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# Magic bullet or shot in the dark? Potential and limits of biological control for experimental ecology

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David Muru. Magic bullet or shot in the dark? Potential and limits of biological control for experimental ecology. *Vegetal Biology*. Université Côte d'Azur, 2021. English. NNT : 2021COAZ6008 . tel-03272503

**HAL Id: tel-03272503**

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# THÈSE DE DOCTORAT

## Solution miracle ou coup dans l'eau ? Potentiel et limites de l'utilisation des programmes de lutte biologique en écologie expérimentale.

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**Présentée en vue de l'obtention du grade  
de docteur** en Sciences de la Vie et de la  
Santé  
d'Université Côte d'Azur

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# Magic bullet or shot in the dark? Potential and limits of biological control for experimental ecology

Solution miracle ou coup dans l'eau ? Potentiel et  
limites de l'utilisation des programmes de lutte  
biologique en écologie expérimentale.

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## Résumé de la thèse

La lutte biologique (ou biocontrôle) est l'utilisation d'organismes vivants pour lutter contre les populations de ravageurs des cultures en réduisant leur densité et les dommages qu'ils causent. L'auxiliaire de lutte biologique peut interagir, directement ou indirectement avec d'autres organismes en plus du ravageur ciblé et inversement. Le suivi de ses populations, en plus du suivi d'autres variables écologiques, pourrait donc permettre de confirmer ou réfuter des théories écologiques ou découvrir de nouvelles interactions avec les facettes biotiques et abiotiques de l'écosystème. De plus, les aspects méthodologiques de la phase de suivi post-lâcher et ceux de l'expérimentation en écologie partagent de fortes ressemblances. Dans ce travail, j'explore comment ces deux disciplines peuvent être conciliées et comment les données qui découlent de la lutte biologique peuvent être optimisées pour leur utilisation en écologie.

J'utilise des données issues de programmes de lutte biologique pour traiter des questions en relation avec les dynamiques d'invasion, l'écologie des communautés et l'écologie du paysage. Dans le premier chapitre, je détaille les cas d'études utilisés : (i) l'introduction du parasitoïde *Torymus sinensis* contre la guêpe galligène *Dryocosmus kuriphilus* ; (ii) l'introduction de l'ectoparasitoïde *Mastrus ridens* contre le carpocapse de la pomme *Cydia pomonella* ; (iii) un inventaire des *Trichogramma* de France ayant pour objectif de caractériser les zones écologiques de chaque espèce ; (iv) la description des parasitoïdes oophages associés à *Iphiaclides podalirius* à fine échelle temporelle. Le chapitre 2 est centré sur l'étude de la dispersion de *T. sinensis* à l'échelle de plusieurs zones productrices de châtaignes. Dans ce chapitre, j'utilise des données de suivi sur les sites de lâcher pour en faire un modèle de croissance des populations de *T. sinensis* et ainsi inférer les dates de colonisations de sites naturellement colonisés. Dans le chapitre 3, je recherche les impacts du succès du contrôle biologique de *D. kuriphilus* par *T. sinensis* sur la structure de la communauté de parasitoïdes natifs, récemment associée au ravageur. Le chapitre 4 est dédié aux cas d'études où la valorisation scientifique varie d'un échec complet (primo-introductions de *M. ridens*), la diffusion de connaissances naturalistes (suivi d'*I. podalirius* et des parasitoïdes oophages associés) en passant par l'identification de motifs écologiques à l'aide d'outils statistiques spécifiques (suivi des espèces de *Trichogramma* à échelle nationale).

Enfin, en compilant les connaissances disponibles dans la littérature et mon expérience sur le terrain, je discute ensuite du potentiel et des limites de l'utilisation de la lutte biologique en tant qu'expérimentation en écologie. Je conclus que bien que la lutte biologique fournisse un contexte écologique à l'expérimentation en permettant la manipulation de plusieurs facteurs, le contexte et les organismes impliqués ne peuvent pas être adaptés à n'importe quelle problématique écologique. Par exemple, le parallèle évident entre la biologie de l'invasion et la lutte biologique fait de ce dernier un fort atout pour étudier les procédés qui régissent le succès des invasions. Cependant, les facteurs comme les faibles seuils de détectabilité d'un auxiliaire de lutte biologique à faible densité (couplé à la sensibilité des méthodes de suivis) peuvent faire de l'étude des dynamiques et interactions lors des stades précoces de l'invasion, une entreprise périlleuse.

Mots clés : Lutte Biologique, Ecologie expérimentale, Ecologie des communautés, Dynamique des populations, Acclimatation.



## Thesis Abstract

Biological control (or biocontrol) is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be. The biological control agent may directly or indirectly interact with more than just the target pest and vice versa. Therefore, monitoring its populations, in conjunction to other ecological factors, may allow to confirm or discard ecology theories or unveil brand new interactions with both abiotic and biotic facets of the recipient ecosystem. Moreover, the methodological aspects of the post release monitoring phase and those of ecological experimentations sometimes do share similarities. In this work I explore how both disciplines are reconciled and how the resulting data from biocontrol could be optimized for its use in ecology.

I use data from biological control programs to address questions related to invasion dynamics, community ecology and landscape ecology.

In chapter 1, I detail the case studies: (i) the introduction of the parasitoid *Torymus sinensis* (Hymenoptera: Torymidae) against the Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) ; (ii) the introduction of the ectoparasitoid *Mastrus ridens* (Hymenoptera: Ichneumonidae) against the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) ; (iii) a nation-wide survey of *Trichogramma* species in France in order to characterize the ecological ranges of each species; (iv) the description of egg parasitoid species associated with *Iphiclides podalirius* (Lepidoptera: Papilionidae) at a fine temporal scale. Chapter 2 is focused on understanding the dispersal of *T. sinensis* at the scale of several chestnut producing areas. In this chapter, I use monitoring data from release sites to fit a growth model for populations of *T. sinensis* in order to infer the time since colonization in naturally colonized sites. In chapter 3 I investigate the impacts of the successful control of *D. kuriphilus* by *T. sinensis* on the structure of native parasitoid community that recently became associated with the pest. Chapter 4 is focused on cases where scientific valorization ranges from a complete failure (primo-introduction of *M. ridens*), the diffusion of naturalist knowledge (survey of *I. podalirius* and related oophagous parasitoids) and/or the identification of some patterns using specific statistics (national survey of *Trichogramma* species).

Finally, by compiling knowledge from the extensive literature on biological control and field experience I then discuss on the potentials and limits of biological control programs for experimental ecology. I conclude that although biological control gives an ecological context to experimentation by allowing to manipulate a wide variety of factors, the context and the organisms at play may not be compatible with any ecological issue. For example, the obvious parallel between classical biological control and invasion biology makes the former extremely useful to study ecological processes that drive the success of invasions. This in turn could yield knowledge that may have implication in other disciplines such as the preservation of endangered species. However, factors like the low detectability of a biological control agent at low densities (coupled with varying sensibility of monitoring methods) may render the study of early stages dynamics and interactions too much of a daunting endeavor.

Key words: Biological control, Experimental ecology, Community ecology, Population dynamics, Classical biocontrol.

*“ Même sans même sang, on s’aimera. “  
A Géraldine, Iléna et ma famille.*

*“L’enfer des Hommes, c’est leur Amour des Dieux. “  
Mass Hysteria. – L’enfer des Dieux.*

*“Feeding on my tolerance is all you f\*\*\*in' do  
No looking back, no regrets, no apologies  
What you get is what you see”  
Slayer - Repentless.*

*“And I've Had Recurring Nightmares  
That I Was Loved For Who I Am  
And Missed The Opportunity  
To Be A Better Man.”  
Muse – Hoodoo.*



## Acknowledgements

I would first of all like to thank my partner **Géraldine Groussier** for supporting me during the last few months and for bearing that she would not see me a lot during the day. It is going to change, even though I always enjoy my long nights doing anything else than writing a thesis! But in any case, thank you for your help and support! Thank you **Iléna** for accepting me as a parental figure and allowing me to give you the love you deserve.

Next, I would like to acknowledge my family and especially **my Parents** and **my Abuela**, that also gave me their love and support not only through these past few months but during my whole life. I have a special thought for my little brother **Kevin** that will be finishing his thesis in about a year or so. Keep it up!!

Up next, my best friend **Jonathan “Sean” Cacheiro**, we have been friend for so many years that I cannot even count them anymore. Thank you for your support and for being there so often when I need it. Oh, and give me that gold now! Thank you, **Astrid Chesnay**, as well for taking good care of my bro, now for life! And **Julien Crine**, I am glad you are back.

Thank you, **Camille Duraj**, my dear friend and in a way, quite awkwardly, daughter. You are the only person that helped for some technical aspects of my thesis. Thanks for your company during field trips, it was quite fun!

I suppose next on the list is my closest work friend **Didier Crochard**, always available for coffee and the fun we had together was a serious relief during hard times. I am going to miss our beloved Wamps club!

Thanks, **Madalina Viciriuc**, for becoming one of my closest friends in the past few years and making me laugh so much. We shared many memories together with Géraldine: Romania, so many trips in the French countryside, swimming lessons, and so much more. Once this ongoing world mayhem has stopped, I hope we can start it all over again!

Thank you, **Ignacio Ruiz**, for becoming a good friend and for sharing some evenings playing 7D2D. That was cool, we need to do this again sometime soon Bro! Maybe someday in the same office who knows...!

Thank you, **Nicolas Borowiec**, for letting me in in your circle of friends and for introducing me to your Volley-Ball club. Can't wait to go and drink a few pints of Guinness!!

Thank you, **Elodie Vercken**, for giving me the chance to carry out this PhD. Thank you for your extremely useful insights, always top notch! And thank you for the time and faith you invested in me through the past 3 years.

Thank you, **Nicolas Ris** for your advice during these three years.

Thank you, **Sylvie Warot** for all your work with the molecular data through this whole manuscript.

Thank you, **Pieter and Brigitte Kan** for your insane field work contribution and, maybe the most important, awesome *raclette* parties!

Thank you, **Thierry Groussier**, for your help with the final SIG maps. Thank god you retired!

Thank you, **Alexia Crézé**, for your amazing drawings of some case study life cycles! Check her out on Instagram at *Maile98600*, wonderful stuff!

Now in a bunch because... Well... I don't need no reason! So, there you go:

Thank you, Laurent Kremmer, Laurent Lamy, Alexandra Auguste, Thomas Guillemaud, Eric Lombaert, Emeline Deleury, Silène Lartigue, Flora Aubree, Antoine Pasquier, Aurélie Blin, Thibaut Malausa, Marcel Thaon, Gabriel Zizzo, Nadine Sellier, Pierre Frendo, Benoit Industri, Olivia et Marc Magliano, Sandra Castellano, Marie-José Oddone, Séverine Burtey, Annick Groussier, Joël Bouchaya, Maxime Jacquot.

A special thanks to **Seán McLoughlin** and **GrayStillPlays** for being my nightly windows of fun and entertainment during the redaction of this work. **Seán**, I am truly sorry for your loss. I would like to offer you my deepest and most sincere condolences and may the soul of your father rest in peace.

And I guess, thank you **Covid19** for the extra months you gave me to finish the thesis, even though we would all have done better without you.

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# Introduction

## Experimental Ecology

### Observation, theory and experimentation: the triplets of Natural Sciences

Ecology, like all natural sciences, started with observations of intriguing patterns in nature that motivated the development of general theories of mechanisms and processes shaping these same patterns. For example, one of Darwin's major theoretical contributions, was based on patterns he observed by travelling around the world. For example, the fact that fossils of mylodons and sloth from our era shared astonishing similarities, or the fact that Galapagos turtles showed unique characteristics depending on the island they lived in (e.g., elongated necks in dry islands). It is because of such observations that he defied the theological theory of creation and proposed his theory of evolution (Darwin 1859). Theories ensure that scientific activity is guided by our previous knowledge about the world, allowing researchers to improve on past work and allowing new research fields to be developed. For example, Darwin's work presented above (and more) was the foundation for evolutionary ecology. However, experimentations need to be designed to challenge or validate these theories and improve our understanding of the complex systems that are ecosystems. Several decades ago, and for about 30 years, some authors criticized Darwin's theory because it makes untestable predictions (Waddington 1957, Birch and Ehrlich 1967, Peters 1976, 1991). However, experimentally validate or challenge a theory could be done by testing prediction or testing the assumptions on which the theory was based (Dayton 1973, Holt 1977). If all assumptions are proved to be true, then the theory may be validated. Darwin's theory assumptions are as follow: (1) there is variation within the traits that individual possess, (2) the variation is heritable and (3) natural selection exists (i.e., different traits equal different fitness). All three assumptions were overwhelmingly tested and supported (see Futuyama et al 1986 for (1) and (2), Endler 1986 for (3)) leading to the global acceptance of the theory of evolution.

Most of the time, experiments may be tricky to implement and carry out because of the need to control (and sometimes manipulate) ecological factors in order to measure their impact. For example, the enemy release hypothesis (ERH) predicts that the success of an invader in a new environment is due to their release from co-evolved natural enemies (Maron and Vilà 2001). In this case, validating the theory would require the comparison of the invader performances between invading populations and native ones while controlling other confounding factors (e.g., climat, differences in recipient communities etc.) Schoeman et al (2019) recently conducted an experiment to test ERH for a globally invasive amphibian, *Xenopus laevis* (Anura: Pipidae). By comparing the metazoan parasite communities of *X. laevis* from 20 invasive and 27 native sites in five countries and three continents they showed that invasive *X. laevis* harboured impoverished parasite communities that were distinct from those of native *X. laevis* from undisturbed habitats. Their study supported the ERH in terms of metazoan parasites as natural enemies, irrespective of the geographical origin, climatic conditions and invasion history of the host populations. In the absence of this theoretical link, they would have shown that parasite

communities are different between invasive and native populations of *X. laevis* and maybe propose ERH as a new theory.

Sometimes ecosystem may naturally undergo perturbations that locally modify ecological factors. Such situations (as it was the case for the work of Schoeman et al, 2019) could be used as an advantage as it renders possible the comparisons with locations that remain unperturbed. In some occasions, perturbations can be anthropogenic as, for example, urbanization, deforestation, or more importantly for us in this work: biological control.

### Biological control: an opportunity for experimental ecology

Biological control (or biocontrol) is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg et al 2001). To achieve such goals, it relies on modifications of the ecosystem, sometimes by introducing organisms (i.e., classical and augmentative biological control), or else, by modifying the environment (i.e., conservation biological control).

Both classical (CIBC) and augmentative biological control (AuBC) relies on the deliberate introduction of a natural enemy to control pest populations. In the case of CIBC, the natural enemy is a new addition to the recipient ecosystem whereas in the case of AuBC, the natural enemy is already present. For ease of reference, we will use the definitions of Eilenberg et al (2001) that build up onto previous definitions of each concept. Therefore, we will define CIBC as “*the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control*”. By adding a new link in the ecosystem’s food-web, the deliberate introduction of an exotic biological control agent has the potential to disrupt existing interactions, the same way natural invasion may do (see Louda et al 2003, Ehler 1998, Ewel et al 1999, Strong and Pemberton 2000). For instance, CIBC essentially aims at carrying out a “controlled” invasion. The term “controlled” refers to the facts that some parameters – e.g., the choice of the biological control agents (species and, sometimes, its genetic background), the introduction’s date and location(s), the modalities of introductions (local propagule size, spatial and temporal maps distribution of introductions), or the genetic background of biological control – can be *a priori* decided. Thus, CIBC may allow to compare metrics between invasive and noninvasive alien species, such as those related to translocation bias, propagule pressure, and foraging/reproduction/dispersal traits. This would allow to characterize the propensity of alien species to invade (Pyšek and Richardson 2007). Similarly, experimental work on CIBC has shown that all ecosystems are not equally vulnerable as they vary in features that determine their vulnerability to invasion such as community diversity, composition and assembly (Lonsdale 1999).

The two sub-concepts (i.e., inoculation and inundation BC) united under the AuBC banner need to be defined separately as both the practical approach and ecological implications distinguish these two strategies (Eilenberg 2001). Inoculation biological control will be defined as: “*The intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently*” and inundation biological control as “*The use of living organisms to control pests when control is achieved*

*exclusively by the released organisms themselves*". Similarly to CIBC, opportunities to conduct AuBC have been used to carry out experimental ecology. For example, Fournier and Boivin (2000) studied the functional relation between environmental conditions (i.e., wind strength and direction, accumulated solar radiation etc.) and the dispersal of two parasitoids: *Trichogramma evanescens* and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). They showed that accumulated solar radiation had different impacts on the two species. While it had no effect on *T. pretiosum*, when >15,000 kJ/m<sup>2</sup> were accumulated, more parasitism was observed for *T. evanescens*. Moreover, *T. evanescens* showed higher sensitivity to wind. The authors suggested that this may be related to adaptation to aggregated or rare hosts, which require more flight control for location. Kazmer and Luck (1995), used AuBC with *Trichogramma pretiosum*, to test the hypothesis of size-fitness that states that fitness following dispersal from the natal host increases with body size. They found that, contrary to the hypothesis expectations, wasp size is not a reliable predictor of individual or average cohort fitness. Consistent size-fitness relationships only emerge when fitness is averaged over many genotypes and environments.

The last strategy from biological control to cover here is conservation biological control (CBC). Again, we will follow the definition from Eilenberg et al (2001) that states: "*Conservation biological control is the modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests*". In this section, although CBC is extremely relevant as a framework for ecological experimentation, I will be more concise as all chapters are dealing with either CIBC or ABC. In CBC, unlike the other strategies, there is no releases of natural enemies. Nonetheless, the whole premise of CBC is based on modifications of the environment and therefore on stringent knowledge about the roles of habitat features (Hanski 1999, Hubbell 2001, Resasco et al 2017).

Overall, biological control strategies fulfill the requirements to carry out experimental ecology: the ability to control a perturbation and to monitor other response variables at the same time. It may therefore be used as a playground to investigate ecological processes.

## Biological control as a playground for experimental ecology

### Population dynamics

Classical and augmentation biological control programs rely on the stability of the host parasitoid interaction and have been central in host density dependence experimental research. They are basically invasions in the form of replicated experimental introductions to natural communities that enable to detail the population dynamics and habitat occupancy patterns associated with the initial establishment of an invasive species (CIBC) or a spike in natural enemy abundances (AuBC).

The field of population dynamics focuses on the distribution and growth of populations and how ecological interactions may shape them. Predators and parasitoids being an important component of terrestrial communities, they are a central interest to ecologists and their pursuit of unravelling the complexity of factors driving the dynamics of species interactions. Population dynamics have a long history and extensive literature, including many studies on insects (Price 1997, Murdoch 1994, Gotelli 1995, Murdoch & Briggs 1996, Speight et al. 1999).

More particularly about, predator-prey or host-parasitoid interactions and the forces that stabilize them. Historically, the rise of the field of population dynamics have occurred in conjunction with mathematical models such as the renowned work of Lotka (1925) and Volterra (1926). Mathematical models of population dynamics are of considerable interest because of their proven power to make important insights into the dynamics of real populations and communities (May, 1974; Nisbet and Gurney, 1982; Murray, 1989; Shigesada and Kawasaki, 1997).

Spatial and temporal patterns of interactions between species (e.g., host-parasitoid) such as host density dependence may determine the stability of such interaction. For instance, the tendency for parasitoids to aggregate in patches where host density is high ("aggregation to host density") has been suggested as a powerful stabilizing force in host-parasitoid systems (Hassell and May 1973, 1974, Beddington et al. 1978, May and Hassell 1981, Heads and Lawton 1983, Waage 1983, Hogarth and Diamond 1984). Recently, Morgan et al (2017) found that *Chrysonotomyia pulcherrima* (Hymenoptera: Chalcidoidea), a specialist parasitoid of mango gall fly *Procontarinia matteiana* (Diptera: Cecidomyiidae), exhibits an inverse host-density dependence and a density-independent aggregation. This aspect of population dynamics is important as it may regulate the size and growth of the population (Hawley 1985a, 1985b; Service 1985, Murdoch 1994) and therefore, could have profound impacts on biological control success. Moreover, Morgan et al (2017) found higher parasitism rates near vegetation. Thus, they concluded that natural vegetation plays a role in promoting stabilizing aggregation of parasitoids, possibly through provision of non-host resources (e.g., nectar, pollen).

As a result of stochastic or demographic changes in abundance or density, a population may experience a decrease in density. At low population densities, some species are subject to the "Allee effect". An Allee effect is a positive relationship between population size or density and a positive association between absolute average individual fitness over a finite interval (Stephens et al 1999). In some cases, the population cannot persist below a critical population size (Stephens and Sutherland 1999). The Allee effect is studied at any moment of a population's dynamics but a large majority of studies focus on a specific stage: population establishment (e.g., Drake and Lodge 2006, Fauvergue et al 2007, Gertzen et al 2011). In fact, invading populations are usually small and therefore may exhibit an Allee effect and collapse. Therefore, deliberate introductions realized in the frame of biological control seem naturally adapted to study the Allee effect in invading populations and its relation to establishment success.

Invading populations may differ in the number of individuals entering the new environment and the number of discrete invading events (Lockwood et al. 2005). These features are addressed together in the concept of propagule pressure. Propagule pressure may shape the patterns of interactions between the invasive species and the native community. Reviews have suggested that invasion success can be primarily attributed to propagule pressure (Lockwood et al. 2005; Hayes and Barry 2008), especially during early stages such as persistence at introduction sites (Mikheyev et al. 2008) and colonization of new sites (Jeschke and Strayer 2006). Releasing higher numbers of individuals or repeated releases over several years have been shown to increase the chances of getting the biological control agent established (Beirne 1975, Cameron et al 1993). However, it is unclear how the components of propagule pressure

combine to determine invasion success. Nonetheless, how they determine invasion success is also addressed as optimal release strategy theory in biological control (Shea and Possingham 2000). In natural invasions, the components from propagule pressure are usually unknown, making it often impossible to use them to relate propagule pressure and establishment success. In this context, CIBC represents a good opportunity to gather empirical data on how propagule pressure shapes the establishment success (see Memmott et al 2005, Yeates et al 2012, Borowiec et al 2018).

### Community ecology

When it involves the introduction of an exotic (or native) organism in a new ecosystem, biological control may impact ecological processes that drive ecosystem stability. For instance, the biological control agent may instigate, or be a target, of new interactions that may destabilize the recipient (agro)ecosystem (e.g., competition or predation). Conveniently, community ecology studies interactions between populations of different species such as predation and competition and evaluates their impact on the stability of ecosystems.

Predation is the consumption of all or part of one living organism by another. Predator-prey interactions involve species that reside on many different trophic levels, including the impacts of herbivores on plants, carnivores on herbivores, carnivores on other carnivores, and parasitoids on hosts. As mentioned above, predation shapes population dynamics of both protagonists (predators and preys) and is at the heart of both CIBC and AuBC. As a matter of fact, biological control has even been used to study predation among species from the same trophic level (Rosenheim et al 1995). This interaction is called intraguild predation. A textbook example would be intra-guild predation involving the biological control agent and a naturalized (non-native but long established) natural enemy (Chacon et al 2008) which can, in turn, induce the displacement of native species (Dixon 2000, Koch 2003). In the study from Chacon et al (2008), *Aphidius colemani* (Hymenoptera: Braconidae) was used as a surrogate for Asian aphidiine braconids such as *Binodoxys communis* (the biological control agent).

One way to define interspecific competition is as a mutually negative interaction between two or more species within the same guild or trophic level. Negative competitive interactions manifest themselves as reduced abundance, decreased fitness, or a decrease in some fitness component, such as body size, growth rate, fecundity, or survivorship and may even lead to the niche displacement of species. Resource competition between several biological control agents or between a biological control agent and the native community (Schellhorn et al 2002) has been extensively documented through biological control programs. For instance, Schellhorn et al (2002) empirically showed that an introduced parasitoid could extirpate a native parasitoid of the same pest. In this case, extirpation occurred because of the synergy between the better foraging abilities of the exotic parasitoid and frequent disturbances due to farming practices. Apparent competition is an indirect interaction between two species that is mediated by a third species (Holt 1977, Bonsall and Hassell 1997, van Veen et al 2006). In extreme cases of resource competition, species may undergo a niche displacement, being forced to change host or prey in order to still persist in the ecosystem (Messing et al 2006) due to the high performances of competitors. A well-known example is the work of Murdoch et al. 2003 on the wasp *Aphytis melinus* used to control California red scale *Aonidiella aurantii*, a pest of multiple

species of citrus. The more effective natural enemy *Aphytis melinus* competitively displaced a less effective natural enemy *Aphytis lingnanensis*. If biological control programs were adapted to study resource competition, they are also vastly used to study apparent competition. For example, in recent years, Jaworski et al (2017) tested the occurrence of apparent competition between the major tomato pests *Tuta absoluta* and *Bemisia tabaci* when preyed on by a generalist mirid bug *Macrolophus pygmaeus* (an AuBC agent), by monitoring pest and predator population dynamics in a greenhouse experiment. Although the importance of apparent competition in structuring insect communities was discussed since the early 80s (e.g., Lawton and Strong 1981, Freeland 1983, Jeffries and Lawton 1984) its first empirical evidence came from biological control with the works of Settle and Wilson (1990) and Evans and England (1996) on the grape leaf hopper and the alfalfa weevil respectively.

The diversity of species and interactions between them has been argued to be a determinant factor in ecosystem stability. Robert MacArthur (1955) and Charles Elton (1958) suggested several reasons why more complex communities might be more stable than simple ones. Here stability means a range of things, but can refer to both the tendency for populations to persist while showing low levels of temporal variation, and to the tendency for community composition to remain unchanged. Theory on species diversity and species coexistence has outpaced experimentation for a long time, but several empirical works have been carried out in the last decade or so (e.g., Stachowicz et al 2008, Rogers et al 2014). As I have already discussed, biological control programs have been extensively used to study interactions between species. However, experimental evidence for the diversity-stability theory is rarely obtained from biological control (but see: Ong and Vandermeer 2015). In fact, a large amount of the literature comes from communities such as grasslands (Tilman and Downing 1994, Tilman 1996) or aquatic microbial food webs (Mc Grady-Steed et al 1997, Mc Grady-Steed and Morin 2000).

### Landscape ecology

Biological control is usually deployed over multiple locally restrained areas, granting the opportunity to investigate the role of landscape features on population interactions and dynamics. Furthermore, agroecosystems, which is the target ecosystem for biological practitioners, are a particular kind of landscape. In fact, anthropogenic actions simplify the landscape by, among other things, increasing fragmentation of natural habitats (Tschardt et al 2005, Baessler and Klotz 2006). Furthermore, agroecosystems are composed of farmlands of which management can be actively modified (Carcamo 1995, Bengtsson et al 2005) and need sustainable regulation of pest populations (Lawton and Brown 1993, Swift et al 1996, Koss et al 2005). Overall, the lower complexity of ecological interactions within agroecosystems and the ability to replicate experimentation, and control ecological variables motivates the use of biological control for investigating landscape ecology.

Landscape structure has been shown to affect community structure, species richness and abundance, population dynamics and interactions within and between trophic levels and naturally the efficiency of biological control (Kareiva 1987, Marino and Landis 1996, Zabel and Tschardt 1998, Tschardt and Brandl 2004, Bianchi et al 2006, Finke and Denno 2006, Woodcock et al 2007). In fact, communities, are made up of species with different spatial

strategies (Ettema and Wardle 2002, Kareiva 1990, Steffan-Dewenter et al 2002) and the spatial scale of population processes is contingent on the species' trophic level (Holt et al 1999, Lawton 1995, Pimm 1991). Hence, decreasing size and connectivity of habitats as well as changes of the landscape type between habitats may not only decrease population densities and species richness, but also disrupt plant-herbivore, herbivore-enemy, and plant-pollinator interactions (Didham et al 1996, Matthies et al 1995, Steffan-Dewenter and Tschamntke 1999, Tschamntke and Kruess 1999). In the context of pest biological control, landscape structure also mediates the interactions between native and introduced biological control agents (Didham et al 2007). It is known to influence natural enemy abundance and pest control in other agricultural systems (Bianchi et al. 2006, Chaplin-Kramer et al. 2011, Rusch et al. 2016). For example, biological control has been shown to be hindered by landscape simplification correlating with increased pest numbers and significantly lower yield (Grab et al 2018). Semi-natural habitats have been considered as key landscape features involved in biological control success. For instance, in mango orchards, parasitism rate of the mango gall fly (*Proncontarinia mattheiana*) by its parasitoid *Chrysonotomyia pulcherrima* decreased as distance from natural vegetation increased (Morgan et al 2017). Tomasetto et al (2017) have even suggested that the changes in landscape (mainly the loss of natural habitat acting as refuge sites for natural enemies), mediated by the intensification of agricultural practices, causes the agroecosystem to evolve a resistance towards biological control

### Evolutionary Ecology

As detailed above, biological control subjects both biological control agents and native species to modifications of a variety of ecological interactions. In response to this change in environment, some protagonists may be subject to evolutionary forces.

Firstly, as the result of specific antagonistic coevolution, which is vastly illustrated with host-parasitoid models, species may evolve to exploit resource or avoid predation more efficiently. This is a process referred to as « arms race ». For example, the parsnip *Pastinaca sativa* is known to increase in toxic furanocoumarins as they coevolve with their major specialist herbivore, the parsnip webworm, *Depressaria pastinacella* (Zangerl and Berenbaum 2005). Here the increased toxicity of the weed was systematically observed after reassociation with its coevolved herbivore in non-native areas. In fact, after invading new areas in the world the weed reallocated its resources from chemical defense into growth and reproduction. However, after its herbivore resumed the interaction with the weed in the invaded areas decades later, weeds developed unexpectedly high levels of toxic furanocoumarins (ibid.). In classical biological control, pest may experience evolution driven by the introduction of a biological control agent. In fact, sometimes pests outperform their natural enemies in this arms-race and lead to significant decrease of biological control success. For instance, Tomasetto et al (2017) showed that parasitism rates of an introduced biological control agent may decrease over time due to growing resistance to parasitism among pest populations. In their case, this is supported by the fact that the parasitoid undergoes parthenogenic (thelytokous) reproduction, whereas the pest reproduces sexually. Similarly, Stastny and Sargent (2017) reported that the chrysomelid beetle *Neogalerucella calmariensis*, introduced into Canada for control of invasive *Lythrum salicaria* can rapidly select for increased resistance (increased antiherbivore defenses) and tolerance

(faster regrowth). Sap feeders such as whiteflies, aphids or mealybugs are known to have coevolved (each separately) with eubacterial endosymbionts (Clark et al 1992) providing essential nutrients to the host (Srivastava 1987). This background, in relation to pest biological control, resulted in the first report of parasitism resistance in aphids induced by a coevolved endosymbiont (Oliver et al 2003).

Secondly, evolutionary forces could stem from a response to an environmental perturbation. Invading species (e.g., CIBC agents) experience novel abiotic and biotic conditions in their introduced environments that can include climates that differ from what they are adapted to, altered availability, distribution, genetic composition, defense, or phenology of their hosts or novel predators, parasitoids, and competitors. These novel ecological conditions may impose strong natural selection, which can lead to evolutionary change (Reznick & Ghalambor, 2001, McEvoy et al 2012). Natural selection is the differential fitness of individuals due to variations in phenotype that lead to the spread of advantageous traits through heritability in a population (Endler et al 1986). For example, biological control agents have been shown to experience changes in critical daylength for diapause inductions (Bean et al 2012) or increasing development speed and survival when exposed to shorter growing seasons (McEvoy et al 2012, Szucs et al 2012).

