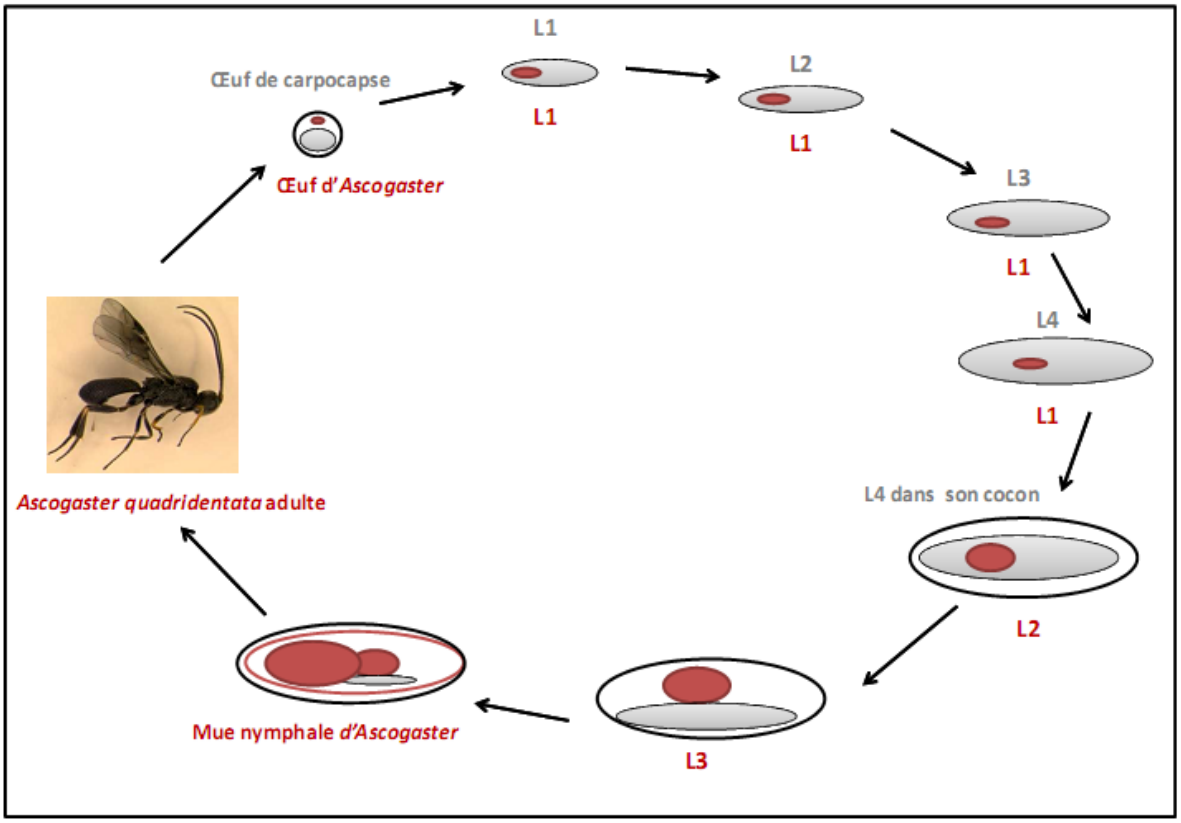

Figure 13 : Cycle de développement d'*Ascogaster quadridentata* (en rouge) sur le carpocapse des pommes (en gris).

2.2. *Perilampus tristis*

Perilampus tristis Mayr (Figures 14 et 15) est un hyménoptère chalcidien de la famille des Perilampidae qui mesure 3 à 4 mm. C'est un hyperparasitoïde (endoparasitoïde secondaire) qui se reconnaît à son thorax très puissant (Athanassov *et al.*, 1997). Les adultes sont souvent trouvés sur les fleurs particulièrement de la famille des Compositae ou sur des colonies de pucerons où ils se nourrissent du miellat. Les femelles de *Perilampus* sont dépourvues d'œufs à l'émergence (Clausen, 1940).

C'est un parasitoïde généraliste qui attaque certains lépidoptères de la famille des Tortricidae comme *Cydia pomonella* et *Rhyacionia buoliana* à travers leurs parasites primaires, essentiellement les Braconidae (Bouček, 1977). Toutefois, *P. tristis* se développe aussi sur des parasites primaires de la famille des Ichneumonidae (Clausen, 1940). Dans nos

échantillonnages, les individus de *P. tristis* se développaient sur les parasitoïdes primaires du carpocapse *Pristomerus vulnerator* (Figure 16) et *Ascogaster quadridentata* (Figure 17).

Perilampus tristis

Figure 14: *P. tristis* adulte mâle (12-259, cliché M. Maalouly).

Figure 15: *P. tristis* adulte femelle (12-785, cliché M. Maalouly).

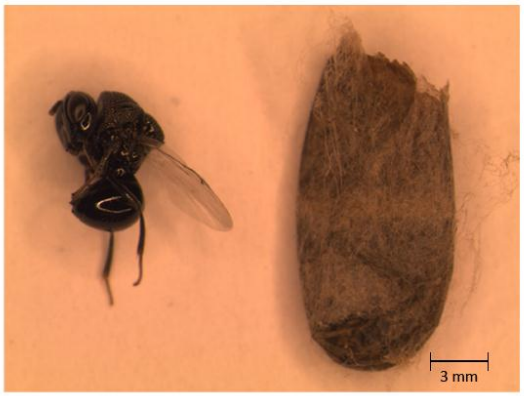

Figure 16: *P. tristis* se développant sur *P. vulnerator* (cocon) (11-1-908, cliché M. Maalouly).

Figure 17: *P. tristis* se développant sur *A. quadridentata* (cocon) (12-259, cliché M. Maalouly).

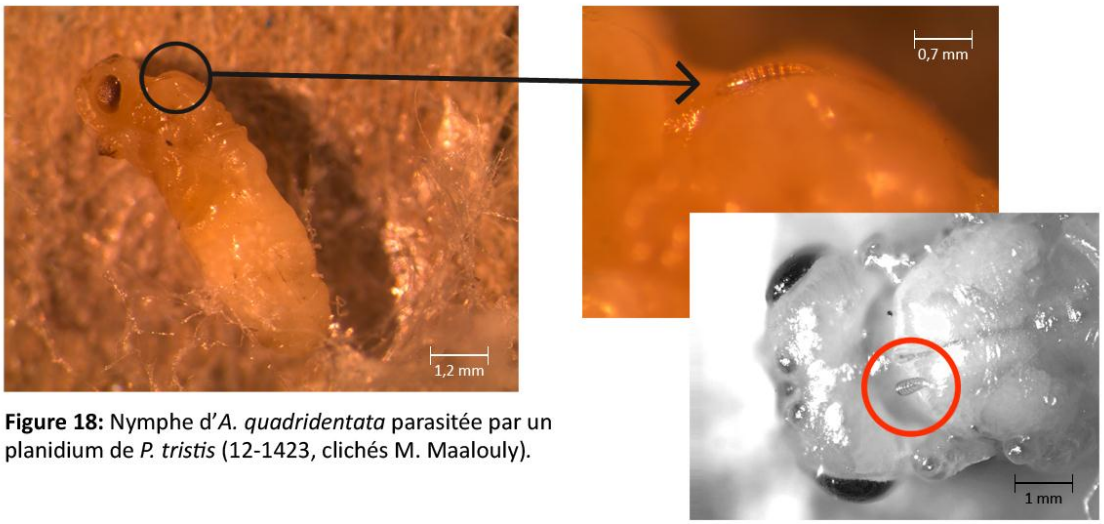

Figure 18: Nymphe d'*A. quadridentata* parasitée par un planidium de *P. tristis* (12-1423, clichés M. Maalouly).

Les femelles du genre *Perilampus* ne pondent pas leurs œufs dans ou sur leur hôte ni même dans la chenille parasitée par leur hôte. Il est probable que *Perilampus* pond ses œufs à proximité des œufs pondus par le papillon (Smith, 1912). Le développement des *Perilampus* est caractérisé par la présence d'un planidium, type spécialisé de larve de premier stadecaractéristique de certains hyménoptères et diptères parasitoïdes. Les planidia sont aplatis, sclérotisés et très mobils pour pouvoir atteindre leur hôte. Dans la famille des Perilampidae et des Eucharitidae le planidium reste dans une position debout en attendant passivement le passage d'un hôte potentiel (Figure 19) (Clausen, 1940). Cette phase de recherche d'un hôte est caractérisée par une grande mortalité des planidia (Smith, 1912).

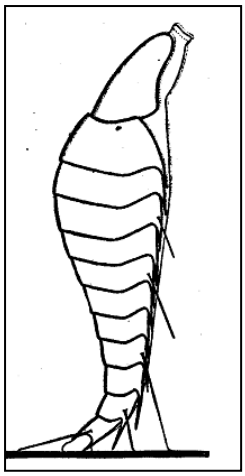

Figure 19: Planidium de *Schizaspidia manipurensis* Clausen (famille des Eucharidae), en position debout attendant le passage de son hôte (Clausen, 1928).

L'œuf de *P. tristis* est cylindrique et mesure 2 mm de long et 0.07 mm de large. Il a un pédicelle court à l'une de ses extrémités. Clausen (1940) a décrit le développement de *P. tristis* sur les larves diapausantes de *Rhyacionia buoliana*, la tordeuse des pousses de pin. Le planidium de *P. tristis* entre dans la jeune chenille en été et se trouve en hiver dans le corps gras ou les glandes salivaires de la larve. Quand la diapause est levée au printemps, le planidium s'attache extérieurement à la jeune larve du parasitoïde primaire puis y pénètre en attendant le stade nymphe de son hôte. Quand le stade nymphe est atteint par le parasitoïde primaire le planidium devient ectoparasitique (Figure 18). *P. tristis* complète son développement en un mois. Les changements histolytiques lors de la nymphose de l'hôte sont responsables du déclenchement du nourrissage rapide et du développement de *Perilampus* (Clausen, 1940). *P. tristis* passe par 4 stades larvaires avant d'atteindre l'âge

adulte (Figure 9) (Bergold and Ripper, 1937). Suite à nos observations au laboratoire des larves de carpocapse diapausantes et de saison parasitées par *P. tristis* le développement de ce parasitoïde sur le carpocapse correspondrait à la description faite par Clausen (1940).

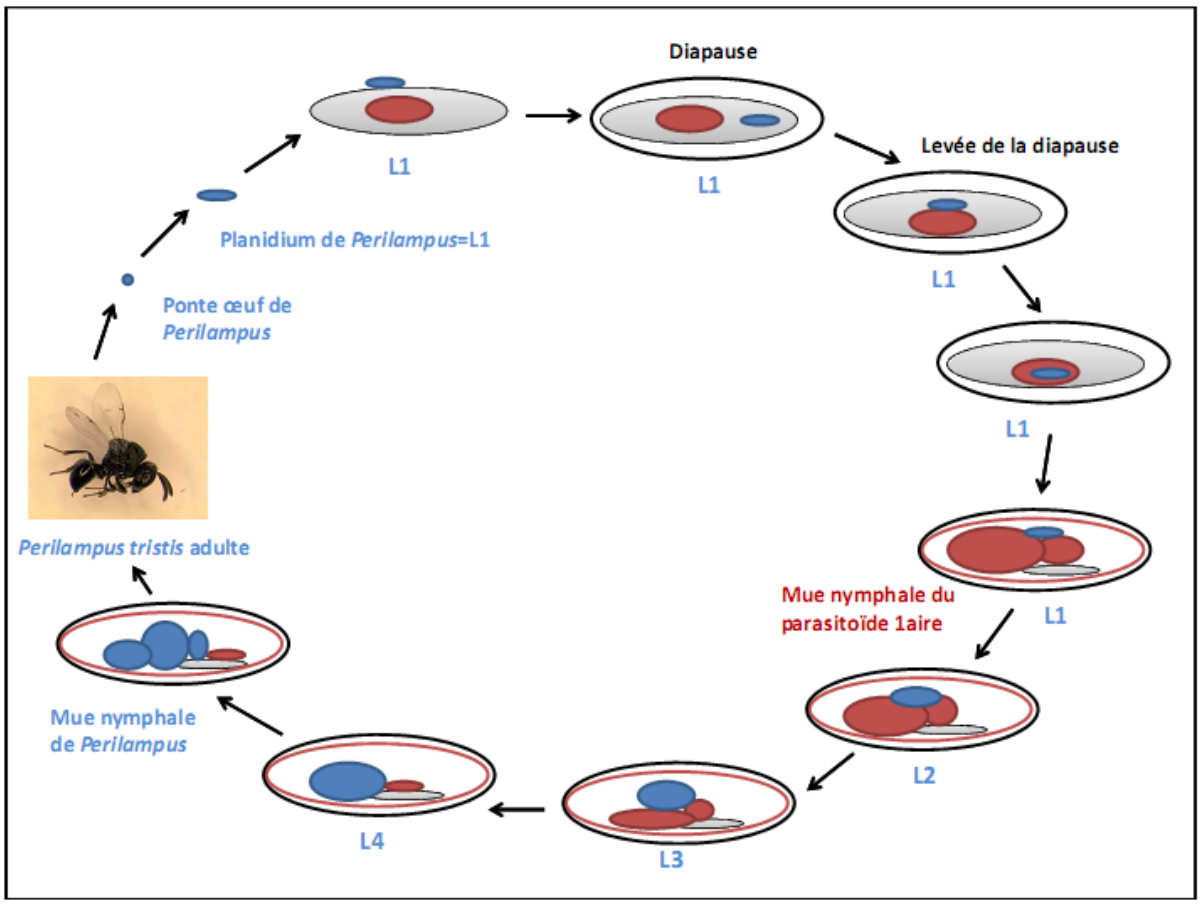

Figure 20: Cycle de développement de *Perilampus tristis* (en bleu) sur son hôte, parasitoïde primaire (en rouge) d'une chenille de lépidoptère diapausante (en gris). Le stade larvaire de la chenille n'est pas précisé.

2.3. *Pristomerus vulnerator*

Pristomerus vulnerator Panzer (Figures 21 et 22) est un parasitoïde de la famille des Ichneumonidae (sous-famille des Cremastinae) d'environ 9 mm de long (Athanassov *et al.*, 1997). C'est un parasitoïde généraliste qui se développe sur plusieurs espèces de

lépidoptères. *P. vulnerator* est un endoparasitoïde primaire du carpocapse du pommier (Rosenberg, 1934).

Les femelles pondent un œuf dans les jeunes larves de carpocapse directement après la pénétration de cette dernière dans le fruit. La femelle parasitoïde insère son ovipositeur dans plusieurs directions dans la chair du fruit. L'œuf de *P. vulnerator* mesure 0.24 à 0.28 mm de long et 0.05 à 0.06 mm de large. La larve du parasitoïde reste dans la larve de son hôte durant la période où le carpocapse est dans le fruit (Rosenberg, 1934). Quand la chenille quitte la pomme et tisse son cocon pour former sa nymphe la larve mature du parasitoïde émerge de son hôte. Elle tisse un cocon dur allongé présentant différents degrés de pigmentation où elle se développe jusqu'au stade adulte (Diaconu *et al.*, 2000). Le cocon de *P. vulnerator* est de couleur gris-brun (Geiger, 1957). Sur les tordeuses des pousses du pin les espèces du genre *Pristomerus* sont cléptoparasitiques. Elles parasitent préférentiellement les larves déjà parasitées par d'autres espèces de parasitoïdes (Schröder, 1974). Dans la communauté des parasitoïdes du carpocapse il n'est pas connu si *P. vulnerator* se comporte comme un cléptoparasitoïde (Mills, 2005).

2.4. *Bassus rufipes*

Bassus rufipes ou *Microdus rufipes* Nees (Figures 23 et 24) est un braconide qui se distingue par ses ailes sombres, son abdomen rougeâtre et par le long ovipositeur des femelles. C'est un endoparasitoïde larvaire (Athanassov *et al.*, 1997). Il est généraliste mais parasite plusieurs espèces de lépidoptères de la famille des Tortricidae. Plusieurs aspects de la biologie et de l'écologie de ce parasitoïde ressemblent à ceux d'*Ascogaster quadridentata* (Diaconu et al., 2000).

2.5. *Hyssopus pallidus*

Hyssopus pallidus Askew (Figures 25 et 26) est un ectoparasitoïde larvaire grégaire de la famille des Eulophidae. Il est natif d'Europe et est spécialisé sur *Cydia pomonella* et *Cydia molesta* dans cette région (Askew, 1964; Zaviezo and Mills, 1999; Tschudi-Rein *et al.*, 2004).

Les femelles d'*H. pallidus* entrent à travers le tunnel de nourrissage creusé par la larve de carpocapse ou à travers le calyx de la pomme. Une fois la larve trouvée, la femelle du parasitoïde la paralyse et pond plusieurs œufs dessus (Dorn and Beckage, 2007).

2.6. *Dibrachys cavus*

Dibrachys cavus Walker (Figures 27, 28 et 29) est un hyménoptère chalcidien de la famille des Pteromalidae d'environ 2 mm. C'est un ectoparasitoïde grégaire (Athanassov *et al.*, 1997). C'est généraliste qui peut se développer comme parasitoïde primaire du carpocapse ou comme hyperparasitoïde. Il attaque les cocons du carpocapse parasitant les chenilles ou les chrysalides (Rosenberg, 1934; Geiger, 1957).

2.7. *Trichomma enecator*

Trichomma enecator Rossi (Figure 30) est un hyménoptère de la famille des Ichneumonidae de 12 mm de long. Il est caractérisé par un corps très mince (Athanassov *et al.*, 1997). C'est un généraliste qui attaque plusieurs espèces de Tortricidae. Il parasite les jeunes larves de carpocapse suite à leur pénétration dans le fruit comme dans le cas de *Pristomerus vulnerator*. La larve se développe en endoparasitoïde et les adultes émergent des chrysalides du carpocapse en les perforant (Rosenberg, 1934; Athanassov *et al.*, 1997). *T. enecator* s'avère plus abondant sur le carpocapse sur noyer que sur le pommier (Geiger, 1957).

Nous avons analysé les variations du sexe ratio et nous avons cherché la présence de particules symbiotiques, les polyDNAvirus, chez les parasitoïdes du carpocapse. Les résultats de ces travaux sont présentés dans les annexes I et II.

Pristomerus vulnerator

Figure 21: *P. vulnerator* adulte mâle (12-1971, cliché M. Maalouly).

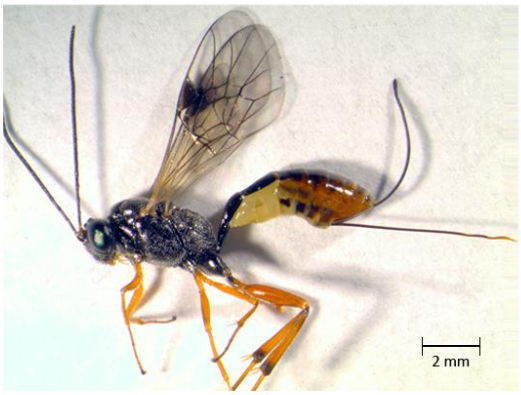

Figure 22: *P. vulnerator* adulte femelle (12-1978, cliché M. Maalouly).

Bassus rufipes

Figure 23: *B. rufipes* adulte mâle (12-799, cliché M. Maalouly).

Figure 24: *B. rufipes* adulte femelle et son cocon (12-468, cliché M. Maalouly).

Hyssopus pallidus

Figure 25: Larve mature de carpocapse parasitée par des larves d'*H. pallidus* (11-1-1376, cliché M. Maalouly).

Figure 26: *H. pallidus* adulte (11-1-1376, cliché M. Maalouly).

Dibrachys cavus

FIgure 27: Larve de carpocapse parasitée par des larves de *D. cavus* (11-3-4347, cliché M. Maalouly).

Figure 28: *D. cavus* adultes et restes de la larve de carpocapse (11-3-4347, cliché M. Maalouly).

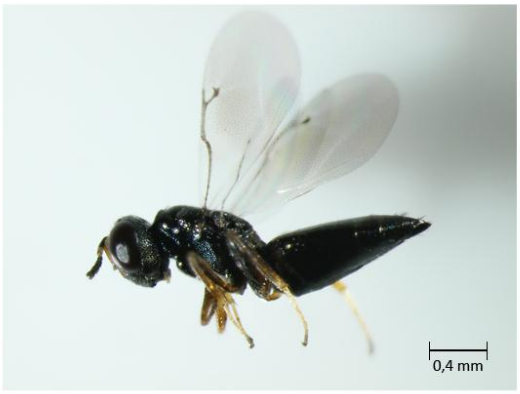

Figure 29: *D. cavus* adulte (11-3-4347, cliché M. Maalouly).

Trichomma enecator

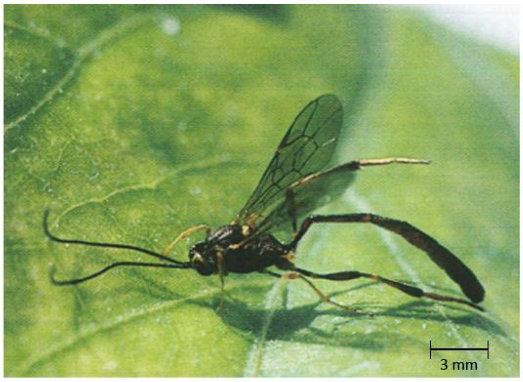

Figure 30: *T. enecator* adulte (d'après Athanassov et al, 1997).

Matériels et méthodes

1. Présentation et description des zones d'étude

Les différentes zones d'étude définies pour ce travail de thèse sont situées au Sud Est de la France. Tous les sites d'échantillonnage du carpocapse correspondaient à des vergers de pommiers. Ces zones peuvent être divisées en deux classes suivant la question abordée.

Pour l'étude des variations temporelles du parasitisme du carpocapse (Chapitre 1) nous avons étudié des vergers expérimentaux non traités situés dans deux sites différents de la vallée du Rhône (Figure 31). Ces vergers sont plantés de plusieurs variétés de pommes. Le premier site est situé dans la région d'Avignon dans une plaine agricole à 20m au dessus du niveau de la mer. Ce site englobe deux vergers proches (distance approximative de 500 m) et sera nommé 'Montfavet' (43°54'22"N, 4°53'7"E; 43°54'51"N, 4°52'56"E). Le climat est méditerranéen avec une température moyenne journalière de 15,37 ± 5,74 °C, des précipitations moyennes de 1,76 ± 3,00 mm et une vitesse moyenne du vent de 2,02 ± 1,12 m/s (en 2011). Le deuxième site est situé dans la région de Valence à une altitude de 190 m au dessus du niveau de la mer. Il englobe un seul verger et sera nommé 'Gotheron' (44°58'21"N, 4°55'39"E) vu qu'il est situé sur le domaine expérimental de l'UERI INRA Gotheron. En 2011, la température moyenne journalière était de 13,43 ± 5,81 °C, le niveau moyen des précipitations était de 2,09 ± 3,36 mm et la vitesse moyenne journalière du vent de 2,65 ± 0,99 m/s.

Ces deux sites diffèrent par le nombre de générations annuelles du carpocapse. Les populations de carpocapse sont multivoltines avec trois générations par année à Montfavet. A Gotheron, les populations de carpocapse complètent deux générations par an.

Figure 31 : Localisation géographique des sites d'étude. Le site de Montfavet est en rouge et le site de Gotheron en vert.

Pour l'étude des variations spatiales du parasitisme du carpocapse (Chapitre 2) la zone d'étude définie est un bassin de production de pommes d'environ 80 Km^2 situé dans la basse vallée de la Durance (coordonnées géographiques en WGS84 : de 43°47'11''N à 43°51'10''N et de 4°51'29''E à 4°59'25''E). La majorité des vergers de pommes sont conduits en agriculture conventionnelle (environ 90 %). Le reste de la production de pomme provient de l'agriculture biologique (environ 2 %). A cause des faibles prix actuels des fruits certains vergers sont abandonnés chaque année et peuvent rester non cultivés pendant plusieurs années avant d'être déracinés (environ 8 %). Le paysage est caractérisé par un dense réseau de haies brise-vents essentiellement monospécifiques constituées par des cyprès ou des peupliers. Elles protègent les vergers du mistral, vent qui souffle dans la vallée du Rhône du Nord au Sud. Il est caractérisé par la présence de haies composites présentant une grande diversité florale (arbres de *Prunus* et de *Cornus*). De plus, de nombreux canaux d'irrigation traversent cette zone.

Tous les vergers, les haies et les bois dans cette zone ont été cartographiés avec ArcView (Version 9.1, ESRI) à partir d'une photo aérienne de l'IGN prise en 2004 (BD ORTHO, IGN, 2004-pixel size: 0,5 m) et des observations de terrain. Les canaux ont été récupérés de cartes IGN (BD CARTO IGN) (Figure 32). Les pratiques agronomiques ont été enregistrées par année

pour un ensemble de vergers échantillonnés à partir d'entretiens avec les agriculteurs et du recueil de leurs calendriers de traitements.

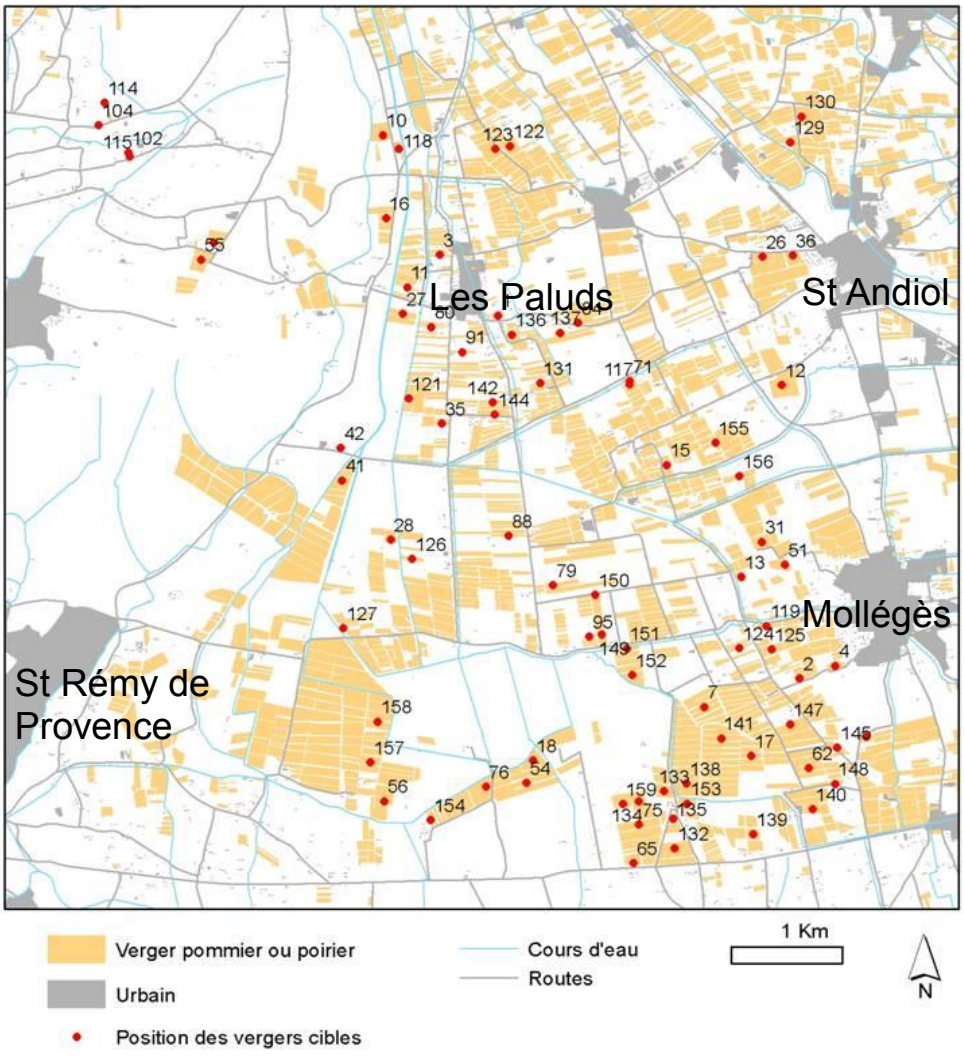

Figure 32: Cartographie de la zone de la basse vallée de la Durance effectuée sous ArcView (source: Equipe EPI, INRA)

2. Méthodes d'échantillonnage et identification des parasitoïdes

Nous avons échantillonné des jeunes larves et des larves âgées de carpocapse pour étudier les variations temporelles du parasitisme de ce ravageur. Pour l'étude des variations spatiales du parasitisme nous avons échantillonné des larves diapausantes de carpocapse.

Les jeunes larves de carpocapse attaquent les fruits. Ainsi, nous avons échantillonné les jeunes larves dans les fruits. Les fruits attaqués (présentant des traces de piqures) sont collectés et disséqués pour chercher la jeune larve de carpocapse à l'intérieur (Figure 33).

Les larves âgées quittent le fruit et descendent le long du tronc de l'arbre pour tisser leur cocon sur l'arbre ou dans le sol afin d'émerger durant la saison ou pour diapauser. L'échantillonnage des larves âgées et des larves diapausantes s'est fait en utilisant des bandes en carton ondulé de 10 cm de large enroulées autour des troncs des arbres (Figure 34).

Figure 33: Pomme coupées pour chercher la jeune larve de carpocapse (source : http://extension.usu.edu/boxelder/htm/fruit/insectspray/codling)

Figure 34: Bande-piège autour des troncs des arbres pour capturer les larves âgées de carpocapse (source: Equipe EPI, INRA)

Nous avons suivi toutes les larves échantillonnées jusqu'à l'émergence. Les larves collectées dans les fruits ont été placées dans des piluliers individuels avec un milieu nutritif à base de soja (Stonefly Heliothis diet, Ward's, NY) préparé dans une solution aqueuse avec 0,2 % d'acide acétique. Les larves collectées dans les bandes-piège ont été placées dans des piluliers individuels avec un morceau de carton ondulé. Toutes les larves collectées en saison ont été placées au laboratoire à 24°C avec un éclairage naturel. Les larves diapausantes ont

été stockées dans un insectarium extérieur durant l'hiver. Les émergences des adultes de carpocapse ou de parasitoïdes ont été suivies.

Nous avons identifié et sexé les adultes parasitoïdes suivant de clés d'identification basées sur des critères morphologiques à l'aide d'un microscope binoculaire (Athanassov *et al*., 1997).

