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Gestion du carpocapse du pommier : impact du changement climatique sur la lutte contre *Cydia pomonella* dans les vergers de Provence

Marie Perrin

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Présentée par
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Gestion du carpocapse des pommes :
Impact du changement climatique sur la lutte contre
Cydia pomonella dans les vergers de Provence

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Résumé de la thèse

Le changement climatique induit par les activités anthropiques est à l'origine de perturbations dans tous les écosystèmes dont les agroécosystèmes. La majorité des études s'accordent pour prédire une augmentation des dégâts des ravageurs sur les cultures avec l'augmentation annoncée des températures. Étudier les effets des changements climatiques sur l'efficacité et la pérennité des différentes méthodes de lutte utilisées pour contrôler ces ravageurs est donc primordial.

L'objectif de cette thèse est de comprendre comment le changement climatique pourrait influencer les différentes méthodes de lutte utilisées contre un ravageur des pommiers, le carpocapse des pommes, *Cydia pomonella* (Lepidoptera : Tortricidae), dans la Région PACA, particulièrement touchée par le réchauffement climatique. Ces méthodes de lutte intègrent l'utilisation d'insecticides, la lutte biologique et la confusion sexuelle.

Pour répondre à cette problématique, les effets croisés température/insecticides sur la mortalité de *C. pomonella* ont été étudiés. Ces deux stress ont diminué la sensibilité de *C. pomonella* à deux insecticides. Pour comprendre l'origine de ces effets, les mécanismes biochimiques et moléculaires susceptibles d'être activés par ce double stress ont été étudiés. Ainsi, une augmentation de l'activité des enzymes de détoxification et une surexpression des gènes *Cyp9A61* et *Gst1* ont été observés lorsqu'un seul stress thermique a été appliqué mais pas avec les deux stress appliqués simultanément. Les gènes *Hsp70* et *Cyp6B2*, pourraient être impliqués dans la tolérance à ces deux stress chez *C. pomonella*.

Avec la volonté grandissante de réduire l'impact des insecticides de synthèse, l'utilisation des ennemis naturels de *C. pomonella* comme agents de lutte biologique suscite un intérêt croissant. Cependant ceux-ci pourront également être affectés par un effet croisé entre les températures et les insecticides. Ainsi, une température plus élevée a diminué l'efficacité du spinosad et de l'emamectine contre *C. pomonella*. Elle a augmenté cependant la toxicité de ces insecticides sur un de ses parasitoïde, *Mastrus ridens* ; et de l'emamectine sur un de ses prédateur, *Forficula auricularia*, pouvant causer un déclin des populations d'ennemis naturels.

D'autre part, le succès des ennemis naturels dépend aussi de leurs optimums thermiques qui détermineront leur capacité à se développer et à être performant contre le ravageur cible. Les modèles phénologiques basés sur les données de développement de *C. pomonella* indiquent que la Région PACA pourrait voir apparaître une 4^{ème} génération dans les prochaines années. Les résultats montrent également que *M. ridens* a été capable de se développer et se reproduire à 28°C sans impact sur son potentiel de parasitisme. *F. auricularia* n'a pas été capable de se développer au-delà de 25°C et a perdu de son potentiel de régulation à 30°C. D'après ces résultats, la biologie de ces espèces et leur saisonnalité, le potentiel de régulation de ses deux ennemis naturels devrait rester relativement stable dans le climat futur.

Enfin, une dernière partie s'intéresse à la lutte par confusion sexuelle. La perception antennaire par *C. pomonella* de composés phéromonaux utilisés dans les diffuseurs à différentes températures est abordée. Les résultats montrent que les mâles de *C. pomonella* qui ont été élevés à 35°C perçoivent moins bien les composés phéromonaux que ceux qui ont été élevés à 20°C.

Les résultats de cette thèse montrent qu'il est nécessaire de prédire l'émergence des générations de carpocapse pour utiliser les méthodes de lutte adaptées avec précision. S'il est trop spéculatif de statuer sur l'efficacité de la confusion sexuelle dans le futur, il semble possible d'adapter l'application de différents insecticides au fil des saisons selon l'effet de la température sur leur efficacité contre *C. pomonella* et leur toxicité sur les ennemis naturels. Il serait ainsi possible de combiner la lutte biologique et l'utilisation de certains insecticides dans le respect de l'environnement et de la santé humaine dans un contexte de réchauffement climatique.

Abstract

Climate change, induced by anthropogenic activities, is causing changes in all ecosystems, including agro-ecosystems, which are essential to human survival. The majority of studies predict an increase in the damage of pests on cultures. Studying the effects of climate change both on the different components of agroecosystems and on the efficiency and sustainability of the different pest management methods used to control crop pests and minimize agricultural losses is essential.

The aim of this PhD is to understand how climate change could influence the different control methods used against an apple pest, *Cydia pomonella*, in the PACA region particularly affected by global warming. These control methods include the use of chemical insecticides and alternative strategies limiting environmental and sanitary impacts.

To address this issue, the cross effects of temperature and insecticides on the mortality of *C. pomonella* were studied.

These two stresses decreased the sensitivity of *C. pomonella* to two insecticides. To understand the origin of these effects, the biochemical and molecular mechanisms that could be activated by this double stress were studied. Thus, an increase in detoxification enzyme activities and an overexpression of Cyp9A61 and Gst1 genes were observed when a single heat stress was applied but not with both stresses simultaneously. The genes Hsp70 and Cyp6B2 could be involved in the tolerance to both stresses in *C. pomonella*.

With the increasing concern to reduce the impact of synthetic insecticides, there is an interest in using natural enemies of *C. pomonella* as biological control agents. However, they might also be affected by a temperature and insecticide cross effect. Indeed, higher temperature decreased the efficiency of spinosad and emamectin against *C. pomonella*. However, it increased the toxicity of these insecticides on one of its parasitoids, *Mastrus ridens*; and of emamectin on one of its predators, *Forficula auricularia*, which may cause a decline in populations.

Moreover, the success of natural enemies also depends on their thermal optimums which will determine their ability to successfully develop and perform against the target pest. Phenological models based on *C. pomonella* development data indicated that the PACA region could face the appearance of a 4th generation in the next few years. Results also showed that *M. ridens* was able to develop and breed at 28°C without impacting its parasitism potential. *F. auricularia* was not able to develop above 25°C and lost its control potential at 30°C. Based on these results, the biology of these species and their seasonality, the regulatory potential of these two natural enemies should remain relatively stable in the future climate.

Finally, a last part investigates the use of mating disruption. The antennal perception by *C. pomonella* of pheromonal compounds used in dispensers at different temperatures is discussed. Our results show that higher developmental temperature decreased the overall electrical response of the antennae in *C. pomonella* males to certain compounds in comparison with lower developmental temperature.

The results of this study show that it is essential to predict the emergence of codling moth generations in order to use the appropriate control methods with precision. If it is too speculative to give an opinion on the efficiency of mating disruption in the future, it seems possible to adapt the application of different insecticides over the seasons according to the effect of temperature on their efficiency against *C. pomonella* and their toxicity on natural enemies. It would thus be possible to combine biological control and the use of specific insecticides in a way that respects the environment and human health in a context of global warming.

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Introduction générale

Les variations climatiques façonnent la biodiversité. Les disparitions, apparitions et évolutions d'espèces sont en effet fortement liées aux variations que le climat terrestre a subies par le passé. Ces variations climatiques sont généralement les conséquences naturelles des fluctuations des courants océaniques, des cycles astronomiques, de l'intensité de l'activité solaire, de l'activité volcanique, des concentrations atmosphériques de gaz à effets de serre (GES) ou plus rarement de la chute de météorites.

Depuis la deuxième moitié du XIX^e siècle, avec la première révolution industrielle, les variations climatiques se sont accélérées. Les révolutions industrielles successives ont considérablement augmenté les émissions de GES, tels que le CO₂, le CH₄, les NO_x et les SO_x. Par exemple, les émissions de CO₂ sont passées d'une concentration de 280ppm dans l'atmosphère au début de la révolution industrielle à 413ppm en 2020 (World Meteorological Organization 2021). Ces augmentations de GES sont responsables d'un réchauffement du climat terrestre. La température globale moyenne s'est ainsi élevée de 1,1°C depuis la période 1850-1900. De nos jours on parle de changements climatiques de manière générale pour englober l'augmentation des émissions de GES et ses conséquences passées et à venir. Les climatologues s'accordent ainsi pour prédire un réchauffement planétaire moyen d'ici à 2100 de 1,8°C dans le meilleur des scénarios à 4,4°C dans le pire (IPCC 2021), une augmentation des précipitations dans les latitudes élevées et une partie des tropiques mais une diminution dans les autres latitudes (Bates *et al.* 2008), la fonte des glaces et l'augmentation du niveau des océans, pouvant atteindre jusqu'à un mètre d'ici la fin du siècle (Ramstorf 2009), ainsi que l'augmentation de la fréquence et de l'intensité des événements extrêmes tels que les canicules (IPCC 2012).

Toutes ces modifications du climat induites par les activités anthropiques sont à l'origine de changements profonds dans l'équilibre des écosystèmes. Parmi ceux-ci, les agroécosystèmes sont à l'origine de nombreuses préoccupations puisqu'ils sont essentiels à la survie de l'Homme. Plusieurs travaux expérimentaux et de modélisation ont montré et/ou prédit un impact fort des changements climatiques sur la production agricole *via* leurs influences multiples sur la croissance des plantes, mais également sur les ravageurs de cultures. Certains ravageurs ont ainsi étendu leur aire de répartition vers des latitudes et des altitudes plus élevées ou ont pu s'établir sur de nouveaux territoires en tant qu'espèces envahissantes en raison d'une augmentation

de la température mondiale (Chuine *et al.* 2010 ; Reineke & Thiery 2016 ; Thomson *et al.* 2010). Les changements dans la phénologie, le voltinisme et la dynamique des populations de ravageurs, tels qu'ils ont été observés pour la plupart des insectes, menacent également la production agricole. Les températures plus chaudes entraînent généralement une augmentation de la survie en hiver, une date d'émergence moyenne avancée, un développement plus rapide, un taux de reproduction plus élevé et une saison de développement prolongée. La majorité des études s'accordent ainsi pour prédire une augmentation globale des dégâts sur les cultures. Les pertes de rendements liées à l'effet positif de l'augmentation des températures sur les populations de ravageurs pourraient ainsi atteindre à l'échelle mondiale 10 à 25% par degré de réchauffement pour les trois céréales les plus consommées, c'est-à-dire le riz, le maïs et le blé (Deutsch *et al.* 2018). De plus, l'augmentation des températures peut accélérer l'apparition de résistances à certaines méthodes de lutte comme l'utilisation d'insecticides chez ces espèces et en amplifier les dégâts. Cela pose de grandes questions sur la pérennité de certaines méthodes de lutte contre les ravageurs. De plus, on assiste depuis quelques années, à une volonté citoyenne et parfois politique de réduire les impacts environnementaux et sanitaires d'une agriculture héritée du XX^e siècle, en développant notamment des alternatives aux molécules chimiques. Il apparaît donc primordial d'étudier les effets des changements climatiques à la fois sur les différents composants des agroécosystèmes mais aussi sur l'efficacité et la pérennité des différentes méthodes de lutte utilisées pour contrôler les ravageurs des cultures et minimiser les pertes agricoles.

Cette thèse cherche à comprendre comment le changement climatique pourrait influencer les différentes méthodes de lutte utilisées contre les ravageurs, en intégrant aussi bien l'utilisation d'insecticides chimiques que des stratégies limitant les impacts environnementaux et sanitaires. Le premier chapitre de ce manuscrit est ainsi une revue générale de cette problématique. Les questions soulevées par ce travail de revue ont été en partie adressées en utilisant comme modèle un ravageur majeur des vergers de pommiers de la Région PACA, le carpocapse *Cydia pomonella*. Après une description de l'agroécosystème dans le bassin méditerranéen, les chapitres suivants se focalisent donc sur les impacts du réchauffement climatique sur l'efficacité des principales méthodes de lutte utilisées contre ce ravageur.

I-1 : Contexte et revue générale des effets du changement climatique sur l'efficacité des principales méthodes de lutte contre les ravageurs

Si le changement climatique constitue une préoccupation majeure au XXI^e siècle, la production alimentaire pour une population mondiale grandissante est un deuxième enjeu d'intérêt pour ce siècle. L'impact futur des ravageurs sur la production alimentaire reste difficile à quantifier et il est important d'adapter efficacement la gestion de ces ravageurs au futur climat.

A notre connaissance, il n'existe à ce jour aucun document qui examine l'influence du climat sur les différentes stratégies de lutte contre les ravageurs. Ce premier chapitre de manuscrit présente une revue scientifique de la littérature relative aux principales stratégies de lutte contre les ravageurs et à leur efficacité dans un contexte de changement climatique. La première partie traite de la lutte par insecticides chimiques et aborde les impacts du changement climatique directement sur leur application, sur la susceptibilité des ravageurs aux insecticides de synthèse, et sur la probabilité d'apparition de résistance. La deuxième partie présente les effets du changement climatique sur la durabilité des diffuseurs de phéromones utilisés pour la confusion sexuelle et sur la réponse des ravageurs aux phéromones sexuelles. La troisième partie recense les effets du climat à la fois sur la survie en champs et sur la pathogénicité des bactéries, champignons, virus et nématodes entomopathogènes. Elle traite aussi de l'effet du climat sur la réponse immunitaire des ravageurs face à une infection par des organismes pathogènes. Enfin, la dernière partie aborde la question de la lutte biologique par les espèces entomophages, que ce soit en termes d'effets directs du changement climatique sur leurs traits d'histoire de vie et leurs comportements, mais aussi de leurs effets indirects sur les relations trophiques ou la composition de leurs communautés. Enfin, nous proposons des pistes d'amélioration possibles afin d'adapter ces différentes stratégies au futur climat.

Influence of climate change on the management of pest insects and potential improvements in a warming world

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Soumission prochaine à Global Change Biology

INTRODUCTION

Humankind is currently facing two major challenges that interact with each other: climate change and food production for a fast-growing population (Dawson *et al.* 2011; Campbell *et al.* 2016). While there is clear evidence that human activities associated with food production are a significant contributor to climate change (Gerber *et al.* 2013), the magnitude and variability of the impact of climate change on food production, especially plants, is still unclear. For example, some studies have predicted increased plant growth and thus crop yields with increasing temperature and CO₂ concentration (Gutierrez *et al.* 2008; Reilly *et al.* 2003) while others predicted the opposite because of increased frequency of critical temperatures or droughts (Gourdji *et al.* 2013; IPCC 2014).

Models assessing the agricultural impacts of climate change mainly focus on plants and rarely consider crop losses due to pests, although many experimental studies are available. Among pests, insects are probably the most studied in this context. Because insects are ectotherms –*i.e.* their body temperature varies with that of the environment—their physiology, life history traits and behavior should be greatly affected by global warming. As for many ectothermic species (Parmesan 2006), some insect pests have expanded their geographic ranges because of increased temperatures (*e.g.* Chuine *et al.* 2010; Thomson *et al.* 2010). Moreover, warmer temperatures commonly result in their increased winter survival, earlier mean emergence date, faster development, higher reproductive rate and extended active season (Bale *et al.* 2002; Caffarra & Eccel 2011; Juszczak *et al.* 2013).

Because of the often-positive influence of temperature on pests' population dynamics, crop losses are overall expected to increase in the future, and may reach 10 to 25% per degree of global mean surface warming for rice, maize and wheat (Deutsch *et al.* 2018). There is thus a need to prepare for pest outbreaks that are likely to occur more frequently (Gutierrez *et al.* 2008) and adapt their management to future climate. However, crop losses to insect pests do not occur in a 'passive' context: they will occur within adaptively managed agricultural systems where multiple pest management interventions are applied in response to changing pest presence and abundance. Thus, it is important not only to understand the impact of climate change on plant/pest systems, but also how it may interact with interventions that are often used to manage changing pest densities and their impacts in these systems. This topic is especially important considering the high cost of pest management, which is expected to strongly

increase in the next decades for many cultures (Diffenbaugh *et al.* 2008; Koleva & Schneider 2009). Understanding the potential effects of climate change on the effectiveness of pest control strategies will pave the way towards identifying possible adaptations or improvements of these strategies to cope with climate change.

SCOPE OF THIS REVIEW

Despite the important impact that climate change may have on pest insects themselves and crop losses to pests, its influence on pest insect's management strategies has never been reviewed to our knowledge, except for biological control by entomophagous organisms (e.g. Schmitz & Barton 2014; Tylianakis & Binzer 2014). The purpose of this paper is precisely to review the influence of climate on the efficiency of the most commonly used methods to control agricultural pest insects, to estimate their sustainability under changing climatic conditions and to identify potential improvements.

The term climate change refers here to any significant change in climatic parameters such as temperature, precipitation, extreme events or greenhouse gas rates, which are sustainable over time (Abbaszadeh *et al.* 2011; Boullis *et al.* 2015). Considering the abundance of literature on this topic, our review mainly focuses on the influence of temperature but the influence of increasing CO₂, O₃ and UV radiation and decreasing humidity is also discussed.

We focused on the most common management methods used in conventional, integrated and organic farming, *i.e.* chemical insecticides, pheromone-based mating disruption and biological control by entomopathogenous (*i.e.* fungi, bacteria, viruses and nematodes) and entomophagous organisms (predators and parasitoids). Direct and indirect influences of climate on the efficiency of these pest control methods have been considered, as represented in

Fig. 1. Other strategies are not discussed in this paper, either because climate is not expected to influence their effectiveness in a significant way (e.g. methods of physical pest exclusion such as nets) or because no or few research studies were available (e.g. the sterile insect technique). Moreover, we only reviewed strategies relying on a direct action on pests, and did not consider indirect approaches such as resistant plant cultivar selection or transgenic plants.

Keywords used for our bibliographic research thus included terms related to climate change (*i.e.* climate change, global warming, temperature, heat, CO₂, O₃, UV, drought, humidity), pest management (*i.e.* pest management, biological control, insecticides, pesticides, mating disruption), functional groups and taxa (*i.e.* insect pests, Lepidoptera, Coleoptera, Diptera, Hemiptera, biological control agents, parasitoids, predators, natural enemies and entomopathogenic fungi, bacteria, nematodes or viruses) and biological characteristics (*i.e.* survival, development, growth, fecundity, life history traits, metabolism, behaviour, mating behaviour, pheromones, virulence, trophic relationships).

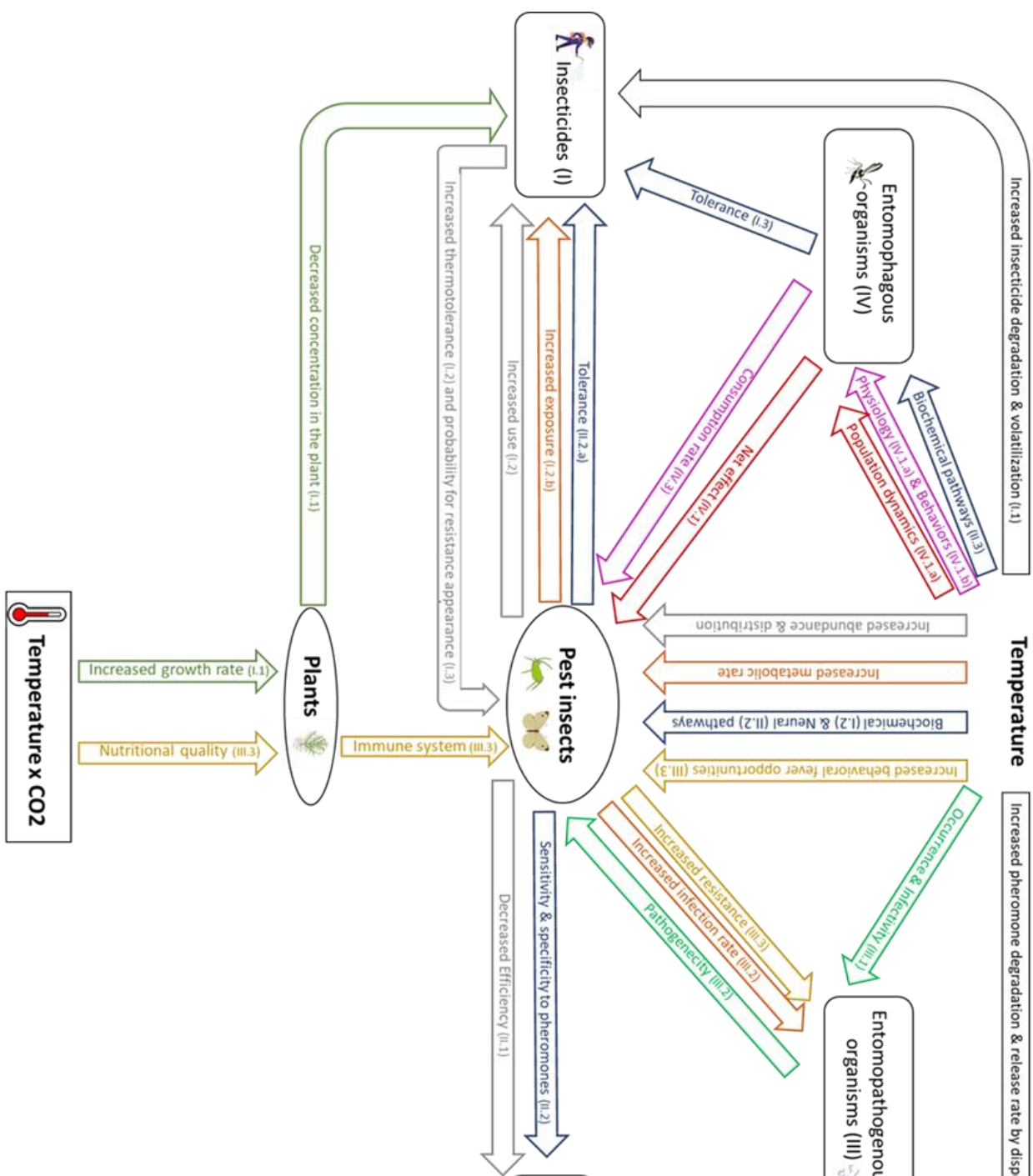


Fig. 1: Influence of increased temperature and CO₂ on four pest management methods through direct and indirect effects. Arrows with similar colours indicate a logical link between elements, representing indirect effects of temperature on pest management (e.g. increased temperature results in increased metabolic rate in insect pests, resulting in higher exposure to insecticides and infection rate by entomopathogenous organisms because of higher activity and feeding rate). The number between brackets indicate the chapter dedicated to this topic in this review. When « increased » or « decreased » is not indicated for a given mechanism, a change is expected with temperature but we could not predict if that would be a rise or a drop. We only represent mechanisms that have received experimental evidence and other hypothetical mechanisms have not been included although they may be discussed in this review (e.g. changes in plant odors with climate which may influence parasitoid or predator orientation towards pests).

I. CHEMICAL INSECTICIDES

Chemical insecticides are widely employed and considered essential to modern cropping systems, despite their sometime negative impacts on the environment (Stehle & Schulz 2015) and human health (Koureas *et al.* 2012). Several models have estimated that pesticide use should strongly increase under climate change and consequently that direct and externalized costs will rise for growers and society (Koleva & Schneider 2009). This overall need for increased pesticide application is predicted to result from the impact of climate on (i) insecticide concentration in crops and plant, (ii) pest abundance, (iii) insecticide toxicity on pests and their natural enemies and (iv) increased probability of insecticide resistance.

1. IMPACTS OF CLIMATE CHANGE ON PESTICIDE APPLICATION AND PERSISTENCE

Warmer years are commonly associated with an increase in pesticide application (Koleva *et al.* 2009). In addition to insect-related explanations that are discussed in the next section, several biological and chemical mechanisms may explain why more pesticides are needed during warm years. These processes have already been reviewed by Delcour *et al.* (2015), and so we only include a brief overview here. First, higher temperatures and CO₂ concentrations commonly result in increased plant growth rate (Gutierrez *et al.* 2008; Reilly *et al.* 2003), decreasing pesticide

concentration (Holland & Sinclair 2004; Zongmao & Haibin 1997). Secondly, plant transpiration is severely limited during hot and dry periods, reducing the intake and transport of pesticides in plants (Keikotlhaile 2011). Thirdly, higher volatilization rates have been observed for insecticides under hot weather (Holland & Sinclair 2004; Navarro *et al.* 2007), especially for moist soils (Johnson *et al.* 1995; Otieno *et al.* 2013). Finally, the chemical and microbial reactions responsible for the degradation of chemical components are accelerated with increased temperature, precipitation and sunlight (Athanasopoulos *et al.* 2004; Rosenzweig *et al.* 2001). All these processes imply that less pesticide will be present in crops and plants for a given application quantity, and that more product will need to be applied to achieve a given level of pest suppression.

2. IMPACTS OF CLIMATE CHANGE ON PEST CONTROL BY CHEMICAL INSECTICIDES

Insecticide application is commonly expected to increase with climate change as distribution and abundance of insect pests should increase with rising temperature and ambient CO₂ concentration (Delcour *et al.* 2015; Deutsch *et al.* 2018; Guru Pirasanna Pandi *et al.* 2018). However, a warmer climate would be expected to have other and more unpredictable consequences on pest control with insecticides *via* its short-term effects on insecticide toxicity and its long-term effects on the evolution of insecticide resistance.

1. Temperature-dependency of insecticide toxicity

Studies on various insect species have shown that the toxicity of a wide range of insecticides strongly depends on temperature (*e.g.* Hodjati & Curtis 1999; Glunt *et al.* 2014; Scott 1995), probably explaining seasonal changes in insecticide susceptibility observed in some pests (Wu *et al.* 2004).

Such temperature-dependency of insecticide toxicity however strongly varies with several factors, including the specific insecticide in question. Although several studies suggested that the influence of temperature on insecticide toxicity may be relatively consistent within an insecticide class (*e.g.* the toxicity increases with temperature for organophosphate insecticides but decreases for pyrethroid insecticides; Satpute *et al.*

2007; Scott 1995), other results indicate that variation also exists within classes, including widely used pyrethroids and neonicotinoids (Boina *et al.* 2009).

The range of temperature tested and the period of exposure to an insecticide also influence its toxicity. Li *et al.* (2006) and Boina *et al.* (2009) reported that toxicity of some insecticides increased with temperature between 17 and 27°C and decreased between 27 and 37°C, while the opposite trend was observed for other products. The vast majority of studies, including all those cited in this section, varied post-exposure temperature but it should be noted that developmental temperature also influences susceptibility to insecticides (Medrzycki *et al.* 2010).

The relationship between temperature and insecticide toxicity is overall strongly species-specific. For example, the toxicity of deltamethrin increases with temperature for the fall armyworm *Spodoptera frugiperda* and the tobacco budworm *Heliothis virescens*, but decreases for the cabbage looper *Trichoplusia ni* and the boll weevil *Anthonomus grandis* (Sparks *et al.* 1982, 1983). The temperature-related toxicity may even vary between insect stages for a species Honeybee adult workers developing at 33°C were significantly more susceptible to dimethoate than individuals reared at 35°C, but the opposite result was observed for larvae (Medrzycki *et al.* 2010).

It is therefore clear that temperature has a strong influence on insecticide toxicity, depending on chemistry, pest species and temperature range. Attempting to make broad generalizations about temperature-dependent toxicity of insecticides is thus problematic and each system needs to be characterized separately. Moreover, other factors that are part of climate change and pest management methods are known to influence insecticide toxicity and complexify projections, although such studies are still scarce. Ge *et al.* (2013a) reported for example that under elevated CO₂, the dissipation of an insecticide was faster in a transgenic Bt rice than in its non-transgenic parent, making transgenic plants less efficient in insecticide-based pest control under such atmospheric conditions.

2. Consequences of climate change on the evolution of insecticide resistance

Short-term conditional resistance of pests to insecticides in response to temperature may help to explain the aforementioned temperature-dependency of insecticide toxicity. Such resistance may arise from phenotypic plasticity or epigenetic modifications (Matzrafari 2018) which would modulate certain biochemical pathways,

described in Box 1. Similar pathways may be involved in the insecticide-induced thermotolerance, as reported for several pest species (Ge *et al.* 2013b; Su *et al.* 2017).

Climate change is also expected to speed up the rate of evolution of insecticide resistance, which despite being a longer-term process than epigenetic or phenotypically plastic responses, can occur relatively quickly. For example, higher temperature is thought to result in shorter generation time of multivoltine pests and thus faster evolution of insecticide resistance (Tobin *et al.* 2008). Among the rare studies investigating this assumption, Maino *et al.* (2018) reported that Australian climatic regions supporting increased voltinism were indeed positively correlated with the presence of pyrethroid resistance in a mite pest.

Box 1. Physiological mechanisms underlying temperature-dependant toxicity of insecticides.

Understanding mechanisms underlying the temperature-dependant toxicity of pesticides may help to design products adapted to the future climate. Although few experimental evidence is available, temperature has been proposed to modulate insecticide toxicity through its influence on:

- Locomotor activity and feeding rate, which are positively correlated with the penetration of the product into pest's body (Boina *et al.* 2009; Petz *et al.* 2004).
- Neural pathways, including changes in the functioning of sodium channels (Narahashi *et al.* 1995; Song & Narahashi 1996) and mitochondrial Na⁺-K⁺-ATPase activity (Li *et al.* 2006).
- The activity of detoxification enzymes, including glutathione S-transferases, carboxylesterases and cytochromes P450 (Okutman Tas & Pavlostathis 2007; Zhang *et al.* 2015). Two studies on mosquitoes however contradicted this hypothesis (Oliver & Brooke 2017; Polson *et al.* 2012).
- Heat Shock Proteins synthesis, which may be stimulated by heat stress and increase pest tolerance to insecticides (Patil *et al.* 1996; Rhagavendra *et al.* 2010). HSP up-regulation has been experimentally identified as responsible for insecticide-induced thermotolerance (Ge *et al.* 2013b; Su *et al.* 2017).

Climate may also induce more complex transgenerational and evolutionary effects. Liu *et al.* (2008) described a sharp increase in the level of susceptibility to methamidophos in *Plutella xylostella* from an insecticide-resistant population exposed to extreme

temperature. Notably, this increased insecticide susceptibility was still observed in the next generation of the insects in the absence of extreme high temperatures. In addition, individuals from a non-resistant population displayed higher up-regulation of heat shock protein expression at high temperature than individuals from the insecticide-resistant population, increasing their thermotolerance. Conversely, Tran *et al.* 2018 observed an increase in chlorpyrifos toxicity with temperature in a mosquito species that disappeared in offspring with a parental exposure to chlorpyrifos and increased temperature. High temperatures thus have a strong impact on the evolution of insecticide resistance, but the evolution of resistance in turn modulates the susceptibility of pests to high temperatures.

II. PHEROMONE-BASED PEST MANAGEMENT

Long-distance communication in insects often relies on pheromones (Boullis *et al.* 2016). Pheromone production (Roeser-Mueller *et al.* 2010), composition (Ono 1993) and duration (van Oudenhove *et al.* 2011) are known to vary with different aspects of climate, especially temperature (Ono 1994; Bontonou *et al.* 2013) and CO₂ concentration (Boullis *et al.* 2016). Climate change thus appears to have the potential to influence insect-induced crop yield losses due to changes in pheromone-dependent behaviours such as mating, aggregation or foraging.

Sex pheromones are commonly synthesized industrially and used to lure pests in traps and monitor their presence or disrupt their mating and control their populations (Cork 2004). Although several studies have reported seasonal variations in capture rate of pheromone-baited traps, these changes are usually explained by seasonal variations in the abundance of pests and not by changes in their attraction by synthetic pheromones (Tinzaara *et al.* 2007). To our knowledge, the influence of climate change on the efficacy of pheromone-based pest management has never been clearly investigated, but existing evidence suggests that there are clearly potential impacts.

1. IMPACT OF CLIMATE CHANGE ON PHEROMONE DISPENSERS

Air velocity and temperature strongly influence the release rate of synthetic pheromones from dispensers (Bierl-Leonhardt *et al.* 1979; Leonhardt *et al.* 1990; Witzgall *et al.* 2010), probably explaining seasonal variations observed for synthetic pheromone concentration in the air (Karg & Sauer 1997). A linear relationship between temperature and pheromone release rate has thus been described both in laboratory (van der Kraan & Ebberts 1990) and in the field across a broad geographic gradient in USA (Tobin *et al.* 2011). It has been estimated that disparlure half-life time at some of the warmer locations sometimes did not exceed the length of time pheromone-baited traps were deployed because of increased release rate (Tobin *et al.* 2011). Moreover, this decreased dispensers' durability may be accelerated by pheromone degradation occurring at high temperature (Atterholt *et al.* 1999). In the future, they may thus need to be replaced more frequently (Nation 1993; Tobin *et al.* 2011) but the increased cost may be at least partially compensated by a lower density, since increased release rate may result in extended distance downwind at which the concentration of the blend is above threshold to induce an insect response (Linn & Roelofs 1991).

2. IMPACT OF CLIMATE CHANGE ON MATING DISRUPTION

Climate change is expected to result in increased pest density, number of generations and advanced phenology, all crucial elements for the success of mating disruption strategy. Pheromone-based pest control indeed frequently fails when population density is too high (Feldhege *et al.* 1993; Louis & Schirra 2001), although such assumption has been contradicted in some species (Onufrieva *et al.* 2019). We may thus expect that the success of this tactics could decrease with climate change, at least for some species, because of the predicted increase in pest abundance. Moreover, this pest management method depends on mathematical models based on degree-day accumulation to predict emergence and periodicity of sex attraction and therefore adjust the optimal time for the use of synthetic pheromone (Boullis *et al.* 2016; Del Tio *et al.* 2001; Gallardo *et al.* 2009). The future success of mating disruption strategy thus relies on the ability of programmers to adapt their models to the changes in voltinism and phenology that have already occurred or are expected to occur in the next years (Juszczak *et al.* 2013; Parmesan 2006).

Such adjustments of models are quite simple, but climate may have hidden physiological consequences that may disrupt pheromone attraction and that would be harder to consider. Temperature and humidity thus both influence pest males' response to synthetic pheromones through different mechanisms. First, warm conditions are known to result in higher electroantennogram responses to sex pheromone and higher expression of pheromone-mediated behaviors in pests (Lemmen & Evenden 2015). Secondly, temperature and humidity may affect the rate at which sensory cells adapt and disadapt to odor, a mechanism essential for pheromone tracking by males. Male antennae of the oriental fruit moth *Grapholita molesta* are for example able to track pulses of pheromone at high temperatures while lower temperatures result in significant adaptation to the odor and loss of response (Baker *et al.* 1988). In the European corn borer *Ostrinia nubilalis*, the proportion of males taking flight and reaching synthetic pheromone source decreases under high humidity conditions, probably because of adaptation of antennal neurons or attenuation of their firing rate (Royer & McNeil 1993). Thirdly, global warming may decrease the degree of specificity to odors, as observed in *G. molesta* and in the pink bollworm moth *Pectinophora gossypiella*, which exhibited peak response to a broader range of blends and doses at the highest temperature in wind tunnel (Linn *et al.* 1988).

Such decrease in specificity with increasing temperature was confirmed in the field for *G. molesta* (Linn & Roelofs 1991). According to the authors, faster disadaptation rates as well as differential adaptation rates for component-specific receptors at high temperature might result in a broader spectrum of response with respect to blend ratios.

Based on these few studies, we may expect that global warming will improve efficacy of pheromone-based pest control due to increased response to odors, increased ability to track pheromone pulses and decreased specificity in pests, but such assumptions require to be experimentally investigated.

III. BIOLOGICAL CONTROL BY ENTOMOPATHOGENIC ORGANISMS

Entomopathogenic bacteria, fungi, DNA viruses and nematodes are commonly used for pest management (Lacey *et al.* 2001; Shah & Goettel 1999), as they allow to target specifically pest species and thus have a relatively low environmental impact (Inceoglu *et al.* 2006; Seiber *et al.* 2018). Climate strongly influences their life cycle and occurrence in agroecosystems, their pathogenicity and the immune response of insects, as discussed in this section.

1. OCCURRENCE OF ENTOMOPATHOGENIC ORGANISMS IN AGROECOSYSTEMS

Temperature, relative humidity and ultraviolet radiation (UV) affect the life cycle and survival of entomopathogenic fungi (Walstad *et al.* 1970; Meikle *et al.* 2003; Dhillon & Gujar 2010), viruses (Asano, 2005; Lacey 2017; Wu *et al.* 2015), bacteria (Leong *et al.* 1980; Myasnik *et al.* 2001) and nematodes (Singh & Prasad 2016; Campos-Herrera *et al.* 2010; Sharmila *et al.* 2018). Relative sensibility to these three climatic factors differs however between these groups (Table 1).

Table 1: Relative role of three major climatic factors on survival and occurrence of entomopathogenous organisms in crops.

	Solar radiation	Relative humidity	Temperature
Bacteria	Major (Leong <i>et al.</i> 1980; Myasnik <i>et al.</i> 2001)	Minor (Ignoffo <i>et al.</i> 1983)	Important but not limiting (Ignoffo <i>et al.</i> 1982, 1983). Growth rate increases with temperature (Adamo & Lovett 2011).
Fungi	Important (Hedgecock <i>et al.</i> 1995; Hussein <i>et al.</i> 2010)	Major (Hunter & Kunimoto, 1974; Bruck & Lewis 2002). High RH promotes conidia germination (Verhaar <i>et al.</i> 1998) and reduces time of germination (Luz & Fargues 1997).	Important. High temperatures decrease conidia viability (Sen <i>et al.</i> 2009); extreme temperatures delay germination and growth (Uma Devi <i>et al.</i> 2005)
Nematode	Important (Sharmila & Subramanian 2015)	Major. RH promotes survival and mobility (Abbaszadeh <i>et al.</i> 2011, Wang <i>et al.</i> 1995)	Important. Most species survive between 0°C and 37°C (Ulu & Susurluk 2014) but interspecific variation is observed for critical limits (Kulkarni <i>et al.</i> 2015; Ali <i>et al.</i> 2007).
Virus	Major (Asano 2005; Wu <i>et al.</i> 2015)	Minor	Minor and not limiting (Abbaszadeh <i>et al.</i> 2011; Lathika & Jacob 1974).
Observation	UV absorber or UV-reflective material are commonly added to increase viability in crops (Asano 2005; Wu <i>et al.</i> 2015)		

2. INFLUENCE OF CLIMATE ON BIOPESTICIDES PATHENOGENICITY

Infection by entomopathogenic bacteria and viruses require their ingestion by the insect while cuticular contact is needed for infection by fungi and nematodes. Considering that metabolic rate and energetic requirements increase with temperature in insects (Brown *et al.* 2004; Deutsch *et al.* 2018), global warming is therefore expected to result in increased pathogen infection because of increased feeding rate and mobility of pests (Elder & Reilly, 2014; Glare & O'Callaghan 2000). This common assumption has been experimentally confirmed in at least one species of mosquito (Walker 1995). Conversely, several nematode species actively search for insect hosts and their mobility also increases with temperature (Chen *et al.* 2003), presumably resulting in higher infection rate.

Temperature, humidity and atmospheric concentration of CO₂ have a strong influence on effectiveness of entomopathogenous organisms (Roy *et al.* 2006; Vega *et al.* 2010; Boomsma *et al.* 2014). A strong positive relationship between temperature and toxicity of entomopathogenic bacteria or their toxins has been commonly described for many pest species (El-Banhawy & Anderson 1985; van-Frankenhuyzen 1990; Glare & O'Callaghan 2000; Li *et al.* 1995). In *Plutella xylostella*, a 32-fold increase in abamectin toxicity was for example observed between 15°C and 32°C (He *et al.* 2001). Increased Bacteria pathogenicity may be partly explained by an increased production of melanin, a pigment that protects organisms against the damaging effects of UV, reported at high temperature (Ruan *et al.* 2004). Rising temperature may also result in an increased number of fungi nodules found in the haemolymph of pests (Mostafa *et al.* 2005) or an increased attachment rate and decreased time required for penetration into pests for nematodes, resulting in higher pathogenicity (Chen *et al.* 2003). High temperatures may however also have deleterious effects on nematodes (El Khoury *et al.* 2018) and fungi pathogenicity (Kistner & Belovsky 2017), probably explaining the strong seasonal variations observed for their success to control pests (Blanford & Thomas 1999). The efficiency of viruses to control pests varies greatly depending on geographical regions and therefore on climate (Abbaszadeh *et al.* 2011) although Shikano & Cory (2015) reported that temperature did not influence the success rate of infection of the cabbage looper *Trichoplusia ni* by a specific and a generalist virus.

The climate-dependency of pathogenicity described in bacteria, fungi and nematodes strongly depend on pest species and temperature range. *Bacillus thuringiensis* is for

example more effective at 30°C to control the Diptera *Sarcophaga africa* whereas it is more effective at 10°C and 20°C to control the honeycomb moth *Galleria mellonella* (Mastore *et al.* 2019). A biocide combining a toxin produced by *B. thuringiensis* and abamectin displayed significantly lower toxicities at 15/20°C than at higher temperatures against four pest species but toxicity increased with temperature above 20°C only in one of the four species (Zhu *et al.* 2005).

Humidity also determines the outcome of pest-bioinsecticide interactions, especially in fungi that require generally at least 95% relative humidity on host-insect cuticles for germination, penetration and infection (Hallsworth & Magan 1999; Verhaar *et al.* 1998). Similarly, high humidity promotes mortality of pests exposed to baculoviruses (Fuxa *et al.* 1999) or nematodes (Grant & Villani 2003; Radová & Trnková 2010).

Moreover, the interactions between climatic factors influence the effectiveness of entomopathogenous organisms. High temperature, decreased humidity and increased CO₂ concentration resulted for examples in decreased growth and sporulation of three entomopathogenic fungi (Borisade & Magan 2015). The combination of a high temperature, low humidity and UV exposure seriously compromised the survival of nematodes on the leaves and thus decreased their efficacy against foliar insects (Kaya 1985; Smart 1995).

3. INFLUENCE OF CLIMATE ON THE IMMUNE RESPONSE OF PESTS TO INFECTION

Although physiological fever is unknown in insects, these animals are able to perform behavioral fever. To do so, animals move to non-lethal temperatures for themselves but deleterious for the pathogen growth, thus limiting an infection (Inglis *et al.* 1997; Kistner & Belovsky 2017). Pathogenicity of entomopathogenous organisms may thus decrease in a warming world since pest insects should be able to perform behavioral fever more frequently. In the lab, the possibility for *Schistocerca gregaria* individuals infected with the fungus *Metarhizium anisopliae* to access thermal regimes allowing heightened fever temperatures decreased pathogen reproduction by 35%, delayed locusts' death and allowed them to produce viable offspring compared to control individuals (Elliot *et al.* 2002). Such a general assumption is however uncertain since the outcome depends on the thermal sensitivities of both partners (Müller & Schmid-

Hempel 1993) and on the complex effect of temperature on insect immune functioning which is quite unpredictable (Murdock *et al.* 2012). Moreover, the ability of a pest to resist pathogens at high temperatures can vary with pathogen species, as observed in the cricket *Gryllus texensis* that is more resistant to a Gram-negative bacterium but more susceptible to a Gram-positive bacterium at high temperature (Adamo & Lovett 2011).

In insects, immune response generally increases with the quality of food resources (Lee *et al.* 2006; Shikano *et al.* 2010). There are complex interactions between food quality and climate that play a determinant role in the pathogen's ability to infect its host and in the host's ability to resist (Triggs & Knell 2012; Shikano & Cory 2015). In the Indianmeal moth *Plodia interpunctella*, activity of phenoloxydase –an enzyme involved in arthropod immune system- increased with increasing temperature when larvae were raised on good food but decreased when poor food was provided (Triggs & Knell 2012). A decreased Protein/Carbohydrate diet, which is a common prediction for plants with climate change (Zvereva & Kozlov 2006; Dieleman *et al.* 2012), resulted in decreased survival of *Trichoplusia ni* larvae challenged with a specific (TnSNPV) or a more generalist baculovirus (AcMNPV), but temperature influenced differently the outcome depending on the virus (Shikano & Cory 2015).

Climate change may thus influence the outcome of entomopathogenous organisms versus pests' interactions in very complex ways, through its influence on occurrence in the field and pathenogenicity of control agents, and on pests immune response, which depends on (i) the direct influence of temperature, (ii) the indirect influence through its effect on plant nutritional quality, and (iii) the interaction between temperature and food quality.

IV. BIOLOGICAL CONTROL BY ENTOMOPHAGOUS ORGANISMS

Among pest management strategies, biological control by entomophagous organisms has received the most attention in the climate change context. Several reviews and conceptual frameworks have already been proposed on this subject (e.g. Aguilar-Fenolosa & Jacas 2014; Castex *et al.* 2018; Chidawanyika *et al.* 2019; Hance *et al.* 2006; Schmitz & Barton 2014 ; Selvaraj & Ganeshamoorthi 2013; Thierry *et al.* 2019; Thomson *et al.* 2010; Tylianakis & Binzer 2014) but considering that an increasing number of studies are published each year and that each review or opinion paper often focuses on a sole aspect, we propose here a non-exhaustive updated overview of the influence of climate change on entomophagous arthropods and their efficiency, focusing more on capturing the range and diversity of effects than on concrete examples. As discussed below, climate may influence pest control by directly affecting life-history traits, behaviors and communities' composition of natural enemies, and indirectly through cascading effects or spatial and temporal mismatch between the two trophic levels. Both biological control by introduction (*i.e.* introduction and acclimation of an exotic species as a biological control agent) and biological control by conservation (*i.e.* conservation of a native species as a biological control agent) have been considered, and predators as much as parasitoids.

1. DIRECT INFLUENCE OF CLIMATE ON NATURAL ENEMIES

a) Life history traits

Biological control by entomophagous organisms depends strongly on their population dynamics, which is determined by their life-history traits (Roff 1992). Almost all of them have been reported to be strongly climate-dependent in many natural enemies.

Development. The relationship between development rate and temperature is commonly bell-shaped in ectotherms (Sinclair *et al.* 2016), development time of most natural enemies should thus decrease with global warming, as long as temperatures do not exceed their critical upper limit. Developmental thermal requirements (*i.e.* thermal limits and optimum) nevertheless vary strongly between natural enemies' species (Furlong & Zalucki 2017) and populations (Traoré *et al.* 2006). Larval and pupal survival, and consequently adult emergence rate, also strongly depend on climatic factors since each species and/or population is able to develop only in a

specific range of temperature (Furlong & Zalucki 2017; Hance *et al.* 2007), humidity (Croft & Copland 1993) and CO₂ (Coehlo Junior *et al.* 2017). Many authors investigated the influence of temperature on emergence rate in natural enemies with various results (*e.g.* Agboka *et al.* 2004; Knapp & Nedved 2013) but only scarce studies are available for humidity (Krammer & Hain 1989; Pappas *et al.* 2008) or CO₂ (Chen *et al.* 2005; Roth & Lindroth 1995). Predictions are thus difficult to generalize and unpredictable consequences may arise when several factors interact. In a parasitoid, elevated temperature increased emergence success while drought had no effect, but the combination of both factors surprisingly produced the lowest parasitoid emergence (Romo & Tylianakis 2013).

Body size. Ectotherms tend to be smaller with increasing developmental temperature (Sibly & Atkinson 1994), as observed in several natural enemies (*e.g.* Knapp & Nedved 2013; Le Lann *et al.* 2011). Such relationship may have great consequences on pest management since body size is usually positively correlated to other major life-history traits such as fecundity (Kajita & Evans 2010), energetic reserves (Ellers *et al.* 2002) or dispersal ability (Ellers *et al.* 1998). Only scarce studies investigated the direct influence of CO₂ or humidity on body size of natural enemies and results varied between species, some authors reporting a decreased body size with elevated CO₂ (Zuo *et al.* 2015) while others reported no effect (Yin *et al.* 2009).

Reproductive traits. The relationship between potential fecundity and developmental temperature is usually bell-shaped in natural enemies (Colinet *et al.* 2007; Ris *et al.* 2004) while heterogeneous results have been reported for the influence of adult temperature on realized fecundity (*e.g.* Huang *et al.* 2008; Liu & Tsai 2002; Wang *et al.* 2013). Timing of reproduction may also be influenced by climate as increased developmental and adult temperature both resulted in earlier reproduction in a parasitoid (Moiroux *et al.* 2018) because of accelerated egg maturation rate (Papaj 2000; Berger *et al.* 2008).

Temperature may interact with humidity and shape natural enemies' reproductive parameters as observed in several parasitoid species (Emana 2007; Kalyebi *et al.* 2006), although several authors reported that temperature was a predominant factor compared to humidity in life-history traits determination (Bruce *et al.* 2009; Mainali *et al.* 2012). One may notice that most studies that we found on the influence of humidity were interested in natural enemies living in Africa, where humidity is a limiting factor, and that little literature was available for temperate latitudes.

Longevity. Finally, longevity commonly decreases as temperature increases in natural enemies although the strength of this relationship may vary between species (Tingle & Copland 1989), populations (Moiroux *et al.* 2013) and thermal regimes (Milosavljevic *et al.* 2019).

b) Behaviors

Abram *et al.* (2017) proposed a framework to classify the influence of temperature on behaviours of ectothermic species that we used here as a guideline to provide examples of the influence of climate on behaviours of natural enemies related to biological control. These authors distinguished two major effects: kinetic effects, which are detailed in Table 2, and integrated effects. Integrated effects occur when “*thermal information received by thermosensory organs is integrated in the central nervous system and used intentionally to modulate behaviours in a top-down fashion*”. Behavioral thermoregulation, *i.e.* the ability to modify performances by behaving in a location with adequate temperature (May 1979; Woods *et al.* 2015), is probably the simplest example. Increased thermoregulation opportunities with climate change may allow natural enemies to select better development (Hance *et al.* 2006; Josso *et al.* 2011) or oviposition sites (Ohashi *et al.* 2005), and thus increase their populations and better control pests oviposition. Other changes in foraging (Amat *et al.* 2006) or reproductive behaviours (Moiroux *et al.* 2014) may also be considered as thermosensory behavioural adjustments, although their interpretation as such is more speculative. To our knowledge, only a few studies investigated direct effects of CO₂, O₃ and humidity on behaviors related to biological control. As for behavioral thermoregulation, entomophagous arthropods select for microhabitats with optimal humidity to live and oviposit (Fink & Vökl 1995; Steidel and Reinhard 2003) and their locomotory activity is also determined by this factor (Rousse *et al.* 2009). O₃ may interfere with their olfactory system and affect their ability to discriminate between different prey densities (Gate *et al.* 1995) while CO₂ is commonly used to erase information from the memory in insects, resulting in modified behaviours (Louâpre & Pierre 2012). Whether they are kinetic or integrated effects, in response to temperature, CO₂, O₃ or humidity, such influence of climate on natural enemies' behaviors may strongly affect the outcome of pest management. Except for the short-term kinetics effects, it is however quite complicated to propose a general trend of the influence of

climate change on biological control through behavioural changes considering the diversity of effects that have been reported.

Table 2: Influence of increasing adult (short-term and medium-term effects) or developmental temperature (long-term effects) on behaviors related to biological control in entomophagous arthropods according to the framework proposed by Abram *et al.* (2017).

Kinetic effects	Definition	Direct influence	Indirect influence
Short-term	<i>Any behaviour whose rate is directly affected by temperature because of the thermal dependency of biochemical and/or neural pathways.</i>	Positive influence on feeding rate (Hansen & Jensen, 2002; Islam & Chapman 2001; Karner & Manglitz 1985)	
		Positive influence on locomotion-based behaviours, e.g. flight and walking speed (Gilchrist, 1996; Suverkropp <i>et al.</i> 2001) or rapidity to handle pests (Sentis <i>et al.</i> 2012)	
Medium-term	<i>Changes in insect behaviour due to the thermal dependency of state variables external to the individual animal itself.</i>	Negative influence on longevity and variable influence on egg load	Consequences on host handling time, clutch size (Rosenheim & Rosen 1991) and oviposition rate (Moiroux <i>et al.</i> 2018)
		Influence on the composition, quantity, and persistence of pheromones (Ono 1993; Roeser-Mueller <i>et al.</i> 2010)	Consequences on oviposition behavior (Sentis <i>et al.</i> 2015) or superparasitism (Moiroux <i>et al.</i> 2016)
		Negative influence on neural pathways involved in host's cues perception	
Long-term	<i>Effects occurring over longer time scales, acting across ontogenetic stages to modify irreversible aspects of physiological state and as a result shaping behavioural phenotype</i>	Negative influence on body size (Sibly & Atkinson 1994)	Consequences on oviposition (Segoli & Rosenheim 2015), flight performance (Fischbein <i>et al.</i> 2018) or conflicts between conspecifics (Guerra-Grenier <i>et al.</i> 2020).
		Negative influence on adult basal metabolic rate (Clarke 1993)	Decreased oviposition rate (Le Lann <i>et al.</i> 2011)

c) Cascading effects

Additionally to the direct effects of climate on entomophagous arthropods, an increasing number of studies underlined cascading effects that may arise with climate change. Climate change may first indirectly influence the fitness of natural enemies and thus their populations through its effects on prey or host quality, which is commonly related to their body size. Warm-developed aphids were for example reported to be less suitable for parasitoid development than cold-developed aphids because of their smaller size (Li & Mills 2004). Changes in plant content with climate change may also decrease prey quality for natural enemies, as reported for the ladybeetle *Harmonia axyridis* that eats more preys in elevated CO₂ treatments probably to compensate for the reduced soluble protein concentration in aphids owing to the decreased foliar N in plants (Chen *et al.* 2005). An increased concentration of allelochemicals in plants and therefore in host's diet with CO₂ or O₃-enrichment may also be toxic for natural enemies or alter the availability of nutrients, resulting in increased mortality (Holton *et al.* 2003; Roth & Lindroth 1995).