Finally, over certain circumstances, the ecological interactions induced by biological control may lead some populations to persist at small sizes, having several potential evolutionary implications. Firstly, the number of founders in a newly introduced population as well as their allele composition may have strong impact on their fitness, population dynamics, dispersal and their ability to coevolve with an antagonistic organism (Briskie and Mackintosh 2004, Hufbauer et al 2013, Szucs et al 2014). In fact, the gene pools of a few individuals (which may not reflect the gene pool of the source population) will restrain the allele composition of the invading population. This evolutionary mechanism is referred to as the “founder effect”. This process may take place into any population that experience a bottleneck (i.e., a drastic reduction in size). CIBC is particularly prone to creating bottlenecks in natural enemy populations before introducing them in the target area (e.g., during sampling, rearing or releasing). This founder effect is at the root of two major evolutionary forces that impacts small populations: genetic drift and inbreeding.

Genetic drift refers to random change in the frequencies of alleles from generation to generation due to stochastic fluctuations (Masel 2011). Genetic drift may cause gene variants to disappear completely and thereby reduce genetic variation (Star and Spencer 2013). It can also cause initially rare or deleterious alleles to become much more frequent and even fixed. This may happen after the population experience a bottleneck and its size is greatly decreased. When populations are small, the rate of inbreeding increases (mating amongst siblings), increasing the damage done by recessive deleterious mutations, in a process known as inbreeding depression (Wright 1977, Shields 1987). Concern about inbreeding is particularly great when population sizes remain small for long periods, as it is often the case for small introduced population that may experience a demographic lag phase (Coutts et al 2018). For instance, some literature (e.g., Baker et al., 2003; Hufbauer et al., 2004; Lloyd et al., 2005) suggest that biological control agents do indeed experience bottlenecks in population size that reduce variation in neutral loci as predicted on theoretical grounds (Hopper et al., 1993). Although the consequences of lower neutral loci variation have not been studied directly in classical

biological control, it has been shown to reduce fitness of a parasitoid used in augmentative biological control (Hufbauer 2002, Hufbauer et al 2004).

## Research question

Despite the number of biological control programs carried out in the last 50 years, few are used as a mean to carry out experimental ecology. For example, Memmott et al (2005) used the classical biological control of broom (*Cytisus scoparius*) to investigate the effect of propagule size on the invasion of the biological control agent *Arytainilla spartiophila* (Hemiptera: Psyllidae). To do so, they manipulated the propagule size of their releases in several locations on a 135 km transect and sampled populations during five years. In the frame of conservation biological control, Ortiz-Martinez and Lavandero (2018) studied the temporal effect of landscape context on the natural enemy assemblages regulating *Sitobion avenae* populations (Hemiptera: Aphididae). On the other hand, most biological control programs are carried out without seeking ecological knowledge and rather detail establishment or pest control without relating to ecological processes (e.g., Garcia-Mari et al 2004, Charles et al 2019).

Biological control programs used as a mean to carry out experimental ecology share some similarities that, I think, are the reason why so few biological control programs yield ecological knowledge. They all start with: (1) a clear ecological hypothesis to test, (2) a fleshed out experimental design to test it and (3) the sufficient funding to carry out a post-release monitoring phase to gather data according to said experimental design. Here it would be easy for one to hypothesize that the lack funding could be the most limiting factor out of the three mentioned above. As a matter of fact, funding supporting the research and development of such programs is, at best, oriented towards agronomic outcome (Fowler 2000) and sometimes even not linked to biology (Schaffner et al 2020). For instance, Julien et al. (1984) pointed out that “financial support varied with nearly every release and possibly affected the outcome”. In addition, post-release surveys of a biological control agent may be a consequent endeavor for research teams and may need to get the necessary funding a few years after the releases, when funding agencies already lost interest on this particular management strategy on a particular crop.

Are biological control programs with enough funding for post-release monitoring an efficient framework to carry out experimental ecology? I will try to answer this question by using case studies focused either on population dynamics, community ecology or landscape ecology. Through them, I will investigate the potential and limits of the data gathered, keeping in mind the realistic constraints inherent to biological control programs.

# Chapter 1: My case-studies

## *Torymus sinensis* and the Asian chestnut gall wasp *Dryocosmus kuriphilus*

### Biology and Ecology

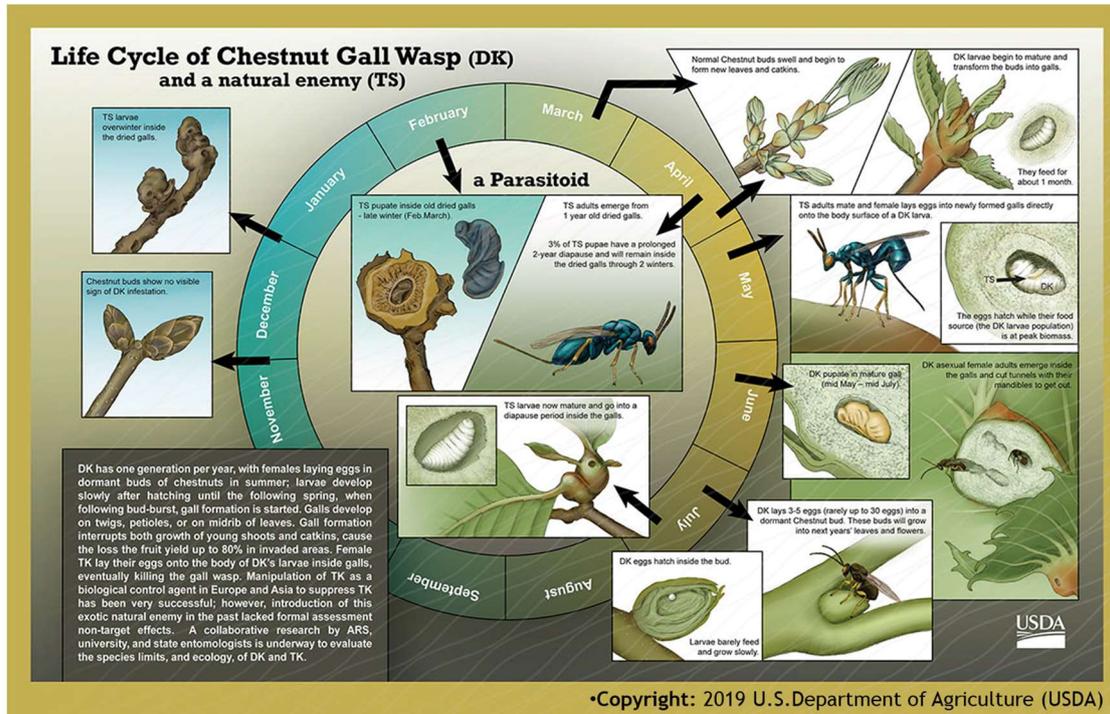


Figure 1 - Life cycle of the chestnut gall wasp and its natural enemy *T. sinensis*. Copyright: USDA. 2019

*Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), also known as the Asian Chestnut Gall Wasp (ACGW), is a small gall-inducing wasp that is specialized on attacking chestnut trees (*Castanea* spp., Fig 1). It is considered as the most important pest on chestnut trees in the world (Brussino et al 2002, Moriya et al 1990) as it can decrease the chestnut yields by 60 to 80 % (EFSA, 2010, Payne et al 1983).

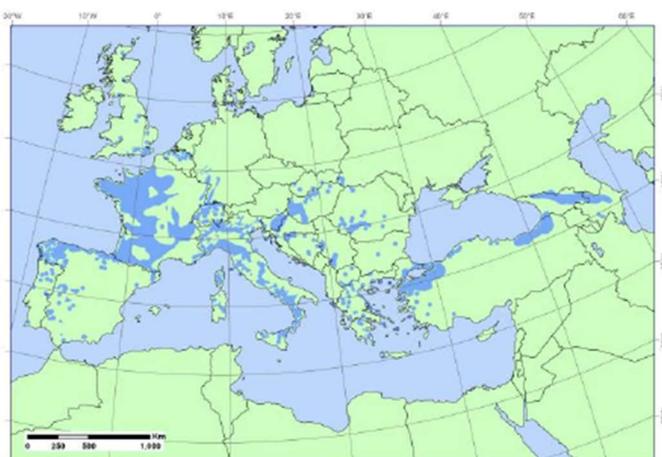


Figure 2 - Distribution map of *Castanea sativa* in Palearctic region (EUFORGEN, 2019)

The ACGW is native to China and first invaded neighboring countries. First noticed in Japan (Okoyama) in 1941 (Murakami et al 1994), its invasion was fast as it was present in the whole country in the late 50's (Moriya et al, 2003). Then, ACGW was monitored in Korea in 1958 and successfully invaded the whole country as well (Murakami et al 1995). In 1999, the ACGW was detected in Népal (Abe et al 2007). In 1974, the ACGW was monitored outside of Asia for the first

time, in the south east off the United States of America, in the state of Georgia and then invaded other states before invading the North of the USA (Rieske, 2007). In 2002, it was detected in Europe for the first time in Italy (Brussino et al 2002, Quacchia et al 2008). From 2005, new populations of the ACGW were discovered in Slovenia (Knapic et al 2009), Hungary (Csoka et al 2009) and Switzerland (EPPO RS, 2009). In France, first isolated spots of ACGW were observed from 2005 close to the Italian border but its pervasive presence in the south of France was only patent from 2010.

The ACGW is an univoltine thelytokous species (Tamura 1960a, 1960b, 1965). Females lay their eggs in summer within chestnut buds and each female can lay up to a hundred eggs. Eggs hatch 30 to 40 days after being laid and larvae spend winter within the buds. Next spring, the presence of larvae triggers the formation of a galls and the larvae finishes its development from May to July and adults emerge from June to August.

*T. sinensis* is a closely associated parasitoid of *D. kuriphilus*. It is a solitary ectophagous parasitoid and realizes only one generation per year, like its host. *T. sinensis* females lay eggs in newly-formed *D. kuriphilus* galls in early spring. The parasitoid larva feeds ectophagously on the host larva. By late spring the mature larva (with characteristic brown stripes on the abdomen) has already stopped feeding, but it does not pupate until winter and the adult emerges the following spring.

#### Classical biological control against the ACGW

Soon after the ACGW began its worldwide invasion, efforts were focused on classical biological control (Murakami et al 1977). Everywhere ACGW invaded, several indigenous species were known to attack the ACGW. However, it was never enough to efficiently regulate populations of ACGW (Murakami and Gyoutoku 1995, Aebi et al, 2006, 2007, Cooper and Rieske 2011, Murakami et al 1994, Ôtake et al 1982). The inventory of ACGW associated parasitoids in China has allowed the identification of a candidate for classical biological control: *Torymus sinensis* (Hymenoptera: Torymidae).

This parasitoid was successfully introduced in Japan (1975), USA (1977) and Italy (2005) (Rieske 2007, Quacchia et al 2008). At least in Japan and Italy, *T. sinensis* was successfully

established and the ACGW infestations were significantly reduced after around 8-10 years (Moryia et al 2003, Quacchia et al 2014).

In France a classical biological control program against the ACGW was started in 2011. The main objective was to durably regulate the population of the ACGW and thanks to the establishment and naturalization of *T. sinensis*. In addition, this was also the opportunity to experimentally manipulate the number of propagule (of the introduced *T. sinensis* populations) along with their size, and

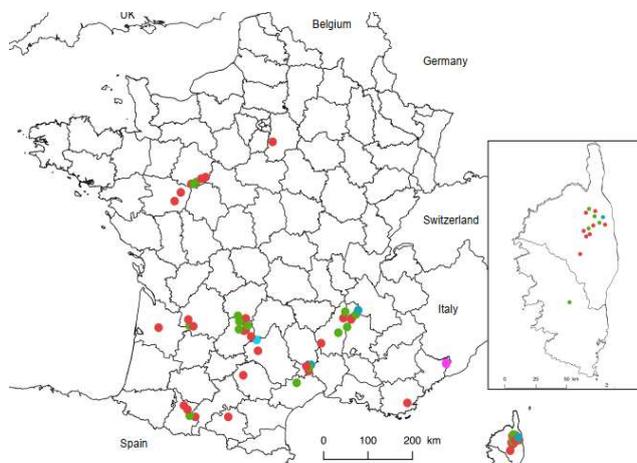


Figure 3 - Map of the releases of *T. sinensis* in France. Colors refer to the initial propagule pressure used in the releases. See Borowiec et al 2008 for more information.

describe their impact on the establishment and spread of the biological control agent. To do so, the project was separated in two main phases: (i) the introduction of *T. sinensis* in France and, (ii) the monitoring of *D. kuriphilus* populations and those of its parasitoids. Releases were carried out at a national scale within 58 sites between 2011 and 2015 (Fig 3). Eleven sites were used as control and were monitored even though no releases were carried out.

### *Mastrus ridens* and the codling moth *Cydia pomonella*

#### Biology and Ecology

The apple codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), is a worldwide distributed pest of apple, pear, walnut and quince trees. Everywhere it is established, it has a serious influence on the productivity and selling of the fruits it attacks. Indeed, as an internal feeder, it makes the fruit unacceptable to the consumer and can cause a decrease in apple harvest from 30% up to 50% (Balasko et al 2020).

The codling moth originated in central Asia (Mills 2005) which is the area where apple trees originated as well (Geibel et al 1999, Harris et al 2002). It is currently present everywhere apples are grown with exception of eastern China, Japan and western Australia (Mills 2005).

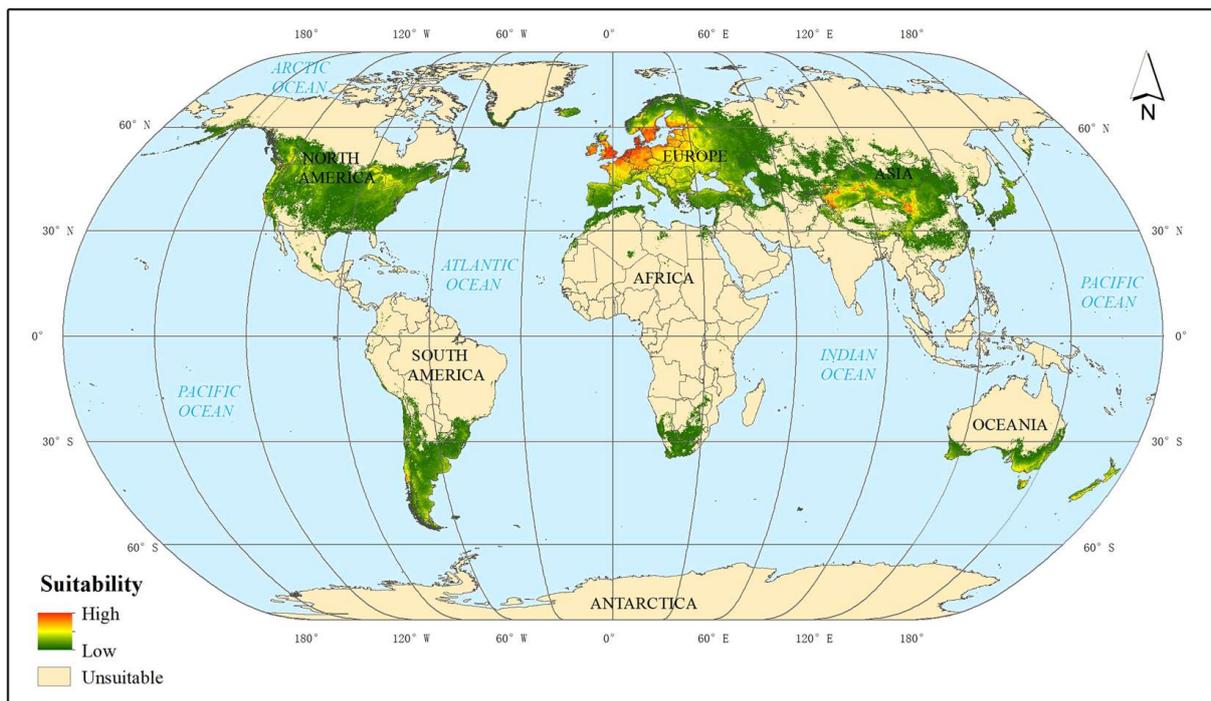


Figure 4 - Map of the suitable areas for *Cydia pomonella* realized by using MAXTENT machine learning. From Jiang et al 2018

Codling moth, *Cydia pomonella* (L.), deposit most eggs on leaves near apples but few on the fruits themselves (Jackson 1979). As soon as it emerges from the egg, the first instar larva searches a fruit in which it will eat its way in. The larvae will grow inside the fruit until the fifth

instar, when it leaves the fruit to pupate either in tree bark or in the soil. In France, the codling moth realizes 2 to 3 generations and diapauses through winter.

Until 2009, *Mastrus ridens* is native from Kazakhstan and was referred to in the literature as *Mastrus ridibundus* (Horstmann 2009). *M. ridens* is an idiobiont ectoparasitoid specialized on *C. pomonella* (Fig 5). Females locate codling moth during their last larval stage from a kairomone in its silken cocoon (Jumean et al 2005).

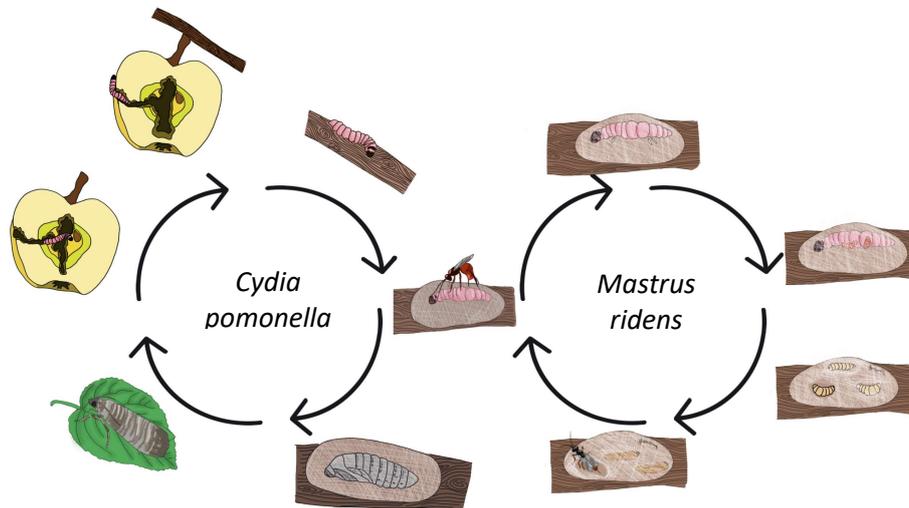


Figure 5 - Life cycle of *Cydia pomonella* and its specific parasitoid *Mastrus ridens*. Drawing by Alexia Crézé.

#### Classical biological control against *C. pomonella*

The use of chemicals against *C. pomonella* in apple integrated pest management programs have been a driving factor in secondary pest outbreaks (Hoyt 1969). Therefore, efforts have been oriented towards alternative solutions such as the use of entomopathogens (see Lacey and Unruh 2005 for a review). Among these alternative solution, classical biological control has led to field studies of the parasitoid complexes in its native area (Mills 2005). Over 100 parasitoids were recorded on *C. pomonella*, yet parasitism rates have often been reported as low (Labanowski 1981, Mills 2005). However, in Asia, area from which *C. pomonella* is originated, parasitism rates often exceed 20% (Mills 2005). This high parasitism rates are attributed mostly to the presence of *Mastrus ridens* (Hymenoptera: Ichneumonidae) and other specialist parasitoids (Mills 2005). *M. ridens* has, since 1998, been used as a biological control agent many times and, in some cases, parasitism rates exceeding 40% have been observed (Mills 2005).

Following the introductions of several specialist parasitoids in the United-states (Kuhlmann et Mills 1999, Mills 2005), it appeared that *Mastrus ridens* was the candidate that showed the highest probability of becoming permanently established. *M. ridens* has, since 1998, been used as a biological control agent many times and, in some cases, parasitism rates exceeding 40% have been observed (Mills 2005). It has become established everywhere it was released and thus constitutes a promising asset to control *C. pomonella* populations (D'hervé et al 2012, Tortosa et al 2014, Charles et al 2019).

The work presented in chapter 4 about *M. ridens* and the development of a classical biological control program has led to the publication of an article in a French technical journal called *Phytoma*, that publishes mainly for farmers and technical institutes (Muru et al 2018).

### *Trichogramma* species: stars of augmentation biological control

*Trichogramma* are small (about 0.5mm) wasps from the Trichogrammatidae family (Fig 6). Their pre-imaginal development occurs inside the host eggs (Fig 7), the host embryo being



Figure 7 - Adult of *Trichogramma gicai* on *Foeniculum vulgare*. All rights to Brigitte Kan-Van Limburg Stirum

usually quickly killed. According to current taxonomy, this genus contains about 210 described species worldwide, 40 of which occurring in Europe. At the genus level, the host range of *Trichogramma* covers ten insect orders, mainly Lepidoptera (Consoli et al 2010).

*Trichogramma* species are studied for two main reasons. They are conveniently easy to rear and manipulate in laboratory conditions and they are used as biological control agents worldwide. For instance, in the early 2000 it was estimated that *Trichogramma* were used for biological control in more than twenty million hectares (Smith 1996). Several *Trichogramma* species are commercialized for the control of crop pests (*T. brassicae* against *Ostrinia nubilalis*), greenhouse productions (*T. evanescens* against Noctuidae), fruit orchards (e.g., *T. cacoeciae* against *Cydia pomonella*) (see Websites of biocontrol agents' manufacturers Smith et al 2008, van Lenteren 2012). This demonstrates the potential of *Trichogramma* to provide efficient and economically competitive pest control. However, the current situation is still unsatisfactory as inter

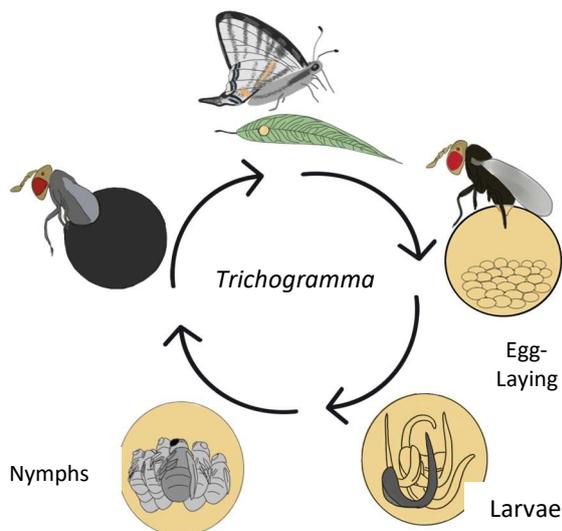


Figure 6- Life-cycle adapted to *Trichogramma* species. Drawing by Alexia Crézé.

and intra-specific biodiversity is poorly documented. Many *Trichogramma* species are described as highly polyphagous and habitat-generalists, which has been presented as potential drawback for their use in biocontrol (Babendreier et al 2003, Yong and Hoffman 2006, Paraiso et al 2013).

Smarter biological control: Learning more about *Trichogramma*'s ecology

Efficient biological control stems in major part from the understanding of the biology and ecology of the biological control agent. In the case of *Trichogramma*, only a few species are used and their ecology (e.g., host range, ecological distribution, etc.) or taxonomy (e.g., species delimitation) are usually poorly known. Moreover, information obtained on the local biodiversity of *Trichogramma*, non-intentional impacts and their geographical/ecological distributions is essential for regulation agencies to more objectively evaluate requests for the introduction of new species. That is why the INRAE developed a nation-wide initiative to survey the species of *Trichogramma* in various habitats and host plants. In this endeavor, the survey was carried out exclusively by using surrogate eggs of *Ephestia kuehniella* through two different methods. Indeed, the eggs were either sprinkled under the leaves or introduced as a “manufactured” patch. In complement to this wide-scale standardized survey, we attempted to document more closely the natural diversity of egg parasitoids in a single location, but using naturally occurring eggs. We focused our efforts on the eggs of *Iphiclides podalirius*, a common Rhopalocera that is endangered in some parts of Europe (e.g., Belgium, Fichet et al 2008).

#### The hidden side of the moon: Sampling wild populations

The scarce swallowtail butterfly (Fig 8), *Iphiclides podalirius* (Lepidoptera: Papilionidae) is found in a larger geographical area, from south Europe to Western China (Mazel, 2014). It feeds on plants from the Rosaceae family, with a preference for the *Prunus* genus in Europe (Tolman and Lewington 1997). Eggs are laid singly, mostly under the leaves of the plants mentioned above. They hatch after one to four weeks, depending on temperature. Caterpillars are highly sedentary, especially in the first instars. They spend most of their time on a silk cushion spun on the surface of the leaf selected as a resting site normally the one on which the egg was laid and move only to feed upon nearby leaves. The closely related species *Iphiclides feisthamelii* is parasitized by several species of *Trichogramma* (Stefanescu et al 2010).



Figure 8 - *Iphiclides podalirius* on Lavender. All rights to Géraldine Groussier.

This project had three main objectives. Firstly, we wanted to describe the egg-parasitoid complex associated with a wild species of butterfly. Secondly, we hoped to get a better understanding of how the different species of egg-parasitoids are distributed as well as the relationships between host eggs, and parasitoids in a natural situation. Thirdly, we aimed at comparing the diversity of *Trichogramma* obtained from wild eggs with the results collected using sentinel eggs of *E. kuehniella*.

In addition to the work described in Chapter 4 on this topic, the data acquired allowed the redaction (in preparation) of another manuscript dedicated to the first recording of

*Trichogramma gicai* on *Iphiaclides podalirius* and other hosts. In this article, one-of-a-kind data on the wasp's behavior are provided, as long as new molecular data, wild footage and information about the holes *T. gicai* leaves behind after emergence with a comparison between host species.

## Chapter 2: Population Ecology

### The *Torymus sinensis* case study: some background

In this chapter, I use data from a classical biological control program aimed towards the release of *Torymus sinensis* against the Asian chestnut gall wasp *Dryocosmus kuriphilus* in France. This particular biological control program has been carried out since 2010 with both scientific and socio-economical stakes in mind. Indeed, the program included a temporally and spatially large post-release monitoring phase. By developing a multi-site, five-years long monitoring, the program aimed at: (1) precisely evaluate the efficiency of the biological control agent and its impact on native communities and (2) acquire knowledge on underlying ecological processes that allow establishment, dispersal and expansion.

To achieve this goal, populations of the biological control agent, the pest, and the whole associated native community of parasitoids were monitored each year starting one year before the releases to get an initial state. Therefore, data on population dynamics were acquired from the beginning of the invasion, providing a detailed growth rate of *T. sinensis* for very small populations.

In the article below, we tried to take advantage of such an original dataset to understand better the dynamics of introduced populations at early invasion stages. Our data involves yearly-monitoring of *T. sinensis* populations within 2 different kinds of locations: (1) release sites, and (2) naturally colonized sites of which we know exactly the year of colonization.

Other sites, prospected as potential release sites for *T. sinensis* during the course of the program, were found as already colonized, so that their colonization date was unknown. As such information on colonization would give valuable data on the expansion dynamics of the biological control agent in agricultural landscapes, we built a population growth model based of the dynamics observed in release and naturally colonized sites to infer the time since colonization of our punctual observations.

## Manuscript 1: When did you get there? Inferring time since colonization in naturally colonized locations. (*in prep.*)

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### Abstract

Inferring when and where an ongoing invasion first took place by using base growth models has received little attention, especially when considering smaller spatial scales. Indeed, population dynamics of invasive species are often studied long after establishment, when populations are big enough to have significant negative impacts on farmlands or on the environment. This implies that the extrapolation of such population dynamics to initial establishment conditions would probably be deceitful due to processes such as invasion lags, density dependence and Allee effects when populations are smaller. In this work, we used field data on the early stages of an invasion from a post-release monitoring of a biological control agent to create a model that is able to infer the time since colonization of naturally colonized sites.

We performed a model validation to ensure that our reference growth model was capable of inferring known dates of colonization. In more than 75% of cases, the inferring error was inferior to one year.

Overall, the base growth model predicted years of colonization that are mostly anterior to the release years of close sites. There are two non-exclusive possible explanations for this. First, wild populations of *T. sinensis* were numerous already prior to the release and we “got lucky” when prospecting for uncolonized sites for release. Alternatively, maybe the model tends to overestimate the time since colonization because dynamics within primo-introduction was different than that of secondary colonization events. We argue that our model may be context dependent, and may not be adapted to describe the dynamics of secondary colonization events.

### Introduction

The study of invasion dynamics is a major topic in invasion biology, that may directly help preventing or managing invasions. Invasion dynamics can be investigated either in a forward way (i.e., predicting the size or area of an invasive population in the future), or in a backward way (i.e., inferring when or where an invasion started).

Most studies in invasion biology take on a forward approach. Usually, their aim is to predict when or where new invasions will occur through the understanding of the processes that facilitate the dispersal and establishment of a given species in a new area (Carlton 1996, Andrade-Restrepo et al. 2019, Novoa et al. 2020). Other studies rather predict the success or failure of a still hypothetical invasion in a given geographical area (Lambdon and Hulme, 2006, Leung et al. 2004, Lantschner et al 2017, Liang et al. 2018, Lins et al. 2018).

On the other hand, inferring when and where an ongoing invasion first took place has received less attention, especially when considering smaller spatial scales. However, inferring the time since an invasion started could help to parametrize predictive models, identify biological or environmental features that are important during the early stages of colonization and anticipate

the responses of native ecosystems. Time since colonization (TSC) is one of the numerous factors that influences the success and properties of a nascent invasion (Barney, 2006) and could help understanding if the invasion was correlated with some other anthropologic event (Lombaert et al 2017). In addition, TSC has recently been considered as a predictor of spatial extent of marine invasions, based on data spanning diverse life histories and ecological characteristics (Byers et al, 2015). A similar result was obtained with invasive terrestrial plants in China (Huang et al. 2009). Furthermore, predicting the moment when an invasive species spread to a new habitat is important as the intensity of negative competitive effects has been showed to depend on the time since the invasion took place (Iacarella et al, 2015). In a similar vein, the time elapsed since a disturbance occurred has been shown to deeply affect population dynamics (Mutz et al. 2017). Although in the case of Mutz et al (2017) the disturbance was the occurrence of a fire, the same could be expected of a biological invasion. Indeed, biological invasions also disturb local populations or communities even when they are transient (Mallon et al. 2017, Muru et al. 2020). However, the exact TSC is rarely known because the species is usually detected once its abundance is high enough to be sampled. Therefore, it remains largely unaccounted for in most works on biological invasions (Barney and Whitlow 2008).