Il est important de noter que les bandes-pièges pourraient servir de refuge pour les larves de carpocapse les protégeant des ectoparasitoïdes. Cette méthode ne serait pas adaptée pour l'échantillonnage de parasitoïdes de cocons ou de nymphes.

L'ensemble des données pour l'étude des variations spatiales du parasitisme (provenant de la zone d'étude dans la basse vallée de la Durance) a été récolté de façon collective avec la contribution des membres de l'équipe EPI. C'est un grand travail de planification, de collecte et de dépouillement sur plusieurs années.

3. Infestation d'œufs de carpocapse par *Ascogaster quadridentata*

Nous avons exposé des œufs de carpocapse provenant de souches élevées au laboratoire au parasitisme d'*A. quadridentata* (parasitoïde ovo-larvaire) provenant du terrain afin d'étudier les interactions hôte-parasitoïde.

Nous avons ainsi exposé des œufs de carpocapse provenant de deux souches différentes (une souche résistante au virus de la granulose et une souche sensible) au parasitisme et nous avons élevé les larves jusqu'à l'émergence des adultes pour déterminer s'il existe un coût à la résistance pour le carpocapse. Nous avons aussi exposé des œufs de carpocapse (souche sensible) au parasitisme et nous avons prélevé des larves à différents stades de développement pour faire de la détection de parasitisme par des outils moléculaires que nous avons développé (PCR-RFLP et marqueurs spécifiques).

Chapitre 1
Dynamiques temporelles des complexes de parasitoïdes du carpocapse du pommier

Dans ce chapitre nous cherchons à connaître les variations temporelles du parasitisme du carpocapse du pommier au cours d'une saison de production.

Nous avons voulu déterminer la composition de la communauté des parasitoïdes qui attaque les différentes générations du carpocapse pour comprendre comment cette composition changeait au cours de la saison. Nous avons analysé la phénologie des parasitoïdes du carpocapse afin de déterminer leur degré de synchronisation avec leur hôte et voir si cette synchronisation variait au cours de la saison. Finalement, nous avons testé les variations temporelles du taux de parasitisme du carpocapse.

Pour ce faire, nous avons analysé les dynamiques temporelles de la communauté de parasitoïdes du carpocapse en 2011 dans deux sites expérimentaux au Sud Est de la France différant par le nombre de générations annuelles de carpocapse. Il s'agit de vergers de pommiers non traités. Le parasitisme est estimé par échantillonnage de jeunes larves collectées dans les fruits et de larves âgées dans les bandes-piège placées autour des troncs des arbres. Nous avons analysé les effets des sites d'étude, des cohortes du carpocapse, des méthodes d'échantillonnage (fruits ou bandes-piège) et de l'espèce sur la composition de la communauté de parasitoïdes, le taux de parasitisme et la phénologie des insectes (degrés jour cumulés).

Les résultats montrent que les taux de parasitisme étaient différents entre sites et entre larves jeunes et âgées. Les taux de parasitisme étaient plus élevés dans les jeunes larves que dans les larves âgées et ont globalement augmenté au cours des cohortes des larves âgées du carpocapse. La communauté de parasitoïdes est principalement composée par trois espèces: *Ascogaster quadridentata*, *Pristomerus vulnerator* et *Perilampus tristis*. Ces parasitoïdes ont été observés dans les deux sites, pour chaque cohorte de carpocapse et

dans les larves jeunes et âgées. La proportion relative de *Perilampus* (hyperparasitoïde qui attaque les deux parasitoïdes primaires *Ascogaster* et *Pristomerus*) a augmenté au cours des cohortes du carpocapse alors que la proportion d'*Ascogaster* a diminué. Les émergences des adultes d'*Ascogaster* sont de plus synchronisées avec celles des adultes carpocapses.

Ces analyses et résultats sont présentés dans un article soumis au journal *Biological control.* La suite du chapitre reprend l'intégralité du manuscrit de cet article. Une analyse de l'effet du parasitisme sur la dynamique du carpocapse est présentée dans l'annexe III. Les tests présentés ont été effectués sur les mêmes données d'échantillonnage analysées dans l'article.

Temporal dynamics of parasitoid assemblages parasitizing the codling moth

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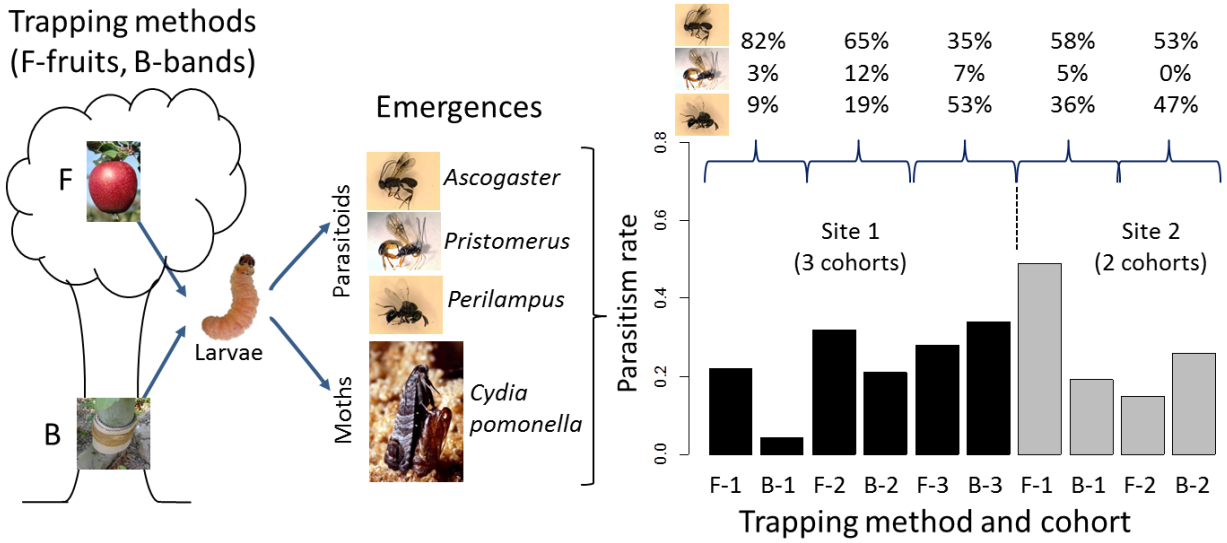
Abstract

Population dynamics of parasitoid-host interaction is primary important knowledge to develop an efficient biological control strategy of insect pests. We analyzed the seasonal dynamic of the parasitoid community of the codling moth in two sites in South-Eastern France, which differed by the number of codling moth annual generations. Parasitism was estimated by sampling both young larvae collected within apple fruits and mature larvae in band traps wrapped around the trunk of the apple trees. Parasitism rates differed between sites and between young and mature larvae. Parasitism rate were higher in young larvae (29% in average) than in the mature ones (21% in average) and globally increased along the season among cohorts of mature codling moth larvae (from 4% to 34%). The three most abundant species in the parasitoid community – *Ascogaster quadridentata, Pristomerus vulnerator* and *Perilampus tristis* – were observed at both sites, in each codling moth cohort and in both young and mature larvae. Among all the parasitoids, the proportion of *Perilampus* – an hyper-parasitoid attacking both *Ascogaster* and *Pristomerus* primary parasitoids – increased among the codling moth cohorts (from 9% to 53%) whereas the proportion of *Ascogaster* decreased (from 82% to 35%). This shed light on the importance to characterize the dynamic of the whole trophic network (including hyperparasitism) to design biological control strategies.

Keywords: *Cydia pomonella, Ascogaster,* life histories, parasitoid wasps, parasitism, *Perilampus,* phenology, *Pristomerus.*

Graphical abstract



Highlights

- We analyzed the seasonal dynamic of the parasitoid community of the codling moth in experimental apple orchards.
- Parasitism rates differed between sites and between young and mature larvae.
- The three most abundant species in the parasitoid community were Ascogaster quadridentata, Pristomerus vulnerator and Perilampus tristis.
- The proportion of Perilampus – an hyper-parasitoid attacking both Ascogaster and Pristomerus primary parasitoids – increased with time.

1. Introduction

To ensure the success of biological control programs it is important to understand the ecology of beneficial parasitoids, notably how parasitoids interact with biotic and abiotic parts in the agroecosystems as these interaction factors have huge impact on the population dynamic of their hosts (e.g. Godfray, 1994; Pearce et al, 2006; Quicke, 1997; Van Driessche and Bellows, 1996).

First, understanding the synchrony between parasitoids and their hosts is important for successful introduction, propagation and release of biological control agents (Messenger and van den Bosch, 1971). The level of phenological asynchrony between a parasitoid and its host affects the population dynamics, abundance and distribution of both species (Stenseth and Mysterud, 2002; Voigt et al, 2003). For a stable host-parasitoid interaction it is required that all the hosts within a population are not parasitized (Van Nouhuys and Lei 2004). Phenological asynchrony can create a temporal refuge for hosts when the risk of parasitism is low allowing stabilizing the interactions. The synchrony between the phenology of the parasitoids and their hosts may be modified by both climatic changes (Hance et al, 2007; Henri et al, 2012; Voigt et al, 2003) and selective pressures in the host-parasitoid interaction (Forbe et al, 2004; Sait et al, 1997). High synchronization of the phenology of the parasitoid on this of its host increases the parasitism pressure, which may drive to local extinction of both host and parasitoid populations. In case the host does not go extinct, the small size of parasitoid populations in the following generation may provoke a parasitoid population crash or local extinction followed by an outbreak of the host (Hance et al, 2007). Contrarily, phenological asynchrony may expose an insufficient number of hosts to parasitoids causing the decline of parasitoid population to extinction and host demographic outbreak (Godfray et al, 1994). As insect phenologies mainly depend on temperature, with specific levels of accumulated degree days required for reaching each developmental stage (Hodgson et al, 2011; Nylin and Gotthard, 1998), we hypothesize that both seasonal and geographical variations in temperature may largely impact the synchrony of the parasitoids with their host and consequently the level of their interaction.

Second, parasitism rates may depend on the composition of the parasitoid community. Parasitism rates can be promoted by a higher parasitoid diversity if species have additive or

synergistic interactions. Alternatively, negative interactions between parasitoids (*e.g.* hyperparasitism), may reduce the control of insect pests (Rosenheim, 1998). Parasitoids that attack their hosts at an early stage of their development access to a more abundant host resource than those attacking their host later (Chesson, 1991; Price, 1972), which may also result in exploitative competition between parasitoids (Harvey et al, 2013; Hawkins, 2000; Schoener, 1983; Teder et al, 2013;). Finally, specialist parasitoids of a given host are known to contribute more to mortality of this host than generalist parasitoids (Elzinga et al, 2007; Godfray et al, 1995; Stefanescu et al, 2012).Because parasitoids occupy a relatively high position in food chains and because of their sometimes high level of specialization they are directly sensitive to variation in the population dynamics of their host and indirectly to those of other parasitoids with which they compete (Hance et al, 2007; Stireman et al, 2005; Van Baaren et a,l 2010; Walther, 2010). In conclusion, we hypothesize that seasonal variation in parasitism rates largely depend on variation in the composition of the parasitoid community and the level of specificity of the parasitoids to their hosts.

Third, parasitoids are engaged in an obligate association with their hosts. Their fate is tied to that of their hosts, possibly under varying environmental conditions. Parasitoids perceive and respond to the physiological status of their host (Tauber et al, 1983). Many parasitoids larvae detect the fluctuations in endocrine titers in their host enabling them to assess their host's developmental stage (Brown and Friedlander, 1995). Some parasitoids regulate the induction and the termination of their diapause according to the physiology of their host (Fisher, 1971). Furthermore, many parasitoids are known to also induce behavioral and physiological changes in their host. These changes are thought to increase the parasitoid survival by decreasing the risk of predation (Adamo et al, 1997; Brodeur and Vet, 1994; Brodeur and McNeil, 1989; Godfray, 1994; Grosman et al, 2008) or winter mortality (Quan et al, 2013; Whaton, 1999). For example, the braconid *Glyptapanteles* sp. induce their host *Thyrinteina leucocerae* (Lepidoptera: Geometridae) to fight predators. After the parasitoid egress from their caterpillar host to pupate , the host stops feeding and moving, but knocks off predators with violent head-swings to protect the parasitoid cocoons until their emergence and dies before reaching adulthood (Grosman et al, 2008). These examples show how important are the changes in the physiology and the behavior of the parasitized insects in comparison with their uninfected congeners. Our hypothesis was thus that

parasitized larvae may have modified dispersal ability and/or behavior, which may affect their trapping according to the sampling methodology (*e.g.* Josso et al 2011 for an example). We also postulated that estimations of the parasitism would depend on the age of the sampled host since each species in the parasitoid community is likely to attack different instars of their host. Consequently, we assumed that the estimation of parasitism rate may highly depend on the trapping methodologies. Here, we studied the temporal dynamics of the parasitism in the codling moth (*Cydia pomonella* L., Lepidoptera, Tortricidae). The codling moth is the major insect pest in apple orchards (Barnes, 1991; Franck et al, 2007). Damages are caused by young larvae, which attack the apple fruits. At the end of their development, the mature larvae leave the fruits and migrate downwards following the tree trunk, seeking for a shelter. Depending on temperature and photoperiod conditions, the mature larvae pupate to produce adults or diapause into a cocoon. The life cycle of the codling moth varies from one to four annual generations according to geographical (latitude and altitude) and seasonal variations of the temperatures and according to the host-plants (Shel'Deshova, 1967; Barnes, 1991).

In a recent study (Maalouly et al, 2013), we observed that three parasitoid species mainly parasitize the overwintering codling moth larvae in commercial apple orchards from southeastern France: *Ascogaster quadridentata* (Braconidae), *Pristomerus vulnerator* (Ichneumonidae) and *Perilampus tristis* (Perilampidae), which was in agreement with previous observation of codling moth parasitism in Europe (Athanassov et al, 1997; Diaconu et al, 2000; Mills, 2005; Rosenberg, 1934). However, no study has reported observation on the composition of the parasitoid community parasitizing non-diapausing codling moth larvae within season so far.

The braconid *A. quadridentata* is an ovo-larval endoparasitoid specialized on tortricid moths (Athanassov et al, 1997). Females are ready for oviposition soon after emergence. Parasitized codling moth larvae are only one-third of the size of non-parasitized larvae at the maximum of their development. The *A. quadridentata* larva emerges from the mature codling moth larva and entirely consumes the body contents of its host (Clausen, 1940). The ichneumonid *P. vulnerator* is a solitary endoparasitoid. The female deposits one egg in the young host larva after it entered the fruit. The parasitoid larva remains in latent state until the codling moth larva leaves the fruit and builds shelter to pupate (Coutin, 1974). The

mature parasitoid larva leaves the host's body and weaves a hard elongated cocoon where development continues up to the adult stage (Diaconu et al, 2000). The ichneumonid *P. vulnerator* is known as a generalist parasitoid developing on several host species among the Lepidoptera (Diaconu et al, 2000; Rosenberg, 1934). The chalcidoid *P. tristis* was noted as a hyperparasitoid on the tortricids *via* their primary hosts, mainly Braconidae (Bogenshütz, 1991; Boucek, 1977). The chalcidoid female does not oviposit directly in its host or within the caterpillar host of the primary parasitoid. It might lay its eggs near the caterpillars but nothing is clearly known about how the *P. tristis* planidium reaches its caterpillar host (Smith, 1912). . Clausen (1940) described the development of *P. tristis* In *Rhyacionia buoliana*, the *P. tristis* planidium penetrates the young diapausing caterpillar and spends the winter in its fat body or its salivary glands. In spring, it enters within the body cavity of the primary host and waits that it pupates. Afterwards, *P. tristis* evolves as an ectoparasite.

Former observations about the composition of the parasitoid community and parasitism in the codling moth only concerned overwintering mature larvae. In the present study, we monitor the seasonal dynamic of parasitoid assemblages in apple orchards from southeastern France within both young and mature codling moth larvae collected with two different trapping methods in order to enhance our global understanding of the pest biocontrol. Our aims was i) to increase our knowledge of the parasitoid community attacking the codling moth during the growing season, ii) to assess the effects of trapping methods and of instars of the codling moth collected on the observed composition of the parasitoid community and the estimation of parasitism rate, and iii) to assess if interactions between parasitoid species, and particularly hyperparasitism, affect the ability of the parasitoid community to control the codling moth.

2. Materials and methods

2.1. Study sites

The study was carried out in experimental orchards planted with several apple varieties situated in two different sites along the Rhône valley, southeastern France.

The first site was located in the region of Avignon in an agricultural plain at an altitude of approximately 20 m above sea level. This study site encompassed two orchards, 950 m away

and it is hereafter referred to as “Montfavet” (43°54'22"N, 4°53'7"E; 43°54'51"N, 4°52'56"E). The climate is Mediterranean with monthly mean temperature of 15.4 ± sd 6.4 °C, mean precipitations of 54 ± sd 46 mm and mean wind speed of 2.0 ± sd 0.4 m/s in 2011. Three generations of codling moth per year were observed in Montfavet. First generation larvae were defined as the progeny of adult codling moths that developed from the diapausing larvae of the previous year. Fifth instar larvae enter in diapause either at the second or the third generation.

The second site was situated in the region of Valence, at an altitude of 190 m above sea level. It encompassed a single orchard hereafter referred to as “Gotheron” (44°58'21"N, 4°55'39"E). In Gotheron, codling moth populations complete only two generations a year. The climate was about 2°C colder, 10-30 mm wetter and 0.5 m/s windier at Gotheron than at Montfavet.

The first codling moth generation was treated with granulovirus once in one of experimental orchards at the Montfavet site. No insecticide was used in the other apple orchards. The fruit load per tree was estimated at the beginning of each codling moth generation in each study site on all the sampled trees.

2.2. Sampling of codling moth larvae

2.2.1. Sampling of young and mature codling moth larvae

Codling moth larvae were sampled in 2011 from mid-May until the end of October (Figure 1). In each studied site a set of trees was selected to sample young larvae in apple fruits while another set was selected to collect mature larvae in band traps. The sets of trees were interchanged at each codling moth cohort. Codling moth captures were performed for each set on 38 and 9 trees at Montfavet and Gotheron respectively. For each sampling date, all the attacked fruits that we observed in a tree were collected and dissected to search for a young codling moth larva inside. A total of 1879 attacked apple fruits were collected in Montfavet and 982 fruits in Gotheron. Mature larvae leave the fruits and follow down the tree trunk to spin their cocoons on the tree or in the ground to emerge during the growing season or to diapause. Mature larvae were sampled using 10-cm-wide corrugated cardboard band traps wrapped around tree trunks, 20 cm above the ground.

2.2.2. Sampling dates and cohorts of codling moths larvae

The temporal dynamics of the different parasitoid species within the communities may be different and parasitoid community composition may vary over time. We thus adapted our sampling dates along the growing season to match the phenology of the codling moth (*i.e.* the target host) and thereafter grouped our samples in cohorts of larvae likely to have been parasitized at approximately the same dates.

For both sites, sampling dates were determined using a phenological model providing the daily proportion of each codling moth instars according to accumulated degree days (Boivin et al, 2005). The model was originally developed based on data from the study area and is now used locally for providing advice to apple growers. It was run on the CTIFL web platform INOKI® (http://www.fruits-et-legumes.net/Inoki). Decision rules were as follows for each codling moth generation: young larvae were sampled in fruits when the model predicted that 50% of the codling moth eggs had hatched until it predicted that 100% of eggs had hatched; mature larvae were sampled in band traps when the first adult codling moth began to emerge until 100% of the adults of that generation had emerged. Resulting sampling dates are provided in Figure 1.1.

At some sampling dates, we may have captured, with a single trapping method, larvae from two different codling moth generations because of generation overlap and it appeared difficult to group larvae per generation. To still keep track of the temporal dynamic of sampling and also have sufficient sample sizes, we thus grouped larvae in three cohorts at Montfavet and two cohorts at Gotheron, each cohort corresponding only approximately to larvae from a single codling moth generation. Individuals were classified in cohorts as follows. Larvae from Gotheron were classified in two cohorts depending on whether they entered diapause or not. Indeed, generations have little overlap and diapause induction largely depends on the photoperiod incurred by first instar larvae (Riedl and Croft, 1978) thus informing on the period when larvae were parasitized. At Montfavet, the 2^{nd} and 3^{rd} generation overlapped largely and part of the 2^{nd} generation larvae enter diapause. Classification of larvae from Montfavet in cohorts was thus based strictly on sampling periods. We then used the phenological model to estimate risks of missing some larvae with

our sampling strategy and risks of miss-classification of collected larvae. Details of calculations of sampling errors are provided in Supplementary material (Table 1.S1).

2.3. Monitoring of codling moth and parasitoid emergences

2.3.1. Rearing of codling moth larvae

Each collected larva was individually monitored until adult emergence. Larvae collected from fruits were placed in individual vials with nutritive soybean instant diet (Stonefly Heliothis diet, Ward's, NY) prepared in aqueous solution with 0.2 % acetic acid. Larvae collected from band traps were stored in individual vials with a piece of cardboard. All the collected larvae were stored at 24 °C with natural photoperiod. The larvae collected in fruits that did not emerge by the beginning of September (*i.e.* potentially diapausing larvae) were stored in individual vials with a piece of cardboard and on the 9th of September 2011, all larvae that had not emerged were transferred to an outdoor insectarium for the winter season.

2.3.2. Adult emergence and calculation of accumulated degree days

Adult emergence was monitored daily from early June until early December 2011 (non-diapausing larvae) and from early April until the end of June 2012 (diapausing larvae). Accumulated degree days (ADD) were calculated at the emergence of each adult sample from daily maximum and minimum temperatures records with the single sine method (Brunner and Hoyt, 1987) and using the Statewide Integrated Pest Management Program calculator of the University of California Agriculture and Natural Resources (http://www.ipm.ucdavis.edu/WEATHER/index.html). ADD were computed separately for each emerging year using January, the 1st as the starting date year (Jones et al, 2008; Juszczak et al, 2012). The temperature and the time period during which the larvae were stored in the laboratory were considered in the ADD calculation. The lower and upper thresholds considered for the codling moth development were respectively 10 °C (Shel'Deshova, 1967) and 35°C (Howell and Neven, 2000). The same developmental constants were used for all the parasitoid species as they are not documented and likely to depend on these of their host.

2.3.3. Parasitoid identification

Emerging adults and their cocoons were kept individually in 90% ethanol. Adult parasitoids were identified at species level according to morphological diagnostic identification key criteria (Athanassov *et al.*, 1997) using a binocular microscope. Primary and secondary host species were verified by inspecting the Lepidoptera and parasitoid cocoons after parasitoid emergences (Diaconu et al, 2000).

2.3.4. Statistical analyses

Factors affecting the trapping dynamics of the codling moth were assessed with a linear model on the log transformed abundance of the codling moth larvae per tree. The effects of the study site (Montfavet or Gotheron), the codling moth cohort, the sampling method (in fruits or in band traps) and the tree fruit load were analyzed.

Comparisons of the composition of the parasitoid community between the two study sites, generations, trapping methods, diapausing and non diapausing larvae, and the secondary host preference of the hyperparasitoid were conducted using Chi-square tests. P-values were calculated using Monte Carlo simulations to account for small sample sizes.

The phenologies of the codling moth and of its parasitoids were compared by analyzing variation in accumulated degree days at adult emergence as function of the study site, the insect species that emerged and their interaction. Analyses were performed using linear models on the log transformed accumulated degree days either for each generation separately or for diapausing larvae. Since the composition of the parasitoid community observed on diapausing larvae differed between Montfavet and Gotheron only species that were common to the two sites were included for this analysis.

Finally, factors affecting the parasitism rate in Montfavet and Gotheron were assessed using generalized linear models with a binomial variable that took the value of 1 if the larva was parasitized and 0 otherwise and a logit link function. The effects of the codling moth cohort, of the sampling method and of their interaction were analyzed using likelihood ratio chi-square tests (LR Chi^2) of model comparisons. Codling moth abundances in band traps at tree level were used as a covariate.

The statistical analyses were performed using the lme4 R package (R 2.15.1, R Core development Team).

3. Results

3.1. Definition of the codling moth cohorts

At Gotheron, sampling began in mid-June and lasted until mid-November 2011 (Table 1.1 and Figure 1.1). Some individuals from the two cohorts were sampled at the same time in August 2011 (weeks 33-35). For that period, 11 out of the 20 codling moth adults emerging from larvae collected in fruits and 210 out of the 217 codling moth adults emerging from larvae collected in band traps had entered diapause at the larval stage and were classified in the second cohort.

At Montfavet, larvae sampling began in mid-May and lasted until the end of October 2011 (Table 1.1 and Figure 1.1). Cohorts were defined based on the sampling scheme determined by predictions of the INOKI phenological model (see materials and methods). Sampling lasted 64 days for the first codling moth cohort, 44 days for the second cohort and 49 days for the third cohort (Table 1.1). Overlap of codling moth generations caused some mismatch between cohorts and generations. This mismatch was, however, small. It mainly concerned the misclassification of the approximately 10% latest second generation larvae in the third cohort (Table 1.S1).

Study site	Trapping method	Sampling dates	Cohort	# Number of CM	# Number of parasitoids
Montfavet	Fruit	05/16/11 to 06/06/11	1	138	39
Montfavet	Fruit	07/25/11 to 08/03/11	2	115	53
Montfavet	Fruit	09/05/11 to 09/14/11	3	21	8
Montfavet	Band trap	05/30/11 to 07/18/11	1	577	27
Montfavet	Band trap	08/01/11 to 09/07/11	2	457	118
Montfavet	Band trap	09/12/11 to 10/24/11	3	129	66
Gotheron	Fruit	14/06/11 to 31/08/11	1	122	116
Gotheron	Fruit	08/16/11 to 08/31/11	2	11	2
Gotheron	Band trap	06/21/11 to 08/31/11	1	71	17
Gotheron	Band trap	08/02/11 to 10/17/11	2	297	105

Table 1.1: Sampling dates of the codling moth (CM) larvae in fruits and band traps and cohort assignation at Montfavet and Gotheron. The number of emerging moths and parasitoids in each cohort are reported.

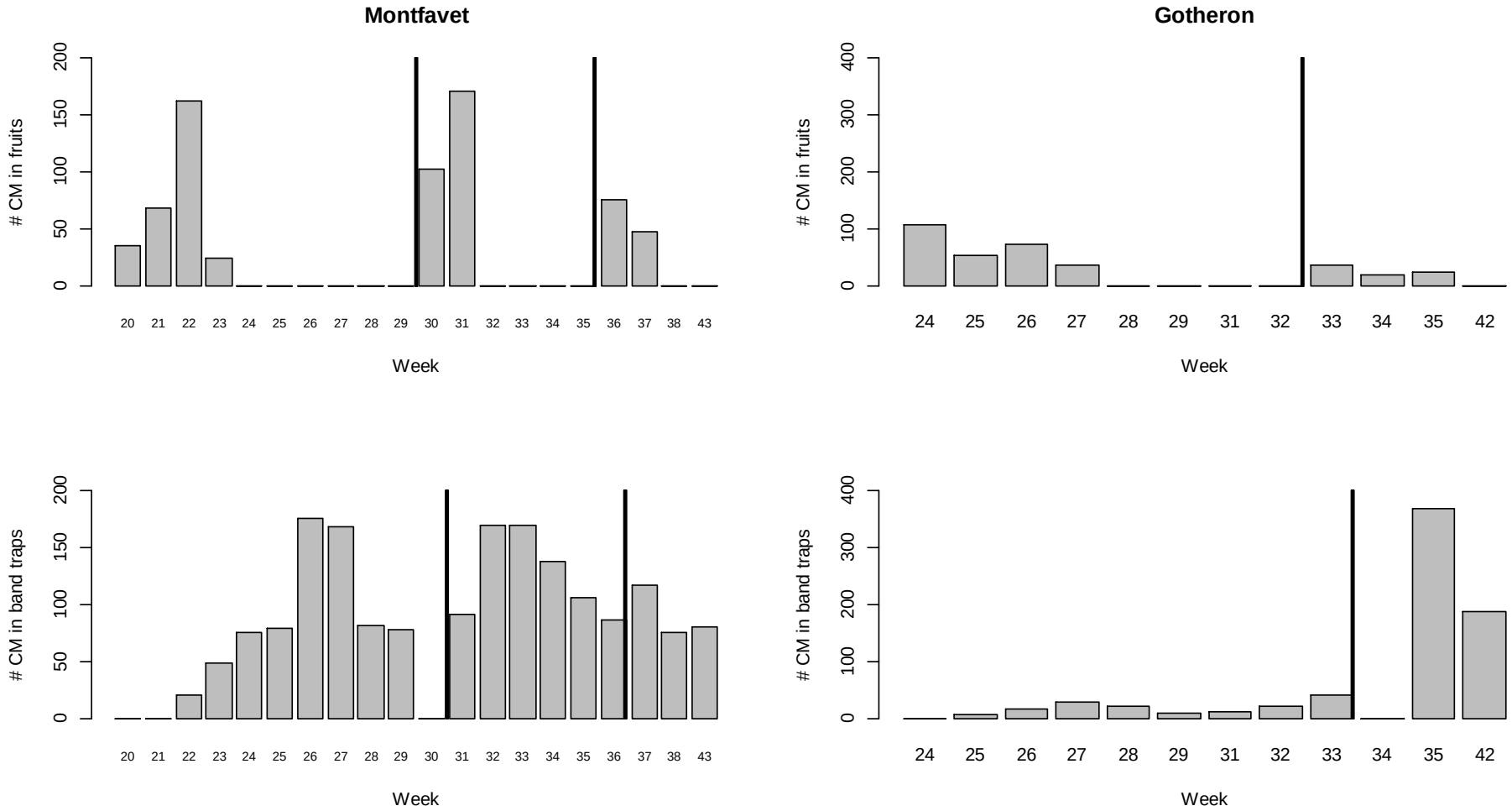


Figure 1.1: Number of collected codling moth larvae per week in fruits and band traps at Montfavet and Gotheron over the whole sampling period. The vertical lines indicate the sampling period defined according to the INOKI phenological model. Week numbers start 1st January. No sampling was performed for the weeks reported without bar plot.

