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# An integrative eco-genomic modelling perspective on the invasion of the Pyrénées-Orientales chestnut tree forests by the global insect pest, *Dryocosmus kuriphilus*

Jean-Loup Zitoun

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# THÈSE

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Docteur

Délivré par

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Présentée par Jean-Loup Zitoun

**An integrative eco-genomic modelling perspective  
on the invasion of the Pyrénées-Orientales  
chestnut tree forests by the global insect pest,  
*Dryocosmus kuriphilus***

Soutenue le 6 avril 2023

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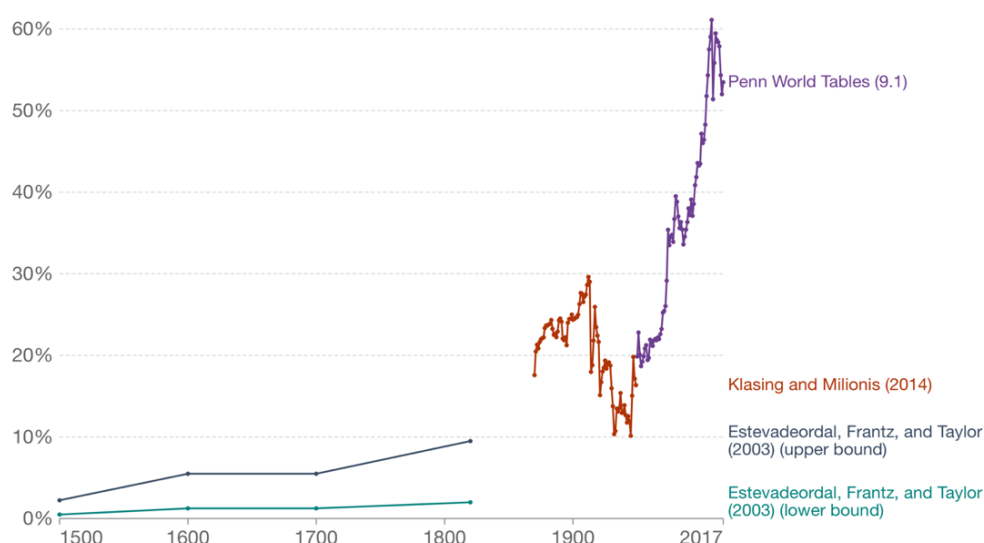
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## **General introduction**

# I. Biological invasions: a major threat associated with global changes.

## I.1. Globalization and its impact on the rise of invasions.

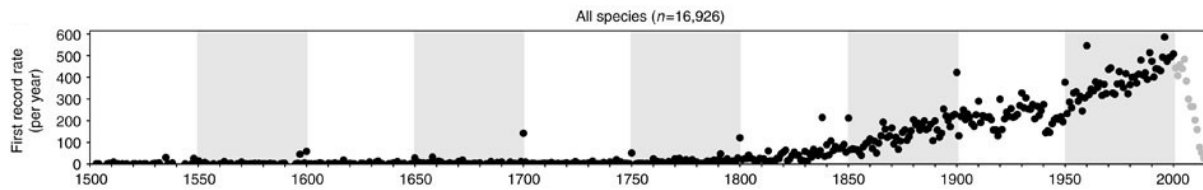
Since the 19th century, the world has entered a globalization era associated with an increase of international exchanges (Ortiz-Ospina and Beltekian, 2014). The integration of national economies into a global economic system has indeed enabled countries to experience sustained economic growths associated with a rise in trading. Figure 1 clearly highlights such a tendency by demonstrating the exponential evolution of trade openness index, defined as the sum of world exports and imports, divided by world gross domestic products (GDP).



**Figure 1.** Globalization over 5 centuries. From Ortiz-Ospina and Beltekian, Trade and Globalization, 2014.

Such a fast increase in international exchanges has been shown to have various negative effects on local environments, a major one being the accidental transportation of species (Yan et al., 2001). While the invasion of long-established ecosystems by ‘alien’ organisms is a natural phenomenon, human-facilitated introductions have greatly increased the rate, scale, and geographic range of such invasions. The biogeographical barriers delimiting the distribution areas of the endemic species are indeed easily crossed via such transportation, leading to the colonization of new environments by so called ‘invasive’ species, which the National Wildlife Federation defined as introduced species that grow and reproduces quickly, spread aggressively with potential to cause harm in their new environment (NWF, 2022). As illustrated in Figure 2, an increase in the frequency of biological invasions has been observed since the 19th century

(Seebens et al., 2017) and has gradually followed the intensification of international trade illustrated in Figure 1.



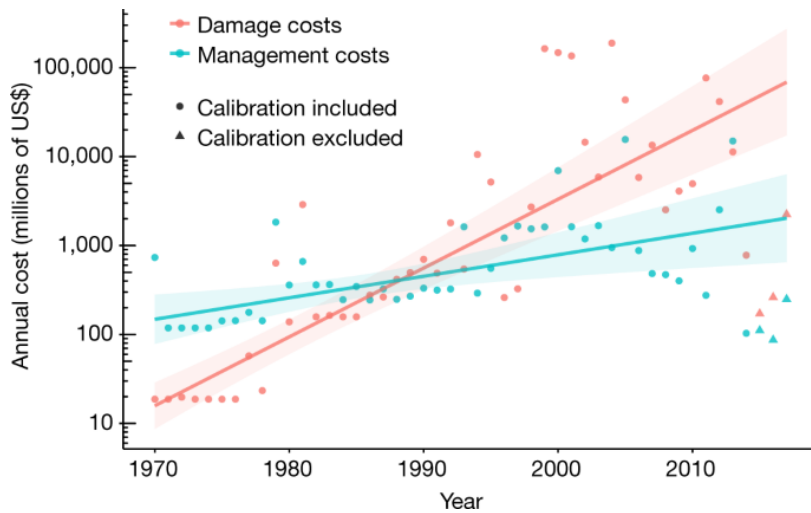
**Figure 2.** Evolution of the number of biological invasions recorded each year worldwide since the 16<sup>th</sup> century. From Seebens et al. 2017.

Merchant ships travelling through international waters constitute the main vector of biological invasions worldwide (Bailey, 2015; De Castro, 2017). Indeed, they are transporting a vast diversity of exploited natural resources that typically come with incidentally associated plant, insect and/or parasitic species, and they also commonly carry species in their ballast tanks, sucking them up whenever they take on ballast water, and then releasing them on other sites of the world. Invasions with important effect on human activities have been linked to this pathway, such as the introduction of the North American comb jellyfish that has devastated the Black Sea fishing industry (Kideys, 2002) and the spread of the Chinese mitten crabs that accelerated the erosion of banks in the Thames estuary (Rudnick et al., 2000). Alternative modes of transportations such as trains, motor vehicles, aircraft have been documented (Hulme, 2009) and, whatever path it takes, the anthropogenic spread of invasive species can have strong economic and ecological impacts, which make it a global issue associated with the globalization era (Meyerson and Mooney, 2007).

## **1.2. Economic impact on natural valued resources.**

Damages caused by invasive species induced high economic losses and the implementation of strategies to control their populations and/or reduce their impacts come at further costs. The overall cost of invasions was reported to reach an annual mean of US\$26.8 billion over the period 1970–2017 (Diagne et al., 2021). Interestingly, the exponential increase in the costs associated with damage over this period has hardly been matched by a similar increase in the budget allocated to control strategies targeting invasive species, the annual geometric rate of increase of the former (+19%) being about 3 times larger than those of the latter (+6%) (Figure 3). The impact of such species on ‘natural valued’ resources represents one of the main causes

of economic losses (Pagad et al., 2018). Such resources are described as economically accessible sources of matter and energy in the natural environment (INSEE, 2021) and, when it comes to plant species, can have diverse purposes such as agronomical, cultural or ornamental.



**Figure 3.** Temporal trends of global damage and management costs (in millions of US dollars) based on both mean annual costs for each decade and model prediction between 1970 and 2017. From Diagne et al. 2021.

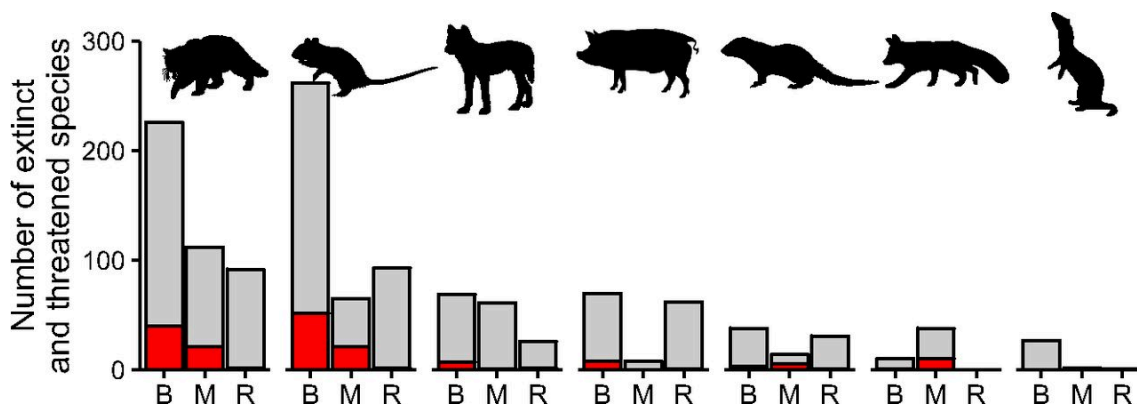
Damages induced by invasive species on agronomical valued resources constitute the prominent part of invasion's costs, especially in countries that are large agricultural producers such as China, United States, India, and Brazil (Paini et al., 2016). For example, crop and forest production losses and damages from invasive insects have been estimated to reach almost US\$120 billion per year only in USA, US\$36 billion in India, US\$18.9 billion in China, and US\$12 billion in Brazil (Pimentel et al., 2005; Dhaliwal et al., 2015; Wan and Yang, 2016; Oliveira et al., 2013). Natural resources with cultural or ornamental values also suffer from damages caused by invasive species. For example, *Paysandisia archon*, a pest butterfly originating from south America has been introduced into the Mediterranean basin since the mid-1990s and is decimating numerous palm tree populations, which are of high cultural relevance in Mediterranean regions and essential component of the urban landscape (Muñoz-Adalia and Colinas, 2020; MacLeod and Hussein, 2017). The cost of removal and replacement of dead palms has then been estimated at more than 500 million in France (Ferry, 2010).

Invasive species affecting agronomical resources can also represent an indirect risk for human health, which comes at further costs to our societies that are hardly ever quantified. A well-known example corresponds to *Drosophila suzukii*, a quarantine pest native from East Asia.

This spotted-wing drosophila is one of the most important invasive pests of fruit and wine production worldwide, with a large host range of 84 species of plants in 19 families (cherry, blueberry, strawberry, peach, grape, and other soft fruits). It causes significant damages by ovipositing and feeding on healthy fruits, inducing high losses of production (Bieńkowski and Orlova-Bienkowskaja, 2020). However, the harmful effects of fruits flies are not limited to economic damages as the consumption of fruits containing drosophila larvae can also cause intestinal myiasis in humans (Hall and Smith, 1993), with indirect costs that remain to be quantified.

### I.3. Ecological impact on biodiversity.

Biological invasions also are one of the major threat for biodiversity (Pejchar and Mooney, 2009), natural ecosystems (Simberloff et al., 2013) and ecosystem services such as resource provisioning or their regulating and cultural aspects (Shackleton et al., 2018; Pejchar and Mooney, 2009). Invasive species are indeed a major cause of species extinctions worldwide (Bellard et al., 2016), causing homogenization of fauna and flora (Winter et al., 2009) and changes in biogeography (Olden et al., 2008). When an invasive species reaches a new environment, it is typically integrated into the local ecological network and is therefore likely to have an impact on the populations of native species through predation or interspecific competition for feeding resources or specific habitats (David et al., 2017). Figure 4 illustrates how much invasive species can then affect biodiversity, by summarizing the number of threatened or extinct species negatively affected by invasive mammalian predators, which are responsible of the majority (58%) of modern bird, mammal, and reptile extinctions (Doherty et al., 2016).



**Figure 4.** Numbers of threatened and extinct bird (B), mammal (M), and reptile (R) species negatively affected by invasive mammalian predators. Gray bars are the total number of extinct and threatened species and red bars are extinct species. Predators (L to R) are the cat, rodents, dog, pig, small Indian mongoose, red fox, and stoat. From Doherty et al. 2016.

Extinction events of native species can further have cascading ecological impacts. Indeed, such species often are longstanding pieces of local food webs or constitute typical habitat for other native species. When their population are affected by invasive species, invasion can then indirectly affect more distant parts of the ecosystems and biodiversity. Such cascading effects are generally related to introduced top-predators (Gallardo et al., 2016), whose top-down impact is more likely to step down the food chain and finally affect primary producers. Considering that the environment complexity can lower the probability of such trophic-cascade effects (David et al., 2017), the artificially simplified ecosystems use in agriculture could then be particularly susceptible to these disturbances of the ecological balance.

Invasive species can also have such indirect impacts by facilitating the spread of pathogens. Stricker et al. (2016), suggest that invasive species can facilitate pathogen emergence and amplification, raising concerns about movement of pathogens among agricultural, horticultural, and wild stands. Such phenomena could be due to the importation of exogenous parasite associated to the invasive species and therefore identified as co-invaders (LyMBERY et al., 2014) or by the modification of the native host community that could facilitate the transmission of local parasites (Kelly et al., 2009).

Finally, another consequence of the introduction of an alien species on a local ecosystem is the potential of hybridization with a closely related native species. This has for instance been observed for red deer (*Cervus elaphus*) and introduced sika deer (*C. nippon*) in Poland (Biedrzycka et al., 2012). Such hybridizations may constitute a threat for biodiversity as they can have a large effect on the genetic structure and conservation status of native populations (Todesco et al., 2016). Indeed, hybridization may drive rare taxa to extinction through genetic swamping, where hybrids replaced the endemic species (Rhymer and Simberloff, 1996), or by demographic swamping, where population growth rates are reduced due to the wasteful production of maladaptive hybrids (Wolf et al., 2001). The former has been observed following the introduction of smooth cordgrass into the range of California cordgrass; a subset of hybrid genotypes outcompeted the native species, overgrows its niche space, produces much seed, and sires the majority of seed on native flowers, resulting in extinction events of the native

cordgrass plants (Ayres et al., 2004). Demographic swamping has also been observed following the introduction of the invasive *Senecio madagascariensis* in the ecological range of the native *S. pinnatifolius* in Australia. While their hybridization rates are very high, hybrids are not viable and it leads to waste reproductive effort of the native whilst the abundance of the invasive plant increase. (Prentis et al., 2007).

With the increasing realization that invasive species have strong economic and ecological impacts, it has become essential to identify the main determinants of their installation success and of the subsequent population growth of these species in their new environment.

## **II. The fate of an invasive species: a balance between ‘Bottom-up’ and ‘Top-down’ regulations, key species effects and control strategies.**

When invading a new environment, an alien species integrates an existing ecological network that defines regulation factors which impact on the invasive population dynamic and therefore its expansion. These regulation factors are distributed into two main categories; the ‘bottom-up’ regulation, which is imposed by the resources used by the invasive species, and the ‘top-down’ regulation, which is dependent upon its predators and parasites. Meanwhile, some other ‘key’ species of the ecological network can have indirect impacts on the invasive population and modify its efficiency to colonize the new environment. Finally, diverse control strategies can be implemented to reduce the spread of invasive species, especially when they have significant impacts on the local environment and/or natural valued resources.

### **II.1. Bottom-up regulation: demographic, genetic and physiological characteristics of the host resource.**

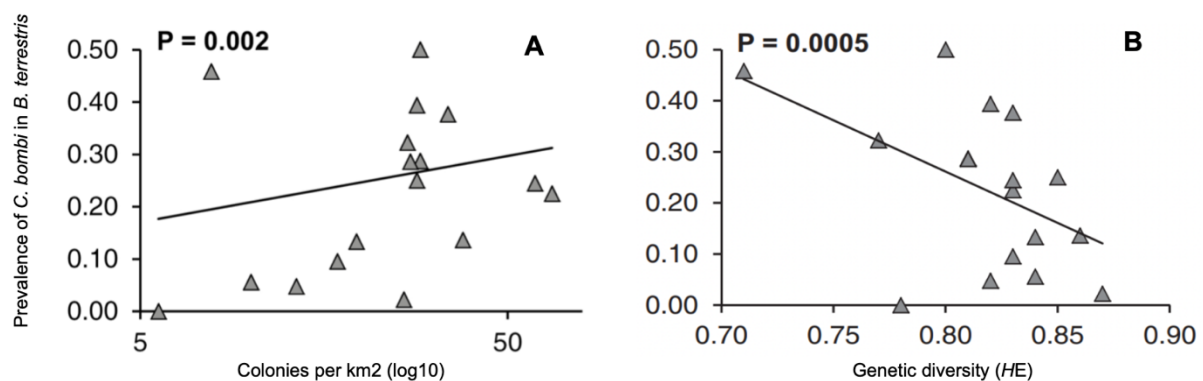
Bottom-up regulation is associated with the species representing resources used by the invasive species. Such resources can be food or habitat and therefore variation in their presence or abundance can impact the growth of the invader population. Interspecific competition for resources is the dominant force determining the distribution of biomass within networks under bottom-up control, so that increasing resources and therefore nutrients and energy availability facilitates the installation and persistence of the invasive species (David et al., 2017).



Meanwhile, one should also expect the genetic and physiological status of the resource to have an impact on its fate, especially when the invader corresponds to a parasitic species.

*Resource distribution.* The resource distribution clearly impacts the installation and development of invasive species, as suggested by Lotze et al. (2001) who observed that in the Baltic Sea, a nutrient enrichment strongly stimulated recruit density and growth of annual algae, thereby highlighting that the bottom-up control on early stage may be a predominant structuring force. Moreover, for invasive parasites, an increase in the host density lead to a higher prevalence of infection by parasite species. In fact, Parsche and Lattorff (2018) found evidence that *Bombus terrestris* colony density promoted infection prevalence by the intestinal protozoan *Crithidia bombi* (Figure 5A), highlighting that density-dependent effects promote parasite transmission.