While the forward predictive approach essentially relies on the use of mathematical modelling of species distribution (Peterson et al 2003, Ficetola et al 2007, Giovanelli et al 2007, Loo et al 2007, Ward 2007, Evangelista et al 2008, Medley 2009), the inferring approach has relied on a variety of methods depending on the invasive species. For example, in the context of mangrove colonization, the time since invasion is derived from the maximum age of trees which can be estimated using the sequence of internodes in the main stem (Panapitukkul et al 1998). This method is very specific to mangrove formations and cannot be transposed to arthropods. Lombaert et al (2017) used random forest approximate Bayesian computation analyses on populations genetic structures. This let them conclude that the invasion of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in the United States of America and the early history of maize expansion from Mexico were clearly not associated. In some even older cases such as the house mouse (Auffray and Davidian, 1991) in Europe or humans in the Hawaii islands, paleontological and archeological data are used. When considering the invasion by alien plants, herbarium records can be used to infer invasion periods (Fuentes et al 2008). In contrast with predictive approaches, population dynamics models are almost never used to infer TSC because of the lack of reliable data at low population densities. Early dynamics at the beginning of an invasion are often badly known and may include long periods of lag at very low density before the population grows enough to be detected and monitored (Coutts et al 2018). In the field of invasion biology, population dynamics of invasive species are often studied long after establishment, when populations are big enough to have significant negative impacts on farmlands or on the environment. In this case, extrapolation of such population dynamics to initial establishment conditions would probably be deceitful due to processes such as invasion lags (Coutts et al 2018), density dependence (Sullivan et al 2017) and Allee effects when populations are smaller (Shaw et al 2016). One of the rare uses of population dynamics to infer TSC has been based on surface coverage. Winogron and Kiviat (1997) used GIS photographs to determine changes in phragmites (Cyperales: Poaceae) distribution over time within four sites and then used expansion rates to extrapolate the initial year of invasion. However, they

used the data for each site separately, meaning that only data from site A was used to extrapolate initial invasion of site A and so on. Population dynamics models can thus be used to predict time since colonization at a larger scale only when enough data on small population dynamics are available and growth dynamics are generalizable across populations. Such stringent requirements might be met when an introduction is known to occur and can be monitored in real time, as is the case for instance with biological control introductions.

Classical biological control is the deliberate introduction of an exotic biological control agent to durably regulate a target pest (usually invasive) (Eilenberg et al. 2001). Once the biological control agent is established locally, it is expected to spread on its own and expand into a larger geographical area. Because the ecological processes at work in these introduced populations are strongly similar to those affecting invasive populations, classical biological control introductions can provide valuable empirical data to investigate invasion dynamics, especially the early stages for which data from natural populations is strongly lacking (Fauvergue et al 2012, Marsico et al 2009). Indeed, biological control practitioners have the control of the temporal and spatial dimension of the introduction of the biocontrol agent and can start monitoring its dynamics as soon it is released into the native ecosystem. Following this initial phase of establishment, secondary colonization events will eventually occur in agricultural ecosystems, yet these new sites will be detected only when their populations reach a given level of density so that TSC will, again, not be known. However, in this case, the detailed early population dynamics monitored within the release sites might in theory be used to infer with better confidence the founding date of the secondary colonization sites. If the method proves valid, the inference of TSC for secondary colonization events of biocontrol agents might help identifying landscape features that facilitate or hinder expansion at a fine spatial scale by pairing colonized sites with release sites that would act as known source populations.

The case study of the introduction, establishment and colonization of *Torymus sinensis* (Hymenoptera: Torymidae), a biological control agent used against the Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) provides an ideal framework to test whether early population dynamics in release sites may be used to infer TSC in secondary colonization sites. The Asian chestnut gall wasp, native to China, was accidentally introduced in Italy in 2002 (Brussino et al. 2002) and is now distributed throughout Italy and other European countries. In response to damage observed on chestnut production, classical biological control programs were quickly implemented in newly infested countries, and the parasitoid *T. sinensis* was released in multiple sites in Southern France between 2011 and 2014. Establishment and population dynamics of *T. sinensis* was closely monitored on these sites during 1 to 5 years, and population growth was found to be highly similar across sites (Borowiec et al 2018), so that a lot of data is available to parameterize a general model of post-introduction population dynamics. In addition, 49 sites prospected between 2011 and 2014 were found to be naturally colonized by *T. sinensis*. The TSC of these sites, if known, could help understanding better how this biocontrol agent has spread at the scale of several chestnut-producing areas.

## Methods

### *Biological material*

*D. kuriphilus* is a specialist attacking only chestnut trees. Eggs are laid within the buds during summer. Next spring, the presence of larvae triggers the formation of galls. At this stage, females of *T. sinensis* parasitize the *D. kuriphilus* larvae within the galls. The *T. sinensis* larvae develop during spring and summer and will undergo metamorphosis during winter as the galls take a brown coloration. The next generation of *T. sinensis* will emerge next spring, one year after eggs are laid.

In France, first isolated spots of *Dryocosmus kuriphilus* were observed from 2005 close to the Italian border but its pervasive presence in South of France was only patent from 2010. *Torymus sinensis* was introduced in France between 2011 and 2014, on a total of 59 sites (chestnut orchards) separated by at least 4 km. The introductions covered a wide geographical area (920 km from North to South, 1 030 km from East to West) in mainland France and Corsica. Four propagule pressures (A:1\*100 females, B:1\*50 females, D:1\*1000 females, E: 2\*110 females) of *T. sinensis* were introduced in separated sites. However, it was found that propagule pressure has no influence on establishment as *T. sinensis* successfully established in all sites (see Borowiec et al. 2018 for more details). The study included control sites (labelled as “C” in the data) that were monitored but where *T. sinensis* had not invaded yet. These sites were all naturally colonized by *T. sinensis* and have therefore be included in the dataset. Additionally, during the same time frame, some prospected sites seemed to have undergone natural colonization of *T. sinensis*. Indeed, these 49 extra sites (labelled as “F” in the data) contained *Torymus sinensis* individuals without our intervention.

As detailed in Borowiec et al. 2018, the exotic *Torymus sinensis* from all sites (i.e., A, B, D, E, C and F) were counted from winter dry galls which are easily distinguished as they are brown and dry. In France, the Asian chestnut gall wasp is the only gall wasp on chestnut trees, therefore confusion with other species was impossible. We collected 2 000 to 5 000 galls per site during the two first years and then only 500 to 2 000. Galls were gathered on several trees. Once collected, galls were put in hermetic boxes placed outdoors from January to October so that parasitoids could develop and emerge in natural conditions at our laboratory (located at Sophia Antipolis, France). Each box referred to one site and contained 500 galls which constitutes a good compromise between the number of galls and the ability of parasitoids to emerge and get into the collecting vial placed at the extremity of the boxes. All emerged insects were collected and then stored in 96% ethanol and kept at -22°C.

Overall, 58785 galls were sampled for a total of 71494 individuals of *T. sinensis*.

### *Inferring dates of colonization*

Sites labelled as E were put aside as there were only two instances of such propagule pressure. “D” sites have been characterized as slightly different from others in previous work (Borowiec et al 2018) and were therefore discarded from the model as well. To create the growth model, we used sites “A”, “B” and “C” (later referred to as ABC dataset). The final model is as follows:

$$\text{Year} \sim \log(\text{Abundance of } T. \text{ sinensis})$$

Borowiec et al (2018) did study the growth of *T. sinensis* and showed that growth dynamics were exponential. Therefore, we used the log of the abundance so that we could use a linear model. Furthermore we made sure that the growth curve of each modalities (A, B and C) did not behave differently from one another by using the `glht()` function from the “multcomp” R package. The intercept of the model was set to 0 because the “year 0” happens before the colonization (or releases) of *T. sinensis*, when the population is not there yet. We also tested if the model was improved by introducing a random effect on the slope. As a matter of fact, introducing a random effect was not meaningful for the model.

We performed a model validation to ensure that the model was able to infer known dates of colonization with relative accuracy. To do so, we used a random sample (n=40) from our “ABC” dataset (n=265) to investigate the precision of our base growth model. This sample was then not used to fit the base model.

We used bootstraps to sample a thousand times 40 random lines from our ABC dataset. Each time, the base model was refitted without the 40 extracted lines and time of colonization was inferred for each of them. Then the differences between known and predicted (known minus predicted) year of colonization were recorded and compiled for the 1000 repetitions. Positive values are obtained when the model underestimates the time since colonization (i.e., predicted time since colonization is smaller than actual time). Conversely, negative values translate an overestimation of the time since colonization by the model.

To infer dates of colonization for naturally colonized sites we used the reference growth model we fitted on the whole ABC dataset. However, instead of using a generalized least square model, we used a simple linear model to be able to predict a confidence interval rather than just one value. Inferring time since colonization was made from sites labelled as “F” by using the `predict()` function. Predictions were categorized depending on whether the confidence interval overlaps an integer value or not. For example, a prediction confidence interval from 0.9 to 1.2 was categorized as 1 year, meaning that the prediction is that colonization occurred one year prior to the first sampling of *T. sinensis*. Conversely, if the prediction is from 1.3 to 1.8, we categorized the prediction to “between 1 and 2 years”. The estimated year of colonization was then obtained by simply subtracting the estimated time since colonization to the year *T. sinensis* was first sampled.

## Results

### *Model validation*

The Figure 9 shows the errors made by the model when trying to predict the time since colonization for a subsample of sites where *T. sinensis* was introduced. In more than 75% of cases, the error is inferior to one year (dashed lines). We can also note that the errors are slightly biased towards positive values, which implies that the model tends to underestimate the time since colonization.

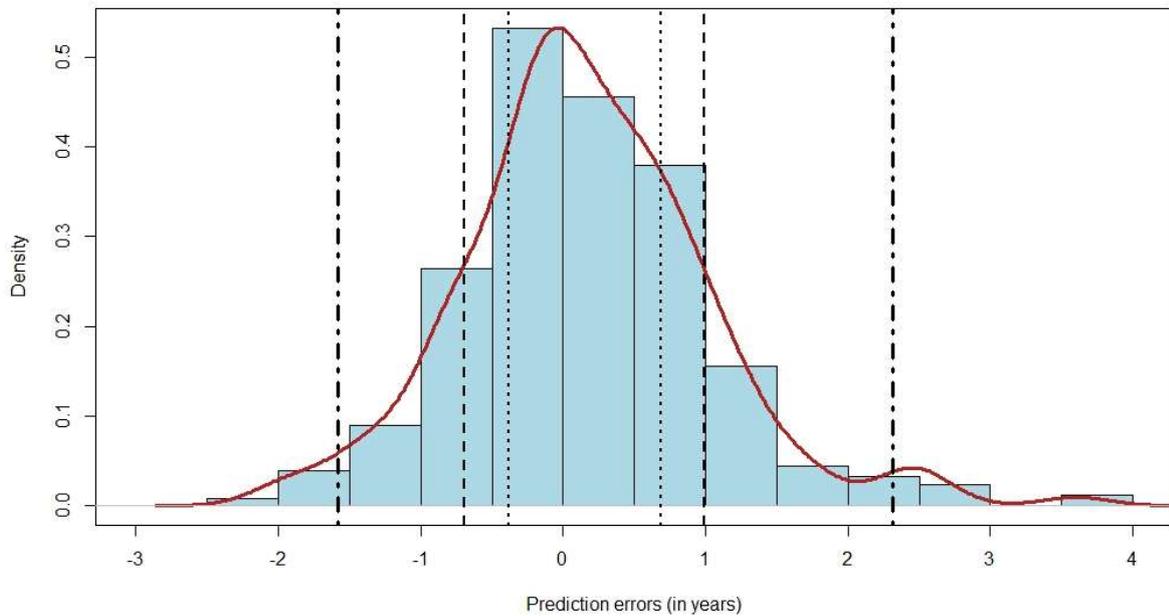


Figure 9 - Histogram of the prediction errors made by the model. 50% of the errors are contained within the dotted line, 75% are contained between the dashed lines and 90% are contained between the dashed and dotted lines.

### *Inferring dates of colonization*

Overall, the base growth model predicted years of colonization that are mostly anterior to the release years of close sites. First, in the Alpes Maritimes region (Figure 10) there is only one release site (2011) and several colonization sites predicted to be invaded from 2008-2009 until 2012-2013. In this region, most of colonization sites are predicted to be invaded by *T. sinensis* prior to its release in the region. Conversely in Corsica (Figure 11), there is a great number of release sites for only one colonization site. Here the colonization site is predicted to be invaded between 2011 and 2012 for a first release within the island in 2011.

The region of Ardèche (Figure 12) is the region with the most balanced numbered of colonization versus release sites. In Ardèche, the first release happened in 2011 and 13 colonization sites are predicted to have been invaded prior to 2011 with the first invasion predicted in 2009.

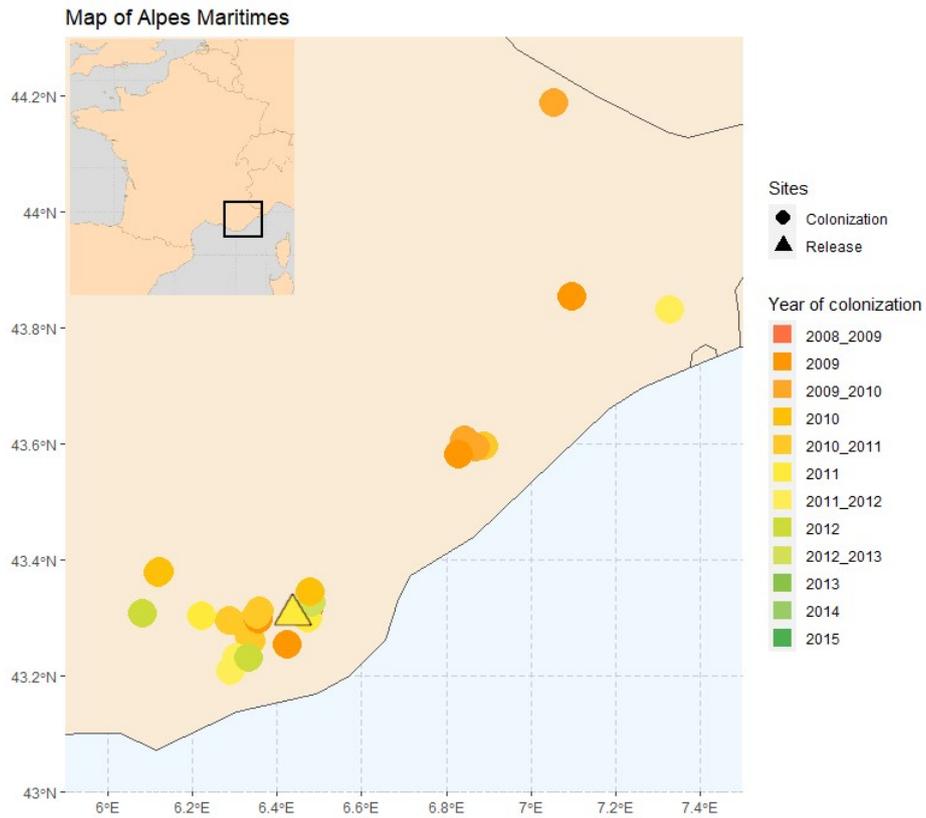


Figure 10 - Map of the release and naturally colonized sites in the region of Alpes Maritimes. Colonization spots are based on prediction from the model.

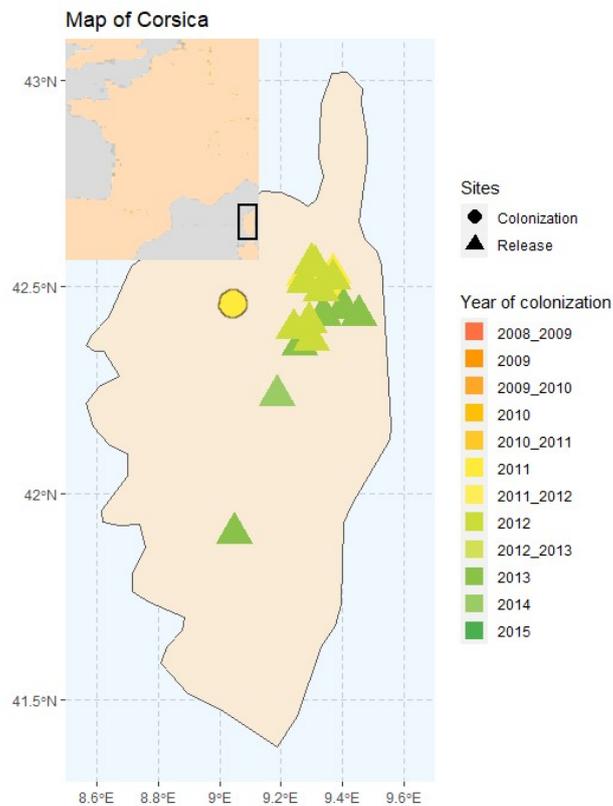


Figure 111 - Map of the release and naturally colonized sites in Corsica. Colonization spots are based on prediction from the model.

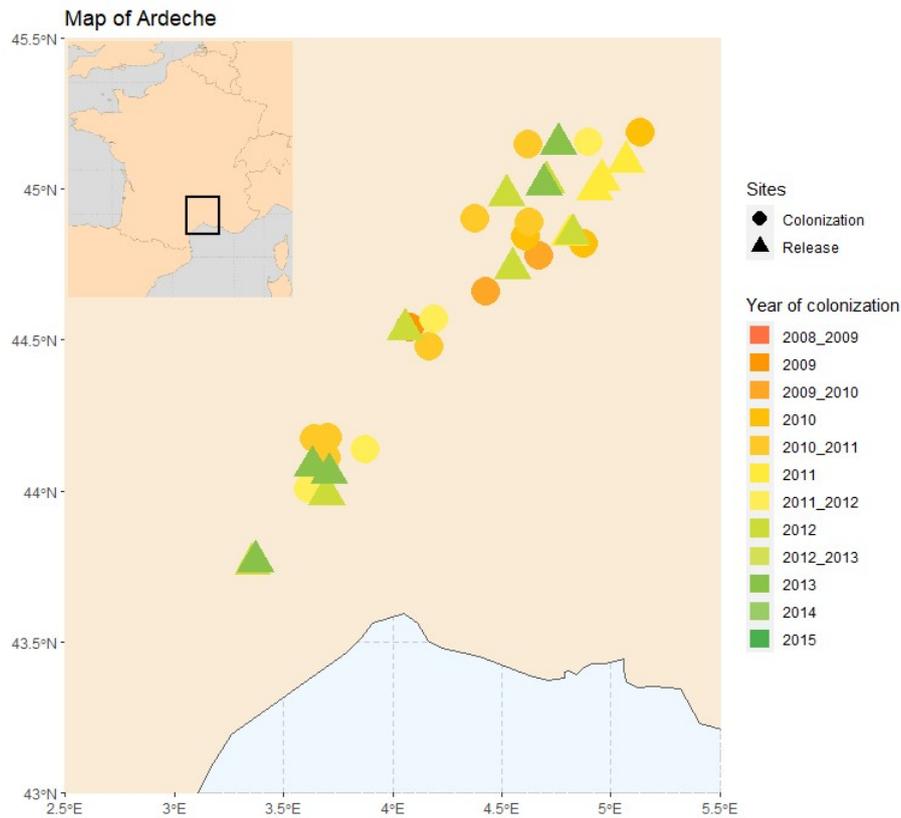


Figure 12 - Map of the release and naturally colonized sites in the region of Ardèche. Colonization spots are based on prediction from the model.

## Discussion

To our knowledge, this is the first attempt to infer the time since colonization based only on population abundances. We took advantage of the large amount of data available from the very first steps of the *T. sinensis* invasion. Understanding what happens during the small period of time following the arrival of the first propagule is crucial to be able to precisely date the origin of an invasion. In our case, the colonization dates inferred for the naturally colonized sites are mostly anterior to the releases from our biological control program. This result is apparently incompatible with our *a priori* hypothesis that initial releases would serve as propagule sources for secondary colonization events within agricultural landscapes.

If we consider that our model gives an accurate inference of the time since colonization, it implies that *T. sinensis* was present virtually everywhere in the release areas, excepted in the monitored release and control sites. This is highly improbable except in the Alpes Maritimes, where only one release was done and where some populations of *T. sinensis* were already known. These populations were the result of natural migration from Italy, where *T. sinensis* was released in 2005 (Quacchia et al 2008). At least in this area, we suggest that the natural colonization of several locations (F sites) could have been substantially helped by demographic

reinforcements from nearby wild populations. Furthermore, natural spread and colonization of *T. sinensis* may be enhanced by the omnipresence and high levels of infestation of *D. kuriphilus*. Indeed, higher resource availability leads to higher survivability in migration events (Bowler and Benton 2009a).

Alternatively, a second interpretation would be that the model is not accurate and tends to overestimate the time since colonization. Several factors not exclusive to one another could cause natural colonization and our releases to have different dynamics. Firstly, this could be the consequence of differences in dispersing abilities. Indeed, dispersers are often a non-random draw from a population (Bowler & Benton 2005 Clobert et al 2009, Cote et al 2017) and therefore natural colonization could have been done by individuals with different phenotypic traits (e.g., morphology, physiology, behavior...). However, wild and released populations originate from the same populations. In fact, due to the biology of the host, *D. kuriphilus*, the biological control agent was impossible to rear in the laboratory. Therefore, released individuals were collected in the wild making them as able to disperse as wild populations. Another explanation could be that sites selected for release were not as optimal as sites chosen by wild populations (Mortier et al 2018). However, sites where natural colonization was observed were selected following the same technical specifications as the release sites. Thus, it is unlikely that site quality differed drastically between naturally colonized and release sites. Our last explanation would be that populations dynamics are highly influenced by the numbers of individuals immigrating and the frequency of migration events (Wittman et al 2014). In other words, a population surrounded by many others would display a different demographic dynamic than an isolated population. In our case the model was built on data from the very first of the invasion. This means that populations resulting from primo introductions were of small size with probably a low migration rate. On the other hand, populations resulting from natural colonization are surrounded by established population that act as a continuous source of propagule. This supply of individuals may accelerate and therefore modify the population dynamics within these sites.

We argue that the inference quality from our model is probably strongly context-dependent. For example, it is probable that using data from primo-introduction demographics is not accurate when trying to infer the time since colonization for subsequent invasions. Indeed, the latter is most probably taking place in an environment where source population may modify the growth rate (Fahrig and Merriam 1985) and therefore populations may display more complex dynamics (Doebeli 1995, Ruxton and Rohani 1999). Furthermore, we suggest that the dispersal abilities of the invasive organism could also play a role in the inference quality made by the model. In fact, any populations from an organism dispersing gradually will have multiple close population that would impact its demographics. Therefore, an organism with larger dispersal distances or with unique or rare dispersal events could be more suited for the model. In our case, the fact that the host populations are virtually continuously distributed and that our biological control agent has very strong dispersing abilities most certainly implies that dynamics will be greatly influenced by neighboring populations (Bowler and Benton 2009b). In such a context, a model fitted on isolated primary introductions could not correctly describe the dynamics of secondary colonization events subjected to continuous propagule pressure from several close sources. In contrast, our approach would be better adapted to infer colonization

history in a more fragmented context, where single migration events can be related to secondary colonization events.

A limit to our study and the interpretation we have of our data is our inability to discriminate between founding individuals from "wild populations" naturally expanding in France from Italy, "released populations" from our biological control program, and "additional populations" from "unofficial" releases carried out by farmers. We propose that the ability to discriminate between released and wild populations should be essential to the realization of a biological control program including a post-release monitoring. This could be achieved by characterizing specific molecular signatures for example (Schwartz et al 2007, Malausa et al 2010a). This could greatly increase the value of the data collected by knowing exactly where each individual collected would come from, or how much different sources of individuals contribute to an expansion in progress.

In conclusion, our work helps answering a fundamental question during the invasion of an exotic pest: When and where was the invader firstly introduced? Indeed, by realizing a fine and extensive post monitoring release of a biological control program, we were able to obtain rare data about the early stages of an invasion and use them to create a model that infers the time since colonization. Although the optimal use of this model is context-dependent and more adapted to fragmented populations, this work emphasizes the importance of creating and using data from the monitoring of biological control programs to eventually be able to predict and prevent invasions. Indeed, knowing the time and location of introductions could then allow for the identification of the factors that influence the occurrence of novel introductions.

### Assets and Limits of the Dataset

Regarding population ecology, the main asset from our dataset is that we obtained precise data on the dynamics of *T. sinensis* during the earlier stages of the invasion. This data allowed us to create an accurate base growth model of *T. sinensis* populations during these stages. However, when we tried to use it to determine when the local invasion of neighboring sites happened, we realized that the model is mostly suited to describe situations of primary introductions, where the population is not exposed to continuous propagule pressure from population reservoirs in the environment. In consequence, secondary colonization events of *T. sinensis* cannot be attributed to single release sites with accuracy, but might rather result from multiple migration events from several release sites and/or previously established populations. In order to better understand how these different components contribute to the expansion dynamics in agricultural landscapes, being able to genetically characterize and discriminate between different sources populations would be a significant asset.

In conclusion, it is clear that such a dataset has a great potential as a rare documentation of the very first stages of population growth following an introduction. However, merely monitoring species abundances locally without finer insight into larger processes at the meta-population scale might not be enough to really understand the processes that affect the expansion of a biological control agent.

## Chapter 3: Community Ecology

### The *Torymus sinensis* case study: some more background

In this chapter, I will again use data from the classical biological control program involving *Torymus sinensis* against the Asian chestnut gall wasp *Dryocosmus kuriphilus* in France (See Chapter 1 Section I).

The long post release monitoring of the biological control agent, its target pest and the native community of parasitoids recently associated with *D. kuriphilus* represents an ideal situation in which to study non-intentional impacts of the biological control program. In fact, the population of native parasitoids has associated itself with *D. kuriphilus* only a few years prior to the release of *T. sinensis* when the pest invaded France. Therefore, the stability of such system may be deeply impacted by the perturbation that would represent the introduction of an exotic super-efficient biological control agent.

**Manuscript 2: The open bar is closed: restructuration of a native parasitoid community following successful control of an invasive pest. (Recommended by Peer Community In Zoology)**

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#### Abstract

The rise of the Asian chestnut gall wasp *Dryocosmus kuriphilus* in France has benefited the native community of parasitoids originally associated with oak gall wasps by becoming an additional trophic subsidy and therefore perturbing population dynamics of local parasitoids. However, the successful biological control of this pest has then led to significant decreases in its population densities. Here we investigate how the invasion of the Asian chestnut gall wasp *Dryocosmus kuriphilus* in France and its subsequent control by the exotic parasitoid *Torymus sinensis* has impacted the local community of native parasitoids.

We explored 5 years of native community dynamics within 26 locations during the rise and fall of the invasive pest. In an attempt to understand how mechanisms such as local extinction or competition come into play, we analyzed how the patterns of co-occurrence between the different native parasitoid species changed through time.

Our results demonstrate that native parasitoid communities experienced increased competition as the *D. kuriphilus* levels of infestation decreased. During the last year of the survey, two alternative patterns were observed depending on the sampled location: either native parasitoid communities were represented by an extremely limited number of species occurring at low densities, in some cases no native parasitoid species at all, or they were dominated by one main parasitoid: *Mesopolobus sericeus*. These two patterns seemed to correlate with the habitat type, *M. sericeus* being more abundant in semi-natural habitats compared to agricultural lands, the

former known to be natural reservoirs for native parasitoids. These results highlight how the “boom-and-bust” dynamics of an invasive pest followed by successful biological control can deeply alter the structure of native communities of natural enemies.

*Note: The supplementary material from this manuscript is available at the end of the thesis.*

## Introduction

Biological invasions are defined as the introduction, establishment and expansion of populations outside of their native area. Apart from their well-known effects on agricultural production, biological invasions are also identified as major drivers of global changes in biodiversity worldwide. More generally, invasions are known to have a diversity of direct and indirect negative effects on native ecosystems (see McGeoch et al 2015 for a review of environmental impacts caused by invasion). In particular, they can deeply alter interspecific interactions and restructure native communities, often with negative consequences (Ricciardi and Isaac 2000, Ricciardi 2001, Carroll 2007). For instance, invasive predators, parasitoids or pathogens were proven to drastically reduce the size of resident prey or host populations (Daszak et al 2000) or to compete with other species from the same trophic level (Hamilton et al 1999, Grosholz 2002, see David et al 2017 for a review of the impacts of invasive species on food webs).

While much research has focused on invasive top-consumers (predators, parasitoids, etc.), a more restrained amount of literature examines the impact of primary-consumer invasive species as a new resource for the native community (Carlsson et al. 2009). The situation where a successful invasive species becomes a resource for native species of a higher trophic level is referred to as a form of facilitation and the invasive species acts as a trophic subsidy (Rodriguez 2006). The ecological impact of invasive species acting as a trophic subsidy has been shown across a variety of model systems such as invasive macroalgae (Olabarria et al. 2009, Rossi et al. 2010, Suarez-Jimenez et al. 2017), some phytophagous insects (Barber et al. 2008, Girardoz et al. 2008, Jones et al. 2014, Haye et al. 2015, Herlihy et al. 2016, Noyes 2017), *Drosophila suzukii* (Mazzetto et al. 2016) or four invasive gall wasp species (Schonrogge and Crawley 2000). Hence, trophic subsidies, possibly in pair with changes in population densities of local populations (Eveleigh et al. 2007) may change the established dynamics within recipient communities through, for example, apparent competition (Settle and Wilson 1990, Holt and Bonsall 2017) or niche displacement (Mallon et al. 2007). Even when the invasion is only transient and therefore the trophic subsidy finally disappears, the consequences on native communities can be lasting on the recipient community. Indeed, recovery from the disappearance of the alien species is not systematic and may thus lead to further disequilibrium within the community (Courchamp et al. 2003). For example, Mallon et al. (2017) have recently reported a permanent niche displacement of native species caused by a failed invasion by *Escherichia coli* in soil microcosms, and referred to it as a “legacy effect”. Such lasting effects of transient invasions on species niche breadth and space for instance, may as well occur at the community level, thus impacting overall community structure by forcing species to modify the way they exploit available resources. However, empirical data on the response of native

community dynamics to transient invasion remain scarce, as there is a lack of studies exploring multi-year community dynamics during the rise and fall of an invasive pest.

Classical biological control – i.e., the deliberate introduction of an exotic biological control agent to durably regulate a target (usually exotic) pest (Eilenberg et al. 2001) - can provide valuable empirical data on the dynamics of communities disturbed by two successive invaders, the pest and its introduced natural enemy. Indeed, during a biological control program, the dynamics of local communities are disturbed twice consecutively. Firstly, the arrival of the invasive pest decreases the level of primary resource and can alter the abundances of its native competitors and natural enemies (Jones et al. 2014, Haye et al. 2015, Herlihy et al. 2016). Then, the establishment of the biological control agent will again modify the community structure by strongly reducing the abundance of the invasive pest and potentially interacting with native species. Long-term direct and indirect effects of either the pest or its natural enemy on the recipient community have been documented (Henneman and Memmott 2001, also see Louda et al. 2003 for a review of 10 case studies with quantitative data), and the most obvious mechanisms that impact recipient communities appear to be extreme polyphagy in pair with the ability to infiltrate natural areas away from targeted agroecosystems. Interactions such as those existing between parasitoids and their hosts may also be impacted by the dynamics inherent to classical biological control. If they are able to, native non-specialist parasitoids may be displaced from their native hosts to an invasive one. However, the native community of parasitoids can be outcompeted from the exotic pest by the exotic parasitoid (Naranjo 2017) that has been chosen for its efficiency exploiting the exotic pest. Firstly, this could lead to local extinction of native populations of parasitoids, especially if the exotic parasitoid outcompetes them on the native host(s) as well (Bengtsson 1989). On the other hand, the introduction of an exotic parasitoid to control an exotic pest often leads to a displacement of the native community of parasitoids that have become associated with the exotic pest (Bennett 1993, Lynch and Thomas 2000, van Lenteren et al. 2006). This happens logically when the introduced parasitoid is specialized on the exotic pest and is a superior competitor or more adapted to find and exploit the pest than its native counterparts (Naranjo 2017). The resulting displacement might only be a step backwards, bringing the system back to the previous pattern of host-parasitoid dynamics (before the pest invaded the area), or a novel state might emerge, depending on the resilience of the native species.

However, the temporal dynamics and spatial variability of these processes remain poorly understood and empirical data are greatly lacking at this point with, to our knowledge, no reports of such non-intentional effect in the context of biological control. Therefore, here we use successful classical biological control of an invasive pest as a framework to properly investigate how these two subsequent invasions impact the structure of native communities.

The Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), native to China, was accidentally introduced in Italy in 2002 (Brussino et al. 2002) and is now distributed throughout Italy and other European countries (EPPO, 2014). *D. kuriphilus* is a specialist attacking only chestnut trees. In absence of competitors (Bernardo et al. 2013) and specialized antagonists, *D. kuriphilus* was able to proliferate quickly and massively. Therefore, it became a trophic subsidy for several native parasitoids previously associated to gall wasps from other plants/trees (Matosevic and Melika 2013, Panzavolta et al. 2013, Francati et al. 2015, Noyes 2019). In response to damage observed on chestnut production and also apiculture, classical

biological control programs were quickly implemented in newly infested countries. *Torymus sinensis* was chosen as a biological control agent due to its high specificity of parasitism (Quacchia et al. 2014, Ferracini et al. 2017) and its previous effective control of the target pest outside Europe (Gyoutoku and Uemura 1985, Cooper and Rieske 2007, 2011). In France, *T. sinensis* has been proven established with fast and significant impacts on the targeted pest in the subsequent years (Borowiec et al. 2018). This thus led to the quite unique opportunity to investigate how local communities evolve with regard to the deprivation of their trophic subsidy whereas most scientific work usually studies the recruitment of native parasitoids by the exotic biological control agents and its impact on food webs (Henneman and Memmott 2001, Eveleigh et al. 2007, Barber et al. 2008, Girardo et al. 2008, David et al. 2017).

## Methodology

### *Biological control introductions*

In France, first isolated spots of *Dryocosmus kuriphilus* were observed from 2005 close to the Italian border but its pervasive presence in South of France was only patent from 2010. *Torymus sinensis* was introduced in France between 2009 and 2014, on a total of 59 sites (chestnut orchards) separated by at least 4 km. In each site, the monitoring of native parasitoids started one year prior to *T. sinensis* release. The introductions covered a wide geographical area (920 km from North to South, 1 030 km from East to West) in metropolitan France including Corsica. Two propagule sizes (100 and 1 000 individuals) of *T. sinensis* were introduced in separated sites but *T. sinensis* established itself in all sites whatever the initial propagule size was (see Borowiec et al. 2018 for more details). For this study we kept only the 26 sites for which at least five consecutive years of monitoring were available (Figure 13), 58785 galls were sampled for a total of 31301 individuals of *T. sinensis*.

### *Sampling of insect communities associated with chestnut galls*

#### *Estimation of *D. kuriphilus* levels of infestation*

Ten chestnut trees per site per year were sampled and, for each of them, ten totally random twigs were selected at human height and inspected for galls as explained in Table 1. The number of galls in a twig can therefore be zero. From these, the infestation levels of *D. kuriphilus* were estimated, by combining information on the mean percentage of buds with at least one gall and on the mean number of galls per bud as shown in Table 1. Because of the difficulty of this task (geographical cover, meteorological contingencies, staff's availability and skills), infestations were finally not available for some sites and/or dates.

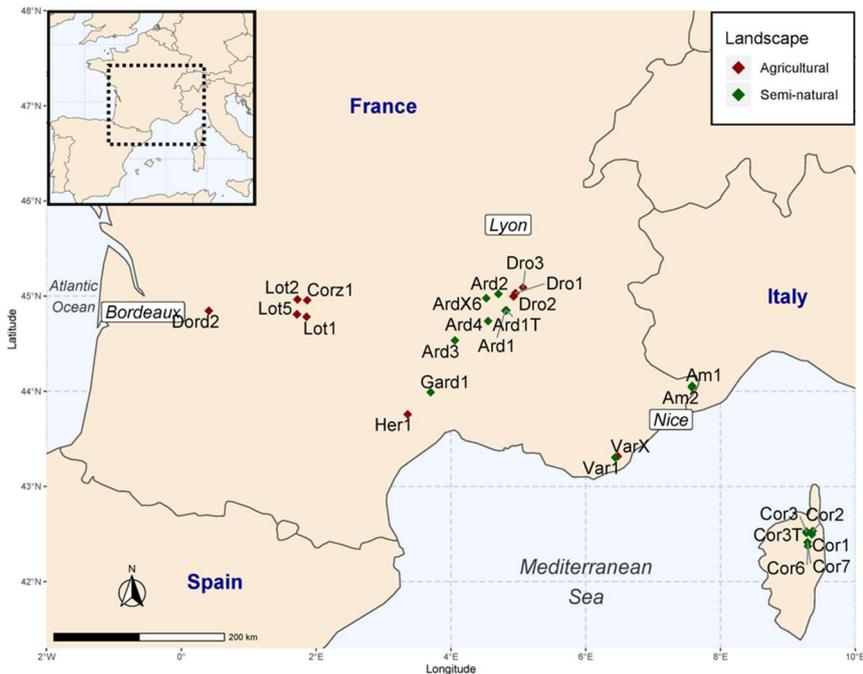


Figure 12 - Map of the survey. Red points correspond to sites within an agricultural landscape (mainly apple orchards) whereas green points correspond to sites within a semi-natural landscape (mainly forests). *Torymus sinensis* was released in 2009 (Am1, Am2), in 2011 (Ard1, Cor1, Cor2, Cor3, Dro1, Dro2, Dro3, Var1) and in 2012 (Ard1T, Ard2, Ard3, Ard4, ArdX6, Cor3T, Cor6, Cor7, Corz1, Dord2, Gard1, Her1, Lot1, Lot2, Lot5, VarX).

Table 1 - Table showing how the classes of infestation by *D. kuriphilus* were determined. Classes are created from 1 to 5 depending on the mean number of galls per bud and the mean percentage of buds with at least one gall. The higher these features are, the higher the infestation is estimated.

		Mean number of galls per bud		
		<1	[1-2[	2≤
Mean % of buds with at least one gall	[0-33%]	1	2	3
	[34-66%]	2	3	4
	[67-100%]	3	4	5

### Diversity and abundance of associated parasitoids

As detailed in Borowiec et al. 2018, both the exotic *Torymus sinensis* and the native parasitoids (see Table 2) were counted from winter dry galls which are easily distinguished as they are brown and dry. Our work focuses on these galls and we deliberately omitted fresh spring galls for logistical reasons. In France, the Asian chestnut gall wasp is the only gall wasp on chestnut trees, therefore confusion with other species was impossible. We collected 2 000 to 5 000 galls

per site during the two first years and then only 500 to 2 000. Galls were gathered on several trees. Once collected, galls were put in hermetic boxes placed outdoors from January to October so that parasitoids could develop and emerge in natural conditions at our laboratory (located at Sophia Antipolis, France). Each box referred to one site and contained 500 galls which constitutes a good compromise between the number of galls and the ability of parasitoids to emerge and get into the collecting vial placed at the extremity of the boxes. All emerged insects were collected and then stored in 96% ethanol and kept at -22°C. In addition to the exotic and ubiquitous *T. sinensis*, nine main native parasitoids were identified (Table 2). All identifications were based on morphological characters. *Eupelmus* species were identified using the latest descriptions of the *Eupelmus urozonus* complex (Al Khatib et al. 2014, 2016). Other species were identified by using an unpublished key to chalcidoid parasitoids in oak cynipid galls (Askew and Thuroczy, unpublished). DNA barcoding was used for a representative set of each morphologically-described genus and/or species to ascertain their identifications (see Molecular analyses section below).

### *Molecular analyses*

The DNA extraction was performed using commercial kits (Zygem PIN0500 or Quick extract Lucigen) according to the manufacturers' recommendations in a total volume of 30µL, without crushing the insect. PCR targeted a small portion of the mitochondrial gene Cytochrome Oxidase I (COI), the standard barcode region (Hebert et al. 2003). We thus used the primers LCO 1490 (5'-GTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer et al. 1994) or the related degenerated primers HCO\_PUC (5'-TAAACTTCWGGRTGWCCAAARAAATCA-3') and LCO\_PUC (5'-TTTCAACWAATCATAAAGATATTGG-3'). When unsuccessful of PCR products or sequencing reaction, the primers COI pF2 (5'-ACCWGTAATRATAGGDGGDTTTGGDAA-3') and COI 2437d (5'-GCTARTCATCTAAAWAYTTTAATWCCWG-3') (developed by Simon et al. 1994 and modified by Kaartinen et al. 2010) were tried. The PCR conditions were as follows: 95°C for 5min, followed by 40 cycles of (i) 95°C for 30s, (ii) 48°C for 90sec, and 72°C for 1min with a final extension at 60°C for 30min. PCR products were shipped to Genewiz (Radolfzell, Germany) for their Sanger sequencing. The obtained sequences were checked and compared to either reliable sequences in GenBank (*Eupelmus* species – see Al Khatib et al. 2014, 2016), either to unpublished ones (see supplementary material). Molecular analyses were carried out with MEGA-X software.

Table 2 - List of native parasitoids with their known host range. \* This species may be a complex of cryptic parasitoid species but all specialized on Cynipidae. References: (1): Askew, R. R. and Thuroczy, (unpublished)– (2 Al Khatib, F. et al. (2014) (2016) – (3): Murakami et al. (1994) – (4): Noyes (2019).

<i>Native species</i>	<i>Host range</i>	<i>Refs</i>
<i>Aulogymnus spp.</i>	Hymenoptera : Cynipidae	1
<i>Eupelmus azureus</i>	Hymenoptera : Cynipidae	2
<i>Eupelmus kiefferi</i>	Coleoptera Diptera Hemiptera Hymenoptera Lepidoptera	2
<i>Eupelmus urozonus</i>	Diptera Hymenoptera Neuroptera	2
<i>Eurytoma setigera</i>	Hymenoptera : Cynipidae : Cynipinae	1, 3, 4
<i>Megastigmus dorsalis*</i>	Hymenoptera : Cynipidae	1
<i>Mesopolobus sericeus</i>	Hymenoptera : Cynipidae : Cynipinae : Cynipini	1
<i>Torymus auratus</i>	Hymenoptera : Cynipidae : Cynipinae	1, 4
<i>Sycophila biguttata</i>	Hymenoptera : Cynipidae	1

## Statistical analyses

### *Native parasitoid species co-occurrence analysis*

To assess the patterns of co-occurrence between parasitoid species, and their evolution over time, we used the C-score (Stone and Roberts 1990) from each annual matrix of presence-absence of the nine native species. As *T. sinensis* was always present and here we only consider species occurrences (presence/absence), it was excluded of the analysis.

The C-score is a measure of average pairwise species segregation. It measures the mean number of checkerboard units between all pairs of species in a data matrix. The number of checkerboard units for each pair of species *i* and *k* is calculated as follows:

$$Cscore_{ik} = (S_i - Q)(S_k - Q) \quad (1)$$

where *Q* is the number of shared sites, *S<sub>i</sub>* and *S<sub>k</sub>* are the number of sites in which species *i* and *k* are respectively found. In equation (1) the C-score will be equal to zero if species *i* and *k* share

all sites. Conversely, C-score will be equal to one if species species  $i$  and  $k$  are never found together. The overall C-score for the community is then calculated as follows:

$$Overall\_Cscore = \sum \frac{(S_i - Q)(S_k - Q)}{\frac{R(R-1)}{2}} \quad (2)$$

where  $R$  is the number of rows (=species) in the matrix (Stone and Roberts 1990, Gotelli 2000). When compared to other co-occurrence indices such as CHECKER (Diamond 1975), V-ratio (Robson 1972, Schluter 1984) and COMBO (Pielou and Pielou 1968), C-score has the smallest probability of type I and II errors (Gotelli 2000). However, because the value of the C-score depends on the frequency of occurrence of the species, inter-annual comparisons cannot be performed directly. We thus used the co-occurrence null model from the EcoSimR package (Gotelli 2015) of R (R Development Core Team 2018) to create null assemblages based on our observed presence-absence species matrices. This was done by randomizing (by transposing sub-matrices) species occurrences but keeping row and columns totals fixed (Gotelli 2000). Thus, differences between sites are maintained, making this method appropriate to detect patterns of species interactions (Gotelli 2000). Each randomization produces one matrix in which a ‘simulated’ C-score is calculated. Such randomization is replicated ten thousand times. The significance of the observed C-scores was computed as the proportion of simulated values equal to or more extreme than the observed C-score value.

In order to graphically compare each year, all c-score values were normalized by using:

$$Adjusted\ C\_score_t = \frac{x_t - \mu(Simulated\ C\_score)_t}{\sigma(Simulated\ C\_score)_t}$$

where  $x$  takes the values of observed and simulated C-score and  $t$  refers to the year after release of *T. sinensis* (from 1 to 5). A low value of C-score is indicative of an aggregative pattern, while a high value is indicative of an exclusion pattern.

#### *Native parasitoids community structure*

We described the community structure each year after the release of *T. sinensis* by using the R package ‘pheatmap’ (Raivo 2019). We created clustered heatmaps with the ‘pheatmap’ function to visualize how communities of native parasitoids are structured during the survey. Sites were clustered depending on their native parasitoid absolute abundance using aggregative clustering. As a distance measure between clusters  $x$  and  $y$  we used the Euclidean distance which is calculated as follows:

$$d_{euc}(x, y) = \sqrt{\sum_{i=1}^n (x_i - y_i)^2}$$

As a linkage function, we chose the complete linkage which takes the maximum distance between the two clusters:

$$f = \max (d(x, y))$$

Aggregative clustering starts by computing a distance between every pair of units to be clustered and creating a distance matrix. The two clusters with the smallest value (e.g., A and B) are then merged into a new cluster (e.g., AB). The matrix is then recalculated and as we use the complete linkage the distance between the new cluster and the other clusters (e.g., C and D). The distance between AB and C will be equal to the maximum value between C and A and between C and B.

### *Landscape context*

In an attempt to evaluate the potential role of habitat on the community structure during the last year of survey, we used a Principal Component Analysis (PCA) considering the abundances of each native species using the ‘FactoMineR’ package of R (Husson et al. 2019). More precisely, sites were plotted in the first dimension of the PCA depending on the abundance of native parasitoids in each of them. We then plotted the two main categories of habitat: (i) orchards located within an agricultural landscape with a poor amount of semi-natural habitat; (ii) orchards located within semi-natural habitats (mainly forested areas). The habitat categorization was made based on qualitative estimates first during field work and confirmed later on satellites views. Satellite views were necessary in order to confirm habitat type within the 1-km radius of field observations for all areas belonging to private owners, preventing our access and direct categorization of habitats.

To do so, we used the Open-source platform QGIS (<https://qgis.org/en/site/>) with the satellite view of the OpenLayers plugin. We also used the land registry layer of the French government land registry website (<https://www.geoportail.gouv.fr/donnees/registre-parcellaire-graphique-rpg-2017>).

We also tested with a generalized linear model whether the abundance of *M. sericeus* was significantly different in agricultural and semi-natural habitats. However, given the numerous zeros within the agricultural category, we settled with a generalized linear model testing whether the occurrence (modelled as binomial) of *M. sericeus* was significantly different between the two habitats.

#### a. Results

##### *i. Control of *Dryocosmus kuriphilus* by *Torymus sinensis**

A fast increase of the *Torymus sinensis*’ relative frequency was observed during the 5 years of survey, 90% of galls being finally parasitized by *T. sinensis* (Figure 14). This was easily monitored as each gall can only contain one *T. sinensis* inside. In parallel, the infestation levels

of *D. kuriphilus* decreased markedly, based on the sites in which infestation data was available for each year of the survey (Am1, Am2, Ard1, Gard2, Var1).

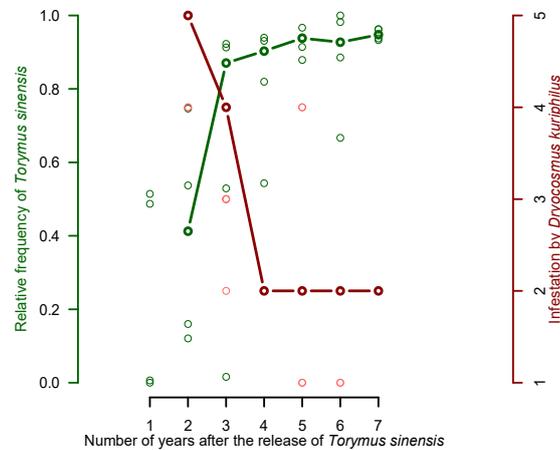


Figure 13 - Infestation levels of *D. kuriphilus* and Relative frequency of *T. sinensis* in galls each year of the survey. Here are only shown the sites where the infestation was consistently measured for all years of the survey.

### Molecular-assisted species identification

The identification of individuals was realized in routine using morphological characters. The molecular characterization was however necessary for taxa in which a species' complex is known (for instance, in the *Eupelmus* genus – see Al Khatib et al. 2014 and 2016) or for which few information is available. As shown in the Figure S2, the COI sequenced (between 550 and 612pb) were informative enough to distinguish closely related species, as in the *Sycophyla* and *Torymus* genera. For some taxa (*Aulogymnus arsamis*, *Eurytoma setigera*, *Megastigmus dorsalis*, *Torymus affinis*), the within molecular diversity may suggest the presence of sister species and/or a marked intraspecific variability

### Abundances and occurrences of native species

Overall, 71 494 specimens of *T. sinensis* and 12 016 specimens of native parasitoids were obtained from 284 425 galls from the 26 sites during the 5 years of the survey. Galls from all locations were monitored for emerging parasitoids at our laboratory.

In terms of abundance, the native species were ordered as follows: *Mesopolobus sericeus* (n= 3 792, 31.6%), *Eupelmus urozonus* (n= 2 069, 17.2%), *Megastigmus dorsalis* (n= 1 877, 15.6%), *Eupelmus azureus* (n= 1 752, 14.6%), *Eurytoma setigera* (n=586, 4.9%), *Eupelmus kiefferi* (n=491, 4.1%), *Aulogymnus* spp. (n=403, 3.4%), *Sycophyla biguttata* (n=116, 1%), *Torymus auratus* (n=19, 0.1%). Nine hundred and eleven (7.5%) individuals remained undetermined using both morphological and molecular identification and were thus discarded from the analysis.

In terms of occurrence, *Torymus sinensis* was observed in the 130 possible site-by-year combinations. In comparison, the results for native species were as follows: *Eupelmus urozonus* (n=111), *Eurytoma setigera* (n=86), *Eupelmus kiefferi* (n=74), *Megastigmus dorsalis* (n=59), *Mesopolobus sericeus* (n=50), *Eupelmus azureus* (n=49), *Aulogygnus* spp. (n=33) *Sycophyla biguttata* (n=26), *Torymus auratus* (n=7).

The mean abundances of all nine native parasitoids are given for each year in Figure S1 (Supplemental Figure S1). Overall, they peaked during the second and/or third years of the survey.

A potential pitfall with such survey could be to miss some other relevant species because of some insufficient sampling effort. However, we are quite confident that this is not the case so far as, excepted for the rarest species (*T. auratus*), all other species were found at least common or even very common in some site-by-date combination, well above a potential detection threshold (Figure 15).

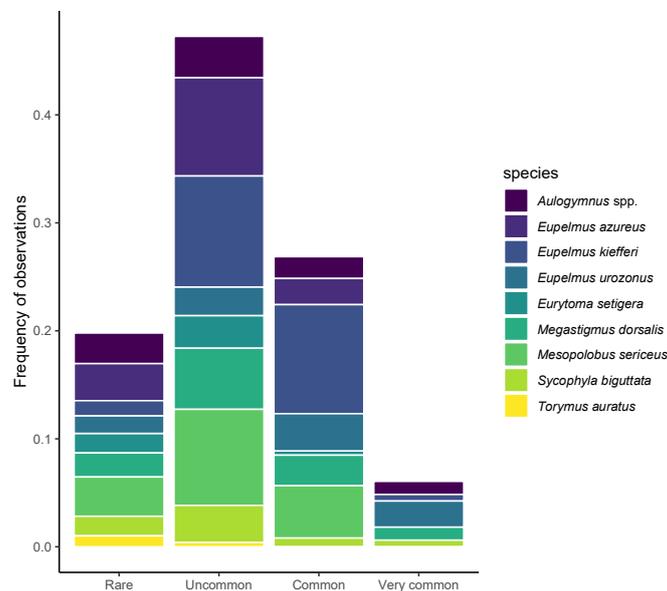


Figure 14 - Frequencies of observations the different species of native parasitoids. Site-by-date-by-species combinations were sorted according to four classes of abundance: Rare (only 1 individual of the species), Uncommon (from 2 to 10 individuals), Common (from 11 to 100 individuals) and Very common (From 101 to max abundance sampled).

### Co-occurrence null model analyses

Starting the third year, native parasitoids species co-occurred less frequently than expected by chance with increasing odds from the first to the fifth year (Figure 16). Therefore, as years passed, there was a decreasing chance of observing co-occurrence of native parasitoids by sampling *D. kuriphilus* galls. More details about which pairs of species were co-occurring more

frequently across the years can be extracted from the heatmaps made for each year of the survey (supplementary material Figure S2-S5).

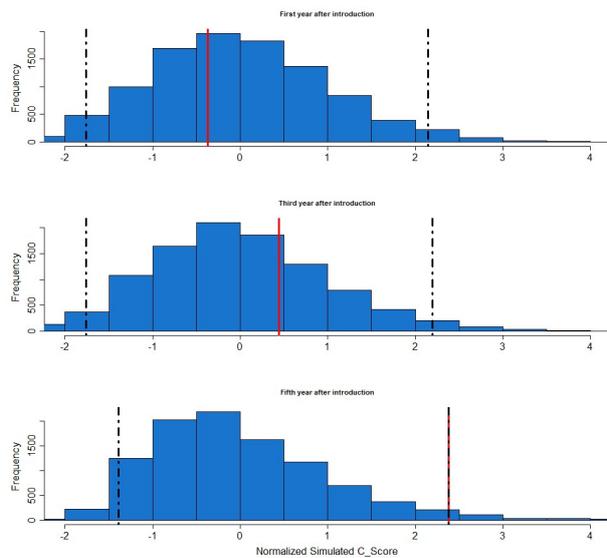


Figure 15 - C-score values for the native community of parasitoids for the first (A), third (B) and fifth year (C) after the release of *T. sinensis*. Blue histogram represents the simulated values, the red bar represents the observed C-score and the dotted lines represent the 95% confidence interval.

### Native parasitoid community structure

During the fifth year of the experiment, we observed two different patterns among sites (Figure 17). In the first category (19 sites, top cluster), the community of native parasitoids was represented by just a few species occurring at low abundances. Furthermore, in a few sites (Lot5, Corz1, Dord2), not even a single native parasitoid was sampled. However, in the second category (7 sites, bottom cluster), the community was dominated by *Mesopolobus sericeus*, a specialized parasitoid of the Cynipini tribe (Table 2) that was not sampled anywhere the first year of the survey (supplementary material, Figure S2).

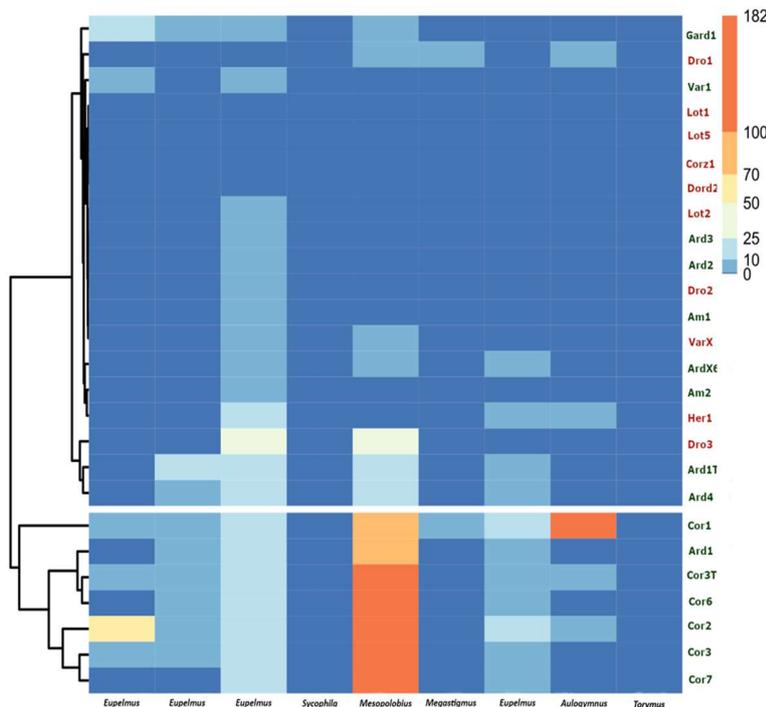


Figure 16 - Heatmap representing the abundances of all native parasitoid species during the fifth year after the release of *T. sinensis*. Abundances are represented in log scale by a color gradient from blue to red. The colors of locations' names refer to the type of habitat (red: agricultural habitat – green: semi-natural habitat). To help the reader, species names are ordered as follows (from left to right): *Eupelmus azureus*, *Eupelmus kiefferi*, *Eupelmus urozonus*, *Sycophila biguttata*, *Mesopolobus sericeus*, *Megastigmus dorsalis*, *Eupelmus setigae*, *Aulogymnus* spp. *Torymus auratus*.

### Landscape context

The Principal Component Analysis on native parasitoid abundances confirmed that, five years after the release of *T. sinensis*, communities were mostly structured by the local presence of *M. sericeus* (Figure 18A). The analysis of the projection of the different sites highlighted that the abundance of *M. sericeus* was correlated with the type of habitat, semi-natural orchards being more likely to host this particular species (Figure 18B). As the PCA suggested an effect driven mostly by the response of the species *M. sericeus*, we showed that this species was indeed occurring more frequently within semi-natural orchards (p.value=0.0396).

To verify whether the strong pattern driven by *M. sericeus* might have obscured responses in other species, we also ran the analysis by excluding *M. sericeus*. We found that the native parasitoid community still evolves towards segregation, although it is only significant the fifth year of the survey (supplementary material Figure S6).

The clustering was significantly different with virtually no difference between sites. Here all sites (excepted Cor1) are grouped in the same big cluster (supplementary material Figure S7). Likewise, the PCA showed a weaker differentiation between our two types of habitat (supplementary material Figure S8).

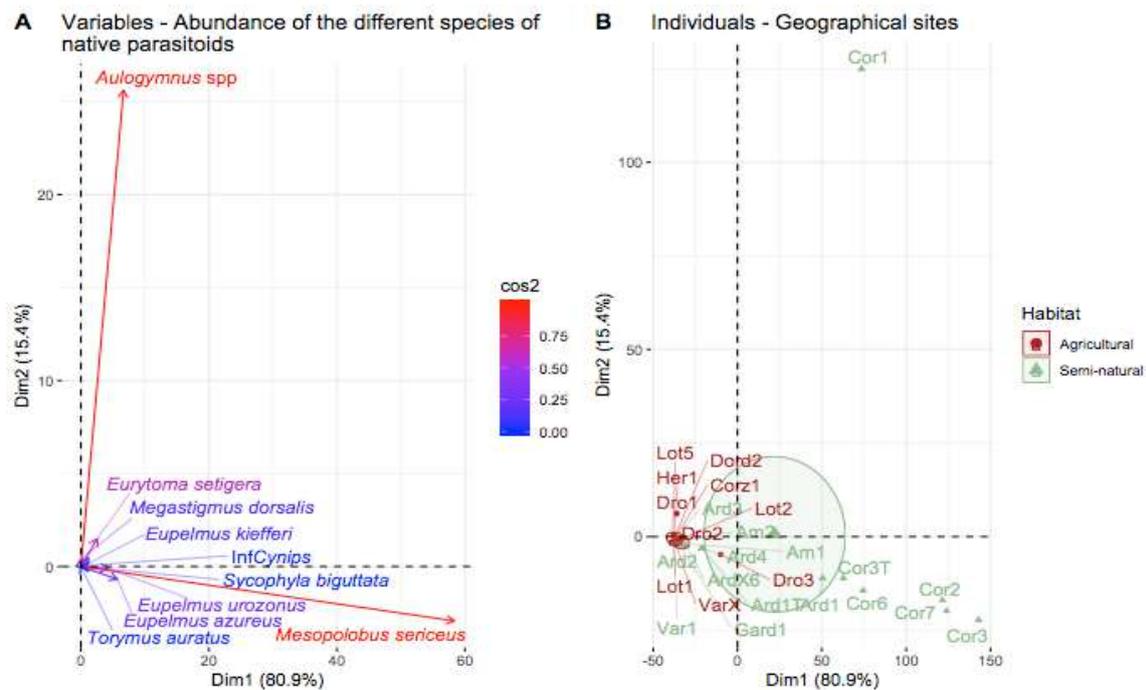


Figure 17 - A: Variables correlation plot of the PCA built from the abundance of native parasitoids. Colors represent the contribution of the different species to the structure of the variation within each axis. B: Projection of the different geographical sites on two principal component axes of the PCA. The colors discriminate the two main habitat: Agricultural (red) and Semi-natural (green).

## Discussion

Our work highlights the restructuring of native parasitoid communities following the successful control of *Dryocosmus kuriphilus* by the biological control agent *Torymus sinensis*. Our results suggest that communities evolved differently mostly depending on the landscape context of the sampling site.

The nine native parasitoids that appeared to use *D. kuriphilus* as a trophic subsidy in our survey are related to oak gall wasps, although their degree of generalism is highly variable (Table 2). Some species such as *Eupelmus kiefferi* and *E. urozonus* are indeed extremely polyphagous whereas, in contrast, *Mesopolobus sericeus* is substantially more specific, being specialized on only one tribe of Cynipids (Noyes 2019). The main result of the co-occurrence model analyses is the increasing exclusive competition through time (Figure 16). This means that the native community underwent a significant restructuring, which was correlated in time with the rarefaction of *D. kuriphilus*. The first parasitoids actually exploiting *D. kuriphilus* were *E. urozonus*, *E. azureus* and *M. dorsalis* (Figure S1), those species combining (i) a wide host range, (ii) a known affinity with several Cynipidae and (iii) pre-existing resources in such kinds

of habitats (Al khatib et al. 2014, Al khatib et al. 2016). They were thus therefore likely to shift relatively easily on the new invasive host (Cornell and Hawkins 1993, Hawkins 2005). However, in the following years, *M. sericeus*, which was not detected during the first year of survey (Figure S1), was the sole species able to markedly increase in abundance and to persist on this trophic subsidy (Figure 17). And this, despite the fact that it was not detected once at the beginning of the survey in any site (Figure S1). It seems to be the same for *Aulogymnus* spp. except that these are much less abundant and include all species from the *Aulogymnus* genus. The examples of native species displaced by invasive species are numerous (e.g., Rowles and O’Dowd 2007, Bohn et al 2008, Inoue et al 2008, Sebastian et al 2015) therefore we are not surprised to observe such outcome for most of our native species, which are outcompeted by the introduced specialist *T. sinensis*. Only *M. sericeus* appears to be able to coexist at significant levels with *Torymus sinensis* on *D. kuriphilus*. Furthermore, the most generalist parasitoids of our system probably exhibit a switching behavior (Murdoch 1969). This behavior refers to a situation where a predator (or here a parasitoid) exhibits adaptive, flexible choices between all available preys (or hosts). This choice entails positively frequency-dependent predation. In our study abundance of other hosts are unknown. It is therefore possible that *D. kuriphilus* eventually becomes rarer than other hosts, forcing generalists to switch to more abundant hosts (Pelletier 2000). However, switching preys can have stabilizing or destabilizing effects, even possibly causing extinction of the predator (Van Leeuwen et al 2007).