3.2. Population dynamics of the codling moth and its parasitoids

3.2.1. Abundance of codling moth larvae

In total, 2449 codling moth larvae were collected at Montfavet and 1075 at Gotheron. The number of codling moths per tree ranged from 0 to 156 with a median of 5 and a mean of 14 for both study sites. In the Montfavet orchards, the fruit load ranged from 0 to 540 apples per tree with a median of 50 and a mean of 83. In the Gotheron orchard the fruit load ranged from 0 to 500 apples per tree with a median of 60 and a mean of 118. The proportions of sampled trees without apple fruit increased along the season, but did not significantly differ between sites (Chi^2=2.54, p=0.13).

The abundance of codling moth per tree was higher at Gotheron (in average 28.1) than at Montfavet (in average 10.1) ($F_{1, 249}$=18.8, p=2.1x10^{-5}). The number of larvae per tree significantly differed between cohorts ($F_{2, 249}$=11.5, p=1.6x10^{-5}). It slightly decreased along the season except at Gotheron where the numbers of larvae collected per band traps were higher at the second (in average 61.7) than at the first cohort (in average 17.7). More larvae were also captured in band traps (in average 17.9) than in fruits (in average 7.3) ($F_{1, 249}$=11.0, p=1.1x10^{-3}). As expected, the number of codling moth sampled per tree increased with fruit load (estimate= 7.25x10^{-3}, $F_{1, 249}$=95.3, p=2.2x10^{-16}).

3.2.2. Composition and structure of the parasitoid community

A total of 311 parasitoids emerged at Montfavet and 240 at Gotheron over the sampling period. 547 parasitoids were identified. Most of these parasitoids belonged to four different Hymenoptera genera: 327 *Ascogaster quadridentata* (Wesmael) (Braconidae), 9 *Bassus rufipes* (Nees) (Braconidae), 34 *Pristomerus vulnerator* (Panzer) (Ichneumonidae), and 177 *Perilampus tristis* (Mayr) (Perilampidae). Furthermore, three other species were observed once or twice: one Ichneumonidae, possibly *Mastrus rudibundus*, and one Eulophidae, possibly *Hyssopus pallidus* (Askew) at Gotheron and two Pteromalidae possibly *Dibrachys cavus* (Walker) at Montfavet. These three additional species were designed as other parasitoids and pooled in the subsequent analyses.

The parasitoid community composition (Table 1.2) significantly differed between the two study sites (Chi^2=31.3, p=5x10^{-4}). The braconid *A. quadridentata* was the most frequent taxon in both sites but *P. vulnerator* was more frequent at Montfavet than at Gotheron

while *P. tristis* showed the opposite trend. The braconid *B. rufipes* was only observed at Montfavet. Furthermore, the community composition differed between the codling moth cohorts at both Montfavet (Chi^2=60.6, p=5.00x10^{-4}) and Gotheron (Chi^2=8.3, p=0.03), but not between diapausing and non-diapausing larvae from the second cohort at Montfavet (Chi^2=1.36, p=0.71). In both sites, the proportion of *A. quadridentata* progressively decreased (from 82% to 35% overall) in parallel of an increase of the proportion of *P. tristis* (from 9% to 53% overall) and the proportion of *P. vulnerator* remained low. The braconid *B. rufipes* was sampled in low proportions in all cohorts. Finally, the parasitoid community composition did not differ between larvae collected in fruits and in band traps except for the second cohort at Montfavet (Chi^2=9.9, p=0.02).

All the *P. tristis* samples were hyperparasitoids and developed either on *A. quadridentata* or on *P. vulnerator* primary parasitoids. The proportions of *P. tristis* on *A. quadridenta* differed between Montfavet (64% of 78 individuals) and Gotheron (97% of 92 individuals) but this difference only reflected differences in the composition of the community of primary parasitoids in the two sites. The proportions of parasitized and un-parasitized *A. quadridentata* did not significantly differ between the two sites (Chi^2 homogeneity tests, p>0.05).

Study site	Cohort	Adult CM	*Ascogaster*	*Perilampus*	*Pristomerus*	*Bassus*	Others
Montfavet	1	715	0.82 (54)	0.09 (6)	0.03 (2)	0.06 (4)	0.00 (0)
Montfavet	2	572	0.66 (113)	0.20 (33)	0.12 (21)	0.02 (3)	0.00 (0)
Montfavet	3	150	0.35 (26)	0.53 (40)	0.07 (5)	0.02 (2)	0.03 (2)
Gotheron	1	193	0.58 (77)	0.36 (48)	0.05 (6)	0.00 (0)	0.01 (2)
Gotheron	2	308	0.53 (57)	0.47 (50)	0.00 (0)	0.00 (0)	0.00 (0)

Table 1.2: Proportion of each parasitoid species and numbers of emerging adults (in brackets) per cohort at Montfavet and at Gotheron.

3.2.2. Seasonal phenology of the codling moth and its parasitoids

There was no difference between sites on the emergence of codling moth and parasitoid adults of the first cohort ($F_{1, 1059}$=0.3, p=0.6). On the contrary, the degree days accumulated by diapausing larvae before emergence differed between study sites ($F_{1, 1182}$=134.8, p=2.2x10^{-16}). This difference between sites depended on species (study site x insect species interactions, $F_{2, 1147}$=3.4, p=0.03). Adult codling moth and *A. quadridentata* parasitoids from Montfavet emerged earlier than those from Gotheron but *P. tristis* adults from Montfavet and Gotheron developing on these *A. quadridentata* emerged simultaneously (Figure 1.2). The study site x insect species interaction was not significant for individuals of the first cohort ($F_{4, 1059}$=0.7, p=0.6).

Furthermore, emergence times differed between species ($F_{4, 1182}$=120.5, p=2.2x10^{-16} for diapausing larvae, $F_{4, 1059}$=12.0, p=1.6x10^{-9} for first cohort larvae and $F_{4, 206}$=12.0, p=1.6x10^{-9} for second cohort larvae). Codling moth and *A. quadridentata* adults belonging to the first cohort or emerging from diapausing larvae emerged simultaneously whereas *A. quadridentata* of the second cohort at Montfavet emerged earlier than the codling moth (Figure 1.S1). The earliest parasitoid emerging from the diapausing larvae was *P. vulnerator*. Finally, *P. tristis* adults emerged later than other species and their emergence time also partly depended on their hosts. Hyperparasitoid *P. tristis* from the codling moth diapausing cohort emerged significantly earlier when they developed on *P. vulnerator* than on *A. quadridentata* primary parasitoids (Figures 1.2 and 1.3, 1.S1).

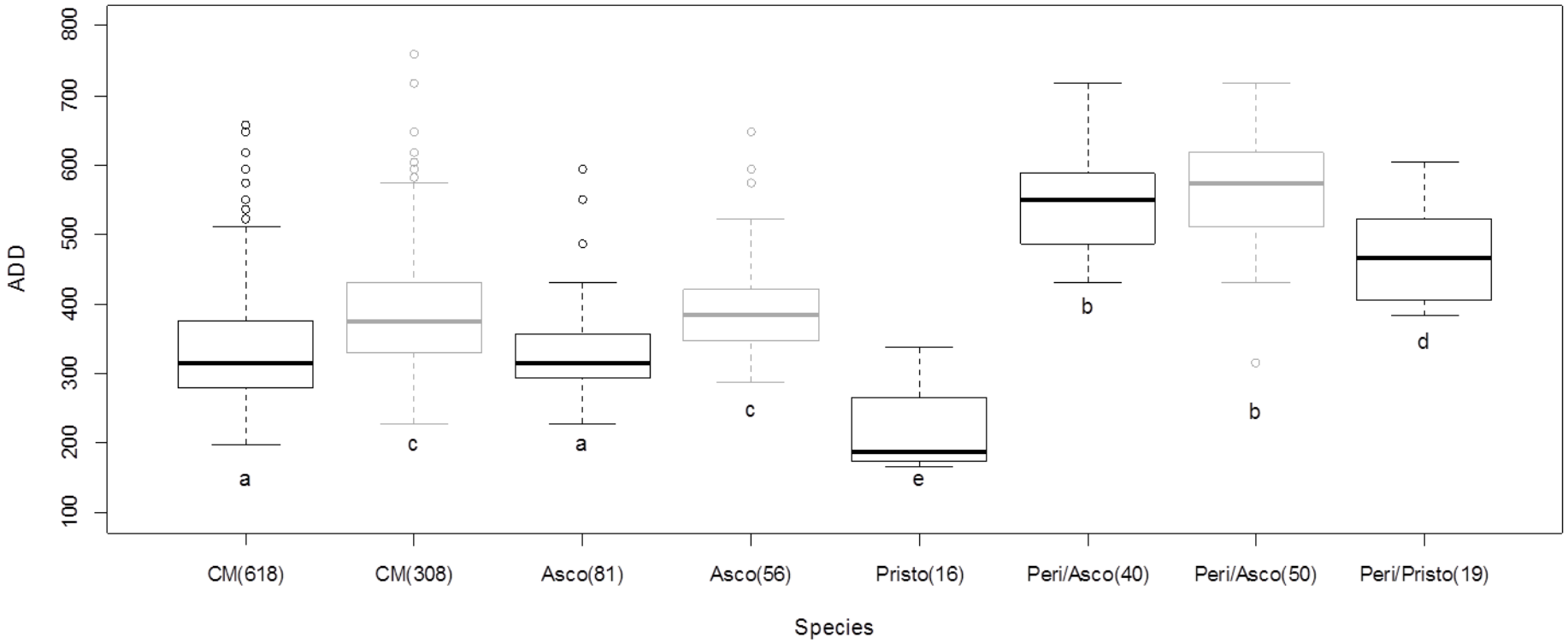


Figure 1.2: Box-plots of the accumulated degree days per species for the diapausing larvae collected at Montfavet (in black) and at Gotheron (in grey). The number of emerging adults per site and per species is referred in brackets. Different letters under box-plots indicate significant differences between species (multiple mean comparisons based on Tukey tests).

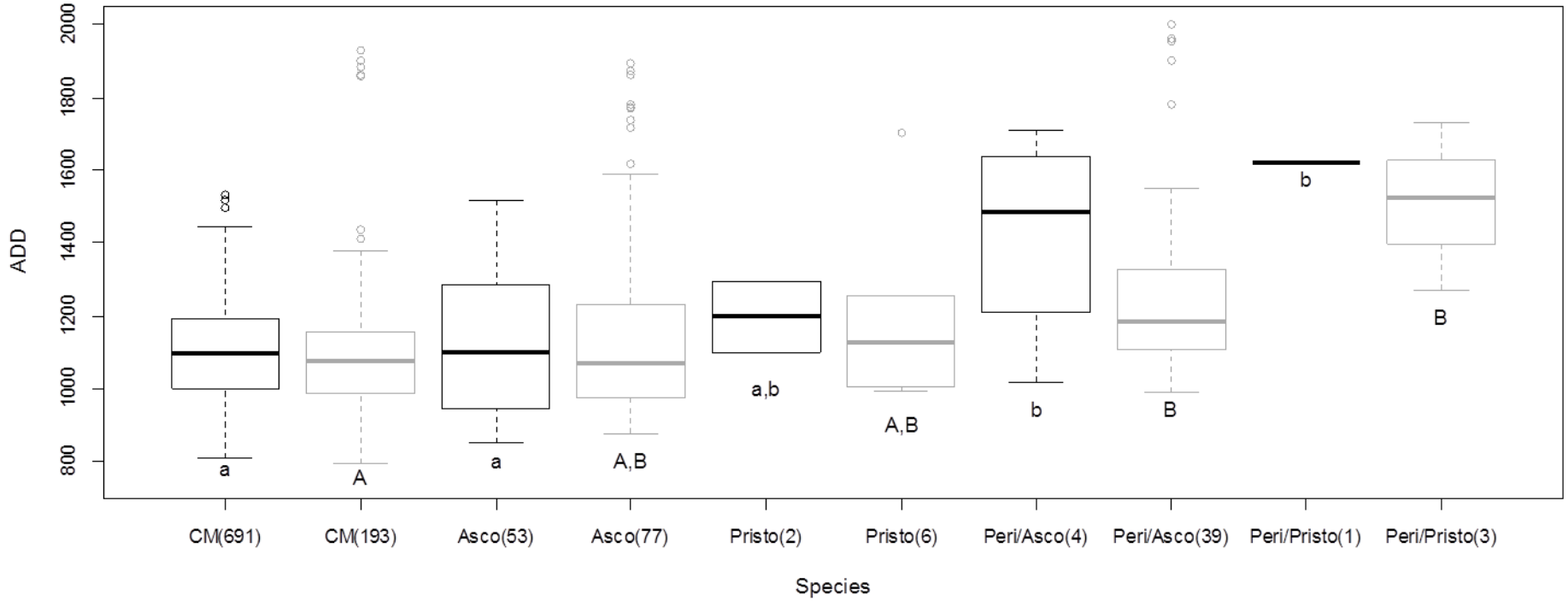


Figure 1.3: Box-plots of the accumulated degree days per species for the emerging adults of the first codling moth cohort at Montfavet (in black) and Gotheron (in grey). Numbers in brackets are those of emerging adults. Different letters under box-plots indicate significant differences between species (multiple mean comparisons based on Tukey tests). Comparisons were performed for each study site independently (lowercase: Montfavet, uppercase: Gotheron).

3.3. Codling moth parasitism rate

The overall parasitism rate reached 18% at Montfavet and 32% at Gotheron, being of 29% for larvae collected in fruits and 21% for larvae collected in bad traps. It significantly increased over the cohorts at Montfavet while it decreased at Gotheron (LR Chi^2=52.9, df=2, p=3.2x10^{-12}, at Montfavet, LR Chi^2=4.0, df=1, p=4.5x10^{-2}at Gotheron). The parasitism rate was different in fruits and band traps at Montfavet (LR Chi^2=10.9, df=1, p=9.5x10^{-4}) but not at Gotheron (LR Chi^2=1.7, df=1, p=0.2). The interaction between trapping method (fruits or band traps) and generations was significant at both Montfavet and Gotheron (LR Chi^2=23.2, df=2, p=9.0x$10^{-6}$and LR Chi^2=4.1, df=1, p=4.2x10^{-2} respectively). The parasitism rate was higher in fruits than band traps for the first and second cohorts at Montfavet and the first cohort at Gotheron. The parasitism rate in fruits and band traps did not differ for the last diapausing cohorts (second and third cohort respectively) at Gotheron and Montfavet (Figure 4). At Gotheron, parasitism rates were significantly higher for higher density of codling moth per tree (LR Chi^2=10.9, df=1, p=9.7x10^{-4}). However, the density of codling moths per tree had no significant effect on the parasitism rate at Montfavet (LR Chi^2=1.9, df=1, p=0.2).

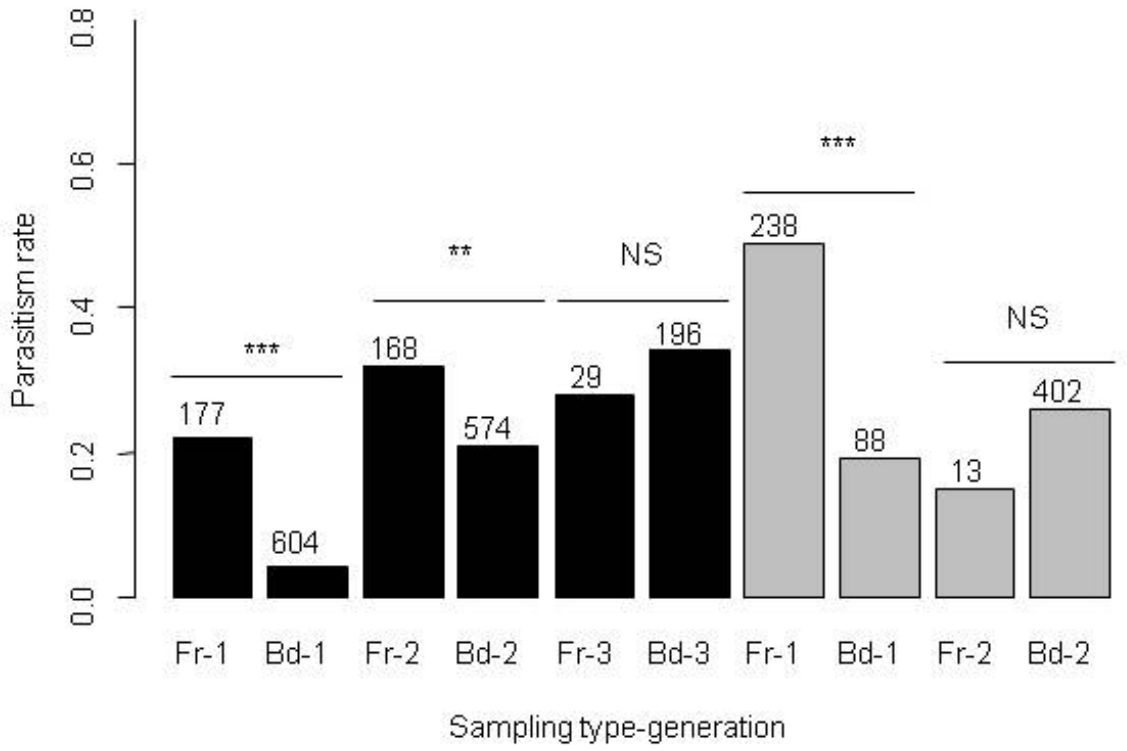


Figure 1.4: Parasitism rate at Montfavet (Black) and Gotheron (Grey) according to the codling moth cohorts (1 to 3) in fruits (Fr) and band traps (Bd). The number of emerging adults (moths and parasitoids) is represented above each bar. Statistical difference between fruits and band traps for a given cohort at each study site are indicated as follow: NS: $p>0.05$, **: $p<0.01$, ***: $p<0.001$.

4. Discussion

We determined the seasonal variations in the parasitoid community of the codling moth, the species phenology and parasitism rates in two study sites in southeastern France using two trapping methods. First, estimations of parasitism rates and community composition differed partly depending on whether they were assessed by collecting fruits or by setting band traps. Second, we determined the phenology and composition of the parasitoid community attacking the codling moth during the growing season at each site, which

differed by the number of codling moth generations. Third we showed that hyperparasitism probably affects the ability of primary parasitoids to durably control the codling moth.

The parasitism rate in the two experimental study sites was relatively high and reached 49% for larvae collected in fruits at Gotheron. Previous studies on the codling moth parasitism showed that parasitism rates in commercial orchards from the same area were less than 5 % (Maalouly et al, 2013) mainly due to pesticide applications that negatively affect hymenopteran parasitoids (Mates et al, 2012). So far, high parasitism rates reaching up to 40% were only observed in non-commercial apple orchards (Diaconu et al, 2000; Minarro and Dapena, 2004). Estimates of parasitism rates were higher in larvae collected in fruits than in band traps in both study sites except for the diapausing cohorts. This indicates that the use of band traps, which is the easiest way to collect codling moth larvae, may lead to an underestimation of codling moth parasitism for the within-season cohorts. Such underestimation could be explained by a behavioral change induced by parasitism to codling moth larvae. A reduction of movement of parasitized larvae was also observed in mosquitoes (De Valdez et al, 2006) and two *Drosophila* species (Josso et al. 2011). Such reduction of movement would make non-diapausing parasitized larvae less likely to reach the band traps. It could be due to host manipulation by parasitoids to reduce the risk of predation or to increase host nutritional acquisition for a successful parasitoid development (De Valdez et al, 2006; Josso et al. 2011). Developmental constraints are likely to be different for the over-wintering parasitoid larvae, which also need to preserve their hosts against the freeze (Quan et al, 2013).To our knowledge, however, such behavioral change induced by parasitism has not been previously reported on the codling moth. On the contrary, the composition of the parasitoid community was globally similar using both trapping methods. A higher proportion of *P. tristis* in band traps than in fruits was only observed in the second codling moth cohort at Montfavet. This result indicates that hyperparasitism may differ between young and mature larvae and that trapping method should be adapted to the targeted parasitoid species (Athanassov et al, 1997).

The composition of the parasitoid community also varied little between sites and among cohorts. This composition is very similar to that previously reported by Maalouly et al. (2013) in codling moth diapausing larvae collected in commercial orchards in southeastern France. Three main parasitoids attacked the codling moth larvae: *A. quadridentata*, *P. vulnerator* and

P. tristis. Four other parasitoid species were found in this study but were present in low numbers and only in one of the two study sites. *B. rufipes* and *D. cavus* were only found in Montfavet while *H. pallidus* and *M. rudibundus* were found in Gotheron. *A. quadridentata* is specialized on tortricid host. On the contrary, *P. vulnerator* and *P. tristis* are generalist parasitoids, known to attack several Lepidoptera families (Rosenberg, 1934). Comparisons on the emergence times of the codling moth and of its parasitoids provide some information on the phenology of the studied species. Our results confirm the observations by Athanassov et al (1997), Diaconu et al (2000), Geier (1957), and Rosenberg (1934) on the emergences of codling moth parasitoids from diapausing larvae. They also extend them to the emergence of parasitoids from within-season codling moth cohorts. These two results were consistent across sites. First, *A. quadridentata* emergence was synchronized with the emergence of the codling moth, except for the second cohort at Montfavet. However, emergence distributions still largely overlapped for that cohort. This synchrony may be due to the ability of the braconid parasitoid to assess the physiological status of it host. The braconid larva is able to detect some variations in the concentration of ecdysteroids within its host to initiate its final development (Reed-Larsen and Brown, 1990). Second, the hyperparasitoid *P. tristis* emerged later than the codling moth whatever its primary parasitoid hosts (*A. quadridentata* or *P. vulnerator*). It suggests that *P. tristis* emergence depended on the development of its secondary host. In agreement with that hypothesis, *Perilampus hyalinus* initiated its development when the dipteran primary parasitoid *Varichaeta aldrichi* reached a pupal stage in the the fall webworm (*Hyphantria cunea*, Arctiidae) (Smith, 1912). This may explain the delay in emergence of *P. tristis* in comparison with other codling moth parasitoidsin our study. Furthermore, this can explain the difference in emergence between *P. tristis* developing on *P. vulnerator* and on *A. quadridentata* since these two species may have different development times. Finally, *P. vulnerator* emerged earlier than the codling moth from the diapausing cohort but approximately at the same time than it within the season. This generalist ichneumonid species is likely to be less sensitive to its host physiology and abble to adjust its emergence to other more abundant lepidopteran hosts.

Contrarily to the composition of the parasitoid community, the proportions of the different species differed between the two study sites. The braconid *A. quadridentata* was dominant in the parasitoid community in both study sites. In agro-ecosystems dominated by apple

orchards, *A. quadridentata* has been shown to have an almost specialist behavior and parasitize preferentially the codling moth (Frilli, 1968). The particularly good synchrony of the emergence of the codling moth and of *A. quadridentata* in both sites is an indication that *A. quadridentata* may also behave as a specialist on *C. pomonella* in the study area. Its dominance is thus consistent with the fact that specialist parasitoid species of a given host species contribute more to host mortality than generalists (Elzinga et al, 2007; Godfray et al, 1995; Stefanescu et al, 2012). Overall, more primary parasitoids were found at Montfavet (*A. quadridentata*, *P. vulnerator* and *B. rufipes*) while more hyperparasitoid *P. tristis* were found at Gotheron. The composition of parasitoid communities of herbivore species differ among altitudes (Kato, 1996; Randall, 1982). Further, the coexistence of parasitoid species in the parasitoid community can be explained by the presence of alternative hosts (Van Baalen et al, 2001), food resources (Landis et al, 2000) or overwintering sites (Thies et al, 2013). Certain local orchard characteristics can thus promote the abundance of some parasitoids species (Maalouly et al, 2013). In Gotheron, the nearby forest and semi-natural habitats may be a source of alternative hosts for *P. tritis* or of complementary food resources. Further, *P. tristis* developed more frequently on *A. quadridentata* than on *P. vulnerator* overall and more frequently on *P. vulnerator* at Montfavet than at Gotheron but these differences were simply explained by differences in the proportions of the two primary parasitoids between the two sites.

Proportions of the parasitoid species also varied from the beginning to the end of the growing season. Studying the variability of the community structure along time proved hard because parasitoids detection only occurred at their emergence whereas they attack young codling moths larvae or eggs. We thus adapted our sampling scheme and classified our samples in cohorts likely to have been parasitized at the same period in the growing season. This classification was not easy, notably for the 2^{nd} and 3^{rd} codling moth generations at Montfavet since these generations overlap. The definition of the codling moth cohorts at Montfavet was based on the codling moth phenological model (INOKI). This model considers the development of the codling moth but does not take into account change in development possibly due to parasitism. Parasitized larvae with particularly slow development may thus have been classified in a later cohort. Despite these difficulties, changes in the community composition appeared clearly. The relative proportions of *A. quadridentata* decreased in

parallel with an increase of the proportions of the hyperparasitoid *P. tristis* over the cohorts in both study sites. Contrarily to what may have been expected, this increase of the proportion of the hyperparasitoid wasp was related to a small increase in the parasitism rate at Montfavet over time. Primary parasitoid species invulnerable to hyperparasitism can take over the function of vulnerable ones in parasitoid assemblages with strong interactions among species, which finally results in an insignificant impact of the hyperparasitism on primary parasitism levels (Nofemela, 2013). In the present study, the two mainprimary parasitoids parasitizing the codling moth *A. quadridentata* and *P. vulnerator* were both attacked by *P. tristis*. Changes in the assemblage of the primary parasitoid community may not compensate the negative impact loss of *A. quadridentata* individuals. The increase of the parasitism rates may rather result from a faster decrease of the codling moth population than of the parasitoid populations. At Gotheron, the parasitoid populations slightly decreased and the codling moth population increased largely, resulting in a decrease in parasitism along cohorts. In both situations, the decrease of the total number of primary parasitoids sampled may result from the strong control of *A. quadridentata* by *P. tristis*. In the last cohorts, 25 out of 51 and 50 out of 106 *A. quadridentata* individuals were parasitized by *P. tristis* at Montfavet and Gotheron, respectively. It is thus likely that *P. tristis* prevented a high control of the codling moth by *A. quadridentata* in these untreated orchards.

Finally, the conservation of abundant parasitoid populations also requires a sufficiently abundant host population. It is possible that codling moth abundance would have limited parasitism at Gotheron but not at Montfavet since the parasitism rate depended positively on the host density at Gotheron only. However, it is unlikely that *A. quadridentata* suffered from host limitation since the species attacks early stage of its host and a large number of hosts were available (Mills, 2001), and specialist parasitoids are generally able to track scattered host population (Kato, 1996).

To conclude, our results show that *A. quadridentata* is a serious candidate for codling moth biological control in apple orchards in southeastern France with potentially high parasitism rates even early in the season when pest control is the most efficient. However, in our study, although codling moth hosts were abundant and *A. quadridentata* populations were well established, a further increase of parasitism by *A. quadridentata* was likely limited by *P. tritis* hyperparasitism. This chalcidoid wasp was reported in numerous countries in Europe and

North America and lack of effectiveness codling moth biocontrol by its parasitoids because of hyperparasitism is likely to be very widespread (Clausen, 1978). Despite this limitation, improvements of codling moth biological control may be obtained by a better targeting of pesticide treatments in commercial orchards where parasitism rates by *A. quadridentata* are much lower than those observed in the present study (Maalouly et al, 2013). Avoidance of sulfur treatments in organic orchards during early season should notably be considered because egg parasitoid as *A. quadridentata* seems particularly sensitive to this pesticide (Thomson et al, 2000). We showed that this period is short and that it coincides with codling moth emergence, which is usually monitored by farmers. To further increase codling moth biocontrol by parasitoids, it would be also important to reinforce or introduce in parallel other primary parasitoids less vulnerable to hyperparasitism by *P. tristis* and, more generally, to reduce the use of pesticides that affect these species. Furthermore, biological control by parasitoids should be considered within a combination of methods to achieve sustainable crop protection in orchards.

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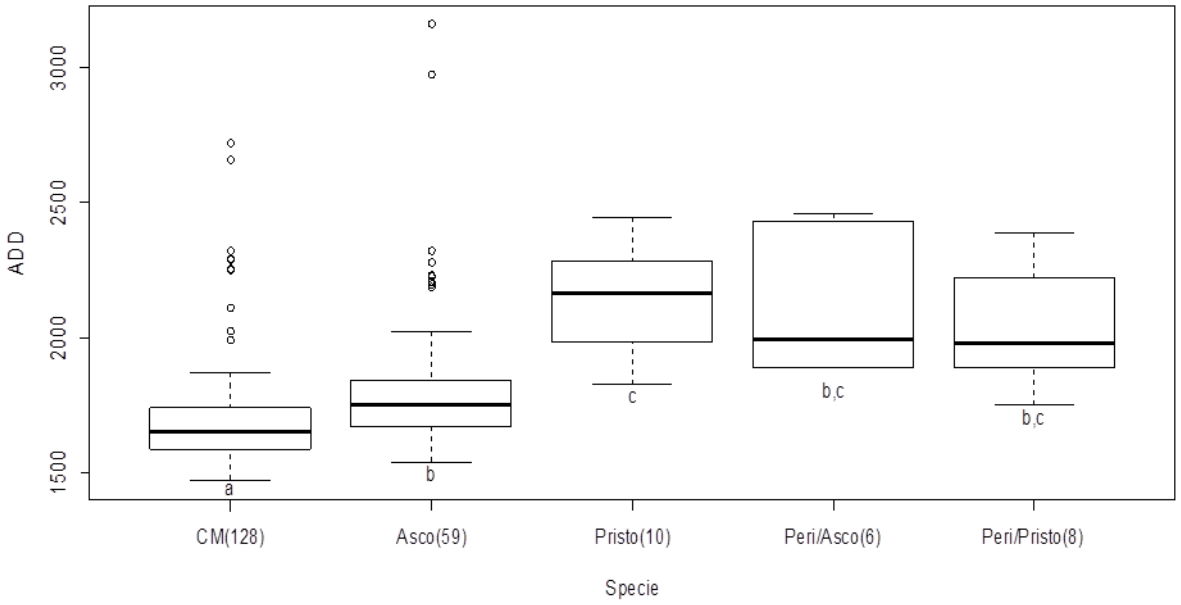
Supplementary material

Table 1.S1: Estimates of sampling and classification errors for larvae collected at Montfavet and Gotheron according to the codling moth phenological model. Sampling errors were of two types. First, for a given cohort, we may have missed some of the earliest individuals of the corresponding generation. This error was estimated as the proportion of individuals of generation *n* that have emerged as adults according to the model before we started sampling individuals of cohort *n*. Second, we may have missed the latest individuals. This error was estimated as the proportion of individuals of generation *n* that emerged as adults according to the model one week or more after we finished sampling individuals of cohort *n*. Finally, some collected individuals may have been misclassified, *i.e*. classified in a cohort that did not correspond to their actual generation, notably because codling moth generations may overlap during the sampling periods. Two types of errors were possible when building cohorts: (i) individuals were classified in cohort *n+1*, while they were of generation *n* and (ii) individuals were classified in cohort *n*, while they were of generation *n+1*. These errors were estimated based on the overlapping of generations according to the model during the sampling periods.