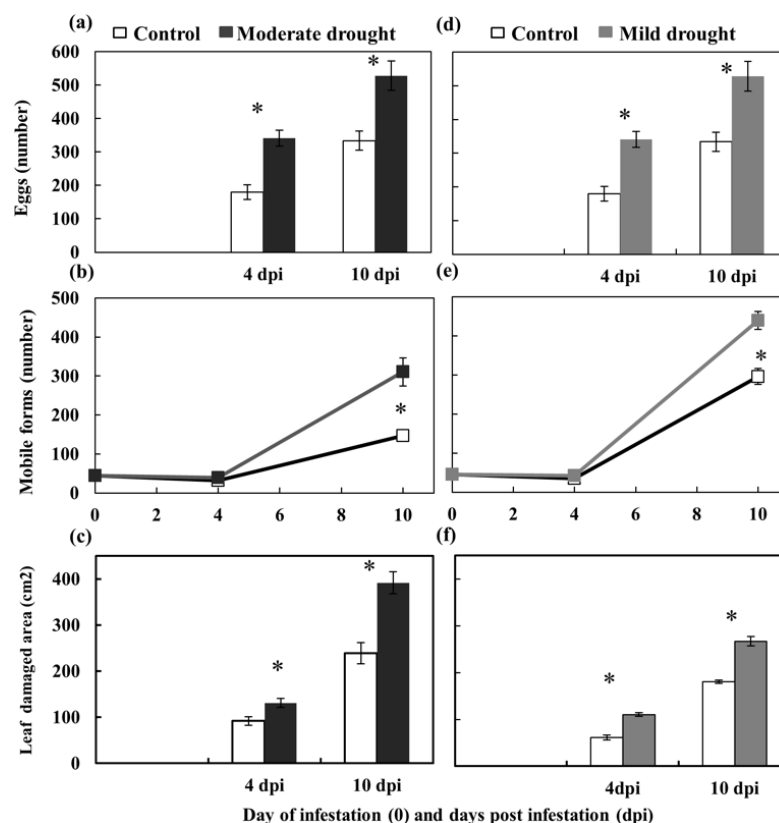
*Genetic resistance.* Population genetic diversity and genetic resistance mechanisms can induce variations on the susceptibility of infection of the host species, and thus affect the ability to spread of an invasive pest. As an example, Parsche and Lattorff (2018) observed a negative relationship between genetic diversity of *Bombus terrestris* populations and their prevalence of infection by *Crithidia bombi* (Figure 5B). A higher genetic diversity can then allow some individuals to be excluded from the compatibility filter of the parasite, and therefore reduce the global spread (prevalence) of the parasite, as observed in this study.



**Figure 5.** Prevalence of *Crithidia bombi* in *Bombus terrestris* ( $N = 17/20$  locations) in relation to (A) density of colonies and (B) genetic diversity ( $H_E$ ). Regression lines with associated  $P$ -values is derived from multiple regressions. From Parsche and Lattorff (2018).

*Physiological characteristics.* The physiology of the host species can also lead to a bottom-up regulation of its invasive pest. Ximénez-Embún et al. (2016) evidenced an increased susceptibility of drought-stressed tomato plants to the invasive tomato red spider mite,

*Tetranychus evansi*. They observed more leaf damage and an enhanced mite performance (increase in egg and mobile forms number) due to the higher level of several amino acids and free sugars in leaves induced by drought (cf. Figure 6). Although water deficit stress also increases the abundance of some defense proteins, *T. evansi* was found to circumventing their adverse effects by downregulating the expression of these genes. Accordingly, an invasive pest could have a better infection and growth rate in stressed-host due to their higher nutritional value and in accordance with the “Plant stress hypothesis” (Bauerfeind and Fischer, 2013).




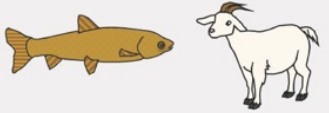
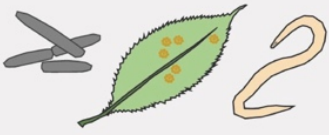
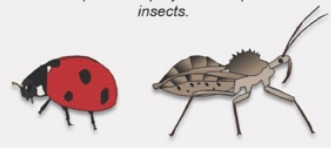


**Figure 6.** Performance of *Tetranychus evansi* in the moderate (a,b,c) and mild (d,e,f) drought stress experiments. The number of eggs (a,d), total mobile forms (b,e) and leaf damaged area (c,f) on control and drought stressed tomato plants were measured at 4 and 10 days post infestation (dpi). Statistical significant difference within each time are represented with \* (Two-way ANOVA, Bonferroni *post hoc* test,  $P < 0.05$ ). From Ximénez-Embún et al. (2016)

*Bottom-up impact on emigration.* While the bottom-up regulation associated with resource species can directly reduce the invasive population growth as illustrated above, it is interesting to point out that indirect impacts of the resource limitations are also observed. Indeed, Fronhofer et al. (2018) suggests that in a low resource environment, the individual dispersal is higher with an average increase of emigration rate of 9% to 16% compared to an environment

with standard resources (Figure 8, Section 2.2). Such a resource-mediated emigration could then favor the colonization of high-resource area and lower levels of intra-specific competition in low-resource environments, promoting the population development and dispersal of the invasive species at a wide spatial scale.

## **II.2. Top-down regulation: predators and parasite species**

Native or introduced predators, parasite and pathogens can be responsible for a ‘top-down’ regulation of an invasive species. The magnitude of their impact may vary depending on the invasive species, as shown in Figure 7. Phytophagous arthropods and herbivorous vertebrates are the main antagonist species effective in limiting the development of invasive plants, while insect invasions are most likely to be impeded by parasites or parasitoids than by generalist predators. This explains why parasitic species are introduced three times more often and exhibits better establishment and control rates on invasive insects (Kenis et al., 2017). Invasive vertebrates, whose important damages come from birds and mammals (Witmer, 2007), are most likely to be regulated by natives or introduced predators. Indeed, several studies highlight the significant effect of specialist predators on the regulation of invasive rodents (Labuschagne et al. 2016), birds (Shwartz et al., 2009) or fishes (Iguchi et al., 2004). Along this line, Twining et al. (2022) suggested that invasive vertebrates could be successfully controlled by the restoration of native predator populations, which constitutes a promising nature-based solution against this threat. More recently, the use of pathogens has received increasingly great attention for the biocontrol of all invasive taxa. Indeed, bacteria and viruses have been shown to be effective control agents against invasive insects (Lacey et al., 2015), plants (Harding and Raizada, 2015) and vertebrates (McColl et al., 2014). Overall, naturally occurring predator or parasites can effectively decrease the growth rate of invaders, eventually leading to their extinction (Twining et al., 2022), so that such antagonist species are also widely used to limit invasions through their introduction as control agents (Bale et al., 2008) or ecological engineering (Josephraj Kumar et al., 2022). While the basic ecological principles for the existence of a ‘top-down’ regulation are obvious, it is hard to anticipate the actual response of natural predators/parasites to the spread of an invasive species through a given ecosystem, and to predict the effect of a control agent once it has been introduced into an invaded natural ecosystem. I will thus now attempt to illustrate a bit further the potential outcomes of natural predators/parasite on invasive species.

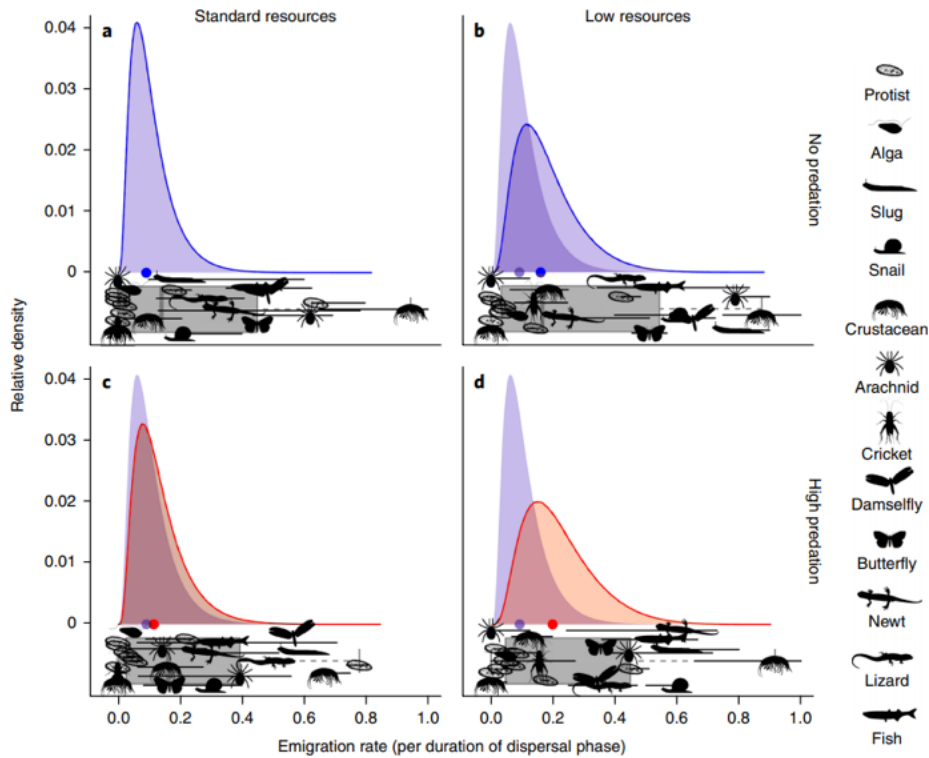
		Nonnative, invasive plants	Nonnative, invasive insects
Types of Antagonists	Phytophagous Arthropods	<p>Phytophagous arthropods include insects and other arthropods that feed on plant material (i.e., leaves, roots, stem, seeds, flowers). They are sometimes effective biological control agents for plants.</p> 	Predators
	Herbivorous Vertebrates	<p>Herbivorous vertebrates are animals that consume plants, often whole, rather than individual parts of the plants. They can be effective biological control agents for plants in small patches.</p> 	Parasitoids and Hyperparasitoids
	Pathogens	<p>Bacteria, fungi, nematodes, and viruses are microorganisms that can infect and help reduce populations of nonnative plants.</p> 	Pathogens
			<p>Predatory insects, such as lady beetles, kill and feed on other insects, such as herbivorous aphids. Generalist predators prey on other predacious insects.</p> 
			<p>Parasites, or parasitoids, are organisms that live and feed in or on a host. Hyperparasitoids parasitize and sometimes kill other parasitoids.</p> 
			<p>Bacteria, fungi, nematodes, protozoa, and viruses are microorganisms that can provide control for populations of nonnative insects.</p> 

**Figure 7.** Five major types of antagonists that may affect nonnative, invasive plants and insects. From Schulz et al. 2019.

*Regulation by native predators.* Native predators can potentially be effective in regulating an invasion, although their top-down effect on the exotic species strongly depends on their ability to consume the new food resource. As an example, Parker and Hay (2005) observed that native generalist crayfishes fed three times more on exotic than on native plants. Such a preferential consumption of the invasive species by native generalist herbivores typically results in a biotic resistance to plant invasions. Another interesting and slightly different example comes from Pio et al. (2019). They observed that the native gastropod predator *Trophon geversianus* readily feed on the invasive barnacle *Balanus glandula*, but only at low rates (4% of their diet) as compared to its native mussel prey. This native species competing with *B. glandula* for space, the difference in the top-down effect exerts on these species finally favor the invasion success of the alien species through a predator-mediated competitive process (Mittelbach, 1986). These two examples illustrate that the effect of endemic predators remains actually hard to anticipate since they could restrain or promote the biological invasion success depending on the model studied.

*Control by parasites and pathogens.* Native parasite species are also able to slow the growth of an invasive population, and even to drive it to extinction. Such a native parasite-mediated regulation was reported by Prider et al. (2009) who observed strong parasitism rate of the native stem parasite, *Cassytha pubescens*, on the invasive host plant *Cytisus scoparius* in South Australia. While this endemic parasite reduces the photosynthetic biomass and survival rate of the invader, this introduced resource also promotes the parasite population development. Indeed, the latter exhibits greater growth rates on introduced hosts rather than on native, which may be due to their higher abundance and lower resistance to infection. Similarly, the accumulation of pathogens, e.g. bacteria or viruses, in the environment can be an advantage for the regulation of aggressive invaders, potentially reducing their impacts on local communities (Flory and Clay, 2013; Stricker et al., 2016).

*Top-down impact on emigration.* As observed with bottom-up regulation factors (See section 2.1), top-down control can have indirect impact on individual dispersal. Figure 8 presents variations in emigration rates of several species in different environmental conditions (standard or low resources, standard or high predation). Fronhofer et al. (2018) observed an increase of 9% to 12% of emigration when individuals are under high predation pressure. Such a predator-mediated emigration could promote the invasive species dispersal at a wide spatial scale when its population experiments strong top-down pressures by native enemies, and raises potential concerns about the indirect impact of ‘top-down’ biological control in facilitating the spatial spread of the invasive species from release sites.



**Figure 8.** Effect of bottom-up resource limitation and top-down predation risk on emigration across 21 species, ranging from protists to vertebrates. Posterior predictive distributions (continuous lines and colored shaded areas; the dots represent the medians of the distributions) of the additive model are shown (lighter shades indicate resource limitation (b,d); blue : without predator cues (a,b); red : with predator cues (c,d). Observed median emigration rate (black animal symbol) and quartiles (corresponding black error line) per study species are shown, as well as box-plots across all species. From Fronhofer et al. 2018.

The bottom-up and top-down regulation processes illustrated in II.1 and II.2 could be interdependent (Carpenter et al., 1985) and determine the success of a biological invasion. Indeed, Lamelas-lópez and Santos (2021) found that the food resource availability and top-down pressures exerted by native predators are the major factors influencing the relative abundance of invasive species on islands. Understanding the natural determinants of an invasion is an obvious step to predict its dynamics and optimize the implementation of the relevant control strategies as reviewed in this field (Buchadas et al., 2017) and, e.g., in the control of infectious diseases (Huppert and Katriel, 2013). Nevertheless, the study of these regulation factors does not take into account all the biological interferences that can affect the invasive species. Taking into account potential indirect interactions between the invader and more distant species within the ecological network could then help to decipher complex dynamics patterns such as cascading effects that could induce “regime shift” in community structure and functioning.

### **II.3. Key species indirect impacts on invasion and control successes**

While species that are resources, predators, parasites or pathogens of an exotic species lead to bottom-up and top-down regulation forces, other ‘key’ species in the local ecological network can have additional impacts on the alien species population. Root et al (1973) indeed predicted a better regulation of invasive species in diverse and heterogeneous environments, according to two main ecological hypotheses.

First, the ‘enemies’ hypothesis states that predatory insects and parasitoids are more effective at regulating invasive population in ecosystems characterized by a higher diversity of primary producers, which are typically associated with larger overall biodiversity. Indeed, a diverse and heterogeneous environment provides more alternative host/prey as well as more wintering or refuges sites for predators and parasite species that contribute regulating the invasive species (Russell, 1989). The distribution of such resources/habitats of native species having antagonistic effects on the invasive species can therefore affect their abundance and specific richness, and subsequently influence the top down pressure they exerted. Such variation could also affect biocontrol agents establishment, especially when they are generalist predators and thus more likely to use alternative prey in complex environments. These introduced agents may then more easily persist during oscillation of their target host/resource population, and could therefore restrain the potential re-emergence of the invasive species. Thus, although ‘key’ species interacting with both endemic and introduced predators have no direct ‘bottom-up’ or ‘top-down’ effects on the invasive, they can influence the establishment and impact of invasive species agents (Pschorn-Walcher 1977; Dahlsten and Mills 1999).

Second, the ‘resource concentration’ hypothesis states that herbivores find more easily their resource, and stay and reproduce at higher rates in monocultures than in heterogeneous environment. According to the plant (visual) apparency and the semiochemical redundancy underlying hypotheses (Castagneyrol et al., 2013; Beyaert and Hilker, 2014; Randlkofer et al., 2010), the presence of a greater diversity of plant species in complex environments can lead to visual and chemical barriers, lowering the ability of the invasive to detect its resource. Furthermore, the phylogenetic proximity of these plants species with the resource used by the invasive could influence this dilution effect. Indeed, Coley and Kursar (2014) state that chemical diversification resulting from herbivore selection may be greater within a plant genus than for more phylogenetically distant species. Thus, beyond the plants diversity, some ‘key’ species could determine the level of interference in host/resource location.

The variations of the bottom-up and top-down pressures described above are therefore dependent upon species that do not directly interact with the invasive and are then defined as key species. These biotic interferences are more likely to occur in perennial forest ecosystems, which exhibits a higher degree of specific diversity, as compared to managed agro-ecosystems. Meanwhile, these complementary mechanisms provide an ‘associational resistance’ leading to a higher reduction of the number of herbivores in diverse agricultural systems (Russell, 1989). Such a phenomena of ‘associational resistance’ has also been observed in protected areas where they serve as natural filters against biological invasions and provide refuges for native biodiversity. Gallardo et al. (2017) investigated the potential distribution of the 100 most invasive species and found that only a quarter of Europe’s protected areas have been colonized by any of these invaders, despite climatical conditions suitable for invasion. In addition, the predicted richness of invaders was found to be 11%–18% significantly lower inside than outside protected areas, which the authors attributed to their lower density of human population and transportation networks.

#### **II.4. Control strategies**

Despite their bottom-up and top-down regulation and possible interactions with key species, many invasive population succeed in colonizing their new environment, and therefore can have economic and ecological impacts. According to the species to be controlled, different strategies are used to reduce the invasive population growth, which can be classified into five main categories: environmental, mechanical, chemical, genetic and biological control strategies.

*Environmental control.* A common strategy to reduce the growth rate of invasive species consists in limiting the number of their breeding sites. This has been widely used for *Aedes* mosquitoes which are the invasive species with the most important cost for human society (Juliano and Lounibos, 2005). Indeed, they constitute a primary health threat since they are the most prominent group of insect vectors of disease, contributing to outbreaks in human and animal diseases (Lounibos, 2002) and altering the transmission cycles of native or invasive pathogens. (McMichael and Bouma, 2000). Since these insects use backwater to reproduce and lay their eggs, temporarily removing or turning over these water reservoirs acts as a source reduction by limiting the available sites for oviposition and is a widely used strategy around the world, although this method may affect native mosquitoes species using the same breeding sites (Baldacchino et al. 2015).