Most natural enemies rely on chemical cues emitted by pest-damaged plants to find their prey or host (Holopainen *et al.* 2013; Vet & Dicke 1992) and several reviews predicted an influence of climatic factors on the orientation behaviour of entomophagous arthropods (e.g. Bidart-Bouzat & Imeh-Nathaniel 2008; Boullis *et al.* 2015) since CO₂, O₃ and water influence the nature and/or ratio of compounds emitted by pest-infested plants (Percy *et al.* 2002; Vallat *et al.* 2005). Experimental evidence to support these projections are however quite limited. Predators (Takabayashi *et al.* 1994) and parasitoids (Pinto *et al.* 2007a) may prefer the odor of pest-infested plants exposed to low humidity or increased ozone concentration to the odor of plants grown in current climatic conditions. However, most studies reported that host location ability or discrimination ability between pest-infested versus control plant odor were not affected by increased ozone (Pinto *et al.* 2007b; Pinto *et al.* 2008; Vuorinen *et al.* 2004a) and CO₂ concentration (Himanen *et al.* 2009) or drought (Weldegergis *et al.* 2015). Some authors observed that odor discrimination ability may decrease with future atmospheric conditions but only for some plant cultivars (Himanen *et al.* 2009) or entomophagous species (Vuorinen *et al.* 2004b) and not others. All these studies suggest that despite the influence of atmospheric conditions on plant volatile compounds (Himanen *et al.* 2009; Weldegergis *et al.* 2015), natural enemies probably

rely on atmospherically stable volatile compounds to locate pest-infested plants and preys (Pinto *et al.* 2007b).

d) Changes in communities

Structure of entomophagous arthropods communities commonly vary temporally throughout the year (Andrade *et al.* 2016; Tougeron *et al.* 2020) and spatially (Meineke *et al.* 2017; Sorribas *et al.* 2010) with climate, mainly because each species differs in its climatic niche (Le Lann *et al.* 2011). The composition and functioning of these communities are thus expected to evolve with climate change, as reported for the French winter aphid parasitoids community that became more diverse recently because of increased winter temperature (Tougeron *et al.* 2018). Global warming may notably unbalance current entomophagous communities through (i) a differential influence of increased temperature on competition ability between species (Kfir *et al.* 1988), (ii) the appearance of new competitors (Vayssade *et al.* 2012) or predators and hyperparasitoids (Tougeron & Tena 2018), (iii) decreased interspecific differences in functional traits reported at high temperature (Le Lann *et al.* 2014) or (iv) an increased microhabitat overlapping to avoid stressful temperatures (Barton & Schmitz 2009). All these effects may result in competitive exclusion or intraguild predation and therefore alter food-webs and reduce pest control (Tylianakis & Binzer 2014). On the opposite, Cebolla *et al.* (2018) predicted that interspecific competition may stimulate some species to oviposit and counterbalance the negative effect of global warming on reproductive rate of natural enemies.

2. IMPACT OF CLIMATE CHANGE ON PEST CONTROL BY ENTOMOPHAGOUS ORGANISMS

a) Spatial and temporal mismatch

Development time commonly decreases (Furlong & Zalucki 2017) and distribution expands (Vuarin *et al.* 2012) with global warming in both pests and their natural enemies. The main concern about such modifications is whether climate change will result in altered spatial and temporal synchronization between the two trophic levels if they display differential response to climatic factors, as it may decrease biological control and even cause local extinction of specialist natural enemies. Several reviews on this topic have already been published (e.g. Damien & Tougeron 2019; Gerard *et*

al. 2013; Hance *et al.* 2007; Thomson *et al.* 2010) and spatial or temporal mismatch have been predicted in some systems (Wetherington *et al.* 2017) but not in others (e.g. Gerard *et al.* 2013; Klapwijk *et al.* 2010), as thermal dependency of development is typically species-specific.

Moreover, a differential influence of temperature on development rate of pests and natural enemies does not necessarily result in asynchrony, and increased asynchrony may not result in specialist natural enemies' extinction (Gebauer *et al.* 2015) or decreased pest control (van Nouhuys & Lei 2004). On the contrary, a reasonable advanced phenology of entomophagous arthropods may help to reduce pest populations as their control early in the year is crucial to avoid high densities later (Langer *et al.* 1997; Neuville *et al.* 2015).

Considering that thermal performance curves of developmental rate have been obtained for most of pest insects and their natural enemies, research on synchrony should now focus on optimising mathematical models that would help to predict accurately risks of spatial and/or temporal mismatch (Castex *et al.* 2018; Gebauer *et al.* 2015; Tonnang *et al.* 2017) for each plant-pest-natural enemy system. Such projections would be helpful to predict short-term changes occurring in agrosystems, although they do not consider long-term evolution of phenology with climate change that has been reported in several species and trophic relationships (e.g. Forrest & Miller-Rushing 2010; Visser & Gienapp 2019). Such issue may be especially relevant for biological control by introduction for which low genetic diversity in natural enemies' populations caused by a limited number of individuals released in the environment may result in an inability of natural enemies to evolve and adapt to a phenological or spatial shift of their prey/host (Aguilar-Fenollosa & Jacas 2014; Gerard *et al.* 2013). Moreover, other climatic factors should also be included in future mathematical projections since some of them, especially CO₂, are known to influence growth rate and thus phenology of some natural enemies' species (e.g. Chen *et al.* 2005; Sun *et al.* 2011).

b) Influence of climate change on the outcome of pests-natural enemies' interactions

The various effects of climate on natural enemies that are reported in previous sections imply that climate change may strongly shape the outcome of pest management by entomophagous arthropods, and several studies experimentally measured such

influence, investigating especially changes in pest abundance caused by changes in predation or parasitism rate. CO₂ concentration and temperature probably received the most attention in this context and heterogeneous results have been reported. Increased CO₂ concentration or temperature commonly result in increased pest abundance because of an increased pest fecundity but a decreased or an unchanged parasitism and predation rate by natural enemies (Bezemer *et al.* 1998; Chen *et al.* 2007; Klaiber *et al.* 2013; Stacey & Fellowes 2002). However, an increased parasitism or predation rate may compensate for the increased pest fecundity and keep pest control stable under elevated CO₂ concentration or temperature in some systems (Chen *et al.* 2007) although such result may depend on entomophagous species (Zamani *et al.* 2006) or plant cultivars (Hentley *et al.* 2014).

Changes in predation or parasitism rate may result from changes in natural enemies' behaviors but also in pests' behaviors. Water-deficit stress or high temperature indeed result in increased aggressive defensive behaviors in aphids, decreasing their control by natural enemies (Ahmed *et al.* 2019; Moiroux *et al.* 2016). Defense behaviors may moreover evolve with climate change (Roitberg & Myers 1979) through selection of adaptations or transgenerational phenotypic plasticity and result in long-term decreased pest control efficiency although such result may depend on natural enemy species and climatic factor studied (Mondor *et al.* 2004).

In an attempt to predict more accurately the influence of climate change on the efficiency of natural enemies to control pests, several authors recently simulated interacting climatic factors (*e.g.* Guyer *et al.* 2020) and reported unexpected results. The parasitoid *Diaeretiella rapae* was thus more effective at suppressing populations of the cabbage aphid under warmer or drought conditions but these effects were reversed when the drivers were expressed in concert, mainly because natural enemies suffered high mortality only when both stresses were applied (Romo & Tylianakis 2013).

Such experimental studies underline the importance of combining drivers to offer a realistic view but they still simplify agro-ecosystem, and provide therefore a limited insight of what biological control may be in the future. Large-scale field approaches or meta-analysis of studies quantifying biological control under different latitudes are powerful tools that may provide better projections since they directly look for the influence of climate on pest abundance in crops (*e.g.* Diehl *et al.* 2013). Field surveys of kale crops across New-Zealand thus revealed that average site temperature did not

influence the abundance of the main aphid pest while it was positively correlated to the number of parasitised aphids, resulting in stable pest control under climate promoting pest fecundity (Romo & Tylianakis 2013). A meta-analysis of 30 studies on the influence of climate on biological control by *Trichogramma* egg parasitoids across the world showed that parasitism (i) decreased with increasing absolute latitude, (ii) was higher in regions with less climate seasonality in continents other than the Americas, (iii) was negatively correlated with the frequency of extreme high temperature events for almost all datasets although this depended on crop type, (iv) was negatively correlated with the daily maximum temperature in corn, but positively correlated in vegetable crops (Wu *et al.* 2016). Such studies may thus help to predict accurately changes in biological control with climate change and should be developed in the future.

c) Interacting effects of climate change and other factors on the outcome of pests-natural enemies' interactions

Climate change may influence differently pest control by entomophagous arthropods depending on cultural practices, especially when considering that toxicity of insecticides is commonly temperature-dependent in natural enemies (Abbes *et al.* 2015; Mansoor *et al.* 2015; Michalko & Košulič 2016). As for pests, contrasting results have been reported depending on insecticides and species but global warming may indirectly reduce biological control by enhancing their toxicity on natural enemies. A 2°C warming thus reduced efficacy of predaceous ladybeetles to control pests in conventional alfalfa fields while there was no influence in organic fields (Murrell & Barton 2017). Cultures relying on insecticides to control insect pests may thus be more severely influenced by climate change than others because of decreased biological control by entomophagous species.

PERSPECTIVES IN A CHANGING CLIMATE

In a near future, pest outbreaks are expected to occur more frequently with global warming (Gutierrez *et al.* 2008) and several models predicted an increased use of chemical insecticides to cope with such issues (Koleva *et al.* 2009). In this section, we review potential solutions to optimize pest management to the upcoming climate while limiting the massive use of insecticides, in order to decrease related environmental and health issues and production costs for farmers.

- Combination of biological control agents

Entomopathogenic and entomophagous organisms are all influenced by climate but their relative sensitivity to temperature, UV radiation, humidity and CO₂ level differ between and within each group. Using combinations of biological control agents is thus an interesting way to deal with such influence of climate, especially when synergy emerges from this combination. For example, Inglis *et al.* (1997) reported that the fungus *Metarhizium flavoviride* was more efficient at hot temperature than *Beauveria bassiana* to regulate the grasshopper *Melanoplus sanguinipes* while the opposite was observed at cool temperature. However, application of both pathogens simultaneously resulted in a greater pest mortality than that for *M. flavoviride* alone in the hot temperature environment and equal to that for *B. bassiana* in the cool temperature environment. Increased daily temperature variation, a common prediction with climate change, did not influence the toxicity of an organophosphate pesticide or of *Bacillus thuringiensis* on the mosquito *Culex pipiens* when applied separately but surprisingly resulted in increased toxicity of a mixture of these two insecticides (Delnat *et al.* 2019). Using cocktails to overcome the constraints of future climate is thus an interesting possibility that should be investigated thoroughly since studies are still scarce.

- Compounds and strains selection

As discussed in the dedicated section, insecticide toxicity strongly varies with temperature, but this relationship depends on both the insecticide (*e.g.* Musser & Shelton 2005) and the target species (*e.g.* Sparks *et al.* 1982, 1983). Investigating specifically the temperature-dependency of insecticide for a given pest may thus help to select the most effective ones. Understanding underlying biochemical mechanisms may also be helpful to design efficient insecticides in a climate change context.

Similarly, intraspecific variability in the sensitivity to temperature, humidity or CO₂ concentration has been described in several entomopathogenic (e.g. Borisade & Morgan 2015) and entomophagous organisms (e.g. Moiroux *et al.* 2010). A positive relationship between heat tolerance and mean or highest annual temperature at place of origin of the strains has for example been reported in nematodes (Mukuka *et al.* 2010; Ulu & Susurluk 2014). Temperature-dependency of life history traits involved in biological control also depend on climate of origins. Parasitoids sampled in warm climate thus developed faster (Traoré *et al.* 2006) or had a higher fecundity (Moiroux *et al.* 2010) than parasitoids from cooler climates at high temperature. Screening strains from different geographical regions, especially from warm and dry areas, may thus allow for selection and eventually cross-breeding of biological control agents adapted to the upcoming climate (Kalyebi *et al.* 2006).

- Genetic improvement of biological control agents

Cross-breeding of tolerant strains may be combined with artificial selection to improve efficacy of commercialized lines (Ehlers *et al.* 2005). Mukuka *et al.* (2010b) were thus able to achieve an increase of 3°C for heat tolerance in a nematode by cross-breeding tolerant parental strains selected among 60 populations and then performing an eleven steps artificial selection. High heritability (*i.e.* 0.68) reported for this trait (Ehlers *et al.* 2005) probably explains such success but other trait may be harder to select. Such artificial selection for heat or desiccation resistance may even sometimes result in improvement of other interesting life history traits. Selection for improved desiccation tolerance resulted for example in increased heat resistance in the nematode *Steinernema feltiae* (Salame *et al.* 2010) while selection over 50 generations for heat resistance resulted in a 60% rise in fecundity for the parasitoid *Trichogramma chilonis* (Srivastava & Singh 2015). Genetic improvement should however be investigated thoroughly since artificial selection for tolerance to a given temperature may be traded-off against tolerance to other temperatures (Lirakis & Magalhaes, 2019) or against other fitness-related traits (Carriere & Boivin 2001). Moreover such procedure is long, expensive and failures have been reported for some traits such as desiccation resistance (Mukuka *et al.* 2010b) or minimal temperature required for activity (Ehlers *et al.* 2005) in nematodes.

Some studies mentioned in the “Chemical Insecticides” section highlight that climate change may influence pest control through its effect on insecticide effectiveness but

also through increased insecticide-induced mortality and sublethal effects in their natural enemies. In this perspective, Kumar *et al.* (2008) developed genetic improvement of the egg parasitoid *Trichogramma chilonis* for combined tolerance to both insecticides and high temperatures. After 81 generations, they obtained strains that would be more performant with global warming when insecticides are applied.

QUE RETENIR ?

Cette revue de littérature a permis de mettre en évidence de nombreux effets directs et indirects du climat sur l'efficacité des principales méthodes de lutte utilisées contre les ravageurs des cultures. A défaut de pouvoir établir des tendances générales en raison de la spécificité de chaque système, elle nous a permis de dégager plusieurs questions qui restent aujourd'hui en suspens ou peu explorées et pourraient s'avérer utiles pour adapter les stratégies de contrôle des ravageurs au futur climat. Les paragraphes qui suivent sont une synthèse non-référencée des principaux questionnements de cette revue de littérature.

Les insecticides. Modèles mathématiques et approches expérimentales indiquent qu'il faudrait appliquer globalement plus d'insecticides chimiques avec le réchauffement climatique en raison (i) d'une réduction de leur rémanence dans les agroécosystèmes, (ii) d'une dilution accrue dans les plantes et (iii) d'une augmentation des populations de ravageurs. Bien que peu de données existent à ce sujet, il est également communément admis que des résistances aux insecticides devraient apparaître plus fréquemment en raison de l'augmentation des populations de ravageurs et de leur nombre de générations.

L'aspect le moins prédictible semble être l'effet de la température sur la toxicité des produits phytosanitaires sur les ravageurs, puisque cette relation peut varier du tout au tout en fonction de l'insecticide, de l'espèce cible, de l'âge d'exposition et de la gamme de température étudiée. Aucune grande tendance n'a pu être dégagée *et il est par conséquent important de considérer chaque système indépendamment afin d'identifier les insecticides restant les plus efficaces avec une élévation des températures pour chaque ravageur, et éventuellement de pouvoir adapter leur utilisation en fonction de la saison d'application.* De plus, les mécanismes physiologiques, biologiques et moléculaires impliqués dans cette variation de la toxicité avec la température n'ont été que très peu étudiés. *Comprendre ces mécanismes pourrait être une étape importante pour développer ou sélectionner des insecticides étant le moins affectés par le réchauffement climatique.*

La confusion sexuelle. Des changements saisonniers ont été relevés dans l'efficacité de la lutte impliquant l'utilisation de phéromones sexuelles de synthèse mais ceux-ci sont généralement expliqués par une variation saisonnière dans l'abondance des ravageurs. Il a également été établi depuis plusieurs décennies que l'augmentation de la température réduit la durabilité des diffuseurs de phéromones de synthèse et accélère la dégradation de ces dernières, mais nous n'avons pas trouvé de preuve que ces effets réduiraient l'efficacité réelle de cette stratégie en champs.

Afin de fiabiliser cette méthode dans un contexte de changements climatiques, deux aspects semblent importants. *Optimiser les modèles phénologiques permettra de prédire au mieux les dates d'émergence des ravageurs selon le futur climat, et donc de mettre en place la confusion sexuelle lorsque les populations de ravageurs sont encore faibles. Comprendre l'effet du climat sur les réponses sensillaires et neuronales, physiologiques et comportementales aux phéromones représente un autre point important.* Les très rares études que nous avons trouvées ont mis en évidence une diminution du degré de spécificité aux odeurs et une augmentation de la réponse antennaire et comportementale aux phéromones sexuelles avec l'élévation de la température chez quelques lépidoptères ravageurs.

Le réchauffement climatique pourrait donc augmenter l'efficacité des méthodes de lutte basées sur l'utilisation des phéromones de synthèse. *La diminution du degré de spécificité aux odeurs à haute température permettrait notamment le développement de formules moins spécifiques que celles disponibles actuellement, afin de viser un groupe de ravageurs plutôt qu'une seule espèce.*

Les organismes entomopathogènes. Si l'élévation des températures et une diminution des précipitations pourraient avoir des effets délétères sur la survie et le potentiel infectieux des nématodes et champignons entomopathogènes en champs, les bactéries et virus ne devraient être que peu affectés par le changement climatique. Bien que leur pathogénicité augmente généralement avec la température, cet effet semble dépendre de l'espèce cible. L'issue de la relation ravageur/pathogène dépendra également de la possibilité pour les premiers d'accéder plus facilement à des situations leur permettant d'effectuer de la « fièvre comportementale » afin de limiter les infections, bien que cet effet puisse être contrebalancé par une diminution de leur système immunitaire liée à une diminution de la qualité des plantes induite par le changement climatique. Parmi les améliorations possibles, il a été proposé qu'un

criblage de différentes populations et/ou lignées permettrait de sélectionner les organismes entomopathogènes les plus adaptés aux futures conditions climatiques pour chaque ravageur.

Les organismes entomophages. Les effets du climat sur la lutte biologique réalisée par les organismes entomophages sont de loin les plus étudiés mais également les plus complexes à projeter dans le futur. Le climat influence les traits d'histoire de vie et les comportements de ces organismes, mais ces effets dépendent trop fortement de l'espèce et de la gamme de température considérées pour permettre une généralisation. *Il semble donc important de décrire l'effet du climat sur les dynamiques de populations des entomophages et sur leurs comportements de prédation et de parasitisme pour chaque système d'intérêt.* Des effets en cascade du changement climatique depuis la plante jusqu'aux ennemis naturels pourraient également intervenir, bien que l'hypothèse classique d'une diminution de leur capacité à trouver les ravageurs en raison de modifications des composés volatiles émis par les plantes ne semble pas vérifiée expérimentalement.

Au-delà des effets directs et indirects sur les entomophages utiles en lutte biologique, il apparaît essentiel de s'intéresser à l'effet du climat sur le couple ennemi naturel /ravageur. Des décalages spatiaux et temporels pourraient ainsi survenir avec le changement climatique. *Améliorer les modèles phénologiques pour comparer les deux niveaux trophiques semble donc un point majeur. De même, un effet différentiel entre niveaux trophiques de la température sur la toxicité des insecticides pourrait avoir des conséquences fortes sur le rôle des entomophages.* Ce type de projection, peu considéré dans la littérature, est particulièrement important pour les producteurs mettant en place des stratégies de protection intégrée reposant en partie sur la lutte biologique mais aussi sur des insecticides lorsque la pression des ravageurs s'avère trop importante.

I-2 : Problématique, objectifs et hypothèses de travail

Nous avons cherché à répondre à certains questionnements soulevés par cette revue des effets du changement climatique sur les méthodes de lutte en nous focalisant sur le principal ravageur en cultures de pommes, *Cydia pomonella* (L.). Ce modèle biologique est particulièrement intéressant car une diversité de stratégies de lutte sont utilisées pour limiter ses effectifs en vergers dont des insecticides de synthèse appartenant à différentes familles, la confusion sexuelle, un virus entomopathogène et la lutte biologique. La Région PACA représente une zone d'étude critique de par l'impact marqué que le changement climatique devrait avoir dans le bassin méditerranéen.

Nous avons ainsi cherché à répondre à la problématique suivante : **Quels seront les impacts du réchauffement climatique sur l'efficacité des principales méthodes de lutte contre le carpocapse des pommes, *Cydia pomonella* (L.), en Région PACA ?**

Cette thèse s'articule autour de l'efficacité de trois méthodes de lutte contre le carpocapse des pommes : (i) les insecticides de synthèse, (ii) la lutte biologique par acclimatation ou conservation d'espèces et (iii) la confusion sexuelle.

La première partie des expérimentations (**Chapitre II**) s'intéresse aux effets croisés température/insecticides sur la mortalité du carpocapse des pommes face à différentes substances insecticides, dont une substance homologuée pour une utilisation en agriculture biologique.

L'augmentation des températures, dans une limite acceptable par rapport à la température optimale de développement, modifie-t-elle les capacités de détoxification de *C. pomonella* et par conséquent sa capacité à se défendre contre des insecticides chimiques ?

Les mécanismes biochimiques et moléculaires susceptibles d'être impliqués dans des variations de toxicité avec la température ont été également étudiés.

Nous avons notamment cherché à déterminer si la température, l'exposition à un insecticide et l'action conjointe de ces deux facteurs induit des changements dans

l'expression de gènes de : (i) protéines de choc thermique, (ii) cibles moléculaires ou (iii) enzymes de détoxification, et dans l'activité spécifique de ces dernières ?

L'hypothèse principale de ce chapitre est que l'augmentation des températures engendrera des variations de mortalité chez *C. pomonella*, accompagnées de changements dans l'expression de certains gènes codant pour des enzymes de détoxification, des protéines de choc thermique et/ou des cibles moléculaires des insecticides.

Le **Chapitre III** s'inscrit dans la volonté de comprendre les effets possibles du réchauffement climatique sur la gestion du carpocapse dans les vergers s'appuyant à la fois sur la lutte biologique réalisée par les insectes entomophages et l'utilisation d'insecticides. Un effet différentiel de la température sur la toxicité des insecticides entre ravageurs et ennemis naturels pourrait ainsi avoir d'importantes conséquences sur les populations de carpocapse. L'objectif de ce chapitre est donc de déterminer les effets croisés de la température et de différentes substances insecticides, dont une homologuée en agriculture biologique, sur la mortalité de *C. pomonella* et de deux de ses ennemis naturels, le prédateur *Forficula auricularia* et le parasitoïde *Mastrus ridens*.

La combinaison de méthodes de lutte biologique et chimiques sera-t-elle encore viable après un accroissement des températures ? Peut-on adapter la période d'utilisation de différents insecticides selon l'effet différentiel de la température sur leur toxicité chez un ravageur et ses ennemis naturels ?

L'hypothèse principale de ce chapitre est que la température affectera différemment la sensibilité des espèces aux substances insecticides étudiées, et que cela aura des conséquences sur les populations du carpocapse des pommes.

Dans le **Chapitre IV**, un intérêt est porté à l'utilisation d'ennemis naturels de *C. pomonella* en arboriculture. Pour garantir le succès des ennemis naturels, les questions de synchronicité phénologique avec le ravageur cible sont primordiales. Les objectifs de ce chapitre sont d'évaluer les effets d'une augmentation des températures sur la phénologie de *C. pomonella* et la phénologie, les traits d'histoire de vie et le

comportement de prédation ou de parasitisme de deux ennemis naturels, *F. auricularia* et *M. ridens*.

Ces différentes espèces ont-elles les mêmes courbes thermiques de développement et les ennemis naturels seront-ils présents en vergers en même temps que *C. pomonella* avec le réchauffement climatique ? Le contrôle futur de *C. pomonella* par chaque ennemi naturel restera-t-il équivalent à celui d'aujourd'hui avec le changement climatique actuel et futur ?

L'hypothèse de ce chapitre est que *C. pomonella* et ses ennemis naturels vont se développer plus vite lorsque la température augmente mais n'auront pas les mêmes optimums et limites de température. La température pourra augmenter le taux parasitisme ou de prédation des deux ennemis naturels via l'augmentation de leur métabolisme, dans la limite de leurs optimums thermiques.

Le **Chapitre V** porte sur la lutte contre le carpocapse des pommes par confusion sexuelle. Il s'intéresse à la perception par les mâles de *C. pomonella* des phéromones commerciales dans un contexte de réchauffement climatique. L'objectif de ce chapitre est de déterminer si une augmentation de température peut perturber la détection par les récepteurs olfactifs antennaires de *C. pomonella* de molécules volatiles seules ou en mélange.

L'augmentation de la température de diffusion des composés volatiles phéromonaux entraîne-t-elle leur meilleure détection par *C. pomonella* ? Le développement du carpocapse des pommes en condition de stress thermique modifie-t-il sa capacité à détecter les odeurs ?

L'hypothèse principale de ce chapitre est que l'augmentation des températures pourrait engendrer chez les mâles de *C. pomonella* une réponse électrique antennaire plus forte aux composés phéromonaux, qu'ils soient seuls ou en mélange.

Enfin, le **Chapitre VI**, présente une discussion générale de l'ensemble des questionnements et résultats présentés dans ce manuscrit. Il amène à d'autres questionnements mis en perspectives.

I-3 : Contexte de l'étude

Climat de la Région PACA et compartiments biologiques présents dans les vergers de pommiers

I-3-1 : Agroécosystèmes : vergers de pommiers de la région PACA

- *Un contexte climatique particulier*

La Région PACA est dominée par un climat de type méditerranéen (Fig. 2) caractérisé par un été chaud, un hiver doux, une sécheresse estivale importante et des précipitations irrégulières, un fort ensoleillement ainsi que des vents puissants et secs (Briche *et al.* 2017). Ce climat est très sec avec très peu de précipitations pendant la période chaude que représente la fin du printemps et l'été mais aussi pendant la période froide avec quelques événements de sécheresse hivernale possible (Raymond *et al.* 2018). Les précipitations sont plutôt marquées mais irrégulières en automne et au début du printemps (Briche *et al.* 2017).

Cependant, il existe beaucoup de nuances de ce climat en fonction notamment du contexte topographique. En effet, les reliefs vont bénéficier d'une diminution de la température avec l'altitude et de précipitations plus abondantes. A contrario, les vallées dans lesquelles s'engouffrent les vents permettent de maintenir un temps sec et ensoleillé. C'est le cas de la vallée du Rhône, dans laquelle s'engouffre le Mistral, vent violent et sec qui prend son origine en haute vallée du Rhône, qui s'intensifie le long de la vallée du Rhône, jusqu'en basse vallée. Il souffle en moyenne 100 jours par an et est à l'origine du temps sec et ensoleillé caractéristique du climat provençal.

En moyenne sur la Région, sans tenir compte des nuances locales du climat méditerranéen, les températures sont comprises entre 5°C en hiver et 25°C en été. Les hivers sont relativement froids avec 70 jours maximum de gel. Comme la période estivale reste très chaude, les températures maximales atteignent souvent les 35°C avec l'occurrence de certains pics journaliers encore plus chauds ces dernières années pouvant être imputables aux changements climatiques.

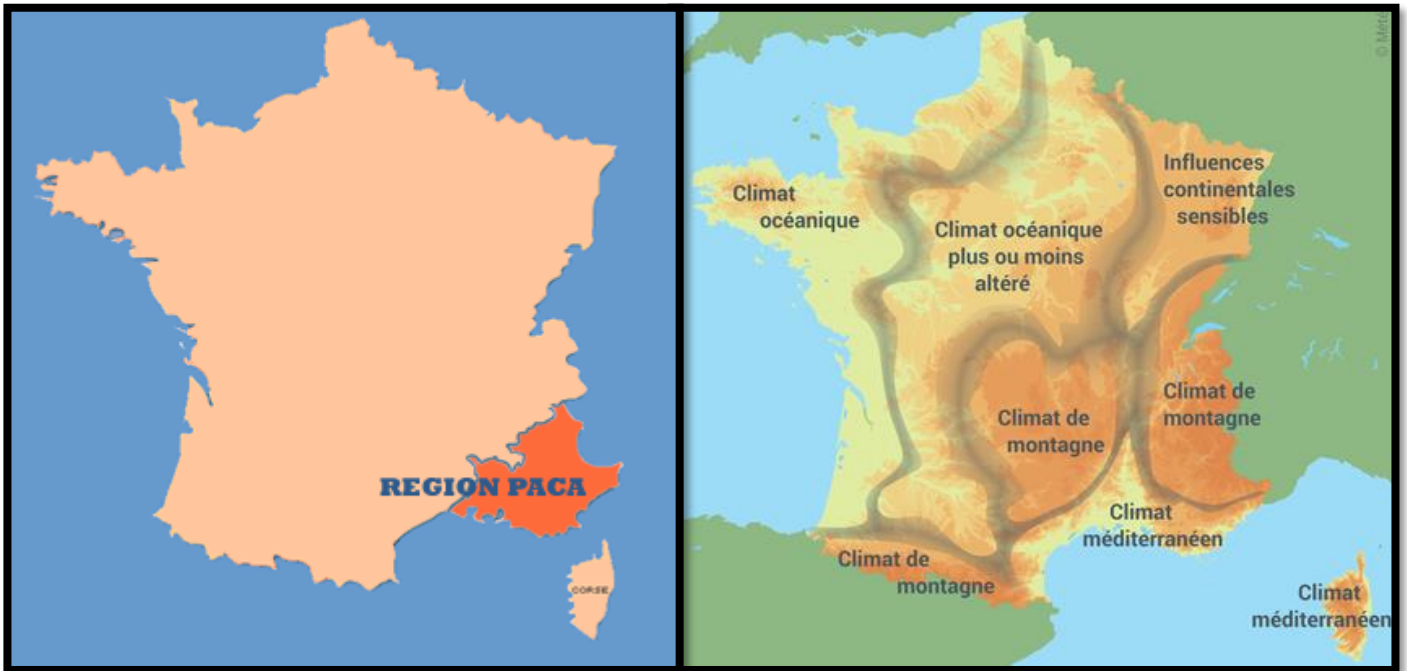


Fig. 2 : Localisation de la Région PACA en France à gauche et répartition des climats en France à droite

- *Culture du pommier en méditerranée*

La France est le troisième producteur européen de pommes derrière la Pologne et l'Italie avec 1,4 millions de tonnes de pommes produites en 2019 (FranceAgriMer 2019). En France, la pomme représente 26% des surfaces de production fruitière (FranceAgriMer 2014). Selon les rapports des stations expérimentales de la Pugère et de la Tapy, la Région PACA représente 25% de la production nationale de pommes. En 2016, en termes de surface de production, le Vaucluse est le premier département producteur de pommes avec 3631 ha de vergers de pommiers, suivi des Bouches du Rhône (2566 ha), des Alpes de Haute Provence (1932 ha) et des Hautes Alpes (1822 ha). En surface totale, la Région PACA enregistre 9822 ha de vergers de pommiers représentant 387 562 tonnes de pommes produites en 2016 (Chambre d'agriculture PACA). Etant donné l'importance de la production des pommes dans la région PACA, les enjeux que représentent le réchauffement climatique et les ravageurs des pommiers à l'échelle régionale sont conséquents.

Présent en région méditerranéenne depuis l'antiquité, le pommier s'est adapté à ce climat et est relativement résistant aux variations climatiques et à l'occurrence de pluies irrégulières (Juniper *et al.* 1998). Certaines variétés seront plus résistantes à ce climat que d'autres (Díez-Palet *et al.* 2019). Cependant, le pommier reste vulnérable à la sécheresse prolongée qui va fortement contribuer à l'arrêt de sa croissance (Šircelj

et al. 2007). Il est donc nécessaire de contrôler l'irrigation en vergers. Bien qu'il soit résistant aux hivers rudes, des chutes de grêles pendant l'hiver ou des épisodes longs de gel peuvent lui être dommageables, certains arboriculteurs prennent donc le parti de couvrir les vergers de filets anti-grêle. Une fois bien installé, un pommier peut produire pendant de nombreuses années, entre 16 et 70 ans dépendant de la variété et de l'environnement d'après Ruth & Kelley 1932. Pendant son installation, le jeune pommier est lui plus vulnérable aux conditions climatiques. En Région PACA, avec une sécheresse estivale marquée, les jeunes pommiers sont généralement plantés en Novembre, ils pourront ainsi bénéficier des pluies hivernales et se développer suffisamment jusqu'au retour de la sécheresse estivale. Il est à noter que le jeune arbre prendra au moins 5 ans à produire ses premières pommes (Ruth & Kelley 1932). Le cycle de vie du pommier se caractérise par une phase de dormance et une période d'activité. De la chute des feuilles en automne au début du débourrement printanier, c'est la phase de dormance hivernale durant laquelle l'arbre va arrêter sa croissance (Gautier 1987 ; Bentayeb 1993). Au débournement et jusqu'à la chute des feuilles, c'est la période d'activité durant laquelle le pommier va produire de nouvelles feuilles et grandir, produire des bourgeons puis des fruits et se lignifier (Gautier 1987). Bien que conditionnées par des facteurs endogènes, l'entrée et la sortie de dormance sont aussi déterminées par des facteurs exogènes tels que la température, la photopériode et le stress hydrique (Wareing 1956 dans Herter 1992). En Région PACA, l'hiver doux et les températures clémentes du mois de mars permettent un allongement de la période d'activité du pommier. Ce climat très particulier qui semble favorable à la culture des pommes va présenter une limite assez importante à la pérennité de ces vergers dans un contexte actuel de changements climatiques.

- *Contrôle phytosanitaire des ravageurs en vergers*

Les vergers de pommiers sont soumis à plusieurs ravageurs. Les deux principaux, qui impliquent un traitement, sont le carpocapse des pommes, *Cydia pomonella*, et le puceron cendré, *Dysaphis plantaginea*. Pour lutter contre ces ravageurs et contre d'autres bioagresseurs phytopathogènes tels que les champignons *Venturia inaequalis* et *Podosphaera leucotricha* (Laget 2012), de nombreux traitements phytosanitaires sont appliqués (entre 23 et 53 traitements par an). En 2015 dans la Région PACA, 28 traitements phytosanitaires en moyennes sont appliqués dans les vergers de pommiers (Voir Fig. 3).

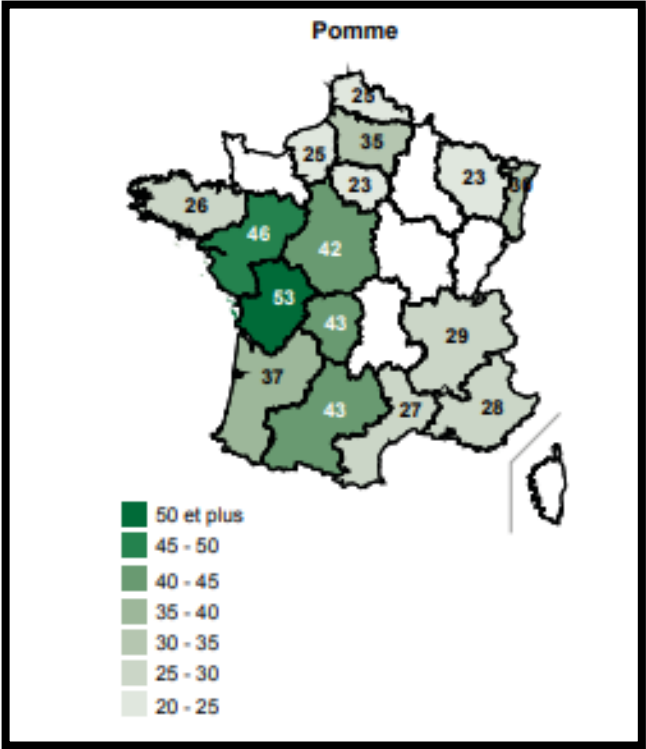


Fig. 3 : Nombre moyen de traitements phytosanitaires appliqués sur les pommiers en fonction des régions en 2015. D'après Agreste 2021.

I-3-2 : Le carpocapse des pommes en Région PACA

Le carpocapse, *C. pomonella* (Lepidoptera : Tortricidae, Linné), est un des principaux ravageurs des pommes, mais aussi des poires et des noix dans le monde. Il est responsable de pertes économiques majeures dans le domaine de la production fruitière (Ciglar 1998). Il est originaire d'Eurasie, provenant sûrement du Kazakhstan, et s'est dispersé à travers le monde au cours des deux derniers siècles (Frank *et al.* 2007). Sa présence sur chaque continent et sa dispersion relativement récente sont reliées à l'intensification des cultures de pommes et de poires à l'échelle mondiale (Fig. 4). Cela en fait un des ravageurs les plus néfastes connu à ce jour (Thaler *et al.* 2008).

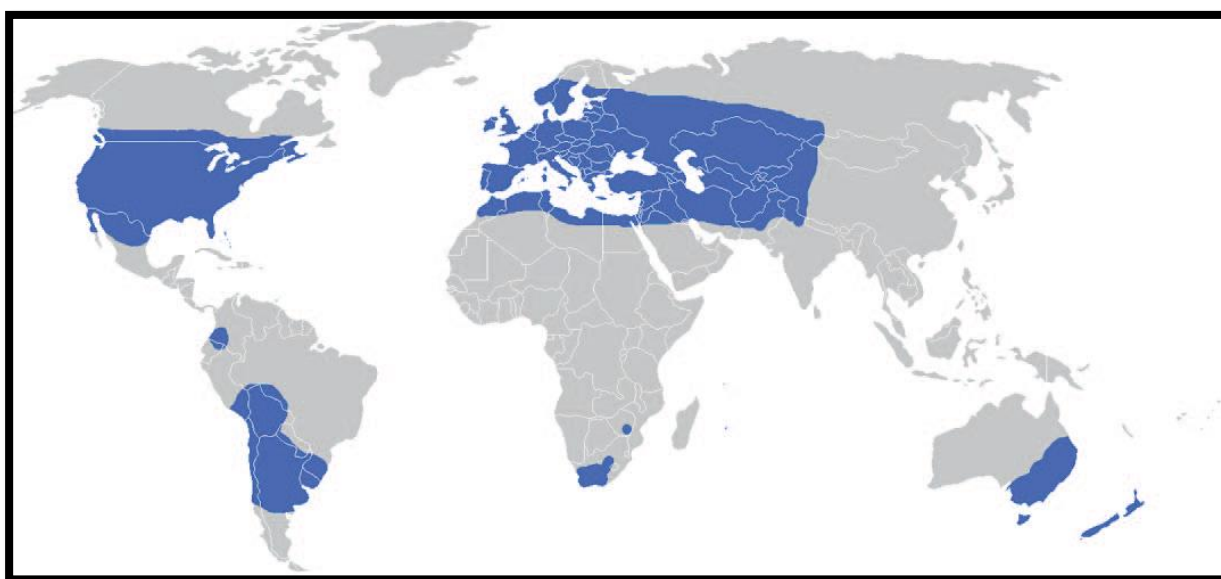


Fig. 4 : Carte de répartition de *Cydia pomonella* (d'après Pajač *et al.*, 2011)

De plus, une problématique de la production fruitière est que les fruits sont commercialisés en frais, ils ne doivent donc présenter aucune imperfection. La qualité du produit est donc au centre des attentions. Dans un verger le seuil d'acceptabilité de dégâts est de 1% de fruits piqués (Pajač *et al.* 2011). Malgré un grand nombre de traitements chimiques pour contrôler les populations de *C. pomonella*, il est difficile de rester en dessous de ce seuil (Pajač *et al.* 2011).

Le carpocapse adulte est de couleur cendrée avec l'extrémité des ailes marron foncé. Il possède une envergure d'environ 18 mm les ailes déployées (Pajač *et al.* 2011). Les œufs sont sphériques, d'environ 1 mm de diamètre et de couleur blancs translucides quand ils viennent d'être pondus. Ils évoluent ensuite vers une couleur laiteuse avant de développer un anneau rouge au centre puis l'apparition de taches noires appelées « têtes noires » (correspond à la tête en formation de la larve) vers la fin de leur

développement. De ces œufs vont émerger des larves qui compteront 5 stades de développement larvaire. Elles mesurent environ 1 mm à la sortie de l'œuf et sont entièrement blanches, à l'exception de leur tête noire. Au fur et à mesure de leur développement, à chaque stade larvaire les larves vont perdre leur capsule céphalique avant d'en recréer une pendant le stade suivant (Fig. 5). Cela permet de suivre précisément le nombre de jours de développement pour chaque stade. Plus les larves se développent, plus elles sont grandes et peuvent se teinter d'une couleur rose (Fig. 6). Au 5^{ème} stade larvaire la larve peut mesurer jusqu'à 20 mm de long (Fig. 6B) (Maceljski, 2002). A ce stade elles tissent un cocon puis se transforment en chrysalide. La chrysalide est marron clair et mesure environ 10 mm de long.

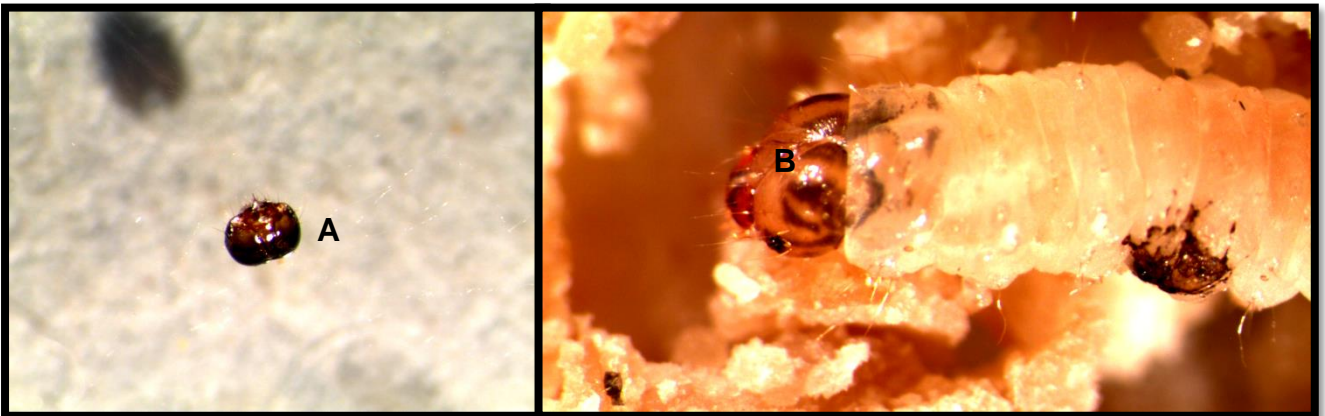


Fig. 5 : capsules céphaliques de larves de carpocapses : tombée au cours du développement larvaire (A) ou reformée sur la larve après une mue (B)

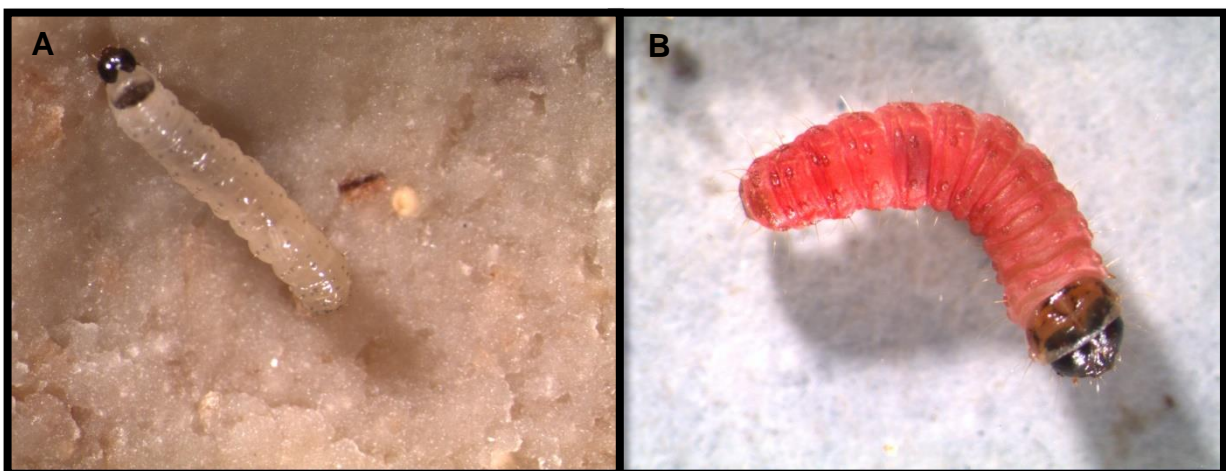


Fig. 6 : Larves de carpocapses aux stades larvaires 3 (A) et 5 (B)

Le carpocapse passe l'hiver sous forme de larve diapausante dans le sol, sous l'écorce des pommiers ou dans les débris se trouvant aux alentours des arbres. Au début du printemps, quand les conditions climatiques dépassent les 10°C et qu'elles sont plus favorables, la larve se nymphose (Pajač *et al.* 2011). Le temps de développement de la chrysalide dépend des conditions climatiques. Au bout d'un certain nombre de jours (entre 7 et 30 en fonction de la température), la chrysalide est formée. Il faut ensuite une certaine quantité de chaleur pour que les adultes émergent des chrysalides. Cette quantité de chaleur nécessaire est calculée en degrés jours (Fig. 7). Calculer les degrés jours permet d'avoir un nombre donné d'accumulation de chaleur pour passer d'un stade de développement à l'autre chez les plantes et les organismes ectothermes (la température corporelle est fonction de celle du milieu dans lequel il vit). Pour que le carpocapse passe de nymphe à adulte il faut 100°J (Wildbolz 1965 ; Ciglar 1998). Le temps que prend l'accumulation de chaleur pour atteindre 100°J dépend des conditions climatiques. Plus il fera chaud, plus le temps nécessaire pour atteindre 100°J sera court et inversement quand il fait plus froid. Généralement, ce cumul de 100°J est atteint courant Avril.

$$^{\circ}J = \frac{T_{max} + T_{min}}{2} - T_{base}$$

Fig. 7 : équation de calcul des °J.

Tmax = température maximale ; *Tmin* = température minimale
Tbase = température minimale nécessaire à la croissance d'un organisme (= 10°C pour le carpocapse)

Le premier vol des adultes commence entre Avril et Mai et dure environ jusqu'à fin Juin. Les adultes volent à l'aube et au crépuscule quand les températures journalières sont moins élevées. Pendant cette période de vol, ils s'accouplent. Chaque femelle va ensuite pondre jusqu'à 200 œufs sur les pommes ou sur les feuilles et branches à proximité des pommes. Après incubation des œufs, les larves nouvellement émergées pénètrent dans les fruits ou elles resteront pendant tout leur développement larvaire. Les larves se nourrissent des levures qu'elles élèvent dans les galeries des pommes (Witzgall *et al.* 2012). Le temps que prend une larve à effectuer tout son développement larvaire dépend aussi des conditions climatiques et notamment de la température. Plus il fera chaud, plus le développement larvaire sera rapide. Une fois

pleinement développées, les larves de stades 5 sortent des fruits et recherchent un site de nymphose (ou de diapause si les conditions ne sont plus favorables). Pour réaliser une génération complète (de l'œuf à l'adulte), il faut un cumul de 610°J (Wildbolz 1965 ; Ciglar 1998). Tant que les températures sont favorables, les générations vont se succéder jusqu'à ce que la totalité des larves qui sortent des pommes entrent en diapause. Il est estimé que seulement 10% des larves entreront en diapause au cours de la première génération, 40% à la fin de la deuxième génération et 100% des larves seront diapausantes à la fin de la troisième génération. Ainsi, une deuxième génération de carpocapse apparaît généralement au mois de Juin et dans la Région PACA, une troisième génération est présente en Septembre. Il est aussi à noter que les générations concordent également avec l'apparition des fruits sur les pommiers. La dernière génération présente en Région PACA est possible grâce à la présence de pommiers produisant des variétés de pommes dites « tardives » qui apparaissent au début de l'automne alors que les conditions climatiques en Provence permettent encore le développement du carpocapse. Le cycle de vie complet du carpocapse en Provence est résumé en Fig. 8.

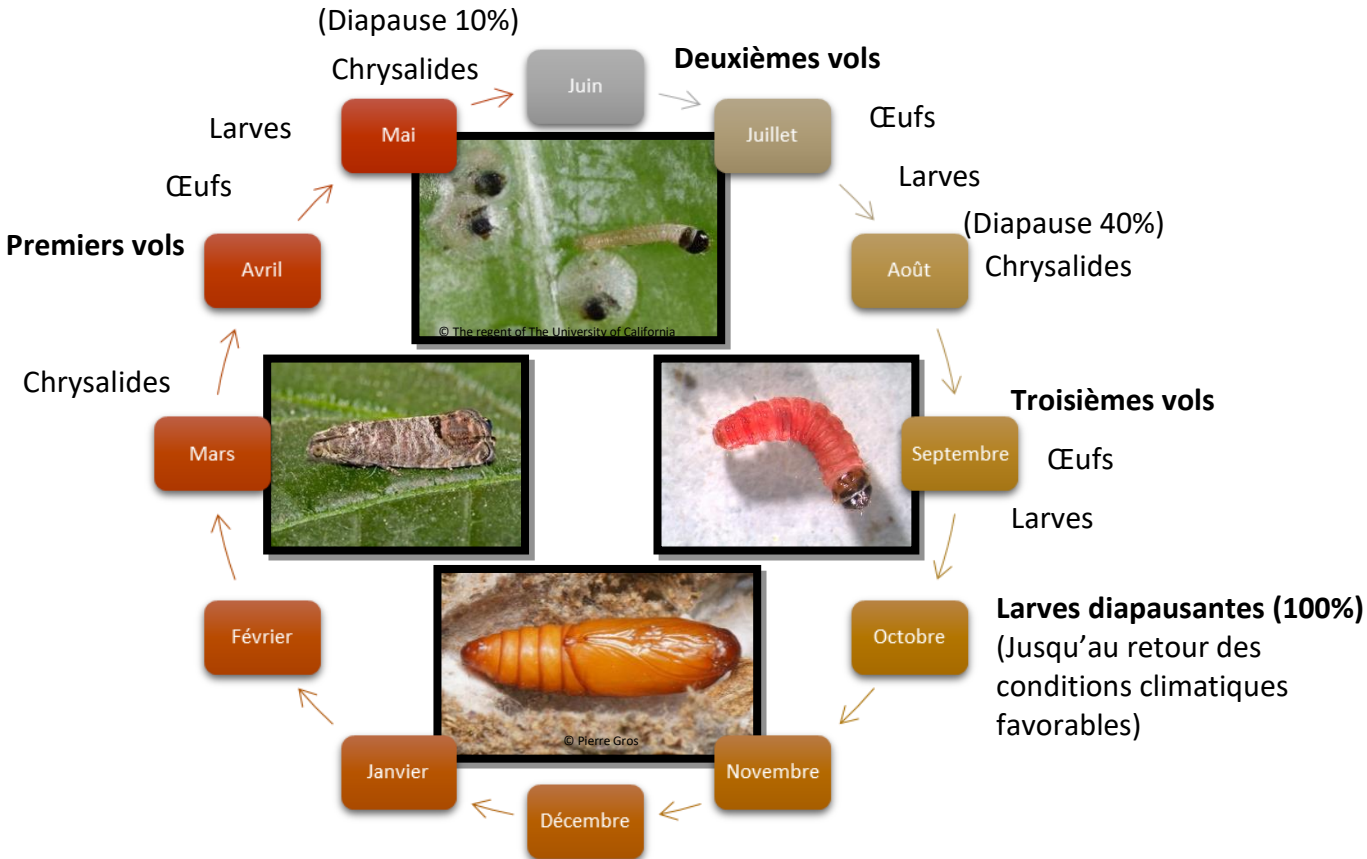


Fig. 8 : Cycle de vie du carpocapse en Provence

La période de vol des adultes permet la dispersion de l'espèce. En effet, bien que les larves ne puissent se déplacer que très peu dans un verger de pommiers, les adultes peuvent se déplacer sur de plus longues distances. Cependant, *C. pomonella* est plutôt une espèce sédentaire (Mani & Wildbolz 1977), il est estimé que seuls 10% des individus d'une population de carpocapse sont des individus mobiles capables de vols sur une plus longue distance (Gu *et al.* 2006). Ces individus peuvent parcourir plusieurs kilomètres, la plus longue distance de vol mesuré à ce jour est de 11 km (Mani & Wildbolz 1977). Ce pourcentage d'individus mobiles suffit tout de même à générer un flux de gènes entre populations. Il est donc admis que ce sont ces individus qui sont responsables de la colonisation de nouvelles parcelles et qui participent activement au brassage génétique inter-populations (Keil *et al.* 2001).