Study site	Cohort	Probability of missing the earliest larvae	Probability of missing the latest larvae	Probability of misclassification
Montfavet	1	0.000	0.102	0.000 G2 classified in cohort 1
	2	0.001	0.096	0.060 G1 classified in cohort 2
				0.051 G3 classified in cohort 2
	3	0.052	0.050	0.094 G2 classified in cohort 3
Gotheron	1	0.030	0.050	0.03 G2 classified in cohort 1
	2	0.000	0.050	0.02 G2 classified in cohort 1

Figure 1.S1: Box-plots of the accumulated degree days per specie for the emerging adults during the second generation in Montfavet. Multiple mean comparisons were analyzed with Tukey tests.



Chapitre 2
Effet du paysage et des pratiques agricoles sur le parasitisme du carpocapse du pommier

Dans ce chapitre, nous cherchons à mieux comprendre les facteurs agronomiques et écologiques qui affectent les populations de parasitoïdes et le taux de parasitisme des larves diapausantes de carpocapse en vergers commerciaux.

Nous avons voulu déterminer la composition de la communauté des parasitoïdes des larves du carpocapse du pommier. Nous avons testé les effets des pratiques agricoles et des caractéristiques des éléments semi-naturels au niveau du verger et du paysage sur la composition de la communauté des parasitoïdes ainsi que sur le taux de parasitisme du carpocapse.

Pour répondre à ces questions nous avons analysé les données issues d' un échantillonnage de larves diapausantes de carpocapse effectué dans des vergers commerciaux de pommes dans un bassin de production (la basse vallée de la Durance) situé au Sud Est de la France durant cinq années consécutives (2006-2010). Nous avons évalué l'effet des pratiques agricoles et les caractéristiques des éléments semi-naturels sur les taux de parasitisme du carpocapse et la composition de la communauté de ses parasitoïdes. Les pratiques agricoles testées étaient la protection phytosanitaire au niveau du verger (nombres de traitements insecticides et fongicides, verger en agriculture biologique ou conventionnelle) et au niveau du paysage dans un voisinage de 250 m des vergers cibles (proportion de vergers en agriculture biologique, des vergers en agriculture conventionnelle, des vergers abandonnés). Les caractéristiques des éléments semi-naturels analysées concernaient les haies au niveau du verger (présence de haies brise-vent de haies composites, diversité floristique des haies) et les haies, les forêts et les rivières au niveau du paysage (longueur et orientation des haies, proportion de forêts, longueur des rivières).

Nous montrons que la communauté de parasitoïdes du carpocapse était constituée de deux parasitoïdes primaires (Braconidae et Ichneumonidae) et un hyperparasite (Perilampidae). La composition de la communauté des parasitoïdes a changé en fonction des années en terme d'abondances relatives des parasitoïdes primaires. La présence des haies brise-vent et des haies composites autour des vergers a favorisé l'abondance relative des parasitoïdes primaires par rapport à l'hyperparasitoïde. Les taux de parasitisme étaient globalement faibles dans tous les vergers pour chacune des années de l'étude. Ces taux étaient plus élevés dans les vergers en agriculture biologique que dans les vergers en agriculture conventionnelle ainsi que dans les vergers entourés d'une faible proportion de vergers conventionnels dans un voisinage de 250 m.

Ce travail est présenté dans un article publié dans le journal *Agriculture, Ecosystems and Environment*: Mariline Maalouly, Pierre Franck, Jean-Charles Bouvier, Jean-François Toubon, Claire Lavigne. *Agriculture, Ecosystems & Environment*, *Volume 169*, *1 April 2013*, *Pages 33-42.*

La suite de ce chapitre reprend l'intégralité de l'article.

Codling moth parasitism is affected by semi-natural habitats and agricultural practices at orchard and landscape levels

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Abstract:

Pest control that results from the activity of naturally occurring parasitoids is an important service that could help reduce pesticide use. We analyzed parasitism in codling moth diapausing larvae from a total of 122 apple orchards in southeastern France during five consecutive years (2006-2010) in relation to the agronomic and land cover characteristics at both the local and landscape levels. Three species of hymenoptera parasitoids were observed, including two primary (Braconidae and Ichneumonidae) and one hyperparasitoid (Perilampidae) wasps. Parasitoid community compositions differed according to the year (in term of the relative abundance of the primary parasitoid species) and the presence of windbreaks and spontaneous hedgerows around the orchards (in terms of the relative abundance of primary *vs.* hyperparasitoid species). The parasitism rates were globally low in all orchards each year (< 4.5% in average), but they were significantly higher in organic orchards than in conventional orchards as well as in orchards surrounded by a low proportion of conventional orchards in a 250 m vicinity. These results are discussed here in terms of biocontrol enhancement and conservation.

Keywords:

biological control, *Cydia pomonella,* Lepidoptera, parasitoid wasp, hyperparasitoid, trophic interaction

Highlights:

- A community including only two primary and one hyper- hymenoptera parasitoids.
- Parasitoids *vs* hyperparasitoid relative abundances enhanced by hedgerow presence
- Low parasitism rates on codling moth larvae in commercial apple orchards
- Parasitism rate depending on protection practices at local and landscape levels

1. Introduction

The intensification of agriculture during the last century resulted in a general increase in crop yields and an increase in field chemical inputs (Matson *et al.*, 1997), a reduction of crop diversity and the simplification of landscapes (Robinson and Sutherland, 2002; Benton *et al.*, 2003). The negative effects of agricultural intensification on biodiversity have been documented (Donald *et al.*, 2001; Tilman *et al.*, 2001; Tscharntke *et al.*, 2005; Geiger *et al.*, 2010; Flohre *et al.*, 2011). Recurrent pesticide exposures also impact the health of agricultural workers (Lee *et al.*, 2004). In the future, more sustainable agricultural systems based on an intensification of ecological processes may enhance the services provided by biodiversity and reduce negative agricultural impacts (Doré *et al.*, 2011). The ecological intensification of agriculture requires a good understanding of the agronomic and ecological factors that affect the populations that are responsible for these services.

Natural enemies of pest species are one benefit of biodiversity, and their abundance and diversity are also affected by agricultural intensification, along with the pest control service that they provide to farmers (Roschewitz *et al.*, 2005; Winqvist *et al.*, 2011; Monteiro *et al.*, 2013; Veres *et al.*, 2013). Both the use of (broad spectrum) pesticides and landscape simplification affect communities of natural enemies. Landscape simplification is associated with a reduced proportion of semi-natural areas such as hedgerows that can provide resources and refuge for a diversity of pest enemies, notably parasitoid wasps (Forman and Baudry 1984; Landis *et al.*, 2008). Landscape simplification is also associated with reduced diversity in the crops and cropping systems that create large homogeneous areas over the landscape, which do not contain the diversity of habitats that are necessary for some species to complete their life cycles through habitat complementation (Tscharntke *et al.*, 2007;

Médiène *et al.*, 2011). Because landscape simplification and high pesticide use often covary within or among landscapes, their effects on biodiversity and ecosystem services have proven difficult to disentangle. Similarly, semi-natural habitat diversity correlates with field crop diversity in most agricultural landscapes (Fahrig *et al.*, 2011).

We investigated how local and landscape-scale agricultural practices and the characteristics of semi-natural elements affect the parasitism of codling moths (*Cydia pomonella* L., Lepidoptera, Tortricidae) and the composition of the associated parasitoid community in an apple-growing basin in southeastern France. The codling moth is a major insect pest of various perennial cultivated plants, notably apple and pear (Barnes 1991), in the temperate regions of the world (Shel'Deshova, 1967). The larvae attack fruits and damages can reach up to 90% of non-treated apple orchard products (Audemard 1991). The codling moth is consequently the target of approximately 90% of insecticide treatments in apple orchards within the study area (10 to 20 treatments per year, Monteiro *et al.*, 2013). In response to this intensive management, the codling moth has developed resistance to most of the insecticides that are used in this area (Reyes *et al*., 2007; Franck *et al*., 2012), as in other regions of the world (Reyes *et al.*, 2009). These insecticides include the granulosis virus, which is the main insecticide for use in organic orchards (Asser-Kaiser *et al.*, 2007). This critical situation requires the development of alternative means of control. The conservation of natural enemies that are already present in orchards is one method that may enhance the biological control of codling moth populations.

Within the guild of natural enemies, hymenopteran parasitoids are usually considered essential agents for the control of agricultural pests (Schmidt *et al.*, 2003; see also Sydmonson *et al.*, 2002). Codling moth parasitoids have been identified in a number of regions worldwide. Assemblages of parasitoid wasps differ between North America and Eurasia (Mills, 2005). In Europe, several primary parasitoids (in the Braconidae and Ichneumonidae) and a hyperparasitoid (Perilampidae) were recorded in a collection of codling moth diapausing larvae (Rosenberg, 1934; Athanassov *et al.*, 1997; Diaconu *et al.*, 2000; Mills, 2005). The dominant species in this community was the braconid *Ascogaster quadridentata* Wesm., an ovo-larval parasitoid specializing in tortricid moths, and two less specialized ichneumonids, namely *Pristomerus vulnerator* Panz. and *Trichomma enecator* Rossi (Athanassov *et al.*, 1997; Mills, 2005). Another braconid, *Microdus rufipes* Nees, has rarely been observed. The chalcidoid *Perilampus tristis* Mayr has been associated with

numerous Lepidoptera and Hymenoptera species (Noyes, 2012) and was noted as a hyperparasite of tortricids (Bogenshütz, 1991). Adult females of *P. tristis* actively engaged in foraging (Clausen, 1940). However, knowledge of parasitoid biology and the ecology remains limited, and their habitat specificities within agricultural landscapes are unknown.

Both agricultural practices as well as local and landscape-scale resources usually affect the abundance and composition of parasitoid communities in a given agro-ecosystem (*e.g.,* Jonsson *et al.*, 2012; Mates *et al.*, 2012; see also Macfadyen *et al.*, 2009). Intensive agricultural practices are expected to negatively affect hymenoptera parasitoids, which are particularly sensitive to pesticides (Thomson and Hoffmann, 2006). The presence of semi-natural areas at a local or landscape scale should *a contrario* promote parasitoid populations (Thies and Tscharntke, 1999; Landis *et al.*, 2000). Flowering habitats provide complementary resources to the parasitoids, notably pollen and nectar, which frequently increase longevity and fecundity in female wasps, in particular for synovigenic species that produce and/or mature their eggs after emergence (Schmale *et al.*, 2001). Hedgerows are known to shelter very abundant and diverse communities of hymenoptera parasitoids (Forman and Baudry 1984) and may provide refuge to their populations during pesticide treatments. The scale at which parasitoids respond to these local and landscape factors depends on their biology, particularly their dispersal ability, as well as the structure of landscape heterogeneity. Furthermore, it has generally been observed that specialist species respond to landscape compositions at smaller spatial scales than generalist species (Chaplin-Kramer *et al.*, 2011) and primary parasitoids may also respond at smaller scales than hyperparasitoids (Holt *et al.*, 2002; Zhao *et al.*, 2012).

The link between diversity and abundance in the parasitoid community and pest parasitism rates is not straightforward because the parasitism rate may also depend on host abundance (Costamagna *et al.*, 2004; Thies *et al.*, 2005; Jonsson *et al.*, 2012). For example, a negative density dependence is expected if the number of hosts encountered by parasitoids exceeds parasitoid egg production (Morrison and Strong, 1980) or if the handling time is increased in large host populations (Hassell and May, 1973). Detecting such density-dependence further requires that studies be conducted at the pertinent spatial scale (Ray and Hastings, 1996). Furthermore, parasitism rates may also depend on the composition of the parasitoid community. Indeed, parasitoid diversity may promote parasitism if species are complementary (with either additive or synergistic effects), but it may also decrease

parasitism if negative interactions occur among species, as in the case of hyperparasitism (Rosenheim, 1998).

For this study, we first characterized the composition of a codling moth parasitoid community in the apple orchards of an intensive agricultural area in southeastern France. We then assessed how the local and landscape scale intensity of agricultural practices and the characteristics of semi-natural areas impacted the parasitoid community composition. We then assessed whether parasitism depended on these same factors or if it was also influenced by the abundance of codling moth larvae. Finally, we investigated how the composition of the parasitoid community, and in particular the presence of a hyperparasitoid, related to parasitism.

2. Materials and methods

2.1. Study area

This study was carried out in an apple production area of approximately 80 km^2 in the lower Durance valley in southeastern France (with coordinates in the WGS84 system from 43°47’11’’N to 43°51’10’’N and from 4°51’29’’E to 4°59’25’’E). Apples are mainly grown in conventional orchards (~90% of orchards), with the remaining production coming from organic orchards (~2%). Due to the currently low fruit prices, some orchards are abandoned every year and may remain uncultivated for a few years before being uprooted (~8% of orchards). The landscape is further characterized by the presence of a dense network of windbreak hedgerows (mainly monospecific hedgerows of Cupressaceae or *Populus* trees, approximately 10 m high and 3 m wide) that protect orchards against the prevailing northern winds (Ricci *et al.*, 2009) and spontaneous hedgerows (based on *Prunus* and *Cornus* species) that display a very diversified flora (Deckers *et al.*, 2004). Finally, numerous irrigation channels are laid out across the area in both north-south and east-west orientations.

All of the orchards, channels, hedgerows and woods in this area were manually digitalized with ArcView (Version 9.1, ESRI) from aerial photographs (BD ORTHO, IGN, 2004-pixel size:

0.5 m). Furthermore, agronomic practices were recorded yearly in a set of sampled orchards after farmer surveys and treatment calendar analysis.

2.2. Sampling and parasitoid detection

Overwintering codling moth larvae were sampled over five consecutive years (2006-2010). Thirty-seven to forty-nine randomly distributed apple orchards in the area were sampled each year (Table 2.1). These individual orchards were not always the same from one year to the next.

Overwintering codling moth larvae were sampled using 10-cm-wide corrugated cardboard band traps wrapped around tree trunks. This sampling method allows for the trapping of healthy and parasitized codling moth larvae that are searching for a winter shelter (Athanasov *et al.*, 1997; Diaconu *et al.*, 2000). A minimum of 30 evenly distributed band traps were set out for each orchard, and this number was increased proportionally up to 50 according to the orchard area. Traps were installed in mid-July and collected in mid-October at the end of the growing season (approximately one month after the last insecticide treatment each year). The larvae were stored in individual vials in an outdoor insectarium during the winter and adult or parasitoid emergence was monitored daily from mid-April until mid-July each year. Emerging adults and their cocoons were kept in 90% ethanol. The species of adult parasitoids were identified according to morphological diagnostic identification key criteria using a binocular microscope (Athanasov *et al.*, 1997). The primary and secondary host species was verified by inspecting the cocoon after parasitoid emergence (Diaconu *et al.*, 2000) and using DNA diagnostic markers when the determination was doubtful (Boreau de Roincé *et al.*, 2012).

In each orchard under study, the mean number of overwintering codling moth larvae per tree (*DensCM*), the parasitism rate (ratio of emerging parasitoids to the total number of adult individuals) and the composition of the parasitoid community were recorded annually.

Year	Sampled orchards	Orchards with codling moths	Orchards with parasitoids	Diapausing CM larvae	Adult individuals (CM/parasitoid)
2006	49	46	10	4853	2815 /112
2007	47	45	7	3133	2753/89
2008	48	40	20	3239	2687/ 80
2009	37	33	13	4786	3990/ 181
2010	47	38	12	7595	5124/ 85

Table 2.1: Numbers of sampled apple orchards, numbers of codling moths (CM) and parasitoid individuals (larvae and adults) collected per year.

2.3. Characteristics of sampled orchards

For each sampled orchard, a set of variables describing the agronomic practices and landscape features that may affect codling moth parasitism were recorded at two different spatial levels.

2.3.1. Local orchard characteristics

Protection practices were described by the number of insecticide (*Insect*) and fungicide (*Fung*) treatments per year and whether the orchard was organic or conventional (crop management: *CropM*). The proportion of organic orchards that were sampled increased from 17% in 2006 to 50% in 2010. Characteristics of the hedgerows bordering the orchards were described by the presence or absence of a windbreak hedgerow at the north of the orchard (*WbH*), the presence or absence of a spontaneous (multi-species) hedgerow at the border (*SpH*), the average hedgerow floristic diversity (*Hdiv*) and the proportion of the orchard perimeter that included hedgerows (*HPeri*). Over the study years, 72% of orchards were protected by a windbreak hedgerow (*WbH*), and 47% had a spontaneous hedgerow (S*pH*). For each hedgerow, a Shannon index was calculated based on the species composition from five positions that were regularly distributed along the hedgerow (Ricci *et*

al., 2011). The *Hdiv* was calculated as the average Shannon index of the hedgerows surrounding the orchard and weighted by their length. Finally, because they affected the average distance of apple trees to the orchard hedge, we recorded the area (*Area*) and shape (*Shape*) of the focus orchards. The shape was calculated as the ratio of the orchard perimeter over the square root of its area.

2.3.2. Landscapes characteristics around the orchards

Landscape characteristics were calculated in areas that were 250 m wide (hereafter called "buffers") around each sampled orchard. This width was the most pertinent to analyze landscape effect on codling moth population in the study area (Ricci *et al.*, 2009). First, the proportions of the buffer areas that were covered by woodland (*Wood*) and by organic (*Org*), conventional (*Conv*) and abandoned (*Abd*) orchards around each sampled orchard were calculated. Second, the hedgerow network (excluding the orchard hedgerows) was characterized as done by Ricci *et al.* (2009) by calculating both the ratio of its total length over the buffer area (*Hlg*) and its overall windbreak effect towards northern winds (orientation index: *HOI*). Finally, the ratio of the total length of water channels over the buffer area (*Channel*) was calculated.

2.3.3. Variable pre-selection

We performed a first selection of local and landscape variables by removing those that strongly covaried with other variables. For this purpose, we assessed the Spearman correlations between quantitative variables by characterizing the orchard and its surrounding landscape. We conducted chi-square independence tests between qualitative variables and Kruskal-Wallis rank sum tests between the quantitative and qualitative variables. We repeated these tests for all study years. We considered two variables to correlate when they had a p-value < 0.01 over the five study years. Correlation tests showed that the proportion of organic orchards (*Org*) in the buffer surrounding an orchard was significantly higher for organic than conventional orchards (*CropM*). Furthermore, the average hedgerow floristic diversity (*Hdiv*) was positively correlated with the presence of a spontaneous hedgerow (*SpH*) surrounding the orchard (Tables 2.S1, 2.S2 and 2.S3).

Consequently, we excluded the *Org* and *Hdiv* variables from any statistical analyses. Table 2.2 shows the local and landscape variables that were retained and their variability among the sampled orchards.

Local orchard variables		
Variable	*Description*	*Mean±sd*
CropM	Crop management (1 = organic)	qualitative
WbH	Windbreak hedgerow (1 = presence)	qualitative
SpH	Spontaneous hedgerow (1 = presence)	qualitative
Insect	Number of insecticide treatments	14.10±4.64
Fung	Number of fungicide treatments	15.51±6.52
Area	Orchard area (m^2)	8100±6200
Shape	Orchard shape	5.13±1.05
Hperi	Hedgerow length/orchard perimeter	0.65±0.25

Landscape variables (250 m)		
Variable	*Description*	*Mean±sd*
HOI	Hedgerow orientation index	0.84±0.09
Hlg	Hedgerow length (m/m^2)	1.17±0.28
Abd	Proportion of abandoned orchards (%)	2.17±3.19
Conv	Proportion of conventional orchards (%)	25.73±18.38
Wood	Proportion of woodland (%)	3.67±5.09
Channel	Length of water channels (m/m^2)	0.12±0.13

Table 2.2: Description of local and landscape variables and their variations among 202 analyzed orchards. Annual variations in the qualitative variables to describe local orchard characteristics are reported in the text.

2.4. Statistical analyses

2.4.1. Composition of the parasitoid community

The local and landscape variables that explained the parasitoid community composition were assessed using direct gradient analysis with a redundancy analysis (RDA) that considered all sampled orchards from which at least one parasitoid had been collected (R 2.15.1, R development Core team, Vegan package). A manual backwards stepwise approach was used to determine the best explanatory variables in the RDAs, thereby testing the significance of the each variable with permutations (100 replicates). A first RDA was performed with all the local variables, the study year, and the density of codling moths (*DensCM*) as covariate. Landscape variables were then added to the significant variables in this first RDA, and a second RDA was performed to keep only the significant variables using the same stepwise approach. Finally, biplots of partial RDAs were drawn to visualize the effects of significant variables on the composition of the parasitoid community.

2.4.2. Codling moth parasitism rate

First, a simple linear model was computed by relating the log transformed parasitism rate per orchard to the presence of at least one hyperparasitoid in the orchard to test the impact of parasitoid community composition on the codling moth parasitism rate.

Second, the relative effects of the local and landscape variables on the parasitism rate were assessed using a multimodel approach (R package MuMin, Calcagno and de Mazancourt, 2010). This approach was based on generalized mixed models and used a binomial variable that took a value of 1 if the larva was parasitized and 0 otherwise, with a logit link function. Models were fitted to local and landscape variables by Laplace approximation (R package lme4). All of the models included the codling moth density (*DensCM*) as a covariate, and the orchards and trap identities were random variables that accounted for within-orchard correlations. Correlations between years were not considered, and orchards sampled at different times were considered in a statistically independent manner.

The multimodel approach was based on the fit of all possible variable combinations. Candidate models were then ranked using the corrected Akaike information criterion (AICc). The importance values for each variable and their coefficient estimates (Burnham and Anderson 2002) were calculated using only the set of models that differed from the best model by a difference of AICc that was lower than 20.

The multimodel approach allows variable rankings, but no formal test of the significance of their importance values has been proposed. We therefore designed a permutation test to assess the significance of the importance for each independent variable. The test was based on 100 permutations of the variable vectors (except the covariable *DensCM*) to keep correlations between variables similar to those in the observed data set. Importance values that were outside the unilateral 95% empirical confidence interval that was built from permutations were considered significant.

Although multimodel approaches can handle large numbers of variables (Calcagno and de Mazancourt, 2010), it is not recommended to blindly include all variables in this analysis (Burnham and Anderson, 2002). We therefore proceeded in two steps, giving priority to local orchard variables over landscape variables. First, we designed *orchard local models* in which we tested the importance of the study year and the eight orchard local variables (*CropM, Insect*, *Fung, WbH, SpH*, *HPeri*, *Area, Shape*). In a second step, we designed *landscape models* by including the significant variables in the *orchard local model* (*CropM, Insect*, *SpH*, see Section 3) and the six landscape variables (*Conv, Abd, Hlg*, *HOI, Channel, Wood*).

2.4.3. Codling moth density

We followed the two-step approach described above to test the effect of orchard and landscape variables on the density of codling moth larvae (*DensCM*) and to verify the impact of these variables on host dynamics. We first analyzed log transformed *DensCM* with a linear model as a function of the study year and the orchard local variables. We then kept only the significant variables in this *orchard local model* (*CropM,* see results) and included them in a *landscape model* in which we added all of the landscape variables.

3. Results

3.1. Collected codling moths and parasitoids

Approximately 80% of sampled orchards had at least one codling moth larva. The density of codling larvae moth per tree (*DensCM*) ranged from 0.02 to 65.28 with a median of 0.77 and a mean of 3.47. A total of 4721 ± sd 1803 codling moth larvae were collected on average per year (Table 2.1). Of these, a total of 3474 ± sd 1068 codling moths or parasitoid adults emerged per year on average.

We collected a total of 547 parasitoids over five years, which were assigned to three different hymenoptera species: *Ascogaster quadridentata* Wesm. (Braconidae), *Pristomerus vulnerato*r Panz. (Ichneumonidae) and *Perilampus tristis* Mayr (Perilampidae). Nine parasitoids were collected dead in their cocoons and could not be identified with certainty. They were discarded from the dataset that was used to analyze the parasitoid community. The *Perilampus* parasitoids were very similar to *P. tristis* and may encompass different but similar species within this group (Argaman, 1991). The *Ascogaster* and *Pristomerus* parasitoids were unambiguously determined. Hereafter, these three parasitoids were referred to by their genus names. They were found in seven to 20 orchards, depending on the study year (Table 2.1). The overall mean parasitism estimated for emerged adults was low, ranging from 1.6% to 4.5% depending on the year (the mean over all five study years: 3.3% ± sd 1.1%).

3.2. Parasitoid community

The parasitoid community composition differed during the study years (Table 2.3). The parasitoid proportions of the community were, on average, 0.61 ± sd 0.29 for *Ascogaster*, 0.22 ± sd 0.30 for *Pristomerus* and 0.17 ± sd 0.08 for *Perilampus* each year. *Ascogaster* was the dominant species from 2007 to 2010, but the community was dominated by *Pristomerus* in 2006.

A final RDA analysis resulting from the stepwise approach explained 47% of the total variation, of which 70% was explained by the first axis and 24% by the second axis. The first axis mainly discriminated between communities as a function of *DensCM.* The three

parasitoid species were more present in orchards with more codling moth larvae, also meaning that the species richness of the community increased with the abundance of codling moth larvae. The second axis mainly discriminated between communities as a function of the study years and the characteristics of orchard hedgerows (*SpH* and *WbH*). These variables had a significant effect on the composition of the parasitoid community (Table 2.3). To better investigate the effects of orchard hedgerows, we performed a partial RDA (pRDA) and factored out *DensCM* and the study years. The two hedgerow variables explained only 13% of the total variation (Figure 2.1). The *Pristomerus* and *Ascogaster* parasitoids were associated with the presence of a windbreak and spontaneous hedgerows. On the contrary, the *Perilampus* hyperparasitoid was mainly associated with the absence of a windbreak hedgerow.

	Df	F	# Permutations	P-value
Year 2006	1	8.90	199	0.005**
Year 2007	1	4.08	199	0.013**
Year 2008	1	2.25	599	0.140
Year 2009	1	4.68	199	0.005**
WbH	1	3.15	599	0.020*
SpH	1	2.78	3599	0.046*
Residual	53			

Table 2.3: Significance of variables explaining composition variation in the parasitoid community in a partial redundancy analysis, factoring out the density effect of diapausing larvae (assessed with 100 permutations). F, pseudo-F (ratio of constrained and unconstrained inertia); p<0.05*; p<0.01**; p<0.001 ***. *WbH* and *SpH* correspond to windbreaks and spontaneous hedgerows, respectively.

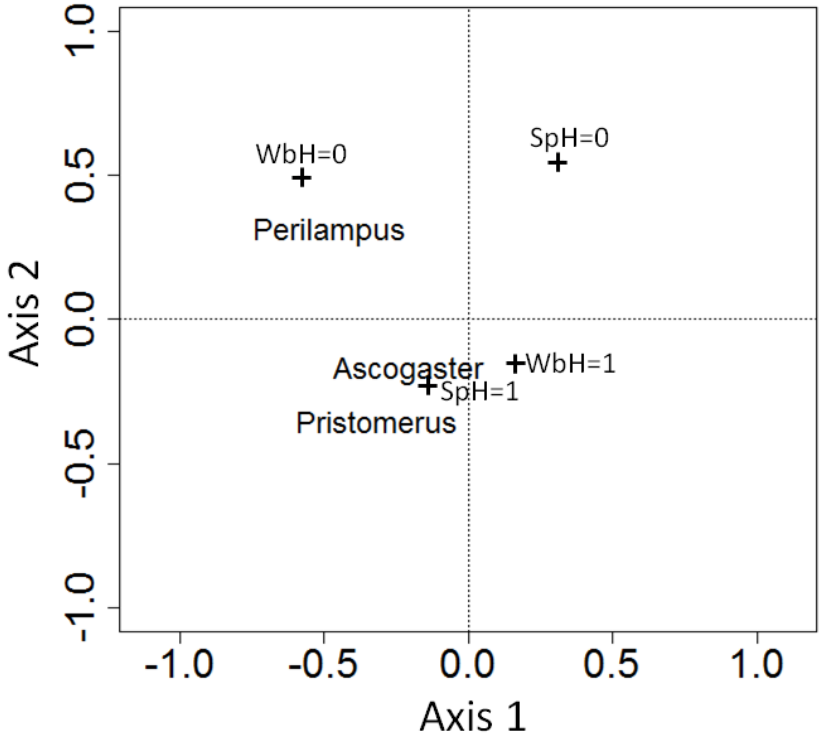

Figure 2.1: Biplot of partial RDA based on parasitoid community composition, factoring out the effect of diapausing larvae density and of sampling years. Species centroids and significant hedgerow variables (cross) are represented. *WbH* and *SpH* correspond to windbreaks and spontaneous hedgerows, respectively.

3.3. Codling moth parasitism rate

Three variables (*CropM*, *SpH*, and *Insect*) significantly explained the codling moth parasitism rate in the *orchard local model* (Table 2.S4). In the *landscape model* (which included these local significant variables and all of the landscape variables), only two variables had significant importance values to explain the parasitism rate: crop management (*CropM*) and the proportion of conventional orchards (*Conv*) in the surrounding landscape (Figure 2.2). These two variables were also significant in the multimodel inference (Table 2.4). The parasitism rate was higher in organic than in conventional orchards or in orchards surrounded by a low proportion of conventional orchards. All of the other variables were not

significant (Table 2.4). In particular, there was no evidence of a relationship between parasitism and codling moth density (*DensCM*).

The linear model that analyzed parasitism as a function of the presence of hyperparasitoids showed that the parasitism rate (Figure 2.3) was significantly higher in orchards in which the hyperparasitoid *Perilampus* was found ($F_{1,60}$ = 9.843, *p*-value=2.64×10^{-3}).