*Mechanical control.* Mechanical control is principally used against invasive plants and consists in detecting and cutting young plants to prevent them from growing and spreading their seeds. In a review of 355 studies of different management methods following plant invasions, Kettenring and Adams (2011) showed that cutting and hand pulling represent 34% and 20% of control strategies tested and that those reduce significantly the percent cover and biomass of invasive plants in the environment. However, such strategies require significant human resources and are therefore very expensive.

*Chemical control.* Chemical products affecting the development or survival rate of pests have been widely used to control insect populations. Insect growth regulators (IGRs) and pyrethroids are indeed used in Europe as larvicides and adulticides, and have been shown to be especially effective when targeted at the most productive breeding sites (Ocampo et al., 2014). Moreover, a new strategy using autodissemination exploiting wild adult mosquitoes as carriers of insecticide compounds has been shown to induce high mortality at the pupal stage for *Aedes* mosquitoes (Devine et al., 2016). Similarly, herbicides are key chemicals used in 55% of management strategies targeting invasive plants as they remain the most efficient in reducing their percent cover, biomass and density (Kettenring and Adams, 2011). However, chemical products are known to have negative impacts on native biodiversity (Klingelhöfer et al., 2022; Silva et al., 2016), one of the most alarming threats being the mortality induced in bee populations following the use of glyphosate as herbicide (Seide et al., 2018).

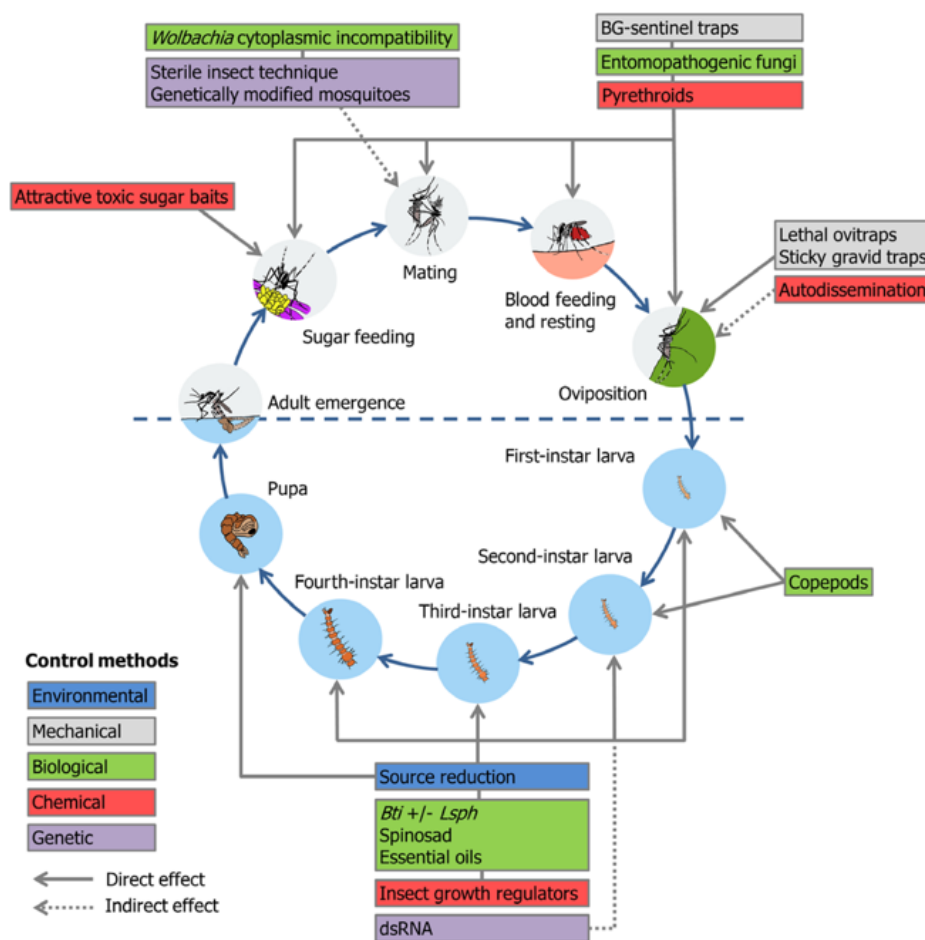
*Genetic control.* Genetic pest management is the most recently developed set of strategy that consist in introducing genetically engineered DNA sequences into wild insect pest populations by exploiting their mating behaviour. The introduction of genetically modified sterile individuals then typically aims at reducing the population density by affecting their reproduction, as successfully implemented in screwworm which are a devastating pest of livestock (Scott, 2018). Interestingly, such transgenes can have additional effects on their ability to vector diseases that have been shown to effectively reduce disease transmission rate (Harvey-Samuel et al., 2017), a successful example being the development of refractory transgenes in *Ae. aegypti* inhibiting the transmission of the avian malaria parasite *P. gallinaceum* to chickens (Kokoza et al., 2010). Such a genetic control strategy, primarily used against mosquitoes vectoring human infectious diseases (Gorman et al., 2016), is now being studied to target other invasive species such as Sea lamprey in the Great lakes in North America as it could be efficient and environmentally safe. Indeed, while the risk of undesired spread of

deleterious alleles is of major concern in a genetic control context, the use of self-limiting genetic control options and confined gene drives could help to avoid it (Ferreira-Martins et al., 2021).

*Biological control.* As shown in section II.2, local or introduced predators, parasite or pathogens have the potential to reduce or eventually suppress an invasive species population, with well-established examples of successful controls of invaders (Myers and Cory, 2017). A key decision in implementing such a strategy is the choice of the control agent, which is crucial for both the success of control and to limit its potential side effects. Introduced generalist consumer may indeed switch to feed on native species, as it has been observed in Hawaii with 22% of 243 introduced control agents documented to attack non-target species (Funasaki et al., 1988). The obvious complication in such case, is that the introduced control agent could itself become an invasive species persisting in the environment, as for instance observed for the cane toad introduced as a control agent in different sugar cane ecosystems, which now has unanticipated effects on Australian ecosystems (Shine, 2010). While specialist predators or parasites seem more adapted agents to introduce as they are expected not to switch on alternative local preys and hosts, such a choice also has drawbacks. First, a predator specialist of an alien species will not exist in the native community and therefore has to be taken from the invasive range, which carries the risk of a further invasion in case of adaptation to local resources (Hoodle, 2004). Second, theoretical models predict boom and crash cycles for specialized consumers (Hanski et al., 1991, 2001), which might not guarantee the maintenance of the control agent for a sufficient time to succeed. In all cases, it should always been bear in mind that ‘specialized’ species may turn to have larger host or prey ranges than initially thought because they were constrained to a specialized niche in their local environment (Jones and Gomulkiewicz, 2012).

The control strategies described above are most often used separately, i.e. one at a time, while, at least hypothetically, joining them would maximise control efficiency as they can act at different stages of the life cycle of the invasive species. Figure 9 illustrates how the five types of control strategies could be combined to target different parts of the life cycle of *Aedes* species and highlights the numerous combinations that could therefore be implemented. While some managements methods like environmental and mechanical control only act at specific stage of *Aedes* life cycle, the use of chemicals for example could be efficient at any time. In addition, the optimal biological agent or insecticide that need to be used also depends on the development

stage of the mosquito. Such combinations of control strategies have been shown to be an effective way to manage invasive weeds by Lake and Minter (2018), especially with the association of biological control and herbicides applications or plant competition. Indeed, their simultaneous implementation could result in a synergistic effect, enhancing the efficacy of both methods. Despite these encouraging results, the authors observed no increase in the number of studies published annually during the last 30 years evaluating the benefits of integrating multiple managements techniques, raising questions about how to promote more widespread use of these methods.



**Figure 9.** Control methods available against *Aedes* species. (*Bti*: *Bacillus thuringiensis* var. *Israelensis*; *Lsph*: *Lysinibacillus sphaericus*; dsRNA: double-stranded RNA). From Baldacchino et al. 2015

To conclude, the identification of various bottom-up and/or top-down factors determining the population growth of an invasive species is an obvious and critical step to understand and

anticipate the local fate of an invasion, and to be in a position to identify and optimize the potential strategies (or their combinations) that could limit such an invasion. Importantly, all these regulatory forces are likely to vary between the various places where a given invasive species is introduced or spread. To understand and deal with an invasion then require a proper assessment of the different features introduced in this section, and the integration of these various empirical findings into dynamics models could then be powerful approach as it allows making quantitative predictions on the spatial and temporal spread of the invasive population size and to anticipate the potential impact of control strategies (Shigesada and Kawasaki 1997, Lewis et al., 2016). This is the integrative approach that I have intended to better understand the ongoing invasion of chestnut tree (*Castanea sativa*) forests in the Pyrénées-Orientales (France) by a worldwide invasive pest; the gall wasp *Dryocosmus kuriphilus*.

### **III. The study system: the invasive Asian Chestnut Gall Wasp, its chestnut tree hosts and its native and introduced hyperparasites**

During this 3-years research study, I assessed the current invasion of natural chestnut tree populations of the French department of Pyrénées-Orientales by the Asian Chestnut Gall Wasp (ACGW). In this last part of this introduction, I therefore aim at giving a brief overview of this biological system that is composed of the host resource, the invasive pest hymenopteran species, its potential predators and pathogens, as well as the key species present in the invaded ecosystem and with which this worldwide invasive species could interact.

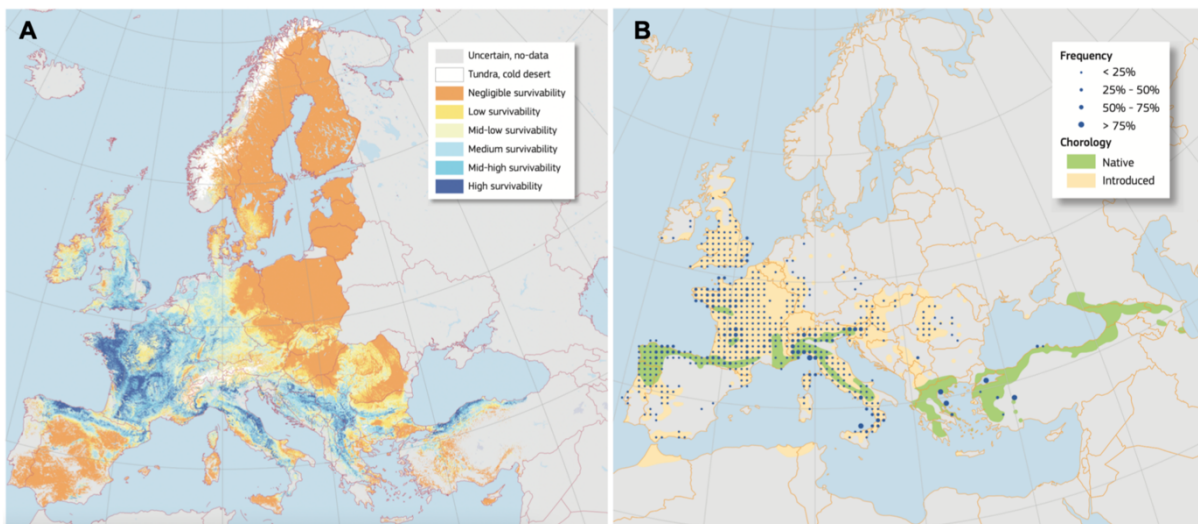
#### **III.1. The resource: the European chestnut, *Castanea sativa*.**

The European chestnut or sweet chestnut (*Castanea sativa* Mill.) is a medium-large deciduous tree species from the *Fagaceae* family. *C. sativa* reach a height of 30–35 m with a mostly straight trunk with branching starting at low heights. Sweet chestnut trees can live to an age of 500 to 600 years in the wild, and some individuals as old as 1000 years have been kept in cultivation (Conedera et al., 2016). Sweet chestnut trees are naturally self-incompatible, and cross-pollination is necessary for reproduction of this monoecious species.

*Habitat and ecology.* *C. sativa* is a warm-temperate tree species adapted to mean annual temperatures that range from 8°C to 15°C and minimum rainfall of 600-800mm (Conedera et al., 2016). Its altitudinal range between 200 and 1800m. It is very sensitive to droughts resulting

from high temperatures combined with lack of precipitations (Conedera et al., 2004). Trees grow better in nutritionally poor and well-drained soils, ranging from very acidic to neutral whereas they do not thrive in limestone.

*Origin and demography.* In Europe, the current distribution of the sweet chestnut covers an area of more than 2.5 million hectares. The comparison of the maximum habitat suitability (Figure 10A) and actual distribution of *C. sativa* in Europe (Figure 10B) highlights that this species spread to the current limit of its potential ecological range. It is however difficult to trace back the species colonization history of Europe because of its broad diffusion and active management that have resulted in its today's widespread distribution.



**Figure 10.** (A) High resolution map estimating the maximum habitat suitability (B) Map of plot distribution and simplified chronology for *Castanea sativa*. From Conedera et al. (2016).

*Economic and recreational services.* The sweet chestnut is a valued natural resource with important economic and environmental roles in many agroforestry systems. With a large genetic diversity, its cultivars are used in different management systems according to the targeted products and services. Because of its high re-sprouting capacity, coppices represent the main type of forest management, supplying firewood, charcoal, poles and wood for small products (Conedera et al., 2016). Moreover, a naturally high tannin content protecting against decay makes chestnut wood particularly suitable for external use such as wooden fences set up to preserve sand dunes. (Tomak and Gonultas, 2018). Traditional orchards used for fruits production represent about 20% of chestnut forests worldwide in Europe. They correspond to open stands, usually composed of grafted trees because of the self-sterility of the species

(Conedera et al., 2016). This species is also involved in honey production thanks to flowers which are rich in pollen and nectar and therefore really attractive to bee.

*Threats and diseases.* A strong decline of the chestnut tree population is observed at the national scale which would be due to various factors such as the lack of maintenance of chestnut groves, the climate and pathogens (Mansot and Castex, 2018). Indeed, sweet chestnut management requires continuous cultural inputs as chestnut stands tend to be invaded by other tree species and to evolve towards mixed forests (Conedera et al., 2001, Pividori et al., 2004). Fruit damages could be induced by the presence of insects such as the chestnut weevil (*Curculio elephas*), nut moths (*Cydia splendana*; *Cydia faggladana*; *Pammene fasciana*) and the nut rot diseases (*Gnomonopsis spp.* or *Phomopsis spp.*) (Bounous, 2014), resulting in important economic loss for the chestnut growers. Further threats for chestnut trees include two exotic fungus whose spread have resulted in a severe decline of chestnut population in the 20<sup>th</sup> century. The ink disease caused by *Phytophthora cinnamomi* and *P. cambivora* and the chestnut blight caused by *Cryphonectria parasitica* (see section 3.4.1) have indeed been introduced in Europe and North America in 1800's and their spread have had a strong impact on both wilderness and cultivated chestnut tree populations. More recently, a worldwide insect pest, the Asian chestnut gall wasp (ACGW) (*Dryocosmus kuriphilus*) was accidentally introduced in 2002 in Piedmont (Italy) and is currently spreading in Europe (See figure 12, Section 3.2). This hymenoptera has a strong economic and ecological impact on chestnut tree population through the formation of galls, which decreased the biomass and chestnut production (Battisti et al. 2014).

*The host resource in the Pyrénées-Orientales.* *C. sativa* is the second most common hardwood in the Occitanie region and is widely distributed in the department with volume that can be up to 100 m<sup>3</sup> by hectares locally (Mansot and Castex, 2018). Its population located in Pyrénées-Orientales is composed of different strains, either endemic or introduced for forest repopulation or ornamental purposes. While the chestnut tree is of high cultural relevance in Mediterranean regions (Mansot and Castex, 2018), its population there fits into environments with different uses of the trees. This resource is valued for the production of chestnuts in crops and wood in agrosystems while it is protected in natural reserves.

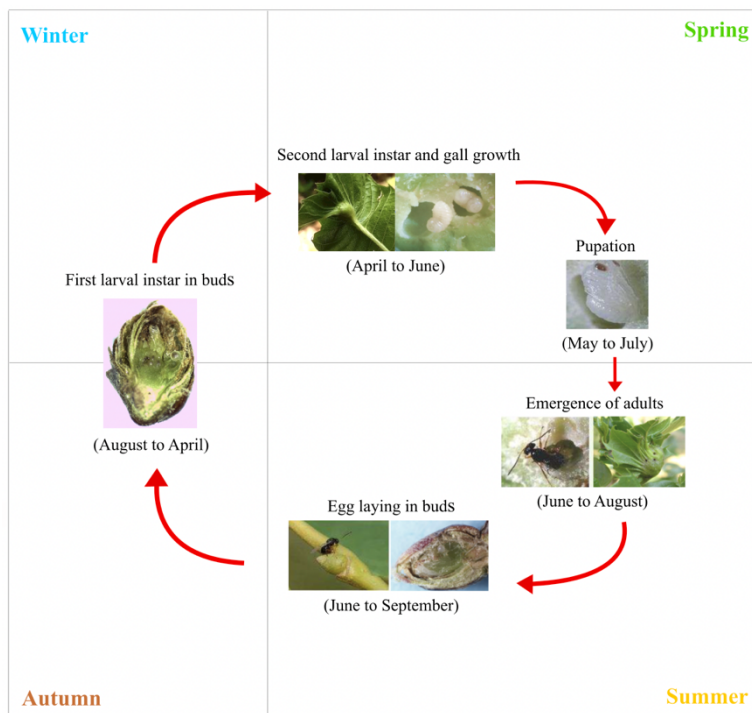
### III.2. The invasive species; the Asian chestnut gall wasp, *Dryocosmus kuriphilus*

The Asian Chestnut Gall Wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu is a micro-hymenoptera belonging to the Cynipidae family. ACGW is classified as a quarantine pest by the European and Mediterranean Plant Protection Organization (EPPO, 2005).