I-3-3 : Gestion et stratégies de contrôle du carpocapse

Plusieurs méthodes de lutte existent actuellement contre le carpocapse des pommes. En début de saison, la plupart des agriculteurs utilisent dans un premier temps ce qui est appelé « l'avertissement agricole ». Cela consiste (1) à piéger les mâles grâce à l'utilisation de phéromones de synthèse afin d'estimer les populations de carpocapse et (2) à contrôler le taux de pénétration de larves dans les fruits sur un échantillonnage de 1000 fruits (Audemard, 1976). Une fois les dégâts constatés, les méthodes de lutte variées peuvent être utilisées. Les insecticides de synthèse, la lutte biologique par acclimatation ou conservation, la lutte autocide (*i.e.* lâchers massifs de mâles stériles), la confusion sexuelle, les insecticides d'origine naturelle tels que le virus de la ganulose (CpGV) ou encore l'utilisation de méthodes de lutte physique tels que les filets Alt'Carpo qui empêchent les carpocapses de pénétrer sur la parcelle cultivée sont les principales méthodes de lutte contre *C. pomonella*.

- *Insecticides de synthèse*

Le contrôle du carpocapse des pommes à travers le monde dépend largement de l'utilisation fréquente d'insecticides incluant des diamides, des hydrazines, des oxadiazines, des pyréthriinoïdes, des spinosynes, des avermectines, des néonicotinoïdes, des organophosphorés et des inhibiteurs et régulateurs de croissance (Bosch *et al.* 2018a ; Bosch *et al.* 2018b ; Voudouris *et al.* 2011 ; Hu *et al.* 2020 ; Reyes *et al.* 2007 ; Reuveny & Cohen 2004). En Vaucluse, en 2015, jusqu'à 9 traitements insecticides contre les ravageurs dont le carpocapse des pommes étaient appliqués dans les vergers de pommiers (Agreste 2021).

Depuis de nombreuses années, avec le brassage génétique entre différentes populations ainsi que l'application toujours plus intensive de traitements insecticides sur les vergers de pommiers, dont 70% sont directement ciblés pour lutter contre le carpocapse, *C. pomonella* a acquis des résistances à plusieurs familles d'insecticides en Europe et aux Etats-Unis (Franck *et al.* 2007). De plus en plus de phénomènes de résistances à divers groupes d'insecticides de synthèse ou d'origine naturelle ont été reportées (Sauphanor *et al.* 2000 ; Reyes *et al.* 2007). En plus de résister à une famille d'insecticides, des résistances croisées à plusieurs insecticides de familles différentes sont apparues, rendant le contrôle de ce ravageur d'autant plus compliquée (Sauphanor *et al.* 1998 ; Dunley *et al.* 2000 ; Reuveny & Cohen 2004).

- *Confusion sexuelle*

Parmi les méthodes de contrôle utilisées, la confusion sexuelle est très répandue. Son utilisation est estimée à plus de 160 000 ha à travers le monde (Witzgal *et al.* 2008). Elle consiste à placer dans les vergers des diffuseurs d'analogues synthétiques de composés phéromonaux émis par les carpocapses femelles. Parmi ces composés, la principale molécule est la codlémone (Witzgal *et al.* 2008). La saturation de l'air en phéromones de synthèses perturbe la structure spatiale de diffusion de la phéromone naturellement produite par les femelles. Les mâles ne sont donc plus capables de s'orienter correctement vers les partenaires sexuels et la reproduction du ravageur sera empêchée, ou au moins retardée, réduisant le nombre d'individus de la génération suivante.

- *Lutte biologique*

La lutte biologique consiste à utiliser d'autres espèces pour réduire les niveaux de populations de carpocapses (Boivin 2001). Les agents de lutte biologique, incluent des espèces entomopathogènes et entomophages.

Il existe trois types de méthodes de lutte biologique, la lutte biologique inondative, la lutte biologique par acclimatation et la lutte biologique par conservation (Boivin 2001 ; Winkler *et al.* 2005).

La lutte biologique inondative consiste à augmenter les populations d'un ennemi naturel en introduisant massivement et ponctuellement dans le milieu des individus issus d'élevage (Eilenberg *et al.* 2001), contrairement à la lutte biologique par acclimatation qui consiste à introduire un ennemi naturel exotique avec pour objectif qu'il s'installe de façon pérenne dans le milieu (Borowiec *et al.* 2011). Enfin la lutte biologique par conservation consiste à manipuler le milieu afin de permettre le développement, la reproduction et l'augmentation des ennemis naturels naturellement présents dans le milieu (Ris *et al.* 2014).

Organismes entomopathogènes. Pour lutter contre *C. pomonella*, le principal micro-organisme utilisé est le virus de la granulose (CpGV) (Lacey & Unruh 2005). Des formulations commerciales de ce virus sont pulvérisées en vergers. Une fois ingéré, le virus va se multiplier rapidement dans la larve et causer sa mort (Grillot 2015). La

première formulation commerciale de granulovirus a été développée dans les années 1980 et aujourd'hui trois formulations commerciales de ce virus sont homologuées en Europe. Cependant, depuis une vingtaine d'années, des résistances au premier isolat viral commercialisé sont apparus en Europe (Fritsch *et al.* 2005 ; Sauphanor *et al.* 2006 ; Schmitt *et al.* 2013). Outre le virus de la granuloose, un nematode du genre *Neoaplectana* et le champignon *Beauveria bassiana* sont également commercialisés pour lutter contre le carpocapse des pommes mais peu utilisés (Hagley 1971 ; Maalouly Matar 2013).

Organismes entomophages. Des ennemis naturels du carpocapse sont également utilisés comme agents de lutte biologique (Lacey & Unruh 2005). Ces ennemis naturels appartiennent à différents ordres d'arthropodes : Hyménoptères, Diptères, Dermaptères, Neuroptères, Coleoptères, Hétéroptères et Arachnides (Mathews *et al.* 2004 ; Nikolic *et al.* 2018). Il peut également s'agir d'oiseaux (Mols *et al.* 2007). Parmi les arthropodes, les ennemis naturels peuvent être des prédateurs, tels que la forficule, qui consommeront les ravageurs à différents moments de leur développement en fonction de l'espèce et qui sont très souvent généralistes (Symondson *et al.* 2002) ; ou des parasitoïdes, qui se développent à la surface ou à l'intérieur de leur hôte jusqu'à sa mort, et qui peuvent être très spécifiques (Maalouly Matar 2013). Par exemple, en Europe, les trois parasitoïdes les plus abondants recensés comme attaquant *C. pomonella* dans les vergers de pommiers sont *Ascogaster quadridentata* (Braconidae), *Pristomerus vulnerator* (Ichneumonidae) et *Perilampus tristis* (Perilampidae) (Athanasov *et al.* 1997 ; Diaconu *et al.* 2000 ; Maalouly Matar *et al.* 2013 ; Rosenberg 1934 ; Cross *et al.* 1999 ; Thorpe *et al.* 2016). Dans une moindre mesure, des attaques par *Bassus rufipes* (Braconidae) et *Trichomma enecator* (Ichneumonidae) ont également été enregistrées (Mills, 2005).

- *Lutte autocide*

La technique de l'insecte stérile (TIS) ou lutte autocide est une méthode de contrôle des populations d'insectes basée sur la réduction de leur descendance. Des mâles stérilisés en élevages et lâchés en grand nombre dans les cultures vont inséminer des femelles sauvages donnant lieu à la ponte d'œufs stériles dont les embryons meurent au début du développement (Oliva *et al.* 2020). De nombreuses espèces de

Lépidoptères ont été étudiées comme candidates à la lutte par la technique de l'insecte stérile. Cependant, à ce jour, seul deux programmes effectifs, en Colombie-Britannique (Canada) et en Argentine, s'intéressent à cette technique pour lutter contre *C. pomonella* seule ou combinée avec de la lutte biologique par utilisation de parasitoïdes (Bloem *et al.* 2005 ; Botto & Glaz 2010). Des recherches expérimentales sur cette technique sont actuellement en cours en France.

- *Filets*

Il existe également une méthode de lutte mécanique contre cet insecte. Elle se présente sous la forme de filets (« AltCarpo ») enveloppant les arbres. Ces derniers perturbent le comportement reproducteur du carpocapse des pommes. On estimait dans le Sud de la France en 2007 que 30000 ha de pommiers étaient protégés par ces filets, dans des vergers conduits principalement en agriculture biologique et en production fruitière intégrée (Romet *et al.* 2010). Ces filets ne font que limiter les dégâts sans cependant totalement les empêcher. Par ailleurs, il semblerait que les coûts des différents filets et structures soient rapidement rentabilisés avec un retour sur investissement intéressant (environ 6000 €/ha, Romet *et al.* 2010).

I-3-4 : Biologie des ennemis naturels du carpocapse

Comme mentionné dans le Chapitre II-4, un large assemblage d'arthropodes sont considérés comme prédateurs ou parasitoïdes du carpocapse. Parmi ces ennemis naturels, deux exemples seront étudiés dans ce manuscrit, l'un pour évaluer l'impact du réchauffement climatique sur la lutte biologique par conservation et l'autre la lutte biologique par acclimatation. Pour la lutte biologique par conservation, un prédateur naturellement présent dans la Région PACA en vergers de pommiers sera étudié : la forficule, *Forficula auricularia*. Ce prédateur omnivore et généraliste est déjà connu pour aider à la régulation du puceron lanigère du pommier, du puceron cendré ou de la drosophile du cerisier (Mueller *et al.* 1988 ; Piñol *et al.* 2008 ; Dib *et al.* 2011 ; Wolf *et al.* 2018 ; Bourne *et al.* 2019) mais consomme également des œufs de carpocapse (Glen, 1975 ; Unruh *et al.* 2016). Pour tester l'impact du réchauffement climatique sur la lutte biologique par acclimatation, le parasitoïde exotique *Mastrus ridens* introduit dans plusieurs pays et présentant un intérêt particulier pour la lutte contre *C. pomonella* sera étudié (Sandanayaka *et al.* 2018 ; Charles *et al.* 2019). Il est introduit en France pour la première fois en 2018. La biologie de ces deux ennemis naturels est détaillée dans les paragraphes suivants.

- *Biologie de la forficule Forficula auricularia (Dermaptera : Forficulidae)*

La forficule, *Forficula auricularia* (L.), encore appelée perce-oreille commun, est un insecte ubiquiste, puisqu'elle est présente sur l'ensemble des continents. Elle est originaire d'Europe, de l'Ouest de l'Asie et du Nord de l'Afrique mais sa capacité à coloniser des milieux très variés a sans doute facilité sa dispersion (Crumb *et al.*, 1941 ; Albouy & Caussadel 1990). De plus, selon Albouy & Caussanel (1990), cette espèce de forficule présente une forte capacité d'adaptation ainsi qu'un bon potentiel de reproduction. Ceci l'aiderait à coloniser de nouveaux milieux facilement, à tel point que sa présence dans certains milieux a parfois permis sa prolifération au point de lui donner, dans certaines conditions seulement, l'appellation de ravageur. C'est le cas notamment aux Etats-Unis, au Canada, en Nouvelle-Zélande et même en France où elle a pu causer des ravages dans des cultures fruitières, maraichères ou florales et dans les jardins de certains particuliers. Cependant, il semblerait que ces dégâts restent cantonnés à certaines cultures comme par exemple celle de la pêche et de l'abricot et se produisent dans certaines conditions particulières. La plupart du temps,

les Dermaptères, dont fait partie *F. auricularia* sont plutôt connus pour être de bons auxiliaires des cultures. En effet, il existe une large documentation sur le rôle de *F. auricularia* en tant que prédateur notamment des pucerons dans les vergers de pommiers ou dans des vergers de clémentines (Mueller *et al.* 1988 ; Piñol *et al.* 2008). Des études récentes ont aussi montré son potentiel de biocontrôle des populations de *Drosophila suzukii* dans les vergers de cerises (Wolf *et al.* 2018 ; Bourne *et al.* 2019). Elles sont aussi connues pour consommer des œufs de *Cydia pomonella* dans différents vergers (Glen 1975 ; Unruh *et al.* 2016). Elles font donc preuve de polyvalence en consommant différentes espèces de ravageurs à travers différents types de vergers.

La forficule est univoltine même si la femelle peut pondre plusieurs fois en une année (Moerkens *et al.* 2010). Les individus de *F. auricularia* adultes mesurent entre 10 et 20 mm et sont de couleur brun-roux. Les adultes possèdent une paire de cerques bien formée, et présentent un fort dimorphisme sexuel sur cette paire de cerques permettant de reconnaître facilement une forficule adulte mâle d'une forficule adulte femelle. La reproduction de *F. auricularia* est sexuée et est surtout permise par le comportement grégaire en journée de cette espèce. Si la forficule a plutôt un rythme de vie nocturne, elle sécrète en journée des phéromones d'agrégation permettant aux différents individus de se retrouver en très grand nombre à l'abri de la lumière de la levée à la tombée du jour (Sauphanor 1992). Ce comportement permet dès le raccourcissement de la photopériode, à l'automne, un large choix de partenaires sexuels, des accouplements multiples avec plusieurs partenaires et les premières pontes vers Novembre (Meunier *et al.* 2012 ; Sandrin *et al.* 2015). La femelle s'isole rapidement après l'accouplement dans un nid sous terre ou sous l'écorce d'un arbre à l'abri de la lumière. La taille de la ponte varie beaucoup d'un individu à un autre allant d'une trentaine d'œufs en moyenne à près d'une centaine pour certaines femelles. La forficule est connue également pour être une des seules espèces d'insectes à avoir un comportement maternel avec ses œufs. Elle peut passer beaucoup de temps à l'entretien de ses œufs et va également passer quelques semaines avec ses juvéniles qui arriveront à l'éclosion des œufs au début du printemps (Costa 2006). Les soins maternels que la femelle apporte à ses œufs sont très importants pour permettre leur bonne éclosion (Boos *et al.* 2014). Ils vont notamment limiter les développements

microbiens. La femelle pourra éventuellement pondre une deuxième fois au milieu du printemps pour une éclosion en été (Meunier *et al.* 2012).

La forficule possède quatre stades larvaires présentés en Fig. 9 (Albouy & Caussanel 1990). Chaque stade larvaire prend environ 25 jours à se développer (Le Navenant 2019). Bien sûr le temps de développement des stades larvaires est fonction de la température et plus il fera chaud, plus les stades larvaires se développeront rapidement. Cependant, les températures optimales de développement de *F. auricularia* se situeraient autour des 25°C (Aroga & Coderre 2001). Des températures plus élevées engendrent un taux de mortalité élevé chez les œufs et les premiers stades larvaires. Les forficules sont hémimétaboles c'est-à-dire que les juvéniles ressemblent fortement aux adultes hormis l'absence d'ailes qui apparaissent sous forme d'ébauche à partir du quatrième stade larvaire et qui seront complètement articulées et formées au stade adulte. D'ailleurs, si les forficules possèdent une paire d'ailes parfaitement fonctionnelle, ils ne s'en servent que rarement (Albouy & Caussanel 1990 ; Deiters *et al.* 2016). Comme les deux premiers stades larvaires restent avec leur mère, ils se développent à proximité du nid, sous le sol ou à la surface du sol. Les deux stades suivants se retrouvent plus souvent à la surface du tronc et au niveau des feuilles notamment dans les vergers et pourront, comme les adultes, avoir un rôle de prédateur de certaines autres espèces d'insectes dont certaines espèces de ravageurs (Dib *et al.* 2011). Il est connu que la forficule sera plus active en vergers à des températures avoisinant les 20°C, des températures trop fluctuantes ou beaucoup plus fortes limiteront leur activité (Bourne *et al.* 2019). Le cycle de vie complet de *F. auricularia* est présenté en Fig. 10.

F. auricularia est donc une espèce d'insecte qui vit relativement longtemps, avec des mâles vivant en moyenne 6 mois puisqu'ils vont mourir peu de temps après l'accouplement et des femelles qui peuvent vivre jusqu'à un an et pondre deux à trois fois.

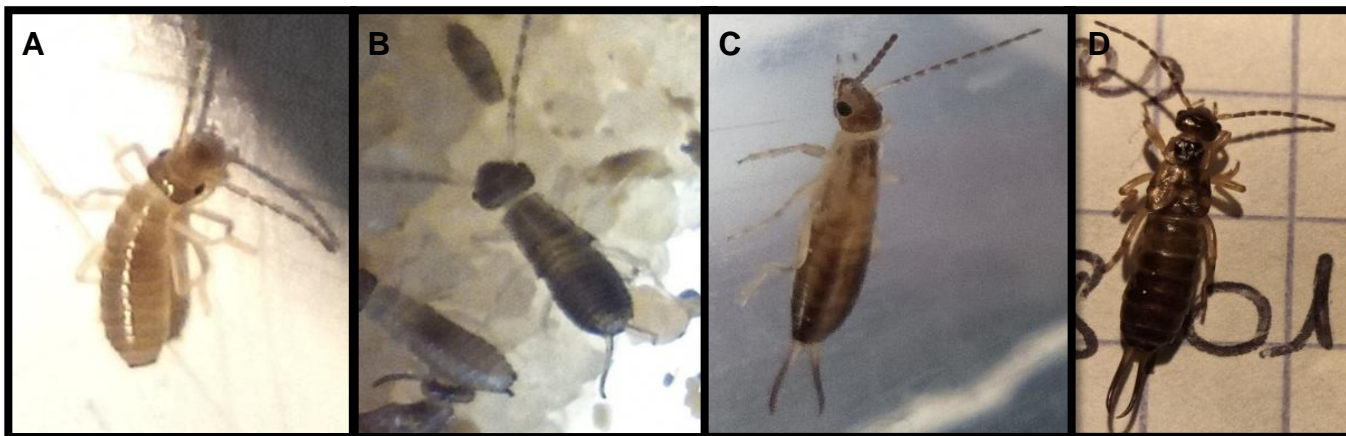


Fig. 9 : Larves de *F. auricularia* aux stades larvaires 1 (A), 2 (B), 3 (C) et 4 (D)

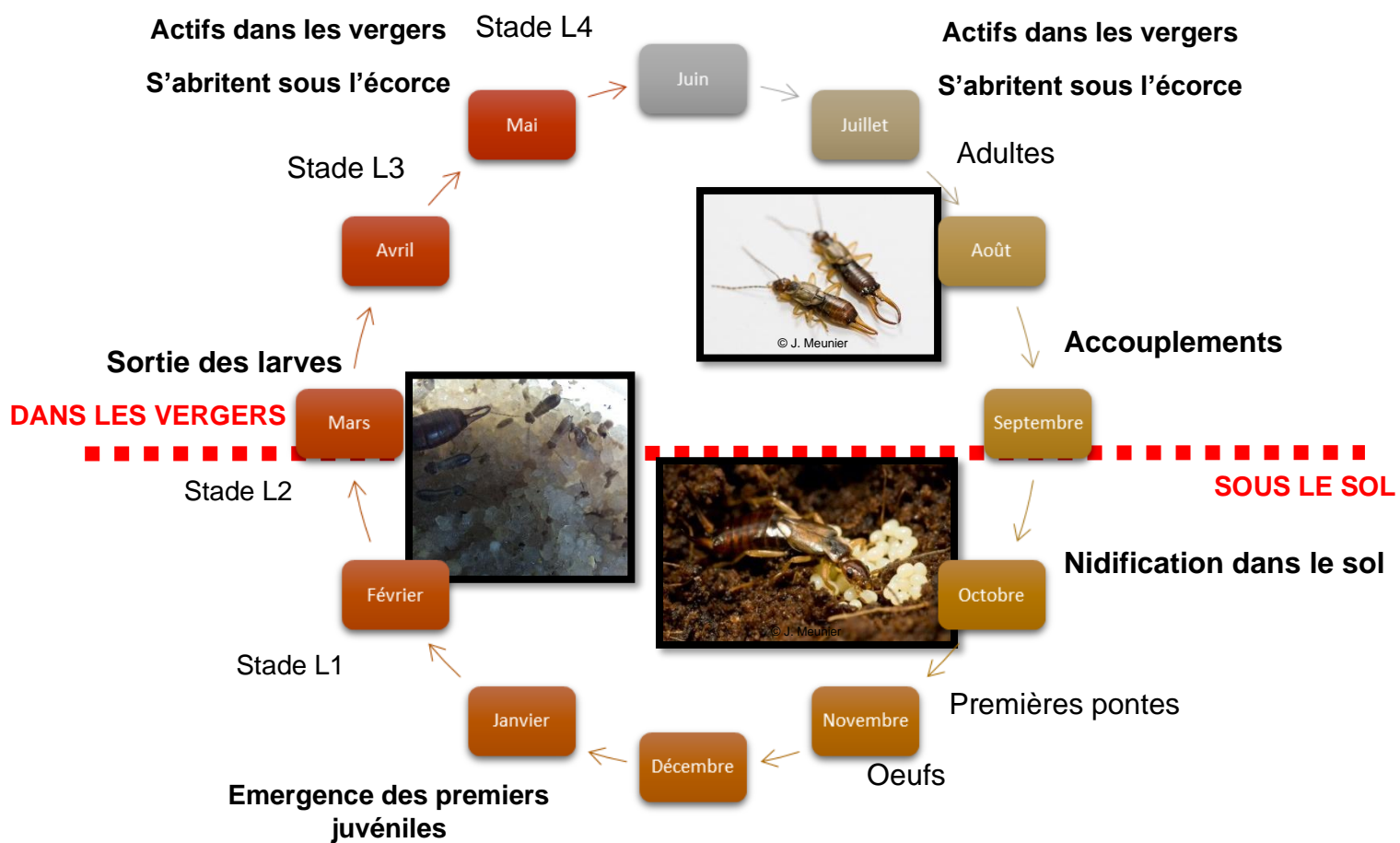


Fig. 10 : Cycle de vie de *F. auricularia*

- *Biologie de l'hyménoptère parasitoïde Mastrus ridens (Hymenoptera : Ichneumonidae)*

Mastrus ridens (Horstmann), anciennement considéré comme la même espèce que *Mastrus ridibundus* (Gravenhorst), n'a été catégorisé comme une espèce différente que récemment, en 2009 (Department of Agriculture, Fisheries & Forestry 2013, Australie). Cependant, une large partie de la littérature concernant *M. ridibundus*, est en réalité attribuable à *M. ridens* puisque les premiers prélèvements de cette espèce datent en réalité de 1994. De plus, comme les deux espèces sont très proches, les caractéristiques biologiques de *M. ridibundus*, référencées dans la littérature sont les mêmes pour *M. ridens* et la seule discrimination des deux espèces qui peut être faite est basée uniquement sur des critères morphologiques.

M. ridens est un Hyménoptère ectoparasitoïde idiobionte des cocons de *C. pomonella*. La femelle va donc pondre dans le cocon, à la surface de la larve de carpocapse grâce à son ovipositeur. Elle va aussi paralyser son hôte pendant la ponte et rester sur le cocon fraîchement parasité pendant plusieurs heures afin de protéger sa ponte des autres femelles. C'est une espèce de parasitoïde plutôt spécifique de son hôte, puisque parmi les quinze espèces de lépidoptères présentés lors de tests, seuls quatre sont attaquées de manière très ponctuelle (Devotto *et al.* 2010 ; Department of Agriculture, Fisheries & Forestry 2013). Cette spécificité en fait un agent de bio-contrôle du carpocapse des pommes particulièrement intéressant. La première colonie de *M. ridens* décrite et prélevée en 1994 provient du Kazakhstan, pays dont est également probablement originaire *C. pomonella*, ce qui pourrait expliquer la forte affinité du parasitoïde pour son hôte (Devotto *et al.* 2010 ; Sandanayaka *et al.* 2018). De cette colonie descendent toutes les lignées de *M. ridens* utilisés pour les premiers lâchers inondatifs aux Etats Unis en 1998, puis en Argentine (2003), au Chili (2006), en Nouvelle Zélande (2012) et plus récemment en Australie en 2015. *M. ridens* est introduit en France et autorisé pour des lâchers à des fins de contrôle du carpocapse des pommes depuis 2018. Si ce parasitoïde est relâché dans autant de pays pour le contrôle de *C. pomonella* c'est grâce à sa très bonne spécificité pour son hôte, car il présente peu de risques pour les espèces de lépidoptères indigènes, mais surtout pour sa très bonne capacité de dispersion qui a été relevée dès le premier lâcher aux Etats Unis. *M. ridens* est d'intérêt général pour la lutte contre le carpocapse, son taux de parasitisme est plus que satisfaisant en conditions naturelles en avoisinant les 40% en

Californie (Mills 2005) et jusqu'à 70% au Kazakhstan (ANSES 2017). Des études expérimentales ont également obtenu des taux satisfaisants en conditions de laboratoires compris entre 13% et 32% (Bueno *et al.* 2017 ; Bezemer & Mills 2003). De plus, il serait capable de se disperser sur plusieurs kilomètres, puisqu'il a été retrouvé à plus de 4 km du point de lâcher quelques semaines seulement après (Devotto *et al.* 2010). La période optimale de lâcher correspond évidemment au moment où le carpocapse est en diapause et où le nombre de cocons disponibles est maximal, à la fin de l'été (Sandanayaka *et al.* 2017). De plus cette période est propice à la survie de *M. ridens*, puisque cette espèce tolère mieux les températures fraîches que les températures trop chaudes (Hougardy & Mills 2007).

Cet ectoparasitoïde grégaire repère son hôte grâce aux substances sémiocchimiques que produisent les larves de carpocapses en tissant leurs cocons pour médier l'agrégation de recherche d'un site de nymphose (Duthie *et al.* 2003 ; Jumean *et al.* 2004 ; Charles *et al.* 2013). En fonction de la taille du cocon, la femelle va pondre un ou plusieurs œufs au sein du même cocon, ce qui jouera sur la taille de l'adulte ou des adultes émergents. Une des particularités de ce parasitoïde est que les femelles sont synovigéniques, c'est à dire qu'elles vont continuer à produire des œufs tout au long de leur cycle de vie à maturité (Sandanayaka *et al.* 2011). Une femelle peut pondre environ 60 œufs sur sa durée de vie et peut parasiter jusqu'à quatre cocons par jour (en conditions de laboratoire : Hougardy *et al.* 2005 ; Bezemer *et al.* 2005). Cependant, très peu d'études s'intéressent à son cycle de vie. Devotto *et al.* (2010) ont étudié les effets de plusieurs températures ainsi que d'une absence d'alimentation sur plusieurs de ses traits d'histoire de vie. Plus récemment Bueno *et al.*, (2017) ont publié une note scientifique sur l'influence des méthodes d'élevage sur la fitness de *M. ridens*. Ces études donnent quelques informations sur la durée de vie des adultes qui avoisinerait les trois semaines dans des conditions climatiques stables et n'excédant pas les 25°C. Il est cependant compliqué de connaître les différents stades de développement de ce parasitoïde car il se développe sous la surface d'un cocon relativement opaque. Selon des observations empiriques personnelles, des données de suivis phénologiques (Perrin *et al.* in prep, [Chapitre IV](#)) et les informations contenues dans un rapport de Jumean (2004), il est tout de même possible d'estimer son développement du stade d'œuf à l'émergence de l'adulte. Les premières larves de *M. ridens* éclosent entre un à trois jours après la ponte. Elles vont ensuite se développer en se nourrissant de leur

larve hôte pendant une durée comprise entre quatre et sept jours durant lesquels elles vont passer par cinq stades larvaires différents. Le cinquième stade larvaire tisse ensuite un cocon à l'intérieur du cocon de *C. pomonella* avant de se nymphoser. Si les conditions climatiques le permettent, les adultes émergeront dix à quatorze jours après. Le cycle de vie complet de *M. ridens* est présenté en Fig. 11. Si les conditions climatiques ne le permettent pas, les pupes vont alors entrer en diapause et émerger le printemps suivant, (Fig. 13). Si les larves sont plutôt jaunes orangées en forme de croissant de lune, les adultes présentent une tête et un thorax de couleur noire et un abdomen rouge orangé prolongé par des pattes orange (Fig. 12). Les pupes ressemblent aux adultes à l'exception de la couleur de leur abdomen qui est beaucoup plus claire (Fig. 12). Les femelles adultes émergentes sont déjà fécondes et comme pour la plupart des parasitoïdes, les mâles émergent deux à trois jours avant les femelles pour pouvoir s'accoupler rapidement. Il est estimé que *M. ridens* serait capable de produire un nombre important de générations par an, fonction du nombre de générations de carpocapses et de la température saisonnière, voir Fig. 13. Les femelles arrêteraient de voler quand les températures descendent en dessous des 15°C (Davis *et al.* 2014 ; Charles *et al.* 2019). Dans la nature, si les différents stades larvaires de *M. ridens* se nourrissent de la larve de carpocapse en décomposition, les adultes se nourrissent plutôt de nectar, et ne rentreraient pas en compétition avec d'autres espèces d'intérêt général ou d'autres auxiliaires des cultures.

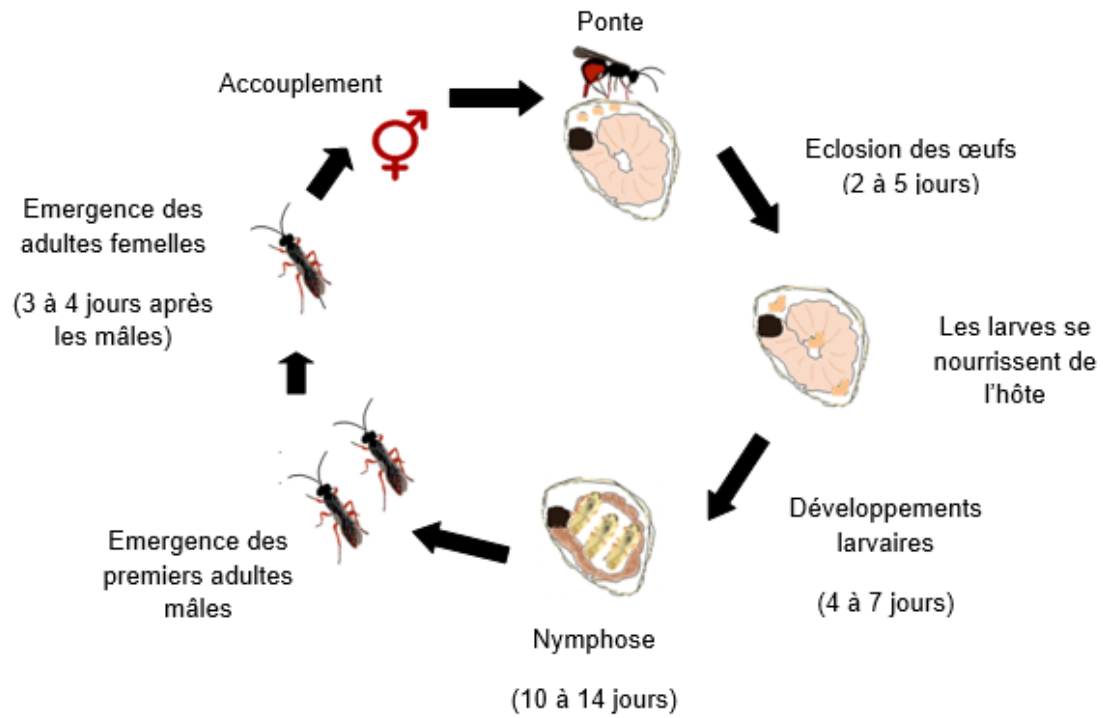


Fig. 11 : Cycle de vie de *M. ridens*

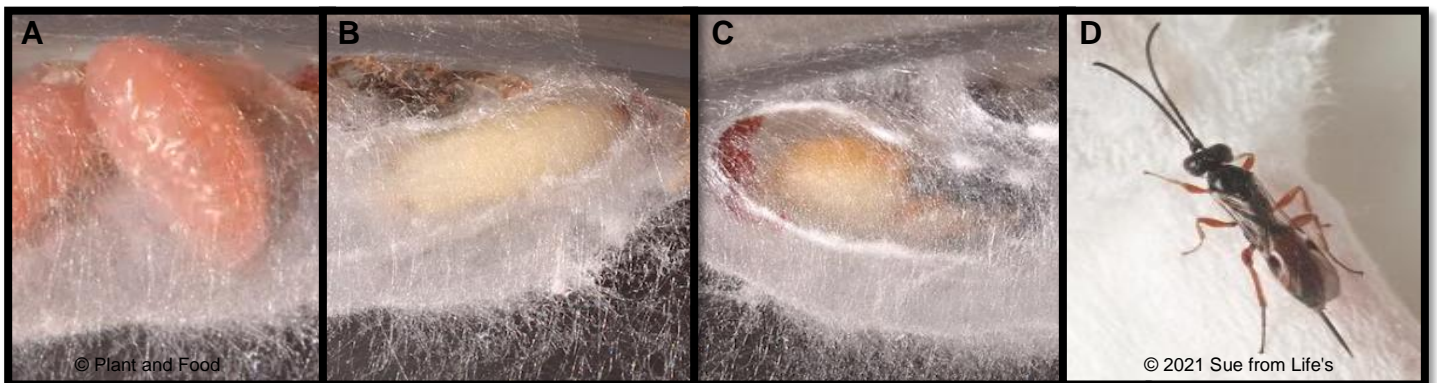


Fig. 12 : Stades de développement de *M. ridens* : larve (A), cocon (B), nymphe (C) et adulte femelle (D)

Période d'activité des adultes et développement des différentes générations

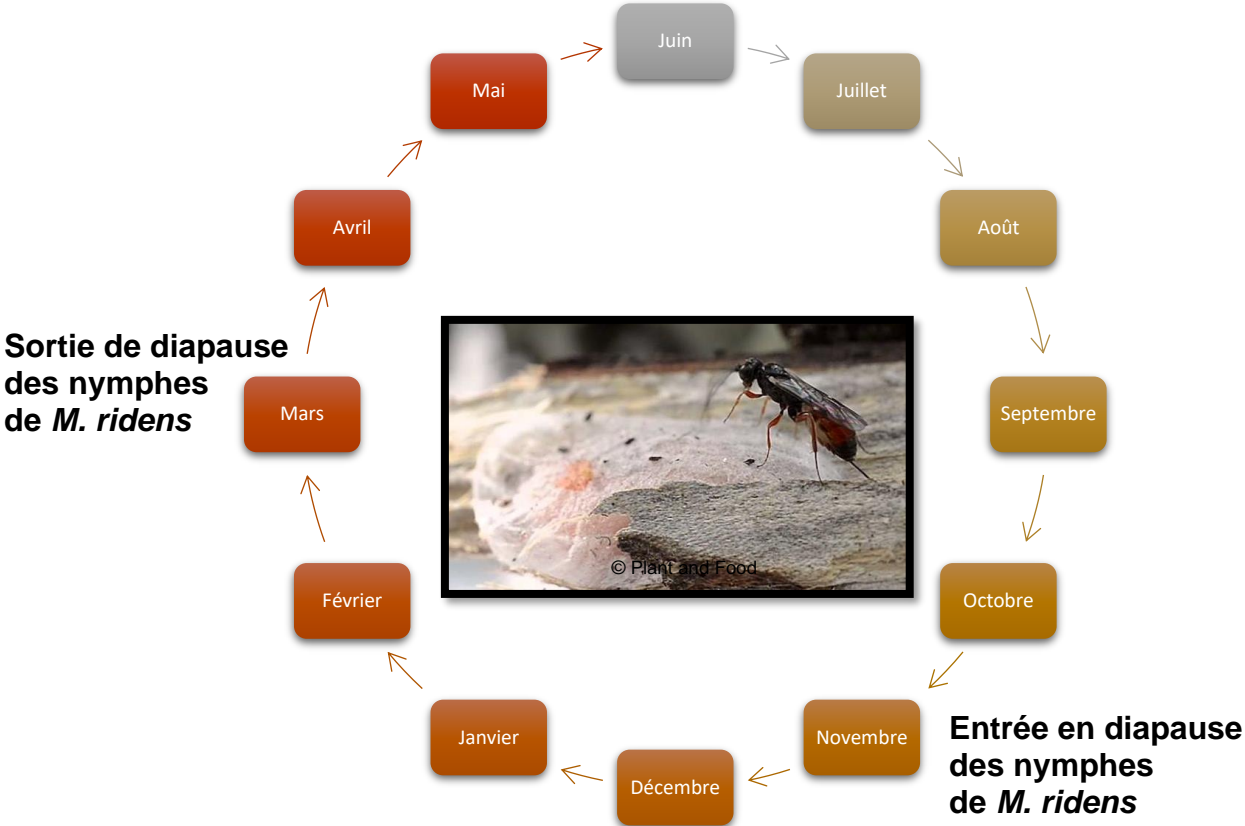


Fig. 13 : Période de présence de *M. ridens* en vergers

I-3-5 : Implications du réchauffement climatique sur les vergers de pommiers et la gestion du carpocapse en Région PACA

Considérant la sévérité des changements climatiques qui ont déjà touché le bassin méditerranéen et qui devraient l'affecter encore plus fortement dans les prochaines années, et considérant l'importance de la production fruitière en Région PACA, les questionnements sur les adaptations des pratiques agricoles et particulièrement arboricoles dans la région sont nombreux.

Il a en effet été avancé que les régions présentant un climat méditerranéen sont et seront les régions les plus soumises aux changements climatiques (IPCC 2013). Pour bien comprendre pourquoi, il est nécessaire de bien apprécier les différents scénarios climatiques. Pour mesurer le climat et donc le changement climatique, l'utilisation d'indicateurs du climat passé est de norme. Ces indicateurs permettent de créer des modèles théoriques qui seront confrontés aux données observées. Les projections du climat futures sont réalisées à partir des modèles climatiques et des estimations du futur niveau de gaz à effets de serre. Ces projections climatiques sont fonctions de la variabilité du climat et permettent uniquement d'estimer plusieurs scénarios possibles (Vert *et al.* 2013). A l'échelle nationale, les projections climatiques, principalement réalisées par Météo-France, prévoient certaines tendances à l'horizon 2050. Parmi ces tendances figurent l'augmentation des températures moyennes, surtout pendant la période estivale avec des canicules et des sécheresses plus fréquentes et une diminution des cumuls de pluie annuels. Trois scénarios principaux sont envisagés : RCP 2.6 (application des politiques de réductions drastiques des GES), RCP 4.5 (diminution des émissions de GES avant 2050), et RCP 8.5 (augmentation constante au cours du 21^{ème} siècle, sans politique de réduction des émissions). Ainsi, tout scénario confondu, le réchauffement climatique en France se poursuit jusqu'en 2050 (Fig. 14). Dans la deuxième partie du 21^{ème} siècle, selon le scénario, l'augmentation des températures moyennes annuelles pourra se stabiliser ou continuera. Selon le scénario 2.6, avec les mesures de restrictions drastiques des émissions de GES, l'augmentation des températures nationales plafonnerait à +1°C en moyenne à l'horizon 2100. Cette augmentation des températures moyennes annuelles pourrait atteindre les +2°C avec le scénario 4.5 (politiques visant à stabiliser les émissions de GES) voir les +4°C en moyenne avec le scénario 8.5 (sans politique de réduction ou de stabilisation des GES) à l'horizon 2100 (Fig. 14).

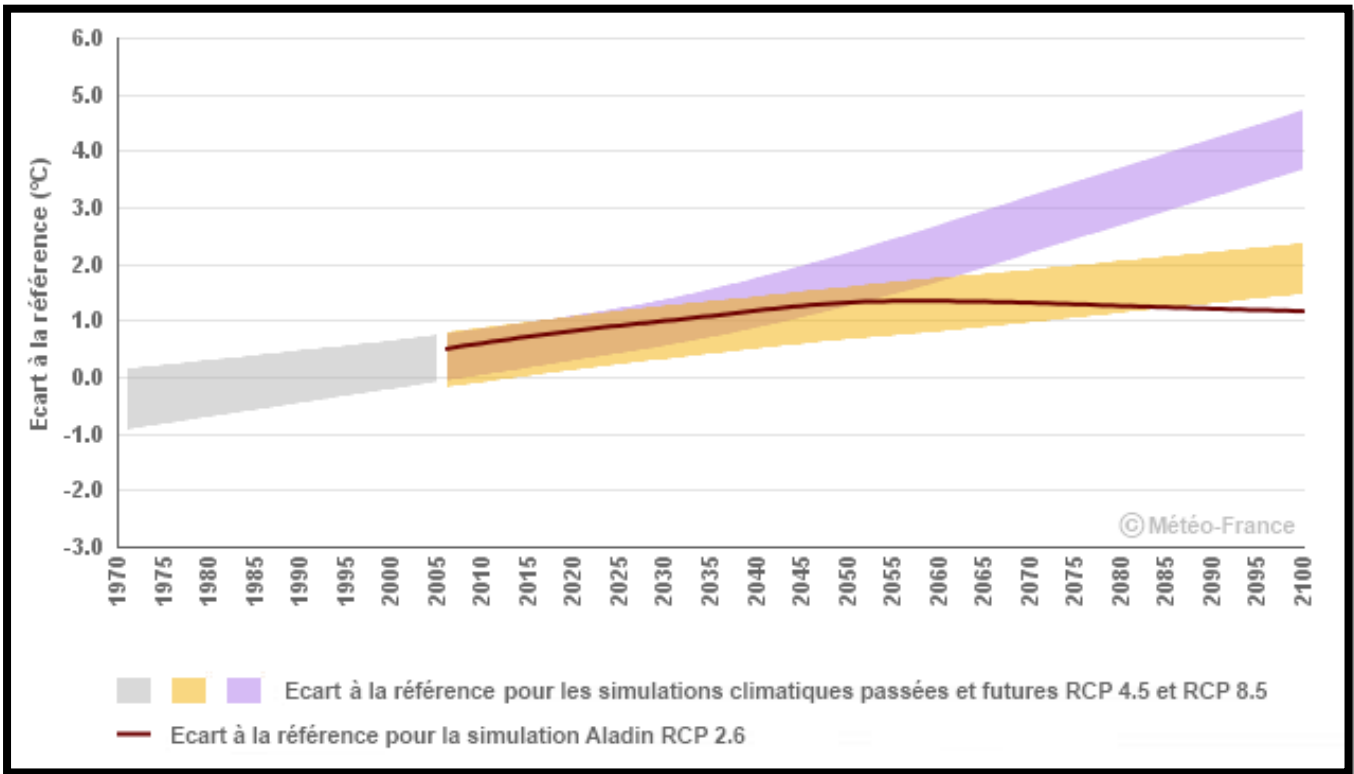


Fig. 14 : Température moyenne annuelle en France. Simulations climatiques passée et future pour trois principaux scénarios d'évolution RCP 2.6, RCP 4.5 et RCP 8.5 (source Météo-France)

Si le réchauffement climatique est déjà observé à l'échelle nationale, il est d'autant plus marqué en région PACA. Si la situation est semblable à la situation nationale pour le scénario 2.6 à l'horizon 2100 (Fig. 15), pour les scénarios 4.5 et 8.5, l'augmentation des températures annuelles serait plus importante qu'à l'échelle nationale à l'horizon 2100 avec respectivement + 2.5°C et + 5°C (voir Fig. 15). La vulnérabilité des climats de type méditerranéen face aux changements climatiques tient à la fois de l'augmentation des températures mais également à la diminution des précipitations et l'augmentation de l'occurrence de périodes de sécheresse et d'évènements climatiques extrêmes (GREC-PACA 2016). Le bassin méditerranéen s'étant déjà grandement asséché au cours des dernières décennies, le GIEC craint que ce type de climat soit d'autant plus soumis aux effets des changements climatiques (IPCC 2014).

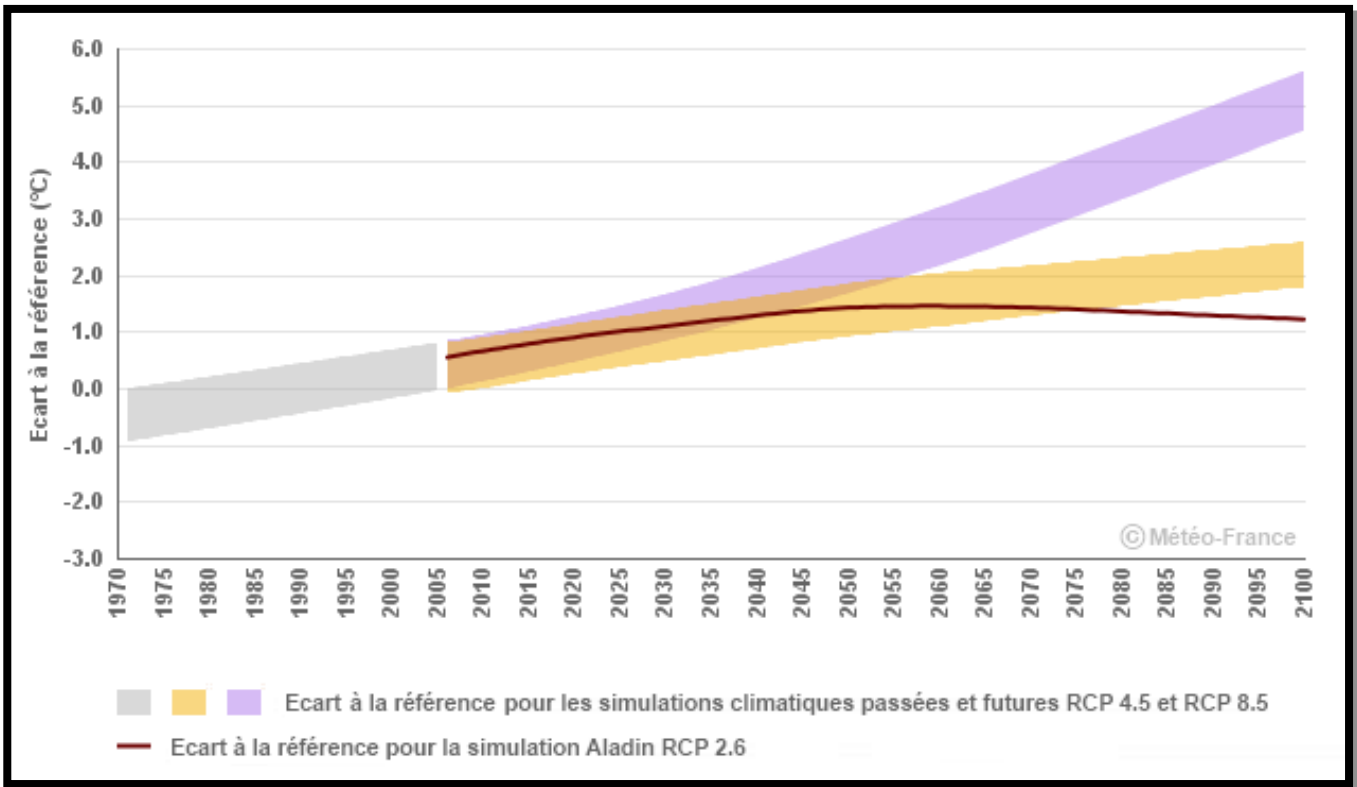


Fig. 15 : Température moyenne annuelle en Région PACA. Simulations climatiques passée et future pour trois principaux scénarios d'évolution RCP 2.6, RCP 4.5 et RCP 8.5 (source Météo-France)

Les pommiers. Les plantes vont être directement impactées par le changement climatique et en particulier par le réchauffement climatique. Parmi les plantes, les arbres fruitiers sont particulièrement vulnérables aux effets du changement climatique du fait de leur cycle de croissance et de développement long et complexe en comparaison avec les cultures annuelles (Legave 2009). Pour produire des fruits, les arbres fruitiers passent par de nombreuses phases coûteuses en énergie qui sont influencées par des facteurs abiotiques, parmi lesquels la température joue un rôle prépondérant. Divers impacts du réchauffement climatique sont visibles tout au long du cycle de développement des arbres fruitiers allant de l'entrée et la sortie de dormance qui peut avoir lieu plus tôt (El Yaacoubi *et al.* 2016) à la fructification qui peut être altérée morphologiquement et qualitativement (Adra 2017), en passant par la croissance végétative et la constitution et l'allocation des réserves en eau et nutriments qui peuvent être fortement affectés par la modification du métabolisme du carbone ou encore par un déficit de pression de vapeur (Wolf *et al.* 1991 ; Teskey *et al.* 2015) (Fig. 16). Le réchauffement climatique peut engendrer un stress thermique affectant négativement l'acquisition de carbone par la plante (Vasseur *et al.* 2012).

L'élévation des températures accélère également la croissance des organes, ce qui a pour effet de raccourcir la période durant laquelle la biomasse s'accumule (Parent *et al.* 2014). Ces deux paramètres peuvent influencer la qualité et la quantité de fruits produits en arboriculture. A ce jour, il existe un manque d'informations sur les effets potentiels du réchauffement climatique sur l'arboriculture fruitière en termes de rendement et de qualité (Fatima 2017).

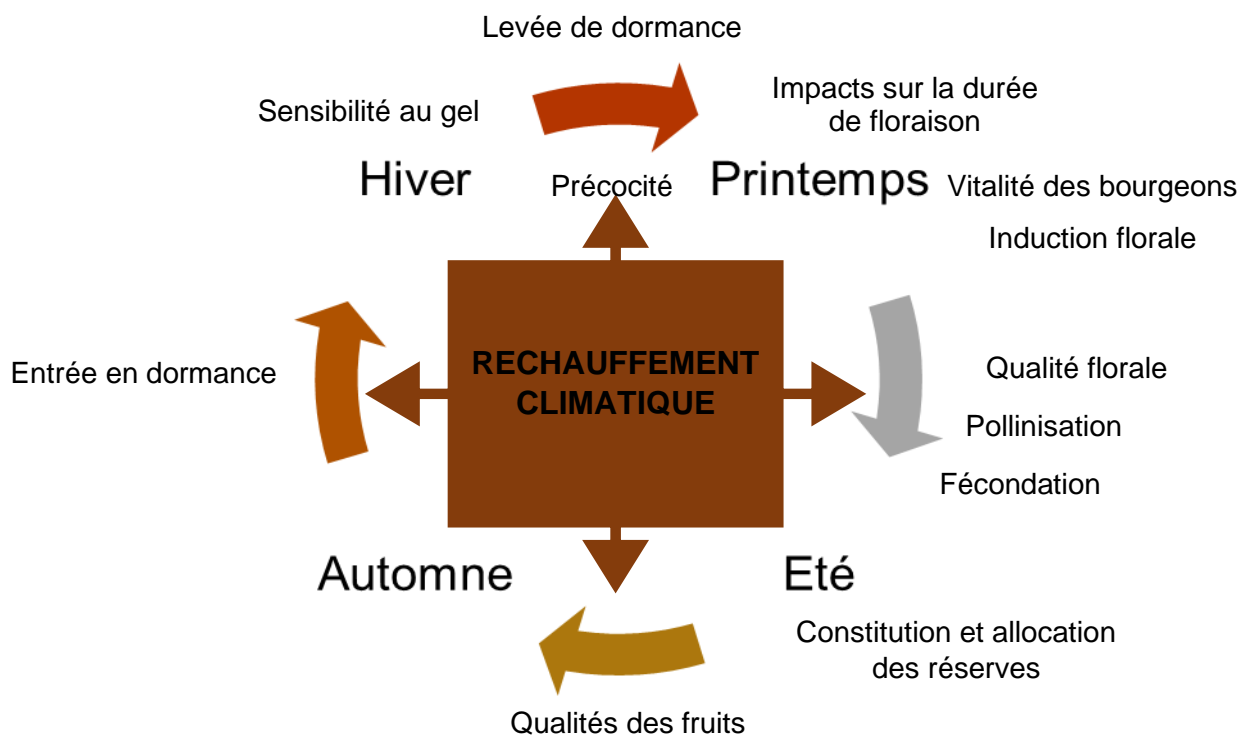


Fig. 16 : représentation schématique des impacts directs du réchauffement climatique sur la croissance d'un arbre fruitier (modifié d'après : Legave 2009)

Le carpocapse. Différents travaux empiriques et de modélisation ont mis en évidence ou prédisent des effets du réchauffement climatique sur le carpocapse des pommes. Les changements phénologiques et de voltinisme sont de loin les plus renseignés. Une troisième génération pourrait ainsi apparaître prochainement dans les régions tempérées où deux générations sont présentes pour l'instant (Juszczack *et al.* 2013 ; Stoeckli *et al.* 2012) alors qu'une quatrième génération est déjà notée en Algérie, où trois générations étaient observées précédemment. Ces changements dans le voltinisme du carpocapse s'expliqueraient par une sortie de diapause qui serait avancée de 15 jours en moyenne d'ici à 2050 et par un raccourcissement du temps

séparant deux générations. Cette augmentation du nombre de générations couplée à l'effet positif de la température sur la fécondité des carpocapses (161.8 ± 24.2 à 24.6°C , 241.9 ± 37.4 à 27.7°C ; Graf *et al.* 2018) devrait donc aboutir à une augmentation forte des populations de ce ravageur et donc des dégâts causés. De plus, l'apparition de résistances aux insecticides et aux entomopathogènes pourrait s'accélérer dans le contexte actuel de réchauffement climatique, en raison de l'augmentation des populations et du nombre de générations produites chaque année

Le réchauffement climatique a déjà et aura dans le futur des effets marqués sur la production fruitière en vergers via un impact direct sur le cycle de croissance des pommiers et sur ses ravageurs, dont *Cydia pomonella*. Il paraît donc primordial d'anticiper les effets du réchauffement climatique sur les méthodes de lutte utilisées contre cette espèce, en particulier dans une région assurant 25% de la production nationale de pommes et étant considérée comme une des plus sensibles au changement climatique. Cette approche permettra de sélectionner et éventuellement adapter les méthodes de lutte les plus efficaces en situation d'élévation des températures pour faire face aux enjeux que sont la limitation de l'impact des changements climatiques et les besoins de produire une alimentation saine pour l'homme et l'environnement.

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En bref :

Ce chapitre est consacré à l'efficacité des insecticides de synthèse. Il s'intéresse aux effets croisés de la température et de trois substances insecticides, dont une homologuée pour une utilisation en agriculture biologique, sur la mortalité du carpocapse des pommes, ainsi qu'aux mécanismes associés.