Variables	Estimate	Std. Error	z value	P-value
DensCM	$2.38\ 10^{-3}$	0.010	0.230	0.818
***CropM* (= organic)**	**1.289**	**0.353**	**3.651**	**$2.62\ 10^{-4}$**
SpH (= presence)	0.472	0.337	1.403	0.161
Insect	-0.045	0.028	1.581	0.114
HOI	2.833	2.677	1.058	0.289
Hlg	0.010	0.498	0.021	0.983
Abd	-0.032	0.057	0.557	0.577
Conv	**-0.025**	**0.011**	**2.159**	**0.031**
Wood	-0.015	0.035	0.425	0.671
Channel	0.409	1.289	0.318	0.751

Table 2.4: Multi-model parameter estimates and associated P-values of variables to explain the variation in parasitism rates among orchards. Significant probabilities are in bold. The covariate *DensCM* corresponds to the density of codling moth larvae per tree. The local (*CropM, SpH, Insect*) and landscape (*HOI*, *Hlg, Abd, Conv, Wood, Channel*) variables included in the models are described in Table 2.2.

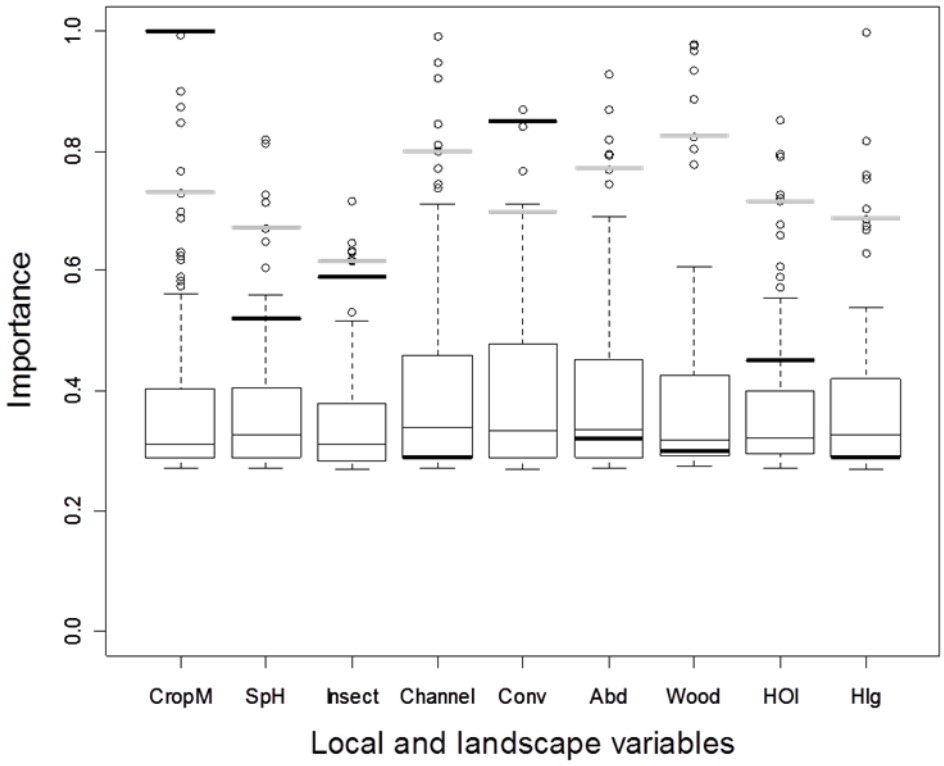

Figure 2.2: Importance values of variables to explain parasitism rates in codling moth larvae. The box-plots (median; box hinges, first and third quartiles; whiskers, 1.5 times the interquartile) represent the distributions of importance values for 100 permutated models. The bolt grey lines represent the 95% quantiles, and the bolt black lines correspond to values for the observed data set. The local (*CropM, SpH*, *Insect*) and landscape (*Channel*, *Abd*, *Wood*, *HOI*, *Hlg*) variables included in the model are described in Table 2.2.

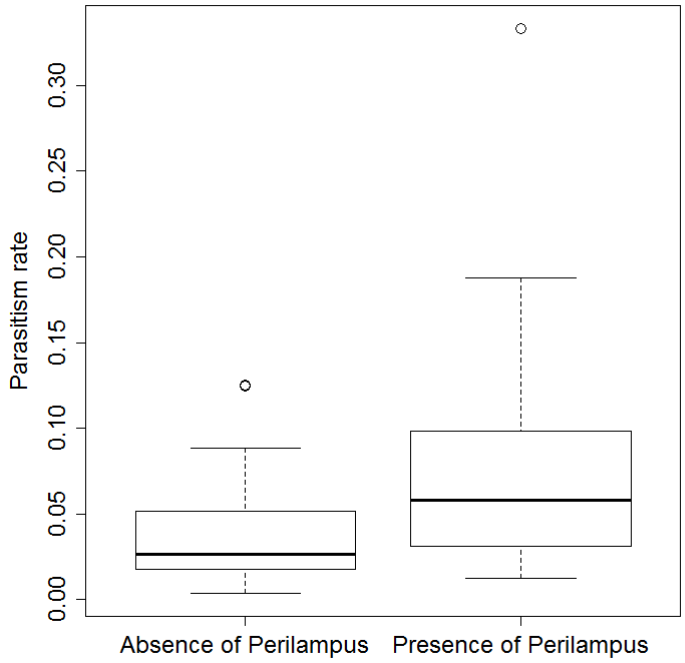

Figure 2.3: Parasitism rate per orchard as a function of the absence/presence of *Perilampus*. The boxplots are characterized by the median, and box hinges corresponding to the first and third quartile and whiskers are equal to 1.5 times the interquartile.

3.4. Density of codling moth

Linear models that were conducted with local orchard variables showed that the density of diapausing codling moth larvae only depended on crop management *(CropM).* The models that included landscape variables revealed two additional significant variables (Table 2.5), namely the hedgerow orientation index (*HOI*) and the total length of water channels (*Channel*). The density of codling moths was significantly higher in organic than in conventional orchards, and it was higher in orchards surrounded by landscapes with a low hedgerow orientation index (*i.e.*, little protection from the wind) and low total water channel length.

Variables	Df	Estimate	Sum of Sq	F value	P-value
CropM	**1**	**-0.629**	**46.31**	**19.8998**	**$1.44\ 10^{-5}$**
Area	1	$-4.1\ 10^{-5}$	5.69	2.4474	0.119
HOI	**1**	**-6.129**	**20.13**	**8.6512**	**$3.70\ 10^{-3}$**
Hlg	1	$-6.62\ 10^{-2}$	0.06	0.0276	0.868
Abd	1	$4.6\ 10^{-3}$	0.03	0.0139	0.906
Conv	1	$-7.8\ 10^{-3}$	1.68	0.7245	0.396
Wood	1	$9.30\ 10^{-3}$	0.20	0.0858	0.769
Channel	**1**	**-3.098**	**20.07**	**8.6251**	**$3.75\ 10^{-3}$**

Table 2.5: Analysis of variance explaining variations in codling moth densities. Significant probabilities are in bold. The local (*CropM, Area*) and landscape (*HOI*, *Hlg, Abd, Conv, Wood, Channel*) variables included in the model are described in Table 2.2.

4. Discussion

We described the parasitoid community and codling moth parasitism rate of apple orchards in southeastern France. We analyzed the effects of crop protection practices and land use characteristics on the parasitism rate with linear models and their effects on community composition with an RDA at both the local and landscape levels. We found that crop protection practices had a major effect on parasitism rates at both levels while the composition of the parasitoid community mainly depended on the presence of local hedgerows and differed between years. Our results highlight factors that may enhance the biological control of this important insect pest in fruit crops.

The parasitoid community that was attacking codling moth diapausing larvae was not very diverse in the study area. Larvae were parasitized by only three hymenopteran parasitoids, namely *A. quadridentata*, *P. vulnerator* and *P. tristis*; moreover, *A. quadridentata* was largely dominant. These three parasitoid species parasitize codling moths all over Europe

(Rosenberg, 1934; Athanassov *et al.*, 1997; Diaconu *et al.*, 2000; Bashir *et al.*, 2010), and the present study also indicates their presence in the western Mediterranean regions. We cannot exclude the possibility that we failed to detect other larval parasitoids. First, nine parasitoids among the 547 that we collected remained undetermined because of their poor conservation condition and host uncertainty. However, because of their low number, it is unlikely that their determination would have significantly modified our results. Larvae in the cardboard traps may also have been protected from ectoparasitoid species that were attacking mature larvae in their cocoons, such as the hymenoptera *Liotryphon caudatus* Ratz. or *Dibrachys cavus* Walk. (Athanassov *et al.*, 1997; Mills, 2005). However, sampling methods cannot explain the absence of the otherwise frequent generalist parasitoid species in attacking codling moth larvae, such as the hymenoptera *T. enecator* Rossi and *M. rufipes* Nees or the diptera *Elodia tragica* Meigen, because these species were observed in other studies with similar trapping methods (Rosenberg, 1934; Athanassov *et al.*, 1997; Diaconu *et al.*, 2000).

The three observed parasitoid species were all described in several Lepidoptera hosts (Rosenberg, 1934); nevertheless, the observed community of parasitoids corresponded to relatively specialized species. The dominant species *A. quadridentata* was reported on several Tortricidae species (Rosenberg, 1934) but preferentially parasitizes codling moth in agro-ecosystems dominated by apple orchards (Frilli, 1968). *P. vulnerator* is reported to have a broader spectrum of Lepidoptera hosts than *A. quadridentata* but was not dominant in the community. Finally, *P. tristis* was mainly reported as a hyperparasitoid (Bogenshütz, 1991). It develops on both *A. quadridentata* and *P. vulnerator*, but it reportedly displays a strong host preference for *A. quadridentata* (Diaconu *et al.*, 2000), which is in agreement with our cocoon inspections (data not shown). These species therefore schematically belonged to a vertical food web with four trophic levels: the apple plant, the codling moth pest, the *Ascogaster* primary parasitoid and the *Perilampus* hyperparasitoid. These trophic interactions should thus largely depend on the availability of the primary apple resource and on orchard disturbances (Polis *et al.*, 1997; Post, 2002).

The parasitoid community composition depended on local orchard hedgerow characteristics. Because only the effects of local variables and no landscape variables were detected, this study was in agreement with the designation of relative specialization for the parasitoid community of codling moth pests in apple orchards (Thies *et al.*, 2003; Rand *et al.*, 2012).

The hedgerow characteristics had a different impact on the primary parasitoids *vs.* hyperparasitoids. The proportions of the two primary parasitoids, *Ascogaster* and *Pristomerus*, were higher in the presence of windbreaks and/or spontaneous hedgerows around the orchard. The hyperparasitoid *Perilampus* was preferentially living in orchards without windbreaks or spontaneous hedgerows. Hedgerows are assumed to play an important role both in maintaining agro-system biodiversity (Andow, 1991; Maudsley, 2000) and enhancing pest control (Bianchi and Van Der Werf, 2003). Prior results from the same study area indicate that windbreaks and spontaneous hedgerows do impact the population structures of natural enemies within orchards (Debras *et al.*, 2008). The higher relative proportions of primary parasitoids to hyperparasitoids in the presence of hedgerows may have resulted from the direct effects of hedgerows on at least some species from the guild. Spontaneous hedgerows provide complementary food resources, and they moderate microclimatic conditions for natural enemies (Landis *et al.*, 2000). They also indirectly inform on the presence of local sustainable pollen and nectar resources, which usually improve the longevity and fertility of hymenoptera parasitoids (Schmale *et al.*, 2001; Géneau *et al.*, 2012). However, such food requirements would be particularly important for synovigenic species such as *Perilampus* in comparison to the proovigenic primary parasitoids *Ascogaster* and *Pristomerus,* which contradicted our results. Nevertheless, the lower proportion of *Perilampus* in the presence of hedgerows is consistent with the foraging behavior of *Perilampus* females that have a preference for heliophilous Asteraceae flowers (Clausen, 1940). The effects of hedgerows may also be mediated by their impact on pesticides. Windbreak hedgerows reduce pesticide drift and increase local pesticide efficiency (Ucar and Hall, 2001). Although we did not detect any significant pesticide treatment effects on community composition, hedgerows may explain differences in the relative proportions of primary and hyperparasitoids in the community because the higher trophic levels are more susceptible to disturbances related to pesticide treatments (Post, 2002; Thies *et al.*, 2003). Finally, the observed effects of hedgerows may not result from the direct effects of hedgerows on parasitoid species but from the modification of their interactions. The higher proportion of primary parasitoids in the presence of hedgerows is consistent with the fact that heterogeneity from the semi-natural habitat structures decreases the interaction strength between parasitoids and hyperparasitoids and reduces intra-guild predation (Polis et al., 1997; Janssen et al., 2007, Thomson and Hoffmann, 2013). Such negative interactions

may also reinforce the spatial segregation between primary parasitoid and hyperparasitoid species.

The parasitoid community composition differed among the five study years. Changes in trophic interactions may initiate temporal oscillations in the dynamics of both prey and predator populations (*e.g.,* Hanski *et al.,* 1993). Consequently, any perturbation that would have affected hyperparasitism, *e.g.*, that would have induced a shift in *Perilampus* preference for *Asgogaster* or *Pristomerus*, may explain the inter-annual variations that were observed in the community composition of codling moth parasitoids. However, a high number of *Pristomerus* emergences and a high proportion of *Pristomerus* in the parasitoid community were both observed in only two orchards during 2006. Changes in the environment or the agronomic practices of these particular orchards may also explain this variation in the parasitoid community. Such temporal variations have so far been rarely analyzed (see Gagic *et al*., 2012) and require further study in the future.

Variables that affected the rate of parasitism were not the same as those affecting the parasitoid community composition. In the present study, parasitism was observed in only a few orchards, and the parasitism rates were very low (<4.5% in average). These low rates were most likely due to pesticide applications that were detrimental to hymenoptera parasitoids in both organic and conventional orchards (Sterk *et al.*, 1999; Mates *et al.*, 2012). In comparison, the parasitism rate in codling moth larvae reached up to 40% in some non-commercial apple orchards (Diaconu *et al.*, 2000). However, parasitism rates in codling moth larvae remain globally low in comparison with other Lepidoptera pests (*e.g.,* Wilkinson et *al.* 2004) and may simply result from the low accessibility of the codling moth host by parasitoid females inside the apple fruit (Mills, 1993; Athanassov *et al.*, 1997).

Agronomic and semi-natural elements that may affect biocontrol at different spatial scales are difficult to disentangle because they often covary over landscapes, with organic fields often surrounded by more semi-natural elements or with organic fields aggregated over the landscape (*e.g.,* Gagic *et al.*, 2012). To attempt to overcome this difficulty, we first removed variables that were strongly correlated and then performed statistical analyses in two successive steps to account for the nested (local, then landscape) structure of the ecological processes (Tyson *et al.*, 2007; Ricci *et al.*, 2009; Franck *et al.*, 2011). Moreover, our approach was based on a multiple models inference to estimate the importance of each variable (Burnham and Anderson 2002). This approach offers superior performance compared with

predictions based on the selection of a single best model because different models may fit ecological data nearly as well (Whittingham *et al.*, 2006; see also Hegyi and Garamszegi, 2011).

Crop management practices were the only variables that significantly explained variations in codling moth parasitism rates. First, parasitism rates were significantly higher in organic than in conventional orchards. This result was somewhat expected because organic crop management is largely based on biological conservation pest control. However, in contrast to pest biocontrol by predators (*e.g.,* Winqvist *et al.* 2012), few published studies have investigated the impact of organic farming on pest parasitism, and their results were inconclusive; parasitoid abundance did not differ between organic and conventional cereal fields (Roschewitz *et al.*, 2005; Macfayden *et al.*, 2009; Gagic *et al.*, 2012). The large crop management effect on parasitism rates in apple orchards may have resulted from the particularly intensive pesticide situation of fruit production. Conventional apple growers have a very low tolerance level for fruit damage, and conventional orchards receive an average of 10.7 insecticide treatments per growing season, some of which are broad spectrum neurotoxic insecticides. It is important to note that organic orchards also had the highest density of organic orchards in the surrounding landscape (which led us to remove this last variable from the analyses, see materials and methods), and it is possible that the observed effect was at least partly due to landscape level organic production. This explanation would also be consistent with our observation that parasitism rates were also higher in landscapes including a low density of conventional apple orchards. Landscape level crop protection effects may result from pesticide drift from surrounding conventional orchards into semi-natural areas or into sampled orchards. Drift may be accentuated by strong winds in the study region and by the small orchard areas (average 0.85 ha). It may affect parasitoids directly or indirectly through the contamination of their food resources. It may also limit their movement between orchards in search of food or hosts (Brown and Glenn, 1999). Large areas of conventional orchards may therefore prove particularly hostile to parasitoids. In any case, these results underline that crop management practices impact parasitism rates over larger areas than the orchard level alone (Jonsson *et al.*, 2012). This finding also confirms previous analyses showing the impact of landscape scale crop protection practices in apple orchards on codling moth egg predation (Monteiro *et al.*, 2013) in the study area. The absence of semi-natural element effects on parasitism rates in the

highly treated apple orchards is additionally consistent with general expectations that establishing semi-natural elements has little impact on cleared landscapes because of the large scale absence of beneficial species (the 'regional pool species hypothesis' by Tscharntke *et al.*, 2005). The presence of semi-natural habitat significantly enhances parasitoid abundance in landscapes dominated by cereal crops (Thies *et al.*, 2005; Gagic *et al.*, 2012) that receive few insecticide treatments in comparison to orchards.

Theoretical models and experimental studies have demonstrated that not only habitat characteristics but also host density may significantly affect parasitoid dynamics (immigration and local population growth, respectively) and consequently influence the sustainability of the trophic interaction in a patch (*e.g.,* Comins and Hassell, 1996; Polis *et al.*, 1997; Borer *et al.*, 2007; Bezemer *et al.*, 2010). In the present study, the covariable that informed codling moth densities was never significant in the analyses that related parasitism rates with local and landscape characteristics. The density-dependence of host-parasitoid interactions are both spatially and temporally structured (Mills and Getz, 1996) and require study at the appropriate scales (Ray and Hastings, 1996). In the present study, codling moth density was estimated at the orchard level based on diapausing larvae, which did not necessarily correspond to the spatial and temporal scales at which the parasitoid may respond to host density. For example, parasitism experiments with *Mastrus ridibundus* (an Asian parasitoid that was introduced to America) showed that it was negatively correlated to the density of codling moth larvae at the tree level, although larval clustering enhanced the probability of parasitoid attack (Bezemer and Mills, 2001). It is possible that the observed parasitism would also respond at this scale. It is also possible that its statistical effect was partly masked by the crop management effect because the codling moth density itself depended on local crop management.

Interestingly, landscape variables that affected codling moth density (*i.e.*, the density of windbreak hedgerows and channel lengths) were not the same as the factors that affected parasitism, although a prior study showed the impacts of landscape level and windbreak hedgerow density on conventional orchards for codling moth density in the same area (Ricci *et al.,* 2009). The major difference is that we only considered orchards where codling moths were present, while the previous authors also considered orchards without codling moths. This discrepancy could therefore suggest that the landscape density of windbreak hedgerows and channel lengths may have a quantitative impact on codling moth density and

the density of conventional orchards would determine the presence of the pest (at a detectable level). Riparian margins host an important and diversified carabid fauna and can enhance functional biodiversity within the adjacent fields (Cole *et al.*, 2012). Numerous ground beetles are generalist predators, notably of the codling moth larvae in apple orchards (Boreau de Roincé *et al.*, 2012). Consequently, a possible explanation of the negative impact of channels on the density of codling moth larvae could result from their role in enhancing predation within orchards.

Finally, the parasitism in each orchard was higher when the hyperparasitoid *Perilampus* was present, which was not expected. In general, a positive relationship is expected between the diversity of natural enemies and biocontrol (*e.g.,* Cardinale *et al.*, 2003; Letourneau *et al.,* 2009), but this relation can be negative in cases where biodiversity is associated with negative interactions between species (intra-guild predation, hyperparasitism) as is the case here (*e.g.,* Finke and Denno, 2004; Gagic *et al.*, 2012). Two processes may explain how we found a positive relationship. First, according to a theoretical model of intra-guild predation, a stable equilibrium that includes the presence of facultative hyperparasitoids requires primary parasitoids to be more efficient than hyperparasitoids at suppressing the abundance of the basal pest resource (Borer *et al.*, 2007), which is in agreement with our observations. Second, in natural conditions, which are not likely at equilibrium, hyperparasitoids are expected to be present in favorable situations where all of the parasitoid species are favored because higher trophic levels are more sensitive to disturbances and a lack of resources (Polis *et al.*, 1997; Janssen *et al.*, 2007).

Although parasitism rates were low in the study area, our results provided some indications of orchard management methods for increasing biocontrol. First, codling moth parasitism could be increased by reducing the intensity of pesticide applications, a result that was consistent with other recent studies (*e.g.,* Jonsson *et al.*, 2012). However, the overall low observed parasitism rates and the absence of positive host density dependence suggest that, even in organic orchards, decreasing the pesticide intensity will not be sufficient to attain high parasitism rates, though it is necessary. Local production methods, the distributions of orchards and the intensity of protection practices in the landscape should most likely be largely modified such that parasitism rates can attain the higher values that were observed in some untreated orchards (up to 40%) and thereby enable parasitoids to efficiently impact codling moth populations. Second, growing windbreaks and/or spontaneous hedgerows on

the orchard borders may be advised because doing so appears to limit codling moth larval density (Ricci *et al.*, 2011) and is associated with higher proportions of the two primary parasitoids, possibly by providing food resources and/or alternative hosts for generalist parasitoids such as *P. vulnerator*. These modifications would also impact other pest or predator species such as carabid beetles and spiders that can predate codling moth larvae (Boreau de Roincé *et al.*, 2012). Furthermore, these predators may also reduce parasitoid populations through direct or incidental predation (Traugott *et al.*, 2012). Their implementation should thus be made with caution. In complement, the reinforcement of local parasitoid populations could be considered, especially of *A. quadridentata,* which is native to many different European regions. In any case, biocontrol by parasitoids can be one among a combination of methods used to achieve sustainable crop protection in orchards.

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Supplementary materials

Table 2.S1: Kruskal-Wallis rank sum test between quantitative and qualitative variables (kruskal-wallis khi-squared values and significance test: p<0.05*; p<0.01**; p<0.001 ***)

2006	*DensCM*	*Insect*	*Fung*	*Area*	*Shape*	*HPeri*	*Hdiv*	*HOI*	*Hlg*	*Abd*	*Conv*	*Org*	*Wood*	*Channel*
CropM	7.34 *	1.28	1.39	4.11 **	0.61	0.43	5.19 **	2.11	4.06 **	6.39 **	1.21	12.44 ***	3.03	0.55
WbH	0.11	0.55	2.14	2.76	3.49	7.35 *	0.01	1.50	0.05	0.51	0.08	1.12	0.11	3.90 **
SpH	1.65	1.70	1.35	0.02	0.91	6.16	9.06 *	6.00 **	1.24	0.55	3.96	1.68	2.26	3.75

2007	*DensCM*	*Insect*	*Fung*	*Area*	*Shape*	*HPeri*	*Hdiv*	*HOI*	*Hlg*	*Abd*	*Conv*	*Org*	*Wood*	*Channel*
CropM	3.24	4.13 *	0.14	6.12 *	0.09	0.05	3.84	2.21	2.86	6.47 *	2.75	12.45***	5.68 *	0.21
WbH	0.08	0.02	4.08 *	2.16	1.88	6.02 *	0.25	1.89	0.08	0.73	0.15	1.02	0.16	2.74
SpH	0.14	0.07	2.18	0.01	0.84	3.34	10.02**	4.01 *	2.37	2.20	2.51	1.75	2.27	2.56

2008	*DensCM*	*Insect*	*Fung*	*Area*	*Shape*	*HPeri*	*Hdiv*	*HOI*	*Hlg*	*Abd*	*Conv*	*Org*	*Wood*	*Channel*
CropM	2.66	0.09	17.69***	2.84	0.25	2.04	1.09	2.38	0.37	11.36**	0.23	14.42***	12.90***	0.14
WbH	0.22	0.01	2.01	3.56	0.68	1.06	0.11	0.59	0.05	0.23	0.19	4.24*	0.01	3.22
SpH	2.95	3.90*	3.39	0.02	3.04	0.57	10.21**	8.85**	6.47*	1.47	0.35	4.48*	9.11**	2.52

2009	*DensCM*	*Insect*	*Fung*	*Area*	*Shape*	*HPeri*	*Hdiv*	*HOI*	*Hlg*	*Abd*	*Conv*	*Org*	*Wood*	*Channel*
CropM	15.42***	16.47***	1.46	2.18	0.03	0.84	2.39	3.33	0.01	14.09***	0.33	6.77**	8.94**	0.00
WbH	0.27	0.45	0.03	0.51	0.14	1.15	0.75	0.86	0.09	1.92	0.51	2.67	0.053	1.37
SpH	1.79	5.29*	0.34	0.88	1.98	0.04	12.48***	4.90*	4.63*	2.81	0.88	2.90	8.26**	0.93

2010	*DensCM*	*Insect*	*Fung*	*Area*	*Shape*	*HPeri*	*Hdiv*	*HOI*	*Hlg*	*Abd*	*Conv*	*Org*	*Wood*	*Channel*
CropM	12.17***	17.26***	0.02	2.17	0.47	1.10	3.91*	2.73	1.03	6.73**	0.02	9.96***	6.52*	0.50
WbH	0.02	0.01	0.82	0.73	0.01	1.23	0.08	0.37	2.06	0.64	0.97	0.90	0.07	0.04
SpH	3.39	4.76*	2.69	2.40	2.31	0.64	11.39**	7.92**	4.94*	10.86***	1.11	6.78***	15.81***	3.79

Table 2.S2: Value and significance of Spearman correlation coefficients between quantitative explanatory variables; p<0.05 *; p < 0.01**; p<0.001 ***

2010	*Fung*	*Channel*	*Conv*	*Org*	*Wood*	*DensCM*	*HPeri*	*HOI*	*Hdiv*	*Area*	*Shape*	*Abd*	*Insect*
Channel	-0.27												
Conv	0.10	-0.09											
Org	0.04	0.25	0.02										
Wood	0.06	0.40*	-0.20	0.49**									
DensCM	0.25	0.08	0.23	0.45*	0.48**								
HPeri	-0.51**	-0.03	0.05	-0.10	-0.02	-0.22							
HOI	-0.02	-0.43*	0.14	-0.28	-0.55***	-0.51**	0.15						
Hdiv	-0.11	0.24	-0.13	0.27	0.39*	0.24	0.02	-0.27					
Area	-0.24*	-0.20	-0.17	0.09	-0.03	-0.07	0.24	-0.01	0.02				
Shape	0.03	0.11	-0.16	-0.02	-0.18	-0.18	0.11	0.25	0.16	-0.11			
Abd	-0.14	0.37*	0.32	0.43*	0.70***	0.40*	-0.09	-0.51**	0.20	0.29	-0.06		
Insect	0.30	0.06	0.19	0.27	0.38*	0.59***	-0.29	-0.44**	0.35	0.07	0.12	0.37*	
Hlg	-0.23	0.20	-0.23	0.13	0.06	-0.17	0.03	0.13	0.21	-0.09	0.45**	-0.03	0.10

Table 2.S2 (continued)

2009	*Fung*	*Channel*	*Conv*	*Org*	*Wood*	*DensCM*	*HPeri*	*HOI*	*Hdiv*	*Area*	*Shape*	*Abd*	*Insect*
Channel	-0.026												
Conv	-0.27	0.01											
Org	0.03	-0.05	-0.15										
Wood	0.39*	0.47*	-0.07	0.26									
DensCM	0.48**	0.17	-0.22	0.34	0.57*								
HPeri	-0.38	-0.21	-0.03	-0.24	-0.202	-0.32							
HOI	-0.26	-0.27	0.19	-0.19	-0.57**	-0.46	0.28						
Hdiv	0.01	0.22	-0.32	0.15	0.52**	0.24	0.069	-0.21					
Area	-0.17	-0.32*	-0.277	0.04	-0.17	-0.08	0.11	-0.02	0.17				
Shape	-0.09	0.21	-0.28	-0.04	-0.08	-0.09	0.20	0.22	0.25	-0.06			
Abd	0.239	0.22	0.07	0.55**	0.49**	0.42*	-0.08	-0.17	0.06	0.07	-0.27		
Insect	0.59***	0.06	-0.16	0.32	0.52**	0.76***	-0.14	-0.44*	0.17	0.04	0.17	0.45*	
Hlg	0.06	0.10	-0.21	-0.04	-0.06	0.18	-0.02	0.14	0.36*	-0.05	0.55***	-0.21	0.21

Table 2.S2 (continued)

2008	*Fung*	*Channel*	*Conv*	*Org*	*Wood*	*DensCM*	*HPeri*	*HOI*	*Hdiv*	*Area*	*Shape*	*Abd*	*Insect*
Channel	0.01												
Conv	0.17	-0.29											
Org	0.59***	-0.01	0.10										
Wood	0.57***	0.38*	-0.05	0.47**									
DensCM	0.34*	-0.057	-0.06	0.26	0.08								
HPeri	-0.32	-0.08	-0.04	-0.24	-0.22	-0.178							
HOI	-0.10	-0.24	0.27	-0.19	-0.15	-0.37*	-0.04						
Hdiv	0.24	0.06	-0.06	0.18	0.38*	0.21	-0.01	-0.16					
Area	0.17	-0.27	-0.23	0.05	0.06	-0.01	0.02	-0.11	0.13				
Shape	-0.01	0.22	-0.26	-0.18	-0.09	-0.08	0.18	-0.01	0.12	-0.28			
Abd	0.47	0.20	0.12	0.55***	0.45**	-0.01	-0.22	-0.01	-0.03	0.16	-0.25		
Insect	0.11	-0.50***	0.19	0.10	-0.24	-0.07	-0.09	0.01	-0.29	0.32*	-0.03	0.19	
Hlg	0.19	0.05	-0.14	-0.02	-0.01	0.06	0.04	-0.03	0.35*	-0.10	0.48**	-0.12	-0.10

Table 2.S2 (continued)

2007	*Fung*	*Channel*	*Conv*	*Org*	*Wood*	*DensCM*	*HPeri*	*HOI*	*Hdiv*	*Area*	*Shape*	*Abd*	*Insect*
Channel	-0.235												
Conv	-0.047	-0.259											
Org	0.183	0.004	-0.133										
Wood	0.097	0.30*	-0.135	0.31*									
DensCM	-0.019	-0.078	-0.119	0.266	0.036								
HPeri	-0.188	-0.139	0.072	-0.044	-0.32*	-0.155							
HOI	0.088	-0.270	0.46**	-0.209	-0.175	-0.148	-0.049						
Hdiv	-0.017	0.119	0.031	0.33*	-0.015	0.095	-0.004	-0.061					
Area	0.181	-0.235	-0.228	0.252	0.120	0.222	-0.098	0.010	0.132				
Shape	-0.230	-0.049	-0.107	-0.078	-0.44**	0.034	0.228	0.000	0.121	-0.053			
Abd	0.044	0.127	-0.110	0.43**	0.45**	0.047	-0.056	-0.085	0.144	0.112	0.046		
Insect	0.30*	-0.188	-0.118	0.40**	0.144	0.131	-0.046	-0.135	-0.061	0.51***	-0.017	0.274	
Hlg	-0.031	-0.34*	-0.090	0.129	-0.38**	0.073	0.158	0.038	0.234	0.060	0.45**	0.081	0.037

Table 2.S2 (continued)

2006	*Fung*	*Channel*	*Conv*	*Org*	*Wood*	*DensCM*	*HPeri*	*HOI*	*Hdiv*	*Area*	*Shape*	*Abd*	*Insect*
Channel	-0.45**												
Conv	-0.132	-0.196											
Org	0.019	0.057	-0.075										
Wood	0.018	0.28*	-0.155	0.278									
DensCM	0.085	-0.094	-0.46**	0.164	0.066								
HPeri	0.118	-0.170	-0.090	-0.062	-0.223	0.203							
HOI	-0.007	-0.232	0.419	-0.201	-0.119	-0.56***	-0.075						
Hdiv	-0.30*	0.140	0.065	0.32*	-0.055	-0.029	-0.070	-0.077					
Area	0.31*	-0.28*	-0.215	0.151	0.093	0.084	-0.103	0.031	0.079				
Shape	-0.199	-0.022	-0.153	-0.095	-0.48**	0.100	0.100	-0.067	0.150	-0.117			
Abd	-0.047	0.158	-0.058	0.47**	0.35*	0.210	-0.087	-0.058	0.142	0.026	0.039		
Insect	0.48***	-0.40**	-0.052	0.212	0.112	0.184	0.042	-0.072	-0.38**	0.272	-0.023	0.231	
Hlg	-0.059	-0.30*	-0.036	0.160	-0.41**	0.29*	0.080	-0.040	0.265	0.127	0.44**	0.021	0.127

Table 2.S3: Independence Chi-square test between qualitative variables; Chi-square values and significance test; p<0.05 *; p < 0.01**; p<0.001 ***

	2006		2007		2008		2009		2010	
	CropM	*WbH*	*CropM*	*WbH*	*CropM*	*WbH*	*CropM*	*WbH*	*CropM*	*WbH*
WbH	0.41		1.09		2.77		2.33		0.63	
SpH	6.01*	0.343	6.43*	0.35	10.10**	0.47	8.93**	1.245	10.55**	2.03

Table 2.S4: Multi-model parameter estimates and associated P-values for the model explaining variation in parasitism rate with only local variables.