(INRA)

*Life cycle and ecology.* ACGW is a gall-forming parasite species specific of the *Castanea* genus. Its reported host are different species of chestnut trees distributed worldwide (*Castanea crenata*, *Castanea dentata*, *Castanea mollissima*, *Castanea sativa*, *Castanea seguinii*). Odor signals are necessary for host location, with a better detection capacity observed for trees presenting old damage and therefore emitting more volatile compound (Germinara et al., 2011). Univoltine and semelparous short-lived adult females produce eggs asexually through thelytokous parthenogenesis and laid them in chestnut tree buds during summer. Adult longevity is generally 3-4 days and each female carries a mean egg load of 268 eggs (Graziosi and Rieske, 2014). Interestingly, egg resorption, i.e. the ability to dissolve mature oocytes and reallocate nutrients (Bell and Bohm, 1975) seems to be significant in this species, so that a decline in the egg amount is observed as *D. kuriphilus* adults age. Graziosi and Rieske (2014) estimated that the amount of eggs not subject to resorption and which can be laid is typically down to approximately 120 eggs. These eggs hatch within 30 days and develop into first instar larvae that stay in a dormant stage during autumn and winter. Each larva develops within an ovoid shaped chamber (Warmund, 2013). They develop into second instar larvae in spring, inducing the formation of galls on stem and leaves. Finally, adults emerge from these galls and can lay the next and non-overlapping generation of eggs. This life cycle is synchronized with the chestnut tree host phenology (Bernardo et al., 2013) and represented in Figure 11.

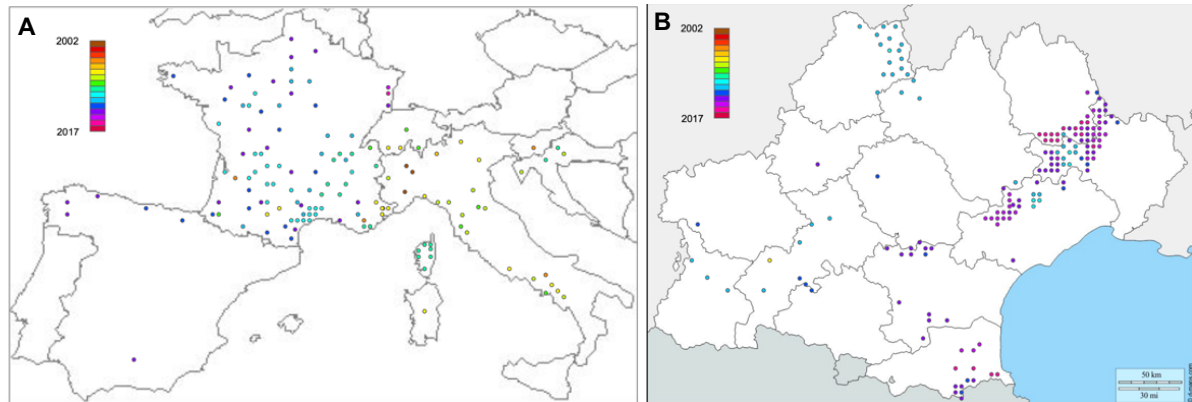


**Figure 11.** Schematic representation of *D. kuriphilus* life cycle.

*Invasion history.* *D. kuriphilus* is an invasive pest native to China which was first reported in Japan in the 1940s (Murakami et al., 1980), then in Korea in 1958 (Cho & Lee, 1963), in USA in 1974 (Payne et al., 1976) and finally in Europe (Piedmont, Italy) in 2002 (Brussino et al., 2002). Following its introduction, *D. kuriphilus* has spread through most of the European *Castanea sativa* geographical range (Battisti et al., 2014). Figure 12 shows the temporal spread of the species in Europe and in French region Occitanie based on scientific articles and reports attesting to its presence (cf. Reference Section 2), highlighting a fast colonization of European chestnut tree populations. Moreover, these spatial dynamics are typically determined by 2 modes of dispersal of *D. kuriphilus*; long-distance dispersal mainly due to anthropic movement of infected plants and scions (Rieske, 2007; Graziosi & Santi 2008) as well as short-distance dispersal depends on active adult flight (about 24-25 km per year, Rieske, 2007), which are relevant at both scales. Indeed, we can observe on Figure 12A that the first entry point of the pest in Italy in 2002 is unlikely to come from natural dispersal. The colonization of isolated areas such as the islands of Corsica and Sardinia then reinforces the hypothesis of an anthropic-facilitated dispersal. On the other part, the short-distance dispersal is clearly visible at the region scale (Figure 12B) where the pest spread to nearby territories from one year to another. The data extracted from numerous reports and scientific articles and which allowed the realization of these maps could, thanks to model selection approaches, allow to estimate the respective contribution of both long and short-range dispersal on the AGCW colonization of



Europe. Such dispersal model based on integrative approaches has been developed by Giliolo et al. (2013) and Gil-Tapetado et al. (2021) and allow to predict realistic dynamics of the chestnut gall wasp colonization in Europe.



**Figure 12.** (A) Map showing the year of first observation of the Asian Chestnut Gall Wasp in the European chestnut tree population based on scientific articles and reports. (B) Map showing the year of first observation of the Asian Chestnut Gall Wasp in the French region Occitanie based on scientific articles and reports.

*Damages.* ACGW is the most important pest of *Castanea* species worldwide (Avtzis et al., 2019). Development of ACGW larvae from eggs deposited in chestnut tree buds induced the formation of galls that can develop on chestnut tree leaves, shoots, dormant buds and stipules. Gall formation has a strong impact on wood and nut production, with losses in nut yield that can reach up to 80% (Battisti et al., 2014). Negative impacts are also observed on the development of plant tissues due to the reduction of leaf photosynthetic area, decrease in the CO<sub>2</sub> assimilation and stomatal conductance by 30% (Ugolini et al., 2014) and female flowers abortion (Conedera and Gehring, 2015). Infested trees have also been shown to be more susceptible to other pathogen such as chestnut blight (Ugolini et al., 2014). An increase in *Cryphonectria parasitica* (See section 3.4.1 for detailed information) prevalence has been observed in chestnut tree stands infested by ACGW (Prospero and Forster, 2011). Abandoned galls could indeed act as an entry point for the fungus and therefore be colonized by virulent strains of *C. parasitica* (Meyer et al., 2015). In addition, the diffusion of a nut rot of chestnut tree induced by *Gnomoniopsis castaneae* may have also increased in ACGW invaded area (Lione et al., 2016). Although ACGW infestations typically cause damages without being lethal, repeated severe infestations may lead to the death of young trees when combined with abiotic and/or biotic stress factor (Moriya et al., 2003).

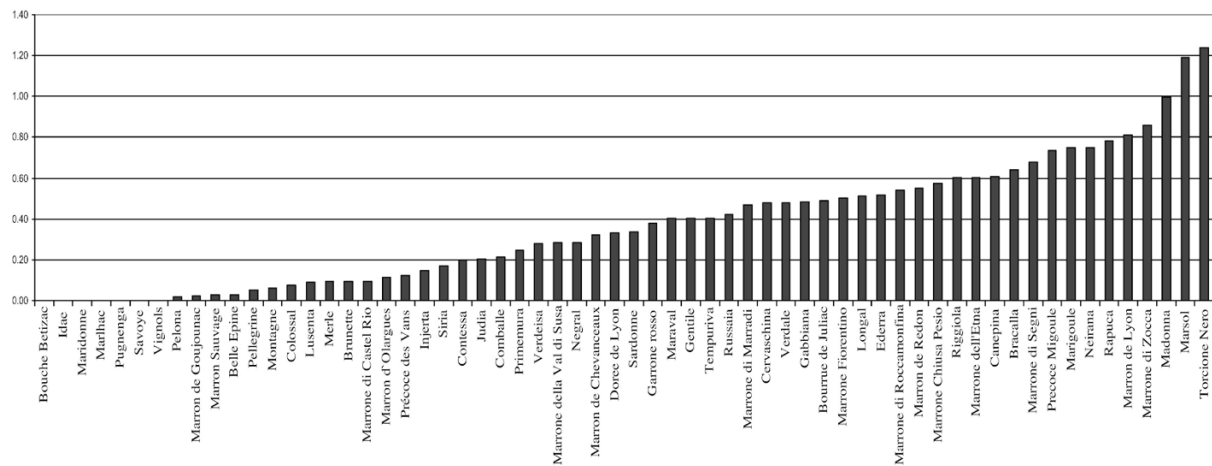
*The Asian chestnut gall wasp in the Pyrénées-Orientales.* At the beginning of my PhD, the presence of the invasive pest in the Occitanie region had been reported by the FREDON (Mansot and Castex, 2018) and, while the actual level of invasion of the chestnut tree had been estimated in orchards located in several department of the Occitanie region, no information was available to quantify its abundance, spread and impact in the Pyrénées-Orientales. To provide such knowledge was one of the objective of my PhD funded by the Occitanie region, and we intended to do so in semi-natural and natural chestnut tree forests, as an understanding of the determinants of *D. kuriphilus* invasion in those environments is generally lacking.

### **III.3. The ‘bottom-up’ and ‘top-down’ regulation and control strategies**

In the field of biological invasions, few studies are devoted to the identification of their ‘bottom-up’ and ‘top-down’ regulation factors in semi-natural or natural environments. Indeed, the majority of such research is devoted to exploited areas as invasive pests generally affect valued crop species, which only loosely inform on the presumably more complex interactions emerging as a result of the invasion in natural ecosystems. Furthermore, the economic loss associated to such pests has led to develop control strategies, whose efficiency might or might not be conserved in these alternative environments that I studied during my PhD. I thus aim to review here, not only the regulation factors that are typically looked at in studies focused on orchards, but also the additional determinants of the ACGW invasion and its control that could be relevant in semi-natural or natural chestnut tree forests.

Numerous attempts were made to control the spread of ACGW populations. Chemicals insecticides were shown ineffective against ACGW larvae as they are protected by the gall they live in, and applications would have to be perfectly timed to kill adults during their 3-4 days lifetime (Bosio et al., 2010). Agrotechnical measures have been intended that consist in the mechanical removal of galls and some pruning methods to control the pest in chestnut orchards (Maltoni et al., 2012). However, this management method is only effective when very few trees are infected (Payne and Johnson, 1979) and is labor-intensive and economically unfeasible for large commercial growers (Payne et al., 1983). In such circumstances, the control of ACGW relies on two alternatives; the use of resistant cultivars and of a biological control agent (EFSA, 2010; Avtzis et al., 2019), which are briefly documented below.

*Varietal selection of low susceptibility to ACGW.* Although most of the chestnut cultivars are sensitive to *D. kuriphilus*, a strong variability in ACGW susceptibility has been observed in *C. sativa* and among the interspecific hybrids. Sartor et al. (2015) estimated the susceptibility of infestation of 64 chestnut cultivars (Figure 13) and recorded an important heterogeneity in infestation rate, with cultivars classified as very susceptible (i.e. with more than 0.6 galls per bud) and some being resistant (i.e. no gall development). The latter could be *C. sativa* cultivars, *C. crenata* cultivar or *C. crenata* x *C. sativa* hybrids, which is the case for the totally resistant strain “Bouche de Bétizac” that is now favored by growers (Dini et al., 2012).



**Figure 13.** Mean infestation rate (number of galls per bud) assessing varietal susceptibility in 62 cultivars of chestnut trees. From Sartor et al. 2015

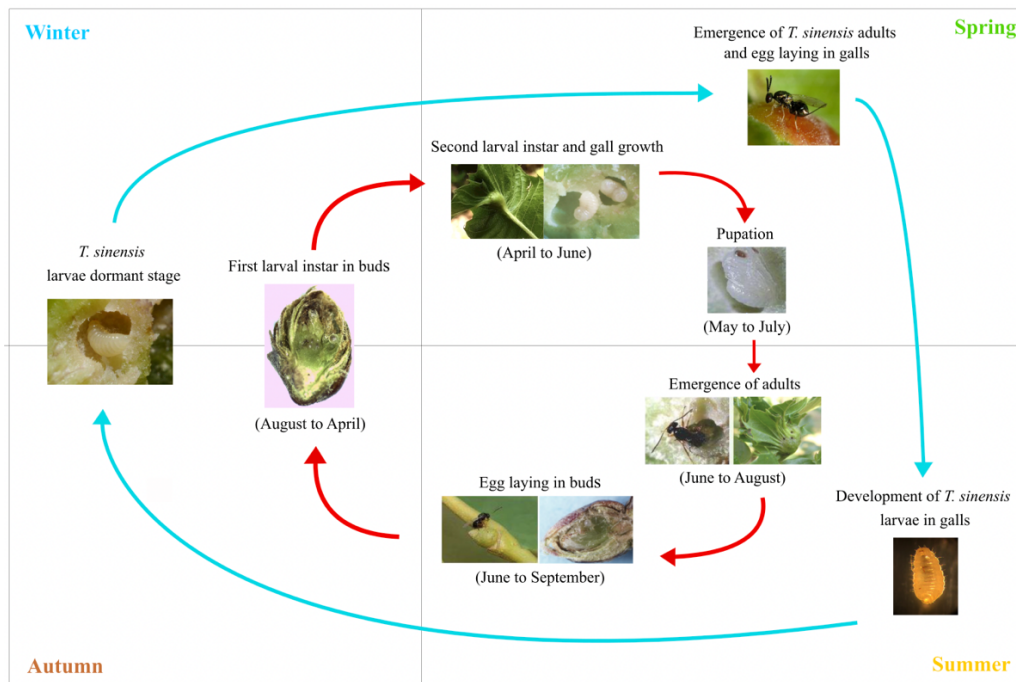
Breeding programs leading to resistant chestnut varieties took place in Japan and was initially very effective (Kajiura and Machida, 1961). However, the resistance was overcome in less than 20 years by an adapted biotype of the wasp which proliferated and rapidly took over (Shimura, 1972). Thus, although varietal selection seems to be a promising method to reduce the impact and spread of *D. kuriphilus*, it may not be effective in the long term in the case of pest adaptation, as somehow expected in any form of (evolutionary) host-parasite interactions and probably favored by the massively different generation time of the two partners.

*The biological control.* *Torymus sinensis* Kamijo is a micro-hymenoptera of the *Torymidae* family. This specialized parasitoid chalcid was introduced in Europe from Asia as a biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*.



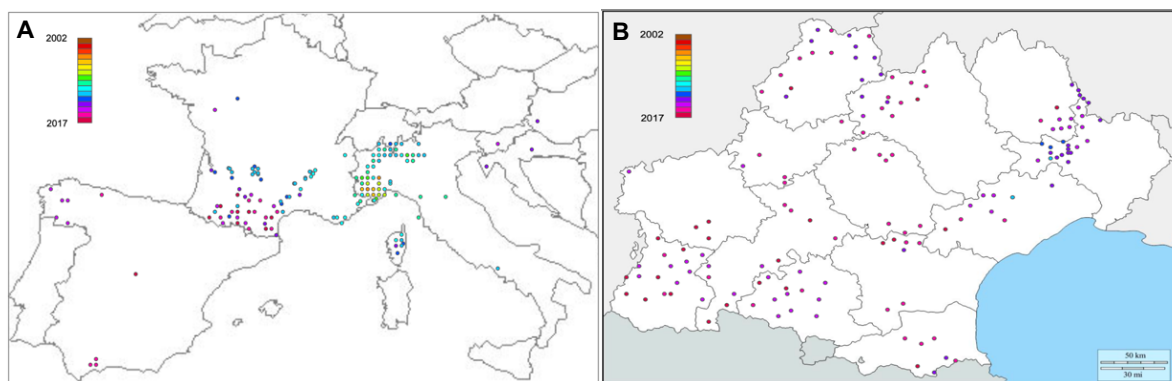
*Torymus sinensis* at larvae and adult stage (INRA)

*Life cycle and ecology.* *T. sinensis* is a hyperparasite described as specific to *Dryocosmus kuriphilus*, i.e. the invasive ACGW, as it uses odor and visual clues to select its host (Graziosi and Rieseke, 2013). Univoltine and semelparous adults mate and females lay their eggs in chestnut tree galls induced by ACGW in spring (See Figure 14). Adults longevity can be up to 31 days when well fed. Eggs are produced during this relatively short reproductive lifetime, and the cumulative mean egg load can reach more than a thousand eggs per individual with no signs of eggs resorption (Picciau et al., 2019). One *T. sinensis* egg can develop into larvae per larval chamber, which is associated to the consumption of the corresponding *D. kuriphilus* larvae over the summer. In the autumn, *T. sinensis* larvae enter a dormant phase that allows them to overwinter until the pupation and emergence phase that results in adult individuals able to infest the next generation of *D. kuriphilus* individuals. While this species is supposed to be strictly univoltine, Ferracini et al. (2015) observed a prolonged diapause mainly as late instar larva for this species. Diapause is extended for 12 months with adults emerging in April as usual, and therefore showing a two-year life cycle. These individuals are able to mate and show the same parasitism behaviour than the first-year emergence individuals. Such a prolonged diapause might be expected to have selective benefits in a parasite relationship with a specific host. Indeed, diapausing *T. sinensis* individuals could allow local populations to survive temporary extinction of the host population (Quacchia et al., 2013b).



**Figure 14.** Schematic representation of *D. kuriphilus* and *T. sinensis* life cycles.

*Introduction history.* *T. sinensis* is considered a good biological control agent because of its high specificity to *D. kuriphilus*, its phenological adaptation to the ACGW life cycle, and its ability to produce large amount of eggs per individual (Kamijo, 1982; Moriya et al., 2003). This hymenopteran was thus broadly released in southwestern Japan in the late 1970s and early 1980s (Murakami et al., 1980), where it became the most abundant and successful parasitoid of ACGW (Aebi et al. 2006) reducing ACGW damage to below the tolerable injury threshold of 30% production loss (Gyoutoku and Uemura, 1985). It was then introduced in Georgia, USA in 1977 (Payne 1978), in Italy in 2005 (Quacchia et al., 2008), in France in 2011 (Borowiec et al., 2014) and in Slovenia, Croatia, and Hungary in 2014 (Matošević et al., 2015). Figure 15 shows the releases that were conducted both in Europe and in Occitanie, which typically follows the dynamic of invasion presented in Figure 12, illustrating that such releases have been the main strategy to try limiting the damage associated with *D. kuriphilus*'s invasion. Interestingly, high levels of parasitism by *T. sinensis* before and immediately after the first releases suggested that the parasitoid was already established in eastern Europe presumably because it had spread from Italy where it was first introduced (Matošević et al., 2017).