L'effet de la température, de l'exposition à un insecticide et de l'action conjointe de ces deux facteurs a été déterminé sur (i) la mortalité de larves de *C. pomonella*, (ii) l'activité d'enzymes de détoxification et (iii) l'expression de gènes codant pour des protéines de choc thermique, des cibles moléculaires et des enzymes de détoxification. Les résultats permettent de définir quelles substances sont affectées par les changements de températures et donnent des pistes sur les processus biochimiques et moléculaires impliqués dans les différences de toxicité des insecticides sur *C. pomonella*.

L'hypothèse principale de ce chapitre est que l'augmentation des températures engendrera des variations de mortalité chez *C. pomonella*, accompagnées de changements dans l'expression de certains gènes codant pour des enzymes de détoxification, des protéines de choc thermique et/ou des cibles moléculaires des insecticides.

L'ensemble des résultats de ce chapitre ont été soumis pour une publication dans « *Pesticide Biochemistry and Physiology* » en Novembre 2021.

Cross effects of heat stress and three insecticides on the survival of the codling moth *Cydia pomonella* (L.) : investigating the molecular and biochemical mechanisms

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Résumé

La température va augmenter à l'avenir et comprendre les effets de la température sur la toxicité des insecticides est déterminant pour évaluer l'efficacité du contrôle des ravageurs dans un contexte de réchauffement climatique. L'étude des mécanismes moléculaires et biochimiques associés aux effets croisés de la température et des insecticides sur la tolérance des ravageurs semble nécessaire dans ce contexte. Cette étude avait pour objectif d'étudier les effets croisés entre la température et des insecticides sur la survie d'un ravageur, le carpocapse des pommes, *Cydia pomonella*, et leurs mécanismes sous-jacents.

L'effet de trois insecticides, à savoir le chlorantraniliprole, l'émamectine et le spinosad, a été évalué à différentes températures sur : (i) la survie des larves de *C. pomonella* ; (ii) les activités des enzymes de détoxification (oxygénases multifonctionnelles du cytochrome p450, carboxylestérases et glutathion S-transférases) et (iii) l'expression des gènes de certaines enzymes de détoxification, des protéines de choc thermique et des récepteurs ciblés par les insecticides.

Nous avons observé une diminution de l'efficacité de l'emamectine et du spinosad à haute température pour lutter contre le carpocapse, alors qu'aucune influence de la température sur l'efficacité du chlorantraniliprole n'a été observée. Les activités des enzymes de détoxification ont augmenté avec le stress thermique seul mais pas avec le double stress (température + insecticides). De plus, deux gènes de détoxification (*Cyp9A61* et *Gst1*) ont été surexprimés par un seul stress mais pas par deux stress alors que les gènes *Hsp70* et *Cyp6B2* pourraient être impliqués dans la tolérance aux deux stress chez *C. pomonella*.

Ces résultats ont confirmé les effets croisés de la température et des insecticides sur *C. pomonella* pour l'émamectine et le spinosad et ont fourni des indices pour comprendre comment la température affecte la sensibilité de *C. pomonella* aux insecticides. Ils illustrent cependant la complexité des réponses moléculaires et biochimiques des individus confrontés à des stress multiples.

Mots clés : cytochrome p450, carboxylestérases, glutathion S-transférases, protéines de choc thermique, emamectine, spinosad.

Abstract

As temperature is expected to increase in the future, understanding temperature-mediated toxicity of insecticides is determinant to assess pest management efficiency in a warming world. Investigating molecular and biochemical mechanisms associated with cross mechanisms of temperature and insecticides on pests' tolerance would also be useful in this context. This study aimed to investigate cross effects between temperature and insecticides on the survival of a major pest, the codling moth *Cydia pomonella*, and their underlying mechanisms.

The effect of three insecticidal active ingredients, i.e. chlorantraniliprole, emamectin and spinosad, was assessed at different temperatures on: (i) *C. pomonella* larval survival; (ii) detoxification enzymes activities (cytochrome p450 multi-function oxygenases, carboxylesterases and glutathione S-transferases) and (iii) genes expression of some detoxification enzymes, heat shock proteins and receptors targeted by the insecticides.

We observed a decreased efficiency of emamectin and spinosad at high temperature to control the codling moth while no influence of temperature on chlorantraniliprole efficacy was observed. Detoxification enzymes activities were improved by heat stress alone but not by double stress (temperature + insecticides). Moreover, two detoxification genes (*Cyp9A61* and *Gst1*) were over-expressed by a single stress but not by two stresses while *Hsp70* and *Cyp6B2* genes may be involved in tolerance to two stresses in *C. pomonella*.

These results confirmed the cross effects of temperature and insecticides on *C. pomonella* for emamectin and spinosad and provided clues to understand how temperature affects the susceptibility of *C. pomonella* to insecticides. They illustrate however the complexity of molecular and biochemical responses of individuals facing multiple stresses.

Key words: cytochrome p450, carboxylesterases, glutathione S-transferases, heat shock proteins, emamectin, spinosad.

Introduction

Despite their negative impact on environment (Stehle & Schulz 2015) and human health (Koureas *et al.* 2012), chemical insecticides are widely employed to manage insect pests. Their efficiency is however known to depend on environmental conditions, including temperature (Matzrafi, 2018) and climate change may thus influence their effectiveness through several mechanisms. First, insecticide concentration in crops and plants is expected to decrease with increasing temperature (Delcour *et al.* 2014) because of increased dilution in plants (Holland & Sinclair, 2004) volatilization (Navarro *et al.* 2007; Steurbaut, 2009) and biological or chemical degradation in soils (Athanasopoulos *et al.* 2004). Secondly, rising temperature commonly results in faster development, advanced mean emergence date, higher reproductive rate and increased distribution range in insects (Bale *et al.* 2002; Caffarra & Eccel 2011; Juszczak *et al.* 2013), resulting consequently in increased pests' abundance and occurrence of outbreaks (Gutierrez *et al.* 2008). Thirdly, the increased number of generations reported with increasing temperature may accelerate appearance of resistance to some insecticides in multivoltine pest species (Maino *et al.* 2018).

Such influence of global warming on insecticide use may also result from the temperature-dependency of toxicity that has been reported for a wide range of products (Hodjati & Curtis 1999; Glunt *et al.* 2014; Aizawa *et al.* 2018; Pozidi-Metaxa & Athanassiou 2012; Saeed *et al.* 2018) although this relationship depends on several factors including the insecticide itself. For example, toxicity commonly increases with temperature for organophosphate insecticides and decreases for pyrethroid insecticides (Satpute *et al.* 2007; Scott 1995), but some exception has been reported within several classes, including pyrethroids (Boina *et al.* 2009). Moreover, the relationship between temperature and insecticide toxicity is insect specific, as observed for deltamethrin that is more toxic as temperature increases for the fall armyworm *Spodoptera frugiperda* and the tobacco budworm *Heliothis virescens*, but less toxic for the cabbage looper *Trichoplusia ni* and the boll weevil *Anthonomus grandis* (Sparks *et al.* 1982, 1983).

Biochemical mechanisms involved in insecticide detoxification or in protection against extreme temperatures have been thoroughly investigated individually, but only few studies focused on those involved in the influence of temperature on insecticides' toxicity. Among potential explanations, increased insecticide toxicity may be due to an

increase in locomotor activity and feeding rate occurring at high temperature in insects, therefore increasing the penetration of the product into pest's body (Boina *et al.* 2009; Medrzycki *et al.* 2010). Such mechanism is however unable to explain the negative relationship between temperature and toxicity observed for some products or pest species and other mechanisms should thus be involved. Several enzymes such as glutathione S-transferases (GST), carboxylesterases (CbE) and cytochromes P450 (P450) are known to be involved in insecticide detoxification by organisms and enzymatic activities directly depends on temperature (Okutman Tas & Pavlostathis 2007). They may thus mediate the relationship between temperature and insecticide toxicity. Zhang *et al.* (2015) observed in the diamondback moth *Plutella xylostella* that the activity of these resistance-related enzymes was inhibited at 36 and 40°C compared to 25°C, in conjunction with a reduced resistance to chlorpyrifos. However, changes in activity levels of detoxification enzymes with temperature were not always associated with a change in susceptibility of the mosquito *Aedes aegypti* to four organophosphate insecticides (Polson *et al.* 2012). In *Anopheles arabiensis*, insecticide toxicity decreased with increasing temperature but no changes in the activity of detoxification enzymes occurred (Oliver & Brooke 2017).

Heat Shock Proteins (HSPs) may also be involved in the temperature dependency of insecticides toxicity. They are proteins that have been described in most living organisms and are activated by specific genes in response to a wide range of stressful environmental factors to protect organisms (Chapuis *et al.* 2011; Tutar & Okan 2012). It has been proposed that HSP synthesis in response to one stress, temperature here, may increase tolerance to other stresses, including insecticides (Patil *et al.* 1996; Ge *et al.* 2013). For example, increased tolerance to a carbamate and an organophosphate insecticide in two mosquito species may have been induced by exposure to an HSP-inducing temperature (Patil *et al.* 1996; Raghavendra *et al.* 2010), although HSPs were not quantified in these studies. Conversely transcript levels encoding *hsp70* were up-regulated in individuals of two pest species exposed to insecticide, *Bemisia tabaci* (Su *et al.* 2017) and *Nilaparvata lugens* (Ge *et al.* 2013), resulting in increased thermotolerance.

Detoxification enzymes and HSPs may thus both mediate the influence of temperature on insecticide toxicity and disentangling such mechanisms might help to select or design products adapted to the future climate.

The aim of this study was to investigate molecular and biochemical mechanisms involved in cross-effects of temperature and insecticides on mortality of the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae), a major pest in apple orchards. Three insecticides, commonly used against this species in organic and conventional agriculture, known to be stable at high temperature (Afssa, 2010; Anses, 2010, 2011) and having different modes of action were chosen: (1) emamectin known to activate glutamate-gated chloride channels (GluCl) specific to invertebrates and also to bind to GABA receptors located at glutaminergic synapses (Wolstenholme 2012; Jansson *et al.*, 1997), (2) chlorantraniliprole that inhibits the ryanodine receptor at the level of the muscular fibrils with a myo-blocking effect (Cordova *et al.*, 2006) and (3) spinosad that has a nicotine agonist effect on acetylcholine receptor sites and an antagonist effect on a lower affinity site blocking the same receptors (Salgado, 1998). We determined if the cross-effects between temperature and insecticides (i) had an effect on codling moth larval mortality, providing information on the efficiency of insecticides when temperature increases, and (ii) induced changes in activity and genes expression of detoxification enzymes (cytochromes P450, glutathione S-transferase and carboxylesterases), as well as in gene expression of HSP (hsp70) and receptors targeted by the insecticides (GABA receptor & Ryanodine receptor).

Material & Methods

Insect sample

A *Cydia pomonella* granulovirus susceptible strain (CpNPP) originating from northern France and reared in laboratory since 1995 was used for this study. In our study, CpNPP was reared on artificial diet at INRAE Avignon (France) without insecticide pressure at 25°C; 16:8 h L:D. After few days of mating, eggs were collected, washed with a water-based solution of dishwashing liquid and bleach and then dried. The neonates emerged are then used for the different experimentations

Bioassays and sample preparation

We tested the influence of both temperature and insecticides on mortality of *Cydia pomonella* larvae. Commercial formulations of Emamectin benzoate: Affirm® 0,95%, Spinosad: SUCESS4® 480g/L and Chlorantraniliprole: CORAGEN® 200 g/l were supplied respectively from Syngenta (France), Dow AgroSciences Distribution (France) and FMC Agricultural solutions (France).

For bioassays, 96 multiwell plates containing 150µL of artificial diet (Stonefly diet Industries Ltd, Rochester, NY) per well were treated with different concentrations of insecticides diluted in osmosed water (Table 3). A 6µL volume of insecticide solution, or water for control, was deposited per well on diet surface. After 20 minutes of drying, a neonate larva was deposited, and wells were closed with parafilm strips (Reyes *et al.* 2007). The plates were then placed in climatic chambers at four different temperatures: 20°C, 25°C, 30°C or 35°C ($\pm 1^\circ\text{C}$). Mortality rate was assessed at 105-degree days, i.e. 10 days at 20°C, 7 days at 25°C, 5 days at 30°C and 4 days at 35°C to homogenize the larval stages at notation. A larva was considered as dead when not responding to a probe with dissecting forceps. Missing larvae were subtracted from the initial number. The corrected mortality was calculated using the Abbott formula, which corrects insecticide-induced mortality by natural mortality in same bioassay (Abbott 1925). Each test consisted in 24 individuals per dose (including control), per insecticide and per temperature and was replicated three times. To limit experimental variation between biotests, each replicate started the same day for the different temperatures.

Table 3: Concentrations of insecticide active substance used for bioassays on *C. pomonella* neonate larvae.

Insecticides	Concentrations (mg/L)					
	Affirm® (Emamectin)	0.02	0.03	0.05	0.08	0.14
CORAGEN® (Chlorantraniliprole)	0.3	0.53	0.95	1.69	3	
SUCCESS4® (Spinosad)	0.05	0.15	0.45	1.34	4.01	12

Exposure treatments for enzymatic and biomolecular analyses

Exposure treatments were used to quantify the influence of temperature and insecticide on activity of several detoxification enzymes and molecular analyses. Codling moth individuals were exposed during their development (from neonatal larvae to adult emergence) to two temperatures, 20 or 35°C, and to one of the three insecticides at LD30 concentrations (i.e.: estimated concentration, based on probit regression of bioassay results, resulting in 30% mortality) or water for control individuals (

Table 4). Each neonate larva was individually placed in a plastic hemolysis tube

Insecticides	LD30	
	at 20°C	at 35°C
Affirm® (Emamectin)	0.04	0.11
CORAGEN® (Chlorantraniliprole)	0.66	0.39
SUCCESS4® (Spinosad)	1.34	2.69

containing artificial diet prepared according to Guennelon *et al.* (1981) recipe without formaldehyde. Diet was polluted by dropping 15 µl of insecticide solution on the surface of the diet after its polymerization and dried at room temperature before use.

Table 4: LD30 (mg/L) of 3 commercial insecticides at 20°C and 35°C on a susceptible *C. pomonella* strain, corresponding to the concentration used for enzymatic and molecular experiments.

Insecticides	LD30	
	at 20°C	at 35°C
Affirm® (Emamectin)	0.04	0.11
CORAGEN® (Chlorantraniliprole)	0.66	0.39
SUCCESS4® (Spinosad)	1.34	2.69

Adult emergence was checked every day. As soon as adults emerged, organisms were dissected individually to separate thorax from abdomen. The anterior half of the

abdomen was immediately used to measure P450 activity. Posterior half abdomen was crushed in 110 μ l of Hepes buffer (50 mM, pH 7) on the ice and the obtained homogenate was centrifuged at 15 000 g for 15 min at 4 °C. Supernatant was stored at -80 °C and was later used for CbE and GST measurements the same day (Bouvier *et al.* 2002). The thorax was immediately dived in liquid nitrogen and kept at -80°C for molecular analyses.

Enzymatic activities

Cytochromes P450 (P450) activity

The P450 activity was determined using 7-ethoxycoumarin O-deethylation (ECOD) (Ulrich and Weber 1972) adapted for organic tissues analysis in microplates. Each sample was crushed and homogenized with a mini hand-held homogenizer in an incubation solution containing 100 μ l of Hepes buffer (50 mM pH 7) with 7-ethoxycoumarin (0.4 mM) on the ice. After a 4h incubation at 30 °C, the reaction was stopped by adding 100 μ L of 1.5M glycine buffer (pH 10.3) and the solution was centrifuged at 15 000 g for 2 min at 2 °C. The supernatant was placed in a well of black microplates (96-wells, Corning Costar®, New York, U.S). The 7-hydroxycoumarin (HC) fluorescence was quantified with 380 nm excitation and 465 nm emission filters. Three wells per microplates were left without samples but just the mix receiving glycine buffer previous to incubation to have a blank. A ten-fold dilution for males and a five-fold dilution for females was operated before dosage using the Bradford method (Bradford 1976). P450 dosage was performed on this reaction product after fluorescence measurement. The P450 activity was expressed as pg of 7-Hydroxycoumarine (7-HC)/mg of total protein/min by using a standard curve of 7-HC (0.5-4.5 nmoles/well) to convert fluorescence in 7-HC quantity.

Carboxylesterases (CbE) activity

Total non-specific CbE activity was measured with α -naphthyl acetate (α -NA) as substrate (Reyes 2007). The reaction mixture was 1 μ l of extract and 194 μ l of 30 μ M α -NA in Hepes buffer (50 mM, pH 7.0) in each microplate well. Three wells per microplate were filled with 1 μ l of Hepes buffer (50 mM, pH 7.0) instead of extract as blank. After 20 min of incubation at 22 °C in darkness, the reaction was stopped and colored by adding 55 μ l of 0.2 % Fast Garnet GBC diluted in 2.5 % sodium dodecyl

sulphate solution. Absorbance was recorded at 590 nm, after incubation for 20 min in darkness at room temperature. A 10-fold dilution for males and a 5-fold dilution for females was operated before this dosage using the Bradford method (Bradford 1976). The standard curve with α -Naphthol (α -Nol) (0-18 nmoles/well) was elaborated to express CbE activity in nmoles of product/min/mg of total proteins.

Glutathione S-transferase (GST) activity

GST activity was determined in transparent microplates (96-wells, Sterilin®, Newport, UK) using 2,4-dinitro-chlorobenzene (CDNB) as substrate (Nauen and Stumpf 2002). The reaction mixture consisted of 2 μ l of extract, 198 μ l of a solution containing: 10 μ l of 50 mM glutathione (GSH), 185 μ l of Hepes buffer (50 mM, pH 7.0) and 3 μ L of 50 mM CDNB. Three wells per microplate were filled with 2 μ l of Hepes buffer (50 mM, pH 7.0) instead of extract as blank. Absorbance was measured after 2 min of incubation at 25 °C in kinetic mode every 30 seconds at 340nm. We used the molar extinction coefficient (9.6 mM⁻¹.cm⁻¹) of CDNB-glutathione to convert absorbance in μ mol of CDNB-glutathione. The final specific activity was expressed in μ mol of CDNB-glutathione/min/mg of total protein extracted.

Molecular analysis

We quantified the influence of temperature and insecticide on expression of genes involved in detoxification processes using the thorax kept at -80°C, as indicated previously. Three replicates of 5 individuals per treatment and per sex were performed.

RNA extraction and quality assessment

Total RNA was extracted from frozen samples with the Qiagen Rneasy® Plus Micro Kit (Qiagen). Thorax of 5 individuals were pooled into 350 μ l of Buffer RLT plus and were grounded using a 3,15 mm steel bead on a 1600 MiniG (Spex® SamplePrep) tissue homogenizer at 1500 strokes/min for 15 seconds. Extraction was then continued as described in the Rneasy® Plus Micro Kit Handbook (*“Purification of Total RNA from Animal and Human Tissue”*; p. 30-38). A Dnase treatment was carried out using the

Rnase-Free Dnase set (Qiagen) as described by the manufacturer. Total RNA purity and concentration were evaluated by spectrophotometry using NanoDrop ND-2000 (ThermoFisher) and integrity was verified by 1 % agarose gel electrophoresis.

Genes selection and primer design:

The sequences of *Cydia pomonella* genes involved in response to chemical or thermic stresses were obtained from GenBank (www.ncbi.nlm.nih.gov/genBank) and from the search module available in InsectBase (<http://www.insect-genome.com/cydia/>). According to the literature we have listed 30 genes generally involved in resistance. Among those, we selected the predominant *Cytochrome P450*, *Glutathione S-transferase*, *Heat Shock Protein*, *Hsp*, *Carboxylesterase* genes, as well as those coding for receptors targeting by the three insecticides we tested, i.e. *ryanodine receptor*, *Gaba receptor* and *nicotinic receptors*.

The design of all primers pair was made using the Primer 3 web interface version 4-1-0 (<https://bioinfo.ut.ee/primer3/>) (Rozen and Skaletsky 2000), where possible, within 1kb of the 3' end of the genes or of the poly-A tail for complete cDNA synthesis.

Selected sequences were manually edited and aligned using Bioedit Software (Bioedit v.7.2.1., Hall, T.A. 1999) to check the specificity of the designed primer between same family genes.

Efficiency of all primers pair was assessed by qPCR (see below) against a four points standard curve of serial dilutions (1:5) of cDNA ranging from 2 to 0,016 ng/μl, a melting curve analysis and a 2 % agarose gel electrophoresis were used to check the primers specificity.

All primers pair with a unique PCR product and an efficiency ranging from 1.91 to 2.11 were kept for gene expression analyses: *Cyp6B6*, *Cyp6B2*, *Cyp6B1*, *Cyp9A61*, *Gst1*, *GstD1*, *GABAreceptor*, *Hsp70*, *Ryanodinereceptor* and *Actin*, as the housekeeping gene (supplementary data Table S1). *Carboxylesterases* and *nicotinic receptors* genes had no sufficient efficiency to be kept for gene expression analyses.

Gene expression analyses:

The gene expression was assessed by Reverse transcription followed by quantitative PCR. Total RNA (200ng) was used for first strand cDNA synthesis, using oligo-(dT)15

primers and the Reverse Transcriptase Core Kit (Eurogentec, Liège, Belgium) according to the manufacturer's instructions. qPCR were performed in a 384-well plate using a CFX384 cycler (BioRad, Hercules, CA, USA). qPCR amplification was performed using the Takyon No Rox SYBR Core kit dTTP blue (Eurogentec, Liège, Belgium) in a 10 µl final volume containing 20 ng of cDNA and 250 nM of forward and reverse primers. The amplification program used was: 4 min at 95°C, 40 cycles of 10 sec at 95°C, and 1 min at 60°C. For amplicon specificity confirmation, each qPCR program was followed by a melting curve assay by increasing temperatures from 60 to 95°C, with 5s and each 0.5°C steps. Each biological condition was triplicate and each reaction was carried out in an independent technical triplicate and included a no-template control, a reagent-only control and a no-reverse-transcriptase control. Quantitation cycles (Cq) were defined based on the mean of the three technical replicates and then averaged within biological replicates. The geometric mean of the expression level of actin (ACT) was used as an internal control (reference) to normalize the expression data for the target genes.

The relative expression levels of target genes compared with reference gene were calculated as fold changes of the expression using the $2^{-\Delta\Delta Cq}$ method:

$$2^{[(Cq_{(target, sample)} - Cq_{(reference, sample)}) - (Mean(Cq_{(target, calibrator)} - Cq_{(reference, calibrator)})]}$$

(Livak and Schmittgen, 2001),

where the mean Cq values corresponded to the mean of the three biological replicates. To assess the effect of growth temperature and insecticide, the gene expression of each treatment was related to gene expressions of control individuals grown at 20°C. Moreover, sexes were analyzed separately to avoid differential expression between gender.

Data analyses

In order to calculate the different LD50 for each temperature and each insecticide, dose-response curves were fitted with the package drc using R studio software (Ritz *et al.* 2015; Rstudio team 2020 version 1.3.1073). The function drm of this package was used to calculate the different LD50 presented in results and the function Edcomp of this package has been used to compare each concentration and temperature tested.

For enzymatic activities, differences between insecticide treatments, temperatures and sexes were analysed using a Kruskal-Wallis test followed by a Wilcoxon rank-sum test performed using R software.

For gene expression analysis, the data presented are the averages of three biological replicates (each in technical triplicates). Glm gaussian family tests were used to test for differences in gene expression with temperature and insecticide as factors.

Results

Bioassays

Temperature had a significant impact on *C. pomonella* LD50 for emamectin with respectively twice and thrice the dose of chemical needed to kill 50% of the larvae at 30°C ($p=0.008$) and 35°C ($p=0.009$) compared to 20°C (Table 5). Temperature also had a significant impact on LD50 for Spinosad with fourth the dose of chemical required to kill 50% of the larvae at 25°C, 30°C and 35°C compared to 20°C ($p=0.04$) (Table 5) while no significant difference was observed between the three highest temperatures. Temperature did not significantly influence the mortality of larvae exposed to chlorantraniliprole (Table 5).

Table 5: LD50 measured for three insecticides used against larvae of a *C. pomonella* susceptible strain CpNPP at four different temperatures. Different letters indicate significant differences between treatments. (Edcomp, $\alpha=0.05$).

Temperature	20°C		25°C		30°C		35°C	
	n	LD ₅₀ (confidence interval)	n	LD ₅₀ (confidence interval)	n	LD ₅₀ (confidence interval)	n	LD ₅₀ (confidence interval)
Affirm® (Emamectin)	1427	0.069 a (0.05-0.09)	1491	0.106 b (0.09-0.12)	497	0.134 c (0.11-0.16)	836	0.186 d (0.15-0.22)
CORAGEN® (Chlorantraniliprole)	431	1.052 (0.8-1.3)	572	1.049 (0.77-1.32)	430	0.907 (0.65-1.32)	427	0.703 (0.49-0.91)
SUCCESS4® (Spinosad)	1001	0.792 a (0.31-1.27)	1172	4.583 b (3.25-5.91)	1328	3.617 b (2.38-4.85)	1144	4.737 b (3.45-6.02)

Enzymatic activity

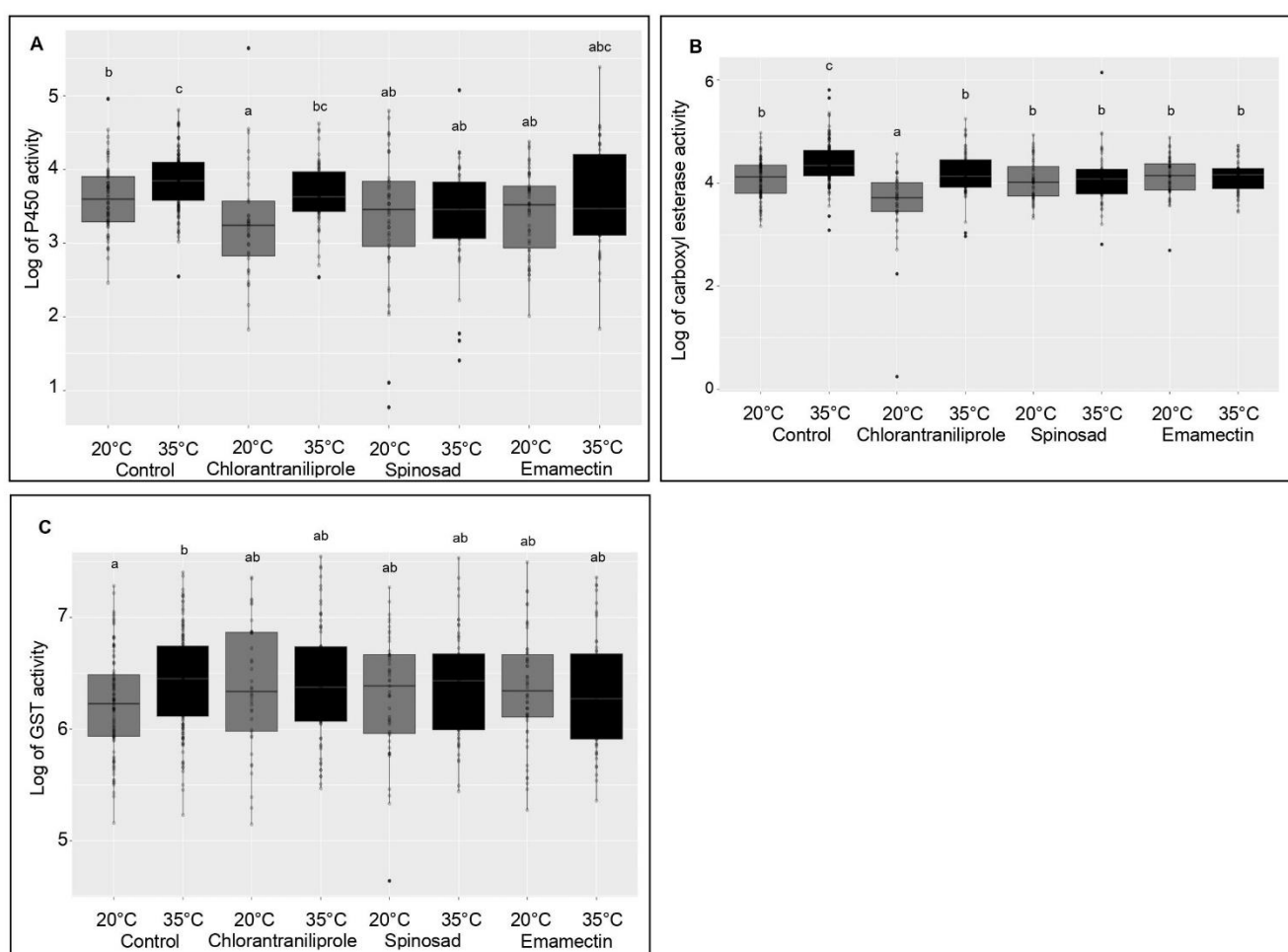


Fig. 17 : Activity of Relative cytochromes P450 ($\text{pg } 7\text{EC}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$) (A), Carboxylesterases ($\text{nmol anaphtol}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$) (B), and Gluthatione S-transferase ($\text{CDNB-GST conjugate}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$) (C) measured in *C. pomonella* adults emerging from substrate treated with LD30 concentrations of three insecticides and exposed to two different temperature. Bars with different letters are significantly different (wilcoxon test, $\alpha = 0.05$).

We did not observe any influence of the sex on enzymatic activities. Both sexes were thus pooled in analyses.

Cytochromes P450 activity was higher in controls and chlorantraniliprole-exposed individuals that developed at 35°C (average comprise between 41.2 and 50.0 pg of $7\text{EC}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$, $n=123$, $n=61$) compared to 20°C (average 36.9 pg of $7\text{EC}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$, $n=123$, $n=61$).

$1.\text{mg protein}^{-1}$, $p=0.0404$, $n=164$). Temperature did not influence P450 activity when individuals were exposed either to spinosad ($n=50$, $n=44$) or emamectin ($n=45$, $n=49$) (Fig. 17A). Regarding the insecticide effect at 20°C , P450 activity was not modified either after spinosad or emamectin exposure, while a significant decrease was observed after chlorantraniliprole exposure ($p<0.01$).

Higher temperature resulted in higher **carboxylesterases activity** for control ($n=144$, 20°C) and chlorantraniliprole-exposed individuals (35°C $n=69$, 20°C $n=38$) but not for those exposed to spinosad ($n=51$, $n=52$) or emamectin ($n=50$, $n=53$, control 20°C $n=103$). The highest carboxylesterases activity was recorded in individuals that developed at 35°C without insecticide spinosad $87.9 \text{ nmol anaphtol.min}^{-1}.\text{mg protein}^{-1}$, $p<0.001$) while the lowest activity was observed in individuals that developed at 20°C and were exposed to chlorantraniliprole (average $78.6 \text{ nmol anaphtol.min}^{-1}.\text{mg protein}^{-1}$, $p<0.001$) (Fig. 17B). No significant difference was observed between other treatments.

Glutathione S-transferase activity was significantly higher in control individuals that developed at 35°C (average $638.8 \text{ nmoles of CDNB-GSH conjugate.min}^{-1}.\text{mg protein}^{-1}$, $p=0.0298$, $n=144$) compared to 20°C ($n=143$). Temperature did not influence GST activity when any of the three insecticides was added to the diet. No significant difference was detected after insecticide exposure compared to the control. Strong variability between individuals was observed for this enzyme (comprised between 103 and 1867 nmoles of CDNB-GSH.min⁻¹.mg protein⁻¹, chlorantraniliprole $n=38$, $n=69$; spinosad $n=51$, $n=52$; emamectin $n=50$, $n=53$) (Fig. 17C).

Molecular analysis

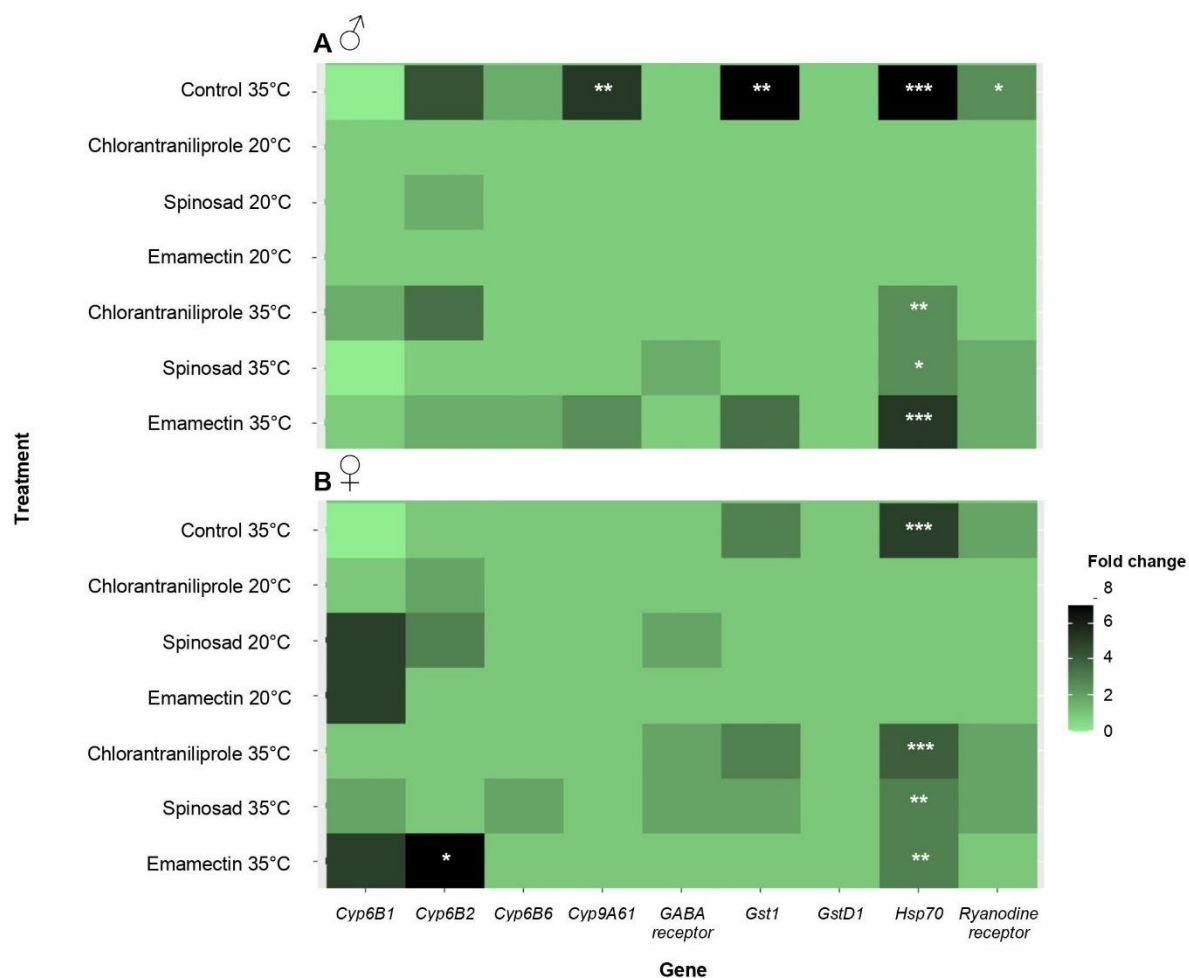


Fig. 18: Heatmap showing fold-change values calculated using the comparative Ct method for males (A) and females (B) using control 20°C individuals as reference (see section “Materials and Methods” for details). Variations in green colors indicate an increased (darkest) or a decreased (lighter) genes expression for 35°C-treated individuals compared to 20°C-control individuals. Significant treatment effects are noted with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) (glm, $\alpha = 0.05$). (Color reproduction web and print).

Cyp. Temperature and insecticides did not significantly influence the relative expression of *Cyp6B1* or *Cyp6B6* although females exposed to spinosad or emamectin tended to over-express *Cyp6B1* gene in comparison with control females. We observed a significant 7-fold higher expression of *Cyp6B2* in females exposed to both high temperature and emamectin in comparison to control females (glm, $p = 0,0462$). No other significant difference was detected between treatments although a higher

temperature resulted respectively in a 5- and 4-fold increase in *Cyp6B2* relative gene expression in 35°C-control or 35°C-chlorantraniliprole exposed males (Fig. 18, Fig. 19). A significant 6-fold over-expression of *Cyp9A61* gene was observed in males exposed to 35°C in the absence of insecticide compared to 20°C-control individuals (*glm*, $p=0,0064$). No other difference between treatments was detected (Fig. 18).

GABA receptor. Temperature and insecticides did not significantly influence the relative expression of *GABA*receptor (Fig. 18).

Gst. In absence of insecticide, exposure to a higher temperature resulted in a significant 8-fold over-expression of *Gst1* gene in males (*glm*, $p=0,0017$) and a 3-fold non-significant over-expression in females. No other significant difference was observed although a 4-fold increase in expression was noted for males exposed to both high temperature and emamectin in comparison with control males and a 3-fold over-expression was noted for females exposed to both high temperature and chlorantraniliprole in comparison with control females (Fig. 18, Fig. 19). Neither temperature nor insecticides influenced the relative expression of *GstD1* (Fig. 18).

Hsp. Exposure to 35°C induced a strong over-expression of *Hsp70* gene in both sexes compared to 20°C (Fig. 18, Fig. 19), whether insecticides were applied or not. Insecticides did not influence HSP gene expression when temperature was kept at 20°C.

Ryanodine receptor. Heat exposure resulted in a significant 2-fold increase in *Ryanodine receptor* gene expression in males compared with lower temperature when no insecticides were applied (*glm*, $p=0,0419$) while no other significant difference was detected between treatments (Fig. 18).

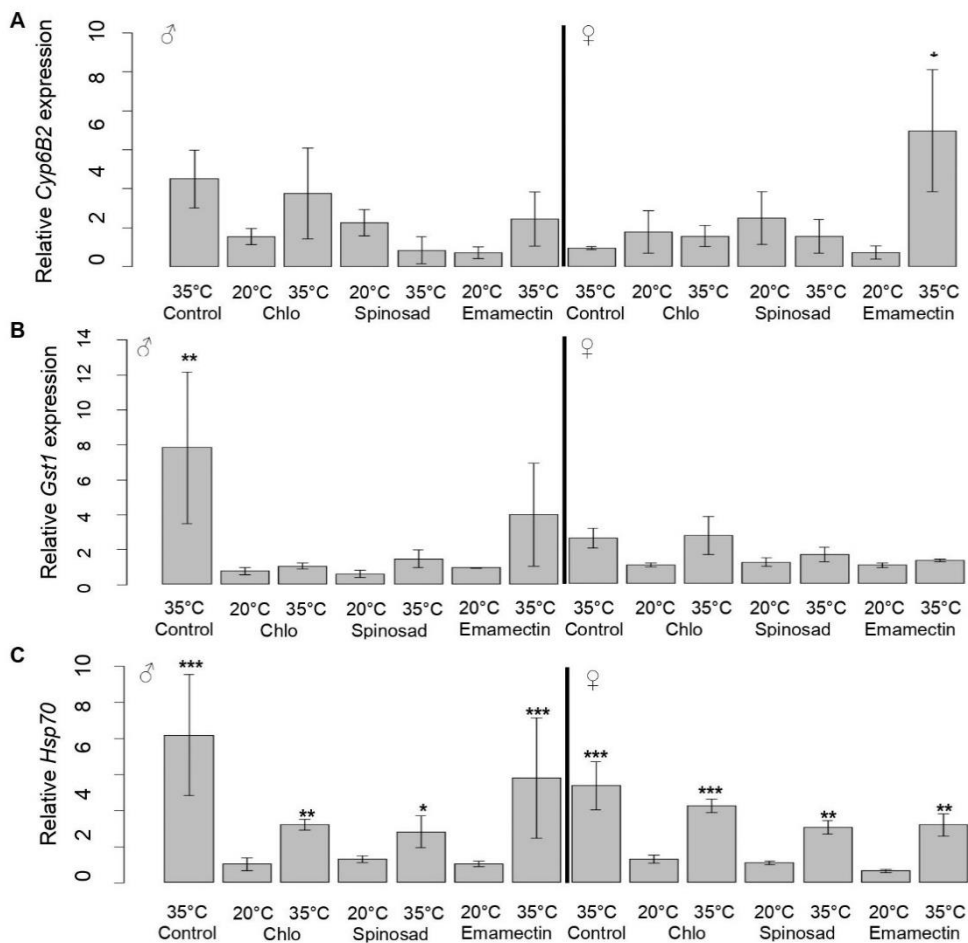


Fig. 19: Relative gene expression (\pm SEM) of *Cyp6B2* (A), *Gst1* (B), and *Hsp70* (C) in *C. pomonella* emerging adults from substrate treated with LD30 concentrations of emamectin, chlorantraniliprole (Chlo) and spinosad at an optimum temperature (20°C) and an extreme temperature (35°C) compared to control without insecticide at 20°C. Significant differences between treatment effects are noted with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$), glm, $\alpha = 0.05$.

Discussion

Understanding mechanisms involved in the temperature-dependency of insecticides toxicity is determinant to assess and improve their efficiency in a warming world. An increase in temperature decreased the toxicity of emamectin and spinosad on our strain of *Cydia pomonella* while it did not influence the toxicity of chlorantraniliprole. Such influence may be partly explained by changes in activity and gene expression of detoxification enzymes and *Hsp70*.

The negative relationship that we observed between temperature and spinosad toxicity on *C. pomonella* is consistent with a study by Mansoor *et al.* (2014) who reported a decrease in the toxicity of this insecticidal active ingredient from 20°C to 32°C in the cotton mealybug *Phenacoccus solenopsis*. In contrast to our results however, toxicity of emamectin was previously reported to increase by 1000-fold from 16°C to 31°C in the diamondback moth *Plutella xylostella* (Li *et al.* 2004) and by a 1.64-fold from 20°C to 32°C in *Phenacoccus selenopsis*. Similarly, a positive relationship between temperature and chlorantraniliprole toxicity was reported in *P. xylostella* (Teja *et al.* 2018) while we did not observe any influence of temperature on the toxicity of this insecticide on the codling moth.

Such differences between species in the temperature-dependency of an insecticide toxicity are well-known (Sparks *et al.* 1982, 1983) but to our knowledge, no hypothesis was previously proposed to explain this interspecific variation. First, the efficiency of biochemical pathways involved in insecticide detoxification may vary between species and with temperature. Secondly, differences in the experimental procedures may induce important differences between studies. Most experiments focused on transient 48h stress effects of temperature and insecticides on mortality (e.g. Li *et al.* 2007; Boina *et al.* 2009). In our study, we exposed *C. pomonella* to those stresses during the complete development of codling moth larvae, which is consistent with what may happen with climate change in agroecosystems since global warming would be effective during the whole life cycle of pests. Recently, Oliver & Brooke (2017) reported a lower mortality in *Anopheles arabiensis* individuals either exposed to deltamethrin and 1-cyhalothrin after a transient 3h heat shock. However, this lower mortality was only temporary as the mortality increased again within few hours for deltamethrin. Medrzycki *et al.* (2010) observed that developmental temperature differently influenced the toxicity of dimethoate in *Apis mellifera* according to their age. Adult workers

emerging at 33°C were indeed more susceptible to this insecticide than those emerging at 35°C while the opposite pattern was observed in larvae. These two studies highlight the importance of the timing and duration of thermal exposure on subsequent susceptibility to insecticide intoxication.

Temperature is known to mediate enzyme activities and gene expression in insects (Swain *et al.* 2009; Yang *et al.* 2016). We observed in *C. pomonella* that a high developmental temperature induced higher carboxylesterases, cytochromes P450 and glutathione S-transferases activities. Such positive relationship between temperature and activity of defensive enzymes is consistent with previous studies on other insect species. High temperatures indeed resulted in increased activity of carboxylesterase in the mosquito *Culex quinquefasciatus* (Swain *et al.* 2009) or glutathione-S-transferase in the silkworm *Antheraea mylitta* (Jena *et al.* 2013). In codling moth, insecticide resistance mechanisms are well known and GST, EST and P450 are major enzymes involved in detoxification (Reyes *et al.* 2007). The simultaneous increase in tolerance to spinosad and emamectin and in the activity of these three enzyme families with temperatures thus suggests that these two observations may be correlated. Changes in insecticide toxicity with temperature were also correlated with changes in activity of the same detoxification enzymes in the pest *Plutella xylostella* (Zhang *et al.* 2015). Such correlations were however not found in two mosquito species. In *Anopheles arabiensis*, insecticide toxicity decreased with increasing temperature but no changes in the activity of detoxification enzymes occurred (Oliver & Brooke 2017). In *Aedes aegypti*, some changes in activity levels of detoxification enzymes with temperature were not associated with a change in susceptibility to insecticides (Polson *et al.* 2012). We observed close results for codling moth tolerance to chlorantraniliprole that did not vary with temperature despite the increase in activity of detoxification enzymes. Such difference with results on emamectin and spinosad may be explained by the non-involvement of those enzymes in chlorantraniliprole tolerance reported recently for this pest in Spain (Bosch *et al.* 2018), and contrarily to observations on three other lepidopterans (Mao *et al.* 2019; Yin *et al.* 2021; Wang *et al.* 2018). These differences underline that stress responses may vary according to the insect species studied but also according to intrinsic properties of the insecticidal active substances and may explain differences observed between studies.

Additionally to the increase in enzymes activity, we observed that genes of at least two detoxification enzymes (*Cyp9A61* and *Gst1*) were over-expressed in males in the presence of heat stress, suggesting that they may be involved in increased tolerance to emamectin and spinosad at high temperature. These genes are indeed commonly associated with chemical detoxification (Zimmer *et al.* 2018). Liu *et al.* (2017) similarly reported that a 40°C heat shock for 4h upregulated genes encoding glutathione S-transferase as well as Cyp450 genes from CYP3, CYP6 and CYP9 clades in the moth *Glyphodes pyloalis* while a Delta class glutathione S-transferase gene was upregulated in response to heat stress in *Apis cerena cerena* (Yan *et al.* 2013). We did not observe any change in expression of other genes coding for detoxification enzymes or molecular targets of insecticidal active substance, except a slight over-expression of the gene coding for the ryanodine receptor, the target of chlorantraniliprole, at 35°C. This change was however not associated with a change in the susceptibility to this insecticide for *C. pomonella*.

As for *Cyp9A61* and *Gst1*, *HSP70* gene was upregulated in response to high temperature. Heat Shock Proteins have a primary function as molecular chaperones, promoting correct refolding and preventing aggregation of denatured proteins (Johnson *et al.* 1998), and their upregulation in response to heat has been commonly reported (Lindquist, 1986; Sorensen *et al.* 2003). Some previous studies also reported HSP upregulation in response to insecticides (Patil *et al.* 1996), resulting in increased thermotolerance of pest species (Ge *et al.* 2013; Su *et al.* 2018). In our study, insecticide exposure did not influence HSP70 expression for individuals that developed at 20°C. Drawing conclusion on the role of HSP70 in insecticide tolerance is thus uncertain for *C. pomonella* considering our results.

Surprisingly, the increase in GST, CbE and P450 activity and in expression of *Cyp9A61* and *Gst1* genes that we observed at high temperature for control individuals disappeared when emamectin or spinosad were applied. One exception to this apparent absence of response to double stress was the *Cyp6B2* gene from CYP6 clade that was significantly overexpressed in 35°C emamectin-treated females compared to females grown at 20°C. This gene, which is involved in detoxification of deltamethine and azinphos methyl in *C. pomonella* (Wan *et al.* 2019), may also have a strong effect on the detoxification of emamectin and its over-expression may be maintained despite the double stress.

Several hypotheses may be proposed to explain the apparent absence of biochemical of molecular responses to two stresses (heat + insecticide) in comparison to one stress. The mobilization of the enzymes on the insecticidal substrates would make them less accessible for the substrates proposed for the analysis. Indeed, insecticides can compete with the substrate for these enzymes when the active site is the same for the two molecules and this phenomenon increases when the enzymes simply do molecular sequestration and remain bounded to the insecticide for a longer time (Kostaropoulos *et al.* 2001; Goh *et al.* 1995; Despres *et al.* 2007). We may also hypothesize that energy may be preferentially allocated to the expression of genes regulating more ubiquitous mechanisms, such as HSP (Sorensen *et al.* 2003), to protect individuals against multiple stresses. *HSP70* gene was indeed still upregulated when two stresses were applied although this upregulation was less pronounced when individuals faced double stress compared to heat stress alone. Increasing the expression of *HSP70* gene is associated with increased energy allocation to transcription, translation and protein function (Hoekstra & Montooth 2013; Liu *et al.* 2008). We may expect that individuals facing multiple stresses would reduce the costly expression of HSP compared to individuals facing one stress to cope with other biochemical and molecular responses involved in stress responses.

Conclusion

Our results highlight the cross-effects of temperature and insecticides on *C. pomonella* mortality for emamectin and spinosad and provide important clues for understanding how temperature affects *C. pomonella* susceptibility to insecticides. *Hsp70* may play a role in dual stress (heat+insecticide) responses, and *CYP6B2* may also be involved in mechanisms of reduced sensitivity to emamectin and high temperatures. If one stress induces overexpression of detoxification genes, two do not. Allocation of cellular energy to more ubiquitous multi-stress tolerance pathways seems likely. We would need to move to more comprehensive RNA seq gene expression techniques to get a more complete picture of the selected defense pathways based on the number and types of stresses. Our observations echo notions of evolutionary biology that define “landscape fitness” in which individuals subjected to environmental changes will be selected on different traits able to respond to stresses without affecting too much its other vital functions, to move towards the best evolutionary compromise (Yang *et al.*, 2021).

Acknowledgments

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Supplementary data Table S1

All primers were designed specifically for this study except Hsp70-2 which are from Yang et al. 2016

Gene ID correspond to the Genbank accession number or InsectBase nomenclature.

Gene ID	Name	Forward primer 5' → 3'	Reverse primer 5' → 3'
CPOM03546	CYP 6B6	ACTGGTAGGTGCAATTTTAAGGG	CTAGCAACCTCTGGAGTTCCG
KC832920	CYP 9A61	CAAATACCGCTCTGGGCATAC	GATACGCCATCACCTTTACTTCG
CPOM05212	CYP 6B2	ATCCTTTCGTGGTTATCAGTGAG	CCCTCCGATTAGCTTGTTGAA
CPOM08435	CYP 6B1	ACTACCCGAACCCTGATGTC	TGAAGGCTCCACCCTGTAG
CPOM06919	GST 1	GATCAATCCACAACATACGGTTCC	GGTGGGAACAGAAATGCTACAATC
CPOM00366	GST D1	CACCACGGAGAACAGCTCAAG	CTGCCCTTGCCATATTTGTTG
CPOM16859	GABA receptor alpha-6	CGTGCATCCTCATCGTAGTCC	GTAATGGACGCCAGCAAATTC
CPOM13918	Ryanodine receptor	GATGAGGTCAACAGGAATTGTAC	TGATGGCGAGCAGGATAATG
JN863695	HSP70-2	GCCAACGACCAGGGTAACAG	CGACCTGTATTTTGGGCTTG
KC832921	Beta-actine	CGAGGCTCTTTTCCAACCC	AGGCGAGGATCGACCCG

En bref :

Ce chapitre est consacré à l'efficacité de la lutte intégrée. Il s'inscrit dans la volonté de comprendre les effets possibles du réchauffement climatique sur la gestion du carpocapse dans les vergers s'appuyant à la fois sur la lutte biologique réalisée par les insectes entomophages et l'utilisation d'insecticides. Il s'intéresse aux effets croisés de la température et de différentes substances insecticides, dont une homologuée en agriculture biologique, sur la mortalité de *C. pomonella* et de deux de ses ennemis naturels, le prédateur *Forficula auricularia* et le parasitoïde *Mastrus ridens*.

L'effet de l'action conjointe de la température et des insecticides a été déterminé sur la mortalité de larves de *C. pomonella*, par ingestion, et sur la mortalité d'adultes de *M. ridens* et *F. auricularia*, par ingestion et contact tarsal. Les résultats permettent d'identifier la possibilité de combiner les insecticides et les ennemis naturels étudiés pour contrôler *C. pomonella* en vergers conduits en production fruitière intégrée dans un contexte de réchauffement climatique et nous permettent de suggérer des préconisations d'utilisation de chaque substance insecticide testée.

L'hypothèse principale de ce chapitre est que la température affectera différemment la sensibilité de ses trois espèces d'insectes aux substances insecticides étudiées, et que cela aura des conséquences sur la régulation des populations du carpocapse des pommes.

L'ensemble des résultats de ce chapitre ont été soumis pour une publication dans « *Journal of Pest Science* » en Novembre 2021.