Factor	Estimate	Std. Error	z value	P-values
DensCM	0.012	0.010	1.150	0.250
Year 2007	-0.033	0.446	0.074	0.941
Year 2008	0.486	0.439	1.107	0.268
Year 2009	0.598	0.436	1.371	0.170
Year 2010	-0.301	0.437	0.688	0.491
CropM	1.336	0.3770	3.545	$3.93\ 10^{-4}$ ***
WbH	-0.370	0.326	1.134	0.257
SpH	0.675	0.320	2.107	0.035 *
Insect	-0.058	0.028	2.025	0.043 *
Fung	-0.010	0.028	0.355	0.722
Hperi	-0.606	0.623	0.973	0.331
Shape	-0.008	0.131	0.059	0.953
Area	[#]			

[#] no coefficient estimate for variable '*Area*' because this variable was not present in models with ΔAICc <20.

Chapitre 3
Interactions intra- et inter-spécifiques au sein de la communauté du carpocapse du pommier et de ses parasitoïdes

Dans ce chapitre, nous avons étudié le parsitisme par *A. quadridentata* pour différentes souches de caropocapse, ainsi que les interactions entre les espèces de parasitoides s'attaquant au carpocapse.

Les résultats présentés dans ce chapitre s'appuient en partie sur un manuscrit en préparation pour *Molecular Ecology Resources.*

1. Interactions carpocapse-parasitoïde

1.1. Effet de la résistance au virus de la granulose sur le parasitisme du carpocapse

L'utilisation massive des insecticides contre les ravageurs a conduit au développement de résistances à ces molécules. Les gènes responsables de la résistance aux insecticides présentent un coût à la fitness qui reste mal compris. Berticat *et al.* (2004) a montré que la présence de ces gènes de résistance augmente la probabilité de prédation du moustique *Culex pipiens*.

Dans ce contexte nous avons voulu savoir s'il y a interaction entre la résistance au virus de la granulose et le parasitisme du carpocapse. Les souches résistantes de carpocapse sont-elles plus parasitées que les souches sensibles?

Nous avons exposé des œufs de carpocapse d'une souche résistante au virus de la granulose (RGV) et d'une souche sensible (SV) à *Ascogaster quadridentata*. Dans une boîte de Petri

nous avons placé deux carrés de papier renfermant 10 œufs de carpocapse de part et d'autre du centre de la boîte où nous avons rajouté une femelle d'*A. quadridentata*. Nous avons laissé parasiter les œufs par la femelle pour environ une heure. Deux modalités ont été testées. Dans la première, nous avons exposé dans une même boîte d'un côté des œufs de la souche résistante (RGV), de l'autre des œufs de la souche sensible (SV) pour analyser le choix de la femelle parasitoïde. Nous avons effectué trois essais de cette modalité de choix. Dans la deuxième, nous avons exposé des œufs de la même souche (RGV ou SV) dans une même boîte. Trois essais ont été effectués avec la souche résistante et deux avec la souche sensible. Nous avons observé et noté le comportement de la femelle. Des carrés d'œufs témoins sont placés dans les mêmes conditions de l'expérience. Nous avons placé six carrés d'œufs de la souche résistante et cinq de la souche sensible. Nous avons élevé par la suite les larves de carpocapse issues des œufs testés jusqu'à l'émergence. Le nombre d'adultes de carpocapse et du parasitoïde ainsi que le nombre de morts sont notés pour chaque essai. Nous avons calculé les taux de parasitisme pour chacune des souches étudiées suivant la modalité avec ou sans choix de souches. Il s'agit du rapport du nombre de parasitoïdes sur le nombre total d'adultes (parasitoïde et carpocapse) qui ont émergé. Nous avons calculé parallèlement la mortalité des souches parasitées (avec et sans choix) et témoins. Le taux de mortalité est le rapport du nombre de morts sur le nombre total d'œufs utilisés. Nous avons analysé ces données à l'aide de tests de Khi2.

Les observations au cours de l'exposition des œufs de carpocapse à *A. quadridentata* dans les boîtes avec choix de la souche ont montré que le parasitoïde explorait les œufs et exhibait un comportement de ponte sans différence apparente sur les deux patchs de souches différentes. Les taux de parasitisme par *A. quadridentata* sont élevés (Tableau 3.1). Les tests de Khi2 ont montré qu'il n'y a pas de différence significative de la mortalité des témoins entre les souches RGV et SV (Khi^2 = 2,95, df = 1, p-value = 0,086). Le parasitisme par *A. quadridentata* n'induit pas une mortalité précoce du carpocapse pour les souches RGV parasitées (pour RGV avec choix de souches: Khi^2 = 0,31, df = 1, p-value = 0,57 ; pour RGV sans choix de souches: $Khi^2 = 6 \times 10^{-04}$, df = 1, p-value = 0,98) et pour les souches SV parasitées (pour SV avec choix de souches: Khi^2 = 0,03, df = 1, p-value = 0,86 ; pour SV sans choix de souches: $Khi^2 = 9 \times 10^{-04}$, df = 1, p-value = 0,97). Il n'y a pas de différence significative des taux de parasitisme entre les souches résistantes et sensibles (pour les

modalités avec choix de souches: $Khi^2 = 5,2 \times 10^{-03}$, df = 1,p-value = 0,94 ; pour les modalités sans choix de souches: $Khi^2 = 0$; df = 1, p-value = 1). De plus, il n'y a pas de différence significative des taux de parasitisme entre les œufs d'une même souche de modalités avec et sans choix de souches (pour RGV: $Khi^2 = 1,3 \times 10^{-03}$; df = 1; p-value = 0,97; pour SV: $Khi^2 = 0$; df = 1; p-value = 1).

	Taux de parasitisme	Taux de mortalité
RGV parasités avec choix	1 (25/25)	0,58 (35/60)
RGV parasités sans choix	0,89 (17/19)	0,68 (41/60)
SV parasités avec choix	0,9 (28/31)	0,48 (29/60)
SV parasités sans choix	0,95 (19/20)	0,5 (20/40)
RGV témoins	X	0,72 (43/60)
SV témoins	X	0,54 (27/50)

Tableau 3.1: Taux de parasitisme par *A. quadridentata* et taux de mortalité des souches résistantes de carpocapse au virus (RGV) et des souches sensibles (SV) parasitées avec et sans choix de souches et témoins.

Ces résultats suggèrent qu'il n'y a pas un coût de parasitisme supplémentaire de la résistance au virus de la granulose du carpocapse des pommes.

1.2. Détection précoce du parasitisme dans les stades immatures du carpocapse

Nous avons voulu savoir à quel stade du développement du carpocapse pourrait-on détecter le parasitisme par des méthodes de biologie moléculaire. Nous avons alors exposé des œufs de carpocapse au parasitisme par *Ascogaster quadridentata* au laboratoire pour estimer la détection du parasitisme à différents stades de développement du carpocapse à l'aide d'une méthode de PCR-RFLP et des marqueurs spécifiques.

Nous avons parasité dix lots de 35 œufs de carpocapse par des femelles d'*A. quadridentata*. Pour chaque lot, une femelle parasitoïde est placée dans une boîte de Petri avec les œufs et laissée pour parasiter pour deux heures à 25°C. Nous avons retiré la femelle et prélevé 5 œufs directement. Par la suite, nous avons prélevé 5 néonates, 5 jeunes larves et 5 larves âgées respectivement après une, deux et trois semaines de développement. Les échantillons

récupérés sont tués et conservés à -20°C. Nous avons suivi les émergences du reste des larves. Afin que les larves complètent leur développement, nous les avons placées dans des piluliers individuels avec 16 h de jour sur un milieu nutritif à base de soja (Stonefly Heliothis diet, Ward's, NY) préparé dans une solution aqueuse avec 0,2 % d'acide acétique. Nous avons utilisé cinq lots de 35 œufs non parasités comme témoins. Ces lots ont été suivis dans les mêmes conditions que les œufs parasités pour estimer la mortalité du carpocapse à chaque stade de développement en l'absence de parasitisme. L'ADN de chaque échantillon (parasité et témoin) a été extrait à l'aide d'un kit d'extraction (DNeasy Tissue kit, Qiagen) et utilisé pour la détection du parasitisme par *A. quadridentata*. Nous avons effectué des tests de Khi^2 pour chaque stade de développement du carpocapse pour comparer la mortalité entre les individus parasités et témoins et pour estimer la détection du parasitisme entre la méthode de PCR-RFLP et la méthode des marqueurs spécifiques.

En moyenne, 85% des œufs de carpocapse étaient parasités (57 *A. quadridentata* ont émergé sur un total de 67 adultes émergeants sur les dix lots d'œufs parasités). La PCR-RFLP a surtout permis de détecter les parasitoïdes dans les larves âgées du carpocapse. La méthode des marqueurs spécifiques a permis la détection de parasitoïdes dans les œufs et les jeunes larves de carpocapse (Tableau 3.2).

Stade	Age	Effectif	Méthode RFLP	Méthode spécifique
Œuf	0	48	0,00	0,90
Néonate	1	44	0,09	0,89
Jeune larve	2	50	0,07	0,80
Larve âgée	3	41	0,76	0,88

Tableau 3.2: Détection du taux de parasitisme par *A. quadridentata* à différents stades de développement du carpocapse (âge en semaine) avecdeux méthodes différentes de biologie moléculaire (PCR-RFLP et PCR utilisant des amorces spécifiques d' *A. quadridentata*).

2. Interactions entre les parasitoïdes du carpocapse

Pour assurer le succes des programmes de lutte biologique il est important de connaitre l'ecologie des parasitoides, de leurs hotes ravageurs et leurs interactions dans les agroecosystemes (Van Driesche and Bellows, 1996). La biologie et les interactions entre les parasitoides du carpocapse du pommier sont encore assez mal connues. Pour davantage les renseigner nous avons développé des marqueurs moléculaires permettant d'apréhender directement le parasitisme dans la larve du carpocapse et ainsi évider l'élevage des chenilles jusqu'à l'émergence des adultes ou bien de fastidieuse dissection larvaire pour apréhender plus précosement le parsitisme (Day, 1994). Une première méthode basée sur la restriction par AluI d'une portion du gène mitochondrial *COI* amplifié par PCR s'avére interessante pour déterminer globalement les parasitoides adultes qui émergent des larves de carpocapse (8 espèces sur les 10 observées). L'utilisation d'amorces PCR spécifiques des quatre espèces de parasitoïdes les plus communes s'attaquant au carpocapse permet de les détecter à un stade précose de dévelopement de leur hôtes. Les estimations de taux de parasitisme ainsi obtenu étaient comparables à celles révellées par des méthodes plus classiques (de l'ordre de 60% dans un verger non-traité de pommiers). Cette approche moléculaire permet en outre d'appréhender les cas de multiparasitisme dans les larves de carpocapse. Il apparait ainsi que le parasitisme du carpocapse dans une parcelle non-traitée de pommiers est essentiellement gouverné par le braconide *A. quadridentata* (92% des *primo* infestion), lequel est surparasité par *P. vulnerator* et *P. tristis* (respectivement 75% et 84% du parasitisme impliquant ces deux parasitoïdes secondaires).

Le manuscript suivant présente les différentes approches moléculaires que nous avons développées pour étudier le parasitisme du carpocapse ainsi que les expérimentions conduites en laboratoire et sur le terrain pour évaluer la pertinence de ces méthodes.

Manuscrit en préparation pour Molecular Ecology Ressources

Molecular tools for the detection and the identification of Hymenoptera parasitoids in tortricid pests

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Running title: Identification of the codling moth parasitoids

Abstract

Biological control requires specific tools for accurate detection and identification of natural enemies, in order to estimate variations in their density according to changes in environmental conditions or agricultural practices. Here, we developed two methods of detection either based on PCR-RFLP with universal primers or PCR with specific DNA primers to identify within an apple pest, *Cydia pomonella* (Lepidoptera: Tortricidae), four frequent Hymenoptera parasitoids in Europe: *Ascogaster quadridentata* (Braconidae), *Pristomerus vulnerator* (Ichneumonidae), *Trichomma enecator* (Ichneumonidae), *Perilampus tristis* (Perilampidae). Both methods were designed following alignment of comparable DNA sequences in the *COI* mitochondrial gene for a range of parasitoids that emerged from *Cydia* and *Grapholita* larvae (102 parasitoids, nine species) and a range of potential tortricid hosts (40 moths, five species) damaging fruits. The PCR-RFLP method (restriction of a 482 bp fragment by AluI) was very powerful to identify adult parasitoids and their hosts, but fail to detect parasitoid larvae in eggs or in young *C. pomonella* caterpillars. The PCRs based on specific primers proved to be also species specific, amplifying DNA fragments of different lengths (131 to 463 bp) for each four hymenoptera parasitoids, and were less sensitive to low DNA amount than the PCR-RFLP method. Molecular estimations of parasitism rates in a natural population did not differ from traditional estimations based on *C. pomonella* caterpillar rearing (about 60% in a non-treated apple orchard). Furthermore, these DNA-based techniques provide information about within-host parasitoid assemblage in several important tortricid fruit pests.

1. Introduction

With the shift towards a reduced reliance on external inputs in agriculture, identifying management options that enhances pest control services has become a critical issue (Scherr and McNeely 2008). The successful implementation of pest management programs requires a better understanding of the ecology behind the provision of ecosystem services (Kremen and Ostfeld 2005) and methods to detect and identify the biodiversity linked with these services. Such methods are crucial when choosing and selecting biocontrol candidates (Hoelmer and Kirk 2005) and when evaluating the efficiencies of biocontrol releases (Van Driesche and Bellows 1996) or the impact of changes in agricultural practices (Tylianakis, Didham *et al.* 2008). They are also very helpful to quantify trophic interaction within the community of natural enemies and the impact of the food web structure on the control of herbivore pests (Montoya, Pimm *et al.* 2006)

The rapid extension of DNA barcode references during the last decade (Hebert, DeWaard *et al.* 2010) favoured the development of numerous DNA-based techniques analysing predation and parasitism in

arthropod pests (Symondson 2002). These techniques have been used to reveal prey in the gut or the faeces of predators (King, Read *et al.* 2008), to determine the host from which emerged parasitoid adult (Rougerie, Smith *et al.* 2011) or to detect parasite larvae inside their host (Agusti, Bourguet *et al.* 2005). They allow accurate estimation of the parasitism rates in field including identification of multi- and hyper-parasitism (Traugott, Bell *et al.* 2008) and they are at least as efficient as traditional method of detection, which requires insect rearing during long periods and careful host dissection (Day 1994).

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is one of the major insect pests in temperate regions, damaging several cultivated fruit trees, notably apple orchards (Shel'Deshova 1967). At early spring, adults rise from overwintering larvae and reproduce. Free neonate larvae penetrate into fruits causing damage. At the end of their development, the larvae leave the fruits and, depending on temperature and photoperiod conditions, either pupate to give adults or enter into diapause. Depending on the latitude, the codling moths complete one to four generations per year. Insecticide treatments remain the major means used to maintain codling moth populations at a low level. As a consequence of these treatments, *C. pomonella* developed resistances to many chemical and biological insecticides (Asser-Kaiser, Fritsch *et al.* 2007; Reyes, Franck *et al.* 2007; Franck, Siegwart *et al.* 2012). This rises up the necessity to develop alternative methods and to diversify management programs to control this pest.

Hymenoptera parasitoids, because of their relatively high specificity to a target species have long been recognized as important agents in the biological control of insect pests in agriculture (De Bach and Rosen 1991; Symondson 2002). Parasitism of the tortricid pests remain globally low (usualy less than 5% in commercial crops), but several hymenoptera parasitoids have been described to parasite various codling moth instars (for a review, see Mills 2005). In Europe, the three most abundant parasitoids that emerged from collections of codling moth diapausing larvae in apple orchards corresponded to: *Ascogaster quadridentata* (Braconidae), *Pristomerus vulnerator* (Ichneumonidae) and *Perilampus tristis* (Perilampidae) (Rosenberg 1934; Athanassov, Charmillot *et al.* 1997; Diaconu, Pisica *et al.* 2000; Maalouly, Franck *et al.* 2013). *Bassus rufipes* (Braconidae) and *Trichomma enecator* (Ichneumonidae) were also frequently recorded, notably from larvae collected on walnut trees (Mills 2005). The pupal or prepupal parasitoids *Dibrachys cavus* (Pteromalidae), *Hyssopus palidus* (Eulophidae), *Mastrus rufipes* and *Liotryphon caudatus* (Ichneumonidae) were infrequently observed (Putman 1963; Mills 2005).

The biology and the ecology of these parasitoids remain largely unknown. The braconid wasp, *A. quadridentata,* is an ovo-larval endoparasitoid specialized on the tortricid moths (Athanassov,

Charmillot *et al.* 1997). The braconid female is ready for oviposition soon after emergence (proovigenic). The parasitized codling moth larvae are about one-third weight of non-parasitized larvae (Suckling, Gibb *et al.* 2002). It is killed at the fourth instar by the parasitoid larva that finalizes its development as an ectoparasite within the codling moth cocoon (Clausen 1940). The ichnomonid wasp, *P. vulnerator* is also an endoparasitoid. The ichnomonid female deposits one egg in the young host larva after it entered into the fruit. The parasitoid larva remains in latent state until the codling moth larva leaves the fruit and builds shelter for pupation (Coutin 1974). The parasitoid larva leaves then the host's body and weaves a hard elongated cocoon to finalize its development as an ectoparasite (Diaconu, Pisica *et al.* 2000). The species is reported as stenophagous developing on several Lepidoptera hosts (Rosenberg 1934; Diaconu, Pisica *et al.* 2000). Cases of super-parasitism and multi-parasitism were reported for some *Pristomerus* species (Schröder 1974) and *P. vulnerator* is likely to be mainly a cleptoparasite. The chalcid wasp, *P. tristis* was described as a hyperparasitoids (Tripp 1962). It parasitizes several tortricid primary parasitoids, but preferentially the braconids (Bouček 1977; Bogenschütz 1991; Diaconu, Pisica *et al.* 2000). A planidium hatches from each *Perilampus* egg and actively search for a caterpillar host (Clausen 1940). The planidium stay latent within the moth larvae. It finally penetrates within the body of its final host when this one becomes ectoparasite.

Here, we report the development of specific primers and a PCR-RFLP method from the COI mitochondrial region to be used in the detection and identification of the most abundant parasitoids in the codling moth larvae. We tested both methods for their ability to detect parasitoid at different stage of their development within the codling moth and their reliability for parasitism evaluation in natural *C. pomonella* populations.

2. Material and Methods

2.1. Biological material used as references

The biological material used to set up the molecular identification tools encompassed 102 hymenoptera parasitoids that emerged from tortricid larvae (92 and 10 larvae were recognized as *C. pomonella* and *G. molesta,* respectively) and 40 non-parasitized tortricids moths considered as potential host species for these parasitoids (Appendix 3.S1). This material was collected in various orchards in western and central Europe between 2002 and 2013. One half of the analysed parasitoids was part of a previously published study on the codling moth parasitism in the Basse-Durance Valley, France (Maalouly, Franck *et al.* 2013). The other half was from larvae collected in organic or non-

treated orchards in France (Rhône-Alpes, Languedoc, Midi-Pyrénée and Normandie regions), the Czeck Republic (Hradec-Králové), and Germany (Bade-Wurtemberg). These parasitoids were morphologically identified using different keys (Goulet and Huber 1993; Athanassov, Charmillot *et al.* 1997) and descriptions (Argaman 1990; Argaman 1991; Simbolotti and van Achterberg 1992; Darling 1996). The 40 tortricid moths emerged from larvae collected on apple (*C. pomonella*, *G. molesta, G. lobarzewkii*), plum (*G. funebrana*) and chestnut (*C. splendana)* trees from the same French regions as the collected parasitoids. Tortricid adults were identified according to male genitalia inspection (Chambon 1986).

2.2. DNA sequencing

DNA barcodes of the 102 hymenoptera parasitoids and of the 40 tortricid moths were obtained with the universal primers *LCO* and *HCO* (Folmer, Black *et al.* 1994). BLASTs were performed to check our morphological identification on the Barcode of Life Data System (BOLD, release 5.25, July 2014) (Ratnasingham and Hebert 2007). Analyses of the DNA sequences were performed with MEGA, version 5.2.2 (Tamura, Peterson *et al.* 2011). Parasitoid and moth nucleotidic sequences were aligned separately using ClustalW, version 2.0 (Larkin, Blackshields *et al.* 2007) and neighbor-joining trees (Saitou and Nei 1987) were computed with the Kimura 2-parameter distance (Kimura 1980) to illustrate nucleotidic diversities within and among parasitoid and moth taxa.

2.3. Diagnosis based on a PCR-RFLP approach

A central part of the COI mitochondrial gene was amplified (476-482 bp) with the primers *Cat0* and *Nancy* labeled with HEX and ATTO565 dyes, respectively (Table 3.3(1)). Amplifications were performed in 12 µL reaction volumes containing 10 mM Tris-HCl, pH 9, 50 mM KCl, 200 µM each dNTP, 0.4 µM each primer, 1.5 mM $MgCl_2$, one unit Taq DNA polymerase (Promega), 0.1 mg/ml BSA with two µL of DNA template. After an initial denaturing step of 2 min at 94°C, 30 cycles were performed consisting of 30 s at 95°C, 45 s of annealing at 48°C and 45 s of elongation at 72°C. Amplified fragments were subsequently cut overnight at 37°C in 20 µL reaction volumes with one unit of the AluI (Biolab) restriction enzyme (AG^CT). Digested fragments were visualized after electrophoresis on an ABI3730 DNA sequencer. This method was developed to co-identify the parasitoids and their hosts in a single PCR run.

Table 3.3 (1). Description of the PCR primers used to amplify different part of the COI gene for the identification of the codling moth parasitoids. Primers were named according to the nomenclature of Simon et al (1994) in Drosophila melanogaster and their alias (in brackets). DNA barcodes were obtained with LCO and HCO. The PCR-RFLP method was conducted based on amplifications with Cat0 and Nancy. Specific parasitoid amplifications were conducted using LCO as forward primer and different specific reverse primers: Asco, Pristo, Tricho, and Peri.

Primers	Sens	5'-3' Sequences	Specificity
C1-J-1464 (LCO)	Forward	GGTCAACAAATCATAAAGATATTGG	Universal
C1-N-2172 (HCO)	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	Universal
C1-J-1757 (Cat0)	Forward	CCTGATATAGCATTTCCTCG	Universal
C1-N-2191 (Nancy)	Reverse	CCCGGTAAAATTAAAATATAAACTTC	Universal
AQ-C1-N-1595 (Asco)	Reverse	ATCATTTCCTAAATAAGAAGTAATTG	Ascogaster
PV-C1-N-1811 (Pristo)	Reverse	TCCTACTCCTTGATTAGTAATTGATC	Pristomerus
TE-C1-N-1927 (Tricho)	Reverse	ATAGCTCCTATAATTGATGATGATC	Trichomma
PT-C1-N-1588 (Peri)	Reverse	CCAATTAATGAACCAGGACATC	Perilampus

2.4. Diagnosis based on specific mitochondrial primers

Specific PCR primers in the *COI* mitochondrial gene were developed for each of the most abundant parasitoid on *C. pomonella*: *A. quadridentata, P. vulnerator, P. tristis* and *T. enecator.* The primers were designed *in silico* with primer3 (Untergrasser, Cutcutache *et al.* 2012) to perform with *LCO.* First, we assessed the specificity of each primer by analyzing site by site similarities with the barcodes of other parasitoid and moth species. Second, we designed the primers with the lowest similarities with all the other species in their 3' part. Finally, we conducted independent PCR tests with each designed primer on moth and parasitoid specimens from the reference set to estimate their reliabilities to amplify the parasitoid species for which they were designed. For each specific primer, PCRs were performed in the same condition as for the PCR-RFLP method, but using 54°C as annealing temperature and with a PCR buffer including 20 mM ammonium sulfate. The *LCO* primer was labeled with FAM dye to visualize the amplified fragments. A PCR test was assumed as positive

for any amplification at the expected size that was higher than 5% of the signal in the parasitoid controls. This method was developed for a more accurate estimation of the parasitism in immature codling moth.

2.5. Early parasitism detection in immature codling moth

First, codling moths were parasitized in laboratory condition to estimate parasitism detection at various codling moth instars with both the PCR-RFLP and the primer specific approaches. We infested 10 sets of 35 codling moths eggs with *A. quadridentata*. For each set, a virgin female wasp was installed in a Petri's dish arena and left with the eggs for approximately 2 hours at 25°C. Then, we redrew the parasitoid and a sample of 5 eggs was immediately collected. We subsequently collected 5 neonates, 5 young, and 5 aged larvae, after respectively one, two and three weeks of development. The collected samples were killed and individually conserved at -20°C. Finally, we monitored the emergence of the remaining materials. In order to complete their development, the collected larvae were placed in individual vials at 25°C with a 16h day length with nutritive soybean instant diet (Stonefly Heliothis diet, Ward's, NY) prepared in aqueous solution with 0.2% acetic acid. Similarly, we used five sets of 35 non-infested eggs as control. These sets were monitored in the same conditions as the infested eggs to estimate mortality of each codling moth instars in absence of the parasitoid. DNA of each codling moth instar – infested and non-infested – was extracted using the DNeasy Tissue Kit (Qiagen) and used for parasitism detection by *A. quadridentata*. For each *C. pomonella* instar, Pearson's chi-square tests were performed with R (*p*-value simulated based on 2000 replicates) to respectively compare *i)* mortality between parasitized and control samples and *ii)* parasitism detection between the PCR-RFLP and the primer specific methods.

2.6. Parasitism detection in natural codling moth populations

Second, we analyzed a naturally infested codling moth population in order to compare parasitism estimates using both molecular and traditional approaches. Mature codling moth larvae were collected in November 2009 with band traps in a non-treated apple orchard (Gotheron, France, 44°58'21.13"N, 4°55'38.70"E). The collected larvae were divided in two sets according to their weight (upper and lower than 30 mg). Half of the larvae of each set were directly killed to estimate parasitism only based on the molecular methods. The other half was stored in individual vials in an outdoor insectarium during the winter and left to naturally emerge (Day 1994). The emerging parasitoids were morphologically identified (Athanassov, Charmillot *et al.* 1997). Their cocoons and those of their host were also carefully inspected to identify primary and secondary hosts (Diaconu, Pisica *et al.* 2000). Each sample (caterpillars, parasitoids and their host cocoons) were conserved in 90% ethanol before DNA extraction. DNA of each sample was extracted separately using 200 µL of

10% Chelex 100 (Bio-Rad) resin solution (Walsh, Metzger *et al.* 1991). The PCR-RFLP method was used to determine the adult parasitoid and their tortricid host. PCRs were also performed with each of the four parasitoid specific primers to identify parasitized cocoons and caterpillars and subsequently determine the parasitoid taxa. Parasitism rates were estimated as the ratio of emerging parasitoids on the total of emerging moths and parasitoids (traditional method) or as the ratio of larvae detected positive for at least one PCR test (molecular method). Pearson's chi-square tests were performed to respectively compare *i)* estimates of parasitism rates and *ii)* distributions of the species within the parasitoid community both between the small and large larvae sets and between the traditional and the molecular approaches.