Increasing landscape complexity (mostly defined as the increase of the proportion of semi-natural habitat) is generally associated with increases in natural enemy abundance and/or diversity (Bianchi et al. 2006, Schmidt et al. 2008, Gardiner et al. 2009a, 2009b). In our study, sites enclosed within large amounts of semi-natural habitats contained the most diverse and abundant native parasitoid communities (Figure 17). In particular, the persistence of *M. sericeus*, the most specialist native parasitoid, was modulated by the local environment (Figure 18B). Indeed, *M. sericeus* was the native parasitoid that seemed to be the best at exploiting *D. kuriphilus*, although only within semi-natural habitat. Among the seven sites showing a marked domination of *M. sericeus* (Figure 17), six of which are located in the island of Corsica (Figure 17). Islands are home to plant and animal communities with relatively little diversification, simplified trophic webs and high rates of endemism (Williamson 1981, Chapuis et al. 1995). In addition to their smaller size, these characteristics (Cassey 2003) partially explain that oscillations (or perturbations) within the resident community are more incline to destructive outcomes such as extinctions (Elton 1958). Nonetheless, five continental sites (Figure 13: ArdX6, Dro3, Ard1T, Ard4, Ard1) also exhibit a slightly less marked but similar increase of *M. sericeus* (Figure 17), four of them being in semi-natural landscapes (Figure 13). We thus think that the final dominance of *M. sericeus* towards other native species is rather explained by differences in the landscape rather than a “mainland versus island” dichotomy. In fact, most of the known hosts of *M. sericeus* are oak gall wasps (Noyes 2019), oak trees being rarer in agricultural landscapes than in semi-natural ones. Large populations of *M. sericeus* acting like sources for the colonization of chestnut orchards are consequently more likely to be sustained in this latter habitat.

In classical biological control, the ecological impacts of a biological control agent are usually explained by the use of non-target hosts/preys (Louda et al. 2003). Although *T. sinensis* has shown a slight host range expansion it appeared to be with minimal impact and no effect

expected on distribution and abundance of non-target hosts (Ferracini et al. 2017). Thus, its impact on native competitors is mediated by its successful control of the shared host.

Our sampling effort was strong enough that we can be confident we did not miss any relevant species. Even if a single individual of a given species was sampled relatively frequently (about 20% of all data), all species, except the rarest one, were also sampled at high density in some cases. This implies that no species in our analyses was so rare that the probability to have completely missed another species with comparable abundance by chance is close to zero. With regard to the rarest species, *Torymus auratus*, it is already known to be rare in winter dry galls but more abundant in spring fresh galls, so that our sampling method might have underestimated its abundance (Kos et al. 2015, Ferracini et al. 2018). Furthermore, species rankings according to abundance and occurrence are not the same, which confirms that our sampling method is robust enough to accurately detect species even when their abundance is low.

We need to point out that although our study contains insightful information on how our native parasitoid community structure evolves, species dynamics we observed the last year of the survey are not fixed but quite the opposite. Species dynamics are most probably still evolving towards a, yet unknown, state of equilibrium. We are still not in measure to predict with certainty what will happen when *D. kuriphilus* will become even rarer. Maybe *T. sinensis* will remain the dominant species or maybe because of the presence of its native hosts, *M. sericeus* will outperform *T. sinensis* at least in semi-natural habitats. Furthermore, although we evidenced a successful host range expansion (by newly including *T. sinensis* into their diet) from the majority of these native parasitoids, nothing is known about how the populations dynamics evolved on their native hosts.

In conclusion, classical biological control offers an exciting frame to investigate real-time population dynamics during invasive processes. Yet, the opportunities remain rare because of various reasons including (i) the quite high rate of establishment's failure, (ii) the temporal frame required for the observation of significant patterns and (iii) the lack of funding for post-release surveys. With regard to this context, the deliberate introduction of *T. sinensis* against *D. kuriphilus* in France thus was a quite unique opportunity. Our work sheds a new light on how the “boom-and-bust” (here defining the situation in which a period of great prosperity is abruptly followed by one of decline) dynamics of an invasive pest can impact the structure of native communities of potential antagonists. Our results evidence a site-specific scenario where a sole native species, *M. sericeus*, dominates the native community on the trophic subsidy and is able to co-exist with the exotic and specialized competitor, *T. sinensis*. *M. sericeus* is now able to exploit both the native gall wasps and *D. kuriphilus*. This extended host range may have lasting impacts on *T. sinensis* populations, all the more so *D. kuriphilus* will reach a low density at a global scale. In turn, the rarefaction of *D. kuriphilus* and the competition with *M. sericeus* might constrain *T. sinensis* to exploit new hosts. Therefore, it would be of particular interest to study the long-term evolution of these two species as the ideal expected outcome of classical biological control is the everlasting control of the host with no unintentional negative impact on the recipient community. Another open perspective of this work is to analyze how the structure of native parasitoids evolves within the oak gall wasp's community.

Data are available online: <https://doi.org/10.5281/zenodo.3929233>

Script and codes are available online: <https://doi.org/10.5281/zenodo.3952462>

### Assets and Limits of the Dataset

The data gathered from the post monitoring release of *T. sinensis* included a long and precise monitoring of the abundances of *T. sinensis*, *D. kuriphilus* and the native parasitoids. On one hand, was enough to highlight evolution of community structure through time following the introduction of a strong competitor. In fact, sometimes null models are not computable if there are not enough sites available. However, although we highlighted an evolution within the community of parasitoids associated with *D. kuriphilus*, we have no data on the impact that such restructuration could have on native hosts. This was not possible to obtain because adding the monitoring of oak gall wasp was too much work for the human resources that were available.

On the other hand, even if some influence of the landscape may be hinted at in this article, detailed environmental structure data and landscape more precise and thorough characterization was lacking. This could have allowed us to make a stronger link between environmental features and the evolution of the community structure.

## Chapter 4: The limits of biological control datasets to investigate ecological processes at fine scale

The ambition of coupling agronomic purposes (initial survey or late field-evaluation of biological control agents) with experimental ecology in the frame of biological control programs relies on very demanding set-ups involving multiple locations, extended time span and/or local sampling efforts (e.g., Malausa et al 2010a, 2010b, Borowiec et al 2018, Goode et al 2019). For example, besides the agronomical objective, Malausa et al (2010a, 2010b) were interested in testing hypotheses derived from invasion biology. To do so, they selected 60 release sites spread in about 400km<sup>2</sup> and released 43 000 individuals. Such exigence requires important resources, be they human, financial or logistic. To some extent, the inflation of the experimental set-up driven by scientific purposes actually benefits to the agronomic purposes by, for instance, allowing to multiply the number of releases or to provide more accurate estimates of the establishment rate and population dynamics of biological control agents. In numerous cases however, this generates “extra-costs” (i.e., investment not justified by the sole agronomic concerns) that financial sponsors may not be enticed to support.

Even when a satisfying level of resource is reached and when an *a priori* adequate frame (in particular time span and number of locations) is accessible, such initiative (coupling agronomic and scientific purposes) may be “unrewarding” as, unluckily, presented in this chapter. Three main causes may explain such disillusion. The first cause may be an incorrect estimate of the relevant temporal and/or spatial scales. This is well illustrated by the first case-study (primo-introduction of *Mastrus ridens* in South East of France) presented here where a very precise experimental set-up has been implemented in vain. Indeed, much effort was invested on the release of *M. ridens* and the monitoring of its spread. However, the biological control agent was not detected at all during our post-release monitoring. If not due to a failure to establish, this could be caused by population migrating out of our restricted monitored area or because population densities remained too low to be detected. A second cause may be sub-optimal sampling methods. This is well illustrated by the second case-study of this chapter (*Trichogramma* sampling at the national scale) where an unprecedented participative field sampling has generated quite disappointing results in term of collected individuals despite *Trichogramma* were proven pervasive. This “sub-optimality” was probably generated here by the combination of (i) an easily available but probably quite limiting substitution host (*E. kuehniella*), (ii) effort during unsuitable period and/or at unsuitable locations, (iii) lack of knowledge about the distribution of natural hosts, etc. Finally, a third cause is the actual rarity of the investigated taxa. This is illustrated here with a third case-study, the very local but intense survey of a “common” Lepidoptera (*Iphioides podalirius*) and its elusive associated oophagous parasitoid complex. In fact, although *I. podalirius* is a common butterfly in south eastern France, its eggs are quite rare and hard to spot (as they are laid individually). This limits the number of eggs (and subsequently of egg parasitoids) that can be collected during a single season. Here the three case-studies highlight the need for considering the detectability of the organisms of interest which is not a step commonly investigated in biological control programs as, in this case, success is not measured by detecting low density populations.

According to the datasets finally generated, the impact in term of scientific valorization ranges from a complete failure (primo-introduction of *M. ridens*), the diffusion of naturalist knowledge (survey of *I. podalirius* and related oophagous parasitoids) and/or the identification of some patterns using specific statistics (national survey of *Trichogramma* species).

### Influence of fine-scale landscape structure on the establishment and early expansion of a biological control agent

In the last decades, the field of agronomy has widened its focus, addressing, among other things, landscape dynamics and how they contribute to deal with issues on agricultural landscapes, such as pest outbreaks and pest population control. In fact, farmers are faced with two main objectives. First, crops need to be protected from pests and diseases in order to maintain high productivity. Second, because of environmental and health issues, we need to bring down the use of pesticides. A promising avenue is to enhance the services provided by the various landscape features within the agroecosystem. Some environmental (or landscape) features could help or hinder the spread of natural enemy populations and therefore increase or decrease their value as biological control agents. For example, complex landscapes with a high density and connectivity of uncultivated, perennial habitats may enhance populations of natural enemies, which immigrate into neighboring annual crop fields, attack pest insects, and contribute significantly to the reduction of pest populations below an economic threshold (Thies and Tscharke 1999). Conversely, landscape features such as urbanized areas can serve as a barrier to insect movement (Faeth and Kane 1978), potentially impeding the spread of a biological control agent. Until now, such studies about landscape ecology are oriented towards conservation biological control considering indigenous natural enemies. However, populations of exotic natural enemies introduced for the first time in an agroecosystem for the needs of a classical biological program are likely influenced by landscape features (Didham et al 2007, Grab et al 2018). This is especially true during the establishment and expansion stages of the introduction. Their ability to control efficiently the pest may depend as well on landscape features such as the presence of semi-natural habitats (Thies and Tscharke 1999, Bianchi et al 2006).

The impact landscape heterogeneity has on the establishment of introduced populations under controlled environments has recently been investigated (see With 2002, O'Reilly-Nugent et al 2016 for reviews of landscape effects on the spread of introduced populations). However, in most cases the landscape structure is simplified, resulting in difficult transposition onto agroecosystems. Therefore, in order to optimize the release strategies of biological control agents, we need to be able to understand the functional structure of agronomical landscapes. This could be achieved by quantitatively evaluating dispersal barriers, demographic sink holes and the influence of the distribution of pests (hosts or preys) on the population dynamics of the biological control agent both during establishment and expansion phases.

The biological control program involving the release of *Mastrus ridens* against the codling moth *Cydia pomonella* was used as an opportunity to document these fine-scale effects of landscape structure on the establishment and early expansion of *M. ridens*

## Release protocol

Releases were carried out in the Basse Durance valley in France. This area is particularly known to be a hot spot of apple production. Conveniently, populations of *Cydia pomonella* along with other generalist predators (e.g., Araneae, Dermaptera etc.) were monitored for years in several orchards (including organic orchards) by researchers from INRAE located in Avignon, just a few kilometers away. Furthermore, cartography of the areas surrounding monitored sites was already available through our collaboration with that team (Contrôle Biologique par Conservation, PSH, INRAE, Avignon, France).

At first, we considered a multi-site release with a post-release monitoring phase at a fine scale around each release site. But the fact that we were introducing small populations meant that we had to detect population expansion and avoid removing individuals at the same time. That is why, in contrast to classical monitoring methods (e.g., malaise traps, D-vac etc.) we opted for the monitor *Mastrus ridens*' expansion by introducing diapausing *C. pomonella* larvae directly in the field. However, the rearing of the larvae for post-release monitoring was limited and part of the production had to be used to produce *M. ridens*. In the end, we had to choose between two main strategies: (1) release within several sites and limit our ability to realize a dense monitoring grid, or (2) go all-in in one site and monitor *M. ridens*' expansion within a denser grid. We chose the latter because we wanted to have the most precise representation of the biological control agent's spread.

Rearing of both *M. ridens* and *C. pomonella* were carried out in our lab at INRAE (Sophia-Antipolis, France), during the whole duration of both the releases and post-release monitoring. Rearings of *C. pomonella* were kept at 19°C and 12D:12L to get diapausing larvae to be used for rearing of *M. ridens* and as sentinel larvae during post-release monitoring. Rearing of *M. ridens* were kept in a separate room at 21°C and 8D:16L.

Two release sessions were carried out in 2018. On the first one, July 27<sup>th</sup>, we released 2000 females and more than 2000 males. The second release occurred on October 13<sup>th</sup> and its purpose was to increase chances of establishing the population before winter. On this second occasion, we released 1000 females and more than 1000 males. Individuals were released at the nymphal stage to minimize perturbation on emerging adults. Custom cages (Fig 19) were built to protect nymphs from birds and ants until the adults emerged. Both releases were done inside the same orchard, with 20 release points distributed over the orchard (Fig. 20). A meteorological station was installed to monitor daily data on weather and wind conditions.



Figure 18 - Custom emergence cage. There is door in the front to introduce the pupae of *M. ridens* and the three other sides are covered with a plastic mesh to allow dispersal after emergence.



Figure 19 - Map of the release site with all release locations (red points)

For the post-release monitoring, sentinel diapausing larvae of *C. pomonella* were exposed for 13 to 15 days in the field, then taken back to the lab to assess whether parasitism by *M. ridens* had occurred. The device (Fig. 21) consisted of a translucent 4-well cell culture plate. The wells were filled with translucent epoxy resin, leaving a shallow and concave space where the larvae were placed. Then, a fine metallic mesh was placed above by melting it on the borders of the plate, preventing larvae from escaping while allowing parasitism by *M. ridens*. During the first and second monitoring sessions, each device contained 5 and 4 larvae respectively. During the latter, numbers were adapted in order to introduce

About two hundred post-release monitoring points were chosen within neighboring orchards and isolated trees that are hosts of *Cydia pomonella* (apples, pears, walnuts, quinces) (Fig. 22A). More than 80 information leaflets were distributed to the neighborhood residents (grey areas on Fig 22A) to ask people if we could put monitoring devices on their garden trees. We only had one positive answer. Post-release monitoring was realized in 2018 once in September and once in October. Traps were placed for 2 weeks and then brought back to the lab for emergence of the parasitoids.



Figure 20 - The monitoring device with four larvae of *C. pomonella* inside.

In 2019, the monitoring of *M. ridens* introduced population was carried out from July to October. Each month, two monitoring sessions were carried out leaving the devices for two weeks at a time. However, the area was reduced and the number of larvae per device was

decreased from 5 to 4 in order to spatially intensify the monitoring (Fig 22B). That way, more devices were deployed closer from each other.

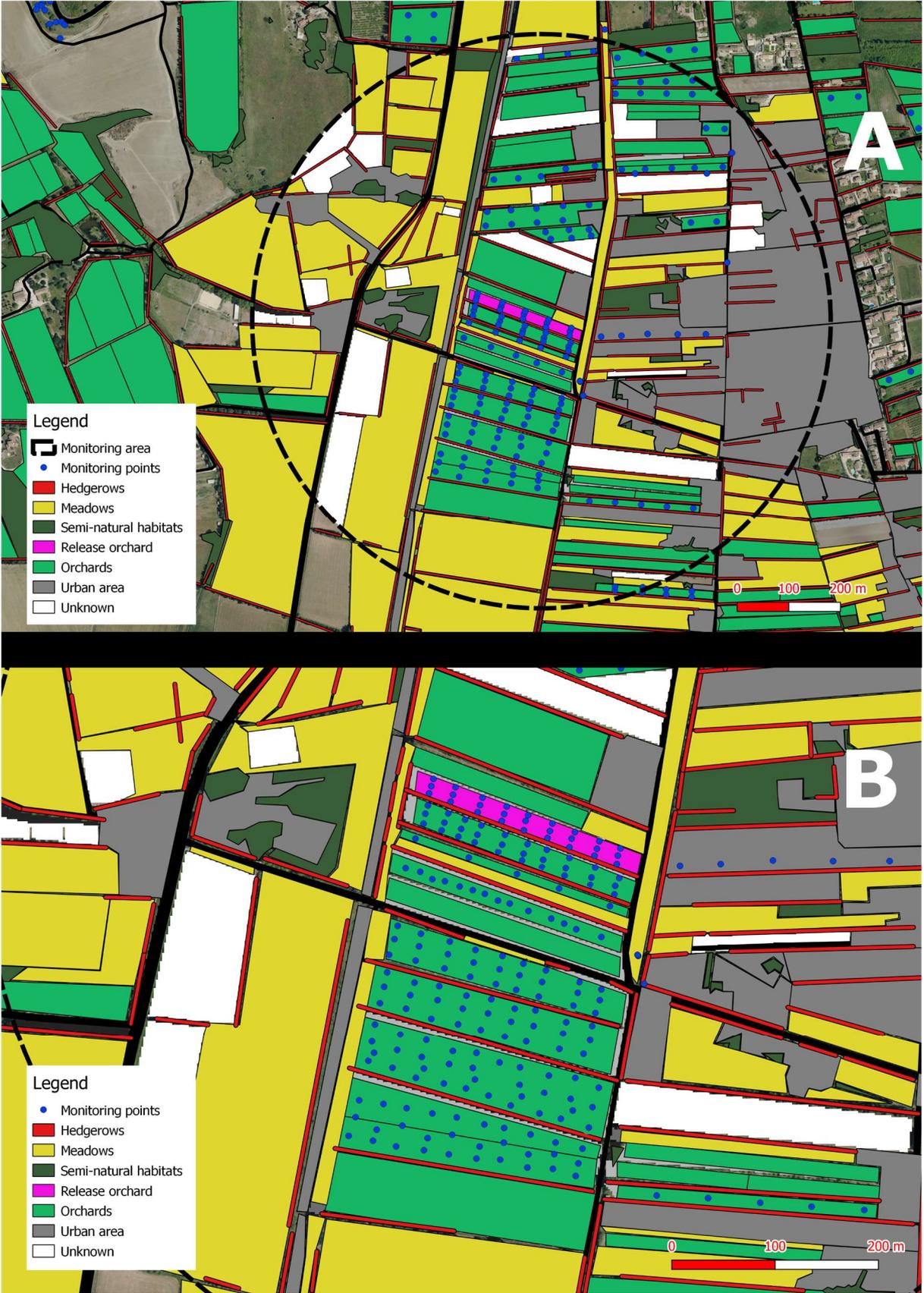


Figure 21 - Maps of the monitoring of *M. ridens*. A: The first monitoring in 2018. B: The second monitoring in 2019

During September of 2019 another monitoring method was used along sentinel larvae in order to increase the chances to detect *M. ridens* at the release site. Using hexane, we captured the chemical volatiles emitted by 10 naïve females of *M. ridens* and 1000mg of silk from *C. pomonella* larvae (collected from the rearing containers). To do so, material (i.e., naïve females and silk) were put in 150mL of solvent (hexane) overnight. Solvent was then left to slightly evaporate at room temperature for 30 min to concentrate the extracted molecules. The hexane solution was then divided in small glass vials and stored at -21°C. In the field, the vials were opened and stuck in the middle of yellow sticky traps (Fig. 23) in order to trap individuals of *M. ridens* attracted by the chemical compounds emitted as the solvent evaporates.



Figure 22 - Picture of the glass vial containing the hexane extract, stuck on a yellow sticky-trap.

During all our monitoring sessions, no individuals of *M. ridens* were detected or collected. However, in October of 2019, one individual of *Mastrus ridens* was collected at 2 kilometres from the release site, outside of our monitored area by colleagues monitoring *C. pomonella* populations.

#### *Mastrus ridens*: a partial failure?

In the first year after the releases, no specimens of *M. ridens* were collected nor detected. As a result, we were not able to investigate how landscape structure affects the spread of a biological control agent at a local scale. In fact, introduced populations often experience an invasion lag, which is a period right after the initial invasion during which the abundance of the population does not increase as it fights to survive stochastic events and the individuals have to make extra efforts to find a mate and reproduce. Furthermore, our monitoring devices were based on the premise that *M. ridens* females would be able to locate them by sensing the cues emitted by the *C. pomonella* larvae (mostly chemical volatiles emitted by the silky cocoons, Jumean et al 2005). Nonetheless, although successful qualitative tests were carried out in the lab, the ability of *M. ridens* to find our devices in the wild was never tested. Thus, there is an argument to be made that the artificial engineering of our monitoring devices decreased the chances of it being found by *M. ridens* in the wild. In that case, there would have been a high-density threshold for the detection of *M. ridens* that was not compatible with our objectives of documenting early establishment and expansion. However, no individuals were collected by this method either. Here, the obvious trade-off between fine scale monitoring and data gathering was considered long before the releases were carried out. We realized that the dense fine monitoring mesh used for monitoring movements at a small spatial scale could not be carried out in several sites, mainly because of our producing capability of both hosts and parasitoids. Furthermore, because

our monitoring was dense, the area covered was relatively small compared to the dispersive abilities of *M. ridens*. Although we tried to ensure that emerging adult would not disperse much after emergence by manipulating their perception of the new environment (following the method from Hougardy and Mills 2005), we cannot be sure that no *M. ridens* individuals flew away from the monitored area. The initial plan was to obtain several data points for different categories of paths between release and capture. Therefore, without enough points, no robust statistical analyses would have been achievable.

Unfortunately, given the short duration of the PhD, after the post-release monitoring phase in 2018, there was no time left to investigate whether the introduced population just failed to established, dispersed out of the monitored area or was undergoing a lag phase. Overall, the whole process of carrying out a classical biological control program from mass-rearing to post-release monitoring in the span of a PhD thesis turned out to be too ambitious. Furthermore, the limitations associated with the biological model *M. ridens* (e.g., low detectability, costly monitoring and probably big population size to achieve establishment), suggest that this species is not ideally suited to be used in fine scale experimentations.

However, the establishment of *Mastrus ridens* seems to be a possible success as a specimen was collected a year after the releases only 2 kilometers from the release site. Therefore, it remains possible that in the future, data on the expansion of *M. ridens* could be acquired through monitoring of the same area. Nonetheless, if the population did not fail to establish, it will remain possible to monitor the spread of the biological control agent at a larger scale (e.g., regional scale).

## Documenting the diversity of native *Trichogramma*: from the description of species to the ecological processes structuring communities

The history between *Trichogramma* species and biological control now lasts for more than 120 years. However, due to the rise of chemical insecticides in the late 1930s, the interest in *Trichogramma* slowly decreased.

Research on *Trichogramma* was revived in the 1970s when European and American researchers started to carry out mass-rearing and releases to regulate populations of several lepidopteran pests such as *Plutella xylostella*, *Ostrinia nubilalis*, *Cydia pomonella* and more (Hassan 1982, Bigler 1986, Hawlitzky 1986, Hassan 1993, Newton 1993). In the following years, they were the most widely produced and released natural enemies in biological control throughout the world (Li 1994, Smith 1996). In the mid-1970s, the only crops targeted by augmentative biological control with *Trichogramma* were sugarcane and corn. During the next decade, cotton, sugar beet, vineyard, cabbage, plum, apple, forests, tomato and rice were added to the list. Surprisingly, despite the number of crops targeted by *Trichogramma* species, the number of pests targeted and *Trichogramma* species used have not increased as significantly as crops targeted did (see Smith 1996 for an exhaustive list at that time).

To this day, only a fraction of described species of *Trichogramma* are being used as biological control agents. Adding to the fact that collecting them is not an easy task due to their minute size, morphological identification is difficult and taxonomy requires improvement. Indeed, the *Trichogramma* genus contains a variety of cryptic species that are referred to as species complexes which are groups of closely related organisms that are so similar in appearance that the boundaries between them are often unclear. Additionally, many species of *Trichogramma* contain mixtures of sexual and asexual populations, and in many, asexual reproduction is induced by the endosymbiont *Wolbachia* (Stouthamer et al 1990, Stouthamer et al 1993). As a result, their ecology is not clear as most *Trichogramma* are reported to be extremely polyphagous (e.g., Knutson 1998). This amount of unclear information leads to a sub-optimized use of *Trichogramma* in biological control.

## Manuscript 3: Survey of *Trichogramma* species in France and neighboring countries: what drives their local presence and diversity? (First Draft)

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Note: The supplementary material from this manuscript is available at the end of the thesis.

### Introduction

Trichogrammatidae is one of the most widely distributed and biologically diverse family of parasitoids with 89 genera and more than 800 species worldwide (Querino et al 2010). The members of this family measure about 0.3mm to 1mm and can be recognized by their three segmented tarsi. The trichogrammatids constitute an economically important group of hymenopteran parasitoids attacking eggs of various orders of insect pests such as Lepidoptera and Hemiptera, and more rarely Coleoptera, Diptera and Thysanoptera (Yousuf and Shafee, 1988). Within this family, *Trichogramma* is the largest genus with approximately 210 species worldwide (Pinto 2006). Many of these species are described as highly polyphagous and habitat-generalists. This assumption should however be taken cautiously. Indeed, the taxonomy of the genus still needs to be clarified, morphological characters used for identification appearing sometimes ambiguous and molecular characterizations remaining fragmentary. As already observed in other hymenopteran parasitoids, some so-called “generalists” could be in fact complexes of stenophagous species, biotypes or host races. A better understanding of species delimitation within the *Trichogramma* genus is thus a first mandatory pre-requisite to understand and improve their roles as biological control agents of agricultural or forestry pests. A second pre-requisite is to more precisely document the geographical and ecological distributions of the *Trichogramma* species. Such knowledge is indeed relevant for, at least, three reasons: (i) Evaluating the functional role of naturally present *Trichogramma* strains (conservation biological control) (Eilenberg et al. 2001), (ii) inferring, from their *in natura* distributions, the potential of some strains in augmentation or even classical biological controls (Eilenberg et al. 2001), (iii) being in capacity to tackle regulation issues - be they at the international (Cock et al. 2010) or national (for France, Decree NOR:AGRG1225395A) levels - about the native and exotic biodiversity. Investigating the presence and local diversity of *Trichogramma* species is however usually a time-consuming and quite unrewarding task without knowing if the mean weak success is due to sub-optimal methods (mainly: exposure of sentinel eggs, collection of naturally parasitized host eggs, collection of adults in traps or through sweeping), a misunderstanding of favorable sampling conditions and/or to actual biological patterns (low density, spatial aggregation, strong temporal variations). With regard to these two pre-requisites (deciphering species delineation within *Trichogramma* and documenting the patterns of local presence and biodiversity), we present here the results of an intensive field sampling covering a large area in France and few sites in Spain and Belgium.

This initiative was made possible through (i) a participative network, (ii) the use of sentinel eggs of the mass-reared *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Honda et al. 1999, Babendreier et al. 2003, Wang and Shipp 2004, Hertz et al. 2007), (iii) the sequencing of part of the mitochondrial gene COI for taxonomic affiliations.

## Methodology

### **Field organization**

The sampling was realized in 2015 and 2016 (from mid-April to early October) on a total of 76 sites (Figure 24). This was made possible thanks to the involvement of more than 40 collectors including INRAE staff but also volunteers (among them, several members of the French Society of Entomology). Each collector was equipped in order to be able to adequately expose sentinel eggs in the field and send them back to our laboratory by snail mail or delivery drivers. The sampling site, the sampling methods (“Sprays” and/or “Egg cards” – see below), the number of “Sprays” and/or “Egg cards” were left to the discretion of collectors as well as the sampled plants, provided that they were able to identify them at least to the genus level. For each combination site-by-date-by sampling-method, the following information was recorded: names of the collectors, dates of hosts’ exposure and removal, name of the location, longitude, latitude, altitude, type of ecosystems (crops, garden or natural habitats), list of sampled plants. From the geographical information (longitude, latitude and altitude), we inferred the local climate (Fig. 24) based on the study of Joly et al. (2010).

### **Sampling methods**

*Trichogramma* were sampled by exposing sentinel eggs of *Ephestia kuehniella* (source: Bioline Agrosiences), those eggs having been previously sterilized by exposure to ultraviolet radiation. Two sampling methods were used: “Sprays” and “Egg cards”.

- *Sprays*. For each sampled plant, the lower faces of leaves were sprayed with water and then sprinkled with sentinel eggs. After 3 days of exposure, the leaves were cut off, kept in an envelope and brought back to the lab where they were transferred into emergence boxes. These boxes were empty cylindrical cardboard boxes (height=15cm -- width=10cm) with a connected transparent tube at its top in which emerged *Trichogramma* stay (positive phototropism).

- *Egg cards*. For each sampled plant, strips of rectangular sticky paper (5cm x 1cm) sprinkled with sentinel eggs were stapled under 4 leaves. The strips were retrieved after 3 days and put in glass vials (length: 7,5 cm, width: 1cm) with a cotton cap.

Both boxes (sprays) and vials (egg cards) were then kept in a temperature-controlled room at 21°C with 70% humidity and 16L:8D photoperiod. For both sampling methods, the emergence of *Trichogramma* was monitored twice a day.

### **Strains’ creation**

The boxes were kept for one month and checked daily for *Trichogramma* wasps (Temperature:22°C, RH:70%, Photoperiod:16L/8D). For each box, all *Trichogramma* that emerged were cumulated into a glass vial with a drop of honey. Once emergences stopped, a

strip of *E. kuehniella* sterilized eggs was provided in order to allow the individuals to reproduce. Based on the molecular characterization (see below), this procedure was then possibly renewed in order to maintain a perennial strain. Each of these strains was then maintained in duplicates (*T. cacoeciae*) or triplicates (other species) within the ISO9010:2015 certified Biological Resource Centre “Egg Parasitoids Collection” (<https://data.inra.fr/dataset.xhtml?persistentId=doi:10.15454/AY4LMT>) under the following conditions (Temperature: 18°C, RH: 70%, Photoperiod: 16L/8D).

### ***Molecular characterization***

After one generation of lab-rearing, three individuals of each strain were molecularly characterized. The identification of the *Trichogramma* strains was achieved through the Sanger sequencing of part of the mitochondrial gene, *Cytochrome Oxidase I*. The use of part of this molecular marker mitochondrial marker is routinely used in our laboratory (Benvenuto *et al.* 2012, Al khatib *et al.* 2014, Correa *et al.* 2016) as well as elsewhere (Zaldivar-Riveron *et al.* 2010, Jinbo *et al.* 2011, Garipey *et al.* 2014).

### ***DNA extraction and amplification***

Genomic DNA were extracted from fresh (recently killed individuals) or frozen (individuals previously kept in ethanol at -20°C) tissues using the prepGEM® Insect kit (ZYGEM, PIN0500). Individuals were placed individually in 15 µL of mix and incubated for 3 hours at 75°C and then for 5 min at 95°C. DNA extracts were stored at -20°C.

The fragment of *Cytochrome Oxidase subunit I* (COI) was amplified using the primers LCO1490 and HCO2198 (Folmer *et al.* 1994). One µL of DNA was used for the PCR reaction (performed in a total volume of 25 µL). PCR was performed with the Multiplex PCR Master Mix QIAGEN (Cat No./ID: 206145) with a final concentration of 3 mM MgCl<sub>2</sub> and 0.125 µL of each of the two primer solutions (100 µM). The PCR conditions were as follows: 95°C for 15 min; 40 cycles at 95°C for 30 s, 50°C for 90 s, 72°C for 1 min; and a final elongation step at 60°C for 30 min. The size of PCR products was analysed using a QIAxcel DNA Fast Analysis Kit (QIAGEN S.A.S) on a Qiaxcel Advanced System (QIAGEN S.A.S).