**Figure 15.** (A) Map showing the year of first release of the ACGW control agent (*T. sinensis*) in Europe based on scientific articles and reports. (B) Map showing the year of first release of the ACGW control agent (*T. sinensis*) in the French region Occitanie based on scientific articles and reports.

*Control efficiency.* After its release, *T. sinensis*, is typically able to get established in chestnut tree orchards and its population to grow exponentially (Borowiec et al., 2018). Such local success is usually related to its high colonizing efficiency (Borowiec et al., 2018), rapid dispersal aided by wind (Colombari and Battisti, 2016a), behavioral adaptation to locate hosts and mates (Borowiec et al., 2018), lack of competition with native parasitoids (Colombari and Battisti, 2016b), and high genetic diversity without a bottleneck-induced founder effect phenomenon (Matošević et al., 2015). The initial field assessment of *T. sinensis* impact on the invasive *D. kuriphilus* populations was achieved in experimental orchards in Japan and showed that 6–18 years after its introduction, the pest population declined to practically undetectable levels, which strongly encouraged its use as a biological control agent (Moriya et al., 1989; Murakami et al., 2001; Moriya et al., 2003). In Northern Italy, 13 years after the first release of *T. sinensis*, there is no longer any report of ACGW resurgence. In their assessment of *T. sinensis* efficacy, Ferracini et al. (2019) concluded that this biological control agent was indeed able to grow large populations and strongly reduce *D. kuriphilus* population, with a reduction of the percentages of gall-forming buds from 62.5% to 0.003% in 7 years. *T. sinensis* has also led to smaller drops of ACGW infestation rates in other places in Europe, such as in Slovenia, Croatia, Hungary (Matošević et al., 2017) and in France (Borowiec et al., 2018). However, the monitoring in one of the first release sites in Japan that was prolonged over 25 years, actually showed three successive peaks in the population of *D. kuriphilus*, shortly followed by peaks in the population of *T. sinensis* (Moriya, personal communication). Such an observation can potentially be explained by cyclic dynamic of *D. kuriphilus* - *T. sinensis* interactions that were first predicted theoretically by Paparella et al. (2016) and confirmed by the integrative

modelling intended in my PhD dissertation and the associated papers (see Chapters 1 and 2). In all those models, the two species are expected to coexist around an unstable fixed point by exhibiting typical host-parasitoid oscillations with period ranging from 5 to 11 years. Accordingly, even when the release of *T. sinensis* successfully lead to a reduction of *D. kuriphilus* populations over the first few years, one cannot rule out periodic reemergence of the invasive follow stasis of apparent elimination.

*Impact on biodiversity.* There are concerns that the non-native *T. sinensis* might hybridize with native *Torymus* species and/or shift to parasitize (non-targeted) native gall wasps. In Japan, hybridization has indeed been observed between *T. sinensis* and the indigenous late-spring strain of *T. beneficus*, with hybrids representing 22% of the population 3 years after the introduction of the control agent. Simultaneously, the indigenous late-spring strain of *T. beneficus* was rapidly displaced by *T. sinensis* (Yara et al. 2000). In Europe, no mate recognition and mating were recorded with native *Torymus* species (Quacchia et al., 2013b), although a suspected case of hybridization between *T. sinensis* and *T. cyaneus* Walker was reported in the laboratory (Aebi et al. 2013). Still, the installation of *T. sinensis* population may have a negative impact on native parasitoids by reducing their abundance and diversity, as suggested by Loru et al. (2021). Indeed, *T. sinensis* adults have been observed to emerge from oaks galls induced by 15 different oaks parasite species (Ferracini et al., 2017), highlighting that some opportunist individuals are able to switch host and therefore might outcompete native parasitoids.

*Hyperparasitism by endemic species.* Parasitoids of oak gall wasps naturally present in Europe have also been reported in ACGW galls (Ferracini et al., 2018). However, these native parasitoids do not maintain ACGW population densities below acceptable economic damage levels (Santi and Maini 2011; Kos et al. 2015). This is mostly because of the low observed levels of such parasitism (2–32%) probably due to asynchronization of their adult emergence with ACGW life cycle (Quacchia et al., 2013a). In addition, native endophytic fungus such as *Fusarium spp.* have also been observed to colonize the ACGW galls and inducing necrosis of the larvae but the low infection rate does not allow the control of the parasite population (Tosi et al., 2015).

*The Bottom-up and Top-down control of ACGW in Pyrénées-Orientales.* The regulation factors dependent of the resource and of predator/parasitic species and their quantitative impact on the ACGW dynamic are unknown in Pyrénées-Orientales. However, some information, especially

on natural enemies and biological control implementation, were extracted from reports made in Occitanie. Although the different chestnut tree strains of the Occitanie region present in cultivated areas exhibits various resistance level to ACGW infestation, the genetic susceptibility of the endemic natural population was unknown in our study area. Emergence experiments were performed in the geographically near departments of Ariège, Haute Garonne et Hautes-Pyrénées and allow to identify that native parasitoids from Eupelmidae, Eurytomidae and Pteromalidae family can infect *D. kuriphilus* larvae at low rates (4%) (Mansot and Castex, 2018). Nevertheless, this top-down impact of endemic species has not been estimated in Pyrénées-Orientales. The biological control with *T. sinensis* was implemented in the Pyrénées-Orientales with first, releases done in 2014. Diffusivity-model then predict its dispersal at the department scale, which have been verified thanks to emergence experiments conducted in 2015, 2016 and 2017 (Mansot and Castex, 2018). However, no quantitative assessment of their abundance or prevalence in chestnut tree galls was performed in our study area.

### **III. 4. The potential key species**

Beyond the bottom-up and top-down regulation associated with endemic species, the indirect effect induced by key species could also influence the dynamic of *D. kuriphilus* invasion. I focus here on the widely present *Quercus pubescens* and *Fagus sylvatica* trees because, as chestnut trees, they belong to the Fagaceae family and can form galls following their infection by parasitic species. They could then potentially i) provide the invasive pest with alternative hosts, and ii) increase endemic parasitoids richness and abundance. I also included the parasitic fungus *Cryphonectria parasitica* as a potential key species because of the possible co-infection of chestnut trees by this fungal species and *D. kuriphilus* and their possible synergetic effects on the hosts.



### 3.4.1 Chestnut blight (*Cryphonectria parasitica*)

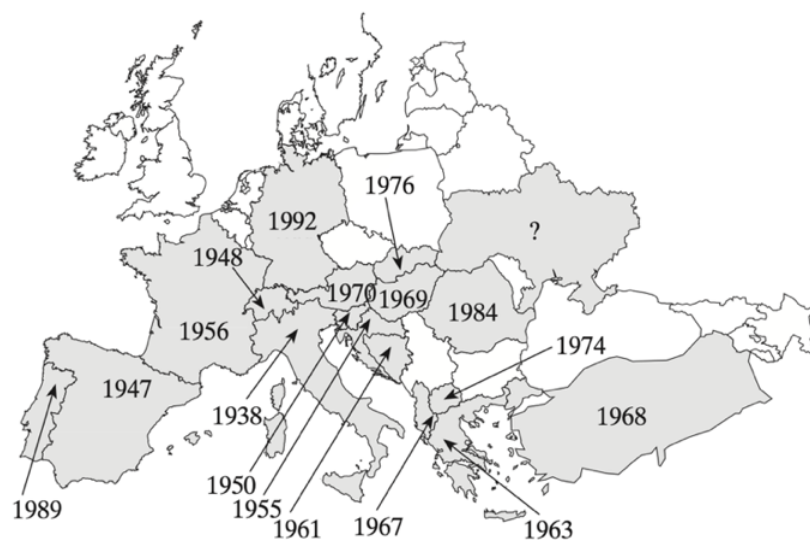
*Cryphonectria parasitica* is a fungus (ascomycetes) and the causative agent of the chestnut blight, a devastating disease infecting European et American chestnut tree populations. Its main hosts are species from the *Castanea* genus (*C. dentata*, *C. sativa*, *C. mollissima*, *C. crenata*, *C. pumila*) even if some minor incidental hosts have been observed like oaks (*Quercus* spp.), maples (*Acer* spp.) and European hornbeam (*Carpinus betulus*). Infected host trees show perennial necrotic lesions on the bark of stems and branches, eventually leading to wilting of the plant part distal to the infection.



Chestnut tree infected by *C. parasitica*. From Rigling and Prospero (2018)

*Ecology and life cycle.* *C. parasitica* is a necrotrophic pathogen that requires fresh wounds or growth cracks in the bark to penetrate into the host tissue (Roane et al., 1986). Drought, fire, natural competition or cutting of trees generate moribund wood tissue that might also serve as an entry point for *C. parasitica* (Prospero et al., 2006). Moreover, abandoned chestnut tree galls induced by the invasive Asian chestnut gall wasp (*Dryocosmus kuriphilus*) have been identified as a new way for the pathogen to penetrate into host tissues (Meyer et al., 2015). Investigations conducted in natural populations have shown that *C. parasitica* has a mixed mating system, with outcrossing and self-fertilization occurring at variable frequencies (Marra et al., 2004). Sexual and asexual spores are infectious and mainly dispersed through rain splash, birds, insects, mites and wind- borne dust (Heald and Studhalter, 1914; Russin et al., 1984; Wendt et al., 1983). An initial lesion is formed after spore germination and it develops into a bark canker. *C. parasitica* may then sporulate on the infected bark, as well as on the bark of recently dead chestnut wood (Prospero et al., 2006).

*Invasion history.* The pathogen is native to East Asia and was spread to other continents via infected chestnut plants. This invasive fungus was first reported on *Castanea dentata* in the United States in 1904, where it led to the quasi-extinction of the host species. It was then accidentally introduced in Europe in 1938 and has since spread in the major part of *C. sativa* distribution area (Figure 16). However, even if disease incidence was high in Europe, the severity remained lower than in the US and it did not lead to the extinction of *C. sativa*. This lower impact is mostly explained by the introduction of a biological control agent, the mycovirus *Cryphonectria hypovirus 1* (CHV-1) that allows for trees to recover. (*cf.* Disease management).



**Figure 16.** Presence and first year of observation of chestnut blight in Europe. From Robin and Heiniger (2001).

*Disease management.* Mechanical control strategies consisting in cutting and burning the *C. parasitica* infected trees have failed to limit the spread of the pathogenic fungus. In Europe, this led to the introduction of the biological control agent, the mycovirus *Cryphonectria hypovirus 1* (CHV-1), which was successful in reducing the impact of chestnut blight by causing hypovirulence (Prospero and Rigling, 2016). The treatment of individual cankers with hypovirus-infected *C. parasitica* strains lead to a reduction of the parasitic growth and sporulation capacity. The agent CHV-1 may then spread to untreated cankers and become established in the *C. parasitica* population. Hypovirulence is now observed in many chestnut tree populations in Europe, as a result of such biological control treatments or after it has naturally emerged (Bryner et al., 2012; Heiniger and Rigling, 1994). In the United States, disease management of chestnut blight was mainly focused on backcrossing the Chinese chestnut's blight resistance into the American chestnut genome (Rigling and Prospero, 2018)

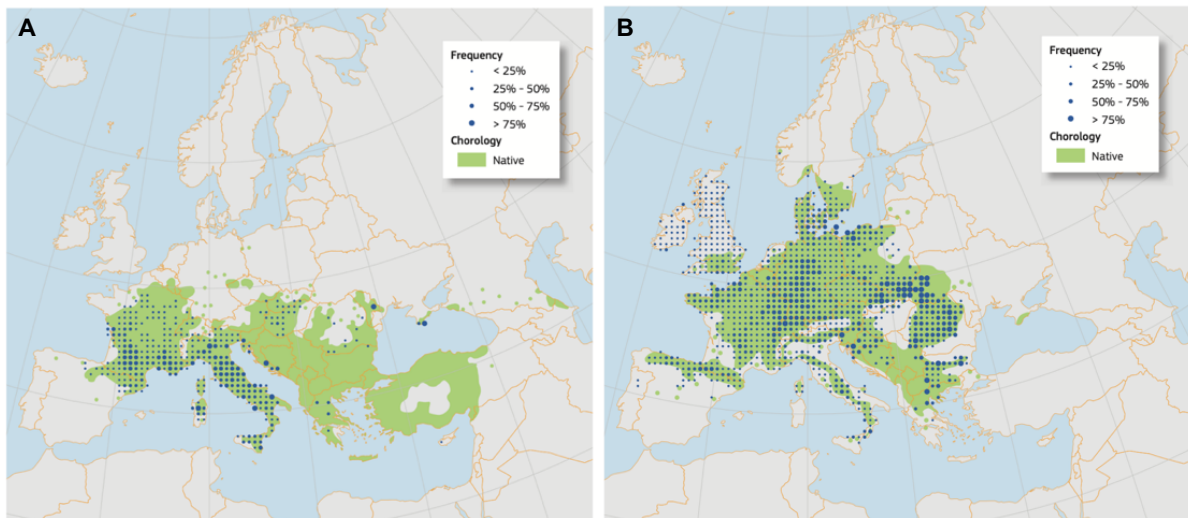
and have resulted in blight-resistant trees with higher survival and lower incidence rates (Bauman et al. 2014).

#### 3.4.2 Pubescent oak (*Quercus pubescens*)

*Quercus pubescens* Willd., is a (semi-)deciduous tree species from the *Fagaceae* family that grows very slowly to end up being up to 15-20m tall and live up to several hundred years (Pasta et al. (2016).

*Habitat and ecology.* Pubescent oaks show a very wide altitudinal range, from 200 to 800m, with some trees recorded up to 1300m in coastal plains. They are indifferent to pH variations and thrive in lime-rich and well drained soils in the northern part of their range, while they may also be common on acidic soils in the warmer countries such as Sicily and Crete. As a heliophilous and thermophilous species, they can tolerate both moderate summer drought stress and low winter temperatures. Being a very poor re-sprouter (Costa et al., 1997), it may be outcompeted by more resilient tree species under intense and frequent anthropogenic disturbance regimes, but efficient seed dispersal allows it to perform rapid colonization after disturbance removal (Sheffer, 2012).

*Origin and distribution.* *Q. pubescens* presents a strong genetic and morphological variability and therefore the species concept is particularly hard to be applied. Indeed, frequent hybridization events with other sympatric deciduous oaks (*Q. pyrenaica*, *Q. faginea*, *Q. petraea*, *Q. frainetto*) have been observed (Himrane et al., 2004, Valbuena-Carabana et al., 2005, Salvini, et al., 2009, Antonecchia et al., 2015, Fortini et al., 2015). Moreover, its complex origin and past distribution due to climate-driven survival, migration during Pleistocene glaciations (Dumolin-Lapègue et al., 1997) and the fragmentation and isolation of its populations due to millennia of deforestation lead to a wide distribution area occupying almost all central and southern Europe (Figure 17A) which is the outcome of a broad ecological niche restricted by human (mostly agronomic) activities.



**Figure 17.** (A) Plot distribution and simplified chronology for *Quercus pubescens*. From Conedera et al. (2016). (B) Plot distributions and simplified chronology map for *Fagus sylvatica*. From Pasta et al. (2016) and Durrant et al. (2016).

### 3.4.3. The European beech (*Fagus sylvatica*)

*Fagus sylvatica* L., or European beech, is a large deciduous tree species from the *Fagaceae* family. It is capable of a high rate of growth until late and commonly reaches 30-40m, reproduces only after 40-50 years and live up to 150-300 years (Durrant et al., 2016).

*Habitat and ecology.* The European beech tolerate very shady situations as seedlings are able to survive and grow below the canopy of established trees, which give them a competitive advantage to other trees species in light deprived understory. It grows on a wide variety of soils with a pH range from 3.5 to 8.5 with preferences for moderately fertile ground, calcified or lightly acidic. Beech requires a humid atmosphere with precipitation well distributed throughout the year and a well-drained soil. It tolerates rigorous winter cold, but is sensitive to spring frost and drought (Durrant et al., 2016). Genetic variability is observed across different climatic zones, with trees more resistant to drought in southern Europe (Packham et al., 2012).

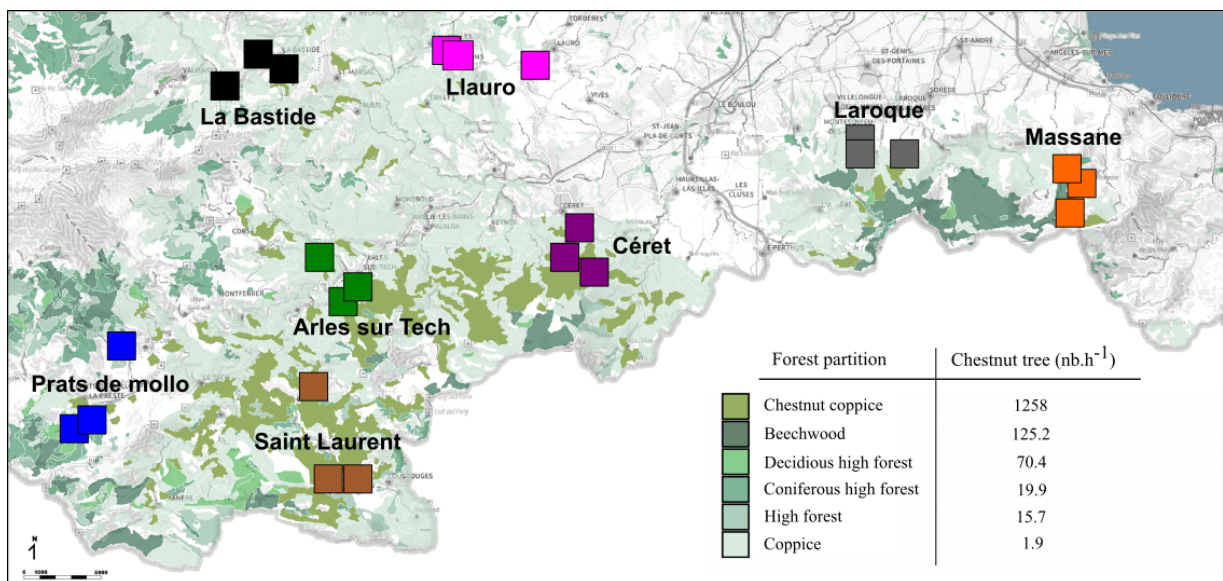
*Origin and distribution.* As represented in Figure 17B, the European beech is one of the most important and widespread broadleaved trees in Europe since it has spread from small scattered populations left after the last glaciation (Magri, 2008). It cannot persist in too septentrional places as it requires a growing season of at least 140 days (Magri, 2008) and it is only present at altitudes of 1 000 m to 2000m at the southern part of its range (Horgan et al., 2003). It is also

highly sensitive to high summer temperatures, drought and moisture that are limiting factors for the distribution of beech in Europe (Fang and Lechowicz, 2006). Meanwhile, global warming has started to affect its distribution as with climate becoming more continental in the eastern parts of Europe, it is replaced by oriental beech (*Fagus orientalis*).