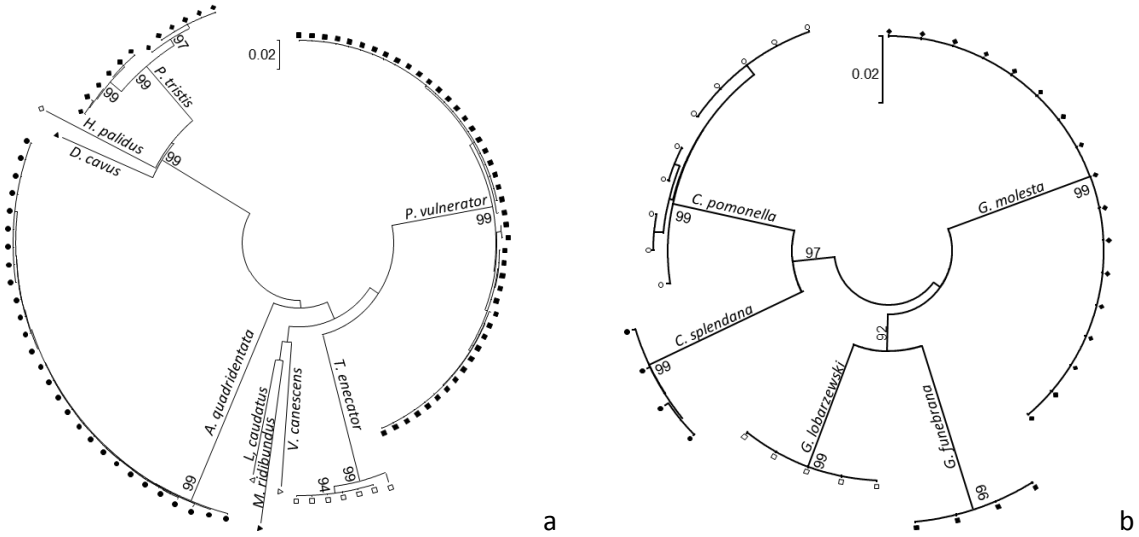
3. Results

3.1. DNA barcodes

The 102 sequenced parasitoids were identified as *P. vulnerator* (Ichneumonidae: Cremastinae; 45 specimens), *A. quadridentata* (Braconidae: Cheloninae; 28 specimens), *T. enecator* (Ichneumonidae: Anomaloninae; 7 specimens), *P. tristis* (Perilampidae; 12 specimens), *B. rufipes* (Braconidae: Agathidinae; 5 specimens), *H. palidus* (Eulophidae), *D. cavus* (Pteromalidae), *M. ridibundus* (Ichneumonidae: Cryptinae), *L. caudatus* (Ichneumonidae: Pimplinae) and *Venturia canescens* (Ichneumonidae: Campopleginae), one specimen for each of these five last species (Appendix S1). The five individuals recognized as *Bassus rufipes* had an identical non-coding *COI*-like sequence (KP402060-KP402063). The 97 remaining specimens had *COI* coding mitochondrial sequences (KP072518-KP072654). All the DNA sequences matched with *COI* sequences recorded in BOLD with similarities upper than 90% (Appendix S1). At the species level, BOLD confirmed the identifications of all the *A. quadridentata*, *D. cavus*, *L. caudatus*, and *V. canescens* specimens (DNA similarities upper than 99.5% with identified sequences). The *T. enecator* DNA sequences matched with non-identified Ichneumonidae (similarities upper than 99.2%). The *M. ridibundus* DNA sequence matched with DNA sequences of several *Isadelphus* (100% similarities), a related genus within the Cryptinae. The net nucleotidic divergences within each taxon ranged between 0.2% in *A. quadridentata* to 1.1% in *P. tristis*. Two mitotype groups differing by less than 2% were detected both in *T. enecator* and in *P. tristis* (Figure 3.1a). Nucleotidic divergences between taxa ranged between 11% (divergence between *P. tristis* and *D. cavus*) to 26% (divergence between *A. quadridentata* and *M. ridibundus*) and exceeded 17% in average between taxa (Figure 3.1a). Comparatively, the nucleotidic divergences among the 40 tortricid specimens (Figure 3.1b, Appendix 3.S1) ranged from 9% to 12% (within and among the *Cydia* and *Grapholita* genus, respectively). The net nucleoditic divergence among the *C.*

pomonella specimens (0.4%) was twice higher than the divergence among specimens within each the other tortricid species. These parasitoid and moth barcodes were used to design two simple molecular methods for estimating parasitism rate in the codling moth and for identifying their parasitoids.

Figure 3.1. Neighbor-Joining trees (Saitou and Nei, 1987) of (a) the codling moth hymenoptera parasitoids (97 specimens, nine different genus) and (b) of their potential tortricid hosts (40 specimens, five different species). The evolutionary distances between coding sequences (651 positions) were computed using the Kimura 2-parameter method (Kimura, 1980). The confidence probabilities that the interior branch length is greater than zero (Dopazo, 1994), were estimated using bootstrap tests (2000 replicates) and were represented next to the branches for probability upper than 90%.



3.2. Parasitoids and moths identification by PCR-RFLP

The PCR-RFLP method was designed in order to identify each parasitoid species and their tortricid hosts (Table 3.4(2)). The diagnosis was based on the sizes of the restriction fragments at both extremities of the amplified fragment thank to marked PCR primers. The *Cat0* and *Nancy* primers and the AluI restriction enzyme were chosen to optimize the diagnosis among species. No intra-specific polymorphism was detected. Different RFLP patterns were observed between the five tortricid

species and between eight out of nine parasitoid species. The PCR-RFLP test was not able to differentiate *P. tristis* from *D. cavus*. The PCR-RFLP pattern observed with *B. rufipes* was specific but corresponded to the restriction of a non-coding *COI*-like sequence. Different RFLP patterns were observed between the parasitoid species and the tortricid species. Differences were mainly due to difference in the number of restriction fragments (1 to 5 fragments in the Hymenoptera species; 3 to 6 and in the Lepidoptera species).

Table 3.4(2). DNA lengths (in bp) for each parasitoid and host species resulting from the PCR-RFLP in the *COI* mitochondrial gene. Restriction fragment lengths were ranged according to their position along the *COI* sequence. The PCR-RFLP diagnosis of the parasitoids and their tortricid hosts and based on the lengths of the two external fragments (labeled with two different dyes). Undetected restriction fragments were provided in brackets.

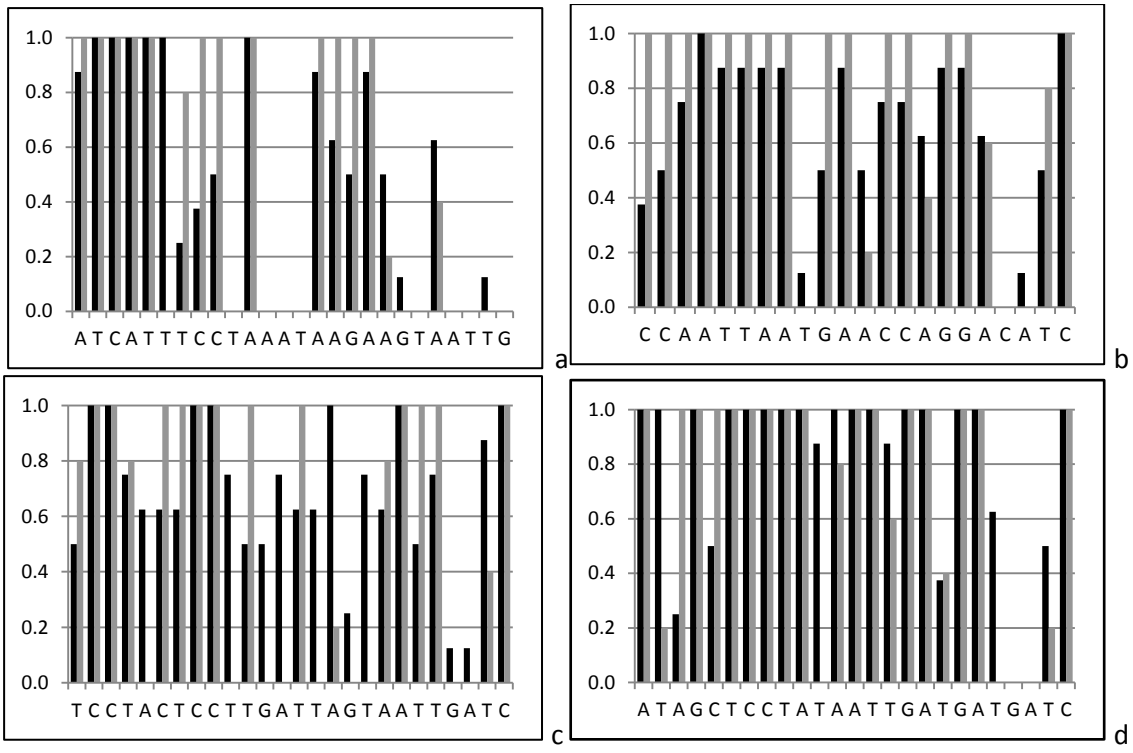
Species	Trophic level	Total length	Restriction fragment lengths
Cydia pomonella	Host	482	170-(45)-(78)-(15)-174
Cydia splendana	Host	482	170-(45)-(78)-(48)-141
Grapholita molesta	Host	482	156-(59)-(78)-(15)-(33)-141
Grapholita funebrana	Host	482	191-(24)-(78)-189
Grapholita lobarzewskii	Host	482	191-(24)-(78)-(15)-174
Ascogaster quadridentata	Parasitoid	482	347-135
Pristomerus vulnerator	Parasitoid	482	191-(135)-156
Trichomma enecator	Parasitoid	482	215-(186)-81
Perilampus tristis	Hyperparasitoid	476	335-(6)-135
Mastrus ridibundus	Parasitoid	482	215-(111)-(15)-(6)-135
Liotryphon caudatus	Parasitoid	482	170-(21)-(135)-156
Dibrachys cavus	Parasitoid	476	335-(6)-135
Hyssopus palidus	Parasitoid	476	335-141
Venturia canescens	Parasitoid	482	482-482
Bassus rufipes	Parasitoid	480	170-(45)-(126)-139[a]

[a] Restriction length of a non-coding *COI*-like gene

3.3. Parasitoid identification using specific PCR primers.

Specific primers in the *COI* gene were designed to perform with *LCO* in the four most abundant parasitoid species in codling moth larvae: *A. quadridentata, P. vulnerator*, *T. enecator* and *P. tristis*. Up to five different primers were tested for each parasitoid species on the set of Hymenoptera and Lepidoptera species in combination with *LCO*. The *Asco*, *Pristo*, *Peri* and *Tricho* primers (Table 3.3(1)) were finally selected for their high specificity (Figure 3.2). They respectively amplified a *COI* fragment of 131 bp, 347 bp, 124 bp and 463 bp in *A. quadridentata, P. vulnerator*, *P. tristis* and *T. enecator*. The four primers were diagnostic. Each primer amplified the expected fragment on the parasitoid species for which it was designed but not on other parasitoid and tortricid species (respectively 9 and 5 species tested). Note, however that the *Tricho* primer amplified a 463 bp fragment on *M. ridibundus* but the signal did not reach 5% of the signal observed in *T. enecator*.

Figure 3.2. Similarity of the specific primers with non-specific parasitoid (black) and the tortricid host (grey) sequences: (a) *Asco*, (b) *Peri*, (c) *Pristo* and (d) *Tricho*.



3.4. Parasitism detection in artificially infested moth

Parasitism by *A. quadridentata* was estimated on ten independent infestation experiments on 35 codling moth eggs. Each parasitoid female always finalized a first visit of the egg patch 40 min after it was introduced in the petri dish arena. In this artificial condition, codling moth mortality rates were significantly higher during the first week of development (mortality rate: mean=0.079, SD=0.013) than later (mortality rate: mean=0.032, SD=0.001, Chi^2=22.5, df=1, P=5x10^{-4}). However, mortality at immature instars did not differ between parasitized and non-parasitized larvae (mortality rate: mean=0.132, SD=0.003, Chi^2=0.0045, df=1, P=0.99). Parasitism rates were estimated with both the PCR-RFLP method and the *Asco* specific primer on the different codling moth instars (Table 3.5(3)). Estimates with the *Asco* primer of the parasitism rate on each immature instar did not significantly differ from 85%, the average proportion of parasitoid adult that emerged from codling moth larvae (parasitism rate: mean=0.867, SD=0.046, Chi^2=0.0651, df=1, P=0.83). Estimates of the parasitism rates on codling moth immature instar were significantly lower with the PCR-RFLP method than with the *Asco* specific primer (Table 3.5(3)).

Table 3.5(3). Average estimates of parasitism rates by *A. quadridentata* in various codling moth instars (age in week) with two alternative PCR methods (diagnosis based on RFLP or with the *Asco* specific primer). Pearson's chi-square tests were performed to compare estimates of the parasitism rate between both methods.

Instar	Age	N	RFLP	Specific	Chi^2	P-value
egg	0	48	0,00	0,90	77.89	5x10^{-4}
neonate	1	44	0,09	0,89	55.71	5x10^{-4}
young larvae	2	50	0,07	0,80	52.60	5x10^{-4}
old larvae	3	41	0,76	0,88	2.03	0.255
adult	>4	67	0,85	0,85	0.00	0.999

3.5. Parasitism detection in naturally infested codling moth population

Parasitism was estimated in a non-treated apple orchard on 184 mature caterpillars (47% with weight lower than 30 mg). 123 of these caterpillars were monitored to determine parasitism based on adult emergences (Table 3.6(4)). Molecular identifications of the parasitoids were performed within their Lepidoptera hosts for the other 61 caterpillar. The PCR-RFLP tests only detected a few caterpillar parasitized by *A. quadridentata* (only 54% of the caterpillar detected positive with the *Asco* primer) and no other parasitoid species. Consequently, molecular identification of the

parasitoids in codling moth larvae was only based on the *Asco*, *Pristo*, *Peri* and *Tricho* specific primers. The same three parasitoid species were both observed to emerge (Table 4) and were molecularly recorded (Table 5). No difference were observed in the parasitism rates (parasitism rate: mean=0.598, SD=0.004, Chi^2=0.0082, df=1, *P*=0.99) and in the distribution of the species (Chi^2=2.67, df=3, *P*=0.43) between the traditional and the molecular methods of detection. 38% of the parasitized caterpillars were determined as parasitized by a unique parasitoid, 95% of which being parasitized by *A. quadridentata*. Hyperparasitism by *P. tristis* accounts for 55% of the parasitized caterpillars, 84% of which being also determined as parasitized by *A. quadridentata*. Parasitism by *P. vulnerator* accounts for 8% of the parasitized caterpillars, 75% of which being also determined as parasitized by *A. quadridentata*. Finally, 5% of the parasitized caterpillars were detected positive for all the three species (Table 3.7(5)). Mortality before emergence did not differ between the small and large caterpillar (mortality rate: mean=0.32, SD=0.01, Chi^2=0.0429, df=1, *P*=0.84, Table 3.6(4)). However, parasitism rates were four times higher in the small caterpillar than in the large ones (all the small larvae were detected as parasitized). The distribution of the parasitoid species also differed between both larval size categories (Chi^2=37.2, df=10, *P*=$5x10^{-4}$, Table 5). 98% of the small caterpillars were determined as parasitized by *A. quadridentata* whereas only 71% of the large caterpillars were determined as parasitized by this species.

Table 3.6(4). Distribution of the parasitoids that emerged from codling moth larvae collected in an apple orchard (traditional method). *N* and *n* respectively indicate the number of codling moth larvae and the number of emerging moths and parasitoids that were analyzed for two classes of caterpillar sizes. Estimates of the parasitism rates correspond to the ratio of the number of emerging parasitoid on *n*.

Larvae sizes	N	n	*Ascogaster quadridentata*	*Pristomerus vulnerator*	*Perilampus tristis* on *Ascogaster*	*Perilampus tristis* on *Pristomerus*	Parasitism rate
>30 mg	66	45	1	1	3	6	24%
<30 mg	57	39	16	3[a]	20	0	100%

[a] One *P. vulnerator* emerged from a larvae also containing an *A. quadridentata* cocoon.

Table 3.7(5). Detection of DNA from multiple parasitoids on 184 codling moth caterpillar collected in an apple orchard (123 out of 184 caterpillars were left to emerge). Estimates of the parasitism rates were based on the ratio of positive PCR for any parasitoids on the total number of collected codling moth caterpillars (N = 97 and 86 for large and small caterpillars, respectively).

Parasitoid combination	> 30 mg	< 30 mg
A. quadridentata	3	37
P. vulnerator	1	1
P. vulnerator + *A. quadridentata*	4	3
P. tristis + *A. quadridentata*	6	42
P. tristis + *P. vulnerator*	6	1
P. tristis + *P. vulnerator* + *A. quadridentata*	4	2
Parasitism rate	25%	100%

4. Discussion

In this study, molecular markers were developed to identify hymenoptera parasitoids in some important tortricid pests attacking fruits. The method based on the restriction of a fragment of the *COI* gene proved to be very useful to conjointly determine the parasitoid and hyperparasitoid adults, and their Lepidoptera or Hymenoptera hosts. The multiplexing of PCRs performed with primers designed to specifically amplify the four most abundant *C. pomonella* parasitoids – *A. quadridentata*, *P. vulnerator*, *P. tristis* and *T. enecator* – provided accurate estimate of the parasitism within the immature Lepidoptera hosts (egg or caterpillar); molecular assessment of parasitism rates did not differ from estimates based on the monitoring of the emergences of parasitoid and moth adults and was able to reveal cases of multiparasitism within the codling moth caterpillars. Enhancement of the biological control of the tortricid pests by their parasitoids requires reducing interactions among species within the hymenoptera parasitoid guild.

4.1. Molecular identification of the Hymenoptera parasitoids

4.1.1. Species delimitation based on barcoding sequences

The ten Hymenoptera parasitoid species observed to emerge from *C. pomonella* and *G. molesta* caterpillars collected in apple orchards from Western and Central Europe were all clearly differentiated according to their DNA barcodes. DNA variations observed within these

morphologically identified species were lower than 2%, the usually accepted threshold to delimit species according to *COI* sequences (Jones, Ghoorah *et al.* 2011; Smith, Fernandez-Triana *et al.* 2013). Note, however, that two infra-specific clusters were observed in both the *P. tristis* and *T. enecator* taxa. These clusters differentiated specimens collected in the same apple orchard and may correspond to two cryptic sympatric species in both taxa. Cryptic species that cannot be differentiated based on their mitochondrial DNA sequences have been identified in numerous taxa (Hebert, Penton *et al.* 2004; Heraty, Woolley *et al.* 2007; Li, Zhou *et al.* 2010). For example, in the *Cotesia melitaearum* parasitoid complex (Hymenoptera: Braconidae), host-associated species were identified only based on microsatellite markers (Kankare, Van Nouhuys *et al.* 2005). Genetic differentiation according to host specialization should be further investigated within these parasitoid taxa, notably in *P. tristis* that parasitizes both *A. quadridentata* and *P. vulnerator* primary parasitoids within the tortricid caterpillars. Similarly, the only collected specimen identified as *M. ridibundus* had a DNA barcode that 100% matched with specimens referenced as other Cryptinae species from the *Isadelphus* genus. Although we cannot exclude some errors in our morphological identification (synonymy of generalist parasitoids), this result suggests that barcoding only based on *COI* sequences would be not efficient enough to differentiate some parasitoid taxa. The sequencing of other mitochondrial and nuclear genes would be useful to confirm species identification and delimitation among the tortricid parasitoids.

4.1.2. Parasitoid identification based on simple diagnostic molecular tests

Anyway, *COI* sequences provided global information to identify the most important functional groups of parasitoids attacking *pomonella* and *G. molesta* caterpillars. The PCR-RFLP method we developed for routine identification was efficient enough to differentiate eight out of the ten morphologically observed parasitoid species. The two non-differentiated species based on this PCR-RFLP test, *P. tristis* and *D. cavus*, belong to two very different chalcid wasps that cannot be mistaken in terms of taxonomy and biology (Perilampidae and Pteromalidae, respectively). The pteromalid wasp is a polyembryonic parasitoid that attacks mature caterpillar within their cocoon. Inversely, *P. tristis* is a solitary hyperparasitoids that parasitize young caterpillar (Mills 2005). The primers designed to specifically amplify *A. quadridentata*, *P. vulnerator*, *P. tristis* and *T. enecator*, the most abundant parasitoid species within the tortricid caterpillars, completed this diagnosis. None of the four designed primers amplified a species other than this for which it was designed. These specific primers were able to detect very early stage of the parasitoids within their Lepidoptera hosts. For example, the *Asco* primer detected the codling moth eggs parasitized by *A. quadridentata*, which confirms the ability of such molecular markers to detect the very first instars of the parasitoids inside their hosts (Derocles, Plantegenest *et al.* 2012). Although this set of specific primers provides a

limited diagnosis the parasitoid species attacking *C. pomonella* caterpillars in apple orchards, the four parasitoids thus detected represented more than 95% of the larval parasitism in the natural codling moth populations.

4.2. Molecular method for parasitoid host identification

Larval determinations of tortricid pests within damaged fruits remain of major concern for quarantine decision both in America and in Europe, notably to differentiate indigenous from exotic species. Several simple methods of identification of the tortricid species based on *COI* sequencing were developed either on the design of specific of DNA primers (Barcenas, Unruh *et al.* 2005) or on PCR-RFLP approaches (Barcenas, Unruh *et al.* 2005; Timm, Warnich *et al.* 2008; Chen and Dorn 2009). Such molecular methods were all very pertinent to differentiate the tortricid species for which morphological larval identification remain problematic because of the lack of diagnostic characters (Guilbot and Goujet 1978). The PCR-RFLP diagnose presented here was based on the restriction by a single enzyme (AluI) and the method was as efficient as the previously published ones to identify the tortricid pests. Furthermore, this method also provides efficient determination of parasitized caterpillars, which cannot be identified morphologically because of size modifications due to parasitism (Suckling, Gibb *et al.* 2002). Consequently, our PCR-RFLP method could be useful to both identify the tortricid pests damaging fruits and the host range of the parasitoids species attacking these moths.

4.3. Molecular estimation of parasitoid communities

The extension of DNA barcode references largely contributed to the development of molecular analyses of the parasitoid food web trophic interactions (Wirta, Hebert *et al.* 2014). Species identifications directly based on barcode sequences were used to detect all the potential links of the parasitoid to their hosts (Rougerie, Smith *et al.* 2011; Santos, Besnard *et al.* 2011) and are likely to quickly increase with Next Generation Sequencing and metabarcoding approaches (Pompanon, Deagle *et al.* 2012). As any molecular methods, the barcoding identification of the parasitoids within their hosts also requires knowledge of potential interaction links between species to valid the observed patterns. A difficulty with such approaches is that they use universal DNA primers for which the specificity require to be tested. Furthermore, accurate species identification also requires long DNA sequences. The simple molecular methods developed here were based on an *a priori* knowledge of the parasitoid community. They are limited to the identification of a predefined set of parasitoids, but can be very useful to understand interactions among species and differences between agronomic or environmental conditions in a well-defined biological system (Hawkins, 1994; Askew, 1986). The molecular methods proposed here to assess codling moth parasitism were as efficient as traditional

methods based on caterpillar rearing, suggesting that their results could compared. Furthermore, these molecular methods confirmed the complex relationships among the parasitoid species inside their codling moth caterpillar hosts. First they revealed that parasitism by *T. vulnerator* mainly required a pre-infestation by *A. quadridentata.* Second, they confirmed that *P. tristis* parasitized both *A. quadridentata* and *T. vulnerator* primary parasitoid inside the codling moth caterpillars (Diaconu, Pisica *et al.* 2000), revealing few cases of parasitized caterpillars by the three parasitoid species. Third, the molecular methods also confirm that size reduction of the codling moth caterpillars was mainly due to parasitism by *A. quadridentata* (Suckling, Gibb *et al.* 2002). We suggest that size dimorphism could be a valuable way to estimate codling moth parasitism since parasitism by *A. quadridentata* accounted for about 92% of the total *C. pomonella* parasitism in the non-treated apple orchard analysed.

4.4. Molecular estimation of parasitism rate

Parasitism rates can be estimated from a collection of insect larvae either based on the ratio of parasitoid adults that emerged, which require insect rearing and monitoring their emergences or the proportion of larvae detected as parasitized, which can be assessed directly within the larvae using molecular markers. We estimated parasitism rate using both methods. Estimates based on specific PCR detection of the four most abundant parasitoid species inside the codling moth caterpillars did not significantly differ from estimates based on caterpillar rearing until adult emergences (Day 1994; Maalouly, Franck *et al.* 2013). This result contrast with previous studies that compared methods of parasitism rate estimations, which usually detected higher parasitism rates with the molecular methods than with the traditional ones (Tilmon *et al.* 2000; Ashfaq *et al.* 2004; Agusti *et al.* 2005). Two main hypotheses were invocated to explain differences in parasitism detection between methods and insect instars. First, the stings and venoms used by the parasitoid wasps to immobilize their hosts frequently induce early-mortality of the parasitized larvae (*e.g.* (Day 1994). Second, the encapsulation of the parasitoid eggs within their hosts constitutes an immune defence developed by numerous insects (*e.g.* Russo *et al* 1996). The main codling moth parasitoid, *A. quadridentata*, directly lays its egg within the egg of its host, which may prevent the encapsulation process. The experiment of artificial infestation of codling moth eggs by *A. quadridentata* did not detect mortality differences between the parasitized and non-parasitized caterpillars, which is also in agreement with the absence of observed differences in the estimates of parasitism rates between methods. Interestingly, these observations suggest that we could compare the various estimates of parasitism rates in the codling moth populations independently of the methodology used. In comparison with previous reports, parasitism of *C. pomonella* caterpillar is significantly higher non-treated apple orchards than commercial ones (Maalouly *et al* 2013; 2015).

4.4. Parasitism identification and codling moth biological control

This study confirm that parasitism of *C. pomonella* caterpillar in the apple orchard is mainly governed by the braconid, *A. quadridentata.* The other observed parasitoid species were either rare or superparasites of previously *A. quadridentata* parasitized codling moth caterpillars (*P. vulnerator* and *P. tristis*). This superparasitism is likely to be detrimental the codling moth biocontrol because it does not enhance pest mortality and it limits growth rate of *A. quadridentata* populations (*e.g.* Clausen 1978; Mills 2005). Consequently, the enhancement of *C. pomonella* biocontrol requires exploring conditions that limit *A. quadridentata* parasitism (Hawkins *et al.*, 1993). Alternative tortricid hosts for *A. quadridentata* may constitute a refuge to its own parasitism. The molecular methods developed here should be very useful to compare more parasitism among the tortricids both in crops and semi-naturals habitats.

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7. Data Accessibility

The contents of Appendix 3.S1 comprise an Excel file that documents collection information, current level of identification at time of submission and various accessions for each individual specimen included in the data release

8. Author Contributions

PF conceived and designed the experiment. PF analyzed and interpreted the data. JO monitored moth and parasitoid emergences, performed the biomolecular analyses and submitted the DNA sequences. MM acquired and analyzed the data resulting from artificial codling moth infestation. PF wrote the paper.

Discussion générale et perspectives

1. Synthèse des travaux

Dans ce travail de thèse, nous avons étudié les déterminants du parasitisme larvaire du carpocapse de la pomme dans un paysage agricole. Nous avons analysé les dynamiques temporelles du parasitisme au cours d'une saison de production. Et nous avons étudié les effets des pratiques agricoles et des éléments semi-naturels au niveau du verger et du paysage sur la composition de la communauté des parasitoïdes et sur le taux de parasitisme.

Nous avons trouvé que les taux de parasitisme augmentaient en cours de saison à chaque génération de carpocapse. Nous avons montré que la proportion relative de l'hyperparasitoïde *Perilampus tristis* augmentait également en cours de saison parallèlement à une diminution de la proportion d'*Ascogaster quadridentata*. Il est probable que *P. tristis* a empêché un plus grand contrôle du carpocapse par *A. quadridentata*.

Nous avons mis en évidence un effet des pratiques phytosanitaires au niveau du verger et du paysage sur le contrôle du carpocapse par ses parasitoïdes. Les taux de parasitisme sont négativement influencés par la densité des vergers conventionnels dans le paysage. Ce résultat est en accord avec une étude qui montre une baisse des effectifs du carpocapse avec l'augmentation de la densité des vergers conventionnels autour des parcelles dans la même zone d'étude (Ricci *et al.*, 2009).

Nous avons également trouvé un effet des caractéristiques des haies locales sur la composition de la communauté des parasitoïdes. La présence de haies brise-vent et de haies composites favorise les parasitoïdes primaires *Ascogaster quadridentata* et *Pristomerus vulnerator* par rapport à l'hyperparasitoïde *Perilampus tristis*. Nous n'avons pas mis en évidence un effet des éléments semi-naturels (par exemple les haies) dans le voisinage de la parcelle sur la composition de la communauté. Cela est en accord avec la spécialisation relative de la communauté de parasitoïdes du carpocapse dans les vergers de pommiers se traduisant par une expérience du paysage à une plus petite échelle.

2. Régulation naturelle des ravageurs par des aménagements du paysage

Les aménagements du paysage peuvent contribuer au contrôle durable des ravageurs. La compréhension des effets des surfaces cultivées et non cultivées au niveau du paysage sur les ravageurs ou leur contrôle par leurs ennemis naturels permet de proposer des solutions adaptées d'aménagements du paysage.

Les habitats seminaturels et le contrôle des ravageurs

La biodiversité et l'abondance des ennemis naturels des ravageurs dans les agrosystèmes dépend de la quantité des habitats non cultivés (Bianchi *et al.*, 2006 ; Tscharntke *et al.*, 2007). Ceci ne garantie pas nécessairement un contrôle plus efficace des ravageurs (Bianchi *et al.*, 2006 ; Straub *et al.*, 2008). Mais il est généralement observé que des communautés d'ennemis naturels plus diversifiées assurent un plus grand contrôle des arthropodes herbivores (Letourneau *et al.*, 2009).

En général, les paysages avec une plus grande proportion d'éléments semi-naturels présentent en moyenne moins de ravageurs dans les parcelles agricoles et/ou plus de contrôle de ces ravageurs par leurs ennemis naturels (Veres *et al.*, 2013). Le taux de parasitisme augmente avec l'augmentation des surfaces non cultivées comme les forêts ou les prairies (Menalled *et al.*, 1999 ; Tscharntke *et al.*, 2002 ;Thies *et al.*, 2003 ; Costamagna *et al.*, 2004 ; Bianchi *et al.*, 2005 ; Bianchi *et al.*, 2008). De même, la prédation augmente en relation avec les zones boisées (Bianchi *et al.*, 2005).