Differential influence of temperature on the toxicity of three insecticides between the codling moth *Cydia pomonella* (L.) and two natural enemies: consequences of global warming on biological control in orchards

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Résumé

La toxicité des insecticides peut varier avec la température et des différences interspécifiques ont été couramment rapportées dans ce domaine. Une influence différentielle de la température sur la toxicité des insecticides entre les ravageurs et leurs ennemis naturels peut avoir des conséquences importantes sur la lutte biologique dans un contexte de réchauffement climatique. Cette étude avait pour objectif d'examiner les effets croisés entre la température et trois insecticides - à savoir le chlorantraniliprole, l'émamectine et le spinosad - sur la mortalité d'un ravageur majeur des vergers, *Cydia pomonella*, et de deux de ses ennemis naturels dans le sud de la France, le prédateur *Forficula auricularia* et le parasitoïde introduit *Mastrus ridens*. Nous avons observé une diminution de l'efficacité de l'emamectine et du spinosad à haute température pour contrôler le carpocapse, alors qu'aucune influence de la température sur l'efficacité du chlorantraniliprole n'a été observée. A l'inverse, l'augmentation des températures a accru la toxicité de tous les insecticides sur *M. ridens*, et seulement de l'émamectine sur *F. auricularia*. Cette étude permet de formuler des recommandations pour l'utilisation de ces insecticides en combinaison avec l'utilisation d'ennemis naturels pour lutter contre le carpocapse dans un contexte de réchauffement climatique. Nos résultats déconseillent l'utilisation du spinosad alors que le chlorantraniliprole semble être adapté aux conditions climatiques futures pour lutter contre *C. pomonella*, conserver *F. auricularia* et faciliter l'établissement de *M. ridens*. Pour la lutte biologique par conservation reposant sur *F. auricularia*, une utilisation alternée d'emamectine au début du printemps, lorsque sa toxicité est la plus faible sur cet ennemi naturel, et du chlorantraniliprole pendant l'été pourrait limiter les risques d'apparition de résistance dans les populations de carpocapse tout en réduisant l'impact des insecticides sur les populations d'ennemis naturels.

Mots clés : Lutte intégrée, changements climatiques, agents de lutte biologique, chlorantraniliprole, spinosad, emamectine

Abstract

Insecticide toxicity may vary with temperature and interspecific differences have been commonly reported for this relationship. A differential influence of temperature on insecticides toxicity between pests and their natural enemies may have important consequences on biological control in a global warming context. This study aimed to investigate cross effects between temperature and three insecticides - i.e. chlorantraniliprole, emamectin and spinosad - on the mortality of a major pest in orchards, *Cydia pomonella*, and two of its natural enemies in southern France, the predatory earwig *Forficula auricularia* and the introduced parasitoid *Mastrus ridens*. We observed a decreased efficiency of emamectin and spinosad at high temperature to control the codling moth while no influence of temperature on chlorantraniliprole efficacy was observed. Increasing temperatures increased the toxicity of all insecticides on *M. ridens*, and only of emamectin on *F. auricularia*. This study provides insight to make recommendations for the use of these insecticides in combination with the use of natural enemies to control the codling moth in a warming world. Our results advise against the use of spinosad while chlorantraniliprole appears to be suitable for future climatic conditions to control *C. pomonella*, conserve *F. auricularia* and facilitate the establishment of *M. ridens*. For conservation biological control relying on *F. auricularia*, an alternating use of emamectin during early spring, when its toxicity is the lowest on this natural enemy, and chlorantraniliprole during summer could limit the risks of resistance appearance in codling moth populations while reducing insecticides impact on populations of natural enemies.

Key words: Integrated pest management, climate change, biocontrol agents, chlorantraniliprole, spinosad, emamectin.

Introduction

Frequent applications of insecticides to control agricultural pests induce side effects on human health, environment and beneficial organisms (Stehle & Schulz 2015; Koureas *et al.* 2012; Pajač *et al.* 2011). Due to climate change, their use is expected to increase significantly in the future, thereby aggravating their impact as well as their economic costs (Chen 2001; Koleva & Schneider 2009). To ensure the transition to a more sustainable agriculture, several solutions have been developed. One of them is Integrated Pest Management (IPM) strategy that is a science-based, decision-making process that combines biological, cultural, physical and chemical tools to identify, manage and reduce risk from pests in a way that minimizes economic, health and environmental risks (Dara 2019; Stark *et al.* 2007).

In IPM strategy, natural enemies, whether they are imported, conserved or supplemented in agroecosystems (Boivin 2001; Winkler *et al.* 2005), play a key role as biological control agents (Lacey & Unruh 2005). Despite their prominent role, natural enemies commonly provide an incomplete control of the pest, and insecticides have to be used sparingly as a complement (Stark *et al.* 2007). Nevertheless, most insecticides cause lethal and sublethal effects on natural enemies, thus indirectly reducing pest control (Desneux *et al.* 2007; Rajak *et al.* 2014). It is then important in IPM strategy to assess the impact of insecticides on natural enemies, in order to select active substances with a low impact on biocontrol agents.

Such ecotoxicological studies have been commonly performed but very few attended to assess insecticides' impact on natural enemies in a climate change context although temperature is known to influence their toxicity on several pests (Glunt *et al.* 2013), predators (Mansoor *et al.* 2015) or parasitoid species (Abbes *et al.* 2015). Contrasted results have been reported, depending on the insecticide class (Boina *et al.* 2009; Musser & Shelton 2005), the active substance itself and the insect species. For example, the toxicity of acetamiprid and chlorpyrifos increases with increasing temperature but decreases for λ -cyhalothrin and spinosad (Mansoor *et al.* 2015) in the predator *Chrysoperla carnea* (Neuroptera, Chrysopidae). On the opposite, λ -cyhalothrin toxicity increases at high temperature while temperature does not influence spinosad toxicity in *Philodromus* spiders (Michalko & Košulič 2016). This species-dependency of temperature-mediated toxicity of insecticides may be especially problematic with global warming in IPM strategy if toxicity decreases with temperature

for the pest but increases for natural enemies. We may thus expect pest outbreaks because of both a decreased efficiency of insecticides and a drop in populations of predators and parasitoids. To our knowledge, and despite such potential influence of global warming, no study investigated the influence of both temperature and insecticides on a pest species and its natural enemies.

The codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) is one of the most important insect pests in apple orchards worldwide, causing serious yield losses (Barnes 1991; Lacey & Unruh 2005). Species from several insects' orders have been reported to be its natural enemies, including predatory Dermaptera, Neuroptera, Coleoptera and Heteroptera (Nikolic *et al.* 2018) and a diverse assemblage of Hymenopteran parasitoids (Athanasov *et al.* 1997; Cross *et al.* 1999; Mills 2005). Their effectiveness in orchards is however quite limited (Maalouy *et al.* 2013; Thorpe *et al.* 2016) and insecticides are commonly used to manage *C. pomonella*, in addition to mating disruption (Witzgall *et al.* 2008; Grigg-McGuffin *et al.* 2015; Ioriatti & Lucchi 2016). The intensive use of insecticides, associated with the multivoltinism and high fertility of the codling moth, has however led to the development of resistance toward several chemical groups of insecticides in wild populations (Sauphanor *et al.* 2000; Boivin *et al.* 2001; Bouvier *et al.* 2001; Brun-Barale *et al.* 2005). There is thus a need to improve alternative or complementary methods to chemical products for the management of this pest.

In this paper, we investigated the insecticide temperature cross-effects of three commonly used insecticides on the mortality of *Cydia pomonella* and two natural enemies present in southern France, the predatory European earwig *Forficula auricularia* (Dermaptera: Forficulidae) and the parasitoid *Mastrus ridens* (Hymenoptera: Ichneumonidae). *Forficula auricularia* is an abundant native predator known to feed on codling moth eggs in apple orchards (Glen 1975; Unruh *et al.* 2016). It thus plays an important role for conservation biological control (CBC). *Mastrus ridens* has been introduced in several countries and is expected to be of particular interest for the control of *C. pomonella* (Sandanayaka *et al.* 2018; Charles *et al.* 2019). It has been introduced in France between 2018 and 2021 in the frame of a classical biological control (CIBC) program against the codling moth aiming at the permanent establishment of this parasitoid to provide a sustainable control of the pest (Borowiec *et al.* 2020). In order to optimize field practices, we tested insecticides that are commonly used against codling moth and are promising for use in conjunction with

biological control strategies: two reduced risk insecticides used in conventional apple orchards, emamectin and chlorantraniliprole (Redmond & Potter 2017; European Food Safety Authority 2012), and one insecticide approved in organic apple orchards, spinosad.

Our experiment was thus performed to investigate changes in insecticides' toxicity on the codling moth and its natural enemies as temperature increases, and discuss the relevance of these substances in IPM strategy relying on both methods to control *Cydia pomonella* in a warming world.

Material & Methods

1. Insect sample

1.1. *Cydia pomonella*

The laboratory strain of *Cydia pomonella* used for this study (later called CpNPP) originates from northern France and has been reared in the laboratory since 1995. It is used on an industrial scale for the production of Carpovirusine™ by Natural Plant Protection Firme, Pau, France. In our study, CpNPP was reared on artificial diet (prepared according to Guennelon *et al.* 1981 recipe without formaldehyde) at INRAE Avignon (France) at 25°C; 16:8 h L:D.

1.2. *Mastrus ridens*

M. ridens was reared at INRAE ISA (Sophia Antipolis, France) in insect-proof cages at 23°C; 16:8 h L: D. This strain results from the mix of several strains: laboratory stock from New-Zealand (2015) and Chile (2016) and individuals collected in Kazakhstan (2018). Each cage contained 50 females and 35 males and was provided with fresh honey and water daily. For reproduction, 50 to 60 overwintering cocoons of codling moth were placed per cage every 3-4 days.

1.3. *Forficula auricularia*

F. auricularia were sampled between March and June 2021 using traps cardboard sheets in peach and apple experimental orchards of INRAE PSH Avignon, southern France, where very few insecticides have been applied for the last 10 years. Adults *F. auricularia* were placed by 50 in ventilated boxes with artificial diet (the same as *C. pomonella*) and cardboard sheets as shelters at 25°C, 16:8 h L:D until experiments.

2. Bioassays

2.1. *C. pomonella* toxicity bioassay by ingestion

After few days of mating, eggs were collected, washed with a water-based solution of dishwashing liquid and bleach, and then dried. Neonatal larvae were used for bioassays since it is the target stage of insecticide treatments. We tested the influence of both temperature and insecticides on the mortality of *Cydia pomonella* larvae. The commercial formulations of emamectin benzoate (Affirm® 0,95%), spinosad

(SUCCESS[®] 4 480g/L) and chlorantraniliprole (CORAGEN[®] 200 g/l) were purchased respectively from Syngenta (France), Dow AgroSciences Distribution (France) and FMC Agricultural solutions (France).

To perform these tests, 96 microwell plates containing approximately 150 μ L of artificial diet (Stonefly diet Industries Ltd, Rochester, NY) per microwell were treated with different concentrations of insecticides diluted in osmosed water (Table 6). A 6 μ L volume of insecticide solution, or water for control, was deposited per microwell on diet surface. After 20 minutes of drying, newborn larvae were individually deposited and microwells were closed with parafilm strips. Plates were then placed in climatic chambers at 4 different temperatures: 20°C, 25°C, 30°C and 35°C. Mortality rate was assessed at 105-degree days, i.e. 10 days at 20°C, 7 days at 25°C, 5 days at 30°C and 4 days at 35°C, to homogenize the larval stages at the time of observation. A larva was considered as dead when not responding to a probe with dissecting forceps. Missing larvae were removed from data. The corrected mortality was calculated using the Abbott formula (Abbott 1925). Each test consisted in 24 individuals per concentration, per insecticide and per temperature and was replicated three times. To ensure that potential differences in toxicity with temperature were physiologically explained, and not the consequences of recording mortality at different times according to temperature (i.e. 105 degree-days), we additionally performed three replicates with mortality observations at 5 days for all temperatures.

Table 6: Concentrations of insecticide active substance used for bioassays on *C pomonella* neonate larvae.

Insecticides	Concentrations (mg/L)					
Affirm [®] (emamectin)	0.02	0.03	0.05	0.08	0.14	0.22
CORAGEN [®] (chlorantraniliprole)	0.3	0.53	0.95	1.69	3	
SUCCESS4 [®] (spinosad)	0.05	0.15	0.45	1.34	4.01	12

2.2. *M. ridens* toxicity bioassay

2.2.1. By ingestion

In this experiment, we tested the influence of both temperature and ingestion of insecticides on mortality of *M. ridens*. Since only adults are exposed to insecticides in orchards (larvae are ectoparasitoids of cocoons), we used freshly (< 48h old) emerged males of *M. ridens* for bioassays. The commercial formulations of the three insecticides were used as for *C. pomonella*. To perform this experiment, little pieces of paper (1 cm²) were soaked with 15 µL of a honey/water solution (3/1) untreated for control or polluted with different insecticides concentration (Table 7). Soaked papers were placed in 10 mL glass vial caps. After preparation of control and treated vials, 5 males were placed per vial and the caps were only partially screwed to allow the air to pass through. Bioassays vials were then placed in climatic chambers at 23°C, 28°C or 33°C. Mortality rate was assessed each day for 72 hours. Individuals were considered as dead when not responding to a probe with dissecting forceps. Missing individuals were removed from the dataset. The corrected mortality was calculated using the Abbott formula (Abbott 1925). Each test consisted in 5 individuals per dose, per insecticide and per temperature and was replicated four times.

Table 7: Concentrations of insecticide active substance used for ingestion bioassays on *M. ridens*. Concentrations are given in mg/L and converted in X dose, corresponding to the field dose used in French orchards.

Insecticides	Concentrations (mg/L and X dose)						
Affirm® (emamectin)	0.4 (X/5)	1 (X/2)	2 (X)	4 (2X)	10 (5X)	20 (10X)	
CORAGEN® (chlorantraniliprole)	0.018 (X/2)	0.035 (X)	0.07 (2X)	0.175 (5X)	0.35 (10X)	1.75 (50X)	3.5 (100X)
SUCCESS4® (spinosad)	0.02 (X/5)	0.05 (X/2)	0.096 (X)	0.2 (2X)	0.48 (5X)	0.96 (10X)	

2.2.2. By tarsal contact

In this experiment, we tested the influence of both temperature and insecticide exposure by tarsal contact on mortality of males. For this test, insecticides were used in the form of pure active substances dissolved in acetone (chlorantraniliprole PESTANAL®, analytical standard purity ≥ 95.0 %; emamectine benzoate PESTANAL®, analytical standard purity ≥ 85.0 %; spinosad PESTANAL®, analytical

standard purity $\geq 95.0\%$). Little pieces of paper (1cm^2) were soaked with $15\mu\text{L}$ of an untreated honey/water solution (3/1) and placed in 10mL glass vial caps. Glass vials were previously treated with $500\mu\text{L}$ of acetone for control or with $500\mu\text{L}$ of insecticide-polluted acetone. One unique dose corresponding to the one used in the field (X dose) was used for each insecticide: 2mg/L for emamectin, 0.096mg/L for spinosad and 0.035mg/L for chlorantraniliprole. In order to homogenize the distribution of the solution throughout vials surface, control and treated vials were gently laid down and rolled until complete evaporation of the solvent. After 2 hours of drying, 5 males were placed per vial. Caps were partially screwed on the vials so that air would always pass through. Bioassays vials were then placed in climatic chambers at 23°C , 28°C or 33°C . Mortality rate was assessed as in the previous experiment. Each test consisted of 5 individuals per dose, per insecticide and per temperature and was replicated four times.

2.3. *F. auricularia* toxicity bioassay

2.3.1. By ingestion

Since *F. auricularia* adults are more active than larvae and the most likely to be exposed to insecticides, we tested the influence of both temperature and ingestion of insecticides on their mortality. The commercial formulations of emamectin benzoate (Affirm[®] 0,95%), spinosad (SUCCESS[®] 4 480g/L) and chlorantraniliprole (CORAGEN[®] 200 g/l) were used as for *C. pomonella* and *M. ridens*. To perform this experiment, individual ventilated Petri dishes were filled with humidified sand and Eppendorf tube caps were filled with artificial diet (Guennelon *et al.* 1981 without formaldehyde). For each cap, a $6\mu\text{L}$ volume of insecticide solution with different concentrations or no solution for control, was deposited on the diet surface (Table 8). After 20 minutes of drying, one cap was placed on a single petri dish and one individual of *F. auricularia* was introduced in it. Petri dishes were then placed in climatic chambers at 23°C , 28°C or 33°C . Mortality was assessed each day at the same hour during five days. Individuals were considered as dead when not responding to a probe with dissecting forceps. Missing individuals were removed from the data. The corrected mortality was calculated using the Abbott formula (Abbott 1925). Each test consisted in 1 individual per dose, per insecticide and per temperature and was replicated fifteen times per sex.

Table 8: Concentrations of insecticide active substance used for ingestion bioassays on *F. auricularia*. Concentrations are given in mg/L and in X dose corresponding to the field dose used in French orchards.

Insecticides	Concentrations (mg/L and X dose)				
Affirm® (emamectin)	4 (2X)	10 (5X)	20 (10X)	40 (20X)	100 (50X)
CORAGEN® (chlorantraniliprole)	3.5 (100X)				
SUCCESS4® (spinosad)	0.096 (X)	0.2 (2X)	0.48 (5X)	0.96 (10X)	2 (20X)

2.3.2. By tarsal contact

We tested the influence of both temperature and insecticide exposure by tarsal contact on mortality of *F. auricularia* adults. Insecticides were used in the form of pure active substances dissolved in acetone (chlorantraniliprole PESTANAL®, analytical standard purity $\geq 95.0\%$; emamectine benzoate PESTANAL®, analytical standard purity $\geq 85.0\%$; spinosad PESTANAL®, analytical standard purity $\geq 95.0\%$). Petri dishes were treated with 115 μ L of acetone for control or with 115 μ L of insecticide-polluted acetone. One unique dose corresponding to the one used in the field (X dose) was used for each insecticide: 2mg/L for emamectin, 0.096mg/L for spinosad and 0.035mg/L for chlorantraniliprole. Following the application, the solvent was spread with a brush over the entire surface of the Petri dish, including the sides. After 30 minutes, 5 individuals of *F. auricularia* were placed in a single Petri dish and artificial diet (Guennelon *et al.* 1981 without formaldehyde) was provided for food. Petri dishes were then placed in climatic chambers at 23°C, 28°C or 33°C. Mortality rate was assessed as in the previous experiment. Each test consisted in 5 individuals per dose, per insecticide and per temperature and was replicated three times per sex.

3. Data analyses

In order to calculate the different LD50 for each temperature and each insecticide, dose response curves were fitted with the package *drc* using Rsoftware (Ritz *et al.* 2015; R core team 2020 version 4.0.2). The function *drm* was used to calculate the different LD50 and the function *EDcomp* has been used to compare each concentration and temperature tested.

For tarsal contact bioassays, corrected mortality was calculated using the Abbott regression (Abbott, 1925) and differences of mortality between treatments, sexes (for *F. auricularia*) and temperatures were analyzed using a χ^2 test.

Results

1. *C. pomonella*

Based on observations at 105 degree-days, temperature had a significant impact on the toxicity of emamectin with respectively one and a half, twice and thrice the dose of chemicals needed to kill 50% of the larvae at 25°C ($p=0.01$), 30°C ($p=0.008$) and 35°C ($p=0.009$) compared to 20°C (Fig. 20A). Temperature also had a significant impact on LD50 for spinosad with fourth the dose of chemicals required to kill 50% of the larvae at 25°C, 30°C and 35°C compared to 20°C ($p=0.04$) (Fig. 20B), but no significant difference was observed between the three higher temperatures (Fig. 20B). Temperature did not significantly influence the mortality of codling moth larvae exposed to chlorantraniliprole (Fig. 20C).

We observed similar results when reading mortality at constant time; i.e. 5 days (supplementary data, table 1). Our results are thus explained by differences in the influence of temperature on insecticides' toxicity and not by differences in reading time.

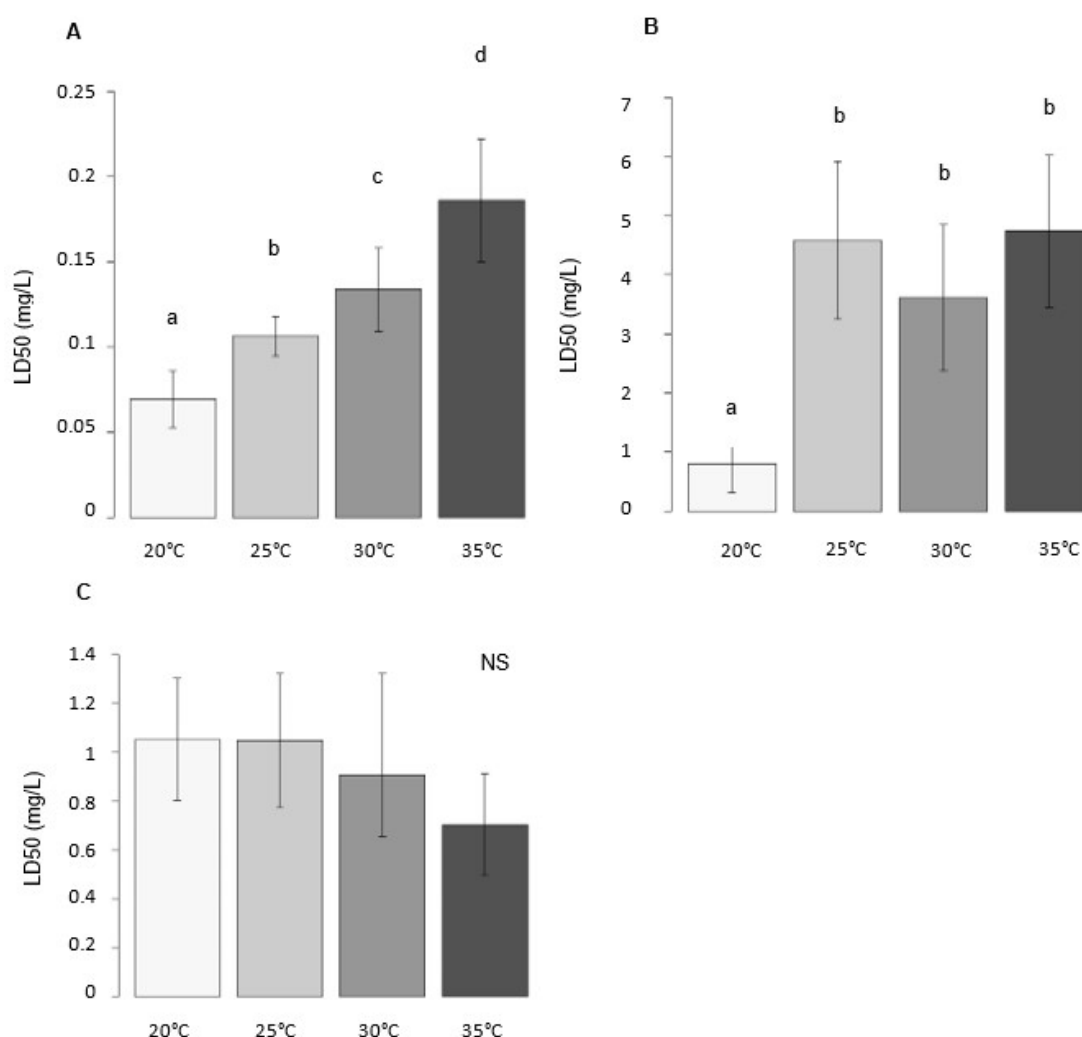


Fig. 20: Effects of temperature on doses of emamectin (A), spinosad (B) & chlorantraniliprole (C) needed to kill 50% (LD50) of codling moth neonatal larvae. Different letters indicate significant differences between temperatures. (EDcomp, $\alpha=0.05$).

2. *M. ridens*

2.1. Toxicity by ingestion

Spinosad toxicity increased with increasing temperatures when *M. ridens* ingested the insecticide. Four times less product was needed to kill 50% of the individuals at 28°C ($p=0.008$) and 6 times less at 33°C ($p =0.009$) compared to 23°C (Fig. 21B). Similar significant results were observed for chlorantraniliprole with respectively 8 and 15 times less product needed to kill 50% of the individuals at 28°C and 33°C compared to 23°C ($p = 0.04$) (Fig. 21C). For emamectin, a similar trend as for other insecticides was observed but differences between temperatures were not significant, probably because of a high variability occurring at 23°C (Fig. 21A).

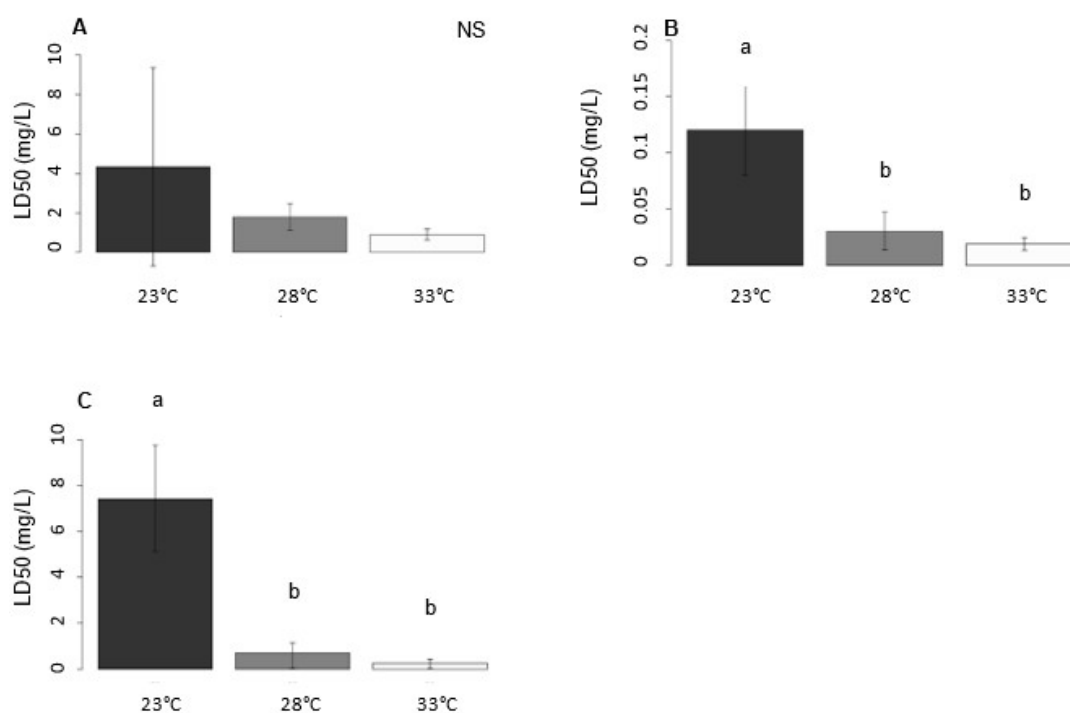


Fig. 21: Effects of temperature on doses of emamectin (A), spinosad (B) & chlorantraniliprole (C) needed to kill 50% (LD50) of *M. ridens* adults. Different letters indicate significant differences between treatments. (EDcomp, $\alpha=0.05$).

2.2. Toxicity by tarsal contact

Table 9 : Effects of temperature and insecticides at field dose (X dose) on the mortality of *M. ridens* adults. Different letters indicate significant differences between treatments. (Khi^2 test).

Insecticide (X dose)	Corrected Mortality (%)		
	23°C	28°C	33°C
emamectin	64.74 ^a ($Khi^2 = 0$)	100 ^b ($Khi^2 = 8.17$)	100 ^b ($Khi^2 = 8.17$)
spinosad	68.27 ^a ($Khi^2 = 0$)	100 ^b ($Khi^2 = 6.97$)	100 ^b ($Khi^2 = 6.97$)
chlorantraniliprole	100 ($Khi^2 = 0.40$)	100 ($Khi^2 = 0.15$)	100 ($Khi^2 = 0.15$)

Toxicity by tarsal contact of emamectin and spinosad increased significantly with temperature as it rose from less than 70% at 23°C to 100% mortality at 28°C and 33°C for both insecticides. Mortality reached 100% when chlorantraniliprole was applied, regardless of the temperature (Table 9).

3. *F. auricularia*

3.1. Toxicity by ingestion

Emamectin toxicity was significantly higher at 28°C ($p=0.008$) and 33°C ($p =0.009$) than at 23°C (Fig. 22A) when earwigs ingested the insecticide. A similar trend was observed for spinosad but differences between temperatures were not significant, probably because of a high variability occurring at 23°C (Fig. 22B). For chlorantraniliprole, no mortality occurred at the single dose tested (3.5 mg/L, corresponding to 100 times the approved field rate), regardless of temperature.

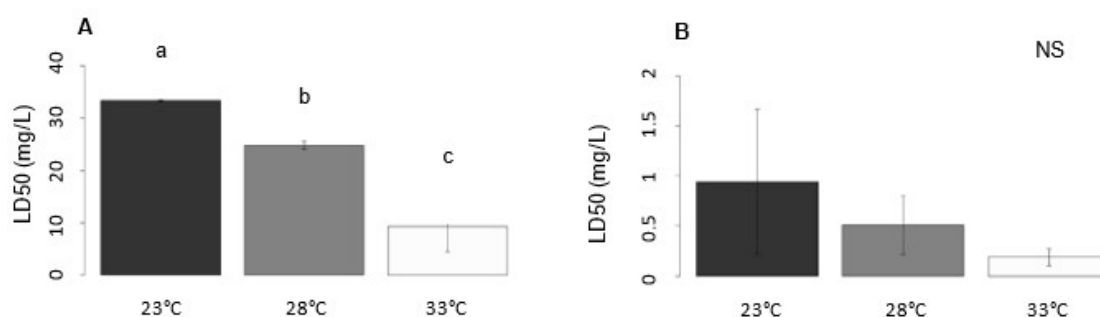


Fig. 22: Effects of temperature on doses of emamectin (A) & spinosad (B) needed to kill 50% (LD50) of *F. auricularia* adults. Results for males and females were pooled since there were no significant differences between sexes. Different letters indicate significant differences between treatments. (ED_{comp} , $\alpha=0.05$).

3.2. Toxicity by tarsal contact

We tested a single dose corresponding to the approved field dose of each insecticide substance. No mortality was observed for *F. auricularia* adults regardless of the insecticide or the temperature tested.

Discussion

The consequences of climate change on biological control caused by a differential influence of temperature on insecticides toxicity between pests and their natural enemies have never been considered to our knowledge, although such influence is known to be species-specific. This topic is especially relevant for organic farming and IPM strategy, that rely on the use of both natural enemies and insecticides. The worst scenario would probably be that insecticide toxicity decreases with global warming for a pest but increases for its natural enemies.

In this study, we observed that higher temperature (i) decreased the efficacy of emamectin and spinosad but did not influence the efficacy of chlorantraniliprole against the major orchard pest *Cydia pomonella*, while it (ii) increased the toxicity of these three insecticides on its parasitoid, *Mastrus ridens*, and (iii) increased the toxicity of emamectin on its predator, the earwig *Forficula auricularia*, when ingested. Our results thus indicate that *C. pomonella* outbreaks may occur more frequently in the future because of both a decreased efficiency of insecticides on the codling moth and a drop or local extinction of natural enemies' populations caused by higher insecticides' toxicity as temperature rises.

Our results on *C. pomonella* are in agreement with those reported for spinosad on another pest species, the cotton mealybug *Phenacoccus solenopsis* (Mansoor *et al.* 2014). On the opposite, the toxicity of emamectin increased with temperature for *P. solenopsis* (Mansoor *et al.* 2014) and for the diamondback moth *Plutella xylostella* (Lepidoptera, Plutellidae), as did chlorantraniliprole toxicity on the last species (Li *et al.* 2004; Teja *et al.* 2018), while it decreased for these two insecticides in the codling moth. Such interspecific differences in temperature-mediated insecticides' toxicity between pests have been frequently reported (Boina *et al.* 2009) although underlying mechanisms still have to be investigated.

In comparison to pests, few studies have been conducted on natural enemies but an increased toxicity at high temperature was reported for chlorantraniliprole in the parasitoid *Bracon nigricans* (Abbes *et al.* 2015), as we observed for *M. ridens* when the substance was ingested. However, spinosad toxicity increased with temperature for *M. ridens* in our study while it was not influenced by temperature in *B. nigricans* (Abbes *et al.* 2015) and decreased with increasing temperature in the lacewing

Chrysoperla carnea (Mansoor *et al.* 2015). The positive relationship between toxicity and temperature that we observed for the three substances in *M. ridens* and for emamectin ingested by *F. auricularia* may result from several mechanisms. First, high temperature may influence gene expression and activity of protective proteins such as detoxification enzymes (Liu *et al.* 2017; Yan *et al.* 2013; Zhang *et al.* 2015) or Heat Shock Proteins (Ge *et al.* 2013; Su *et al.* 2018). Secondly, metabolic rate increases with temperature (Brown *et al.* 2004), increasing consequently food consumption and locomotion rate (Gillooly, 2001; Medrzycki *et al.* 2010), and ultimately the penetration of the substance in insect body (Boina *et al.*, 2009). Thirdly, some insecticides, including emamectin, are known to have deleterious effect on insect feeding behavior (Abdu-Allah and Pittendrigh 2018; Khan *et al.* 2018), sometimes leading to death from starvation (Holmstrup *et al.* 2010). Although our experiment did not allow us to conclude on mechanisms involved, the last hypothesis is unlikely to explain the increased toxicity of emamectin at high temperature for *F. auricularia* considering their ability to starve for several days with no effect on their apparent fitness (Moerkens *et al.* 2009).

Whether the two natural enemies ingested the insecticides or were exposed by tarsal contact, *M. ridens* was more sensitive to substances than *F. auricularia*. The three insecticides indeed caused 100% mortality in *M. ridens* onwards 28°C at the authorized field dose while no mortality was detected in *F. auricularia*. This interspecific difference may be due to differences in composition or thickness of their cuticle (Fernandes *et al.* 2010), insecticide penetration depending notably on the affinity between the cuticle and a substance (Leite *et al.* 1998). The smaller size of *M. ridens* compared to *F. auricularia* may also explain this difference, as the specific target area of the insecticides decreases with increasing body size, resulting in reduced insecticide exposure (Picanco *et al.* 1997; Bacci *et al.* 2007).

Such differences may also explain that toxicity was mediated by temperature for the three insecticides for *M. ridens* while it was only true for emamectin in *F. auricularia*. These interspecific differences in the relationship between temperature and insecticides' toxicity underline the need to study specifically each pest-natural enemy's system. This approach would allow the selection of insecticides that will be the most reliable with global warming. Ideally, insecticides used in IPM programs and in organic

agriculture should be less toxic to natural enemies than to target insect pests (Zhao *et al.*, 2012) and remain harmless as temperature increases.

From our results, spinosad was as toxic to codling moth as it was to the European earwig and was even more toxic to *M. ridens*, regardless of the temperature. It was also more toxic to natural enemies than emamectin and chlorantraniliprole, considering the tiny dose needed to kill 50% of parasitoids compared to the two other insecticides. Lethal and sublethal effects of spinosad and other spinosyns on beneficial arthropods have been previously reported in several studies (Abbes *et al.*, 2015; Biondi *et al.* 2012) and yet, this product is authorized and widely used in organic farming while emamectin and chlorantraniliprole are not (Biondi *et al.* 2012). Moreover, our results indicate that higher temperatures emphasize differences in spinosad toxicity between the two trophic levels. Among the insecticides tested, it would thus probably be the least effective against the codling moth in a climate change context. The use of spinosad in strategies relying on the action of natural enemies should thus probably be avoided in orchards and its approval for organic production may raise questions in the future considering its increased toxicity on some beneficial organisms as temperature increases.

On the opposite, chlorantraniliprole may be the most promising insecticide in a warming world for orchards relying partly on biological control by conservation. The toxicity of this substance indeed remained very effective at high temperature against *C. pomonella* while it did not cause any mortality on *F. auricularia*, regardless of temperature or exposure method. It was moreover relatively safe against *M. ridens* when parasitoids ingested the substance, and despite an increased toxicity of the product at high temperatures, the lethal doses for 50% of individuals remained high (equivalent to 10 to 50 times the recommended field dose). However, the substance caused 100% mortality when tarsal contact occurred for *M. ridens*. This substance may thus be appropriate for classical biological control relying on the introduction of parasitic wasps, although field experiments should be conducted since parasitoids may avoid contact when the insecticide is applied, for example by leaving temporarily the orchard.

For both natural enemies, the LD50 remained very high at low temperature for emamectin, indicating that a high quantity of this insecticide should be applied to kill 50% of the individuals. These results are consistent with the literature on other natural enemies for this substance (Khan *et al.* 2018; Argentine *et al.* 2001). Considering its

low toxicity on beneficial organisms, added to a high selectivity for pests and a rapid degradation in the environment, the use of emamectin has been recommended in IPM strategies for a long time (Lopez *et al.* 2011; Argentine *et al.* 2001). We observed however that its toxicity increased rapidly with temperature for *F. auricularia* and *M. ridens* while it decreased for the codling moth. Its efficacy may thus decrease with global warming although it remained the most effective insecticide against *C. pomonella* among the three tested in terms of effective doses at high temperature. Contrarily to chlorantraniliprole, emamectin toxicity on *F. auricularia* increased strongly with temperature. An alternating use of these insecticides in conservation biological control, with a preferential use of emamectin during the colder periods, early spring notably, and the use of chlorantraniliprole during the summer, may limit the risks of resistance appearance in pest's populations while limiting the impact of chemical substances on the European earwig. Its use in CBC programs involving the introduction of *M. ridens* should however be avoided considering its toxicity at high temperature.

Conclusion

The present study highlights opposite cross effects of temperature and insecticides (spinosad, emamectin, chlorantraniliprole) on *C. pomonella* and two of its natural enemies, one exotic (*M. ridens*) and one native (*F. auricularia*) in southern France. We propose recommendations, according to the future climate, for the use of these insecticides in order to preserve natural enemies associated with *C. pomonella*. The use of spinosad should be avoided while emamectin can be used in conjunction with conservation of *F. auricularia*. Only chlorantraniliprole appears to be suitable in terms of control of *C. pomonella*, conservation of *F. auricularia* and establishment of *M. ridens*. The present study remains a simplified but essential tool to understand the cross-effects of temperature and select insecticides on the target pest and two of its natural enemies, allowing to propose an adaptation of strategies relying on insecticides in apple orchards in a changing climate. We focused on mortality of natural enemies but the effects of sublethal exposures, which affect insects' behavior and life history traits should also be investigated since they may be critical to the long-term establishment or conservation of natural enemies (Poorjavand *et al.* 2014; Saber 2011).

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Statements & Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose

Supplementary data:

Effects of temperature on doses of emamectin, spinosad and chlorantraniliprole needed to kill 50% (LD50) of codling moth neonatal larvae after 5 days of exposure. Different letters indicate significant differences between treatments. (EDcomp, $\alpha=0.05$).

Temperature	20°C		25°C		30°C		35°C	
	n	LD50 (IC)	n	LD50 (IC)	n	LD50 (IC)	n	LD50 (IC)
Affirm® (emamectin)	600	0.060 ^a (0.05-0.07)	599	0.090 ^b (0.07-0.10)	593	0.134 ^c (0.11-0.16)	670	0.188 ^d (0.15-0.22)
CORAGEN® (chlorantraniliprole)	432	0.974 (0.8-1.2)	431	0.853 (0.75-0.95)	430	0.907 (0.65-1.16)	427	0.723 (0.51-0.94)
SUCCESS4® (spinosad)	502	1.977 ^a (1.11-2.83)	503	7.402 ^b (6.33-8.46)	504	5.88 ^b (4.47-7.29)	504	5.31 ^b (3.97-6.65)

En bref :

Ce chapitre est consacré à l'étude des changements d'efficacité de la lutte biologique réalisée par des ennemis naturels en situation d'élévation des températures. Nous avons étudié les effets d'une augmentation des températures sur la phénologie de *Cydia pomonella* et sur la phénologie, les traits d'histoire de vie et le comportement de prédation ou de parasitisme de deux ennemis naturels, *Forficula auricularia* et *Mastrus ridens*.

Les résultats obtenus permettent de discuter du maintien de la synchronisation en vergers entre *C. pomonella* et ses ennemis naturels et de la pérennité du contrôle biologique exercé par ces derniers dans un contexte de réchauffement climatique.

L'hypothèse de ce chapitre est que *C. pomonella* et ses ennemis naturels vont se développer plus vite lorsque la température augmente mais n'auront pas les mêmes optimums et limites de température. La température pourra augmenter le taux parasitisme ou de prédation des deux ennemis naturels via l'augmentation de leur taux de métabolisme dans la limite de leurs optimums thermiques.

L'ensemble des résultats de ce chapitre sont en cours de correction interne et seront soumis pour une publication dans « *Biological Control* » en 2022. Certaines analyses sont encore en cours pour affiner le modèle phénologique, ces analyses sont discutées dans le chapitre VI.

Influence of temperature on biological control of the codling moth *Cydia pomonella* (L.) by two natural enemies, the European earwig *Forficula auricularia* and the parasitoid *Mastrus ridens*.

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Résumé

Le changement climatique devrait avoir une influence considérable sur la biologie des insectes. L'une des préoccupations concernant les agroécosystèmes est que l'augmentation de la température peut influencer de manière différentielle la physiologie et le comportement des ravageurs et de leurs ennemis naturels, entraînant des perturbations de la lutte biologique.

Cette étude avait pour objectif de déterminer si le carpocapse des pommes, *Cydia pomonella*, et deux de ses ennemis naturels, le perce-oreille *Forficula auricularia* et le parasitoïde *Mastrus ridens*, ont des optimums thermiques différents, et si les deux espèces d'ennemis naturels seront toujours capables de fournir un contrôle efficace de *C. pomonella* dans les conditions climatiques futures.

En adaptant un modèle phénologique à nos données sur le taux de développement de *C. pomonella*, nous avons déterminé que sa température optimale de développement serait de 32,3°C et que sa limite supérieure de développement serait de 42,6°C. Nos projections d'une augmentation de +2 ou +4°C de la température moyenne ont abouti à l'apparition d'une quatrième ponte dans le sud de la France pour ce ravageur.

M. ridens a été capable de produire une progéniture viable à 28°C et la température n'a eu aucune influence sur son potentiel de parasitisme. En revanche, *F. auricularia* a été incapable de se développer au-delà de 25°C et a légèrement perdu son potentiel de régulation à 30°C. D'après la biologie de ces espèces, leur saisonnalité et les résultats de notre étude, ces deux ennemis naturels présentent un bon potentiel de régulation de *C. pomonella* dans un contexte de réchauffement climatique.

Mots clés : lutte intégrée, traits d'histoire de vie, comportement, optimum thermique, décalage phénologique

Abstract

Climate change is expected to have a significant influence on insect biology. One concern about agroecosystems is that increased temperature may differentially influence physiology and behaviours of pests and their natural enemies, resulting in altered biological control.

This study aimed to determine whether the codling moth, *Cydia pomonella* and two of its natural enemies, the European earwig *Forficula auricularia* and the parasitoid *Mastrus ridens*, have different thermal optima for development, and whether the two natural enemy species will still be able to provide effective control of *C. pomonella* under future climatic conditions.

By fitting a phenology model to our data on developmental rate for *C. pomonella*, we determined that its optimal temperature for development would be 32.3°C while its upper limit would be 42.6°C. Our projections of a +2 or +4°C increase in mean temperature resulted in the appearance of a fourth egg laying event in southern France for this pest.

M. ridens was able to produce viable offspring at 28°C and temperature had no influence on its parasitism potential. *F. auricularia* was however unable to develop above 25°C and slightly lost its regulatory potential at 30°C. According to the biology of these species, their effective season in orchards and to the results of our study, these two natural enemies present a good potential of regulation of *C. pomonella* in a context of global warming.

Key words: IPM strategies, life history traits, behaviours, thermal optima, phenological mismatch.

Introduction

To ensure effective transition towards a more rational pest management in a global warming context, it is important to understand the impact that future climate will have on the different components of agroecosystems, including insects. Climate change is expected to have a significant influence on their biology since they are ectotherms. In such organisms, the relationship between ambient temperature and the mean value of life history traits or speed of physiological processes and behaviors commonly describe a bell-shaped curve (Abram *et al.* 2017; Duan *et al.* 2018; Fernandes *et al.* 2021), *i.e.* value or speed tend to increase exponentially with temperature up to an optimum temperature, then decrease rapidly down to a critical thermal maximum after which they fall to zero (Angilletta 2009; Huey & Stevenson 1979). Such thermal curves are for example observed for developmental rate in most ectotherm species (Sinclair *et al.* 2016). Insects may thus benefit from global warming since their development time would decrease (Furlong & Zalucki 2017) while their number of generations per year would increase (Juszczack *et al.* 2013; Stoeckli *et al.* 2012).

One major concern about changes in ecosystem functioning with global warming is that temperature may have differential influence between interacting species, considering that thermal curves vary strongly between species. Temperature may indeed exceed thermal limit for one species but remain in the exponential part of the thermal performance curve for others, or exacerbate differences in life history traits between them if the slope of their respective curves differ. Such concern is especially important for pests and their natural enemies in agroecosystems. Global warming may for example result in altered spatial and temporal synchronization between the two trophic levels, and thus result in decreased biological control, if temperature differentially influences their development time (Damien & Tougeron 2019; Wetherington *et al.* 2017).

Differential influence of temperature on life history traits such as fecundity or longevity between pests and their natural enemies may also disrupt population dynamics and ultimately lead to pest outbreaks (Gutierrez *et al.* 2008; Abarca *et al.* 2021). Moreover attack rate by predators and parasitoids is expected to increase with temperature because of an increased metabolic rate (Brown *et al.* 2004) but high temperatures may result in a drop in this physiological trait if the critical limit is reached (Moiroux *et al.* 2012), leading to a decrease in biological control provided by entomophagous species.

Some parasitoids may for example no longer be able to parasitise hosts when the temperature exceeds their thermal range (Devotto *et al.* 2010; Duan *et al.* 2018; Fernandes *et al.* 2021).

Considering that thermal limits differ between species and even between populations (e.g. Moiroux *et al.* 2010, Traoré *et al.* 2006), no general prediction can be drawn. Temporal mismatches have for example been predicted in some systems (Wetherington *et al.* 2017) but not in others (Gerard *et al.* 2013; Klapwijk *et al.* 2010). Each system thus need to be studied specifically. In this study, we tried to get a better understanding of the risks with global warming of altering biological control of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), a major worldwide pest of apple and orchards, by two natural enemies, the European earwig *Forficula auricularia* (Dermaptera: Forficulidae), and the gregarious parasitoid *Mastrus ridens* (Hymenoptera: Ichneumonidae) in Mediterranean orchards.

Forficula auricularia is an abundant native predator known to feed on codling moth eggs in apple orchards (Glen 1975; Dib *et al.* 2011). It thus plays an important role for conservation biological control. *Mastrus ridens* is an exotic parasitoid that has been introduced in several countries and is expected to be of particular interest for the control of *C. pomonella* (Sandanayaka *et al.* 2018; Charles *et al.* 2019). It has been introduced in France in 2018 in the frame of a classical biological control program against the codling moth aiming at the permanent establishment of this parasitoid to provide a sustainable control of the pest (Borowiec *et al.* 2020).

The aim of this study was thus to determine, through measurements of life history traits for the two trophic levels and behavioural observations for natural enemies at different temperatures, (i) whether these different species have different thermal optima for development, and (ii) whether the two natural enemy species will still be able to provide effective control of *C. pomonella* under future climatic conditions. Moreover, the study of the influence of temperature on development time in *C. pomonella* allowed us to project potential changes in voltinism with global warming in south-eastern France for this pest species.

Materials and Methods

All experiments were conducted in the laboratory of INRAE Avignon (France's National Research Institute for Agriculture, Food and Environment), Avignon, France.

1. Insect sampling and rearing

1.1. *Cydia pomonella*

A *C. pomonella* strain susceptible to granulovirus (CpNPP), originating from Northern France and reared in laboratory since 1995, was used for this study. This strain is used on an industrial scale for the production of Carpovirusine™ by Natural Plant Protection, Pau, France. In our study, CpNPP was reared on artificial diet (prepared according to Guennelon *et al.* 1981, recipe without formaldehyde) at $25\pm 1^\circ\text{C}$ with a L16:D8 photoperiod. After few days of mating, eggs were collected, washed with a water-based solution of dishwashing liquid and bleach, and then dried. Eggs were then placed at $25\pm 1^\circ\text{C}$ until neonate hatching.

1.2. *Mastrus ridens*

M. ridens was reared in insect-proof cages at $25\pm 1^\circ\text{C}$; 16:8 h L: D. This strain results from the mix of several strains: laboratory stock from New-Zealand (2015) and Chile (2016) and individuals collected in Kazakhstan (2018). Each cage contained 50 females and 35 males and was provided with fresh honey and water daily. For reproduction, 50 to 60 overwintering cocoons of codling moth were placed per cage every 3-4 days.

1.3. *Forficula auricularia*

Adults of *F. auricularia* were collected from experimental insecticide-free orchards at INRAE Avignon in October 2019 and October 2020 using bands of corrugated cardboard. Couples were isolated in ventilated cages (5cm diameter and 2cm high) with artificial medium at $10\pm 1^\circ\text{C}$ and L16:D8 photoperiod. Wet sand was provided as a substratum for egg laying.

2. Effect of temperature on *Cydia pomonella* development

2.1. Development time and of *Cydia pomonella* larvae

To perform developmental monitoring, neonate larvae were individually placed in a plastic hemolysis tube containing artificial diet prepared according to Guennelon *et al.* (1981) recipe without formaldehyde. Eighty tubes per temperature were then placed in climatic chambers at 20, 25, 28, 30, 32, 35, 38 or 42°C with a L16:D8 photoperiod. Phenological stage was assessed every day according to the presence of cephalic capsules. There are five larval instars in the codling moth and a cephalic capsule can be found after each molting. The development time was calculated from neonatal to final larval instar. Survival rate was assessed every day and summed up at the last larval stage.

2.2. Phenology of *Cydia pomonella*: Developmental Rate and Predictive Models

To determine the relationship between temperature and *C. pomonella* developmental rate, we used the devRate R package (Rebaudo *et al.* 2018) to measure the goodness-of-fit of 37 existing predictive models to our observed developmental time data. Models were selected based on (1) their goodness-of-fit based on R², RMSE and AIC, (2) their ability to provide all phenology parameters of interest (lower and upper temperature thresholds, optimal temperature and the thermal constant) and (3) their successful use to predict *C. pomonella* developmental rate in previous studies.

Based on the selected models, we provided predictions for *C. pomonella* life cycle based on daily temperatures measured by a weather station in an orchard in Avignon (South-eastern France) in 2019, as well as two crude scenarios of warming (the original temperatures +2°C and +4°C). The simulated cycles started at the egg stage, *i.e.* at the first generation produced by individuals emerging from the diapausing pupa stage.

3. Effect of temperature on progeny number, longevity and development time of *Mastrus ridens*

Thirty freshly emerged females per temperature were placed with fifteen males in insect-proof cages at 20, 25 or 28°C (Devotto *et al.* 2010) with a 75% honey and 25% water mixture provided as food. Males were removed after 24h and one *C. pomonella* cocoon was added in the cage and replaced daily throughout the life of each female. Each removed cocoon was placed in a petri dish, kept in similar climatic conditions

than for parasitism and checked daily. Development time was calculated as the number of days from cocoon parasitism to the emergence of *M. ridens*. Daily progeny number was calculated as the cumulated number of *M. ridens* emerging from cocoons proposed to each female during her lifetime. Total progeny number was calculated as the cumulated number of *M. ridens* emerging from the total number of cocoons proposed to each female. Mean progeny number per cocoon effectively parasitized was calculated as the cumulated number of *M. ridens* emerging from cocoons parasitized. These newly emerged individuals were kept in similar conditions than for their development, provided with fresh honey and water and checked daily until they died to determine their longevity.

4. Effect of temperature on the parasitism rate by *Mastrus ridens*

Using the experiment designed to determine the influence of temperature on progeny number, we calculated on thirty females per temperature the parasitism rate as the number of cocoons from which *M. ridens* emerged compared to the number of cocoons initially offered to the mother during her lifetime.

5. Effect of temperature on progeny number, survival and development time of *Forficula auricularia*

Each couple placed at $10 \pm 1^\circ\text{C}$ was checked daily for egg hatching. When a clutch was observed, we recorded the date of egg laying and the number of eggs that were laid per female. The male was then removed, as females naturally do to prevent cannibalistic feeding on eggs (Dib *et al.* 2017), and the cage was immediately placed at 20, 25, 28 or $30 \pm 1^\circ\text{C}$. Ten clutches were placed per temperature. Insects were fed every week with ground pollen and the sand was re-humidified every day. The development time of eggs and each nymphal instar was recorded every day. *Forficula auricularia* goes through four nymphal instars before reaching adulthood (Dib *et al.* 2017). After the second molting, 3rd nymphal instar individuals were transferred with their siblings to a larger ventilated cage (8cm diameter and 8cm high) containing wet sand, cardboard strip as shelter and artificial medium as food. Development time corresponded to the number of days spent between egg laying and adult instar. Survival rate was assessed every day and summed up when the adult instar was

reached. Progeny number corresponds to the number of earwigs that reached the adult stage per mother.

6. Effect of temperature on the predatory behavior of *Forficula auricularia*

After a 12h fast, 4th instar nymphs and adults from both sexes of *F. auricularia*, sampled randomly from the progeny obtained in the experiment on life history traits or from individuals sampled in field, were weighed and placed individually in ventilated boxes containing a small corrugated cardboard band as shelter and two hundred *C. pomonella* eggs were placed in each box. Boxes were then placed in climatic chambers at 20, 25, 28 and 30°C. Fifteen boxes were used for each predator life-stage at each temperature. After 24h, each individual was removed and weighed. The number of eggs remaining on the oviposition leaf cutting was counted to estimate the predation potency of *F. auricularia* nymphs and adults.

7. Statistical analysis

All data analyses were carried out using R software, version 4.0.2 (R Core Development Team, Vienna, Austria 2020). Homogeneity of variance and normality of data were checked using Bartlett's and Shapiro-Wilk tests, respectively.