*Pubescent oak, beech et chestnut blight in Pyrénées-Orientales.* The tree selected as key species, i.e. the pubescent oak and the beech, are with the chestnut tree the 3 most represented deciduous species in terms of area in Occitanie (Mansot and Castex, 2018). Indeed, they respectively represent 23%, 9% and 7% of the forested areas in this region (CNPf Occitanie, 2022). This co-occurrence is also relevant at the European scale where they are among the most represented hardwoods, as highlighted in Figure 10B, 17A and 17B that show their distribution areas. Regarding the third key species, the chestnut blight is widely distributed across European Forest and has been observed in the Pyrénées-orientales department since more than 20 years (Robin et al., 2000). These 3 key species being intimately linked to the chestnut tree host resource in the natural environment, either at the department and Europe scale, they could through indirect interactions, have an impact on the *D. kuriphilus* invasion dynamic and its control by *T. sinensis*.

## Research questions and manuscript structure

Since its accidental introduction in France in 2005, the Asian chestnut gall wasp has successfully spread across the country until its arrival in the Occitanie region in 2007. This global invader had strong economic and ecological impacts on both exploited and natural chestnut tree stands, especially in the Cevennes where the chestnut production was severely impacted (Mansot and Castex, 2018). Although local institutions in charge of crops and plant health reported the presence of the invasive pest *D. kuriphilus* and of the control agent *T. sinensis* in multiple places located in the Pyrénées-Orientales (Mansot and Castex, 2018), no quantitative assessment of their relative abundance were available in 2019, when I started my PhD project. We then set-up ecological and genomic studies to fill that gap and design integrative population dynamic models to allow identifying i) the main determinants of *D. kuriphilus* spread in the (semi-)natural chestnut tree population of the Pyrénées-Orientales that are typically managed for wood (rather than fruits) production, and ii) the ability of the biocontrol agent to limit the local invasion, which were funded by the interdisciplinary mission of the CNRS in its call ‘Modélisation du Vivant’. Over the last three years, I then conducted a fieldwork study in 24 sites located in different vegetal formations within the department in order to allow for the identification of the contributions of ‘Bottom-up’, i.e. the abundance, frequency and genetic susceptibility of chestnut trees, and ‘Top-down’ regulation factors, i.e. the rate of parasitism of the invasive *D. kuriphilus* by endemic parasitoid species.



Map of the different vegetal formations including chestnut trees at different abundance and the sampling sites for my PhD study

In those various sites, I further collected biological samples to allow me, implementing RADseq-like sequencing approaches of chestnut trees and GWAS analyses to identify loci associated with their susceptibility to *D. kuriphilus* infestation, as well as barcoding analyses to identify the endemic species of hymenoptera that actually parasitize *D. kuriphilus*. These ecological and genomic data were all analyzed and integrated into ‘eco-genomic’ dynamic models that were specifically designed to address the points i) and ii), and to provide a quantitative understanding of the *D. kuriphilus* – *T. sinensis* interaction in (semi-)natural tree forests, while most of the current knowledge is focused on such interaction dynamics in orchards.

In this PhD thesis, I thus aimed to improve our knowledge on the determinants of successful biological invasions, the efficiency of biological control strategies and the potential role of key species by studying the diverse interactions that *D. kuriphilus* face in the natural environments represented by various forest formations in the Pyrénées-Orientales. The main outcomes of my research are presented in three chapters corresponding to scientific papers that are being submitted for publication.

In **chapter I**, I identified the ‘Bottom-up’ and ‘Top-down’ determinants of invasion success and control of the invasive Asian Chestnut Gall Wasp in (semi-)natural chestnut tree forests and built a dynamic model of the *D. kuriphilus* – *T. sinensis* interaction integrating these regulation factors in order to quantify their impacts and the potential efficacy of this biological control strategy in such environments.

In **chapter II**, I took the questions about the spread of the invasive pest in chestnut tree forests and its biological control agent a step further by considering their dispersal between chestnut tree sites that shows different levels of ‘bottom-up’ and ‘top-down’ determinants. I then focused on the consequences of *D. kuriphilus* and/or *T. sinensis* dispersal on the ‘global’ scale stabilization of their interaction and the implication for the biological control efficacy.

In **chapter III**, I completed the results of the first two chapters by further considering the impact of suspected key species (*Quercus pubescens*, *Fagus sylvatica*, *Cryphonectria parasitica*) on the *D. kuriphilus* – *T. sinensis* interaction in chestnut tree forests. I tested whether or not the presence of these tree species in the forest assemblage could alter the detection capacity of *D. kuriphilus* for its host, increase the native parasitoid abundance and/or constitute an ecological niche for the biological control agent.

## Chapter I

### **Assessing the invasion of the global chestnut tree pest, *Dryocosmus kuriphilus*, and its bottom-up and top-down control: an integrative eco-genomic modelling study in the natural forests of the Pyrénées-Orientales.**

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## INTRODUCTION

Biological invasions have been steadily increasing over the last two centuries (Seebens et al., 2017, 2020) and have become a major driver of life evolution on Earth by causing species extinction (Bellard et al., 2016), homogenization of fauna and flora (Winter et al., 2009) and changes in biogeography (Capinha et al., 2015). Accordingly, invasive species are increasingly recognized as threats to the biodiversity, functioning and/or productivity of all (agro-)ecosystems (Pyšek and Richardson, 2010; Blackburn et al., 2019) that impair the services they provide to human societies (Walsh et al., 2016). The global economic costs of those impacts have reached a staggering 1.288 trillion of US dollars over the last 50 years, with marked heterogeneities between regions and taxonomic groups (Diagne et al., 2021).

Invasive insects are known to have advert effects on crops (Tonnang et al., 2022), agroforestry (Fleming et al., 2017), animal husbandry (Lantschner et al., 2019), human infrastructure (Buczowski and Bertelsmeier, 2017) and health (Bradshaw et al., 2016; Paine et al., 2016). According to a recent comprehensive study, their average annual cost was around 10.4 billion over the last 60 years (Renault et al., 2022), which represents about 40% percent of the above global expenditures. Three quarters of those costs resulted from direct resource losses mostly incurred to forestry and agriculture in similar proportions (Renault et al., 2022). The number of non-native forest insects that have established outside of their natural range has indeed increased dramatically on all continents (Roy et al., 2014; Hurely et al., 2016; Brockerhoff and Liebhold, 2017; Seebens et al., 2017, 2022; Renault et al., 2022) where they represent, along with invasive pathogens, one of the greatest threats to forestry and biodiversity conservation (Bradshaw et al., 2016; Ghelardini et al., 2017). As invasive pests are expected to keep on having profound impacts on natural and exploited forests (Aukema et al., 2011; Ramsfield et al., 2016; Stenlid and Oliva, 2016), it is essential to identify the determinants that impede or promote the establishment and local spread of such invasive aliens in order to improve risk assessment, anticipate its evolution, and quantify the efficacy of potential forest protection measures (Kueffer et al., 2013).

The dynamic of local emergence of an invasive species is typically governed by forces associated with resources limitation, called ‘bottom-up’ as they emerge from lower trophic level, and natural enemies, referred to as ‘top-down’ as being imposed by a higher trophic level (Frost et al., 2019). Variations in plant palatability, nutritional quality, distribution and abundance are essential bottom-up factors that have long been thought to be prominent in

limiting the populations of herbivore insects (Vidal and Murphy, 2018). Meanwhile, the idea that predators and parasitoids can down-regulate the herbivore populations below densities at which they have major effects on plant biomass has been stimulating plant ecology research on such top-down factors since it was first proposed to provide a tentative answer to the question ‘Why is the world green?’ ( Hairston et al., 1960). To compare the relative importance of bottom-up and top-down forces has naturally emerged as essential to understand the population ecology of herbivores (e.g. Hunter et al 1997; Wilkinson and Sherratt, 2016). In a recent meta-analysis run over 350 herbivorous insect species, top-down forces were found to have about 2-fold higher impacts than bottom-up factors on abundance and life-history traits in both natural and cultivated environments (Vidal and Murphy, 2018), which provided compelling quantitative evidence of the necessity of tri-trophic approach to understand the dynamic of forest invasion by non-native insects (Hunter et al 1997; Fischbein and Corley, 2022).

The Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus*, is the most dangerous insect pest of chestnut trees (*Castanea* spp.) affecting orchards and forests worldwide (Fernandes et al., 2022). Native to China, it was first reported to have spread to Japan in 1941 (Murakami, 1981) and other Asian countries (Tamura, 1962; Ueno, 2006), before to be introduced in North America in 1974 (Payne et al., 1976) and in Europe in 2002, where it has invaded a wide range of region from Italy (Martinez-Sanudo et al., 2019). While this invasive insect pest can affect species of the genus *Castanea* and hybrid clones, there is a broad variability among clones regarding the level of infestation, suggesting a genotype-dependent variation in susceptibility (Sartor et al., 2009, 2015; Castedo-Dorado et al., 2023) that is thought to contribute explaining the variations in infestation observed at the individual tree level within populations of *C. sativa* (Bombi et al., 2018). The individual rate of chestnut tree infestation by ACGW has also been shown to decrease in mixed stands, as compare to pure *C. sativa* monoculture, in line with the general ‘associational susceptibility’ hypothesis (Fernandez-Conradi et al., 2018). Along with these typical bottom-up factors, *D. kuriphilus* recruits native enemies that can contribute to the biotic resistance of the invaded environment. Numerous native parasitoid have been shown to shift onto the invasive chestnut gall wasp, in particular from the Cynipini communities on oaks (Stone et al., 2002, Acs et al., 2007, Quacchia et al., 2013, Gil-Tapedo et al., 2018). While these native parasitoids are readily able to find and lay eggs into *D. kuriphilus* galls, their impact on its invasion has often been shown to be limited (Gil-Tapedo et al., 2018), so that bio-control using a Chinese natural parasitoid, *Torymus sinensis*, has been intended in Japan (Moriya et al., 2003), South-Korea (Murakami et al., 1995), North-America (Cooper and

Rieske, 2007) and Europe (Gibbs et al. 2011). To gain a proper quantitative understanding of the determinants of a *D. kuriphilus* invasion therefore requires to measure the bottom-up and top-down forces at works, and to integrate such empirical findings into specifically designed dynamical models of the tri-trophic interaction *Castanea sativa* - *D. kuriphilus* - *Torymus sp.* While such integrative approaches have proven efficient to assess the spread of plant pests and to anticipate the efficacy of biological control by their parasitoids (Raghu et al. 2007; Steiner et al. 2008), they remain to be implemented to study the invasion of chestnut tree forests by *D. kuriphilus*.

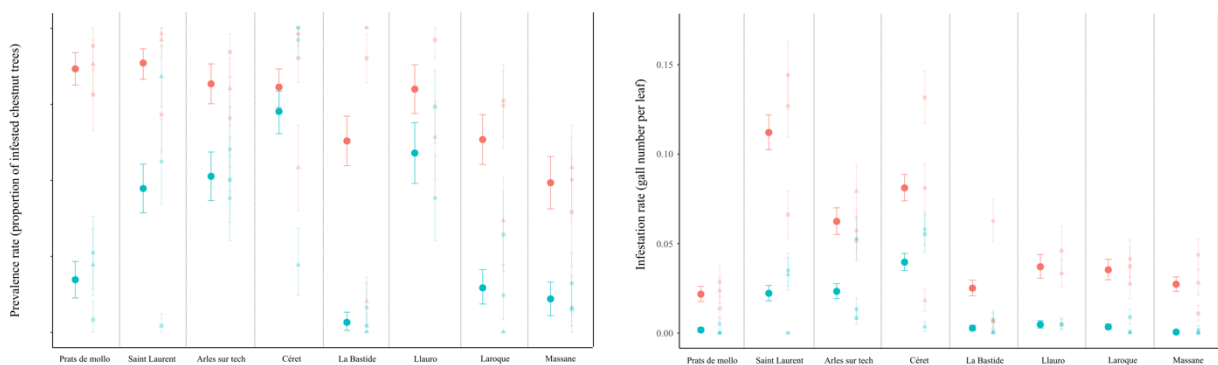
In this contribution, we investigated the spread of *D. kuriphilus* in the natural chestnut tree forests of the Pyrénées-Orientales, an area at the French-Spanish border, where the invasive pest has been informally reported since 2013, and where *T. sinensis* has been sporadically released in several places since 2014 (Mansot and Castex, 2018). We conducted a two-years ecological field study in 24 sites located in different vegetal formations containing chestnut trees in order to provide a comprehensive view of the local infestation of the chestnut tree populations by *D. kuriphilus*. In those sampling sites, we concomitantly implemented RAD sequencing of a hundred chestnut trees and GWAS analyses to identify loci associated with susceptibility to *D. kuriphilus* infestation, and we further performed barcoding analyses of gall contents to identify native hymenopteran parasitoid species as well as the rate of *D. kuriphilus* parasitism by such native parasitoids and *T. sinensis*. Those ecological and genomic data were integrated into a dynamical model of the host-parasite-parasitoid interactions to quantify the importance of bottom-up factors, i.e. the abundance, frequency and genetic susceptibility of chestnut trees, and the top-down factors, i.e. native and introduced parasitoids, on both the invasion potential of *D. kuriphilus* and its control by the biological agent *T. sinensis*.

## RESULTS

### Widespread and heterogeneous invasion of the Pyrénées-Orientales chestnut tree populations by *D. kuriphilus*.

The chestnut tree populations located in the 23 sampling sites were all found infested by *D. kuriphilus* over the 2-years of our field study, highlighting the widespread colonization of the Pyrénées-Orientales by the invasive parasite that was first reported in the area in 2013 (Figure 1, SM 1).

In 2019, 75% of individual chestnut trees were infested with an average of 0.05 galls per leaf, representing a parasitic burden of 1 gall every 20 leaves. The prevalence of infestation (proportion of infested trees) showed spatial heterogeneity between sites ( $\chi^2 = 50.6$ ,  $df = 22$ ,  $p = 4.9 \cdot 10^{-4}$ ) and, albeit to a lower extent, between stations ( $\chi^2 = 13.3$ ,  $df = 7$ ,  $p = 6.4 \cdot 10^{-2}$ ), with larger prevalences observed in the Vallespir ( $0.845 \pm 0.029$ ) than in the Aspres ( $0.702 \pm 0.06$ ) and in the Albères ( $0.565 \pm 0.061$ ) (Figure 1A). The rate of infestation (average number of galls per leaf) showed a consistent heterogeneity between sites ( $\chi^2 = 479.8$ ,  $df = 22$ ,  $p < 2.2 \cdot 10^{-16}$ ) and stations ( $\chi^2 = 287.4$ ,  $df = 7$ ,  $p < 2.2 \cdot 10^{-16}$ ), with higher infestation in the Vallespir ( $0.069 \pm 0.004$ ) than in the Aspres ( $0.03 \pm 0.004$ ) and the Albères ( $0.031 \pm 0.003$ ) (Figure 1B).



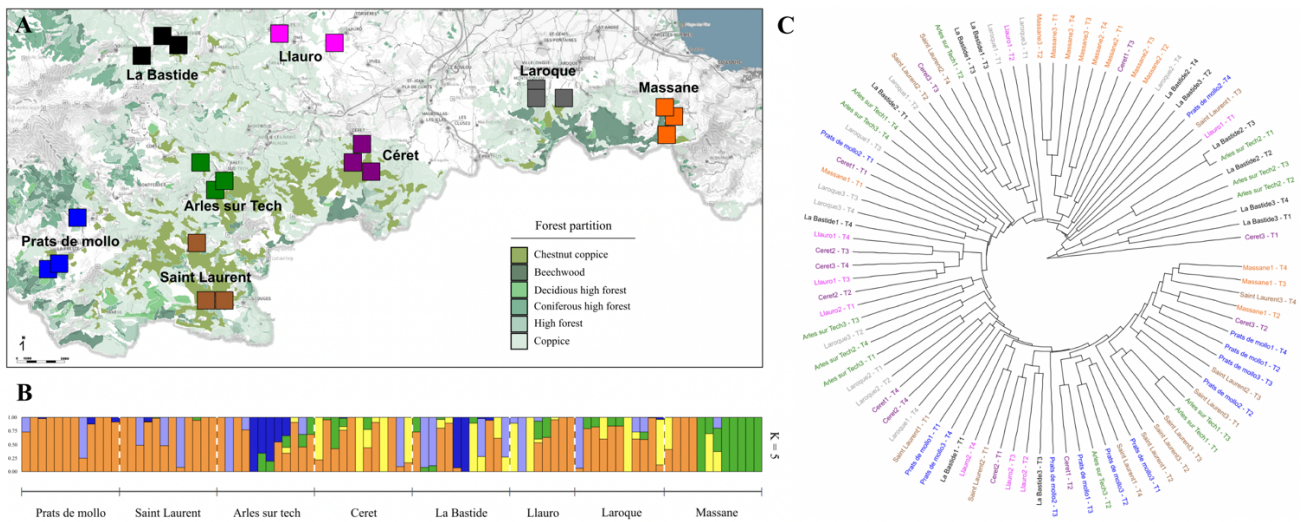
**Figure 1. Infestation of the chestnut tree populations by *D. kuriphilus* in the Pyrénées-Orientales.** Measures of the prevalence (A) and rate (B) of infestation made in 2019 and 2020 appear in red and blue, respectively, with their 95% confidence intervals. Sampling sites were located in 8 stations of the Vallespir (PM-Prats de Mollo, SL-Saint Laurent, AT-Arles sur Tech and C-Céret), the Aspres (LB-La Bastide, L-Llauro) and the Albères (La-Laroque, M-Massane) local massifs.