Parfois, l'effet de la pression phytosanitaire sur les effectifs des ravageurs, la diversité de leurs ennemis naturels et leur potentiel de contrôle biologique est si fort qu'il masque les effets potentiels du paysage (par exemple Ricci *et al.*, 2009 ; Geiger *et al.*, 2010). Jonsson *et al*. (2012) ont montré que les pratiques agricoles (utilisation des insecticides et l'augmentation des surfaces des cultures) ont un impact sur les taux de parasitisme à des niveaux au-delà de la parcelle. Dans certains cas, la relation entre le contrôle des ravageurs et les éléments semi-naturels dans le paysage peut être négative ou inexistante car dans les paysages intensivement traités le pool régional des ennemis naturels des ravageurs n'est pas suffisant pour former des populations d'ennemis naturels capables de contrôler les ravageurs (Fahrig, 2003; Thies *et al.*, 2003; Tscharntke *et al.*, 2005). Sous ces conditions

l'augmentation des surfaces des éléments semi-naturels peut avoir peu ou pas d'effet (Veres *et al*., 2013). Cela pourrait expliquer pourquoi nous n'avons pas trouvé dans notre étude un effet des éléments semi-naturels au-delà de la parcelle sur le parasitisme du carpocapse.

Les habitats semi-naturels et la composition des communautés auxiliaires/ravageurs

La diversité des parasitoïdes peut augmenter le parasitisme si les espèces de parasitoïdes sont complémentaires (Letourneau *et al.*, 2009). Mais dans le cas d'interactions négatives entre les espèces (prédation intra-guilde, hyperparasitisme), une plus grande diversité de parasitoïdes peut aussi diminuer le parasitisme (Rosenheim, 1998). Par contre, l'hyperparasitisme n'est pas toujours considéré comme un facteur de perturbation du contrôle biologique des ravageurs. Dans la communauté de parasitoïdes de la teigne des choux (*Plutella xylostella*) l'augmentation de l'hyperparasitisme sur le parasitoïde dominant dans cette communauté n'a pas eu d'effet négatif sur le parasitisme global par les parasitoïdes primaires. Les parasitoïdes primaires moins vulnérables à l'hyperparasitoïde ont parasité d'avantage les hôtes disponibles (Nofemela, 2013). Nous n'avons pas mis en évidence un effet négatif de l'hyperparasitisme par *P. tristis* sur le parasitisme global du carpocapse.

La diversité des communautés doit être étudiée au-delà de la richesse en espèces en analysant la composition des communautés pour mieux comprendre les effets de l'intensification agricole (Gagic *et al.*, 2012 ; Andersson *et al.*, 2013). Les effets de l'hétérogénéité du paysage et des pratiques agricoles sont moins connus sur la composition des communautés d'arthropodes que sur leur richesse en espèces bien que la composition des communautés soit aussi importante pour les services écosystémiques. Nous avons étudié l'effet des pratiques agricoles et des éléments semi-naturels sur la composition de la communauté des parasitoïdes du carpocapse et montré qu'ils impactaient la composition de la communauté. Nos résultats sont cohérents avec ceux d'une étude récente sur les communautés de pollinisateurs. Andersson *et al.* (2013) ont montré que la composition de ces communautés changeait en fonction de l'hétérogénéité du paysage. Les espèces de syrphes étaient favorisées par les pratiques en agriculture biologique alors que les espèces d'abeilles étaient plus présentes dans les fermes en agriculture conventionnelle.

Il est également important de prendre en considération la quantité et la diversité des proies dans l'étude des processus de régulation. La compétition entre espèces de proies peut parfois réduire le potentiel de régulation d'un ravageur par un auxiliaire donné. On parle alors de compétition apparente (Holt and Lawton, 1993). La régulation du carpocapse (*Cydia pomonella*) et de la tordeuse orientale (*Cydia molesta*) par le parasitoïde *Hyssopus pallidus* n'est pas réduite en présence des deux ravageurs par rapport aux cas où l'un ou l'autre des ravageurs est présent (Häckermann *et al.*, 2007). Nos observations préliminaires sur la zone d'étude n'ont pas montré une compétition apparente entre le carpocapse et la tordeuse orientale par rapport aux parasitoïdes étudiés.

Le paysage peut jouer également directement sur les populations de ravageurs ou sur d'autres communautés d'auxiliaires que les parasitoides. Une étude précédente sur le carpocapse du pommier a montré un effet négatif de la densité des vergers hôtes dans l'environnement des parcelles sur les effectifs du carpocapse, surtout dû aux vergers conventionnels (Ricci et *al.* 2009). Ces études a également mis en évidence un effet négatif du réseau des haies au niveau du verger et du paysage sur l'abondance de ce ravageur (Ricci *et al.*, 2009 ; Ricci *et al.*, 2011). Une autre étude portant sur la prédation d'œufs de carpocapse exposés dans des vergers commerciaux a montré l'effet négatif de la densité en vergers conventionnels et des haies brise-vent dans le paysage sur le taux de prédation du carpocapse. De plus, ce taux de prédation était significativement lié à l'index I-Phy qui mesure l'impact environnemental des pratiques phytosanitaires (Monteiro *et al.*, 2013). Les résultats de ces études sont globalement en accord avec nos résultats.

Echelles d'effet du paysage

L'échelle spatiale à laquelle les parasitoïdes répondent aux facteurs du paysage dépend de leur biologie, particulièrement de leur capacité de dispersion, et de l'hétérogénéité du paysage (Ricci *et al.*, 2013). Rusch *et al.* (2011) ont montré par exemple que les taux de parasitisme du méligèthe du colza (*Meligethes aeneus*) dépendent de variables de pratiques agricoles (travail conventionnel du sol, proportion de parcelles cultivées en colza l'année précédente) et d'éléments semi-naturels (proportions de zones boisées et des prairies) à différents niveaux du paysage. De plus, les espèces spécialistes répondent généralement à la

composition du paysage à une plus petite échelle que les espèces généralistes (Chaplin-Kramer *et al.*, 2011).

Dans le but de réguler les ravageurs il est aussi nécessaire de comprendre les variations temporelles des effets du paysage sur les populations des ravageurs et de leurs ennemis naturels (en termes de contrôle et de composition de communauté). Les études portant sur ces variations ne sont pas fréquentes (Stireman and Singer, 2002 ; Abram *et al.*, 2012 ; Gagic *et al.*, 2012). D'où le besoin de mieux étudier ces variations afin de proposer des solutions adaptées au contrôle des ravageurs.

Des besoins de connaissances sur la biologie et l'écologie des parasitoïdes

Il est important de connaître la biologie et l'écologie des espèces de parasitoïdes afin de mieux comprendre leurs interactions avec leurs hôtes et pour pouvoir proposer des aménagements qui les favorisent. L'écologie des parasitoïdes est généralement mal connue. Nous avons trouvé des informations importantes sur les parasitoïdes du carpocapse dans la littérature scientifique ancienne portant sur la description de ces parasitoïdes, leur spectre d'hôtes et leurs interactions physiologiques avec le carpocapse ou d'autres hôtes alternatifs. Par contre, il faudra être critique par rapport à certaines informations fournies par cette littérature car elles peuvent être basées sur un nombre restreint d'observations et peuvent correspondre à des systèmes très spécifiques. Il serait alors nécessaire d'étudier dans le futur certains aspects de la biologie des parasitoïdes tels leurs besoins en ressources complémentaires ou leurs capacités de dispersion pour concevoir des aménagements paysagers pertinents.

3. Apports de la thèse pour une perspective de régulation des populations du carpocapse

Dans cette thèse nous avons étudié les déterminants du parasitisme larvaire du carpocapse du pommier dans une perspective de proposer des solutions de gestion de ce ravageur par ses parasitoïdes. L'originalité de ce travail réside dans le fait que nous avons étudié le contrôle du carpocapse (taux de parasitisme) et la composition de la communauté de

parasitoïdes au niveau spatial et temporel. Nous avons analysé les effets conjoints des pratiques agricoles et des éléments semi-naturels au niveau local et du paysage. Peu d'études paysagères associent ces effets (voir Rusch *et al.*, 2011; Jonsson *et al.*, 2012). Ceci nous a permis de prendre en compte la protection phytosanitaire au niveau du paysage. Nous n'avons pas mis en évidence un effet des éléments semi-naturels au-delà de la parcelle sur la composition de la communauté de parasitoïdes. Cela est peut être dû en partie à la forte pression phytosanitaire dans la zone d'étude qui peut masquer les effets plus faibles de ces éléments semi-naturels. Mais cela peut être aussi dû à la caractérisation du paysage. Bien que nous ayons pris en compte l'intensité d'exploitation des vergers hôtes (abandonnés, conventionnels, biologiques) il aurait fallu prendre en considération la connectivité des éléments semi-naturels dans le paysage et prendre en compte d'autres vergers non-hôtes présents dans la zone d'étude.

Les ennemis naturels du carpocapse ne suffisent pas à eux seuls pour réduire les populations de ce ravageur. Il est nécessaire de combiner plusieurs stratégies de lutte, qui peuvent concerner des échelles spatiales différentes, pour améliorer durablement le contrôle des populations de carpocapses. Nous avons pris en compte dans ce travail de thèse plusieurs niveaux et plusieurs leviers d'action potentiels sans pouvoir couvrir toutes les possibilités. L'utilisation des parasitoïdes du carpocapse, précisément *Ascogaster quadridentata*, associée à des aménagements des haies des vergers et à une réduction de l'utilisation des pesticides doivent être considérées en tant que pratiques à intégrer dans une stratégie globale. Une étude associant plusieurs stratégies de lutte contre le carpocapse (piégeage de larves diapausantes, utilisation de la confusion sexuelle et récupération des fruits attaqués après récolte) a montré une réduction significative des dégâts alors que ces méthodes utilisées individuellement n'ont pas aboutit à des résultats satisfaisants (Judd *et al.*, 2005).

4. Perspectives

Ce travail ouvre des perspectives de recherche liées à la régulation naturelle du carpocapse et à la gestion des ravageurs de façon plus générale.

Une première perspective est de mieux comprendre les interactions des parasitoïdes du carpocapse avec leurs autres hotes dans un paysage agricole. Il serait intéressant de connaître les espèces alternatives d'hôtes sur lesquelles se développent les parasitoïdes généralistes et de comprendre leur degré de synchronisation avec leurs différents hôtes. Cela permettrait de connaître le niveau de spécialisation des parasitoïdes sur le carpocapse et ces hôtes alternatifs et de comprendre comment s'effectue le choix entre ces différents hôtes potentiels. Les trois espèces de la communauté de parasitoïdes du carpocapse, *Ascogaster quadridentata* étant spécialiste des tortricidés, *Pritomerus vulnerator* et *Perilampus tristis* étant des espèces généralistes, peuvent se développer sur la tordeuse orientale *Cydia molesta* présente dans les vergers de pommiers. Nous pourrons alors effectuer des expériences de choix de ces parasitoïdes entre le carpocapse et la tordeuse orientale afin de voir s'il y a une préférence d'un hôte par rapport à l'autre et s'il y a des différences de développement de ces parasitoïdes entre les deux ravageurs. De plus, le carpocapse peut se développer sur d'autres plantes-hôtes que le pommier tel le poirier ou le noyer. La plante- hôte affecte la reconnaissance par les parasitoïdes de leur hôte, ce qui impacte la composition de la communauté des parasitoïdes sur chaque plante. Il serait alors intéressant d'étudier les conséquences du développement du carpocapse sur différentes plantes-hôte sur les espèces de parasitoïdes en commun. Collatz and Dorn (2013) ont étudié les effets du développement du carpocapse sur la pomme et la noix sur le parasitoïde *Hyssopus pallidus*. Ils ont montré que les adultes parasitoïdes élevés avec des odeurs de pomme répondent aux odeurs de la noix mais pas inversement. L'acceptation de l'hôte n'est pas affectée par la juglone dans le régime alimentaire de l'hôte. Nous avons mené une étude préliminaire sur des larves de carpocapse échantillonnées sur cinq variétés de poires dans un verger proche d'un verger de pommes. Les espèces retrouvées sont les mêmes que celles décrites sur le carpocapse des pommes avec une majorité d'*A. quadridentata* et de *P. tristis* (36 émergeants sur 94 larves collectées ; 10 parasitoïdes émergeant dont 4 *Ascogaster*, 5 *Perilampus* et 1 *Pristomerus*). Nous projetons de poursuivre ce travail sur poirier et de l'élargir sur le parasitisme du carpocapse en vergers de noyers.

Une deuxième perspective de recherche est de prendre en considération les endosymbiotes des parasitoïdes et des hôtes dans l'étude des interactions hôte-parasitoïde dans la perspective de lutte biologique. Les micro-organismes symbiotiques peuvent induire des

manipulations de la reproduction telle l'incompatibilité cytoplasmique et l'induction de la parthénogenèse (par *Wolbachia*) ce qui peut affecter l'élevage d'agents pour la lutte biologique. De plus, les symbiotes peuvent conférer une protection à l'hôte contre ses ennemis naturels tel les parasitoïdes, les bactéries parthénogénétiques, les champignons et les virus (Zindel *et al.*, 2011). Ainsi, il serait intéressant de chercher par des outils moléculaires la présence de symbiotes chez le carpocapse et ses parasitoïdes et d'essayer de voir leurs effets sur l'interaction carpocapse-parasitoïde et sur la régulation du carpocapse par ces parasitoïdes. Ces symbiotes confèrent-ils une protection immunitaire pour le carpocapse contre ses parasitoïdes ?

Enfin, il serait intéressant de poursuivre l'étude des interactions carpocapse-parasitoïdes. Nous pourrons explorer la résistance du carpocapse à ses parasitoïdes en étudiant l'encapsulation des œufs de parasitoïdes par le carpocapse et l'action des polyDNAvirus *d'A. quadridentata* sur cette réaction immunitaire de l'hôte. Nous pourrons continuer à étudier l'interaction entre les résistances du carpocapse aux insecticides et le parasitisme. La problématique émergente de la résistance aux insecticides nous pousse à vouloir connaître les interactions des ces résistances avec le biocontrôle des ravageurs. Nous avons testé cette interaction entre la résistance du carpocapse à un seul produit utilisé dans l'agriculture biologique (le virus de la granulose). Il serait bien d'élargir cette étude sur d'autres insecticides chimiques et de tester l'interaction entre l'infection au virus de la granulose et le parasitisme.

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Annexes

Annexe I Variation du sexe ratio dans les populations des parasitoïdes larvaires du carpocapse

Chez les parasitoïdes hyménoptères, les femelles fécondées peuvent déterminer le sexe de leurs descendants selon qu'elles pondent des œufs fécondés (femelle) ou pas (mâle, voir Introduction, partie 5). Elles peuvent donc ajuster le sexe ratio de leur descendance en fonction de leur environnement ou de la densité de con-spécifiques dans la population pour assurer une transmission maximale de leur patrimoine génétique pour les générations suivantes.

Nous avons analysé le sexe ratio de trois espèces de parasitoïdes larvaires du carpocapse (*Ascogaster quadridentata*, *Perilampus tristis* et *Pristomerus vulnerator*). Tous les adultes ont émergé de larves de carpocapse diapausantes collectées en 2011 dans des vergers expérimentaux non traités (Montfavet et Gotheron) et dans 8 vergers commerciaux en agriculture biologique dans la Basse vallée de la Durance (vergers 51, 124, 145, 162, 164, 165, 167 et 168). Pour chacun de ces vergers nous avons testé la conformité du nombre de mâles et des femelles avec une répartition à 50% à l'aide de tests de Khi2.

Pour *A. quadridentata* les mâles et les femelles se répartissent de façon égale dans la plupart des vergers étudiés à l'exception de Montfavet et des vergers 51 et 165 de la Basse vallée de la Durance. Dans ces vergers le sexe ratio d'*A. quadridentata* est significativement biaisé pour les femelles. Nous n'avons pas détecté de déséquilibre de sexe ratio significatif chez *P. tristis* et *P. vulnerator* (Tableau I.1).

Verger	Proportion de femelles *Ascogater*	Proportion de femelles *Perilampus*	Proportion de femelles *Pristomerus*
Montfavet	**0,63 (51/81)**	0,49 (29/59)	0,50 (8/16)
Gotheron	0,58 (32/55)	0,42 (21/50)	X
51	**0,82 (9/11)**	X	X
124	0,71 (12/17)	X	X
145	0,44 (8/18)	X	X
162	0,63 (19/30)	X	X
164	0,54 (90/168)	0,52 (17/33)	0,57 (12/21)
165	**0,73 (36/49)**	0,38 (5/13)	X
167	0,56 (33/59)	X	0,70 (7/10)
168	0,64 (9/14)	X	X

Tableau I.1: Proportion de femelles des parasitoïdes du carpocapse (*Ascogaster*, *Perilampus* et *Pristomerus*). Les nombres entre parenthèses représentent respectivement le nombre de femelles et le nombre total d'une espèce dans un verger donné. Les valeurs significatives sont marquées en gras.

Plusieurs hypothèses peuvent expliquer des biais de sexe ratio en faveur des femelles du parasitoïde *A. quadridentata* dans certains environnements (vergers) et nous n'avons pas d'élément sur celle qui serait à privilégier. Le biais de sexe ratio peut s'expliquer par de la *local mate competition* (LMC). Chez les parasitoïdes hyménoptères qui se développent de façon grégaire ou quasi-grégaire dans les patches d'hôtes (comme les parasitoïdes des œufs) et quand la reproduction se fait entre des individus apparentés, le sexe ratio des descendants est souvent biaisé du côté des femelles (Godfray, 1994). Pour une femelle qui exploite seule un hôte ou un patch d'hôtes, la stratégie optimale est l'allocation du nombre minimal de fils nécessaire pour inséminer leurs sœurs. Dans le cas où les descendants ne sont que des femelles, ces dernières, non fécondées, ne peuvent engendrer que des mâles, ce qui réduit en théorie fortement leur fitness. Par contre, la production d'un nombre « superflu » de fils va gaspiller les ressources en hôtes qui doivent être mieux allouées pour le développement des descendants femelles (Hamilton, 1967). D'un autre côté, le biais de sexe ratio en faveur des femelles peut être dû à l'action de bactéries endosymbiotiques comme les *Wolbachia*, qui peuvent induire de la parthénogenèse télythoque (féminisation par diploïdisation d'œuf non-fécondé). Certaines lignées du parasitoïde *A. quadridentata* pourraient être infectées par ces bactéries endosymbiotiques.

Annexe II Les polyDNAvirus chez les parasitoïdes du carpocapse

Les parasitoïdes ont développé un ensemble de facteurs de virulence parmi lesquels des facteurs produits par des gènes de polyDNAvirus symbiotiques (PDV). Les PDV sont des virus chimériques composés de particules virales renfermant de l'ADN circulaire qui code pour des gènes de virulence probablement provenant des guêpes parasitoïdes. Le génome des PDV est intégré de façon stable dans celui des guêpes parasitoïdes (Bezier *et al.*, 2013). L'expression des gènes et la production des PDV se fait dans les cellules spécialisées du calyx, structure située à la base des oviductes de la guêpe parasitoïde. Les particules virales sont injectées à l'oviposition de l'œuf du parasitoïde dans l'hôte. L'expression des gènes de virulence entraîne des changements physiologiques de l'hôte comme l'inhibition de l'encapsulation de l'œuf du parasitoïde et des manipulations développementales permettant le développement et l'émergence du parasitoïde (Moreau, 2003; Beckage and Gelman, 2004; Moreau *et al.*, 2009; Beckage, 2012). Il existe deux genres de PDV : les Bracovirus (BV) et les Ichnovirus (IV) associés à plusieurs centaines d'espèces de guêpes parasitoïdes appartenant à six sous-familles de Braconidae et la sous-famille des Campoleginae dans la famille des Ichneumonidae (Strand *et al.*, 2012).

Nous avons procédé à des dissections des ovaires d'*Ascogaster quadridentata* femelles en collaboration avec Marc Ravallec et Nathalie Volkoff. Les ovaires du braconide *A. quadridentata* sont précédés par un calyx (Figure II.1), structure spécialisée située à la base des oviductes. Des photos en microscopie électronique à transmission de coupes au niveau du calyx (Figure II.4 et II.5) témoignent de la présence de bracovirus. La figure II.4 montre une partie de l'œuf d'*A. quadridentata* (structure claire à gauche) et les cellules du calyx remplies de bracovirus (à droite).

La dissection des ovaires de *Perilampus tristis* montre l'absence de calyx (Figure II.2). Aucune particule virale n'a été observée sur les photos en microscopie électronique des coupes au niveau des ovaires.

La dissection des ovaires de *Pristomerus vulnerator* montre qu'il n'y a pas de calyx à la base des oviductes (Figure II.3). Les photos en microscopie électronique des coupes au niveau de ces ovaires montrent l'absence de toute particule virale.

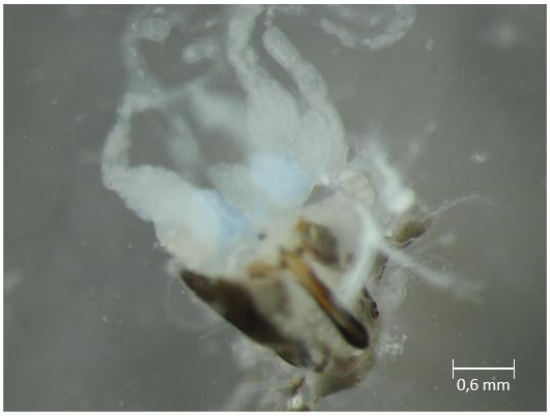

Figure II.1: Ovaires d'*A. quadridentata.* Le calyx est en bleu (11-1-726, cliché M. Maalouly).

Figure II.2: Ovaires de *P. tristis* (11-1-729, cliché M. Maalouly).

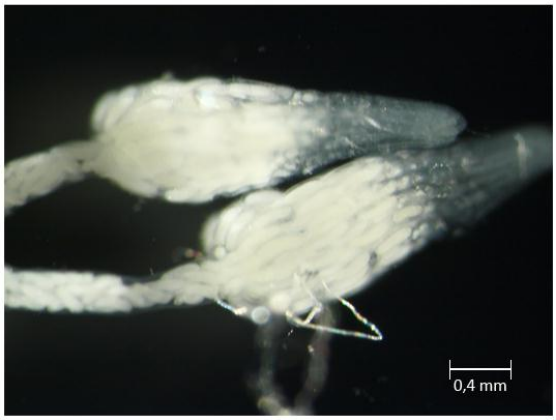

Figure II.3: Ovaires de *P. vulnerator* (11-2-2075, 11-2-2425, clichés M. Maalouly).

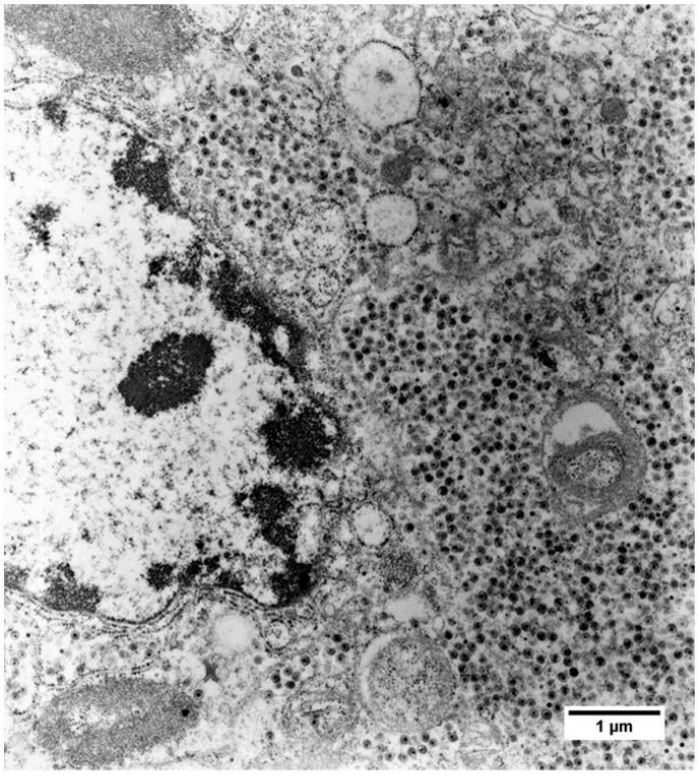

Figure II.4:
Photo en microscopie électronique d'une coupe au niveau du calyx d'*A. quadridentata*
(11-1-726, cliché Marc Ravallec INRA).

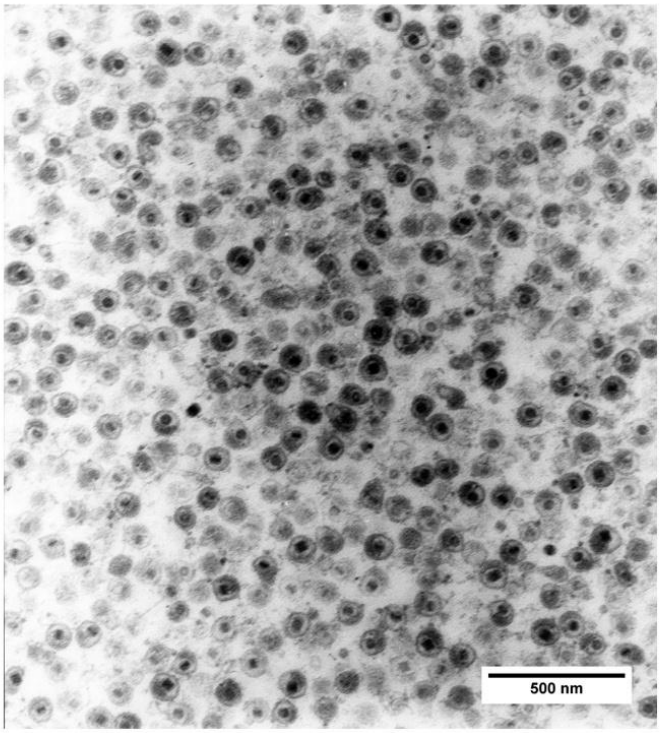

Figure II.5:
Photo en microscopie électronique de Bracovirus au niveau du calyx d'*A. quadridentata*
(11-1-726, cliché Marc Ravallec INRA).

Annexe III Effet du parasitisme sur la dynamique de l'hôte: entrée en diapause de larves saines et parasitées de carpocapse

Nous avons voulu tester l'effet du parasitisme sur l'entrée en diapause du carpocapse. Ainsi nous avons construit des modèles linéaires généralisés pour analyser la variable binomiale (larve diapausante, larve non diapausante) avec une fonction de lien logit pour les données de Montfavet et de Gotheron. L'effet du statut de la larve (parasitée, non parasitée) et de la semaine de l'échantillonnage sur la proportion de larves qui entrent en diapause ont été analysés.

La proportion des larves diapausantes était différente entre les larves parasitées et celles non parasitées à Montfavet et à Gotheron (Figure III.1). Une plus grande proportion de larves non parasitées est entrée en diapause pour les deux sites d'étude (LR Chi^2=48.8, p=2.9x10^{-12}, df=1 à Montfavet, LR Chi^2= 25.7, p=4.0x10^{-7}, df=1 à Gotheron). De manière plus attendue, la semaine d'échantillonnage avait un effet significatif sur la proportion de larves entrant en diapause. Cette proportion a augmenté au cours du temps (LR Chi^2=1563.5, p=2.2x10^{-16}, df=19 à Montfavet, LR Chi^2=802.1, p=2.2x10^{-16}, df=11 à Gotheron).

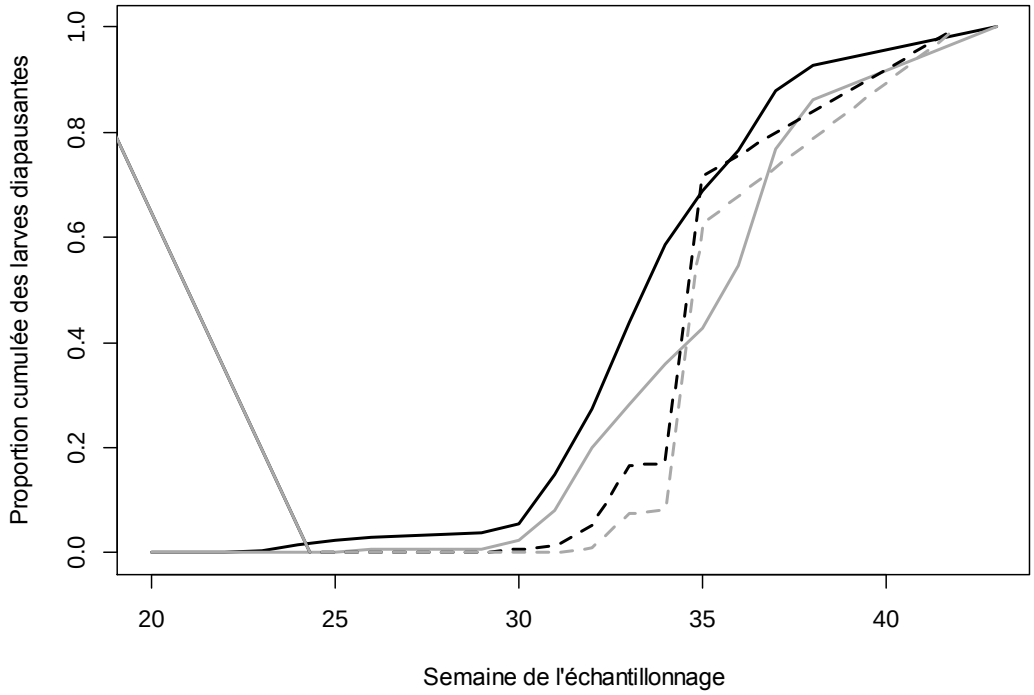

Figure III.1: Proportions cumulées des larves diapausantes à Montfavet (ligne pleine) et Gotheron (ligne en tirets) au cours de la période d'étude (ratio des effectifs cumulés des larves diapausantes par semaine d'échantillonnage et du nombre total de larves diapausantes). Les lignes noires correspondent aux larves de carpocapse non parasitées et les lignes grises aux larves parasitées.

L'entrée tardive en diapause des larves parasitées de carpocapse peut être expliquée soit par une modification du développement de la larve de carpocapse due au parasitisme soit par des différences génétiques des larves. Les parasitoïdes pourraient parasiter de façon préférentielle certains génotypes de carpocapse ou ces génotypes peuvent être moins résistants au parasitisme que d'autres. Et si ces génotypes entraient en diapause plus tard que d'autres génotypes du carpocapse cela entraînerait des entrées tardives en diapause des larves parasitées.