When these requirements were met after a boxcox transformation, differences between temperature treatments were analyzed on the transformed data using one-way ANOVA test followed by *post-hoc* Tukey tests at the significance level of 5%. Differences between temperature treatments and sexes or development stages were analyzed on the transformed data using two-way ANOVA test followed by *post-hoc* Tukey tests at the significance level of 5%. When homogeneity of variance and normality requirement even after boxcox transformation of data were not met, differences between temperature treatments were analyzed using Kruskal Wallis non parametric test followed by *post-hoc* Dunn tests at the significance level of 5%. When data were available for only two temperatures (*i.e.* life history traits of *F. auricularia*), differences between temperature treatments were analyzed using Wilcoxon non parametric tests at the significance level of 5%.

Results

1. *Cydia pomonella*

1.1. Development time and mortality

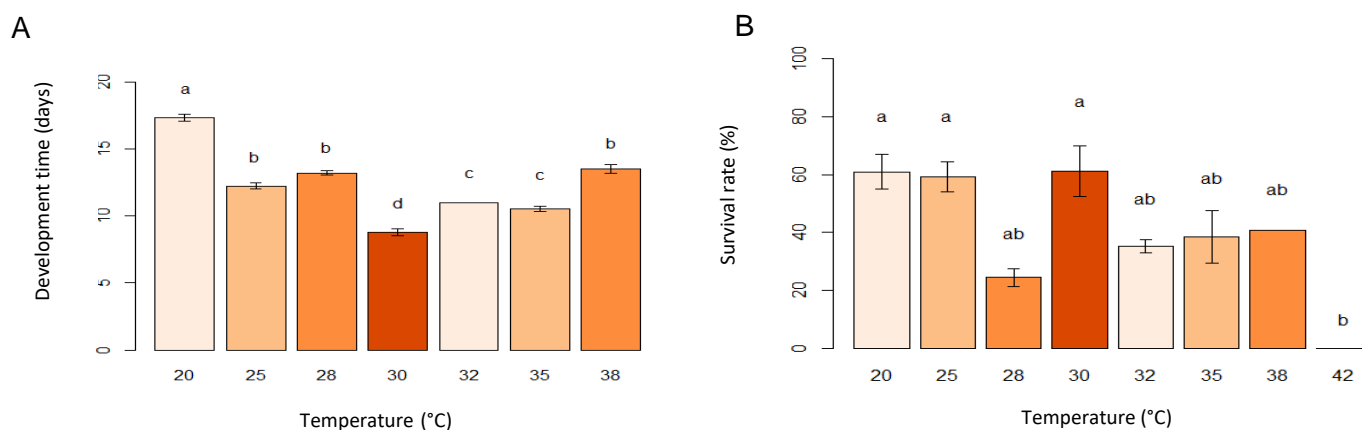


Fig. 23: Effect of the studied temperatures on the mean (\pm SED) of development time (Dunn, $\alpha=0.05$) (A) and survival rate (Tukey, $\alpha=0.05$) (B) of *Cydia pomonella* from first to fifth larval instar. Different letters indicate significant differences between treatments

Temperature had a significant impact on the development time of *C. pomonella*. The shortest developmental time was observed at 30°C ($\chi^2 = 394.2$, $df = 6$, $p < 0.001$) (Fig. 23A) while it was the longest at the coldest temperature, i.e. 20°C ($p < 0.001$). The development time was longer at 25°C, 28°C and 38°C than at 32°C and 35°C (Fig. 23A).

Temperature did not significantly influence the survival rate of *C. pomonella*. It (Fig. 23B) except at 42°C. No larva was able to develop successfully to the fifth instar for this temperature ($F = 4.302$, $df = 7$, $p = 0.01$).

1.2. Phenology

1.2.1. Model fitting

Although 10 of the tested models provided a fit on our development time data, only 6 of those had already successfully been used to predict *C. pomonella* developmental rates. All 10 fitted models had very close AIC (-4000 ± 30) and RMSE (0.0158 ± 0.0002) parameters. Of those, only two were able to provide every phenology parameters of interest: Lactin-2 (Lactin *et al.* 1995) (Fig. 24A) and Holling type III (Hilbert & Logan,

1983) (Fig. 24B). Both were fitted with parameters starting values measured on *C. pomonella* by Aghdam *et al.* (2009), although final model parameters after adjustment to our observed data diverged substantially (Table 10). Both selected models provided good and very similar RMSE, AIC and R^2 (Table 10) although none had normally-distributed residuals (Holling: $W = 0.83074$, p -value $< 2.2e-16$, Lactin-2: $W = 0.83463$, p -value $< 2.2e-16$).

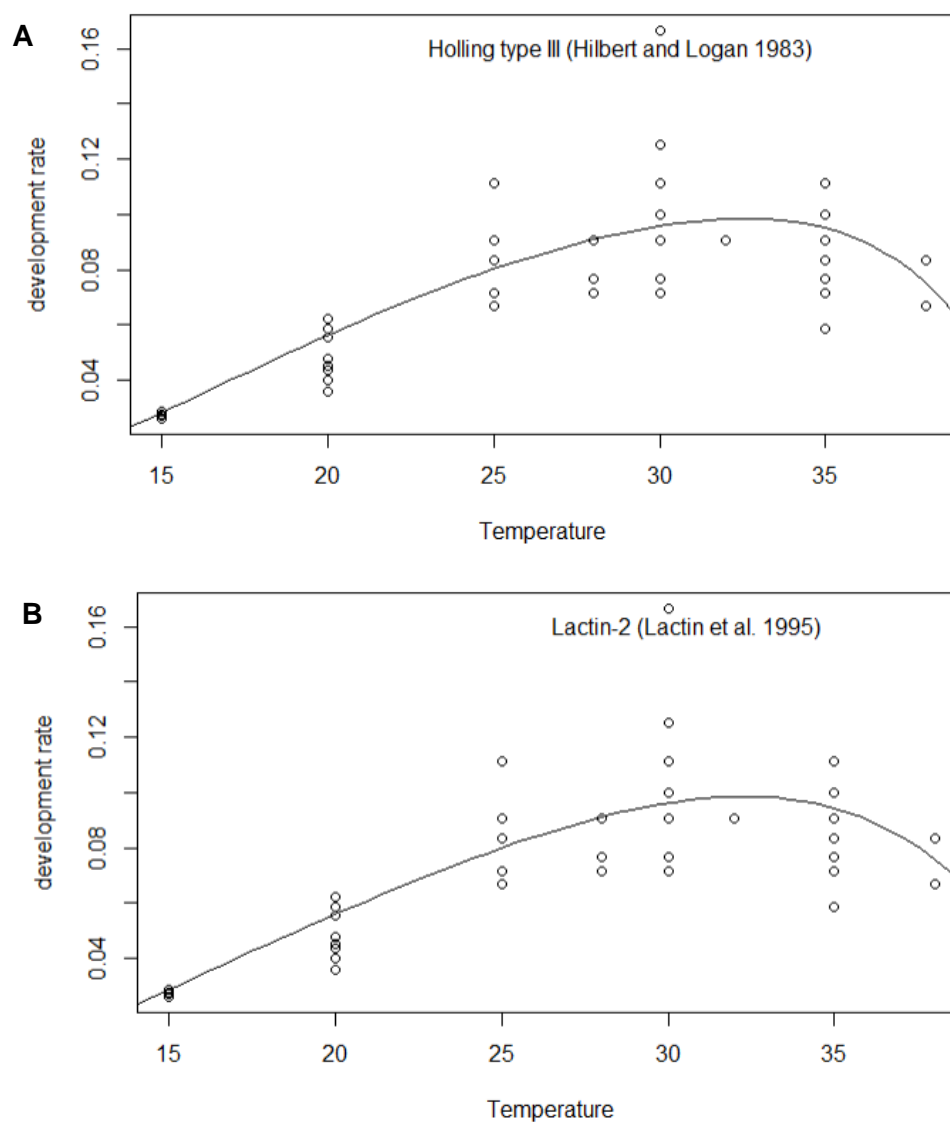


Fig. 24 : Observed developmental rates of *Cydia pomonella* (circles) and fitting Lactin-2 and Holling type III models (lines) at studied constant temperatures.

Table 10: Selected development models, their parameters after adjustment to the experimental data, goodness of fit, and metrics of development

Lactin-2 model :		$rT \sim (aa * T) - \left(\frac{aa * T_{max} - T_{max} - T}{\Delta T} \right) + bb$			
Parameters	aa	Tmax	deltaT	bb	
	0.005534	54.364683	6.356897	-1.055104	
Goodness-of-fit	R ²	RSS	RMSE	AIC	
	0.80	0.118649	0.01562478	-4032.448	
Metrics of development	CTmin	CTmax	Topt	XTmin	XTmax
	9.9	42.6	32.31000	10.0	42.6

Holling type III model :		$\varphi * \left(\frac{(T - T_b)^2}{(T - T_b)^2 + aa^2} \right) - \frac{-(T_{max} - (T - T_b))}{\Delta T}$			
Parameters	φ	aa	Tb	Tmax	deltaT
	0.164	18.639	6.487	36.042	3.569
Goodness-of-fit	R ²	RSS	RMSE	AIC	
	0.80	0.118639	0.01562412	-4030.489	
Metrics of development	CTmin	CTmax	Topt	XTmin	XTmax
	6.6	41.7	32.56188	7.9	41.7

1.2.2. Predicting *C. pomonella* active cycle

The models fitted on our observed data allowed us to consistently predict the three successive egg laying observed each year in the field in the south of France (Fig. 25A). Moreover, the same model run under the scenarios of +2°C and +4°C higher temperatures predicted a fourth egg laying (Fig. 25B and C). Eggs did not hatch before the end of the season at +2°C while larvae were predicted at +4°C.

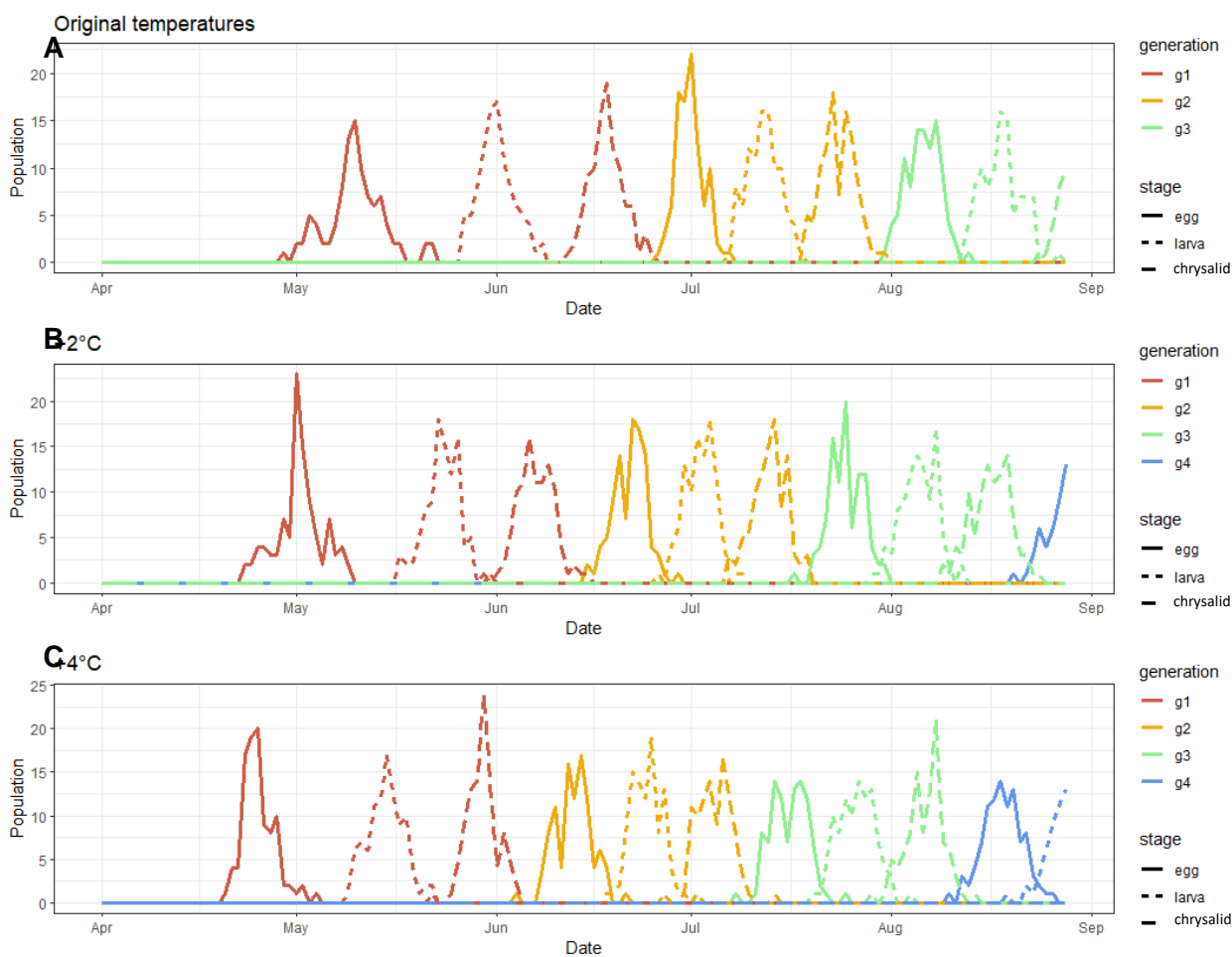


Fig. 25: Predictions of *C. pomonella* active cycle based on the Holling III developmental rate model (*Lactin-2* is not shown because its predictions are indistinguishable) and 2019 temperatures (original, +2°C and +4°C). The active cycle starts at the egg stage after the first adults emerge from winter diapause. G1, G2, G3, G4 indicate the generation number. When taking into account the first diapausing generation, G1 stands for second generation, etc. The simulations start on april 1st and stops 150 days after, taking into account the forced diapause triggered by photoperiod.

2. *Mastrus ridens*

2.1. Life history traits

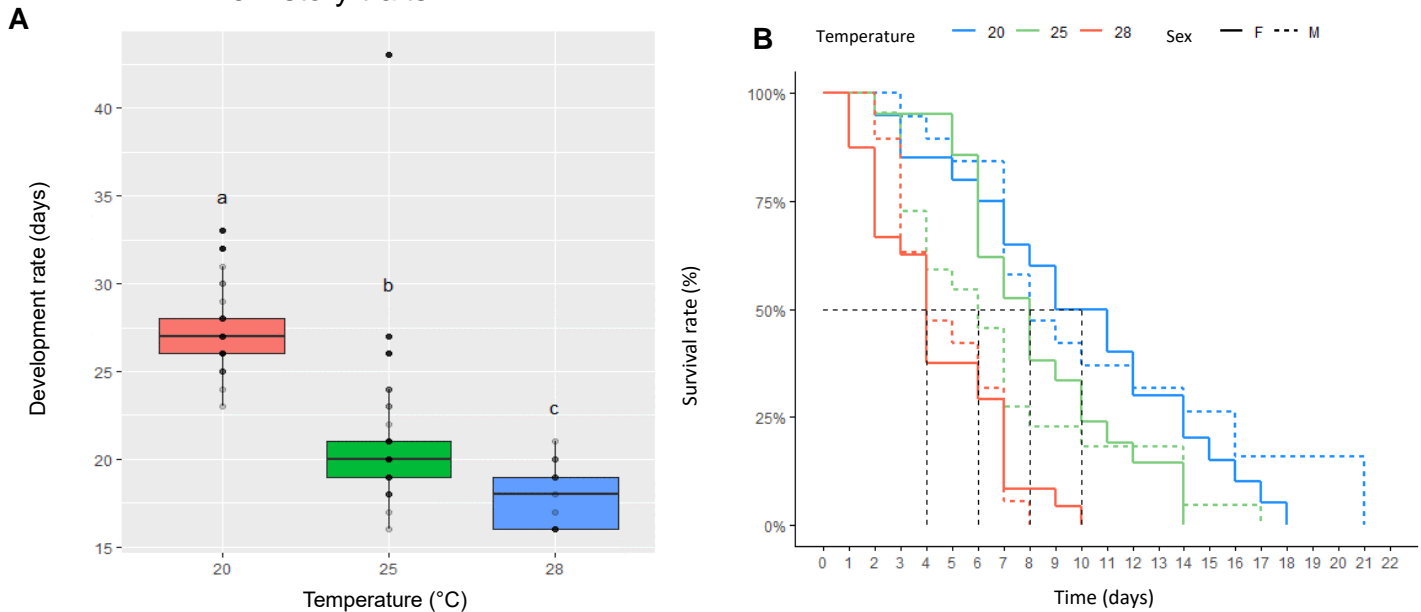


Fig. 26: Effect of temperatures on (A) the development time “from egg hatching to adult emergence” (Dunn, $\alpha=0.05$) and (B) longevity “from adult emergence to death” (Tukey, $\alpha=0.05$) of *Mastrus ridens*. Different letters indicate significant differences between treatments

Temperature had a significant impact on the development time ($\chi^2 = 70.448$, $df = 3$, $p < 0.001$) (Fig. 26A) and adults longevity ($F = 40.776$, $df = 1$, $p < 0.001$) (Fig. 26B) of *M. ridens*. The higher the temperature the shorter the development time and longevity were (mean value for longevity: 20°C: 10.3 days; 25°C: 7.6 days of life span; 28°C: 4.6 days of life span).

Temperature had no significant influence on the progeny number of *M. ridens*, ($\chi^2 = 3.84$, $df = 2$, $p = 0.1$). The mean number of offspring per cocoon proposed to each female was $0.26 (\pm 0.08)$ at 20°C, $0.70 (\pm 0.28)$ at 25°C and $0.51 (\pm 0.13)$ at 28°C. The mean total number of offspring per female was $6.55 (\pm 3.9)$ at 20°C, $6.95 (\pm 7.9)$ at 25°C and $7.06 (\pm 7.2)$ at 28°C. Finally mean number of offspring per cocoon parasitized was $2.98 (\pm 1.2)$ at 20°C, $3.27 (\pm 1.7)$ at 25°C and $3.59 (\pm 1.8)$ at 28°C.

2.2. Parasitism rate

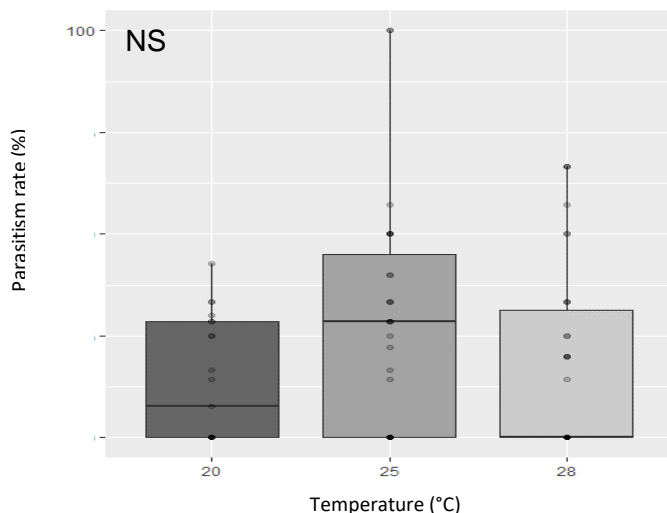


Fig. 27 : Effect of temperatures on the parasitism rate of *Cydia pomonella* cocoons by *Mastrus ridens*. There was no significant difference between temperatures.

There was no significant influence of the temperature on the parasitism rate of *M. ridens* females ($\chi^2 = 4.65$, $df = 2$, $p = 0.1$ (Fig. 27). The mean parasitism rate was 13.6% at 20°C, 28.4% at 25°C and 17.69% at 28°C.

3. *Forficula auricularia*

3.1. Life history traits

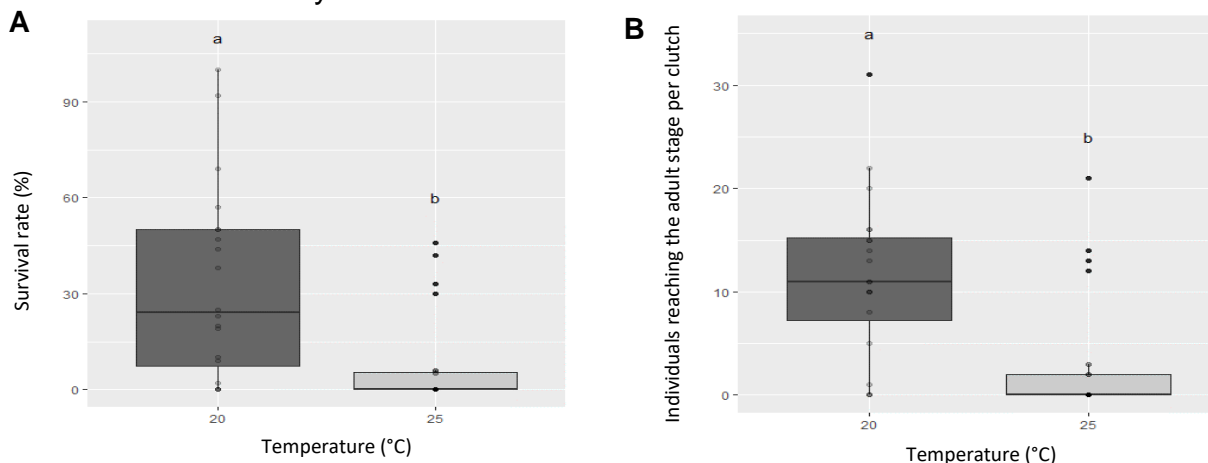


Fig. 28: Effect of temperatures on (A) the survival rate (Wilcoxon, $\alpha=0.05$) and (B) progeny number (Wilcoxon, $\alpha=0.05$) of *Forficula auricularia*, from egg hatching to adult stage. Different letters indicate significant differences between treatments

Temperature had no significant impact on development time of *F. auricularia* between 20°C (average of 83.00 ± 4.57 days from egg to adult, $n = 17$) and 25°C (average of 75.44 ± 5.52 days from egg to adult, $n = 9$) ($W = 107$, $p = 0.1$). Temperature had

however a strong significant influence on larval survival rate for this species ($W = 432$, $p < 0.001$) (Fig. 28). No larva was able to hatch at 28°C and 30°C while it was lower at 25°C (6.6% on average) than at 20°C (32.7% on average). The number of individuals reaching adult stage was consequently significantly lower at 25°C (2.8 individuals on average) than 20°C (11.4 individuals on average) ($W = 421.5$, $p < 0.001$).

3.2. Predation rate

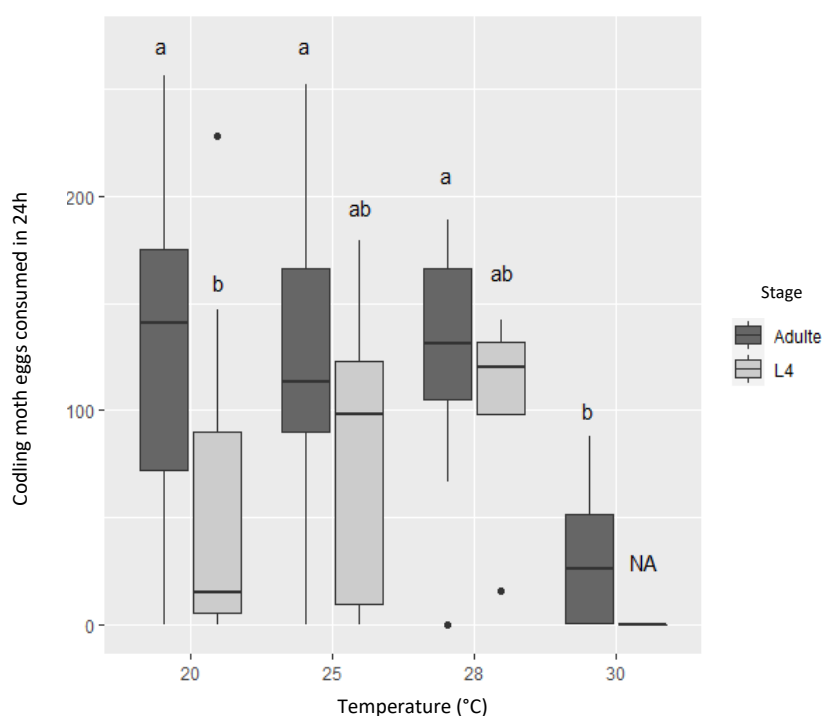


Fig. 29: Effect of temperatures on the predation rate of *Cydia pomonella* eggs over 24 h by the 4th nymphal instars ($n = 17, 17, 5$ & 0 respectively for 20°C, 25°C, 28°C & 30°C and adults ($n = 22, 25, 21$ & 26 respectively for 20°C, 25°C, 28°C & 30°C) of *Forficula auricularia*. Different letters indicate significant differences between treatments (Tukey, $\alpha=0.05$).

Adult individuals of *F. auricularia* consumed significantly less *C. pomonella* eggs at 30°C than at the three lower temperatures ($F = 20.20$, $df = 3$, $p < 0.001$) while there were no significant differences between other temperatures (Fig. 29). At 20°C, the 4th instar nymphs of *F. auricularia* consumed significantly less *C. pomonella* eggs than adults ($F = 20.17$, $df = 1$, $p < 0.001$) while there were no significant differences between instars at other temperatures. Temperature did not influence significantly egg consumption by nymphs ($F = 0.77$, $df = 3$, $p = 0.5$).

Discussion

Temperature influences life history traits and behaviour of insects (Abram *et al.* 2017). The present research explored the influence of temperature on development time in the pest *Cydia pomonella* and two natural enemies, *Mastrus ridens* and *Forficula auricularia*, as well as the regulation potential of these two last species.

Life history traits. As observed in most species (Sinclair *et al.* 2016), the thermal curve for development rate was bell-shaped in the codling moth. A Lactin-2 model applied to our data indicated that optimal temperature for development would be 32.3°C for our strain. A close but smaller value was reported for a strain sampled in East Azerbaijan that had an optimal developmental temperature of 30°C but was not able to develop at 35°C (Aghdam *et al.* 2009), in contrary to our strain. Lactin-2 and Hollin III models both indicated that the critical upper limit for our *C. pomonella* strain would be 42°C, a mean-temperature that occurred only rarely in the vicinity of Avignon but more frequently during the last years (MeteoFrance). Such intraspecific differences in thermal sensitivity are quite common between insect populations (Moiroux *et al.* 2010) and may explain that we calculated a higher optimal and critical temperature. These results indicate that *C. pomonella* would thus benefit from global warming as long as mean temperature does not exceed 30 to 32°C. However it could vary among strains. This would result in an increased number of generations in South-Eastern France, as predicted in more temperate areas such as Switzerland (Stoeckli *et al.* 2012) or Poland (Juszczak *et al.* 2013). Such projections were confirmed in our +2°C and +4°C scenario that indicated that a fourth egg laying may occur in southern France with global warming. Orchards would however suffer additional damages only if temperature increases by 4°C since eggs of this fourth generation were not able to hatch in a +2°C scenario. This may indicate that under global warming or under specific, local and microclimatic conditions (e.g. the face of an apple in direct sunlight, Kührt *et al.* 2006) the number of generations per year may increase substantially.

The thermal curve of *Mastrus ridens* developmental rate seemed to be bell-shaped but temperatures above 28°C should be tested to get a better understanding of thermal constraints on development in this species. Devotto *et al.* (2010) indicated that the optimum temperature for this species would be in a large range between 25°C and 35°C, a hypothesis supported by our results at 28°C. Testing at least one temperature above 28°C would allow us to accurately fit development models and calculate precise

optimal and critical upper limit for this species, a step necessary to predict the influence of global warming on the voltinism of this species. We did not observe any influence of temperature on the number of progeny produced by females, suggesting that global warming may influence population dynamics of this species through changes in the number of generations but not in reproduction. Devotto *et al.* (2010) however reported that *M. ridens* does not produce offspring at 35°C and survives only 5 days at this temperature.

We were not able to get a bell-shaped curve for *Forficula auricularia* developmental rate because no individual developed above 25°C. We thus measured development time only at two temperatures. *F. auricularia* eggs appear to be particularly sensitive to high temperature, which greatly affected survival rate and the number of individuals reaching the adulthood per clutch. This result is in disagreement with previous studies on another sympatric earwig species, the ringlegged earwig *Euborellia annulipes* (Dermaptera: Anisolabididae), which had good egg and clutch survival rates to adulthood at 28°C and 30°C (Rankin *et al.* 1995; Lemos *et al.* 2003). Another study on *F. auricularia* indicated that eggs were able to encounter temperatures up to 36°C without damage (Chauvin *et al.* 1991). However, a recent study of *F. auricularia* life history traits, associated with seasonal temperature variation, showed that eggs from populations that experienced a warm fall suffered greater mortality rates during development (Tourneur & Meunier 2020). These variations could be explained by lower egg quality, less efficient maternal care or predation of eggs by the female to avoid excessive energy loss (Tourneur & Meunier 2020). In our study, females and their eggs were moved from a winter egg-laying temperature (~10°C) to 28°C and 30°C, a temperature variation that may be harmful for the eggs and their mothers. According to Dib *et al.* (2017), once individuals outgrow the second instar of nymphal development, survival is much greater. Here we observed increased mortality of eggs and early nymphal instars that could explain the low proportion of adults at 25°C. However, predation tests using 4th instar nymphs and adults showed that individuals can withstand temperatures of 28°C and 30°C for at least 48h, confirming the hypothesis that eggs and young nymphs are the most fragile stages. As female carries out her first oviposition in winter, underground, the eggs and the first two nymphal instars are then sheltered under the ground and only emerge to the surface in spring. Furthermore, according to the literature (Dib *et al.* 2017), only the last two nymphal

stages and the adults will be present above ground, disperse into the trees (arboreal phase) and will be effective for codling moth control. A temperature of 30°C seemed then appropriate for testing their predation potential in agreement with the findings of Dib *et al.* (2011).

Biological control. The *F. auricularia* consumption rate of *C. pomonella* egg was significantly lower for 4th instar nymphs than for adults at 20°C. The number of nymphs was not sufficient to test their predation potential at 28°C and 30°C. For adults, the consumption of eggs decreased significantly at 30°C, indicating less predation potential at this temperature. It is known that 3rd and 4th instar nymphs of *F. auricularia* have good regulatory potential at 25°C (Dib *et al.* 2011). Many studies on Coleoptera or Hemiptera species indicated that predation rates increases with temperature, even above 30°C (McCaffrey & Horsburgh, 1986; Geden & Axtell, 1988; Grafius & Warner, 1989). In earwigs, a study on *E. annulipes* reported a change in predation behaviour at 32°C, with a shift from an increased predation decelerating to a maximum, to a sigmoidal increased predation to a maximum, yet corresponding to the maximum predation rate among all tested temperatures (Nunes *et al.* 2020). Another study on the striped earwig, *Labidura riparia* (Dermaptera: Labiduridae), indicated that predation rates increased from 25°C to 30°C and decreased at 35°C (Kharboutli & Mack 1993), while our study suggests that the thermal optimum is exceeded at 30°C for *F. auricularia*. However, Kharboutli & Mack (1993) suggested that the feeding rate decreased at 35°C due to a decrease in hunting time to avoid these climatic conditions. In our study, since *F. auricularia* is a nocturnal species, it will rather hunt at dusk when temperatures, even in summer, are milder (Vancassel 1973). Naturally present in abundance in apple orchards, it will be able to guarantee an efficient regulation of the eggs of the first annual generation of codling moth.

The parasitism potential of *M. ridens* was the same in our study regardless of temperature. Our parasitism rates are consistent with several other reports which found ranging from 17% to 28% average value (Bezemer & Mills 2003; Bueno *et al.* 2017). However, in the study of Bezemer & Mills 2003, 8 cocoons were proposed to each female every day. This seems to indicate that whether one or several cocoons are proposed to one female, the parasitism rate remains similar. Other studies investigating the effect of temperature on parasitism rates indicated that temperatures between 20°C and 30°C do not alter parasitism rates. For example, the number of

lepidopteran eggs parasitized by *Telenomus cyamophylax* (Hymenoptera: Scelionidae) remains unchanged at 20°C, 25°C and 30°C (Foerster & Butnariu 2004). Similarly, in *Hyssopus pallidus* (Hymenoptera: Eulophidae), an ectoparasitoid of the genus *Cydia*, temperatures between 20°C and 30°C had no influence on the parasitism rate (Häckermann *et al.* 2008). However, this depends greatly on the parasitoid species and their temperature optimum. For example, in *Microplitis similis* (Hymenoptera: Braconidae), a parasitoid of *Spodoptera litura* (Lepidoptera: Noctuidae), the best rates of larvae parasitism are reported between 27°C and 33°C (Yi *et al.* 2020). Here, as shown by Devotto *et al.* 2010, *M. ridens* ceases to reproduce at 35°C indicating a thermal optimum different from *M. similis* but more similar to *H. pallidus* and *T. cyamophylax*. We can assume that if a daily average of 35°C is not reached, *M. ridens* should be promising candidate for controlling *C. pomonella* in biological control programmes by acclimation, during all its period of presence, with a maximum of effectiveness at the 3rd generation, period when *C. pomonella* larvae enter in winter diapause (Sandanayaka *et al.* 2017).

The two natural enemy species considered in this study could be particularly interesting against the first generation of codling moths and on the diapausing larvae of the previous generation. Indeed, *F. auricularia* targets eggs and is present as early as spring in orchards (Dib *et al.* 2017), the 4th instar nymphs have shown their efficiency to consume eggs at 20 and 25°C which should not be exceeded in April. Similarly, adults should be effective up to 28°C, allowing nocturnal control of eggs during more periods of the codling moth activity. For *M. ridens*, if individuals can protect themselves from the high summer heat, and have sufficient food and water, temperature does not affect their behaviour. It is assumed that this species will effectively regulate *C. pomonella* since the species has been successfully introduced into Argentina and California, regions known to have hot and dry summers. However, a limitation to our study lies in the constant temperature regime tested. While *M. ridens* is not able to produce viable offspring at 35°C (Devotto *et al.* 2010) and *F. auricularia* loses its regulatory potential at 30°C, these measurements were made at a constant temperature regime. Even with global warming, maximum summer temperatures are not expected to rise 30°C mean over a 24-hour period. Furthermore, Tourneur & Meunier (2020) suggested that “adaptation of an insect with a relatively long-life cycle does not necessarily operate in response to the overall mean temperatures of the

invaded environments, but in response to their seasonality and/or mean temperature at a specific time of their life cycle". It would then be interesting to test the regulation potential of these two species at high temperatures but with a fluctuating regime, more reflective of the field reality.

It seems thus unlikely, under the conditions of our study, that a phenological mismatch would occur between the two studied natural enemies and *C. pomonella* at the first generation given their period of activity in orchards. However, for the second generation of codling moths in the peak of summer, this may depend on average daily temperatures even if the regulatory potential of *M. ridens* and *F. auricularia* remains relatively stable as temperature increases. According to the results of our study at constant thermal regime, these two natural enemies would present a good potential of regulation of *C. pomonella* in a context of global warming.

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En bref :

Ce chapitre est consacré à l'efficacité de la confusion sexuelle dans un contexte de réchauffement climatique. Il s'intéresse à l'effet de la température sur la perception par *Cydia pomonella* des composés phéromonaux présents dans les formules commerciales utilisées pour induire une confusion sexuelle chez ce ravageur.

Les effets de la température de développement et d'expérimentation sur la détection de molécules volatiles appliquées seules ou en mélange par les récepteurs olfactifs antennaires de *C. pomonella* ont été déterminés. Les résultats, préliminaires à ce stade, donnent des pistes sur la capacité de *C. pomonella* à détecter les odeurs dans un contexte de réchauffement climatique.

L'hypothèse principale de ce chapitre est que l'augmentation des températures pourrait engendrer une réponse électrique antennaire plus forte chez les mâles de *C. pomonella* aux composés phéromonaux, qu'ils soient seuls ou en mélange.

L'ensemble des résultats de ce chapitre seront complétés par de nouvelles expérimentations en 2022 avant d'envisager une soumission pour une publication scientifique. Les expériences complémentaires prévues sont abordées dans la discussion générale.

High developmental temperature decreases antennae electric response to pheromone components in the codling moth *Cydia pomonella*: potential consequences of climate change on mating disruption in a major orchard pest.

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Résumé

La confusion sexuelle est une technique de gestion économiquement viable utilisée dans le monde entier pour lutter contre *Cydia pomonella*. Cette stratégie peut cependant être influencée par le réchauffement climatique puisque la température est connue pour influencer les réponses physiologiques ou comportementales médiées par les phéromones chez plusieurs espèces de ravageurs. Cette étude avait pour objectif de suivre l'influence de la température de développement et de la température de stimulation sur les réponses électriques globales des antennes (EAG) de carpocapses mâles en réponse à différents composés phéromonaux appliqués seuls ou en mélange. Nous avons observé une diminution significative de la réponse EAG à la codlemone, au (E)-9-dodecan-1-ol, au tetradécan-1-ol et aux composés phéromonaux en mélange avec la codlemone lorsque les carpocapses mâles s'étaient développés à 35°C par rapport à ceux qui s'étaient développés à 20°C. La température pendant la stimulation olfactive n'a cependant pas influencé de manière significative la réponse EAG aux composants de la phéromone, mais l'interaction entre la température de développement et la température de stimulation a eu une influence. Nos résultats indiquent donc que *C. pomonella* pourrait être moins sensible aux composés synthétiques utilisés en confusion sexuelle dans un contexte de réchauffement climatique. Ces résultats, qui devraient être confirmés en augmentant le nombre d'individus testés et en utilisant une analyse mono-sensillaire, remettent en question l'efficacité préventive de la confusion sexuelle ou du contrôle par phéromones du carpocapse des pommes dans les conditions climatiques futures. Ils impliquent également que les mâles pourraient être moins sensibles aux phéromones sexuelles naturelles et par conséquent avoir plus de difficultés à trouver des partenaires.

Mots clés : lutte intégrée, température de développement, température de stimulation, Réponse EAG, codlémone.

Abstract

Mating disruption is a commercially viable management technique used worldwide to control *Cydia pomonella*. This strategy may be influenced by global warming since temperature is known to be involved in pheromone-mediated physiological or behavioral responses in several pest species. This study aimed to monitor the influence of developmental temperature and ambient temperature at the time of perception on the antennae electric global responses of codling moth males to different pheromone components applied alone or in mixture. We observed a significant decrease in EAG response to codlemone, tetradecane-1-ol and pheromone components in mixture with codlemone when codling moth males developed at 35°C compared to 20°C. Temperature during odor stimulation did not influence significantly EAG response to pheromone components however, but the interaction between developmental and ambient temperature did. Our results thus indicate that *C. pomonella* may be less sensitive to synthetic compounds used in mating disruption in a warming world. These results, which should be confirmed by increasing the number of individuals tested and using a single-sensor analysis, question the efficiency of mating disruption prevention or control by pheromones of the codling moth in a warming world. They also imply that males may be less responsive to natural sex pheromones and consequently face more difficulty to locate mates.

Key words: Integrated pest management, developmental temperature, stimulation temperature, EAG response, codlemone.

Introduction

In many insect species, males rely on airborne trails of specific sex pheromones emitted by females to locate sexual partners (Boullis *et al.* 2016). Such communication has been exploited for several decades to develop an efficient pest management strategy, *i.e.* mating disruption (Wright 1965). This strategy relies on the release of a synthetic pheromone in the environment to mask the natural pheromone, disrupt mate localization and/or courtship, and ultimately block the reproductive cycle of several pest species, mostly Lepidoptera. Compared to most pesticides, mating disruption has the advantage of only affecting the target species due to the specificity of the sex pheromones (Witzgall *et al.* 2008). It is thus commonly used in Organic Farming and Integrated Pest Management programs that aim to reduce environmental impact of farming. Although this strategy is most effective when managing low to moderate pest population densities (Louis & Schirra 2001) and commonly associated to other pest control methods such as insecticides, it was successfully used to control several species, such as the Gypsy moth *Lymantria dispar* (Thorpe 2006) or the boll weevil *Pectinophora gossypiella* (Tewari *et al.* 2014).

Several studies suggested that the efficiency of pheromone-based pest control may decrease with global warming because of decreased pheromone dispensers' durability. Higher temperatures indeed commonly result in increased release rate by dispensers and accelerated oxidation of pheromones (Bierl-Leonhardt *et al.* 1979; Leonhardt *et al.* 1990; Nation *et al.* 1993; Witzgall *et al.*, 2010; Atterholt *et al.* 1999). Dispensers may then need to be replaced more frequently during their deployment period in a warming world, as stated by Tobin *et al.* (2011). These authors deployed traps baited with disparlure, the synthetic form of the gypsy moth *Lymantria dispar* sex pheromone, across a broad geographic gradient in USA and determined that disparlure half-life time at some of the warmer locations sometimes did not exceed the length of time pheromone-baited traps were deployed in the field.

In addition to a shorter dispensers' durability, climate change may affect mating disruption through the influence of temperature on neural pathways and behaviors involved in pheromone-based mate location. Several authors reported that pheromone-mediated physiological or behavioral responses could be indeed triggered by high temperatures (Linn *et al.* 1986; Linn & Roelofs 1991) although such studies are scarce. First, rising temperature decreased the degree of specificity to odors in the

oriental fruit moth *Grapholita molesta* and in the pink bollworm moth *Pectinophora gossypiella*. Both species exhibited peak behavioral response to a broader range of blends and doses at the highest temperature in wind tunnel (Linn *et al.* 1988) and in the field (Linn & Roelofs 1991). Secondly, male antennae of *G. molesta* were able to track pulses of pheromone at high temperatures while lower temperatures resulted in loss of response (Baker *et al.* 1988). Finally, warm conditions resulted in higher antennae electric global responses to sex pheromone in the moth *Caloptilia fraxinella* (Lemmen & Evenden, 2015) while a decreased ability of receptor cells to resolve stimulus pulses at low temperatures was observed in *Antheraea polyphemus* by single sensilla stimulation (Kodadová 1996). All these results suggest that the efficiency of mating disruption may increase with global warming in Lepidopteran species because of increased response of males to odors, but decreased specificity, potentially allowing development of formulae affecting several species.

The present study focuses on the codling moth *Cydia pomonella* (L.), a major pest of apple orchards worldwide. Among biocontrol methods available, mating disruption has become a commercially viable management technique used on 160,000 ha worldwide to control this species. It is a sustainable and reliable strategy to control populations at low densities, and its effectiveness is promoted by long-term use and area-wide programs that limit immigration of mated females (Witzgal *et al.* 2008). This approach relies on the introduction in orchards of synthetic analogues of codling moth pheromone components, mainly codlemone, the major molecule involved in males' attraction by females (Roelofs *et al.* 1971). The efficiency and specificity of this control method resulted in several areas in decreased insecticide use and resistance, and increased biological control, thus reducing global costs for growers (Thomson *et al.* 2001).

The aim of this study was to monitor the influence of developmental and experimental temperature on the antennae electric global responses of codling moth males to different pheromone components. We hypothesized, according to previous studies on other Lepidopteran pests, that higher temperatures would result in a stronger antennae electric global response to pheromone components, applied alone or in mixture. These investigations might provide clues on the efficiency of mating disruption to manage *Cydia pomonella* in a context of global warming.

Material & Methods

Insects

Adult males of a *Cydia pomonella* strain (Sv) originating from Northern France and reared in laboratory at INRAE Avignon (France) since 1995 were used for this study. Codling moths were reared on artificial diet under 16L8D photoperiod at two different temperatures: $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ or $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$. After emergence, adult males and females were immediately separated and maintained in the same developmental conditions. All the males were provided with water and sugar solution until the test. Only active males were selected and used for the experiments.

Pheromone components

Three pheromone components were used: (i) codlemone (Purity >95%, Aldrich®), which is the major component of the codling moth pheromone, (ii) (E)-9-dodecan-1-ol (Purity >98%, Aldrich®), which is the second-most abundant compound in female glands (Witzgall *et al.* 2008), and (iii) tetradecan-1-ol (Purity >97%, Aldrich®), which is another minor pheromonal component in codling moth females. They were chosen as they are components of commercial formulation sold to farmers for codling moth mating disruption. Each component was diluted in hexane to get a 0.1 to 1000ng/μl solution or was mixed up with other components and diluted in hexane to obtain 3 solutions (Table 11). A piece of filter paper (6 × 30mm) impregnated with 1μL of tested solution was inserted into a glass Pasteur pipette after the solvent evaporated and used as a stimulus cartridge. Cartridges were made on the day of the experiment and kept in hermetic glass tubes between stimulations. A cartridge with 1μL of hexane was used as a non-stimulation reference.

Table 11: List of pheromonal compound mixtures used to stimulate codling moth antenna

	Mixture 1	Mixture 2	Mixture 3
codlemone	60.9%	60.9%	60.9%
(E)-9-dodecan-1-ol	31.7%		31.7%
tetradecan-1-ol		7.4%	7.4%

Recording and comparison of EAG profiles across individuals

We investigated electrical antennal global responses of *C. pomonella* males developed or stimulated at different temperatures to several pheromonal components, applied alone or in mixture. For odor stimulation, a main airflow (70L/h) was blown towards the individual through a 3cm diameter T-glass tube containing a conductive metal resistance inside followed by a 1.5cm diameter glass tube containing a small outlet allowing to accommodate a Pasteur pipette for odor release (Fig. 30). The conductive metal resistance allowed to heat the air flow arriving on the individual (Fig. 30). An electric current of 0.8 amps was sent to heat the resistance and thus the air blown to the individual at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$. On the contrary, the air flow was not heated for the modality à $20^{\circ}\text{C} (\pm 3^{\circ}\text{C})$ corresponding to room temperature.

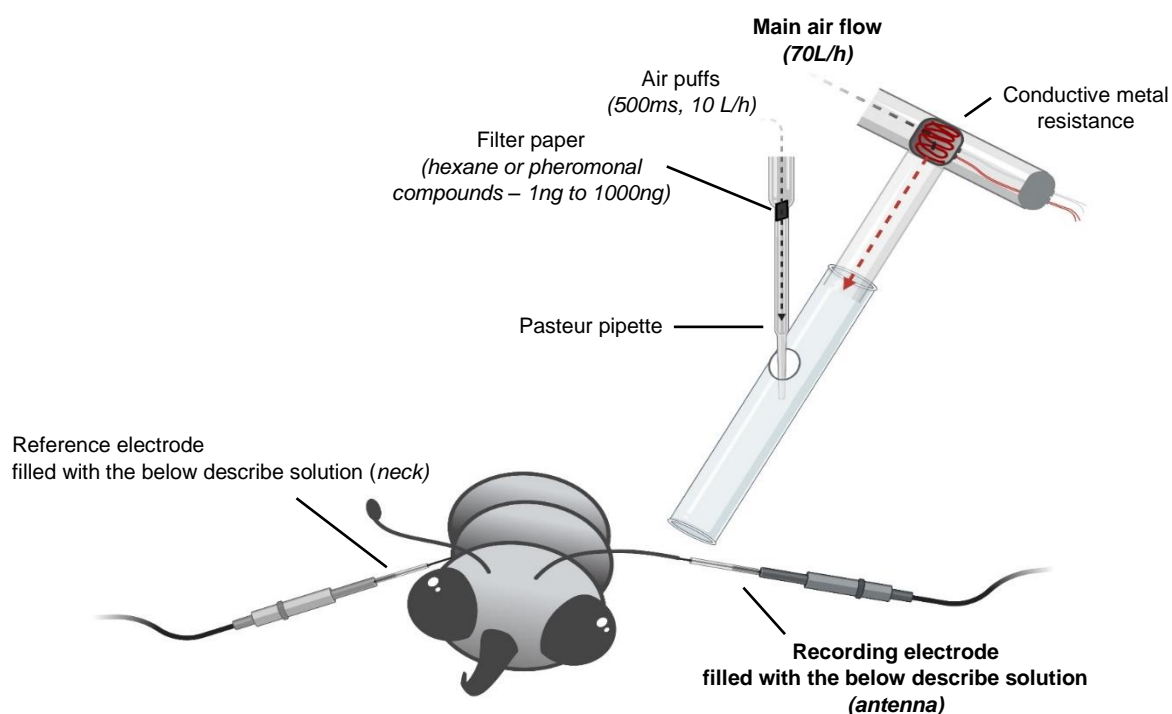


Fig. 30: Illustration of the recording EAG dispositive.

Once the temperature was reached, the insect's body was restrained on a block of Styrofoam excavated according to the dimensions of the insect's body so as not to damage it using small strips of tape (1mm thick). The antennae were carefully removed from the restraining block to allow recording. Electrodes were made from glass capillaries (non-heparinized 75 μ L hematocrit capillaries, Hirschman, Eberstadt, Germany), pulled on a horizontal puller (Narishige PC-10) and filled with a solution containing (in mM): 172 KCl, 22.5 glucose, 10 HEPES, 3 MgCl₂, 1 CaCl₂, 25 NaCl, pH 6.5. The recording electrode was slipped over the cut end of one antenna and the

reference electrode was inserted in the neck using micromanipulators. The antenna was positioned in the middle of humidified, main air stream for stimulation. The entire insect was exposed to a constant charcoal-filtered and humidified air flow (70L/h) through a glass tube (200mm long, 70mm internal diameter). The EAG signal was amplified (x100), low-pass filtered (5kHz) and sampled at 1kHz with a Digidata 1550 acquisition board (Molecular devices, San Jose, CA, USA) under pClamp 10 (Molecular devices). Odor stimuli were applied by puffs (10L/h) in the main air flow with the previously described temperate air stimulation system, blowing onto the antennae with the outlet of the glass tube positioned ~3cm from the antennae. The tip of a Pasteur pipette odor cartridge was inserted into the small outlet on the main airflow tube (Fig. 30) 150mm upstream of its distal end. Odor stimuli were obtained by activating an electrovalve (LHDA1233215H, The Lee Company, Westbrook, ME, USA) that delivered air puffs (500ms, 10L/h) through the pipette. Each stimulation started and ended with hexane. Each pheromone component was tested alone on each individual at all concentrations (1ng to 1000ng for the 3 compounds alone) as well as the three mixtures at 1000ng, in random order. A delay of 30 seconds between each stimulation was observed, except for the highest dose of codlemone and the components in mixtures where the delay was 1 minute between each stimulation. 6 individuals developed at 20°C were tested at 20°C and 6 others were tested at 35°C. Similarly, 6 individuals developed at 35°C were tested at 20°C and 6 others were tested at 35°C. The order in which the individuals were tested and the temperature at which they were tested were also randomized.

Statistical analysis

To analyse results obtained from the Clampfit 11.1 software, EAG response to the hexane control was deducted from the EAG amplitudes elicited by the test odor stimuli. Normality of EAG responses was confirmed using a Shapiro and Wilk test, for each thermal treatment. EAG responses to the different components and mixtures were then analyzed using analysis of variance (ANOVA) followed by a Tukey–HSD multiple comparison test, with dose, developmental and experimental temperature as explanatory variables. All statistical analyses were performed using R software.

Results

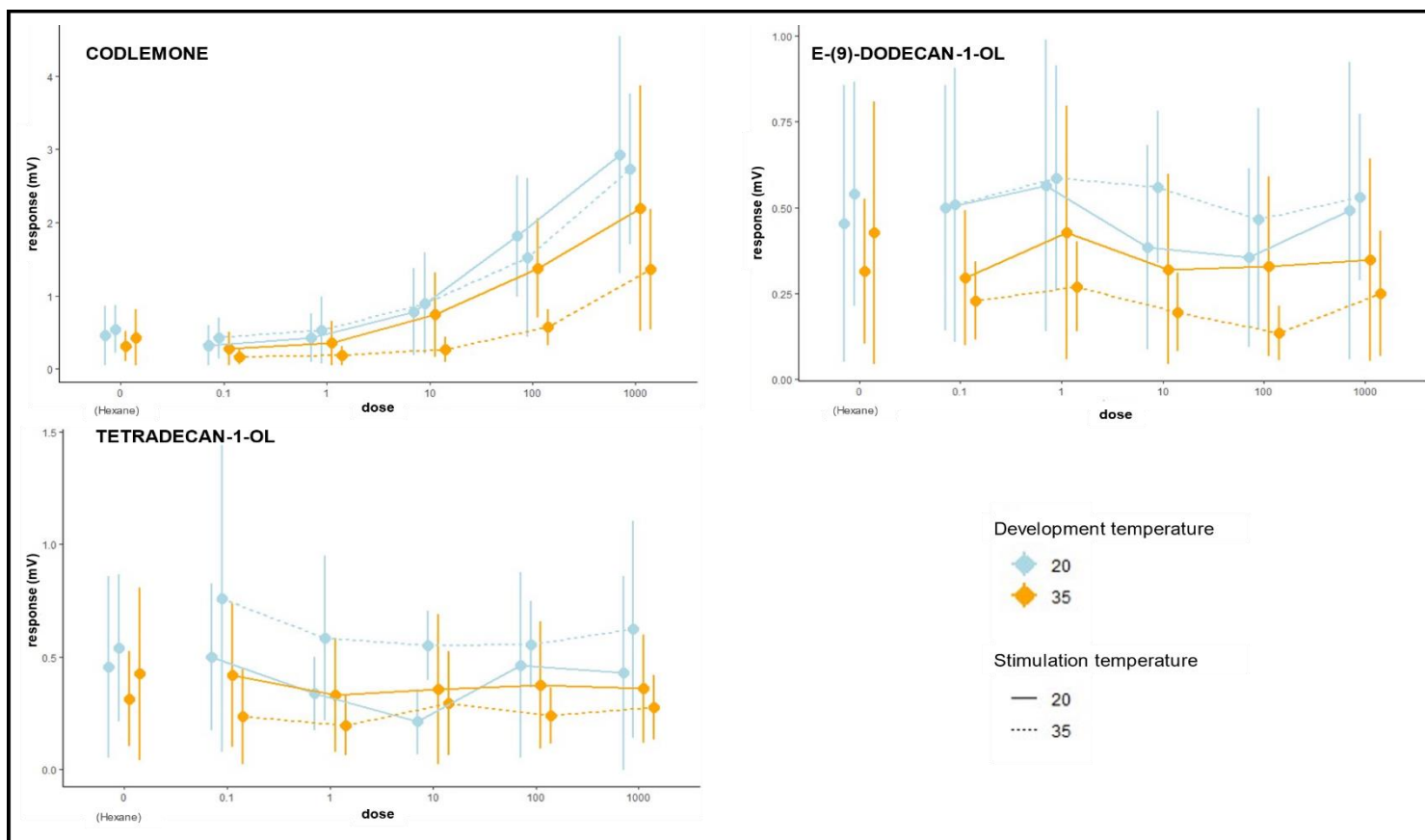


Fig. 31: Effects of stimulation temperature and component doses (0.1, 1, 10, 100 and 1000ng) on *C. pomonella* antennae EAG response (mVolt) whether they developed at 20°C or 35°C to different single compounds. Dose 0 corresponds to the hexane control. Significant differences between development temperatures for the three component, significant differences for interaction of both treatments for codlemone and tetradecan-1-ol.