A decrease in both the prevalence and rate of infestation was observed between 2019 and 2020 across all the studied area. The overall proportion of infested trees dropped to 34% with an average of 0.0132 galls per leaf, representing a reduction of 57.8% and 79.3% in those two infestation measures. Despite significant variations between stations and sites in the amplitude of changes in infestation levels, the spatial patterns observed in 2020 were relatively similar to those observed in 2019. A significant heterogeneity was found at the site and station scales for both the prevalence ( $\chi^2 = 164.7$ ,  $df = 22$ ,  $p < 2.2 \cdot 10^{-16}$ ;  $\chi^2 = 107.2$ ,  $df = 7$ ,  $p < 2.2 \cdot 10^{-16}$ ) and rate ( $\chi^2 = 428.1$ ,  $df = 22$ ,  $p < 2.2 \cdot 10^{-16}$ ;  $\chi^2 = 295.3$ ,  $df = 7$ ,  $p < 2.2 \cdot 10^{-16}$ ) of infestation. The proportion of infested trees and the number of galls per leaf remained on average higher in the Vallespir ( $0.472 \pm 0.04$  and  $0.023 \pm 0.002$ ) and lower in the Albères ( $0.13 \pm 0.04$  and  $0.002 \pm 0.001$ ) and in the Aspres ( $0.256 [0.204; 0.312]$  and  $0.0037 \pm 0.001$ ). Accordingly, the ranking of sites with respect to the level of *D. kuriphilus* invasion was conserved between years when considering either the prevalence ( $\rho = 0.563$ ,  $p = 4.2 \cdot 10^{-3}$ ) or the rate ( $\rho = 0.653$ ,  $p = 5.4 \cdot 10^{-4}$ ) of infestation.

Our two years long field study provided clear evidences that the Asian invasive Chestnut Gall Wasp, *D. kuriphilus*, has widely spread across the chestnut tree populations of the Pyrénées-Orientales with higher levels of infestation in the Vallespir than in the Aspres and the Albères, as well as significant variations at smaller (station, site and individual) scales. To unravel the potential ‘bottom-up’ and ‘top-down’ determinants of those spatial heterogeneities in infestation, we further characterized the ecological and genetic structure of the local chestnut tree populations, and the abundance and species composition of the hyperparasite community.

### **A patchy and heterogeneous chestnut tree distribution with low genetic structure.**

The distribution of chestnut trees in the Pyrénées-Orientales that we characterized from publicly available national inventories (Figure 2A) was found to be patchy as the corresponding populations belong to different vegetal formations whose overall density ranges from 250 to 1570 individual per hectare, and where *Castanea sativa* represent 15.8% to 90.8% of all trees. Our estimates of the species structure in sampling sites were consistent with these large-scale figures. The overall density of trees was found to decrease from the Vallespir ( $1055 \pm 63$ ) and the Aspres ( $1100 \pm 65$ ) to the Albères ( $753 \pm 54$ ), with higher frequencies of chestnut trees found in the ‘chestnut coppices’ and ‘deciduous high forest’ of Vallespir ( $0.639 \pm 0.08$ ), than in the ‘high forest’ of the Aspres ( $0.594 \pm 0.01$ ) and in the ‘Coniferous high forest’ of the Albères ( $0.400 \pm 0.01$ ).



**Figure 2. The heterogeneous distribution and low genetic structuring of the *Castanea sativa* populations in the Pyrénées-Orientales.** (A) Map of the different vegetal formations including chestnut trees at different frequencies and the sampling sites. (B) Genetic structure of the chestnut trees with respect to the 5 ancestral populations identified from RAD sequencing of 91 individuals, and the corresponding (C) phylogenetic tree, that both show a low genetic structure of the chestnut tree populations.

Despite their heterogeneous distribution, the local chestnut tree populations appear to make a well-mixed genetic unit with relatively low genetic structuring. The current genetic structure assessed by nGBS sequencing of 91 individuals located across our 23 sites was best explained as originating from 5 ancestral populations that contributed to the current populations with no pronounced spatial pattern, although one of those populations was over-represented in two sites of the station ‘Massane’ (Figure 2B) where it was first introduced for wood production at the end of world war two. The phylogenetic tree built from the corresponding 449 markers consistently supported this lack of conspicuous spatial genetic clustering (Figure 2C), and Jost’s  $D$  index confirmed the low genetic differentiation between the chestnut tree populations of the 8 stations ( $D = 0.004$ ) and 23 sites ( $D = 0.01$ ), despite clear genetic variations at the individual level (Figure 2B).

To assess the impact of potential ‘bottom-up’ determinants of infestation, we looked at the correlation of the measured prevalence and rate of infestation, with the chestnut tree density, frequency and standard genetic indexes (nucleotide diversity, heterozygosity, inbreeding coefficient, rate of private alleles) calculated at the individual and/or site level from the nGBS sequencing of the 92 sampled trees.

**‘Bottom-up’ control of *D. kuriphilus*: a balance between amplification by host species frequency and susceptibility associated with individual genetic differentiation.**

The generalized linear mixed model (GLMM) regression analysis revealed that the frequency of chestnut tree population in the local forest stand strongly favors its infestation by *D. kuriphilus*. Significant positive correlations were indeed observed between both site prevalence and rate of infestation, and the chestnut tree frequency (Table 3 A, B). Interestingly, the density of trees had no effect on the level of site infestation, reinforcing the idea that the spread of *D. kuriphilus* is modulated by the species structure and diversity of the forest stand (Guyot et al. 2015). The site prevalence of infestation was further negatively correlated with the site rate of private alleles (Table 3 A). Meanwhile, the individual rate of private allele was found to be associated with lower rates of tree infestation (Figure 3C), although this effect did not scale up to the infestation site level (Figure 3B). Noteworthy, neither the site prevalence nor the (site and individual) rates of infestation were correlated to any of the other standard genetic indexes.

Fixed effects	Estimate	Standard error	df	P
<b>A</b> Response variable : Site prevalence rate				
Intercept	-0.560	0.991	42	0.5746
Chestnut tree frequency	2.955	0.817	42	0.0008
Population private allele rate	-8166.863	3871.641	42	0.0409
<b>B</b> Response variable : Site infestation rate				
Intercept	-4.547877	0.6486244	43	0
Chestnut tree frequency	1.561707	0.6191002	43	0.0154
<b>C</b> Response variable : Individual infestation rate				
Intercept	-3.234	0.593	181	0
Individual private allele rate	-11128.909	4729.280	181	0.0197

**Table 1. GLMM analysis of the ‘bottom-up’ control of *D. kuriphilus*.** The best statistical models to explain the prevalence and rate of infestation at the site level (A,B) and the individual rate of infestation (C) are given with their parameter estimates and supports.

### **Immune response genes correlate with *D. kuriphilus* infestation rate.**

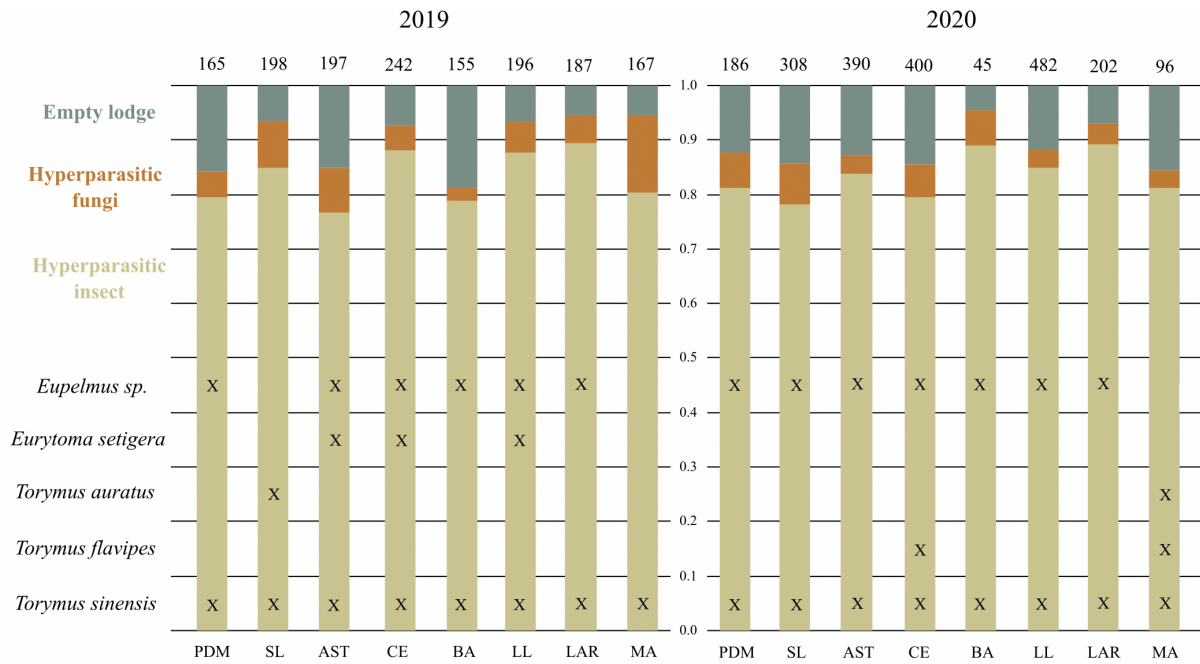
GWAS analyses highlighted that SNPs present in genes related to immune system and response to other organisms are significantly over-represented among loci that are identified. 33 SNPS for the 2019 phenotype and 491 SNPS for the 2020 phenotype are significantly correlated to the infestation rate. Among these SNPS, 22 are significant with both phenotypes and distributed in 18 different loci. Regarding the 502 significant SNPs, they are distributed in 323 different loci among the *C. mollissima* reference genome. 75 of these loci remain uncharacterized and 20 were identified as putative transposon/retrotransposon sequences. Identifications of 228 remaining loci are presented in supplementary material S2. Finally, gene ontology enrichment analysis revealed a significant enrichment in some biological processes. Indeed, genes related to the regulation of immune system process, response to other organisms (biological process involved in interspecies interaction between organisms, response to external biotic stimulus) and cellular process were over-represented. Thus, mutations in these genes could explain observed individual variations in infestation rate and confirms a potential chestnut tree genetic resistance for ACGW infection.

### **‘Top-down’ control of *D. kuriphilus*: a broad spread of the control agent (*T. sinensis*) in the wild and the presence of native hyperparasite species.**

The dissection of 2110 galls collected in the 8 studied localities of the Vallespir, Aspres and Albères massifs showed that only ~5.9% of *D. kuriphilus* larvae developing in chestnut trees galls in 2020 reach the adult stage and emerge, leaving empty lodges with typical emergence holes. Most of the remaining lodges were indeed found occupied by hyperparasitic insects (96.5% and 3.5% being larvae and pupae/adults, respectively), while others were found either filled with fungi or empty, as a result of a failure of *D. kuriphilus* development. The rate of insect hyperparasitism was highly similar in both years (~83.5% in 2019 and ~77.8% in 2020) and across localities (with a Fano factor of 0.003 and 0.002 in 2019 and 2020). The rate of fungi hyperparasitism was also found to be similar in both years (6.7% in 2019 and 4.6% in 2020) and showed little variations between the sampled populations (with a Fano factor of 0.02 and 0.006 in 2019 and 2020). Meanwhile, the proportion of lodges found empty and with no hole of emergence, indicative of a failure of development, were found to be very consistent from one year to another (~9.8% in 2019 and ~11.7% in 2020) and between places (with a Fano



factor of 0.029 and 0.013 in 2019 and 2020).



**Figure 3. Analysis of the ‘top-down’ control of *D. kuriphilus*.** A total of 2110 galls were collected across the 8 localities and the two years of our field study. The corresponding numbers of lodges per locality and year are indicated above each column of the histogram that indicate the proportions of such lodges found with hyperparasite insects and fungi or empty without emergence hole, indicative of a failure of development. Sampling sites are indicated as in Figure 1: PM-Prats de Mollo, SL-Saint Laurent, AT-Arles sur Tech and C-Céret, LB-La Bastide, L-Llauro, La-Laroque, M-Massane.

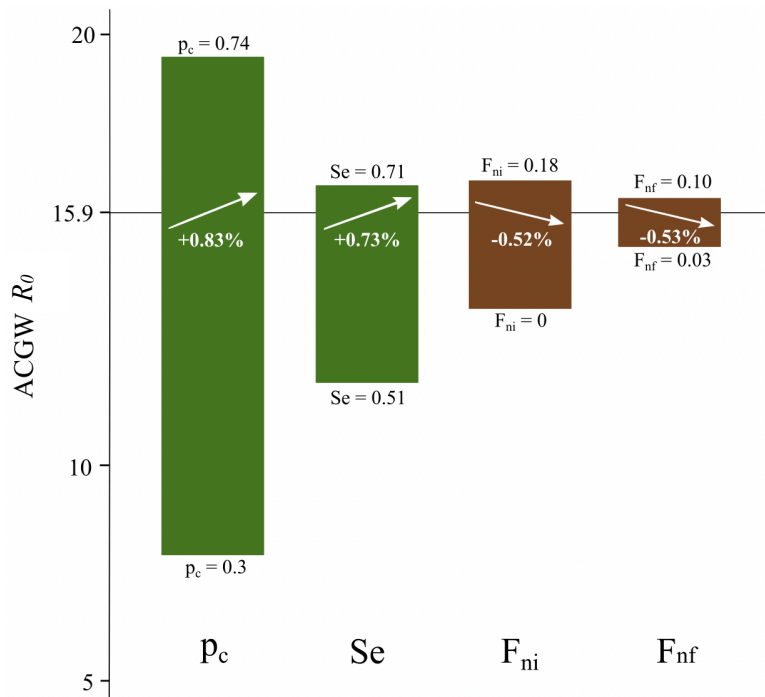
The molecular taxonomic identification based on the DNA barcoding of 3002 hyperparasitic insects found in the collected galls revealed the presence of at least 5 hymenopteran species; the Asian control agent, *Tormus sinensis*, and 4 native species; *Tormus auratus*, *Tormus flavipes*, *Eurytoma setigera*, *Eupelmus sp.* Although the structure of the native community was found to vary, the control agent (*T. sinensis*) was detected in all localities in both 2019 and 2020, demonstrating that it has widely spread and established itself across all the study area since its local introduction in 2014.

To estimate the relative abundance of *T. sinensis* among all hyperparasite species, we collected 45 galls in each of our 8 localities and brought them to the laboratory to conduct an emergence experiment. Among the 404 hyperparasites that emerged within 8 months, 387 specimens were morphologically affiliated to *T. sinensis* (95.5%) while 17 specimens were identified as native hyperparasite species (4.5%) with little variations among the 8 localities (with a Fano factor of 0.048).

Our ecological and genomic study of the ‘bottom-up’ and ‘top-down’ control of *D. kuriphilus* demonstrated that i) the frequency of chestnut trees in the vegetal formations, ii) the tree individual genetic differentiation, i.e. characterized by their rate of private alleles and associated with a lower susceptibility, and iii) the presence of (native and introduced) hyperparasitic insects and fungi, all are influencing the local spread of the Asian Chestnut Gall Wasp. To further develop our quantitative understanding of *D. kuriphilus* invasion, we used a population dynamic modelling specifically tailored to account for the impact of these ‘bottom-up’ and ‘top-down’ factors on the interactions between *C. sativa*, *D. kuriphilus* and its hyperparasites.

### **Bottom-up factors are the main natural determinants of *D. kuriphilus* invasion potential.**

We first used our modelling to identify the expression of *D. kuriphilus* invasion potential as measured by its per capita annual multiplication rate  $R_0$  in the natural environment (See SM 3). The value of such a *D. kuriphilus*'s  $R_0$ , estimated from the average frequency ( $p_c$ ) and genetic susceptibility ( $Se$ ) of chestnut trees and from the average rate of infection by native hyperparasitic insects ( $1-F_{ni}$ ) and fungi ( $1-F_{nf}$ ), was found equal to 15.9. This invasion potential was shown to be about 1.5 more sensitive to the bottom-up than to the top-down factors, as any 1% increase in  $p_c$  and  $Se$  are predicted to increase  $R_0$  by 0.83% and 0.73%, while similar changes in  $F_{ni}$  and  $F_{nf}$  would reduce  $R_0$  by 0.52% and 0.53%. The relative importance of bottom-up and top-down factors in the regulation of the invasion of the local chestnut tree populations by *D. kuriphilus* was confirmed when considering their range of observed variations between our 8 sampling stations. The changes in  $R_0$  induced by spatial heterogeneities in  $p_c$  were indeed found to be 10.1 and 3.9 times broader than those resulting from spatial variations in  $F_{ni}$  and  $F_{nf}$ . Meanwhile, changes in  $R_0$  due to spatial variations in  $Se$  were 4 and 1.5 times broader than those resulting from spatial variations in  $F_{ni}$  and  $F_{nf}$ . The outcomes of our sensitivity analysis of *D. kuriphilus*  $R_0$  are thus consistent with the high spreading potential of this world-wide invasive species (Avtzis et al. 2019) and they provide a clear quantitative assessment of the predominance of the chestnut tree frequency and genetic susceptibility over the native hyperparasite community as main natural determinants of *D. kuriphilus* invasion.