### ***Sequencing and taxonomic affiliation***

PCR products were uni-directionally sequenced (primer HCO2198 only) using the Sanger method by the companies BECKMAN COULTER and then GENEWIZ (Essex, GB). The retrieved sequences were trimmed, cleaned and then compared to previously acquired COI haplotypes available in an internal database (Warot *et al.* submitted). Each PCR product corresponding to a seemingly new haplotype was then sequenced in the second direction in order to define a consensus and verify its originality. The new haplotypes were then added to our database. Details about the observed COI haplotypes are provided in Table 3.

The extrapolation of the taxonomic affiliation from the COI haplotype is detailed in Warot (2018). Briefly speaking, this affiliation does not only rely on the sole clustering observed on COI but also on a multi-locus phylogeny using other genes as well as crossing experiments. Taken as a whole, this integrative approach allowed to delineate several species or species complexes.

### ***Statistical analyses of local presence et diversity***

Three response variables were considered for each combination of “Site x Date x Sampling Method”

- The total number of “sprays” / “egg cards” from which *Trichogramma* individuals emerged
- The species diversity i.e., the number of species (or species complexes) evidenced
- The molecular diversity i.e., the number of COI haplotypes

Six predictors were a priori relevant:

- Sampling method (“Spray” or “Egg card”)
- Sampling effort i.e., the number of “Spray”/“Egg card” locally used
- Date (year - month)
- Climate (from Joly et al. 2010)
- Ecosystem (crops / garden / natural habitats)
- Botanic diversity i.e., the number of sampled plant species

Because “zeros” (no emerged *Trichogramma* from one combination of “Site x Date x Sampling Method”) were over-represented, we used Zero-Inflated Poisson models (ZIP) to explain each of the explained variables with the predictors. Briefly speaking, ZIP can be viewed as a two-step modelling, with, firstly, the fitting a Binomial distribution to deal with the presence/absence of *Trichogramma* and, secondly, the fitting of a Poisson distribution on presence data only. As far as we know, the strategy about model selection with ZIP is not as well-defined as with (generalized) Linear models. We thus tried, for each explained variable, to adjust a ZIP model keeping the maximum of predictors (see § Results and Discussion). The analyses were carried out by using the “pscl” package (Jackman et al 2020) from R (version 3.6.1).

## Results

### ***Sampling’s description***

Taken as a whole, 129 combinations of “Site x Date x Sampling Method” were available and related to 76 different locations (Figure 1). Most of these combinations (108) relied on the use of “Sprays”, the “Egg cards” being used in only 21 combinations. Both methods were simultaneously (same site and date) tested only 13 times. A total of 2019 “sprays” were used and, in 50% of the cases, the number of sprays ranged between 5 and 22 per site-by-date combination. By comparison, a total of 754 “egg cards” were used and, in 50% of the cases, the number of egg cards ranged between 16 and 45 per site-by-date.

In term of climate, all the 8 French climates defined by Joly et al. 2010 were represented: Type 1 = Mountainous: 6; Type 2 – semi-continental: 15; Type 3 – Degraded oceanic: 15; Type 4 – Altered oceanic: 16; Type 5 – Oceanic: 15; Type 6 – Altered Mediterranean: 9; Type 7 – South-Western: 9; Type 8 – Mediterranean: 29. The three types of habitats (Crops, Garden and natural Habitats) were quite equivalently represented with respectively 40, 41 and 48 combinations of “Site x Date x Sampling Method”.

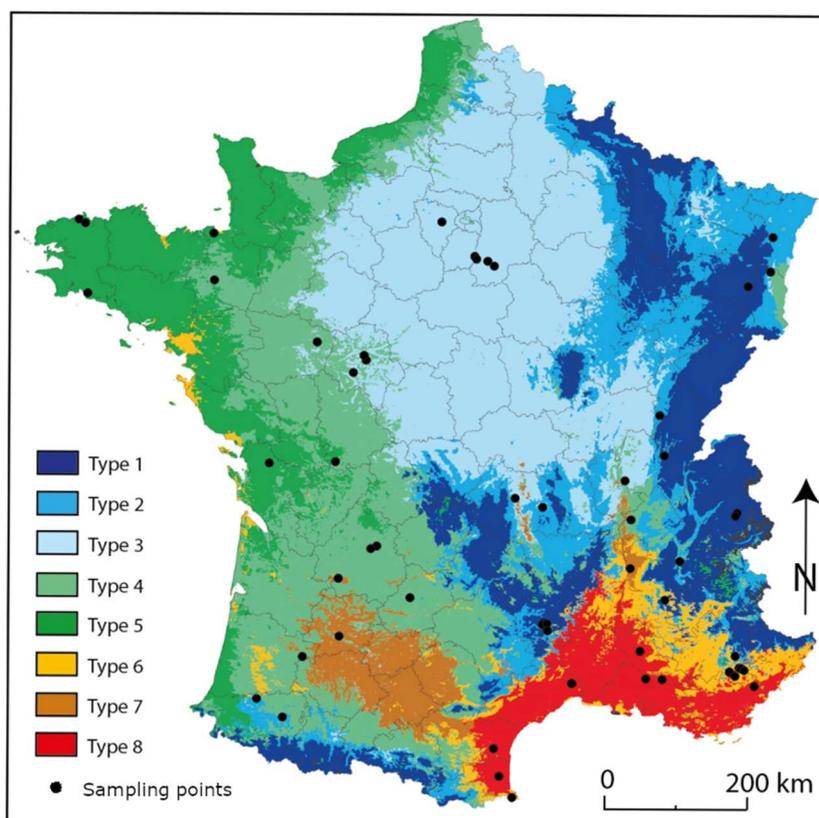


Figure 23 - Map of all sampling points with the 8 main climates of France (from Joly et al 2010). 1: Mountain climates 2: Semi-continental climate 3: Degraded oceanic climate 4: Altered oceanic climate 5: Oceanic climate 6: Altered Mediterranean climate 7: South West climate and 8: Mediterranean climate

### ***COI diversity and taxonomic affiliation***

As shown in Table 3, 35 COI haplotypes were evidenced within this, 22 of them being “new” with regard to our previous knowledge. Three of them (Hap116, Hap020 and Hap101, 1 occurrence each) presented some variations in their amino-acid sequence and were thus probably pseudogenes or technical artefacts, the amino-acid sequence for this COI fragment being, to our knowledge, conserved within the *Trichogramma* genus. These haplotypes can be affiliated to eight taxonomic entities (species or species complexes). By decreasing order of occurrences, those entities are :

- *the “cacoeciae-embryophagum” complex* (76 occurrences for 11 haplotypes). As detailed in Warot (2018), this complex includes two thought to be sister species, the thelytoquous and pervasive *Trichogramma cacoeciae* and the arrhenotoquous and rarer and/or more specialized *T. embryophagum* (not sampled here). We however believe that other sister species could exist within this complex. Those putative species are represented here by the haplotypes Hap076 (provisional name “misG”) and Hap108 (provisional name “misD”).
- *the “daumalae-evanescens” complex* (43 occurrences for 8 haplotypes). As detailed in Warot (2018), this complex includes several strains that were identified as *T. evanescens* based on molecular and or morphological markers and only one strain historically described as *T. daumalae*.
- *T. semblidis* (8 occurrences for 4 haplotypes)
- *T. cordubensis* (6 occurrences for 4 haplotypes)

- an unidentified species provisionally called “*swC*” (4 occurrences for 3 haplotypes)
- an unidentified species provisionally called “*swE*” (3 occurrences for 2 haplotypes)
- the “*brassicae-euproctidis*” complex (2 occurrences for 2 haplotypes). As detailed in Warot (2018), this complex includes strains associated to two valid species names, *Trichogramma brassicae* and *Trichogramma euproctidis*. Representatives of these two species appears to be indeed reproductively incompatible but involved in a complex network of partial compatibilities.
- an unidentified species provisionally called “*misB*” (1 occurrence for 1 haplotype).

id	Reference strain	Strain's origin	Source	Morphologica I expertise	Morphological identification	Taxonomic affiliation	GENBANK Accession	Amino-acid sequence	Occurrences
Hap_011	A1	France, 1989	Historic collections	BP	<i>T. evanescens</i>	I - daumalae-evanescens	MG932147		17
Hap_021	M1	France, 1993	Historic collections	BP	<i>T. evanescens</i>	I - daumalae-evanescens	MG932157		8
Hap_044	V2.12	Moldavia, 2013	BIOLINE Agrosociences	JS	<i>T. evanescens</i>	I - daumalae-evanescens	MG932180		5
Hap_045	V2.16	Moldavia, 2013	BIOLINE Agrosociences	JS	<i>T. evanescens</i>	I - daumalae-evanescens	MG932181		1
Hap_049	V3.6	France, unknown	BIOLINE Agrosociences	JS	<i>T. evanescens</i>	I - daumalae-evanescens	MG932185		9
Hap_050	V3.19	France, unknown	BIOLINE Agrosociences	JS	<i>T. evanescens</i>	I - daumalae-evanescens	MG932186		1
Hap_122	ESP462	Spain, 2016	TRIPTIC project	_	_	I - daumalae-evanescens	MG932241		1
Hap_146	MURU0025	France, 2016	TRIPTIC project	_	_	I - daumalae-evanescens	MG932263		1
Hap_012	TP63	Egypt, 2004	Historic collections	BP	<i>T. cordubensis</i>	III - cordubensis	MG932148		2
Hap_096	CAS134	France, 2016	TRIPTIC project	_	_	III - cordubensis	MG932223		2
Hap_116	PUG191	France, 2015	TRIPTIC project	_	_	III - cordubensis	MG932235	varprot	1
Hap_126	FLO241c	France, 2016	TRIPTIC project	_	_	III - cordubensis	MG932244		1
Hap_138	BL117	France, 2016	TRIPTIC project	_	_	IX - swE	MG932256		1
Hap_139	BL116	France, 2016	TRIPTIC project	_	_	IX - swE	MG932257		2
Hap_073	TSM0016	France, 2015	TRIPTIC project	_	_	V - misB	MG932207		1
Hap_039	E1.7	France, 2013	BIOLINE Agrosociences	JS	<i>T. euproctidis</i>	VI - brassicae-euproctidis	MG932175		1
Hap_124	BSEM-S	France, 2017	TRIPTIC project	_	_	VI - brassicae-euproctidis	MG932242		1
Hap_017	SEMA2	France, 2002	Historic collections	BP	<i>T. semblidis</i>	VIII - semblidis	MG932153		3
Hap_112	FPV034_A	France, 2015	TRIPTIC project	_	_	VIII - semblidis	MG932231		3
Hap_114	AL004	France, 2015	TRIPTIC project	_	_	VIII - semblidis	MG932233		1
Hap_172	BL110	France, 2016	TRIPTIC project	_	_	VIII - semblidis	MG932281		1
Hap_006	TCAL	France, 1987-1989	Historic collections	BP	<i>T. cacoeciae</i>	XII - cacoeciae-embryophagum	MG932142		55
Hap_019	PMBio1	France, 2014	Historic collections	BP	<i>T. cacoeciae</i>	XII - cacoeciae-embryophagum	MG932155		5
Hap_020	ISA15034	France, 2016	TRIPTIC project	_	_	XII - cacoeciae-embryophagum	MG932156	varprot	1
Hap_076	GOT0098	France, 2016	TRIPTIC project	_	_	XII - cacoeciae-embryophagum (misG)	MG932210		1
Hap_101	ACJYR0132	France, 2015	TRIPTIC project	_	_	XII - cacoeciae-embryophagum	MG932224	varprot	1
Hap_106	ACJYR0116	France, 2015	TRIPTIC project	_	_	XII - cacoeciae-embryophagum	MG932227		1
Hap_107	ACJYR0121	France, 2015	TRIPTIC project	_	_	XII - cacoeciae-embryophagum	MG932228		2
Hap_108	NS008	France, 2015	TRIPTIC project	_	_	XII - cacoeciae-embryophagum (misD)	MG932229		1
Hap_117	ESP227	Spain, 2016	TRIPTIC project	_	_	XII - cacoeciae-embryophagum	MG932236		4
Hap_118	PMBIO2	France, 2014	Historic collections	BP	<i>T. cacoeciae</i>	XII - cacoeciae-embryophagum	MG932237		3
Hap_173	FLO218	France, 2016	TRIPTIC project	_	_	XII - cacoeciae-embryophagum	MG932282		1
Hap_016	SEMV	France, 1998	Historic collections	BP	<i>T. semblidis ?</i>	XIII - swC	MG932152		1
Hap_109	NS007	France, 2015	TRIPTIC project	_	_	XIII - swC	MG932230		1
Hap_121	ISA3082	France, 2015	TRIPTIC project	_	_	XIII - swC	MG932240		2

Table 3 - Details about the COI haplotypes found in this study

For each COI haplotype, the following details are provided: (i) the name of its reference strain; (ii) the origin – country (oversea territory in brackets) and date of sampling – of this strain; (iii) the frame within which the strain was obtained; (iv) when available, the identification of the strain based on morphological characters; (v) the name of the identifier, Bernard Pintureau (BP) or Julien Séguret (JS); (vi) our taxonomic affiliations (Warot 2018); (vii) the GENBANK accession numbers; (viii) the observed deviation from the most common amino acid sequence (label “varprot”), (ix) the number of occurrences in this study.

### ***Local presence/absence of Trichogramma***

When all the taxonomic affiliations are considered, 143 occurrences of *Trichogramma* were counted (see Supplement 1). With regard to the total number of “Sprays” and “Egg Cards” (2773), this indicates a success of only about 5%. Yet, at least one occurrence of *Trichogramma* individuals was observed in 41% (31/76) of the sites. With regard to the climatic conditions, *Trichogramma* was observed at least once in 36% (“Type 3 – Degraded oceanic”) to 60% (“Type 2 – semi-continental” and “Type 7 – South-Western”) of the sites except for the “Type 5 – Oceanic” where no *Trichogramma* was collected. *Trichogramma* was observed in each of the three Habitats with the following frequencies: Crops (8/17 sites i.e., 47%); Garden (7/30 sites i.e., 23%) and Natural habitats (16/29 sites i.e., 55%). *Trichogramma* were reported during each of the investigated month (April-September - October discarded because only one site-by-date combination) and the highest frequencies of occurrences were reported in June and July (respectively 8 and 9%). The lowest frequency of occurrences was observed in August (only 2%).

### ***Predicting the distribution of Trichogramma***

The attempts to fit ZIP models led to the production of singularities see Table 4. It was thus not possible to find suitable models by keeping the 6 predictors or even 5 predictors. Models with fewer predictors were thus investigated by discarding the “botanic diversity” (thought to be the less relevant) and even (for COI diversity) the type of habitats (apparently not discriminant) and removing one by one the remaining predictor. Finally, one model was conserved for each explained variable (see Supplement 3). As highlighted in Table 4, the two steps (firstly the presence/absence modelled by the Binomial then the count modelled by the Poisson) considered were not influenced by the same predictors. About the presence/absence (binomial distribution), the sole significant predictor appears to be the sampling effort. It acts however in a counter-intuitive way on the total of occurrences and the species diversity since the probability of *Trichogramma*'s detection was negatively correlated with the sampling effort. About the counting (Poisson distribution), several predictors had significant effects. In particular, the sampling methods with “Sprays” was proven more effective than “Egg cards” for the three explained variables. Quite logically, a positive correlation was found between the sampling effort and each of the explained variables. In addition to the climate “Type 5 - Oceanic” where no *Trichogramma* was found, the climates “Type 3 – degraded oceanic”, “Type 7 – South Western” and “Type 8 – Mediterranean” were proven less favourable for the three explained variables.

Table 4 - Summary of the effects evidenced by the ZIP models

Process	Variables	Total of occurrences	Species diversity	Molecular diversity
<b>Presence / absence (Binomial step)</b>	Sampling method	No effect	No effect	No effect
	Sampling effort	Negative correlation	Negative correlation	No effect
	Date	–	–	–
	Climate	No effect	No effect	No effect
	Ecosystem	No effect	No effect	–
	Botanic diversity	–	–	–
<b>Total count (Poisson step)</b>	Sampling Method	Advantage for “sprays”	Advantage for “sprays”	Advantage for “sprays”
	Sampling effort	Positive correlation	Positive correlation	Positive correlations
	Date	–	–	–
	Climate	Defavourable climates 3, 7 and 8	Defavourable climates 3, 7 and 8	Defavourable climates 3, 7 and 8
	Ecosystem	No effect	No effect	–
	Botanic diversity	–	–	–

### Discussion (bullet points only)

This study is probably the most intensive *Trichogramma* survey in France, the COI-based characterization providing a way to compare with further studies. Several COI haplotypes were generated thanks to this survey and we obtained individuals from 3 (misB, swE and swC) to 5 (misD and misG from the “*cacoeciae-embryophagum*” complex) unaffiliated species that deserve more attention.

Of course, this study does not pretend to be exhaustive and the use of sentinel eggs of *Ephestia kuehniella* probably constraints the *Trichogramma* species that can be recovered. Pintureau (2008) listed for instance much more species present in France and, from our own experience, we have already collected on other Lepidopteran species some *Trichogramma* species (*T. gicai* from *Iphiclides podalirius* or *T. embryophagum* from *Thaumetopoea pityocampa*) that we have never observed from *E. kuehniella*.

Taken a whole, the results indicate a strange pattern, *Trichogramma* being almost pervasive (climates, habitats) but at seemingly low density. This agrees with complementary surveys led at a more local scale (see for instance Ion Scotta’s PhD Thesis). Once again, this pattern may be the consequence of the use of *E. kuehniella* eggs that may be not so attractive. However, climatic conditions appear to be a main driver of the presence/absence and diversity of *Trichogramma*. From a practical point of view, this study was helpful to choose between the two sampling methods (“Sprays” versus “Egg cards”) and to define more suitable dates for *Trichogramma* sampling (June and July).

This study is thus relevant for (i) the integrative characterization of *Trichogramma* species, (ii) the understanding of their geographical and ecological distributions and (iii) regulation issues regarding the status (native, exotic but established, exotic) of *Trichogramma* species evaluated as biological control agents.

## Manuscript 4: Behind the scenes of *Trichogramma*: a close-scale monitoring study of egg parasitism within the natural population of a native butterfly (*in prep.*)

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### Introduction

The collection of living strains of *Trichogramma* species in the wild can be achieved by two main methods: the use of sentinel eggs and the collection of wild eggs. Hegazi et al 2005 used bait traps containing sentinel eggs of *Sitotroga cerealella* (Lepidoptera: Gelechiidae) to survey naturally occurring *Trichogramma* species in olive farms in Egypt. They collected only four species of *Trichogramma*. In 2007, Herz et al extended the survey of *Trichogramma* in olive groves to several countries of the Mediterranean region (Tunisia, Greece, Portugal and Egypt). They added sentinel eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae) to the bait traps and they collected a few eggs of the olive moth *Prays oleae* (Lepidoptera: Yponomeutidae) and the jasmine moth *Palpita unionalis* (Lepidoptera: Pyralidae). In total, six species of *Trichogramma* were collected, of which two were only found in one country: *T. oleae* in Tunisia and *T. nerudai* in Portugal. More generally, the use of sentinel eggs (especially from *E. kuehniella*) is the most used method to survey *Trichogramma* species (Barnay et al 2001). Globally, this method has the inconvenient to greatly restrain the list of *Trichogramma* species that may be collected. Perhaps egg's attributes (e.g., the size of the host egg, or the strength of the chorion) may determine the species that are able to use it as host. In contrast, surveying *Trichogramma* by collecting eggs from wild species broadens the list of *Trichogramma* species. It is not unusual to collect new species of *Trichogramma* when collecting wild eggs, especially in natural habitats (Woelke et al 2018). Furthermore, parasitism rates are usually far superior from eggs collected in the wild than from sentinel eggs. Logan (2019) collected 540 eggs from various Lepidoptera and introduced 2030 sentinel eggs from three species of lepidoptera on O'ahu Island (Hawaï). Wild eggs were parasitized at a much higher rate than sentinel eggs (59.1% and 3.1% respectively).

The present work is complementary to the previous section where many *Trichogramma* strains were collected by using sentinel eggs of *E. kuehniella*. Here we had the chance to collaborate with Brigitte Kan-van Limburg Stirum and Pieter Kan and sample wild eggs of *Iphioides podalirius* (Lepidoptera: Papilionidae) in the wild. B. and P. Kan are naturalists and creators of the website "filming-varwild.com". They own hours of videos that help understanding the behavior of butterflies (and associated parasitoids), not only courtship flight, egg laying, caterpillar habits, and various developmental stages etc., but also how they adapt for survival in often very specific habitats. Therefore, we were able to monitor wild populations of *I. podalirius* and its associated egg parasitoids and acquire extremely rare data. Although *I.*

*podalirius* is common in the studied area, it is an endangered species in other areas such as Romania (Moise 2014), Belgium and parts of France (Fichet et al 2008). Knowledge on parasitoid complexes of European Rhopalocera is, in general, rather scarce and restricted to agricultural pests as *Pieris rapae* (Lepidoptera: Pyralidae, Bisset 1938, Richards 1940) and *Pieris brassicae* (Lep: Pyr, Faure 1926, Bisset 1938, Feltwell 1982). That is why, in order to get a better understanding of the different species of parasitoids as well as the relationships between host eggs, and parasitoids in a natural situation, we set up a monitoring project in which the oviposition in relation to egg parasitism was accurately monitored on a daily base. This allowed us to sample and characterize the parasitoids associated with *Iphiclides podalirius* in natural conditions, a species that is very far from the concerns of crop pests and biological control.

Here the goal was to describe the community of egg parasitoids associated with *I. podalirius* while collecting new strains of *Trichogramma* and study the role of biological control agents that may be collected.

## Methodology

### Site and Sampling

In a field in Callas (Var, France), 10 five-year-old Almond trees (*Prunus dulcis*) from 2 to 4 meters high were selected. All trees are more or less aligned from east to west (Fig 25, total 14 meters) but the distances between trees may vary. The Almond trees grow on a shaggy lawn, with in the west two Apricot trees of about 7 meters high and around 4 meters high vegetation of Mirabelle plum and Fig trees, a house in the south and forest all around. The Scarce

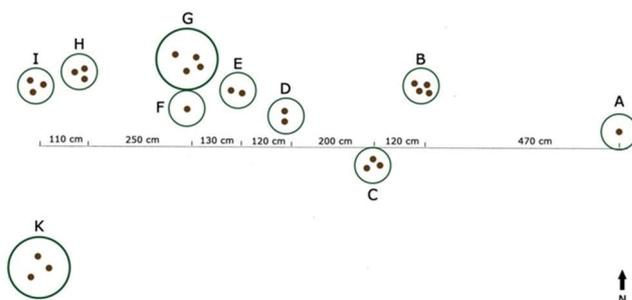


Figure 24 - Simplified map of the research location in Callas with 10 plots of *Prunus dulcis* (A, B, C, D, E, F, G, H, I and K). The dots within the plots represent the main branches of the trees.

swallowtail also regularly deposited eggs on Cherry trees and Blackthorn bushes in the near surroundings.

The monitoring was carried out every day from the first egg found on April 6<sup>th</sup> of 2018 until the end of the second generation of *I. podalirius* on September 13<sup>th</sup>. Each tree was daily examined thoroughly until no more eggs were found, except the heavy raining days. Each daily monitoring session was conducted between 11am and 2pm because we noticed that it

was the butterfly's most active moment of the day. The top part of the tree was examined using binoculars (Pentax Papilio II 8.5x21). The first three days after being laid, the eggs of the Scarce swallowtail of about 1 mm in size, are pearl white. Generally, this is the best time to find them. Eggs that are not parasitized turn gradually brownish and are more difficult to find. The period of hatching can vary between one and two weeks depending on the weather conditions. Eggs were collected when they turned brown. If an instar one caterpillar was observed in the research area, indicating that the egg had not been spotted, it was recorded and removed. Parasitized

eggs can change, after a few days, from marbled white to black, depending on the type of parasitoid. In some cases, the eggs get a more or less transparent appearance, so that the larva or imago can be seen inside. In cloudy and rainy weather, it is more difficult to recognize and find eggs than when it's sunny. But the angle of incidence of the sun also plays a role in relation to the position of the egg, so it depends at what time of the day, research takes place. For eggs on leaves on higher branches, the shadow of an egg can easily be recognized when the light falls from above through the leaf. In many cases the binocular have been used, with which it is possible to have a close look and be able to distinguish details very well. Regularly the trees were observed the same day at different times and from different angles. But despite the fact that trees were regularly observed at daily base, it happened that freshly laid eggs were not seen and sometimes only discovered after a few days. Eight empty parasitized eggs were found at the research location. Collected eggs were placed in glass tubes, closed with cotton-wool. These tubes were placed in a rack and checked daily to monitor the emergence of egg parasitoids. When an egg parasitoid emerged, they were placed in a container, with 70% Ethanol, provided with a label. To compare the coloration and the exit hole of the empty egg shells, the egg was put in a separate container with the same label as the parasitoid. The containers with all parasitoids and associated empty eggs were then transferred to INRAE, Sophia-Antipolis for identification.

#### *Molecular characterization*

The parasitoids were kept in 70% ethanol and brought back to CRB EP-Coll for molecular characterization. First, a non-destructive DNA extraction was performed for each sample with 20µl of QuickExtract™ DNA Extraction Solution kit (Lucigen® QE09050), during 15min at 65°C and 2min at 98°C. Then a portion of the mitochondrial gene Cytochrome oxidase I (COI) was amplified by using the primer pair: LCO 1490 (5'-GTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer et al. 1994). One µL of DNA was used for the PCR reaction (performed in a total volume of 25µL). PCR was performed with the Multiplex PCR Master Mix QIAGEN (Cat No./ID: 206145) with a final concentration of 3 mM MgCl<sub>2</sub> and 0.125 µL of each of the two primer solutions (100 µM). The COI-PCR conditions were as follows: 95°C for 15min, followed by 35 cycles of (i) 94°C for 30s, (ii) 50°C for 1min, and 72°C for 1min with a final extension at 72°C for 10min. PCR products therefore generated were shipped to Genewiz (Leipzig, Germany) for their Sanger sequencing. The obtained sequences were checked and compared to already existing sequences in the international database Genbank® and the internal database of CRB EP-Coll for the *Trichogramma*. The determination of other species has been realized by Lucian Fusu (Romanian taxonomist, Universitatea Alexandru Ioan Cuza). Molecular analyses were carried out with the use of Geneious software version R10 (Drummond et al., 2010).and MEGA software version 7.0.25 (Tamura et al., 2013).

#### *Statistical analysis*

In this work, only descriptive statistics were used as the amount of data limited our ability to conducted robust statistical analyses. The polar plots were realized by using the *polar.plot* function of the *plotrix* package of R (Lemon et al 2021).

## Results

During the whole monitoring period, 179 eggs were collected from the trees. From those eggs 75 were parasitized, allowing us to sample 1188 parasitoids individuals from 4 families (the host egg being able to contain up to 32 adults of *T. gicai*). In total, 7 species of parasitoids were identified: *Anastatus bifasciatus*, *Eupelmus confusus*, *Ooencyrtus* sp., *Trichogramma cordubensis*, *T. evanescens*, *T. gicai* and *Trichogramma* sp. From the 75 parasitized eggs, 55 were parasitized by *Trichogramma* species. Although all 7 species came out of *I. podalirius*' eggs. We have no way to check for eventual hyperparasitoid behavior among them and thus they will be all considered egg-parasitoids in this paper.

We observed an asynchrony between generations of *I. podalirius* and parasitism. Indeed, in the first generation, parasitism rate was low with only 18 % of eggs parasitized. In contrast, during the second generation, the parasitism rate increased up to 63 % (Table 5).

Table 5 - Summary of the outcome of all eggs collected per tree

	Tree ID	A	B	C	D	E	F	G	H	I	K	TOTAL	% TOTAL
Eggs sampled	Caterpillars	40	3	3	1	1	2	1	1	-	11	63	76%
	Parasitized	8	-	3	1	1	-	-	-	-	2	15	18%
	Empty	2	-	-	-	-	-	-	1	-	1	4	5%
	Gone	1	-	-	-	-	-	-	-	-	-	1	1%
	First generation	51	3	6	2	2	2	1	2	0	14	83	
	% TOTAL	61%	4%	7%	2%	2%	2%	1%	2%	0%	17%		
Eggs sampled	Caterpillars	4	5	10	1	1	-	1	-	1	1	24	25%
	Parasitized	16	8	16	6	3	-	6	2	2	1	60	63%
	Empty	3	3	1	-	-	1	-	-	-	1	9	9%
	Gone	2	1	-	-	-	-	-	-	-	-	3	3%
	Second generation	25	17	27	7	4	1	7	2	3	3	96	
	% TOTAL	26%	18%	28%	7%	4%	1%	7%	2%	3%	3%		
Eggs sampled	Caterpillars	44	8	13	2	2	2	2	1	1	12	87	49%
	Parasitized	24	8	19	7	4	-	6	2	2	3	75	42%
	Empty	5	3	1	1	-	1	-	1	-	2	13	7%
	Gone	3	1	-	-	-	-	-	-	-	-	4	2%
	Gen 1 + Gen 2	76	20	33	9	6	3	8	4	3	17	179	
	% TOTAL	42%	11%	18%	5%	3%	2%	4%	2%	2%	9%		



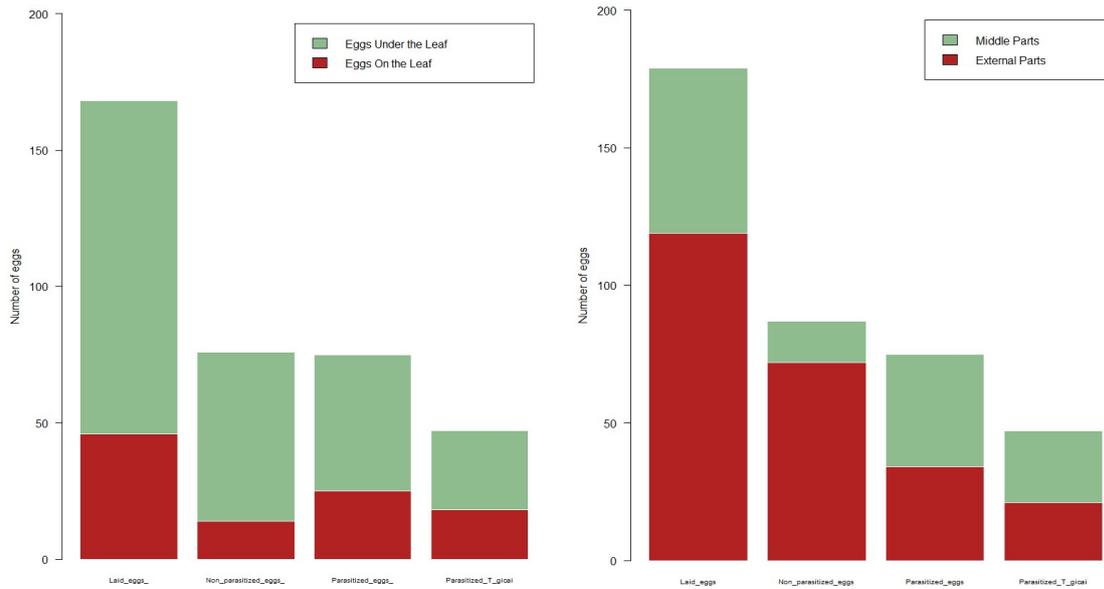


Figure 26 - Stacked barplots of all samples (eggs laid, non parasitized eggs, parasitized eggs and parasitized by *Trichogramma gicai*) with mention of the location at the leaf scale (left) or at the tree scale (right).