Intensity of EAG response by *C. pomonella* increased significantly with the dose applied for codlemone ($F = 136.8$, $p < 0.005$) but not for the two other components (Fig. 31). Moreover, EAG response was lower when males developed at 35°C compared to 20°C for codlemone ($F = 13.25$, $p < 0.005$), (E)-9-dodecan-1-ol ($F = 17.45$, $p < 0.005$), and tetradecan-1-ol ($F = 12.98$, $p < 0.005$), independently of experimental temperature or dose applied. Experimental temperature did not influence significantly EAG response for the three components but we observed a significant influence of the interaction between developmental and experimental temperature for codlemone ($F = 7.11$, $p = 0.008$) and tetradecan-1-ol ($F = 6.16$, $p = 0.014$). For both components, EAG response was the lowest when individuals developed and were tested at 35°C while it was the highest when individuals developed at 20°C and were tested at 20 or 35°C, and was intermediary when individuals developed at 35°C and were tested at 20°C.

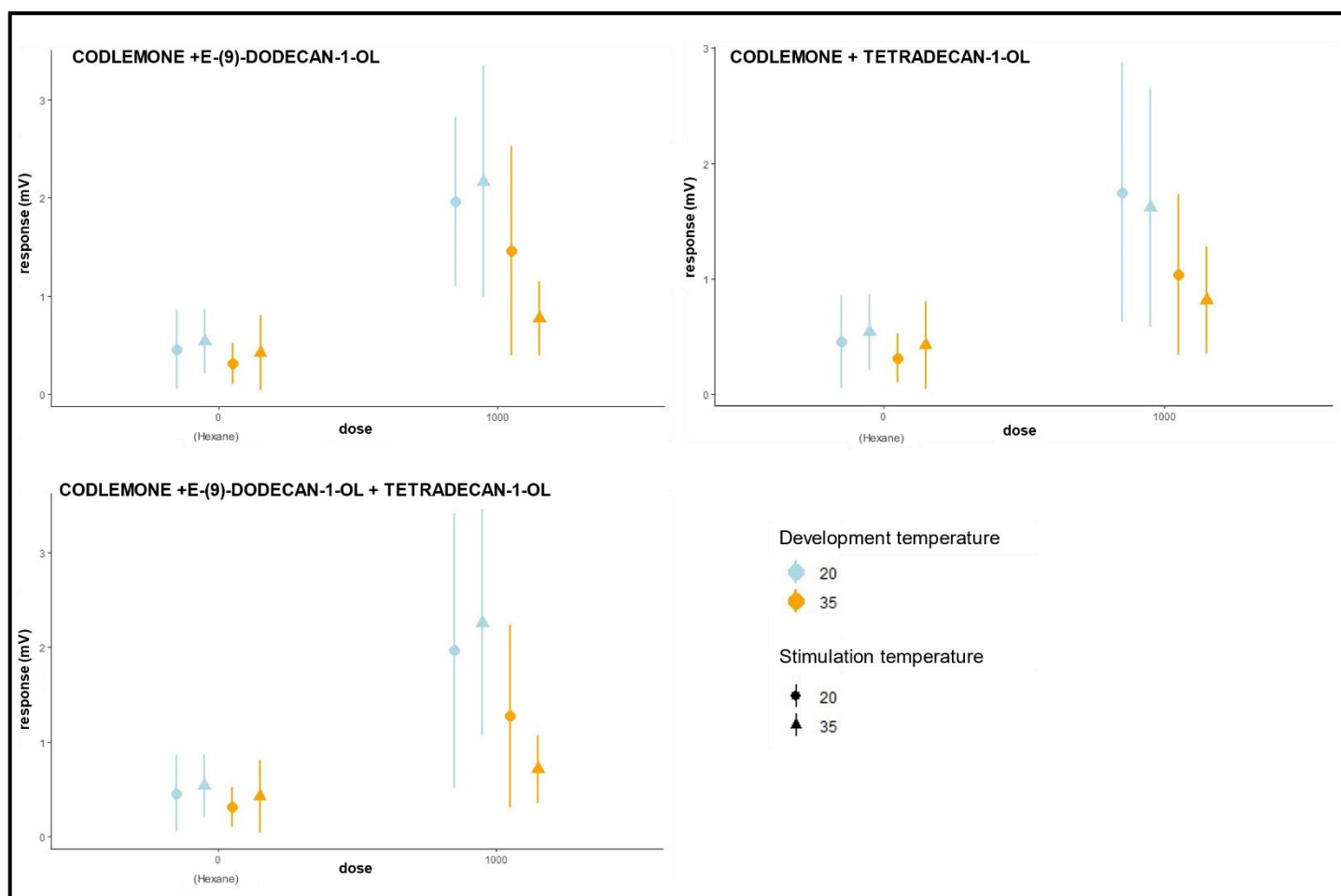


Fig. 32 : Effects of stimulation temperature on *C. pomonella antennae* EAG response (mVolt) whether they developed at 20°C or 35°C to compounds in mixture at one single dose (1000 ng). Dose 0 corresponds to the hexane control. Significant differences between development temperatures for the three mixtures.

EAG response was also significantly stronger when individuals developed at 20°C compared to 35°C for the 3 tested mixtures: mixture 1 (codlemone/(E)-9-dodecan-1-ol); $F = 4.37$, $p = 0.04$, mixture 2 (codlemone/tetradecan-1-ol mixture); $F = 4.27$, $p = 0.043$, and mixture 3 (the three components); $F = 6.07$, $p = 0.028$ (Fig. 32). Stimulation temperature or the interaction between developmental and stimulation temperature did not influence significantly EAG response for any mixture.

Moreover, we observed that EAG response was significantly stronger when codlemone was applied alone at 1000ng/μl than when it was applied in mixture with tetradecan-1-ol mixture ($F = 4.41$, $p = 0.02$) while there was no significant difference when codlemone was applied alone or in mixture with (E)-9-dodecan-1-ol or with the two other components.

Discussion

Temperature is known to influence the production and composition of pheromones in insects (Ono 1993; Ono 1994; Roeser-Mueller *et al.* 2010; Blomquist *et al.* 2012; Bontonou *et al.* 2013). Few studies however investigated the impact of temperature on their perception. Such topic is especially relevant to understand the potential influence of climate change on the efficiency of mating disruption to control pest species. In this study, increased developmental temperature resulted in a significant decrease in EAG response to codlemone, (E)-9-dodecan-1-ol, tetradecane-1-ol and components in mixture in 35°C developed and stimulated males. Moreover, the interaction between developmental and experimental temperature influenced EAG response to odors while experimental temperature alone did not. Our results thus indicate that *C. pomonella* may be less sensitive to synthetic compounds used in mating disruption in a warming world.

Our results are not consistent with previous studies that reported a positive influence of increasing temperature during stimulation on pheromone-mediated physiological and behavioral responses in Lepidoptera pest species. In *Grapholita molesta*, male antennae were able to follow pheromone pulses at high temperatures, whereas at low temperatures, males adapted to the odor, resulting in a loss of response (Baker *et al.* 1988). In the polyphemus moth *Antheraea polyphemus*, sensillae were able to process 5 stimulus pulses per second at 28°C against 2.5 at 18°C, probably increasing their chance of reaching the pheromone source (Kodadovà 1996). Such specific differences in thermal sensitivity of physiological processes are quite common in ectotherms. To better understand the impact of temperature during stimulation on neuronal responses to chemical stimuli in *C. pomonella*, it would be interesting to record the action potentials of a single sensilla in response to the perception of pheromone components. Monosensillar recording, a condition-independent technique compared to EAG response, would allow us to confirm that neural pathways were not influenced by temperature in codling moth males, as would observations of pheromone-based behaviors in wind tunnel or with an olfactometer.

High developmental temperature strongly decreased EAG responses to pheromone components in *C. pomonella*. This result is in opposition with observations reported in the only study that experimented influence of long-term exposure to different temperatures on EAG responses, to our knowledge. In *Caloptilia fraxinella*, individuals

experiencing a long-photoperiod in warm conditions showed a higher electric antennae response compared to individuals placed in long-photoperiod cold conditions (Lemmen & Evenden 2015). Such difference is probably explained by strong differences in the biology of the two species. *C. fraxinella* enters diapause as adult, an increase in temperature and photoperiod would then predispose males to emerge from reproductive diapause and increase their sensitivity to the sex pheromone. In *C. pomonella*, individuals do not go through diapause in the adult stage, so they do not need any particular signals to trigger reproduction other than the perception of the mating pheromones. The decrease in EAG response when developing at high developmental temperature is thus probably not adaptive in the codling moth. We may expect that development at 35°C, which is very close to the critical upper thermal limit for this species, may have affected structure of antennae, as observed in the parasitoid *Aphidius rhopalosiphi* that developed abnormal sensillae after heat exposure (Bourdais *et al.* 2006).

Conclusion

Based on our study, codling moth males were less responsive to odors when they developed at high temperatures while they were not influenced by temperature during stimulation. This original result led to numerous new research questions. In the next months, we will increase the number of individuals tested and use a single-sensor analysis to confirm or not these results. This preliminary experiment questions the efficiency of mating disruption of the codling moth in a warming world, although they could also imply that males may be less responsive to natural sex pheromones and consequently face more difficulty to locate mates.

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DISCUSSION GENERALE

Le changement climatique représente un enjeu de préoccupation majeur de notre siècle. Ils ont et auront un impact important sur la production alimentaire qui constitue un autre enjeu majeur du XXI^e siècle pour nourrir une population mondiale grandissante. En plus des impacts directs sur la production agricole, le changement climatique et particulièrement le réchauffement climatique pourrait être favorable à l'augmentation des populations de ravageurs des cultures, entraînant une diminution des rendements. Ces effets directs et indirects du climat sur la production alimentaire indiquent qu'il est important de prendre en compte ces changements en faisant évoluer nos méthodes de gestion des ravageurs.

Dans ce contexte, ce travail de recherche s'intéresse à un agroécosystème spécifique que sont les vergers de pommiers, qui sont des cultures installées de façon pérenne. La longévité de ces systèmes en fait un bon modèle d'étude des effets du réchauffement climatique. Ce travail s'intéresse particulièrement à un des principaux ravageurs de ces systèmes, le carpocapse des pommes *Cydia pomonella*, qui a étendu sa surface de distribution mondialement et cause de graves dégâts dans les vergers de pommiers notamment. En ce qui concerne le climat de référence choisi, les régions présentant un climat méditerranéen pourraient faire partie des régions les plus soumises aux changements climatiques d'après de nombreuses projections. Etant donné l'importance de la production des pommes dans la Région Provence Alpes Côte d'Azur, les enjeux que représente le réchauffement climatique y sont importants.

Ce travail de recherche avait donc pour but premier de définir quels seront les impacts potentiels du réchauffement climatique sur l'efficacité des principales méthodes de lutte contre le carpocapse des pommes *C. pomonella*, dans la Région PACA.

Changement climatique et méthodes de lutte contre *C. pomonella* en Région PACA : apports de la thèse

Effets du réchauffement climatique sur la lutte par insecticide de synthèse.

Dans un premier temps, les effets croisés température/insecticides sur la mortalité du carpocapse des pommes ont été étudiés (Chapitre II). Trois insecticides de synthèse souvent utilisés contre *C. pomonella* ont été choisis. Ces derniers sont connus pour être stables à températures élevées et ont des modes d'action différents : il s'agit de l'emamectine, du chlorantraniliprole, et du spinosad, homologué en agriculture biologique.

L'hypothèse principale était qu'une différence de sensibilité peut être induite par une augmentation de température chez *C. pomonella*. Nos résultats montrent que les deux stress (thermique et insecticide) ont bien eu un effet croisé sur la mortalité larvaire de *C. pomonella*. De plus, ils donnent une indication sur l'efficacité des insecticides testés à différentes températures. Cela confirme donc l'hypothèse principale. En effet, une augmentation de la température a diminué la toxicité de l'emamectine et du spinosad sur la lignée de laboratoire de *C. pomonella* utilisée, alors qu'elle n'a pas influencé la toxicité du chlorantraniliprole. Si ces résultats sont cohérents avec ceux reportés pour le spinosad chez une cochenille (Mansoor *et al.* 2014), ils sont différents du reste de la littérature pour l'emamectine et le chlorantraniliprole (*i.e.* leur toxicité augmente avec la température chez *Plutella xylostella*. Li *et al.* 2004 ; Teja *et al.* 2018). Ces différences peuvent s'expliquer premièrement par l'efficacité des voies biochimiques impliquées dans la détoxification des insecticides qui peuvent varier entre espèces et avec la température. Ensuite, les différences de procédures expérimentales peuvent induire des différences importantes dans les résultats d'études indépendantes. La plupart des expériences répertoriées dans la littérature se sont concentrées sur des effets transitoires de 48h de la température et des insecticides. Le présent travail s'intéresse quant à lui à un stress développemental permanent sur *C. pomonella* correspondant à ce qui pourrait se produire avec le changement climatique dans les agroécosystèmes.

Les premiers résultats montrant des variations de sensibilité de *C. pomonella* à des insecticides lorsque l'on fait varier la température, un intérêt particulier a été porté aux mécanismes qui expliqueraient ce phénomène. Pour cela l'hypothèse a été émise que

de plus fortes températures, de quelques degrés au dessus de la température optimale de développement (*i.e.* 35°C vs 32,3°C), pourraient augmenter l'activité d'enzymes de détoxification en activant l'expression de certains gènes codant ces enzymes, ou ceux codant des protéines de choc thermique ou encore des cibles moléculaires des insecticides. Les mécanismes biochimiques et moléculaires susceptibles d'être impactés par deux stress (thermique et insecticide) ont donc été étudiés. C'est ainsi qu'une activité plus forte des enzymes de détoxification et une surexpression de deux gènes de détoxification ont été observées lorsqu'un stress thermique seul était appliqué mais ce n'est pas le cas si un double stress (thermique et insecticide) est appliqué. Pour l'activité accrue des enzymes de détoxification, nous avons observé ces résultats pour les trois insecticides. Or pour le chlorantraniliprole, la sensibilité du carpocapse à cet insecticide ne varie pas avec la température malgré l'augmentation de l'activité des enzymes de détoxification. Cette différence avec les résultats sur l'emamectine et le spinosad peut s'expliquer par la non-implication de ces enzymes dans la tolérance au chlorantraniliprole rapportée récemment pour ce ravageur en Espagne (Bosch *et al.* 2018) mais pas chez d'autres espèces de Lépidoptères (Mao *et al.* 2019 ; Yin *et al.* 2021; Wang *et al.* 2018). Ces différences suggèrent que les réponses aux stress peuvent varier selon l'espèce d'insecte étudiée mais aussi selon les propriétés intrinsèques des substances insecticides et peuvent expliquer les différences observées entre les études. Les deux gènes de détoxification (*Cyp9A61* et *Gst1*) qui ont été surexprimés en réponse à un seul stress thermique mais pas à deux stress suggèrent une possible implication de leur part dans l'augmentation de la tolérance à l'emamectine et au spinosad à haute température. Ces gènes sont en effet communément associés à la détoxification chimique (Zimmer *et al.* 2018). Deux autres gènes, *Hsp70* et *Cyp6B2*, ont été surexprimés en présence des deux stress et pourraient être impliqués dans la tolérance à ces stress combinés chez *C. pomonella*. Certaines études ont également rapporté une expression à la hausse des HSP en réponse aux insecticides, entraînant une augmentation de la thermotolérance des espèces nuisibles (Patil *et al.* 1996 ; Ge *et al.* 2013 ; Su *et al.* 2018). Dans notre étude, l'exposition aux insecticides n'a pas influencé l'expression du gène *Hsp70* pour les individus qui se sont développés à température plus basse. Cependant, tirer une conclusion sur le rôle de l'expression du gène *Hsp70* dans la tolérance aux insecticides chez *C. pomonella* serait prématuré au vu de nos résultats. Pour le gène *Cyp6B2*, son expression est connue pour être liée à la détoxification de la deltaméthrine et de

l'aziphos méthyle chez *C. pomonella* (Wan *et al.* 2019). Sa régulation pourrait également avoir un effet important sur la détoxification de l'emamectine comme le suggère la surexpression maintenue avec le double stress. Quelques pistes peuvent être proposées pour expliquer l'absence de réponse biochimique à deux stress (thermique et insecticide) par rapport à un seul stress. La mobilisation des enzymes sur les substrats insecticides les rendrait moins accessibles pour les substrats proposés pour l'analyse puisque les insecticides et les enzymes peuvent entrer en compétition avec le substrat lorsque le site actif est le même pour les deux molécules. Ce phénomène augmente lorsque les enzymes font simplement de la séquestration moléculaire et restent liées à l'insecticide plus longtemps (Kostaropoulos *et al.* 2001 ; Goh *et al.* 1995 ; Despres *et al.* 2007). Nous pouvons également supposer que l'énergie peut être allouée préférentiellement à l'expression de gènes régulant des mécanismes plus ubiquitaires, comme les gènes codant les HSP, afin de protéger les individus contre des stress multiples (Sorensen *et al.* 2003). Le gène *Hsp70* était ainsi toujours surexprimé lorsque deux stress étaient appliqués, bien que cette surexpression fût moins prononcée lorsque les individus étaient confrontés à un double stress par rapport au simple stress thermique. Les individus confrontés à des stress multiples pourraient alors réduire l'expression coûteuse des gènes codant les HSP par rapport aux individus confrontés à un seul stress, pour permettre la mobilisation d'autres réponses biochimiques et moléculaires impliquées dans les réponses aux stress (Hoekstra & Montooth 2013 ; Liu *et al.* 2008).

Ces résultats sont les premiers indices pour comprendre comment la température affecte la sensibilité de *C. pomonella* aux insecticides. Ils illustrent également la complexité des réponses moléculaires et biochimiques mobilisées quand un individu est confronté à des stress multiples. Ils montrent enfin que la toxicité de deux des trois insecticides testés contre *C. pomonella* est bien dépendante de la température et par conséquent du réchauffement climatique. Cela donne une indication sur l'évolution de l'efficacité de ces insecticides de synthèse pour lutter contre *C. pomonella* dans le climat futur.

Effets du réchauffement climatique sur la lutte biologique par acclimatation ou conservation

Cette partie du manuscrit s'inscrit dans la volonté de réduire les impacts économiques, sanitaires et environnementaux des insecticides de synthèse par l'utilisation d'ennemis naturels de *C. pomonella* comme agents de lutte biologique.

Connaître les effets à la fois d'une augmentation des températures et des insecticides sur un ravageur et ses ennemis naturels est primordiale pour le succès d'une lutte biologique (Chapitre III). Les effets croisés de la température et de différentes substances insecticides (emamectine, chlorantraniliprole), dont une homologuée en agriculture biologique (spinosad), sur la mortalité de *C. pomonella* et de deux de ses ennemis naturels ont été déterminés. Ces résultats donnent des informations importantes quant à la possibilité de combiner l'utilisation d'insecticides et d'ennemis naturels pour contrôler *C. pomonella* en vergers dans un contexte de réchauffement climatique.

A la vue des différences de sensibilité de *C. pomonella* à deux des trois substances insecticides étudiées et compte tenu du peu de littérature existant sur ce sujet pour les ennemis naturels, l'hypothèse principale émise était que *Forficula auricularia* et *Mastrus ridens* devraient également présenter les différences de sensibilité aux insecticides. Les résultats de l'étude confirment cette hypothèse. Ils sont cependant opposés à ceux observés pour le carpocapse des pommes. Une température plus élevée augmente la toxicité des trois insecticides testés sur *Mastrus ridens* et de l'emamectine sur *Forficula auricularia*, par ingestion. Ces variations de sensibilité peuvent avoir plusieurs causes. Comme pour *C. pomonella* une température élevée peut influencer l'expression génétique et l'activité de protéines protectrices comme certaines enzymes de détoxification (Liu *et al.* 2017 ; Yan *et al.* 2013 ; Zhang *et al.* 2015) ou les protéines de choc thermique (Ge *et al.* 2013 ; Su *et al.* 2018). Le taux métabolique augmente avec la température (Brown *et al.* 2004), augmentant par conséquent la consommation de nourriture, donc d'insecticide, et la locomotion, donc le contact avec les insecticides (Gillooly 2001 ; Medrzycki *et al.* 2010), et la pénétration de la substance dans le corps de l'insecte (Boina *et al.* 2009). Par ingestion ou par contact tarsal, *M. ridens* était plus sensible aux insecticides que *F. auricularia*. La différence de taille et de cuticule entre ces espèces pourrait expliquer ces différences de sensibilité (Picanco *et al.* 1997 ; Bacci *et al.* 2007 ; Fernandes *et al.* 2010). Il est

également possible que les Hyménoptères parasitoïdes soient plus sensibles à certains insecticides que les prédateurs, comme le suggère les résultats d'une étude qui reprend l'impact du spinosad sur différentes espèces d'ennemis naturels. (Williams *et al.* 2003). Cela pourrait partiellement expliquer la différence de sensibilité aux insecticides de *M. ridens* et *F. auricularia*.

Ces résultats indiquent donc que les ravages causés par *C. pomonella* pourraient être plus importants à l'avenir en raison d'une diminution de l'efficacité des insecticides testés sur ce ravageur couplée à augmentation de leur toxicité sur ses ennemis naturels. Cette étude permet de formuler des recommandations sur la combinaison de méthodes de lutte contre le carpocapse dans un contexte de réchauffement du climat. Ces recommandations sont discutées dans le dernier paragraphe de ce chapitre.

Sans parler des effets directs des insecticides, l'efficacité des ennemis naturels dépend aussi de leur fitness et de leur synchronisme avec le ravageur cible. Il est donc naturel de s'intéresser ici aux effets directs d'une augmentation des températures sur la phénologie de *C. pomonella* ainsi que sur la phénologie, les traits d'histoire de vie et le comportement des deux ennemis naturels étudiés, *M. ridens* et *F. auricularia* (Chapitre IV). L'hypothèse formulée dans ce chapitre était que *C. pomonella* et ses ennemis naturels vont se développer plus rapidement mais n'ayant pas les mêmes optimums de température, le maintien du synchronisme de ces trois espèces sera perturbé. Cette hypothèse a été confirmée pour *C. pomonella* qui voit son temps de développement diminuer avec l'augmentation des températures jusqu'à une température optimale de 32,3°C, déterminée grâce au modèle Lactin-2 appliqué à nos données. Ce ravageur bénéficierait donc du réchauffement climatique tant que la température moyenne de développement ne dépasse pas cette valeur. Il en résulterait une augmentation du nombre de générations dans le sud-est de la France, comme cela est déjà le cas au Maroc avec l'apparition d'une 4^{ème} génération (El Iraqui & Hmimina 2016) ou comme cela a été prédit dans des régions où la température est plus tempérée comme la Suisse (Stoeckli *et al.* 2012) ou la Pologne (Juszczak *et al.* 2013). Ces estimations pourraient être causées par l'augmentation des températures au début du printemps qui pourraient accélérer la fin de la diapause et l'émergence des premières générations d'adultes, et donc augmenter le nombre de générations (El Iraqui & Hmimina 2016 ; Damos *et al.* 2018). En Grèce, des températures de juillet à début août élevées pourraient permettre un développement rapide des différentes

génération avant que la photopériode ne déclenche l'induction de la diapause prolongeant par conséquent les derniers vols. Sur nos données, les modèles Lactin-2 et Hollin III ont tous deux indiqués que *C. pomonella* ne serait pas en mesure de se développer au-dessus d'environ 42°C, une température qui a été dépassée à plusieurs reprises au cours des deux dernières années aux alentours d'Avignon (MétéoFrance). C'est la première fois qu'une si grande température maximale pour l'arrêt du développement de *C. pomonella* est enregistrée et intégrée à plusieurs modèles. La seule autre étude à notre connaissance à enregistrer des températures de développement complet viables supérieures à 36°C (36,5°C) se base sur le modèle Brière-1 qui semble être un des deux plus fiables testés dans cette étude avec le modèle Brière-2 (Aghdam *et al.* 2009). Les modèles phénologiques basés sur nos données et la littérature existante sur le sujet montrent que la Région PACA pourrait voir apparaître une 4^{ème} ponte mais sans larves de carpocapse des pommes dans les prochaines années avec le scénario +2°C, évitant une 4^{ème} génération. Par contre avec le scénario +4°C, cette 4^{ème} ponte évoluerait en larves, laissant craindre des dégâts. Pour le moment, les modélisations ne prévoient pas de 4^{ème} génération complète, celle-ci n'atteignant pas le stade diapausant. Il est probable avec ce dernier scénario que *C. pomonella* ne puisse pas dépasser la 4^{ème} génération dans les conditions climatiques futures, celle-ci étant déjà prédite à la limite de l'optimum thermique du carpocapse des pommes. Sur la base de ces données, nous pouvons émettre l'hypothèse que des températures dépassant celles du scénario +4°C pourraient être délétères à *C. pomonella*.

Notre hypothèse a également été confirmée pour *M. ridens* qui voit son temps de développement diminuer avec la température. Son optimum de température semble se situer entre 25°C et 28°C, en dessous des 32°C d'optimum de *C. pomonella*. La température n'a d'impact ni sur sa fertilité ni sur son taux de parasitisme. Ces résultats laissent penser que si les températures moyennes n'atteignent pas les 35°C, *M. ridens* reste un bon candidat pour contrôler le carpocapse des pommes dans le cadre de programmes de lutte biologique. Les résultats sont plus contrastés pour *F. auricularia*. Les conditions expérimentales de laboratoires testées n'ont pas permis un bon développement des œufs au-delà de 25°C limitant le nombre de températures évaluées sur le développement, la survie et la fertilité de cette espèce. Pour leur comportement de prédation, il est impacté à partir de 30°C, indiquant que si les

couvées se développent à des températures plus basses, les larves plus âgées, à partir du quatrième stade, sont tout à fait capables de se nourrir et de survivre à 30°C. Cette espèce, naturellement présente en abondance dans les vergers, est nocturne et les pontes ont lieu pendant l'hiver ou au printemps. Sa capacité de prédation, bien que réduite, sur les œufs de carpocapse des pommes à 30°C indique que cette espèce pourrait plus efficacement réguler la première génération de carpocapse débutant en mai que les suivantes.

Effets du réchauffement climatique sur la lutte par confusion sexuelle

Cette partie est constituée d'un travail de recherche plus exploratoire. Elle s'intéresse à l'effet des changements de température sur la perception des phéromones utilisées pour confusion sexuelle des carpocapses des pommes en vergers ([Chapitre V](#)).

L'objectif de ce chapitre était d'explorer la réponse électrique antennaire de *C. pomonella* à différentes températures lors d'une stimulation olfactive par plusieurs composés phéromonaux différents, seuls ou en mélange. Le sujet n'est que peu étudié mais d'autres études sur des modèles de Lépidoptère nous ont menés à poser l'hypothèse suivante : l'augmentation des températures engendrerait une meilleure perception des composés phéromonaux seuls ou en mélange par les mâles de *C. pomonella*.

Les résultats de l'étude infirment cette hypothèse. En effet, malgré la grande variabilité des résultats, une température plus élevée de développement diminue la réponse globale électrique des antennes des mâles de *C. pomonella* à la plus forte dose de la phéromone sexuelle principale du carpocapse des pommes (seule ou en mélange). Cette diminution de réponse pourrait être causée directement par une température de développement trop stressante pour les individus s'étant développés à 35°C. De plus, la température de développement a directement un impact sur la taille des individus adultes qui sont plus petits lorsqu'ils se sont développés à 35°C. Cette différence de taille en fonction de la température de développement pourrait également en partie expliquer les différences de réponse aux composés phéromonaux. Cependant, la température de stimulation n'a pas ou peu d'effet sur la réponse électrique globale des antennes. Ces résultats opposés à ceux obtenus sur *Caloptilia fraxinella* (Lemmen & Evenden 2015) pourraient venir des différences biologiques existantes entre ces

espèces. L'absence de différence de perception des composés phéromonaux minoritaires dans les différentes conditions expérimentales testées indique que le composé phéromonal qui induit une réponse des mâles est uniquement la codlemone. Les composés phéromonaux minoritaires testés ne semblent pas être détectés quelle que soit la température dans toutes les conditions. Pour confirmer les tendances obtenues, des manipulations supplémentaires en augmentant le nombre d'individus testés sont nécessaires. D'autres hypothèses pourraient être envisagées pour expliquer les différences de réponses en fonction de la température de développement, notamment au niveau des processus biochimiques et moléculaires neuronaux liés à la perception des odeurs. Des enzymes dégradant les odeurs peuvent métaboliser les molécules volatiles odorantes. Ce métabolisme est crucial pour la sensibilité et la spécificité de la chimioréception chez diverses espèces d'insectes. Une étude sur *Drosophila melanogaster* a montré qu'une estérase dans les antennes avait une activité catalytique élevée contre un groupe chimiquement diversifié de composés odorants de fruits. L'étude n'exclut pas la possibilité de fonctions de dégradation d'odeurs de certaines estérases, P450, GST et UGT exprimées dans les antennes, mais si cela est le cas, ces enzymes ont également des fonctions supplémentaires dans d'autres tissus (Younus *et al.* 2014). Une autre étude sur le rôle des aldéhyde-oxydases, qui sont des enzymes oxydant certains composés aromatiques, donne des pistes sur le rôle qu'elles pourraient jouer dans la détoxification et la dégradation des signaux chimiques environnementaux. Ce rôle a été suggéré sur une aldéhyde oxydase de *Plutella xylostella* qui pourrait être impliquée dans la dégradation de nombreux odorants aldéhydes et participer à la fois à la protection des neurones olfactifs par l'inactivation des odorants redondants et par la détoxification des xénobiotiques (Wang *et al.* 2021). Une étude récente a également suggéré que certaines GST spécifiques aux antennes pourraient dégrader des phéromones. Chez *Plodia interpunctella* notamment, l'expression du gène *PiGSTd1* pourrait être liée à la dégradation et l'inactivation d'une variété de composés odorants, en particulier les phéromones sexuelles (Liu *et al.* 2021). Enfin, une thèse sur l'effet de l'utilisation d'un néonicotinoïde sur le système olfactif d'*Agrotis ipsilon* a permis par identification et clonage de sous-unités des récepteurs nicotiques à l'acétylcholine et étude du taux d'expression des sous-unités en fonction du traitement avec la clothianidine, de dégager des résultats préliminaires sur l'expression des sous-unités dans le lobe antennaire. Ces résultats montrent une baisse d'expression de plusieurs sous-unités

nicotiques après le traitement avec la clothianidine, suggérant qu'elles « pourraient jouer un rôle dans les changements de la sensibilité des neurones du lobe antennaire et du comportement sexuel, observés préalablement après le traitement avec la clothianidine » (Rabhi 2015). Ce travail nous apprend également que certains neurones du lobe antennaire des lépidoptères utilisent le GABA comme neurotransmetteur inhibiteur principal.

De la même façon, si la température a directement un impact sur l'activité de certaines enzymes antennaires de détoxification impliquées dans la dégradation de certaines odeurs ou sur l'expression de gènes associés, la perception des odeurs pourrait-elle être perturbée chez *C. pomonella* ? En effet, si l'existence d'enzymes de détoxification impliquées dans la perception de phéromones est connue et soulignée par plusieurs études, nous avons également observé un effet de la température sur l'activité et l'expression génique de plusieurs enzymes. Cela laisse supposer que l'effet de la température de développement sur l'olfaction pourrait également s'expliquer par un effet de la température sur les enzymes, les récepteurs nicotiques ou GABA au niveau des antennes. Ainsi, la température pourrait-elle avoir un impact important sur l'activité ou l'expression de certaines GST antennaires entraînant une trop rapide dégradation des composés phéromonaux et perturbant leur perception ? Comme pour un néonicotinoïde, qui a un effet sur les sous-unités des récepteurs nicotiques pouvant éventuellement perturber la sensibilité de neurones du lobe antennaire aux odeurs, la température pourrait-elle avoir un effet sur ces sous-unités ou sur le récepteur GABA perturbant la sensibilité neuronale aux odeurs ? La température pourrait-elle également avoir un effet sur la confusion sexuelle *via* une action sur l'expression et l'activité des enzymes antennaires impliquées à la fois dans l'olfaction et dans la détoxification des xénobiotiques ? Si ces hypothèses restent très spéculatives, il paraît évident que la température peut modifier la perception des odeurs par le biais de divers processus moléculaires ou biochimiques encore peu connus.

Quoiqu'il en soit, d'un point de vue purement technique, bien que ces résultats n'encouragent pas l'utilisation préventive de la confusion sexuelle contre cette espèce dans les conditions climatiques futures des vergers, une température de développement constante de 35°C ne reflète pas exactement la réalité de terrain, les individus se développant à température fluctuante en conditions naturelles. De plus,

l'absence d'effet de la température de stimulation semble indiquer que la confusion sexuelle restera au moins partiellement efficace avec le réchauffement climatique.

Changement climatique et méthodes de lutte contre *C. pomonella* : questionnements et perspectives de recherche

Les résultats de ce travail de recherche ont permis de répondre aux questionnements relatifs aux impacts du réchauffement climatique actuel sur l'efficacité des principales méthodes *C. pomonella* en Région PACA. Ce travail a cependant mené à d'autres questionnements et certains points restent à préciser pour statuer sur l'efficacité des principales méthodes de lutte contre le carpocapse des pommes avec le réchauffement climatique.

A court terme, des expérimentations supplémentaires sont prévues pour préciser les expérimentations préliminaires effectuées durant ce travail de thèse sur l'impact des températures sur la perception des phéromones de synthèse par les antennes de mâles de *C. pomonella* (Chapitre V). Des mesures antennographiques monosensillaires sont prévues et devraient permettre de comprendre si des réponses électriques antennaires plus faibles sont dues à un trop grand afflux de signaux nerveux perçus au niveau des neurones des individus. Enfin, pour savoir si une différence de perception au niveau des neurones engendre des réponses physiologiques et comportementales différentes chez les individus, des tests en tunnel de vol ou avec un olfactomètre sont envisagés. Pour statuer sur la spécificité des mâles de *C. pomonella* aux odeurs avec la température, il serait pertinent de tester d'autres composés phéromonaux responsables de l'attraction chez d'autres espèces de tordeuses, seuls ou en mélange avec la codlemone (comme celle de *Grapholita molesta* et *Grapholita funebrana* par exemple). Il paraît en effet peu pertinent de garder les mélanges actuels avec des composés minoritaires. Ces nouveaux composés permettraient d'évaluer si la température de développement notamment permet à *C. pomonella* de détecter les phéromones sexuelles d'autres tordeuses, rendant l'utilisation de diffuseurs de confusion sexuelle ciblant plusieurs espèces (*Ginko duo*) intéressante. Une diminution de spécificité avec une augmentation des températures soulève cependant la question d'un possible impact environnemental de cette technique, dans l'éventualité où des Lépidoptères non ravageurs soient attirés par les formules appliqués en vergers avec le réchauffement climatique.

D'autres expérimentations sont également envisagées pour affiner les résultats obtenus pour répondre aux questionnements de cette thèse. Il s'agirait de tester des

régimes fluctuants de températures. En effet, l'utilisation en laboratoire d'un régime thermique constant constitue une limite dans notre étude. S'il permet d'obtenir des résultats suffisants pour définir la pérennité de l'utilisation des deux ennemis naturels choisis pour ces études, il constitue un biais et ne reflète pas exactement les conditions climatiques naturelles. Un régime fluctuant pourrait notamment permettre une meilleure évaluation des traits d'histoire de vie chez *F. auricularia* qui n'ont pu être évalués au-dessus de 25°C. Si un régime de température plus ou moins constant semble plutôt bien adapté à l'étude des traits d'histoire de vie et du comportement de *M. ridens* puisqu'il s'agit d'une espèce diurne, il n'est pas adapté à *F. auricularia* qui est une espèce nocturne. En effet, les limites thermiques des ectothermes ayant des régimes temporels contrastés seraient adaptées à leurs activités circadiennes. Certaines espèces de fourmis diurnes par exemple toléreraient des températures maximales de développement plus importantes que les espèces de fourmis nocturnes quel que soit leur habitat (Garcia-Robledo *et al.* 2018). Une augmentation des températures nocturnes avec le réchauffement climatique pourrait être délétère pour les espèces nocturnes qui ont une plus faible tolérance aux températures élevées que les espèces diurnes. Pour les insectes nocturnes, des températures plus basses la nuit que la journée permettent de récupérer du stress thermique subi pendant la journée, pour retrouver de meilleures performances développementales et reproductives. Cependant, cette récupération est meilleure si les températures nocturnes sont basses et éloignées des températures journalières. Aussi, si le changement climatique induit un réchauffement nocturne plus marqué que celui diurne comme prédit (Guichard *et al.* 2017), les capacités de récupération des espèces nocturnes pourraient être réduites via une augmentation de leur métabolisme pendant leur période d'activité mais aussi au repos (Bai *et al.* 2019). Ainsi des expérimentations en conditions thermiques fluctuantes permettraient de mieux connaître les optimums thermiques et le potentiel de prédation de *F. auricularia*, espèce nocturne potentiellement plus impactée par le réchauffement climatique que les espèces diurnes. Ces expérimentations permettraient également de confirmer ou d'infirmer la baisse du taux de prédation chez *F. auricularia* à partir de 30°C. Pour *M. ridens*, il va être important de suivre l'installation de cette espèce dans les vergers du Sud de la France ces prochaines années, son introduction en France restant assez récente. Les résultats de recapture obtenus en Californie constituent un retour prometteur quant à l'installation à long terme des populations de *M. ridens* en France. Il serait également

intéressant de connaître précisément le nombre de générations que peuvent effectuer les populations de *M. ridens*, qui seraient plus nombreuses que le nombre de générations de *C. pomonella*, en raison d'une durée de développement plus courte, afin d'évaluer si ces deux espèces restent synchrones dans ce contexte de réchauffement climatique. Des données de développement à températures fluctuantes permettraient notamment de modéliser le développement de *M. ridens* en parallèle avec celui de *C. pomonella* et de plus précisément évaluer la synchronicité des générations de ces deux espèces sur une saison complète.

Pour garantir l'efficacité combinée du chlorantraniliprole et de la lutte biologique par conservation de *F. auricularia* et par acclimatation de *M. ridens* contre *C. pomonella* avec le réchauffement climatique, il serait intéressant de tester l'effet du chlorantraniliprole sur ces trois espèces en conditions réelles de terrain, fluctuantes au niveau des températures quotidiennes. Il pourrait être également approprié dans ce contexte d'utiliser une souche de terrain de *C. pomonella* en plus de notre souche de laboratoire issu d'un élevage qui n'a pas été réalimenté avec des individus de terrain depuis plus de 20ans. Une souche de terrain serait probablement d'autant plus adaptée aux conditions fluctuantes de terrain et aux conditions climatiques futures, surtout si elle provient de vergers méditerranéens subissant déjà des écarts de températures et des températures extrêmes au moins ponctuellement en été. De même il faudrait tester l'effet de l'emamectine et *F. auricularia* contre *C. pomonella* en début de saison, où une utilisation printanière de cette substance semble plus appropriée. Pour garantir la moindre toxicité du chlorantraniliprole sur les deux espèces d'ennemis naturels et de l'emamectine sur la forficule, il serait intéressant d'évaluer certains traits d'histoire de vie ou certains comportements sur les survivants post-traitements. Il faudrait notamment évaluer la fertilité et la capacité de prédation/parasitisme des survivants. Cette expérimentation serait particulièrement intéressante pour *M. ridens*, permettant d'évaluer la toxicité du chlorantraniliprole sur des femelles, n'ayant pas pu être testées dans les expérimentations du [Chapitre IV](#). Particulièrement sur leur fertilité, le sexe ratio secondaire, la taille à l'émergence et leur taux de parasitisme de cocons de *C. pomonella* traités au chlorantraniliprole.

En plus des températures fluctuantes, un paramètre a constitué un biais important lors de nos différentes études à températures stables : l'humidité des enceintes climatiques utilisées. Ce paramètre n'a pas pu être contrôlé de façon précise pendant les

différentes expérimentations et une humidité trop basse peut constituer un facteur particulièrement limitant pour les espèces (voir [Chapitre I-1](#)). Il serait alors intéressant de contrôler et fixer l'humidité relative des enceintes climatiques qui seront utilisées pour les expérimentations en conditions de températures fluctuantes à $60 \pm 5\%$ d'humidité relative afin de limiter ce biais lors des futures expérimentations.

Des expérimentations sur les conditions temporelles et climatiques permettant la sortie de diapause hivernale de *C. pomonella* sont en cours. La connaissance de ce paramètre permettra de compléter et d'affiner le modèle phénologique présenté au [Chapitre IV](#). Ce modèle, complété par ce paramètre permettra de prédire précisément l'émergence des différentes générations de carpocapse des pommes. Ces prédictions aux conditions climatiques permettront d'appliquer les méthodes de lutte les plus adaptées aux différentes générations (période de l'année, densité des populations, températures journalières) au moment précis où elles seront les plus efficaces.

Enfin, une piste n'a pu être explorée dans ce travail de recherche : l'impact de l'élévation des températures sur l'efficacité de la carpovirusine, développé à partir d'un granulovirus spécifique au carpocapse des pommes. En effet, s'il est peu probable que la température ait un effet fortement délétère sur la carpovirusine ([Chapitre I-1](#)), il serait intéressant d'évaluer comment elle influence son efficacité en combinaison avec d'autres méthodes de lutte. En effet, le [Chapitre I-1](#) suggère que toutes les méthodes de lutte seront influencées par le changement climatique. Ces influences seront différentes et plus ou moins marquées en fonction de la méthode de lutte et des espèces/souches dans le cas de l'utilisation d'organismes entomopathogènes et entomophages. Dans les perspectives de ce [Chapitre I-1](#), certains auteurs suggèrent des pistes d'amélioration des différentes méthodes de lutte pour les rendre les plus pérennes possible face aux changements climatiques. Selon leur provenance, il existe des différences de sensibilité à la température entre souches d'une même espèce. Il est donc par exemple possible de sélectionner et éventuellement croiser des souches de parasitoïdes échantillonnées dans des latitudes plus chaudes pour qu'ils soient moins sensibles et plus performant face au réchauffement climatique (Traoré *et al.* 2006 ; Moiroux *et al.* 2010 ; Kalyebi *et al.* 2006). Il est également possible d'effectuer une sélection et une amélioration génétique de souches de parasitoïdes pour les rendre plus résistantes aux insecticides et/ou à la température (Kumar *et al.* 2008, Svrivastava & Singh 2015). Le même processus peut également s'appliquer à

certaines souches d'entomopathogènes pour les rendre plus tolérantes aux différents stress abiotiques (Ehlers *et al.* 2005 ; Mukuka *et al.* 2010b ; Salame *et al.* 2010). Enfin, utiliser des combinaisons d'agents pathogènes ou de méthodes de lutte (insecticides et agents pathogènes) pourrait également constituer une source possible d'amélioration face aux changements climatiques puisque certaines méthodes s'avèrent être plus efficaces contre le ravageur cible en combinaison que seules (Inglis *et al.* 1997 ; Delnat *et al.* 2019). Cela nous amène donc à certaines réflexions. Existe-t-il des interactions positives ou négatives entre température et carpovirusine ? Chez *M. ridens*, certaines populations pourraient-elles être moins sensibles aux fortes températures et avoir une meilleure fitness ? Des populations issues de latitudes méridionales seraient-elles plus adaptées à une augmentation des températures que des populations issues de latitudes plus au Nord ? Existe-il des effets synergiques entre température, carpovirusine et chlorantraniliprole ? Certaines souches de granulovirus sont-elles plus sensibles à la température que d'autres, impliquant la possibilité de sélectionner préférentiellement des souches pour le développement de la carpovirusine de demain ? Est-ce que des stress combinés (thermique et insecticide) déclenchent des voies de défenses chez *C. pomonella* généralistes qui peuvent avoir un impact sur l'efficacité du CpGV ?

Ces questionnements peuvent être une piste de réflexion amenant à l'optimisation des pratiques culturales au climat futur.

Changement climatique et méthodes de lutte contre *C. pomonella* : durabilité dans les conditions climatiques futures

Le bilan des études détaillées dans le [Chapitre I-1](#) de ce manuscrit permet de conclure qu'il faudrait appliquer plus d'insecticides chimiques pour lutter contre les ravageurs en conséquence du réchauffement climatique, sauf pressions sociétales et politiques contraires, en raison (i) d'une réduction de leur durée de vie dans les agroécosystèmes, (ii) d'une dilution accrue dans les plantes et (iii) d'une augmentation des populations de ravageurs. Nos résultats indiquent en plus que si les insecticides testés sont certifiés comme étant stables jusqu'à 50°C, la toxicité de l'emamectine et du spinosad est bien dépendante de la température. Pour ces insecticides, il faudra donc bien appliquer plus de produit pour lutter contre *C. pomonella* en conséquence d'une efficacité réduite directement liée à la sensibilité du carpocapse.

Pour la durabilité de la lutte biologique, nos résultats suggèrent qu'il est peu probable qu'un décalage phénologique survienne entre les deux espèces d'ennemis naturels étudiées et le carpocapse des pommes, alors que l'efficacité de prédation ou de parasitisme par les ennemis naturels concernés ne semble que peu impactée par la température. Leur potentiel de régulation reste relativement stable lorsque la température augmente si on prend en compte leur période d'activité (au printemps et en début d'été pour la forficule et pendant toute la durée de présence de larves diapausantes au-dessus des 15°C pour *M. ridens*). Cette conclusion est soutenue par l'étude de Tourneur et Meunier (2020) qui indiquent que "l'adaptation d'un insecte au cycle de vie relativement long ne s'opère pas nécessairement en réponse aux températures moyennes globales des environnements envahis, mais en réponse à leur saisonnalité et/ou à la température moyenne à un moment précis de leur cycle de vie".

Cependant, nos résultats sur la toxicité de insecticides étudiés sur ces deux mêmes espèces d'ennemis naturels permettent de statuer sur la possibilité de combiner ces insecticides et ces ennemis naturels dans un contexte de réchauffement climatique. Les résultats vont alors alerter sur l'utilisation du spinosad, pourtant homologué en agriculture biologique, tandis que le chlorantraniliprole semble convenir aux conditions climatiques futures pour lutter contre *C. pomonella*, conserver *F. auricularia* et faciliter l'établissement de *M. ridens*. Pour la lutte biologique par conservation reposant sur *F. auricularia*, une utilisation alternée d'emamectine au début du printemps, lorsque sa

toxicité est la plus faible, et du chlorantraniliprole pendant l'été pourrait limiter les risques d'apparition de résistance dans les populations de carpocapse tout en réduisant l'impact des insecticides sur les populations d'ennemis naturels. Il sera alors nécessaire de tester les effets directs et sub-létaux de ces insecticides sur les populations d'ennemis naturels en conditions réelles de terrain. Cependant, les effets différentiels dégagés par nos travaux soulignent d'autant plus l'importance de considérer chaque système indépendamment. Ces résultats confirment également que le changement climatique influence directement le contrôle des ravageurs par lutte biologique en fonction des pratiques culturales.

En revanche, nos résultats sur la confusion sexuelle et la perception de phéromones sexuelles chez *C. pomonella* sont différents de ceux dégagés par le [Chapitre I-1](#). Nos résultats bien que préliminaires peuvent indiquer que si les mâles sont moins sensibles aux phéromones sexuelles des femelles ils auront plus de difficultés à trouver un partenaire pour se reproduire, diminuant alors la densité des populations. Cela pourrait suggérer une diminution de l'efficacité de la confusion sexuelle en vergers avec le réchauffement climatique.

Pour conclure, les résultats de ce travail soulignent que ce type d'étude, dans un système particulier, en ciblant les modèles biologiques, les insecticides de synthèse et les méthodes de lutte préventives reste la solution la plus adaptée pour statuer sur l'impact du réchauffement climatique sur la durabilité des méthodes de lutte. Ainsi, ce cas appliqué permet d'indiquer que l'arboriculture conventionnelle en vergers de pommiers pourrait être d'autant plus touchée par le réchauffement climatique en raison d'une diminution de l'efficacité de certains produits sur le ravageur ciblé et à la perte de contrôle par lutte biologique utilisant des ennemis naturels. De plus, le changement climatique peut avoir un impact direct sur la quantité et la qualité des fruits produits en vergers de pommiers rendant la question de la pérennité des méthodes de lutte utilisées dans ces agroécosystèmes relativement vulnérables d'autant plus importante. Dans une volonté grandissante sociétale et politique de produire de façon plus responsable et de limiter les coûts inhérents à l'utilisation de produits phytosanitaires, ce type d'étude permet de trouver les solutions les plus économiques et durables. Dans une volonté d'adapter au mieux l'utilisation des méthodes de lutte au réchauffement climatique, l'utilisation du modèle phénologique développé à partir des résultats sur le temps de développement de *C. pomonella* permettrait de prédire

l'émergence des différentes générations de *C. pomonella* en vergers et d'utiliser les méthodes de lutte les plus efficaces pour chaque génération. Enfin, en choisissant les insecticides les plus efficaces contre *C. pomonella* tout en prenant en compte les effets différentiels de la température sur la toxicité de ces insecticides sur les ennemis naturels, il semble tout à fait possible, dans un contexte de réchauffement climatique, de combiner la lutte biologique et l'utilisation de certains produits phytosanitaires dans le respect de l'environnement et de la santé humaine.

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Cette partie supplémentaire du manuscrit comprend trois différentes annexes.

L'annexe I présente un article traitant des interactions intra-gilde entre deux prédateurs généralistes fréquemment observés dans les colonies du puceron rose du pommier *Dysaphis plantaginea*. Il s'agit d'un travail réalisé par Hazem Dib, maître de conférences à l'université d'Avignon, pour lequel j'ai eu l'opportunité de travailler durant la phase de conceptualisation des expérimentations ainsi que pour la rédaction d'un article paru en 2020 dans *Biological Control*. Même si cet article n'inclut pas les concepts de changements climatiques, il présente une approche très intéressante pour mesurer l'efficacité et l'optimisation de la lutte biologique via l'utilisation combinée de deux prédateurs des pucerons en vergers.

L'annexe II présente un poster que j'ai eu l'occasion d'exposer et présenter lors du congrès international Resistance 19' (2019) ayant eu lieu à Rothamsted en Angleterre. Ce poster finalisé durant la première année de ce travail de thèse traite de résultats préliminaires des impacts croisés température et insecticides sur la sensibilité de larves de carpocapse. Ces résultats ont été approfondis et sont détaillés dans les Chapitres II et III de ce manuscrit.

L'annexe III présente la liste de toutes les communications passées et prévues qui m'ont permis ou me permettront une valorisation de ce travail de thèse.

Does combining *Forficula auricularia* L. (Dermaptera: Forficulidae) with *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) enhance predation of rosy apple aphid, *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae)?