For all eggs laid on the peripheral leaves, we noted their position on the tree (i.e., North, East, South, West). We found most of the eggs parasitized by *T. gicai* at the North, South and East (Fig 28A) whereas the eggs parasitized by other parasitoids were found mainly at the west of the trees (Fig 28B).

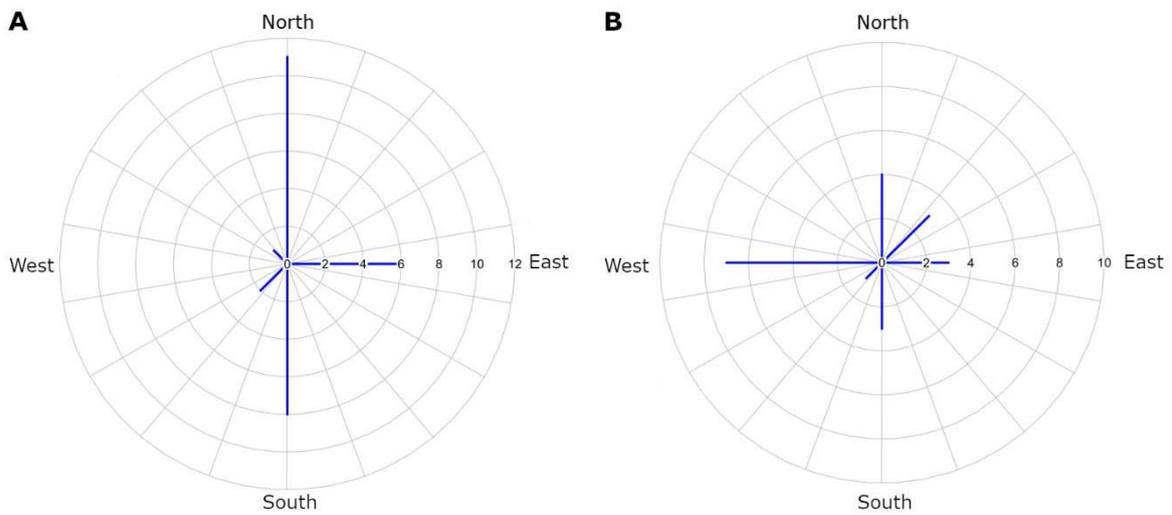


Figure 27 - Polar positions of: A: Eggs parasitized by *Trichogramma gicai*, B: Eggs parasitized by other parasitoid species.

## Discussion

In this work, we acquired rare data about the biology and ecology of *I. podalirius*. In fact, wild insect populations that are not linked to crops are rarely monitored with such detail. We described the egg parasitoid community associated with *I. podalirius*, as well as the egg-laying pattern of females. We observed more eggs laid under the leaves than over the leaves and less eggs laid close to the trunk than on the periphery of the tree. A lot more butterflies exhibit the same behavior of laying eggs under the leaves (LSPN 1987). This is thought to protect them from the weather (e.g., direct sunlight or heavy rains). However, spatial egg laying patterns of Papilionoidea (ex: Rhopalocera) have not received much attention as crop pests (mostly moths) are the most studied species. Here, we can suppose that the inside of the tree is less accessible or that females lay their eggs as soon as they find a suitable leaf. Nonetheless, eggs have been found really close to the trunk in this study. The interesting fact was that parasitism rate was higher closer to the trunk. Here we suggest that this is due to the foraging pattern of *Trichogramma* species (the main parasitoids associated with *I. podalirius*). In fact, once a female of *Trichogramma* has found a suitable host plant, it forages exclusively by walking. Therefore, the trunk may act as a central hub and each dividing branch multiplies the number of paths available. This may decrease the probability to find an egg if it is laid far from the trunk. In summary, the egg laying pattern of *I. podalirius* could evolve in relation of the structure of the tree (e.g., leaf density) or to parasitism (e.g., evasion).

Our results also suggest that *T. gicai* (the main parasitoid) and the other parasitoids may not share some areas of the tree. In fact, we observed that *T. gicai* was found everywhere but the west (n=28), where most of the other parasitoids were found. However, the number of parasitized eggs found was too small to significantly conclude if there was indeed a niche separation at a micro-spatial scale among parasitoids of *I. podalirius*.

When compared to the sentinel eggs used in chapter 3-II, we observe that parasitism rates are much higher with wild eggs. This is consistent with recent study (Logan 2019). However, *Trichogramma* diversity was the same than what is usually observed on sentinel eggs of *Ephestia kuehniella* (up to 5 species, see manuscript 3 above). Indeed, monitoring all eggs of *I. podalirius* laid, within our ten plots during a whole season, has permitted the identification of a regular parasitoid complex including 4 species of *Trichogramma* (from which half are not found on *E. kuehniella*). None of the collected parasitoids are specific to *I. podalirius*. Nonetheless, *T. gicai*, the most predominant species collected, was previously thought specific to *I. feishtmalli* (Stefanescu et al 2010). *Trichogramma evanescens* has been reported here for the first time as a parasitoid of *I. podalirius*.

This dataset has been very time consuming to acquire. Indeed, every day during 6 months all the leaves from ten trees were monitored for *Iphiiclides podalirius* eggs. However, due to the small sample size (e.g., small number of replicates, overall low amounts of eggs collected), we were not able to link environmental features or parasitism rates with the evolution of the community structure. In fact, the very dense temporal scale considered (every day for several months) made it impossible to sample trees within more than one field. As a result, despite the tremendous sampling effort that was put into the study, the data was only able to describe the ecosystem the same way a naturalist description of the ecosystem would. However, as explained in the introduction of this thesis, naturalist observation and description are the starting points

of ecological theories or hypothesis. Here the data seemed to hint at a micro-niche separation among egg parasitoids of *I. podalirius*, but the lack of replications and low amount of data points did not allow for any statistical conclusion. This work strengthens the idea that, when dealing with poorly known organisms or systems as a whole, observation and description are required before carrying out experimental ecology. During future research prospect, it would be interesting to design a robust experiment with the clearly defined goal to validate or discard our hypothesis of: (1) a niche separation at a micro-scale among the egg parasitoids of *I. podalirius* (2) evolution of the egg laying pattern of *I. podalirius* in response of either tree structure or parasitism.

## Discussion

In this work, I used monitoring data from four biological control programs to try to address questions related to ecology and population biology. Two datasets came from classical biological control programs: *Torymus sinensis* against the Asian chestnut gall wasp (chapters 2 and 3) and *Mastrus ridens* against the codling moth (chapter 4). The other two were mainly oriented towards augmentation biological control with *Trichogramma* species (chapter 4). Of the resulting four datasets, the data from *Torymus sinensis* was the most relevant to investigate ecological issues. Indeed, this biological control program allowed to investigate three independent issues, of which two are part of this work: (i) the use of population growth data to infer colonization dates of naturally colonized sites, (ii) the study of the impact of the successful control of *D. kuriphilus* by *T. sinensis* on native community of parasitoids associated with the pest. On the other hand, the three other case studies yielded not enough data, or even no data at all, to be used from an ecological standpoint. From the background literature presented as introduction and the multiple experiences detailed in the several chapter of this work, I will discuss the key elements from biological control programs that, if done correctly, may provide valuable opportunity for experimental ecology.

### Biological control: ecological observations and hypotheses

In the introduction, I put forward the idea that defining a clear hypothesis to test was a key feature for the successful use of biological control programs for experimental ecology. However, it appears that testing hypotheses is not the only way to build ecological knowledge from biological control programs. For example, the *Torymus sinensis* case-study was designed initially to test the hypothesis that propagule pressure impacts establishment success and dispersal, which was done in Borowiec et al (2018). However, this same biological control program also allowed us to provide knowledge based on the observation of the structural patterns of native communities across different localities (chapter 3). In fact, it is the repetition of independent observations that eventually leads to the genesis of hypothesis. In chapter 4, we surveyed the egg parasitoids associated with *I. podalirius* in order to provide a first estimate of the level of parasitism and the diversity of egg parasitoids in a natural context for which little is known. Nonetheless, the data obtained led to some hypotheses that could be explored in the future: (i) the butterfly's egg laying pattern is adapted to tree structure or to their egg parasitoids' distribution and (ii) egg parasitoids of *I. podalirius* display niche separation at a micro scale. In this case, it is not the lack of theoretical background that limited the usefulness of the gathered data, but mostly the lack of replications. In fact, if we had several replicates of the same study, we could have gathered enough data to explore the generality of the observations we made.

This raises awareness about the importance of the experimental/sampling design and its close relation with the quality (e.g., statistical robustness) of the data gathered through a biological control program.

## Rolling out the red carpet: The importance of experimental design.

When biological control programs are paired with a research question in ecology, their experimental design includes three major components: the spatial scale (i.e., the geographical area covered by the experimental design), the implementation of “control” replicates and experimental modalities.

Two of the four case-studies (*T. sinensis* and *Trichogramma*) used in this work involved the implementation of a large-scale experimental design, with multiple location scattered on a large geographical area. In the *T. sinensis* case, such effort was rewarded by the opportunity to investigate several ecological issues that were not initially planned. In contrast, the remaining two case studies (*M. ridens* and *I. podalirius*) involved more localized experimental designs over a single location. Although the *I. podalirius* case-study would have greatly benefited from a bigger experimental design, I am unsure whether it would have been the same for the *M. ridens* case-study or not. In fact, the issue with the latter was not due to analytical strength but rather the lack of overall data. A similar situation occurred several years ago in France. In 2008, *Psytallia lounsburyi* was released in France as part of a classical biological control program against the fruit fly *Bactrocera oleae* (Diptera: Tephritidae). The program was the opportunity to study the genetics and demography of introduced populations (Malausa et al 2010a, 2010b). However, virtually no data was available from the post-release monitoring, although about 42 000 individuals were released over 60 sites. This represented a big investment that did not live up to the scientific expectations, mobilizing more than 10 persons for 4 years.

In biological control, « control » replicates are not routinely included as they require an additional investment for monitoring that does not increase either the control provided or the probabilities of success. Their use is much more common in CBC when there is no easy way to establish direct causal links between pest density, natural enemy density and the strategy deployed (e.g., habitat modification). In such configuration, control replicates may help distinguish the impact of CBC strategies by offering a comparative view with “untouched” systems (e.g., Jacquot et al 2019). During classical biological control of *Fallopia japonica* in England, control sites were monitored to record foliar and ground dwelling arthropods, floral diversity and the response of the target weed (Shaw et al 2011). In the *T. sinensis* case study, control sites were initially monitored but they were rapidly colonized by the biological control agent and therefore could not be used as a comparative treatment. However, the large number of sites monitored and the temporal variation of introductions still allowed to describe the evolution of community structure patterns within native parasitoids without any temporal confounding factor (chapter 3). Moreover, as a silver lining, these initially control sites were used to describe the growth of naturally dispersed populations in chapter 2.

The methodology used to release a biological control agent may constitute an experiment in itself as factors like, for example, the number of individuals and the frequency of introductions - two factors united within the concept of propagule pressure - can be manipulated. As a matter of fact, how propagule pressure relates to the success or failure of invasions has been the object of intense investigations (Williamson and Fitter 1996, Lockwood et al 2005, Britton et al 2013, Cassey et al 2018), to which biological control programs have significantly contributed (e.g., Memmott et al 1998, Memmott et al 2005, Goode et al 2019, Shea and Possingham 2020). By manipulating propagule pressure during the release of *Neodryinus typhlocybae*, a specialist parasitoid of the phytophagous flatid planthopper *Metcalfa pruinosa*, Fauvergue et al 2007

experimentally showed that the biological control agent population did not experience any form of Allee effect. In situations like this where it is hard to establish a clear link between the measured factor and the response variable, there is a clear need to replicate the measure to reduce the importance of confounding variables. That is why, in such cases, the releases need to happen within a large number of sites to have enough replicates, and over an area large enough to avoid cross colonization between sites and therefore biased propagule pressures (e.g., Hayes and Barry 2008, Malausa et al 2010, Davidson and Rieske 2016). In addition to manipulating the raw numbers of adults introduced different development stages can be introduced at once. In fact, some classical biological control agents tend to disperse rapidly following release, which is a well-established driver of Allee effect (Stephens et al 1999, Liebhold and Tobin 2008, Gascoigne et al 2009). A release strategy that increases propagule pressure and diversifies the ways to introduce the biological control agent (e.g., introducing mobile adults and sessile or less mobile pre-imaginal stages) can be used for countering dispersal outside of the considered area (Goode et al 2019) and spread the total population in time, increasing the chances of “fragile” and short-life span organisms to persist. As discussed in the introduction of this thesis, classical biological control programs can be used as a framework to study much more than propagule pressure only. In fact, several issues from the fields of evolutionary, landscape, community and population ecology can be studied through biological control (e.g., Gardiner et al 2009, Thies and Tschardtke 2009, Tschardtke et al 2016, Tomasetto et al 2017, Ortiz-martinez and Lavandero 2018, Escobar-ramirez et al 2019). However, in most of the cases the deployment of biological control needs to be replicated, would it be in terms of landscape context, climate, release strategy, plant communities, habitat modification, native predation pressure etc. Most of the time this would need to happen over large areas to ensure that replicates are independent.

In essence, the experimental design greatly impacts the nature of the resulting data. Thus, I emphasize that, if to be used as experimental ecology, one of the most important characteristics of the experimental design of biological control programs is to have several spatially and temporally independent replicates. However, the bigger the investment, the bigger the disillusion if the biological control agent fails to establish. As a result, in some cases (e.g., when describing the impact of biocontrol practices on biodiversity, see Jacquot et al 2019) the experimental design is reduced and the state of ecological variables (e.g., pest regulation, arthropod diversity, etc.) may be compared in a before-after configuration, highlighting the importance of monitoring the system prior to biological control.

### The calm before the storm: The initial state of the system

Monitoring abundances, diversity, or ecological interactions before the release of a biological control agent is rarely done. However, in many cases of experimental ecology, this appears to be a very important step as it allows for the analysis of dynamical changes in response to an experimental perturbation. In fact, we would not have been able to estimate the growth of naturally dispersing populations in chapter 2 if sites had not been sampled before deploying *T. sinensis*. In contrast, in the *M. ridens* case study, very little was actively monitored prior to the release and I think that it played a significant role in the outcome of the project. In fact, pest densities were surveyed in several orchard by another team and we knew that this orchard was

organic and presented a high pest density compared with the others. However, if we had surveyed pest populations in the surrounding habitat, we might have been able to plan our post-release monitoring better. Indeed, we observed that pest densities within orchards were extremely low and the main populations were limited to big walnut trees in hedgerows. More generally, the initial state is extremely valuable in experimental ecology, both when testing ecological hypothesis or describing interactions. For instance, distributions of both pests and natural enemies are influenced by a variety of factors in addition to the distribution of the lower trophic levels (Berndt et al 2006, Chacon et al 2008, Escobar-ramirez et al 2019, Ortiz-martinez and Lavandero 2018). The initial distributions of pest populations may be important to investigate prior to the releases as continuous pest distribution is one of the attributes leading to successful control (Malecki et al 1993). Moreover, the initial population structure of native communities, landscape structure etc. are important information for assessing impacts of biological control agents and biological control practices in general (Louda et al 1995, Louda et al 2000, Adler et al 2001, Berndt et al 2006, Jacquot et al 2019). Nonetheless, similarly to the use of control sites, measuring ecological variables before the release of a biological control agent is not consistently done even though it may provide biological practitioners with insight on the efficient control of the pest and/ or establishment success of the biological control agent (DeWalt 2006).

As a result of -but not only- the lack of initial state surveys, many biological control agents fail to establish, leaving practitioners unable to provide an ecological explanation for this phenomenon. In fact, being able to predict establishment's probability of success based on previous data would change the way biological control is carried out. However, although establishment is the most important outcome for biological control practitioners, its relevance for investigating ecological issues still needs to be discussed.

### Is establishment the key to successful experimental ecology?

Establishment is at the root of a successful biological control program. However, experimental ecology and biological control share different goals and, although they sometimes measure common variables (e.g., post-release pest densities), they do not need the same degree of precision or statistical robustness.

The *M. ridens* case-study failed to provide the data that should have been used to investigate how the landscape interacts with early dispersal of the biological control agent. The main reason is either that the population failed to locally establish or that it persisted at a sub-detectable density. In this case, although establishment of the biological control agent is a necessary first step for acquiring monitoring data, it does not imply that such data will be sufficiently robust to be analyzed. For example, the *Torymus sinensis* case study was a success, and its establishment rate was of a hundred percent. However, the data gathered only allowed us to investigate the native parasitoid community structure from a presence-absence standpoint and the use of quantitative analyses was limited. Similarly, during the biological control program against the olive fruit fly in France using *Psytallia lounsburyi*, the biological control agent was found the year after the releases (Malausa (2010b). However, the low number of parasitoids collected did not allow to achieve the scientific goal which was to test the effect of hybridization on invasion success (Malausa 2010a).

Overall, biological control and ecological experiments have separate goals that may not be dependent from one another. Indeed, the success of a biological control program is mainly focused on pest control, economic savings for farmers and reduced pesticide use. It therefore, relies mostly on the impact the introduced population has once it is established. On the other hand, experimental ecology may not rely as heavily on establishment as biological control does. Indeed, failures may indeed provide valuable insights on the ecological factors influencing early post-introduction dynamics, provided that relevant data regarding the ecology of the system, the natural enemies, or the pest population dynamics have been acquired. Acquiring this kind of data may be facilitated by the implementation of a post-release monitoring phase.

### Post-release monitoring: the issue of scale

Post-release monitoring is quite easier to implement when its only aim is to detect the populations when they reach a high enough density, as it is usually the case when assessing the success of biological control. However, when post-release monitoring is used to support experimental ecology, researchers are faced with challenges such as: (i) adapting the sampling methodology, (ii) selecting relevant temporal and spatial scales and most of all, (iii) adapting the ecological issues to investigate to fit the characteristics of the species at play.

First, the methodology used to sample the introduced population needs to be sensitive enough in order to account for low-density processes like invasion lags (Couotts et al 2018). More generally, the various methods available for sampling the population should be evaluated and compared to define the limits to the feasibility of the study. Overall, there are three main sampling methods used in post-release monitoring: (i) the use of “destructive”, more generalist methods such as malaise traps, yellow pan traps, vacuum aspiration etc., (ii) the use of sentinel host (or preys) and (iii) the use of wild hosts. Generalist methods are usually used for sampling overall insect (or arthropod) diversity (e.g., Jacquot et al 2019), for instance to describe the structure of natural communities or to compare levels of biodiversity across different habitats. The use of sentinel hosts allows the deployment of a custom sampling grid, provided that the sentinel host is available and easy to rear. In the *M. ridens* case-study, hosts larvae are parasitized for a short period (fifth instar, before pupation) during which the host is hidden in bark or in the soil. Therefore, it was improbable to obtain a regular fine sampling grid relying only on wild hosts. That is why, we chose to use sentinel larvae which in addition would avoid hindering the establishment and spread of the, already small, introduced population. However, this method turned out to be ill-adapted because its detection threshold is most likely higher than the very low densities, we were interested in. Incidentally, it is important to notice, that the only *M. ridens* that was sampled close to our release area was sampled by colleagues trapping wild larvae that go into diapause without the use of sentinel larvae. In contrast with *M. ridens*, in the case of *T. sinensis*, *D. kuriphilus* galls were static, numerous and easy to spot making the use of wild hosts a lot easier. Indeed, the establishment and spread of *T. sinensis* was monitored by sampling natural hosts with no visible negative effect on *T. sinensis* dynamics. In this case however, pest populations had reached very high densities so that we were only sampling a small fraction of the *T. sinensis* population.

In the case of *Trichogramma*, the most common practice to monitor populations is the use of the easy-to-produce sentinel eggs of the surrogate host *Ephesttia kuehniella*. However, in chapter 4 we used both sentinel eggs and wild eggs of *I. podalirius* to sample *Trichogramma*

species and the results were markedly different, with only 50% of the species collected on wild eggs also collected on sentinel *E. kuehniella* eggs. This was due to different levels of host specificities across *Trichogramma* species.

Detection thresholds impose strong limits when studying phenomena that are driven by density fluctuations such as establishment, extinctions, Allee effects etc. In such cases, the question of the spatial and temporal scales used for monitoring populations becomes critical. Scales (temporal or spatial) are composed of two components: grain and extent (Wiens 1989). Wiens defines the grain of spatial scale as the size of the individual unit of observation, which he compares to the quadrat of a field ecologist or the sample units of a statistician. The extent is defined by the overall area (or period) encompassed by the study. For logistical reasons, expanding the extent of a study usually also entails enlarging the grain. The enhanced ability to detect broad-scale patterns carries the cost of a loss of resolution of fine-scale details and detection of low-density populations. Depending on aims, a researcher may deal with different scale layers that have distinct ecological interpretations. For example, spatial distribution of carabid beetles has been shown to differ at various spatial scales with different factors responsible each time (Gongalsky and Cividanes 2008). For example, at the level of plant association, soil factors (e.g., litter depth, micro-climate and vegetation composition) begin to play the role in driving the communities whereas at the landscape level, geological factors (e.g., topography, landscape geochemistry, and history) play the major roles. Bat distribution in agroecosystems is impacted by both small scale (e.g., presence of a suitable landscape element) and landscape scale factors such as resource availability in large regions (Davidson-Watts et al 2006, Flaquer et al 2009, Akasaka et al 2012, Hillen and Veith 2013).

Biological control studies may then be used as a framework to identify the relevant spatial scales on ecological processes such as parasitism rates (Rusch et al 2011), or the impact of land-use intensity on arthropod diversity (Schalkwyk et al 2020). Usually, several spatial scales may explain a given ecological process. For example, Rusch et al 2011 found that that movement of univoltine parasitoids of the pollen beetle occurs at different spatial scales from explorative routine movements at small scales for daily resource-searching, such as host- and food-foraging, to dispersal by fast and long-distance movements for net displacement and settlement at large spatial scales. In some textbook examples, methods already consider these “nested” scales. For example, studying daily movement or the use of space by carabid beetles requires two different spatial scales. The former is based on largescale designs, where pitfall traps are disposed 10m or more apart from each other (e.g., Fournier and Loreau 1999, Olson and Wäckers 2007, Eyre et al. 2009, Smits et al. 2012) whereas the latter is based on a generally much smaller scale, on the order of a few meters (Thiele 1977, Allema et al 2014).

Ideally, the selection of the relevant spatial scale to monitor the biological control agent should rely on the spatial structure of pest populations. Pest populations inside crops are often sustained by immigration from reservoir populations in more natural habitats nearby. Therefore, due to lower habitat quality (because of the use of pesticides for example), crops may act as population sinks (Dias 1996). In the *M. ridens* case study presented in chapter 3, the core of *C. pomonella* populations may be found in wild neighboring tall walnut trees, while cultivated orchards that are frequently sprayed with insecticide (even in organic orchards) act as a marginal, sink habitat. This adds to the detectability issues of *M. ridens* populations induced by the small initial

population size. In this particular case, the potential source habitats that are wild walnut trees were not sampled because of the difficulty to sample the canopy. In this case, shifting our post-release monitoring away from orchards by sampling higher quality, more natural habitats might actually have provided a better outcome.

Overall, there is a desperate need for compatibility between the model organisms considered in biological control and the ecological issue that is explored. In laboratory experiments, researchers have the control over this compatibility as they can choose the correct organisms to study what they want. However, when using biological control as ecological field experiments, it is the ecological issue that needs to be adapted to the biology and ecology of the considered organisms and the technical possibilities deployable to monitor them.

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## Supplementary material

Manuscript 2: The open bar is closed: restructuration of a native parasitoid community following successful control of an invasive pest.

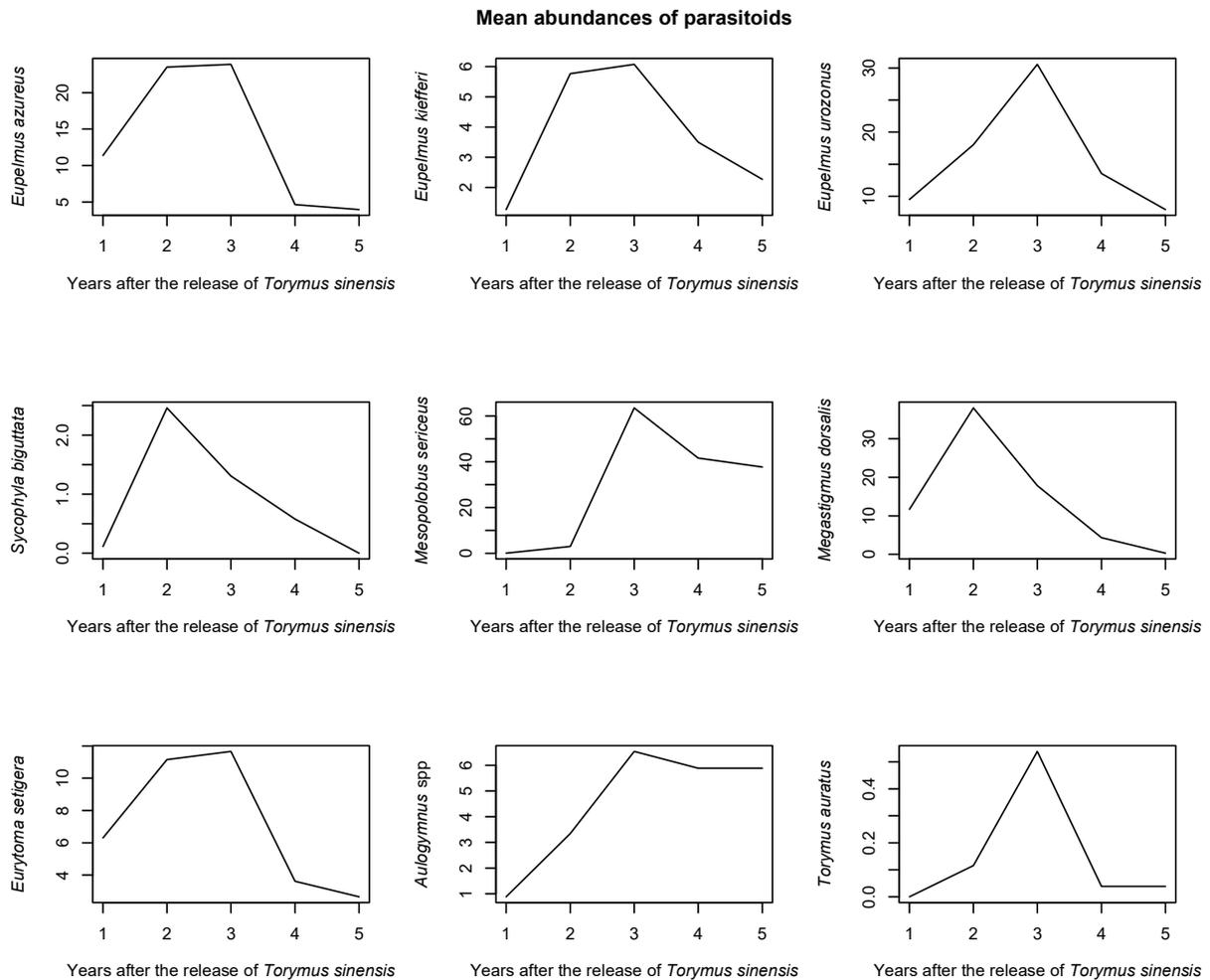


Figure S28 - Mean abundances of all native species during the five years of the study