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Résumé

Une bonne connaissance des interactions entre les espèces d'ennemis naturels est nécessaire pour une mise en œuvre efficace des programmes de lutte biologique. Cette étude de laboratoire avait pour objectif d'évaluer les interactions intra-guildes entre deux prédateurs généralistes fréquemment observés dans les colonies du puceron rose du pommier (RAA), *Dysaphis plantaginea* Passerini (Hemiptera : Aphididae). La complémentarité entre ces deux espèces a été estimée par la comparaison de leur prédation individuelle combinée sur le RAA. La prédation par les larves du 1er (H1) au 4ème (H4) stade d'*Harmonia axyridis* Pallas (Coleoptera : Coccinellidae) et les nymphes du 3ème (F3) et 4ème (F4) stade de *Forficula auricularia* L. (Dermaptera : Forficulidae) a été évaluée pendant 24 heures dans des boîtes de Petri en utilisant sept densités différentes (5, 10, 25, 50, 75, 100 et 200) de nymphes de 3ème stade de RAA. Les deux espèces de prédateurs étaient plus voraces à mesure que leur âge augmentait. Le niveau de satiété, c'est-à-dire le nombre le plus élevé de RAA consommés, a atteint en moyenne 17,8, 36,3, 49,4, 79,6, 62,9 et 84,3 pucerons par jour pour H1, H2, H3, H4, F3 et F4, respectivement. Dans les traitements combinés, il y avait peu de preuves de prédation intra-groupe. Seuls 2,9 % des H1 et H2 sont morts ou ont disparu aux faibles densités de RAA (≤ 25 pucerons). À la densité de RAA non limitante de 200 nymphes, nous avons observé une légère interaction agonistique avec une consommation de RAA inférieure de 7,9 % lorsque les deux prédateurs étaient dans la même arène par rapport à la somme de leurs consommations individuelles. Cependant, cet effet était très faible et a été largement compensé par les avantages de l'utilisation de ces deux prédateurs en combinaison pour augmenter la consommation des proies. Pour les six autres densités de RAA, les prédateurs en combinaison ont consommé une forte proportion ($> 90\%$) de RAA. Nos résultats recommandent l'utilisation de combinaisons de ces deux espèces de prédateurs, en particulier de leurs stades les plus avancés, et indiquent que ces deux prédateurs sont des candidats aphidophages prometteurs au début du printemps contre les RAA.

Mots clés : prédation intra-guilde, compétition, complémentarité, voracité, lutte biologique

Does combining *Forficula auricularia* L. (Dermaptera: Forficulidae) with *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) enhance predation of rosy apple aphid, *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae)?



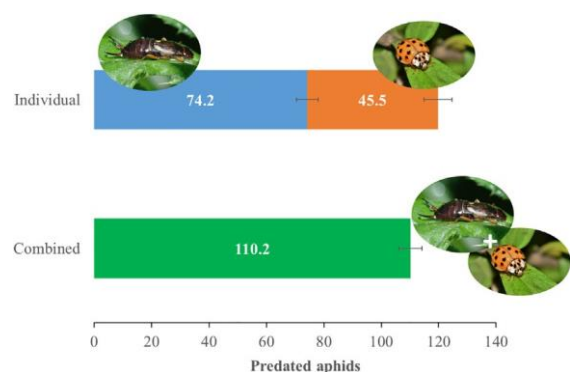
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GRAPHICAL ABSTRACT



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ABSTRACT

A good knowledge of interactions between natural enemy species is necessary for effective implementation of biological control programmes. This laboratory study aimed at assessing the outcome of intraguild interactions between two generalist predators frequently observed in the colonies of the rosy apple aphid (RAA), *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae). The level of complementarity between these two species was estimated by the comparison of their individual and combined predation on RAA. Predation by 1st (H1) to 4th (H4) instar larvae of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) and 3rd (F3) and 4th (F4) instar nymphs of *Forficula auricularia* L. (Dermaptera: Forficulidae) was evaluated for 24 h in Petri dishes using seven different densities (5, 10, 25, 50, 75, 100 and 200) of 3rd instar nymphs of RAA. Both predator species were more voracious as their age increased. The satiation level, i.e. the highest number of consumed RAA, reached on average of 17.8, 36.3, 49.4, 79.6, 62.9 and 84.3 aphids per day for H1, H2, H3, H4, F3 and F4, respectively. In the combined treatments, there was little evidence of intraguild predation. Only 2.9% of H1 and H2 died or disappeared at low RAA densities (≤ 25 aphids). At the non-limiting RAA density of 200, we observed a slight agonistic interaction with 7.9% less RAA consumption when the two predators were in the same arena than the sum of their individual consumptions. However, this effect was very small and was largely outweighed by the benefits of using these two predators in combination to increase prey consumption. For the other six RAA densities, predators in combination consumed a high proportion (> 90%) of RAA. Our results point to the use of

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1. Introduction

Interactions between members of predator guilds and their consequences for prey populations, have become a major topic in biological control studies (Rosenheim et al., 1995; Majerus et al., 2006; Putra et al., 2009; Dib et al., 2016a). These intraguild interactions may result in additive or synergistic predation of target pests (Flowers et al., 2006; Dib et al., 2011) or in agonistic interactions that decrease predation efficiency, releasing the prey from predation pressure when either intraguild predation (IGP), or competition dominates (Rosenheim et al., 1993; Lucas et al., 1998).

IGP is defined as a predation event in which two co-occurring predator species compete for the same prey resulting in the death and eventual consumption of one of the predators. The top predator is referred to as the intraguild predator, the consumed predator is called the intraguild prey, and their common resource is termed the extraguild prey. IGP has been identified as one of the main mortality factors that may destabilise communities, affecting the composition, abundance and dynamics of natural enemy guilds (Polis et al., 1989; Polis and Holt, 1992; Rosenheim et al., 1995; Symondson et al., 2002). To be successful, biological control programmes should take this phenomenon into consideration.

As an important survival tactic, IGP is particularly common among generalist predators; however, it may often interfere with the effectiveness of these biocontrol agents (Polis et al., 1989; Rosenheim et al., 1993; Lucas et al., 1998; Symondson et al., 2002). Aphidophagous guilds are rich in coexisting species, providing ideal systems to study intraguild interactions (e.g., Lucas et al., 1998; Lucas, 2005; Dib et al., 2011, 2016a). In the present work, we studied these interactions in a simple community of three species, a ladybird-earwig-aphid system.

The rosy apple aphid (RAA), *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) is a frequent direct pest causing serious losses in apple orchards (De Berardinis et al., 1994; Qubbaj et al., 2005). RAA has been a major pest on apple trees in Europe, the Middle East, the Caspian region and North America for a long time. It has spread to North Africa, India, Taiwan, Korea and Japan. RAA is unknown in Australia, New Zealand and Oceania, Central Asia, and Central and South America (CABI, 2020). RAA is associated with a large assemblage of natural enemies in orchards. However, the natural populations of these aphid enemies are insufficient to control RAA damage (Wyss et al., 1999; Brown and Mathews, 2007; Dib et al., 2010a; Boreau de Roince et al., 2013). In addition, the increasing releases of many candidate predators have proved to be unsuccessful in controlling RAA, presumably due to IGP and other unknown interactions between the predators (Wyss et al., 1999; Kehrlı and Wyss, 2001; Dib et al., 2016b, 2017a).

It is difficult to study and quantify the factors regulating the population dynamics of generalist predators in the field because of the complex effects of these positive or negative interactions on their survival (Lucas, 2005; Sheppard et al., 2005). Consequently, studies on quantifying the interactions between several species of predators under laboratory conditions are necessary to understand their impact on the regulation of pest aphid populations. The aim of this research is to optimise the use of predators in biological control programmes in the field.

Previous studies have demonstrated that natural enemies of RAA are mainly from two generalist predators, the multicoloured Asian ladybird, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), and the European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae) (Brown and Mathews, 2007; Dib et al., 2010a,b). *Harmonia axyridis*

(Koch, 2003; Majerus et al., 2006; Dib et al., 2010a; Roy et al., 2016) and *F. auricularia* (Brown and Mathews, 2007; Piñol et al., 2009; Moerkens et al., 2011; Dib et al., 2017b) are presently the most common ladybird and earwig species, respectively, found throughout the world. These insects are well-known predators of numerous aphid species, including those present on apple trees (Brown and Mathews, 2007; Dib et al., 2016a; Quarrell et al., 2017; Orpet et al., 2019a), and are good candidates for biological control programmes (Koch, 2003; Lee and Kang, 2004; Labrie et al., 2006; Piñol et al., 2009). Despite its status as an invasive species (Michaud, 2002; Mizell, 2007; Roy et al., 2016), *H. axyridis* is still regarded in many studies as an effective bio-control agent (Koch, 2003; Lee and Kang, 2004; Brown and Mathews, 2007).

There are, however, potential negative and adverse impacts of *H. axyridis* and *F. auricularia*, as they may reduce populations of other aphidophagous natural enemies (Majerus et al., 2006; Dib et al., 2016a). In other words, *H. axyridis* and *F. auricularia*, at specific stages of their development, have the potential to choose other members of the aphidophagous guild as a food source (Snyder and Ives, 2003; Gardiner and Landis, 2007; Putra et al., 2009; Dib et al., 2016a).

Most studies on intraguild interactions concerning *H. axyridis* have focused on its interaction with other coccinellid predators (Yasuda et al., 2001; Snyder et al., 2004; Nóia et al., 2008; Ware et al., 2009). In addition, several field studies have shown a decrease in the abundance of coccinellid predators after the establishment of *H. axyridis* in orchards (LaMana and Miller, 1996; Colunga-Garcia and Gage, 1998; Mizell, 2007). A few reports have investigated the interactions between *H. axyridis* and non-coccinellid natural enemies such as *Episyrphus balteatus* de Geer (Putra et al., 2009; Ingels and De Clercq, 2011), *Metasyrphus corollae* Fabricius (Diptera: Syrphidae) (Putra et al., 2009), *Hyaliodes vitripennis* Say (Hemiptera: Miridae) (Provost et al., 2006), *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) (Gardiner and Landis, 2007), *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) (Phoofolo and Obrycki, 1998; Gardiner and Landis, 2007) and aphid parasitoids (Takizawa et al., 2000; Snyder and Ives, 2003). In the majority of these studies, *H. axyridis* is reported as an intraguild predator. However, very few studies have demonstrated that *H. axyridis* may fall victim to intraguild predation like, for example, to the pentatomid *Podisus maculiventris* Say (Hemiptera: Pentatomidae) (De Clercq et al., 2003).

In contrast, *F. auricularia* has been poorly studied regarding its direct interactions with other aphidophagous predators. To our knowledge, this topic has only been investigated in relation to *E. balteatus* in two laboratory studies. The two predator species did not show negative interactions and had an additive effect on the predation of aphids in the first study (Dib et al., 2011). However, interactions were non-additive and some mortality occurred in younger larvae of *E. balteatus* when aphids were scarce (Dib et al., 2016a).

Harmonia axyridis and *F. auricularia* share the same foraging habitat and food resources, as well as a spatiotemporal overlap in the canopy of apple trees as observed in French orchards by Dib et al. (2010a, 2010b). The rolled apple leaves, which are a symptom of RAA infestation, can be used as shelters by *F. auricularia* during the day (Dib et al., 2017b). Consequently there may be frequent interactions between these two predators in the field, especially when they face low food availability. However, to date, little is known about the intraguild interactions between *H. axyridis* and *F. auricularia*. The likelihood,

Clercq, 2011; Gagnon and Brodeur, 2014). The laboratory experiments reported here were therefore designed to examine the effect of these two factors (i.e. different predatory instars and different RAA densities) on the individual and combined predation of both predator species on RAA individuals. We specifically investigated the complementarity of these two generalist predators by ascertaining if their effects were additive on RAA predation and whether, and to what extent, there was evidence of interference or IGP.

1. Materials and methods

1.1. Insect rearing

All insects used in the experiments were laboratory-reared. The first insects were originally collected from insecticide-free apple orchards in the Latakia region (Syria).

1.1.1. Rosy apple aphid, *Dysaphis plantaginea*

Aphids were mass-reared on detached leaves of the narrow-leaf plantain, *Plantago lanceolata* L. (Lamiales: Plantaginaceae), gathered in insecticide-free orchards, and stuck to agar medium (1 L: 10 g) in ventilated plastic boxes (15 × 15 × 5 cm). *Plantago lanceolata* is the summer host plant of RAA (Dib et al., 2011). RAA were left to reproduce by parthenogenesis in a climatic chamber at 20 ± 2 °C, 65 ± 5% RH, and with a photoperiod L16:D8 h.

1.1.2. Multicolored Asian ladybird, *Harmonia axyridis*

Mass rearing of *H. axyridis* was established in spring from 80 adults divided into five groups of 16 individuals, each in a sex ratio of 1:1. They were reared in ventilated plastic cages (18 × 18 × 9 cm) in a chamber kept in room temperature (25 ± 8 °C), light (approximately L16:D8 h) and humidity (65 ± 15%) conditions. These cages were equipped with moistened filter papers, small bands of corrugated cardboard, diluted honey and an 'ad libitum' supply of aphids of different non-identified species on detached leaves of their host plant. To prevent cannibalistic feeding on the eggs, males were removed after the beginning of egg laying. In addition, every cluster of 20–30 eggs observed was directly transferred to a cardboard cup (40 cL). This cup was covered with insect-proof netting to allow ventilation. It was also provided with a moist piece of filter paper, changed twice a week, to provide suitable local humidity. After the eggs hatched, larvae were fed 'ad libitum' on aphids and diluted honey until they reached the desired age for the experiments.

1.1.3. European earwig, *Forficula auricularia*

Thirty couples of *F. auricularia* adults were isolated in autumn in cardboard cups (20 cL). Upper holes were covered with insect-proof netting for ventilation purposes. Wet sand was put in each cup to provide overwintering refuges and egg-laying sites. Every week, the sand was re-humidified and adults were fed with pieces of bread slightly ground and a small drop of bee honey. Insects were reared in room temperature (20 ± 13 °C), light (approximately L16:D8 h) and humidity (65 ± 20%) conditions. Males were removed from the cups as soon as females laid the eggs to prevent future cannibalism of eggs. After nymph emergence and first moulting, the 2nd instar nymphs were transferred to ventilated plastic containers (0.5 L). Small corrugated cardboard rolls were added to the rearing containers to provide suitable shelters for earwigs to hide during the day (Dib et al., 2017b). They were fed, until they reached the desired age, on an 'ad libitum' supply of ground bread and aphids of different non-identified species on detached leaves of their host plant. Some containers were sometimes transferred to the climatic chamber where the stock population of RAA was

maintained to speed up the time to reach the desired nymphal age. _____

1.2. Experimental preparation

The experimental arena was a plastic Petri dish (15 cm diameter and 3 cm high) with a 5 cm wide ventilation hole in the centre of the lid covered with insect-proof netting. A feeding system made of a plantain leaf with its petiole inserted into a 2 mL Eppendorf tube was set in each Petri dish to guarantee the feeding of aphids throughout the experimental period (Dib et al., 2011). From the bottom to the top, each tube contained 1 mL of water (to keep leaves fresh), a piece of cotton (a separation layer) and a layer of agar medium (to prevent RAA nymphs from hiding in the water-saturated piece of cotton).

RAA has four nymphal stages, but only 3rd instar nymphs (5-day-old) were used in this study because they are easier to manipulate than younger ones; 4th instar nymphs were not used since they may have developed into adults during the experiment, thereby producing offspring (Dib et al., 2011). Similarly, adult aphids were not used because any offspring produced during the experiment would prevent the accurate estimation of predation rates. To establish 3rd instar nymph cohorts, adults were transferred randomly from stock cultures, using a smooth, wet paintbrush, and were allowed to produce offspring on detached plantain leaves stuck to agar medium. After 24 h, the adults were removed to allow the cohort of new-born nymphs to develop and reach the desired age for running the experiments, i.e. at the beginning of the fifth day of adult transfer to detached plantain leaves. The desired density of RAA 3rd instars (see below) was then transferred into the experimental arena and placed on the plantain leaf. Before introducing the predator(s) into the experimental arena, the RAA nymphs were allowed to establish feeding on the plantain leaves for 30 min.

Forficula auricularia and *H. axyridis* have four nymphal (Dib et al., 2017b) and four larval (Koch, 2003) instars, respectively. The two older nymphal instars of *F. auricularia* (F3 and F4) and all larval instars of *H. axyridis* (H1, H2, H3 and H4) were used in the experiments (Table 1). First and 2nd nymphal instars of *F. auricularia* were not studied because the dispersion of nymphs into the tree canopy starts from F3 after the familial phase normally occurring in the soil (Dib et al., 2017b). Before starting the experiments, the predators of the desired age were allowed to feed 'ad libitum' on RAA for 8 h to accustom them to the prey. Then they were starved for 8 h to standardise their level of hunger and increase their motivation to forage prior to being introduced into the experimental arenas. Each predator instar was used only once.

1.3. Experimental design

All experiments were conducted in a climatic chamber at 20 ± 2 °C, 65 ± 5% RH, and with a photoperiod L16:D8 h. The interactions between two different young instars, one of each predator, were assessed using seven densities of 5, 10, 25, 50, 75, 100 and 200

Table 1
Acronyms used in this paper.

Acronym	Full name
RAA	Rosy apple aphid
IGP	Intraguild predation
H1	Treatment with 1st instar larva of the coccinellid, <i>H. axyridis</i>
H2	Treatment with 2nd instar larva of the coccinellid, <i>H. axyridis</i>
H3	Treatment with 3rd instar larva of the coccinellid, <i>H. axyridis</i>
H4	Treatment with 4th instar larva of the coccinellid, <i>H. axyridis</i>
F3	Treatment with 3rd instar nymph of the earwig, <i>F. auricularia</i>
F4	Treatment with 4th instar nymph of the earwig, <i>F. auricularia</i>
H1_2	Group of treatment H1 and treatment H2
H3_4	Group of treatment H3 and treatment H4
F3_4	Group of treatment F3 and treatment F4
F3 + H1_2	Group of treatment F3 + H1 and treatment F3 + H2
F3 + H3_4	Group of treatment F3 + H3 and treatment F3 + H4
F4 + H1_2	Group of treatment F4 + H1 and treatment F4 + H2
F4 + H3_4	Group of treatment F4 + H3 and treatment F4 + H4

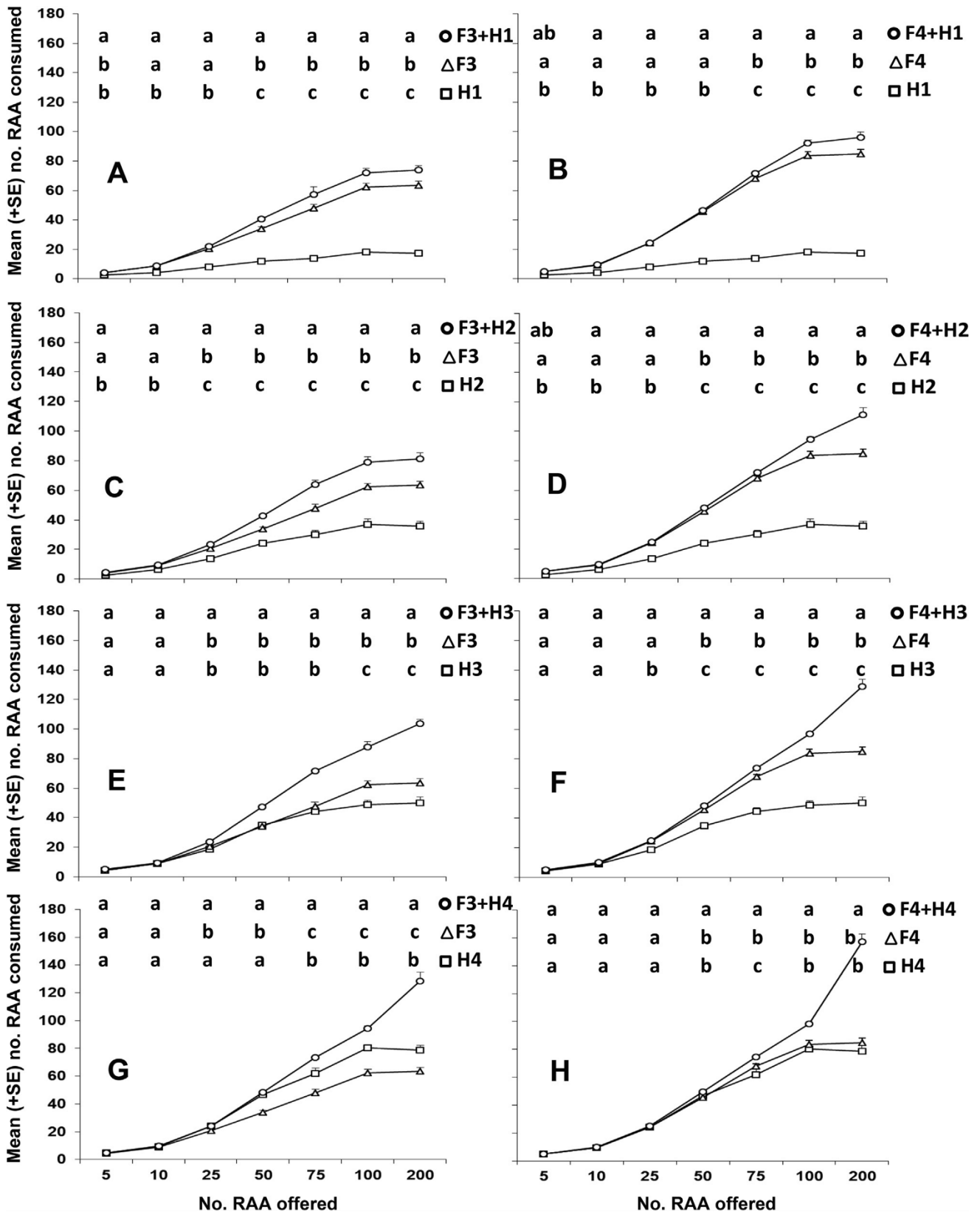


Fig. 1. Mean number (+1 SE) of 3rd instar nymphs of *Dysaphis plantaginea* (RAA) consumed over 24 h in relation to the seven RAA densities (5, 10, 25, 50, 75, 100 and 200) in the 14 predator treatments (six single treatments: 1st (H1), 2nd (H2), 3rd (H3) and 4th (H4) instar larvae of *Harmonia axyridis* and 3rd (F3) and 4th (F4) instar nymphs of *Forficula auricularia* and eight treatments: in combination). Each sub-figure represents the cases of the single and combined treatments of the same two instars: (A) H1 or/and F3, (B) H1 or/and F4, (C) H2 or/and F3, (D) H2 or/and F4, (E) H3 or/and F3, (F) H3 or/and F4, (G) H4 or/and F3 and (H) H4 or/and F4. Different small letters represent significant differences among predator treatments at each initial RAA density (mean separation by Tukey post-hoc, $P < 0.05$). Sample size for each comparison, $N = 3$ (predator treatments) \times 6 (replicates).

individuals of 3rd instar nymphs of RAA. A single F3 or F4 individual was released into the experimental arena containing one of the studied RAA densities immediately after releasing one H1, H2, H3 or H4 individual into the same Petri dish. The two predator instars were allowed to feed and interact for a period of 24 h before being removed. Then, the occurrence of intraguild predation was assessed by counting the remaining live predators and examining the external predation traces on the cadavers. RAA consumption by the two predators was recorded by counting the remaining live aphids in each Petri dish using a lighted head magnifier (3.5X). Four controls were defined to assess: (1) the natural RAA mortality in the absence of predators; (2) the mortality of tested predatory instars measured individually and in the absence of RAA; (3) the consumption of RAA in the presence of only one predator; and (4) the possible direct interaction between the two predators in the absence of RAA. Six replicates were carried out per RAA density and control.

For specific statistical analyses (see below), and to simplify the comparisons between predator treatments, seven groups of two treatments were created from the single and combined predator treatments, based on the age of predatory instars (Table 1): (H1_2), (H3_4), (F3_4), (F3 + H1_2), (F3 + H3_4), (F4 + H1_2) and (F4 + H3_4).

1.1. Data analysis

All data analyses were carried out using RStudio software, version 3.6.1 (2019-07-05) (R Core Development Team, Vienna, Austria).

1.1.1. Statistical models

We first analysed variation in the proportion of predated aphids as a function of predator treatment and initial aphid density with generalized linear models, assuming a binomial distribution of the variable. Secondly, we compared the predator consumption of RAA in the single and combined predator treatments of the same two instars to check the outcome of predatory interactions on RAA predation. The proportion of

predated aphids was thus analysed independently for each initial density with generalized linear models including the predator treatments (three levels: two single treatments and one combined treatment) as independent variable, assuming a binomial distribution of the variable.

Thirdly, to separately test the effects on predation of each of the two factors (initial RAA density and predator species and instar), we then compared the proportion of RAA consumed per predator: (i) between initial RAA densities offered for the same predator species and instar treatment and (ii) among predator groups at the same initial RAA density. As above, the proportion of predated RAA was analysed with generalized linear models, assuming a binomial distribution of the variable.

Finally, we analysed interactions between predators at the initial RAA density of 200 individuals, which was the only non-limiting density. For this purpose, the proportion of predated RAA was analysed with linear models including as independent variables the presence of *H. axyridis* in the arena (binary), the presence of *F. auricularia* (binary), and their interaction. In such an analysis, a significant interaction means either synergistic or antagonistic interactions among predators. As above, we assumed a binomial distribution of the variable. All model residuals were inspected using a QQ plot and a residual versus predicted plot (package DHARMA: Hartig, 2019).

1.1.2. Significance of independent variables

The significance of the predator treatment was assessed by Chi-square tests of model comparison using the drop1 function. Pairwise comparisons between treatment levels were carried out by *post-hoc* Tukey tests (package multcomp: Hothorn et al., 2008) at the significance level of 5%.

2. Results

The RAA mortality, in the absence of predators, was very low ($\leq 0.4\%$) at the different studied RAA densities. There was thus no need

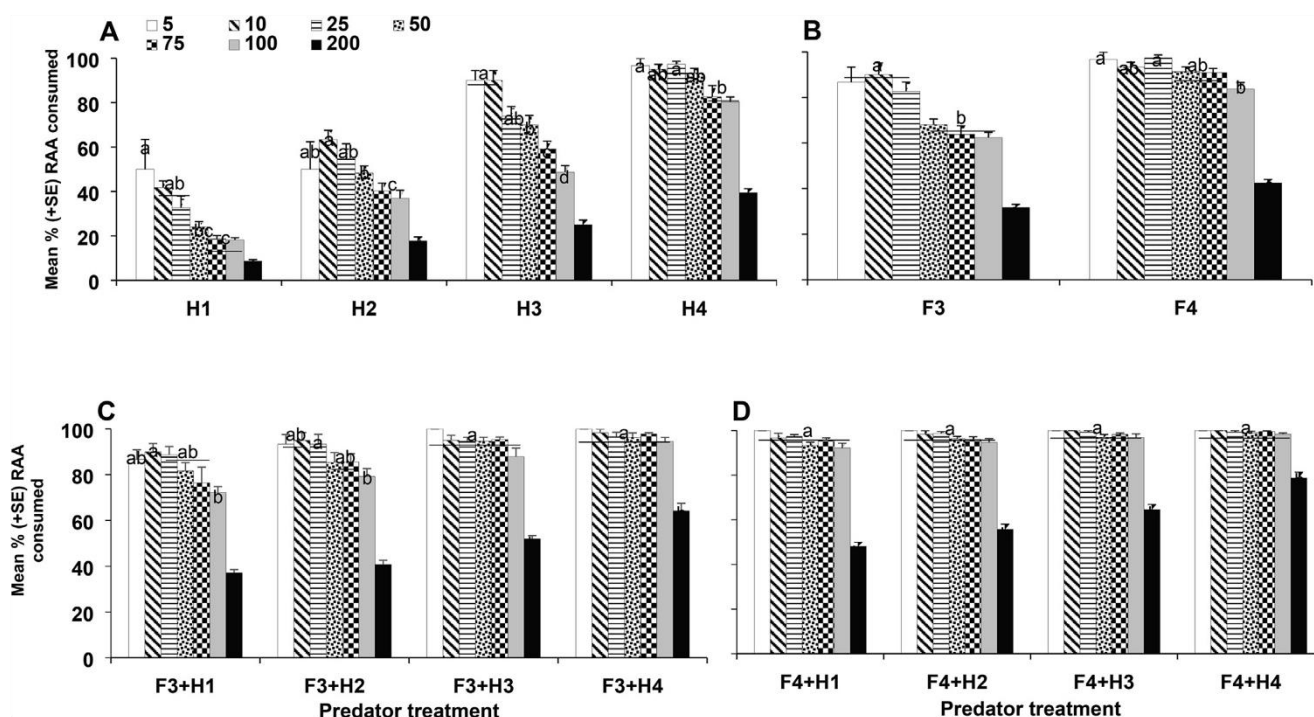


Fig. 2. Mean percentage (+1 SE) of 3rd instar nymphs of *Dysaphis plantaginea* (RAA) consumed over 24 h in relation to the seven RAA densities (5, 10, 25, 50, 75, 100 and 200) in the 14 predator treatments (six single treatments: (A): 1st (H1), 2nd (H2), 3rd (H3) and 4th (H4) instar larvae of *Harmonia axyridis* and (B): 3rd (F3) and 4th (F4) instar nymphs of *Forficula auricularia* and eight treatments in combination: (C) F3 + H1, 2, 3 or 4 and (D) F4 + H1, 2, 3 or 4). Different small letters represent significant differences in percentage of RAA consumption in relation to initial RAA density factor at the same predator species and instar treatment (mean separation by Tukey post-hoc, $P < 0.05$). Sample size for each comparison, $N = 7$ (aphid densities) \times 6 (replicates).

to correct the RAA mortality in the experimental treatments with predators. In the absence of predators: the RAA nymphs showed a 100% survival rate at the lowest three RAA densities studied (5, 10 and 25), 99.7% at the densities of 50, 100 and 200, and 99.6% at the density of 75. No predator instar was found dead after 24 h when they were individually left without RAA.

1.1. Occurrence of intraguild predation

No *F. auricularia* nymph died during the experiments. In contrast, 2.9% (11/384 possible cases) cases of mortality (6 H1 and 2 H2) and disappearance (3 H1) of the *H. axyridis* larvae were observed in the combined predator treatments. However, in the cases of mortality, the cadavers of ladybird larvae were always present and no external predation traces were observed on their bodies. Three larvae were dead in the F3 + H1 treatment at the RAA densities of 0, 5 and 10 (one for each), i.e. 16.7% of cases at only these densities. In the F4 + H1 treatment, three dead larvae were found at the RAA densities 5, 10 and 25 (one for each). In addition, three larvae disappeared at the initial RAA densities 0 (2 larvae) and 5 (1 larva). Twenty-five percent mortality and disappearance cases were therefore observed in the F4 + H1 treatment at low RAA densities (0–25). Two larvae were also found dead in the F4 + H2 treatment, one for each RAA density of 0 and 5, i.e. 16.7% of cases at these two densities.

1.2. Effect of predator species and instar, and initial density of *Dysaphis plantaginea* on predation

RAA consumption depended on predator treatment (Chisq = 157.2, $df = 13$, $P < 10^{-4}$) and the initial RAA density offered (Chisq = 224.3, $df = 6$, $P < 10^{-4}$) (Fig. 1). Multiple mean comparisons indicate that overall the predator species were significantly more voracious as their age increased. Further, younger instars of *H. axyridis* (H1 to H3) were less voracious than the instars of *F. auricularia*, while the oldest (H4) instar individuals were more voracious than F3 and similarly voracious to F4 individuals. Specifically (Supplementary material, Table S1 for detailed *P*-values), earwig nymphs were significantly more voracious than H1 and H2 instars at all initial RAA densities with the exception of one case (at RAA density of 5 comparing F3 and H1) (Fig. 1A, B, C and D). Compared with H3, F3 earwig nymphs predated significantly more RAA at the densities of 100 and 200 (Fig. 1E), and F4 nymphs predated significantly more RAA at the five highest densities (25–200) (Fig. 1F). In contrast, RAA consumption in H4 treatment was significantly higher than in F3 treatment at the five highest densities (25–200) (Fig. 1G) and was significantly lower than in F4 treatment

only at the 75 RAA density (Fig. 1H). The satiation level in our study was determined from the two highest RAA densities (100 and 200), at which the mean number of consumed RAA by predators in the single treatments did not change. It was thus found to be 17.8, 36.3, 49.4, 79.6, 62.9 and 84.3 aphids per day for H1, H2, H3, H4, F3 and F4, respectively (Fig. 1).

The proportion of RAA eaten by the predators (individual and in combination) was always significantly lower when the initial RAA density was 200 ($P < 0.05$ for all predator treatments, see Table S2 for detailed *P*-values) (Fig. 2). In the single predator treatments, the proportion of predated RAA decreased clearly with increasing RAA densities, especially for the younger stages, showing a surplus of prey (Fig. 2A and B). No significant differences were observed in the proportion of predated RAA for the first three (H1, H2, H3, F3), four (H4) and five (F4) initial RAA densities (Fig. 2A and B). The proportion of predated RAA was generally lower at the density of 100 compared to that of 75 and 50, but differences were seldom significant (Fig. 2A and B). For the combined treatments, the proportion of predated RAA was not significantly different at the other six RAA densities considering all F4 treatments with *H. axyridis* larvae (Fig. 2D), and two of the combined F3 treatments (F3 + H3 and F3 + H4) (Fig. 2C). In the other two combined F3 treatments, predation was significantly lower at the RAA density of 100 compared to that of 10 (F3 + H1) and 25 (F3 + H2) (Fig. 2C).

1.3. Individual and combined effects of predator instars on consumption of *Dysaphis plantaginea*

In most cases, when both predators were present, they consumed significantly ($P < 0.05$) more RAA than when only one of them was present, at the same life stage (Fig. 1). This was particularly marked at the higher RAA densities. The RAA consumption in the combined treatments was significantly higher than in each of the single treatments at the highest three (for H1 and F4, and for H4 and F3), four (for H1 and F3, H2 and F4, H3 and F4, and for H4 and F4) and five (for H2 and F3, and for H3 and F3) initial RAA densities (Fig. 1). At the lower RAA densities, RAA consumption in the combined treatments was also significantly higher, but only in one of the two single treatments, with some exceptions (Fig. 1). These exceptions were principally at the two lowest RAA densities (5 and 10), for which RAA consumption was not significantly different between the single and combined treatments (Fig. 1).

The voracity of individual *H. axyridis* younger instars (H1_2 group) was significantly the lowest at all initial RAA densities ($P < 0.001$ for all RAA densities, see Table S3 for detailed *P*-values) (Fig. 3). The

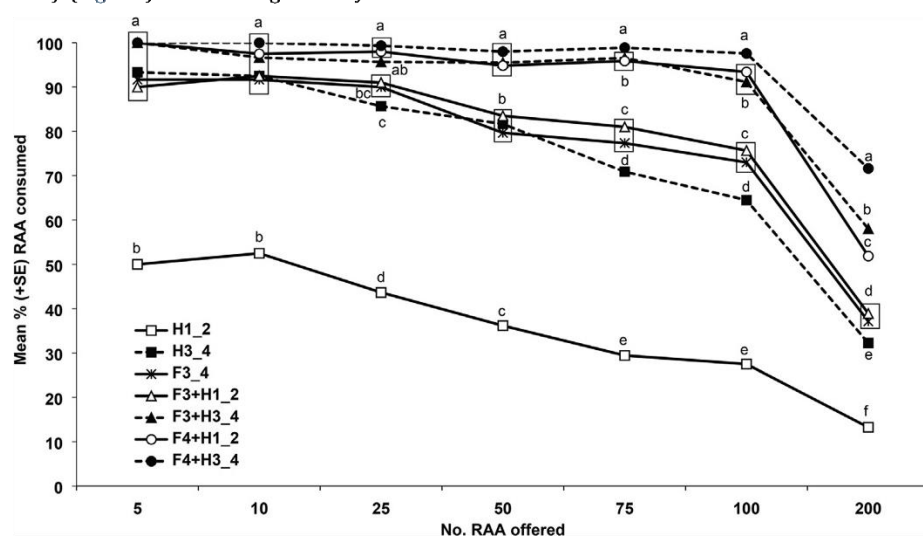


Fig. 3. Mean percentage of 3rd instar nymphs of *Dysaphis plantaginea* (RAA) consumed over 24 h in relation to the seven RAA densities (5, 10, 25, 50, 75, 100 and 200) in the seven groups of two treatments created from the 14 predator treatments (six single treatments: 1st (H1), 2nd (H2), 3rd (H3) and 4th (H4) instar larvae of *Harmonia axyridis* and 3rd (F3) and 4th (F4) instar nymphs of *Forficula auricularia* and eight treatments: in combination). Different small letters represent significant differences among predator groups at the same initial RAA density (mean separation by Tukey post-hoc, $P < 0.05$). Sample size for each comparison, $N = 14$ (predator treatments) \times 6 (replicates).

voracity of other groups was similar at the RAA densities of 5 and 10. At the 25 RAA density, predators ate significantly more aphids in the two groups combining F4 with *H. axyridis* than in the three groups H3_4, F3_4 and F3 + H1_2 (Fig. 3). Predation in these two latter groups was also similar to that in the group F3 + H3_4 (Fig. 3). At the RAA density of 50, predation was significantly higher in the combined treatments with the oldest *F. auricularia* and/or *H. axyridis* instar larvae, and in the combined treatments with younger *F. auricularia* and older *H. axyridis* (F3 and H3 or H4), as compared to all other groups (Fig. 3). At the RAA densities of 75 and 100, predation was highest with combinations of older instars of the two species (F4 + H3_4), followed by the two groups with combinations including older instars of at least one species (F4 + H1_2 and F3 + H3_4), then the two groups with combinations of younger instars (F3 + H1_2), or individual instars of *F. auricularia* (F3_4), and finally the group with older *H. axyridis* instars (H3_4) (Fig. 3). Results were the same at the RAA density of 200 except for the two groups with combinations including older instars of at least one species, with predators in the group (F3 + H3_4) eating significantly more aphids than in the group (F4 + H1_2) (Fig. 3).

The two predators in combination consumed less RAA than the sum of their separated consumptions, showing a significant but weak antagonist effect at the non-limiting RAA density of 200 (significant 'presence of *H. axyridis* × presence of *F. auricularia*' interaction, $P = 9.9 \times 10^{-4}$) (Fig. 4). That effect was, however, very small with 7.9% less consumption when the two predators were in the same arena

than the sum of their individual consumptions (mean consumption in combination ± standard error: 110.2 ± 4.02 aphids; sum of mean consumption of *F. auricularia* and of *H. axyridis* 119.75 ± 6.35 aphids).

4. Discussion

In this laboratory study, the predation of RAA depended on prey density, predator species and developmental stage, and the combination of predator species. The combination of two predators caused a slight agonistic effect but still increased their efficacy in the regulation of RAA, and very few indications of negative interactions were observed between predators (either intraguild predation or indirect interference).

Despite the fact that both *H. axyridis* and *F. auricularia* are generalist predators (Albouy and Caussanel, 1990; Koch, 2003), we observed very little evidence of intraguild predation. Only 2.9% of predators (i.e. 11 predators) died or disappeared. These cases were restricted to the first two larval instars of *H. axyridis* and only observed for the control treatment and at the lowest RAA densities (≤ 25), i.e. where there was no, or little, available food. In contrast, earwig nymphs were always found alive at the end of the experiment. In the aphidophagous assemblages, the feeding specificity of predators is a crucial factor determining the outcome of IGP. In other words, the predator should be sufficiently polyphagous to include non-aphid prey in its diet to play the role of intraguild predator (Lucas et al., 1998; Lucas, 2005; Yasuda et al., 2001). In addition, the relative size of competitors can also influence the direction of the interaction, with larger individuals preying on smaller ones (Lucas et al., 1998; Provost et al., 2005; Putra et al., 2009; Dib et al., 2016a). Lastly, IGP increases when the number of prey is low (Hindayana et al., 2001; N'óia et al., 2008; Ingels and De Clercq, 2011; Gagnon and Brodeur, 2014). Given the small size of the dead or unrecovered ladybirds, the tendency of *H. axyridis* to be less polyphagous than *F. auricularia* and the fact that dead individuals were only found at low RAA densities, it is likely that these ladybirds suffered intraguild predation. Alternatively, they may have died from indirect interference (competition for example) with larger *F. auricularia* nymphs that would have deprived them of food. Such interference is frequently observed among predators (Dib et al., 2016a; Orpet et al., 2019a) and would be consistent with the absence of external predation traces observed on the eight remaining dead ladybirds. It would also be consistent with the position of *H. axyridis* as a top predator, rather than prey, within the guilds of aphidophagous insects (Kajita et al., 2006; dishes. Other contradictory results have been observed in the existing literature concerning the impact of habitat structure and complexity on the frequency of IGP. This factor may be influential (Janssen et al., 2007;

Majerus et al., 2006; N'óia et al., 2008; Ingels and De Clercq, 2011). However, we suggest that this hypothesis (competition for food) played a negligible role in our study because if it was correct we would have observed the same mortality in controls H1 and H2 without a food source.

The confined space and controlled densities of extraguild prey in our laboratory conditions may have increased the possibilities for meeting and contact between these two generalist predators compared to the *in situ* environmental conditions in orchards. In natural settings, the existence of avoidance behaviour of natural enemies coexisting on the same site, by hiding in refuges for example, may be crucial in protecting them from IGP (Janssen et al., 2007; Dib et al., 2016a; Orpet et al., 2019a). In addition, as earwig predators are very polyphagous (Albouy and Caussanel, 1990), they can also eat bits of plants and thus, under natural conditions, they will rarely experience strong fasting conditions as in these experimental conditions. Orpet et al. (2019a) showed that antagonistic interactions between predators in an apple orchard were rare, and earwigs never antagonised other predators. Furthermore, Dib and his colleagues found that when *F. auricularia* and *E. balteatus* were confined together with RAA using the same experimental arenas used here, earwigs were never observed to attack syrphid larvae (Dib et al., 2011). However, when the experimental space was increased using apple seedlings, a few syrphid larvae were not found, or were found to be dead (Dib et al., 2016a). Hindayana et al. (2001) found that IGP still occurred between predators on bean plants, but it

was reduced approximately five-fold compared with that in Petri (Ingels and De Clercq, 2011) or not (Gagnon and Brodeur, 2014). However, by using Petri dishes as an artificial environment, our final goal was to encourage the two predator species to interact to assess the potential magnitude of these interactions.

Although both predator species have many common characteristics which can favour IGP (Albouy and Caussanel, 1990; Ware and Majerus, 2008), predator mortality and disappearance cases were too low to have a limiting effect on the efficacy of their combination. Rather, the combination of these two predators had more predation capacity than a single species, especially at the higher initial RAA densities. Our observations are consistent with the conclusions of Dib et al. (2011, 2016a) who showed that the combination of earwigs with syrphids actually increases the RAA regulation. The significant but small agonistic effect observed when the two predators were combined at non-limiting RAA densities, indicated that the two species express either

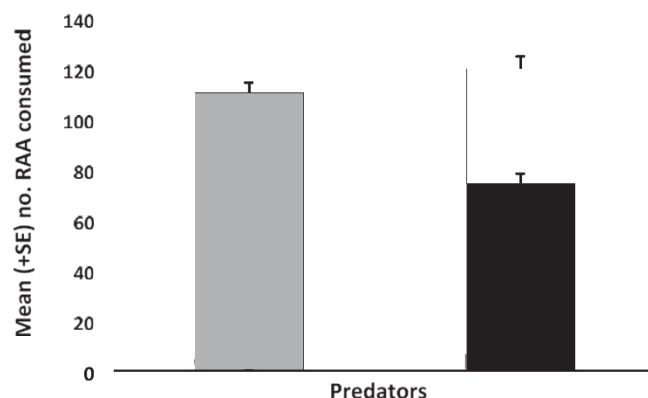


Fig. 4. Mean number (+1 SE) of 3rd instar nymphs of *Dysaphis plantaginea* (RAA) consumed over 24 h at the 200 density in the eight combined predator treatments (grey), four single *Harmonia axyridis* treatments (white), and two single *Forficula auricularia* treatments (black). Values are averaged over all predator stages. There were six replicates for each predator treatment.

intraguild predation or direct competition, possibly preventing each other's access to their prey via aggressive behaviour. That effect, however was very small (7.9%), especially considering the increase in consumption compared to a single predator species. Moreover, this small agonistic effect was probably due to the promiscuity caused by the small arenas.

RAA consumption increased for each successive instar, in agreement with the findings of other authors for *F. auricularia* (Dib et al., 2011) and *H. axyridis* (Koch, 2003). The larger or older life stages have higher food requirements, have faster digestive ability, and thus reach satiation level later than the smaller or younger ones (Mills, 1982; Omkar and Pervez, 2004). Even though the differences in amounts of RAA consumed (satiation levels) at the same instar of both predator species were not significant, earwigs generally showed slightly higher predation rates (62.9 and 84.3 aphids consumed per day by F3 and F4, respectively) compared to ladybirds (49.4 and 79.6 aphids per day for H3 and H4, respectively). This may be attributed to the fact that the ladybird larvae and earwig nymphs naturally differ in their feeding behaviour. In the field, earwigs visit and attack aphid colonies more frequently than ladybirds, and their individual attack duration lasts shorter (Orpet et al., 2019a). However, the foraging efficiency of both predators, especially *H. axyridis*, increased in our study conditions with increasing RAA density and aggregation compared to those in the field. In addition, all experiments were conducted at 20 °C, which is the preferred temperature for RAA development (Graf et al., 2006) and for predation potency of *F. auricularia* (Dib et al., 2011), but not for *H. axyridis*, which is more effective at 25 °C (Koch, 2003; Dib et al., unpublished results).

The initial RAA density had a significant effect on the regulation potential of both studied predator species and their combination. The predation rate was different in the single and combined predator treatments. In the single treatments, the proportion of predated aphids decreased clearly with increasing initial RAA densities, especially at younger predator ages. In contrast, a different trend was observed in the combined treatments, especially for the oldest instars of both predator species and their combinations with other instars. In these treatments, predators consumed a similar and constant proportion (> 90%) of RAA at the first six densities offered (5–100), indicating that saturation wasn't reached and they consumed even more prey, when given the chance. In theory, these combinations would be more effective at regulating RAA numbers in early spring before leaf curl (Wyss et al., 1999; Brown and Mathews, 2007; Dib et al., 2010a, 2010b).

The position of both these predator species as potential candidates in RAA biological control programmes is reinforced by their possession of effective defensive mechanisms and robust physical structures for the attacking and capturing of prey (Albouy and Caussanel, 1990; Labrie et al., 2006; Majerus et al., 2006; Ware and Majerus, 2008). *Forficula auricularia* possesses a volatile aggregation pheromone (Walker et al., 1993). The use of artificial shelters emitting this pheromone can manipulate the level of pest predation by earwigs in augmentative or conservative approaches (Dib et al., 2016b, 2017a; Quarrell et al., 2017). *Forficula auricularia* is also well equipped with strong mouth-parts of the chewing type and is known to defend itself and catch prey by moving their abdomen which is fitted with claw-like forceps (Albouy and Caussanel, 1990). Similarly, in addition to its alkaloid defensive chemistry (Majerus et al., 2006), *H. axyridis* larvae, in particular of 3rd and 4th instars, possess thick defensive dorsal scoli, which could withstand attacks by potential predators (Ware and Majerus, 2008). Such dorsal spines are also present, but not as developed, on younger larvae, which may in part explain why *F. auricularia* nymphs could attack 1st and 2nd larvae, but not those of 3rd and 4th instars in the current study. These latter instar larvae are also known for their greater body size, aggressive behaviour, mobility, and relatively large mandibles compared to the younger ones (Michaud, 2002; Snyder et al., 2004; Majerus et al., 2006; Provost et al., 2006).

This laboratory study showed weak IGP on the younger instar larvae

of *H. axyridis* when RAA were in few numbers or absent. The results indicate that *F. auricularia*-*H. axyridis* combinations, especially of their oldest instars, are promising aphidophagous candidates in early spring against RAA at the beginning of population build-up in the field and when few natural enemies are present. However, the simplified experiments reported here were designed to evaluate the maximum voracity of these predators and the responses of their inevitable enemies with each other, and eventually their effect on RAA regulation. The obtained results should therefore be supplemented by semi-field and field studies to evaluate the real effectiveness of both predators, and their interaction with RAA in more complex habitat structures, with more spatial and temporal refuges and with more diversity of prey and competitors. In such studies, factors affecting RAA regulation, such as environmental factors (Moerkens et al., 2011), principle escaping mechanisms of aphids (Mandour et al., 2006; Provost et al., 2006; Almohamad et al., 2008) and predators (Majerus et al., 2006; Orpet et al., 2019a), the searching behaviour of predators (Yasuda and Ishikawa, 1999; Dib et al., 2016a), and their interaction within the particularly rich aphidophagous guild of RAA (Dib et al., 2010a, 2010b), should be assessed. The development of effective tools, such as molecular tests (Sheppard et al., 2005; Gagnon and Brodeur, 2014; Orpet et al., 2019b) or video recording (Frank et al., 2007; Orpet et al., 2019a), could also be useful in investigating the existence of IGP interactions between these predators and their role in regulating RAA populations.

CRedit authorship contribution statement

Hazem Dib: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. Myriam Siegwart: Writing - original draft, Writing - review & editing, Visualization. Thomas Delattre: Formal analysis, Writing - original draft, Writing - review & editing. Marie Perrin: Writing - review & editing. Claire Lavigne: Formal analysis, Writing - original draft, Writing - review & editing, Visualization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2020.104394>.

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Impacts of cross effects between temperature and three different insecticides on codling moth susceptibility

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Introduction:

Temperature influences absorption, penetration, translocation & detoxification of pesticides by organisms. It is necessary to investigate interactions between pesticide & temperature to understand possible outbreaks of pests in a climate change context. We conducted an experiment to highlight effects of temperature & insecticides on codling moth *Cydia pomonella*, one of the main pests in orchards.

Goals:

Is there a cross effect between temperature & insecticides on codling moth larvae mortality?

→ Are insecticides more or less efficient at high temperature?

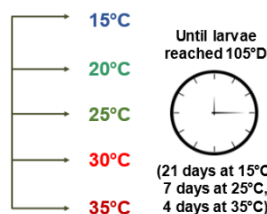
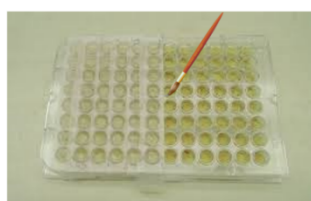
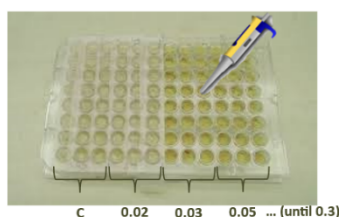
Methods:

Treatment with ≠ concentration of insecticides: Emamectin, Chlorantraniliprol & Spinosad

Deposition of newborn codling moth larvae
 n = 24/treatment

5 ≠ T°

Assessment of larvae mortality

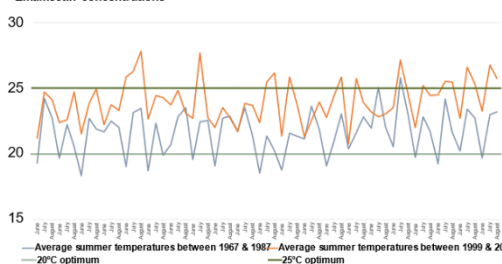
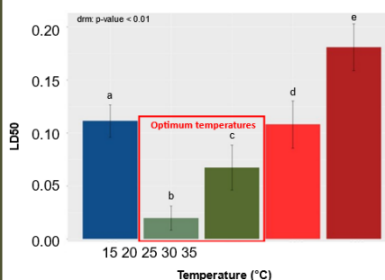
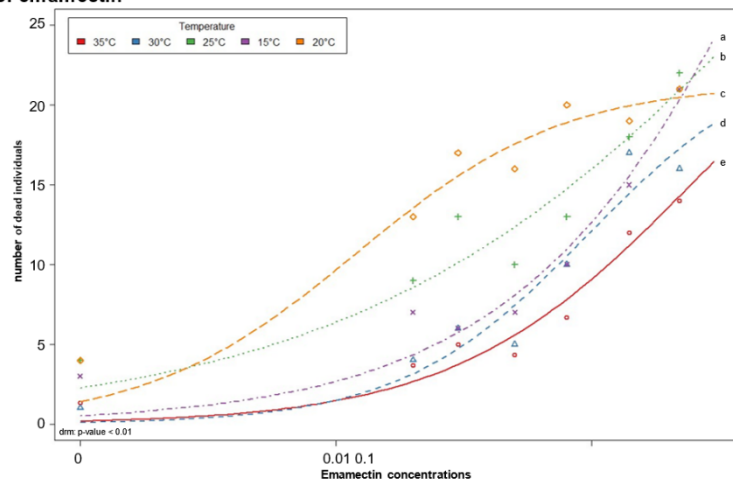


Number of dead larvae
 ↓
 LD50 depending on trial temperature

Exemple for emamectin

Results:

Results for emamectin



Conclusion:

Preliminary results on a susceptible strain of codling moths show:

- An increase in the LD50 at 35°C compared to lower temperatures for one of the three insecticides tested. The other two insecticides are being tested
- Such results have been reported in other pest species with other insecticides & highlight the need for research on this topic in order to adapt their application to future climatic conditions.
- Further research will be conducted to compare a susceptible and a resistant strain of codling moths in order to extrapolate effects of global warming on this adaptation.



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Liste des communications :**Passées :**

- **M. Perrin**. Journée carpocapse. 27 novembre 2018, INRA Avignon.
- **M. Perrin**. Journée Des Doctorants de l'Institut méditerranéen de la Biodiversité d'Ecologie Marine et Continentale (JDD IMBE). 1 juillet 2019, Arbois.
- **M. Perrin**. Resistance 19'. Présentation d'un poster: Impacts of cross effects between temperature and three different insecticides on codling moth susceptibility, du 16 au 18 septembre 2019, Rothamsted research, Harpenden, UK.
- **M. Perrin**. Journée carpocapse. 26 novembre 2019, INRAE Avignon.
- **M. Perrin**. Adalep 2020. 6 & 7 Octobre 2020. CNRS Montpellier.

A venir :

- **M. Perrin**. Journée carpocapse. 04 Février 2022, INRAE Avignon.
- **M. Perrin**. XXVI International Congress of Entomology, juillet 2022, Helsinki, Finlande.