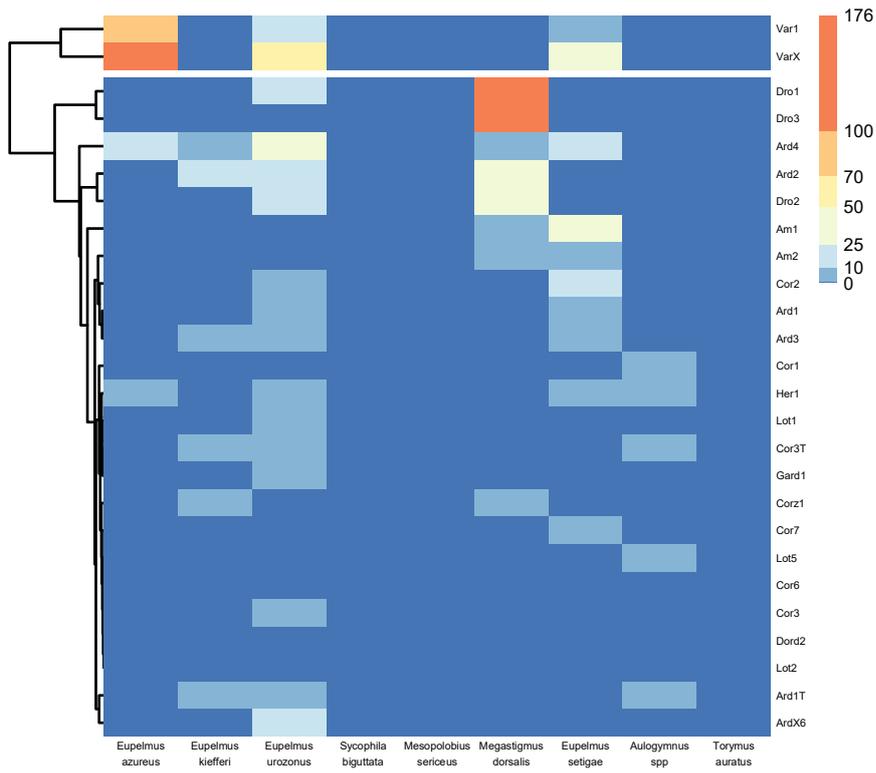


Figure S29 - Heatmap for the first year of the survey

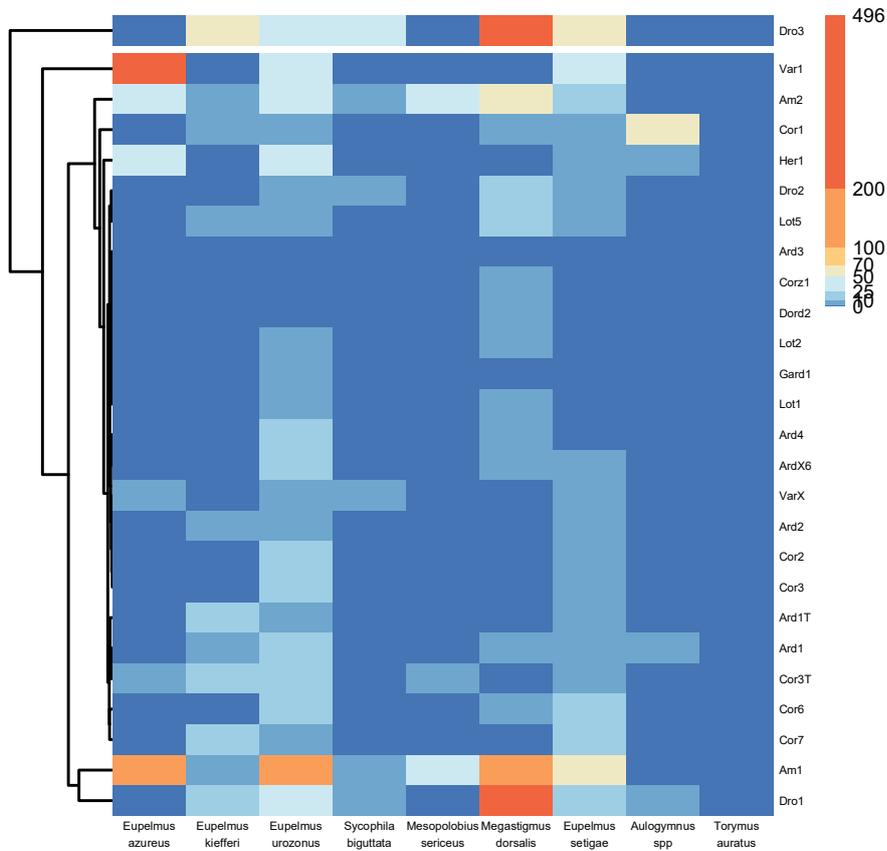


Figure S30 - Heatmap for the second year of the survey

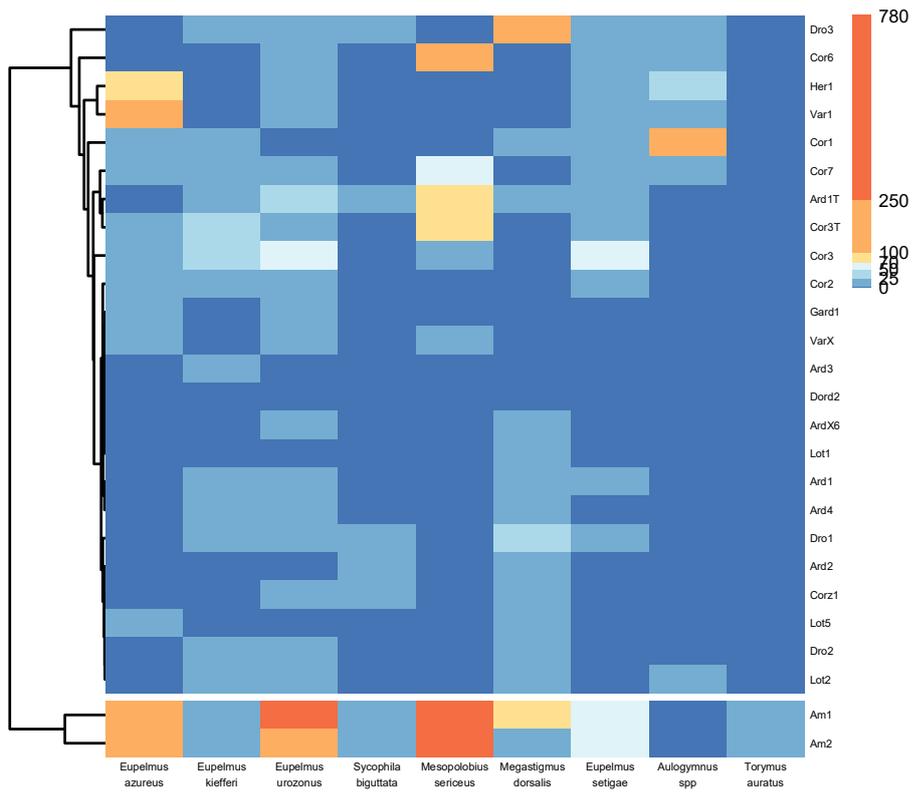


Figure S31 - Heatmap for the third year of the survey

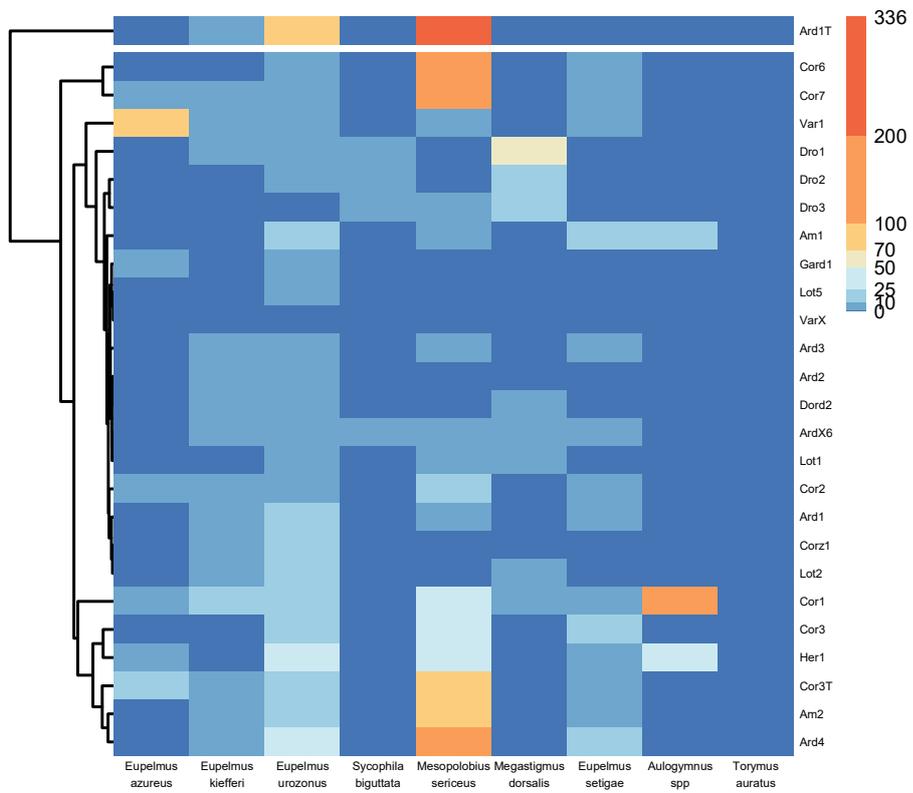


Figure S32 - Heatmap for the fourth year of the survey

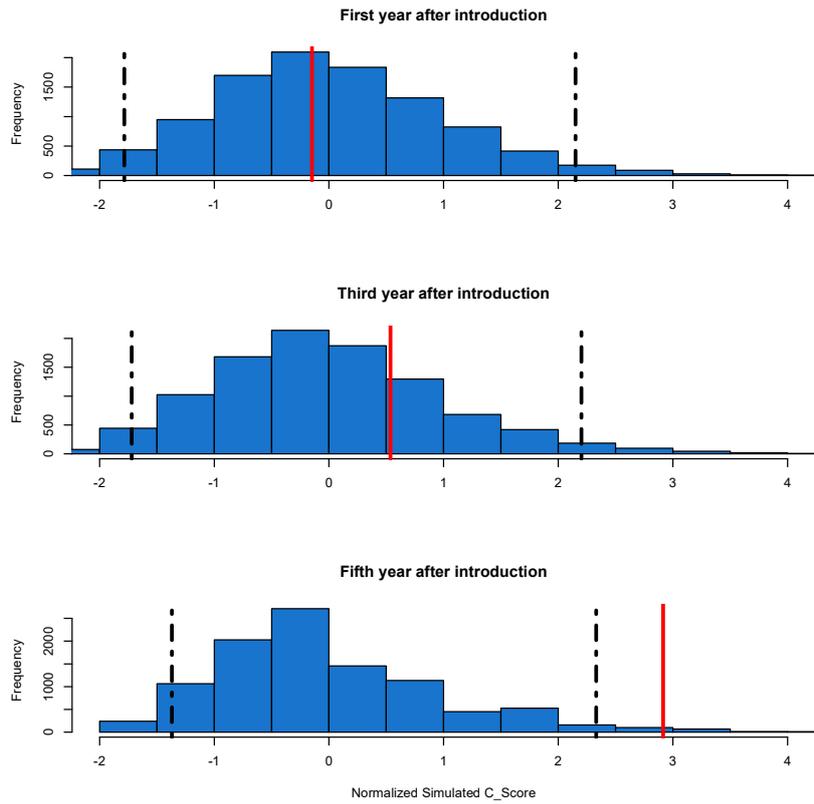


Figure S33 - Co-occurrence analysis excluding *Mesopolobus sericeus*

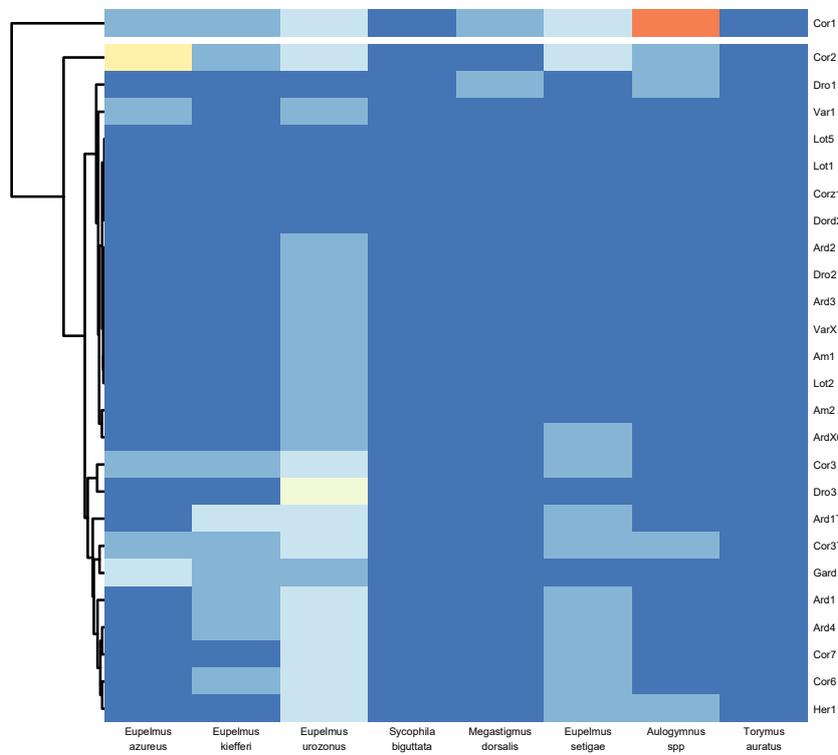


Figure S34 - Heatmap for the fifth year of the survey, excluding *Mesopolobus sericeus*

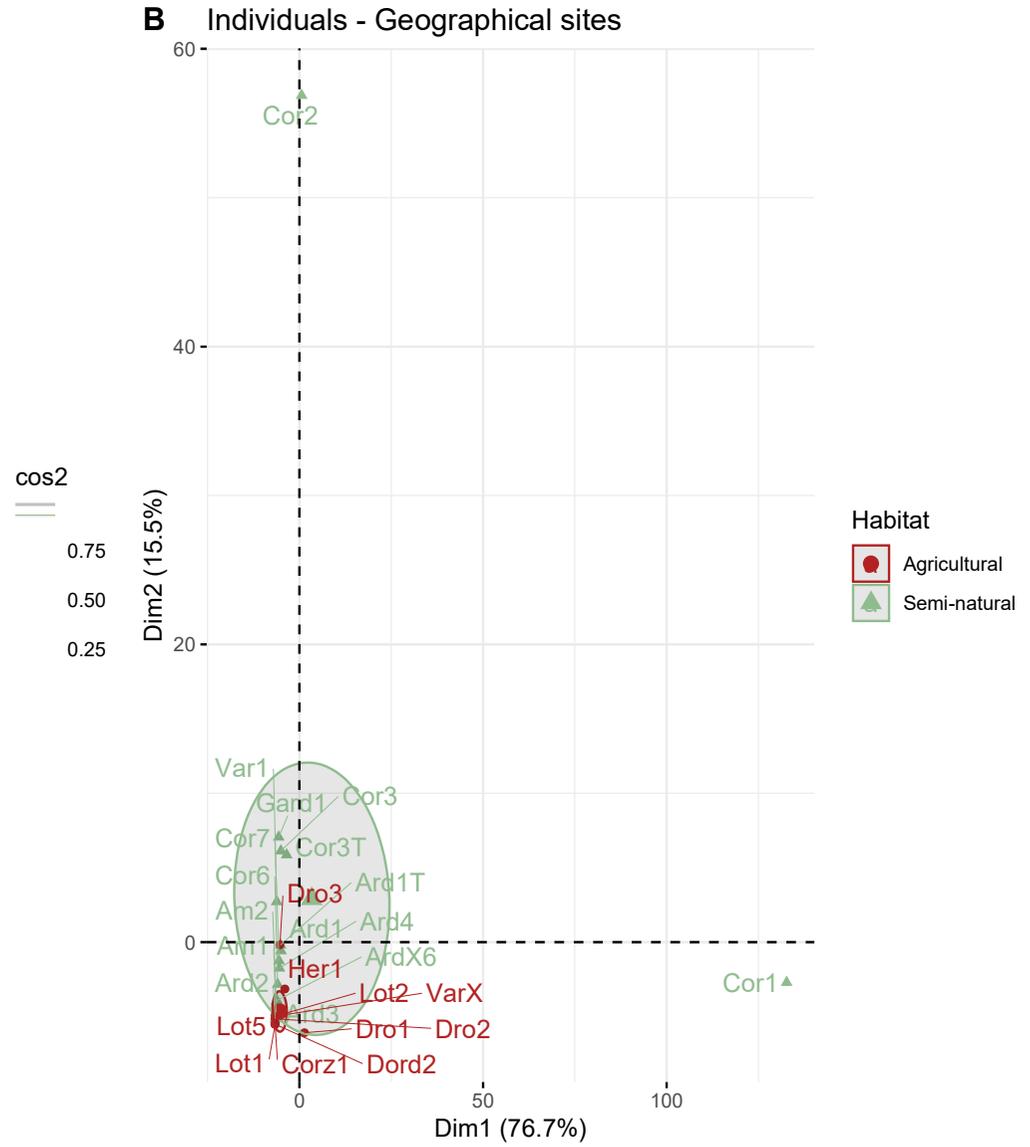
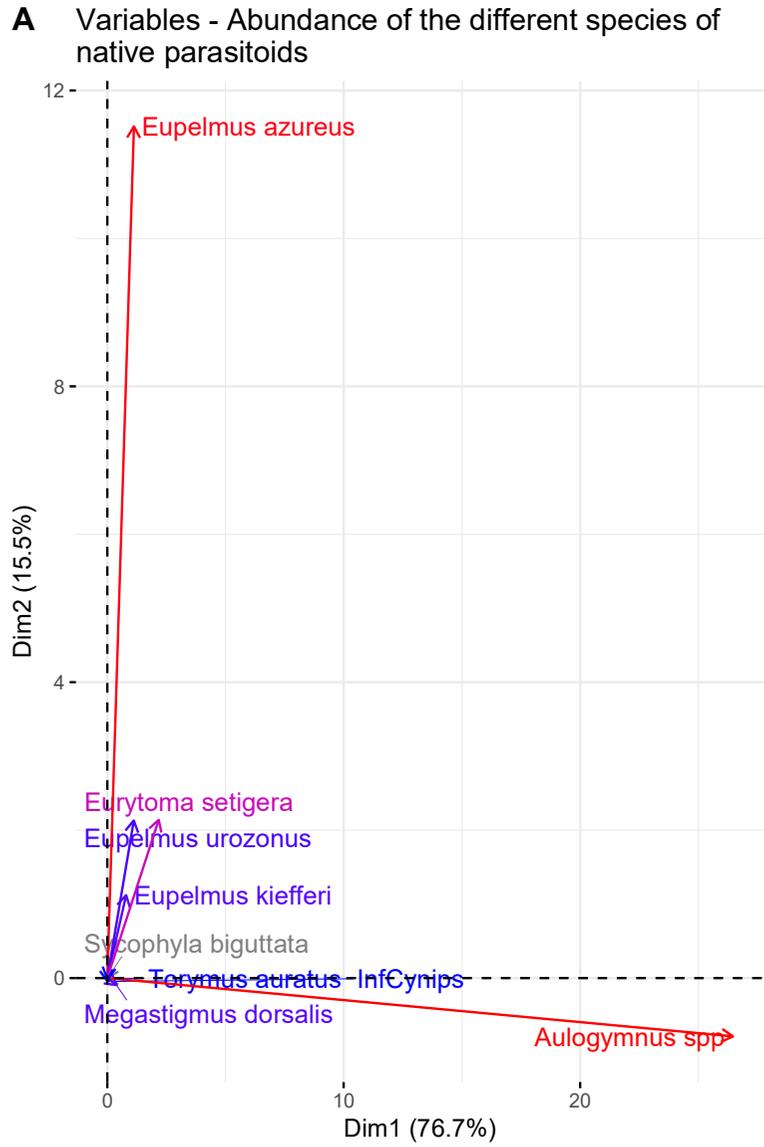


Figure S35 - PCA for the fifth year of the survey excluding *Mesopolobus sericeus*

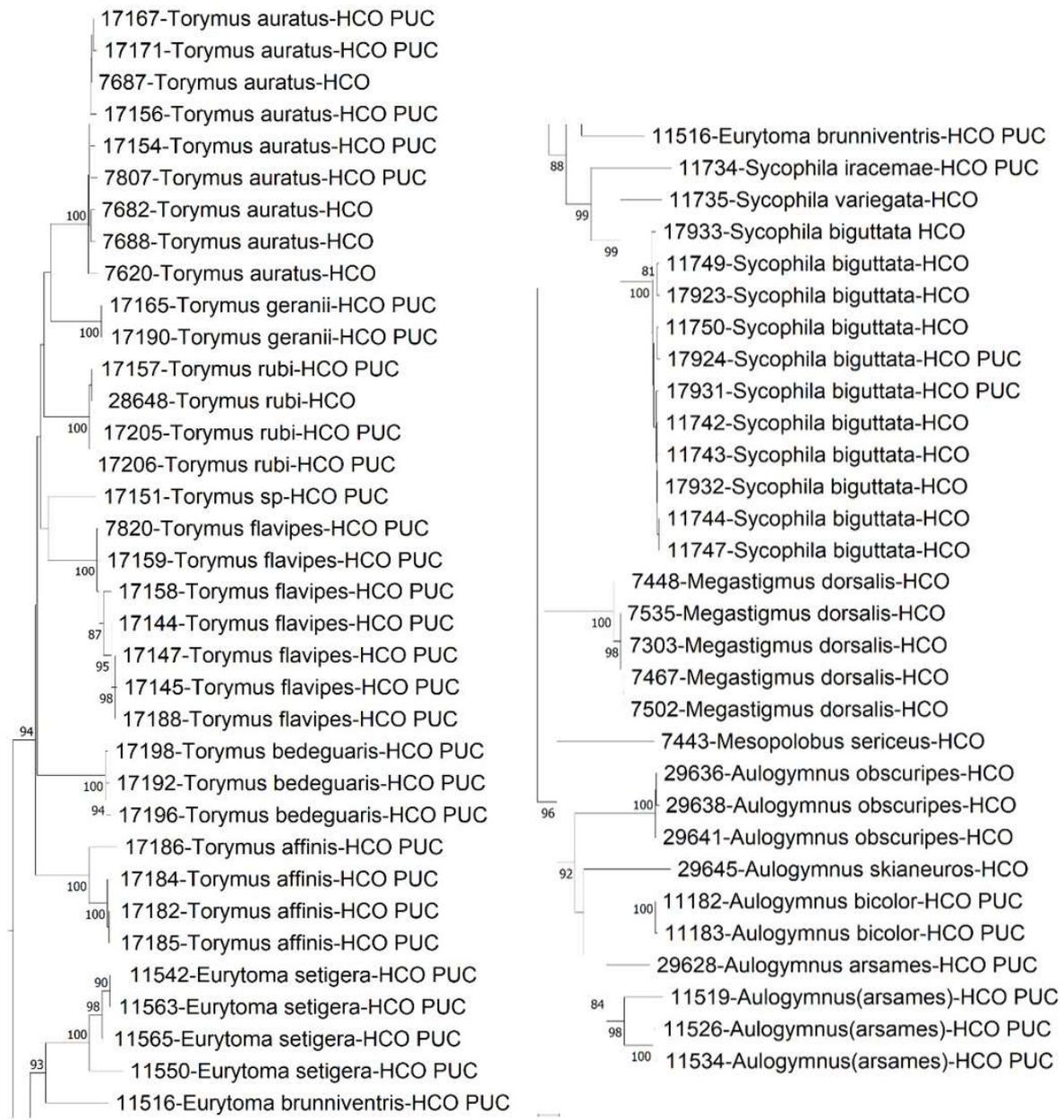


Figure S 36 - Neighbour joining tree obtained from the COI sequences detailed in Appendix 1. This was obtained using MEGA-X with the following parameters: Kimura 2 parameters distance, pairwise deletion (sequences between 550 and 612pb) and 500 replicates for bootstrapping. The labels are organized as follows (from left to right): (1) numeric code: internal code allowing to obtain additional information, (2) genus and species names based on morphological criteria (uncertain species name in brackets), (3) Primer used. The individuals were extracted from a more comprehensive dataset encompassing several hundreds of parasitoids from *Dryocosmus kuriphilus* and other gallwasps. Those selected here illustrate the haplotypic diversity within each taxa.

## Manuscript 3: Survey of *Trichogramma* species in France and neighboring countries: what drives their local presence and diversity?

### Supplement 1: Data records

All data were compiled into a single excel spreadsheet containing the following descriptors in order:

- “name”: First name of Collector(s)
- “surname”: Surname of Collector(s)
- “affiliation”: affiliation of the Collector(s)
- “start\_exposure”: Date on which the exposure began
- “end\_exposure”: Date on which the exposure ended
- “sample\_code”: The sample ID number, composed of letters referring to the site
- “site”: The name of the location
- “lat”: Latitude
- “lon”: Longitude
- “altitude”: Geographical altitude in meters
- “habitat”: We separated the sites in three classes of habitat (wild, crop and garden)
- “plant\_id”: The most precise identification for the plant at the given sample point
- “plant\_family”: The botanical family of the plant for a given sample point
- “plant\_genus”: The plant genus for a given sample point
- “plant\_species”: The plant species for a given sample point
- “height\_quali”: The shortest quantitative distance from the ground to the sample classified in three categories (0cm to 70cm: herbaceous, 70cm to 1.5m: shrubby and 1.5 to 3m: trees)
- “height\_quant”: The shortest quantitative distance from the ground to the sample
- “sampling\_method”: One of the two methods that were used (Sprays or Egg cards)
- “eggs\_retrieved”: Ratio between the amount of eggs that were retrieved and the total amount of eggs introduced categorized as follow: 0: no eggs ; 1: <50% ; 2: 50%<x<75% ; 3: 75%<x.
- “emergence\_of\_trichogramma”: Qualitative binary describing whether at least one *Trichogramma* individual emerged from the sample (1=yes and 0=no)
- “trichogramma\_species”: The name of the species without the genus (e.g. *cacoeciae*)
- “trichogramma\_haplotype”: The haplotype number based on the short COI barcode (Warot et al., submitted)

Supplement 2: Selection of ZIP models

explained variable	nb_variable	method	sampling_effortrt	period	climate	habitat	Plant diversity	
<b>Total of occurrences</b>								
	6	X	X	X	X	X	X	singularity
	5	X	X	X	X	X		singularity
	5	X	X	X	X		X	singularity
	5	X	X	X		X	X	singularity
	5	X	X		X	X	X	singularity
	5	X		X	X	X	X	singularity
	5		X	X	X	X	X	singularity
	4	X	X	X	X			singularity
	4	X	X	X		X		singularity
	4	<b>X</b>	<b>X</b>		<b>X</b>	<b>X</b>		<b>OK</b>
	4	X		X	X	X		singularity
	4		X	X	X	X		singularity
<b>Species diversity</b>								
	6	X	X	X	X	X	X	singularity
	5	X	X	X	X	X		singularity
	5	X	X	X	X		X	singularity
	5	X	X	X		X	X	singularity
	5	X	X		X	X	X	singularity
	5		X	X	X	X	X	singularity
	4	X	X	X	X			singularity
	4	X	X	X		X		singularity
	4	<b>X</b>	<b>X</b>		<b>X</b>	<b>X</b>		<b>OK</b>
	4	X		X	X	X		singularity
	4		X	X	X	X		singularity
<b>Haplotypic diversity</b>								
	6	X	X	X	X	X	X	singularity
	5	X	X	X	X	X		singularity
	5	X	X	X	X		X	singularity
	5	X	X	X		X	X	singularity
	5	X	X		X	X	X	singularity
	5	X		X	X	X	X	singularity
	5		X	X	X	X	X	singularity
	4	X	X	X	X			singularity
	4	X	X	X		X		singularity
	4	X	X		X	X		singularity
	4	X		X	X	X		singularity
	4		X	X	X	X		singularity
	3	X	X	X				singularity
	<b>3</b>	<b>X</b>	<b>X</b>		<b>X</b>			<b>OK</b>
	3	X		X	X			singularity
	3		X	X	X			singularity

### Supplement 3: R outputs of the selected ZIP models

```
> zip_tot10<-zeroinfl(tot~method+sampling_effort+climate+ecosystem, data=data)
> summary(zip_tot10)
```

Call:

```
zeroinfl(formula = tot ~ method + sampling_effort + climate + ecosystem, data = data)
```

Pearson residuals:

Min	1Q	Median	3Q	Max
-1.377e+00	-5.470e-01	-2.763e-01	-5.576e-05	6.590e+00

Count model coefficients (poisson with log link):

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-2.795e+00	1.132e+00	-2.470	0.013502 *
methodspray	3.633e+00	7.337e-01	4.952	7.36e-07 ***
sampling_effort	3.046e-02	5.779e-03	5.270	1.36e-07 ***
climatetype_2	-5.873e-01	3.682e-01	-1.595	0.110680
climatetype_3	-1.178e+00	4.182e-01	-2.818	0.004837 **
climatetype_4	-5.766e-01	4.417e-01	-1.305	0.191747
climatetype_5	-6.462e-05	NA	NA	NA
climatetype_6	-5.462e-01	3.478e-01	-1.570	0.116302
climatetype_7	-1.700e+00	5.503e-01	-3.090	0.002001 **
climatetype_8	-1.660e+00	4.524e-01	-3.670	0.000243 ***
ecosystemgarden	-2.645e-01	4.843e-01	-0.546	0.584974
ecosystemwild	2.818e-01	3.617e-01	0.779	0.435858

Zero-inflation model coefficients (binomial with logit link):

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-13.22490	1722.63303	-0.008	0.9939
methodspray	15.86707	1722.63271	0.009	0.9927
sampling_effort	-0.03403	0.01655	-2.056	0.0398 *
climatetype_2	-1.79619	1.31572	-1.365	0.1722
climatetype_3	-1.26435	1.37803	-0.918	0.3589
climatetype_4	-1.57901	1.36178	-1.160	0.2462
climatetype_5	16.77982	2155.94001	0.008	0.9938
climatetype_6	-1.07678	1.29946	-0.829	0.4073
climatetype_7	-0.64838	1.49901	-0.433	0.6654
climatetype_8	-1.10310	1.23516	-0.893	0.3718
ecosystemgarden	-1.24467	1.19193	-1.044	0.2964
ecosystemwild	-1.53530	0.90991	-1.687	0.0915 .

---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Number of iterations in BFGS optimization: 50

Log-likelihood: -124.3 on 24 Df

Warning message:

In sqrt(diag(object\$vcov)) : production de NaN

```
> zip_div_spe10<-zeroinfl(tot~method+sampling_effort+climate+ecosystem, data=data)
> summary(zip_div_spe10)
```

Call:

```
zeroinfl(formula = tot ~ method + sampling_effort + climate + ecosystem, data = data)
```

Pearson residuals:

```
      Min      1Q  Median      3Q      Max
-1.377e+00 -5.470e-01 -2.763e-01 -5.576e-05  6.590e+00
```

Count model coefficients (poisson with log link):

```
      Estimate Std. Error z value Pr(>|z|)
(Intercept) -2.795e+00  1.132e+00 -2.470 0.013502 *
methodspray  3.633e+00  7.337e-01  4.952 7.36e-07 ***
sampling_effort 3.046e-02  5.779e-03  5.270 1.36e-07 ***
climatetype_2 -5.873e-01  3.682e-01 -1.595 0.110680
climatetype_3 -1.178e+00  4.182e-01 -2.818 0.004837 **
climatetype_4 -5.766e-01  4.417e-01 -1.305 0.191747
climatetype_5 -6.462e-05      NA      NA      NA
climatetype_6 -5.462e-01  3.478e-01 -1.570 0.116302
climatetype_7 -1.700e+00  5.503e-01 -3.090 0.002001 **
climatetype_8 -1.660e+00  4.524e-01 -3.670 0.000243 ***
ecosystemgarden -2.645e-01  4.843e-01 -0.546 0.584974
ecosystemwild  2.818e-01  3.617e-01  0.779 0.435858
```

Zero-inflation model coefficients (binomial with logit link):

```
      Estimate Std. Error z value Pr(>|z|)
(Intercept) -13.22490 1722.63303 -0.008 0.9939
methodspray  15.86707 1722.63271  0.009 0.9927
sampling_effort -0.03403  0.01655 -2.056 0.0398 *
climatetype_2 -1.79619  1.31572 -1.365 0.1722
climatetype_3 -1.26435  1.37803 -0.918 0.3589
climatetype_4 -1.57901  1.36178 -1.160 0.2462
climatetype_5  16.77982 2155.94001  0.008 0.9938
climatetype_6 -1.07678  1.29946 -0.829 0.4073
climatetype_7 -0.64838  1.49901 -0.433 0.6654
climatetype_8 -1.10310  1.23516 -0.893 0.3718
ecosystemgarden -1.24467  1.19193 -1.044 0.2964
ecosystemwild  -1.53530  0.90991 -1.687 0.0915 .
```

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Number of iterations in BFGS optimization: 50

Log-likelihood: -124.3 on 24 Df

Warning message:

In sqrt(diag(object\$vcov)) : production de NaN

```
> zip_div_haplo14<-zeroinfl(div_haplo~method+sampling_effort+climate, data=data)
> summary(zip_div_haplo14)
```

Call:

```
zeroinfl(formula = div_haplo ~ method + sampling_effort + climate, data = data)
```

Pearson residuals:

```
   Min    1Q  Median    3Q   Max
-1.42093 -0.60956 -0.13268  0.05732  3.85924
```

Count model coefficients (poisson with log link):

```
      Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.6786646  0.8983670 -1.869 0.061682 .
methodspray  2.0157156  0.6616849  3.046 0.002316 **
sampling_effort 0.0201890  0.0053719  3.758 0.000171 ***
climatetype_2 -0.1737008  0.4823533 -0.360 0.718764
climatetype_3 -1.2240454  0.5381909 -2.274 0.022944 *
climatetype_4 -0.1329031  0.5375770 -0.247 0.804733
climatetype_5 -0.0001829    NA      NA    NA
climatetype_6  0.0186270  0.5067247  0.037 0.970677
climatetype_7 -1.8524878  0.6156370 -3.009 0.002621 **
climatetype_8 -1.4764060  0.5080073 -2.906 0.003658 **
```

Zero-inflation model coefficients (binomial with logit link):

```
      Estimate Std. Error z value Pr(>|z|)
(Intercept)  14.1567  13.5809  1.042  0.297
methodspray  -6.5499   6.1605 -1.063  0.288
sampling_effort -0.5326   0.4339 -1.227  0.220
climatetype_2 -2.4341   7.5923 -0.321  0.749
climatetype_3 -7.1930   8.4029 -0.856  0.392
climatetype_4 -3.6185   8.1189 -0.446  0.656
climatetype_5  17.4888  2778.1191  0.006  0.995
climatetype_6 -3.2354   7.8652 -0.411  0.681
climatetype_7 -12.6440  137.0296 -0.092  0.926
climatetype_8 -5.2493   7.9087 -0.664  0.507
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Number of iterations in BFGS optimization: 83

Log-likelihood: -95.86 on 20 Df

Warning message:

```
In sqrt(diag(object$vcov)) : production de NaN
```