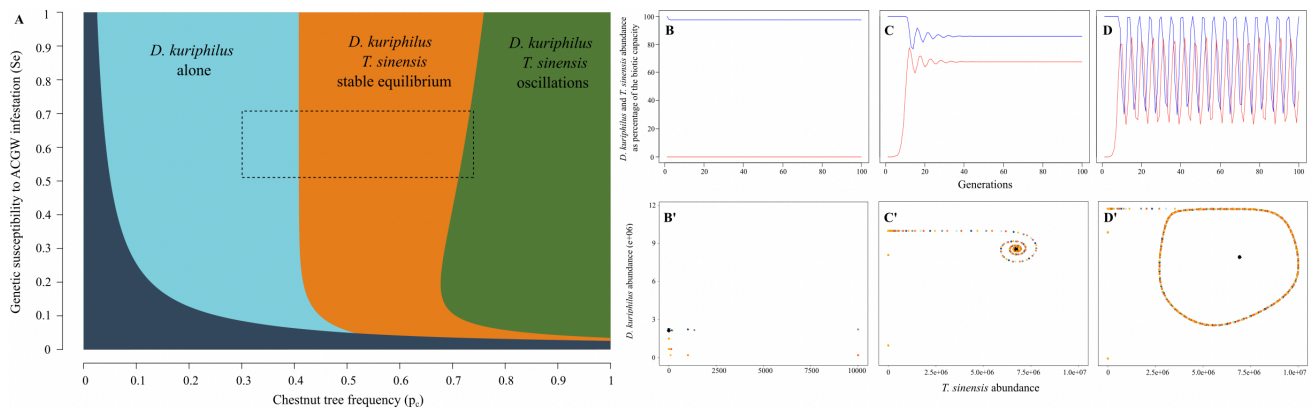


**Figure 4. Sensitivity analysis of the *D. kuriphilus* invasion potential ( $R_0$ ) to the chestnut tree frequency and genetic susceptibility, and to the native hyperparasite community.** The percentages of change in  $R_0$  in response to 1% changes in the average values of  $p_c$ , Se,  $F_{ni}$  or  $F_{nf}$  are represented by rising or down arrows. The ranges of variations in  $R_0$  to be expected in the Pyrénées-Orientales were predicted according to the actual changes in  $p_c$ , Se,  $F_{ni}$  and  $F_{nf}$  that were observed across our sampling stations (SM 1).

**The outcome of the *D. kuriphilus* - *T. sinensis* interaction primarily depends on the frequency of chestnut trees.**

The introduction of the hyperparasite *T. sinensis*, commonly used as a biological control agent, was predicted to lead to four different dynamics according to the frequency of chestnut trees in the vegetal formation and to its genetic susceptibility. As expected, a forest with a very low  $p_c$  or Se, typically lower than 0.1, would not allow for *D. kuriphilus* invasion despite the species high reproductive potential (Figure 5A). However, as soon as  $p_c$  exceeds this threshold, *D. kuriphilus* is able to spread and, wherever  $p_c$  remains lower than  $\sim 0.4$ , the control agent fails to get established so that the population of *D. kuriphilus* is expected to grow towards the biotic capacity sets by the available chestnut trees (Figure 5B). Interestingly, in a mixed forest, where  $p_c$  ranges from 0.4 to 0.7, *T. sinensis* is able to spread by infesting the *D. kuriphilus* population, which lead to dampened oscillations before a stable equilibrium is reached with both the invasive parasite and the introduced hyperparasite settling down in the environment (Figure

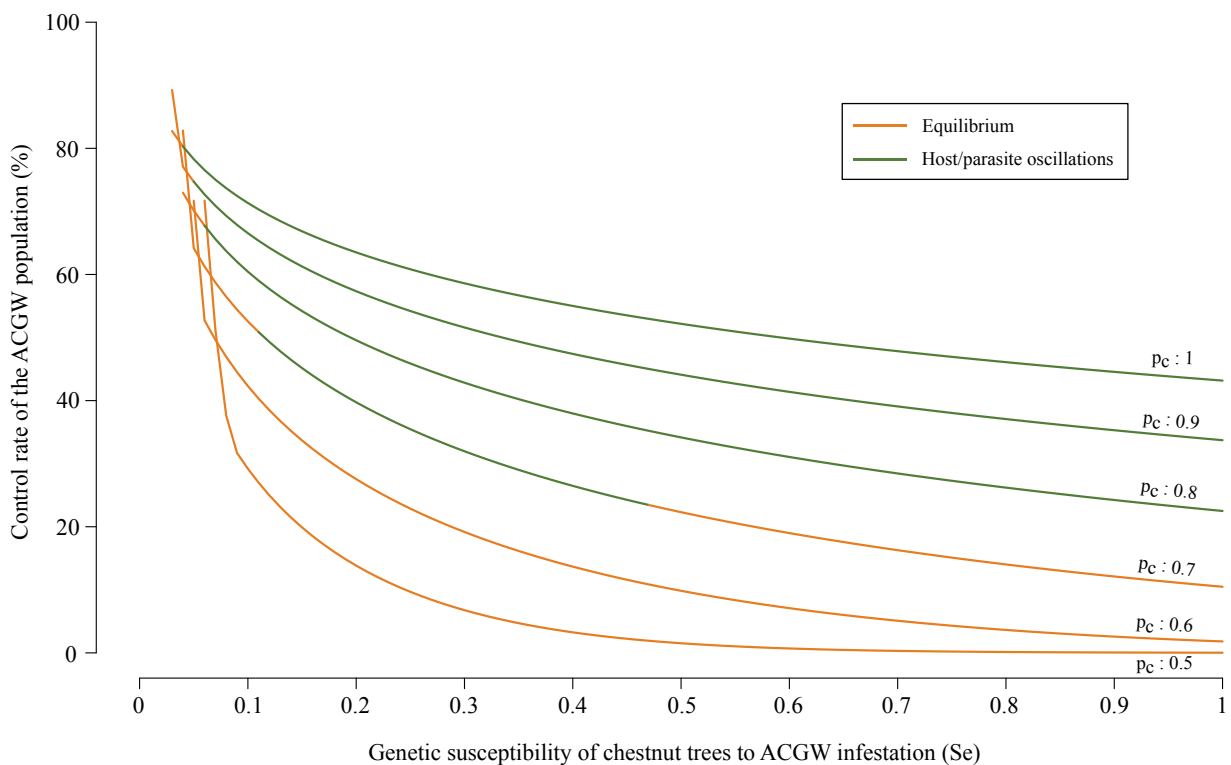
5C). Large oscillations typically expected between a biological control agent and its target, are only predicted to occur in forests dominated by chestnut tree, i.e. when  $p_c$  values are larger than 0.7 (Figure 5D). Finally, none of these asymptotic dynamics were shown to depend on the size of the *D. kuriphilus* population when *T. sinensis* is introduced or on the amount of introduced individuals (Figure 5 B' to D'). This suggests that the timing of *T. sinensis* introduction during *D. kuriphilus* invasion or the release effort have no effect on the long-term outcome of the control intervention. When focusing on the range of variations in  $p_c$  and  $Se$  observed across our 8 sampling stations, the former clearly stands as the main bifurcation parameter whose variations can change the population dynamics from a failure of *T. sinensis* to spread and control the invasion of *D. kuriphilus*, to a persistence of both species at a stable equilibrium or through large oscillations (Figure 5A). On the contrary, the levels of genetic susceptibility measured across our sampling stations were all large enough, to have no effect on the qualitative outcome of the *D. kuriphilus* - *T. sinensis* interaction.



**Figure 5. Impact of the frequency and genetic susceptibility of chestnut trees on the dynamics of interaction between *Dryocosmus kuriphilus* and *Torymus sinensis*.** **A.** Population Dynamics in the  $p_c$  -  $Se$  parameter plane. Dark blue = *D. kuriphilus* fails to invade, light blue = *T. sinensis* fails to spread and control *D. kuriphilus*, orange = stable equilibrium between *D. kuriphilus* and *T. sinensis*, dark green = stable oscillations between *D. kuriphilus* and *T. sinensis*. **B-D.** Chronics describing the typical temporal dynamics of *D. kuriphilus* larvae ( $H(t)$ , blue line) and *T. sinensis* adults ( $P_s(t)$ , red line) observed in light blue, orange and green parts of A. Both abundances are given as percentage of the species biotic limit estimated for a 1km<sup>2</sup> area. **B'-D'.** Phase planes illustrating the lack of sensitivity of the asymptotic dynamics to the initial conditions  $H(0)$  and  $P_s(0)$ , i.e. the abundance of *D. kuriphilus* and *T. sinensis* when the latter is introduced.

**Biological control is most efficient in chestnut tree dominated forests with low genetic susceptibility to *D. kuriphilus*.**

The efficacy of control interventions based on the release of *T. sinensis* is expected to vary according to the species structure and genetic susceptibility of the forest environment. We explored such a dependency by running simulations with and without the control agent, to estimate the percentage of the *D. kuriphilus* population that could be removed by the introduction of *T. sinensis*. This showed that the impact of *T. sinensis* on *D. kuriphilus* populations decreases with the genetic susceptibility of chestnut trees, especially when the latter remains low, while it tends to increase with the frequency of chestnut trees in the local environment (Figure 6). Accordingly, the introduction of the biological control agent is expected to be more efficient in chestnut tree dominated forests exhibiting large oscillations in both species abundance, than in mixed-forest where the interaction dynamics converges towards a stable equilibrium. In the conditions typically encountered in stations of the Pyrénées-Orientales, *T. sinensis* is predicted to lower the abundance of *D. kuriphilus* up to 26.13%, with the proportion of chestnut trees standing as the main cause for such variations as the observed levels of genetic susceptibility typically exceeds 0.51.



**Figure 6. Efficacy of *D. kuriphilus* biological control in different forest environments.** The efficacy is measured as a percentage of reduction in the expected abundance of *D. kuriphilus* in the absence of the control agent *T. sinensis*. Both abundance of *D. kuriphilus* with and without *T. sinensis* were evaluated numerically after a stable (orange) or oscillatory (green) equilibrium was reached according to the value of chestnut tree frequency ( $p_c$ ) and genetic susceptibility ( $Se$ ), as demonstrated in Figure 5.

## DISCUSSION

Our field study provided clear evidence that the chestnut tree pest, *D. kuriphilus*, has widely spread in the Pyrénées-Orientales area, thereby contributing to the global effort of documenting its worldwide invasion. The level of infestation recorded in the first year were relatively high, with 75% of infected chestnut trees and, in 7 out of the 23 studied sites, infestation rates exceeding the threshold of 0.6 galls per bud above which *D. kuriphilus* can induce drastic decrease of tree productivity (Sartor et al., 2015). Such high infestation levels reported 6 years after the first observation of this invasive in the area are consistent with those observed after a similar amount of time in Switzerland and Italy (Gehring et al., 2018). Decreases of 57.8% and 79.3% of prevalence and rate of infestation were observed in 2020, with no site remaining highly infested and 18 sites falling below the threshold of 0.3 galls per bud, where they are considered as weakly infested with no substantial threat for tree production or growth (Sartor et al., 2015). Behind this clear temporal trend, heterogeneities in infestation were observed, at site and individual tree levels, which we intended to exploit to uncover the bottom-up and top-down determinants of *D. kuriphilus* invasion.

Our first aim was then to characterize the bottom factors, i.e. the abundance and genetic resistance of the chestnut tree resource, that could underly the observed variations in *D. kuriphilus* infestation. Data from the national forest inventory (1994) showed that *C. sativa* is one of the most abundant species in the area, represent 8.4% of the woodlands, with a patchy distribution spread in different plant formations where its frequency varied between 16% and 91%, providing a truly heterogeneous ecological context for *D. kuriphilus* invasion (Figure 2A). The heterogeneity in the abundance of its resource was further shown to be associated with genetic disparities between chestnut trees hosts. The genetic structure analysis that we conducted on our 23 sampling sites showed that they correspond to a well-mixed genetic unit of 5 ancestral populations. This low-structured population constituted of a chestnut tree strains

is likely to be explained by the introduction of exotic *C. sativa* varieties for exploitation, ornamental and reforestation purposes followed by hybridization with the endemic population. Such a low genetic differentiation is also consistent with genetic analysis conducted by Frascaria et al. (1993) in natural chestnut tree populations in France. Meanwhile, our GLMM analysis of this bottom-up factors showed that chestnut tree frequency in the site and its private allele rate contributed to explain the observed variations in the prevalence and rate of infestation at the site level. Such a contribution of the abundance and genetic heterogeneities of the resource to the infestation of *D. kuriphilus* confirmed that bottom-up factors are expected to be determinant when the ability of the pest to detect its host is low (Germinara et al., 2011).

During our fieldwork, we observed that even in high-infested sites, some chestnut trees remains uninfected or infected at strikingly low level, which could potentially be explained by genetic resistance mechanisms of the host, as previously suggested by Nugnes et al. (2018) in (semi-)natural chestnut tree populations located in Southern Italy. The GLMM analysis performed at the individual tree level indeed showed that the infestation rate was negatively correlated to the rate of individual private alleles, which is considered to be a proxy of its genetic differentiation from the rest of the population. These unique mutations are indeed more likely to be accumulated by trees that are not part of the endemic population or that have experienced outcrossing with introduced strains, as state by Jiang et al. (2012). Private alleles could indeed be acquired by introgression from coexisting cultivars with their frequency in the genome being then positively correlated to the outcrossing rates of the population. Our results suggest that such introgressions may play a role in the adaptive evolution of local chestnut tree populations, by providing them with greater resistance to *D. kuriphilus* invasion.

We then investigated the genetic basis of the host resistance through a genome wide association study. Although nGBS allowed to randomly sequence only 1% of the *C. sativa* genome, the low expected level of linkage disequilibrium allowed to aim for the identification of genomic regions at the gene level. Indeed, the low linkage disequilibrium value in *Quercus*, a phylogenetically close genus of *Castanea*, is caused by a strong heterozygosity and a low genetic differentiation between populations Plomion et al. (2018), that were both reported in our chestnut tree populations. We identified candidate genes for the chestnut tree resistance to *D. kuriphilus* that were part of biological processes and protein families present in the hypersensitive response pathway which mediates host responses upon pathogen infection. Genes that can act in elicitor recognition, activation of the transcription, salicylic and jasmonic

acid biosynthesis, transport and regulation, and programmed cell death through hypersensitive response with accumulation of reactive oxygens species were over-represented in less infested individuals. Such findings are consistent with results from Dini et al. (2012) who reported a hypersensitive response in the totally resistant chestnut tree strain ‘Bouche de Bétizac’ and with those from Acquadro et al. (2020) who observed a differential expression of leucine rich repeat proteins, receptor-like protein and kinases, cellular death related genes, transcription factor and miRNA target gene comparing chestnut tree varieties infected or not by *D. kuriphilus*.

The resource availability and its genetic resistance were thus identified as bottom-up factors regulating the invasion of *D. kuriphilus* in the forest environment, which is consistent with others studies highlighting the primary importance of the access to the resource for the growth of invasive populations (Vaz-Pinto et al., 2013; Maino et al., 2019; Yam et al., 2016; Costamagna et al., 2007).

Second, we looked at the role of the top-down control of *D. kuriphilus* by native and introduced parasitoids. While endemic species could be identified in the gall formed on infested chestnut trees, they do not represent a strong regulation of *D. kuriphilus* populations. Indeed, molecular taxonomic identification revealed that only 4 hymenopteran species were able to infest *D. kuriphilus* larvae in the Pyrénées-Orientales. These generalist parasitoids, which were previously observed in France (Mansot and Castex, 2018; Muru et al., 2021), typically infest oak gall-forming parasite (Avtzis et al., 2019) that are endemic of our study area. Although, they were able to adapt their diet to this new resource opportunity, as it was observed for others biological invasions (Parker and Hay, 2005; King et al., 2006; Pio et al., 2019), their rate of *D. kuriphilus* larvae parasitism remained low (4.46%), which is also consistent with previous estimates in Occitanie (Mansot and Castex, 2018) and Europe (Kos et al., 2015; Szabó et al., 2014). We also observed hyperparasitism of *D. kuriphilus* by fungal species at a rate that was similarly low (5.44%) as previously observed in other places. Indeed, Tosi et al. (2015) recorded that *Fusarium proliferatum* could infest 0.91% to 11.65% of *D. kuriphilus* larvae, with an average of 3.55% across stations studied in Italy, while Cooper and Rieske (2010) estimated that such rate was of 3% in 2007 and 6.8% in 2009 in the USA.



The barcoding and emergence analyses of the content of *D. kuriphilus* galls collected in our 24 sampling sites provided strong evidence that the biological control agent, *T. sinensis*, had widely spread across all the Pyrénées-Orientales since its first release in 2014 (Mansot and Castex, 2018). The parasitoid was detected during both years of the study and in all 24 sites, with rates of *D. kuriphilus* parasitism that reached 92.8% in 2020. These important rates of control 6 years after the introduction of the control agent, were highly consistent with the >90% rates observed 5-7 years after the implementation of biological control in Italy (Bosio et al., 2013), and likely explain the strong reduction in *D. kuriphilus* abundance observed between 2019 and 2020.

While the bottom-up and top-down factors influencing *D. kuriphilus* infestation levels in the Pyrénées-Orientales were unraveled from our ecological and genomic analyses, we further aimed at providing quantitative insights into their respective impacts on the invasive dynamics of the pest. The implementation of integrative eco-genomic models showed that bottom-up factors, and especially the frequency of chestnut trees in the forest environment, had (1.5 to 4 times) more effect than top-down factors on *D. kuriphilus* growth rate ( $R_0$ ), as previously observed for other invasive species (Arenas et al., 2006; Jamieson et al., 2012). In addition, because of their stronger variability in the (semi-)natural environment as compared to top-down determinants, they were also the major determinants of the outcomes of the interaction dynamic between *D. kuriphilus* and *T. sinensis*, which is consistent with results from David et al. (2021) who showed that the bottom-up factors could determine the local distribution of a control agent. In conditions typically encountered in the Pyrénées-Orientales, our model predicted the long-term coexistence of *D. kuriphilus* and *T. sinensis* around an equilibrium point or through a fluctuations of both species abundances, two typical dynamical behaviors of host-parasitoid population dynamics models (Hochberg and Hassell, 1990). Interestingly, the only other dynamical model of *D. kuriphilus* - *T. sinensis* interaction, also predicted coexistence of the two species through oscillatory population dynamics (Paparella et al., 2016). The outcomes of these models thus contrast with the typical expectation that a biological control agent would contribute eliminating the targeted (invasive) species before to collapse, as predicted by the standard Nicholson-Bailey model commonly used to anticipate the outcome of introduced parasitoid species (Mills and Getz, 1996). The prediction of such regime of persistent oscillations echo the observation from Moriya (personal communication) who observed fluctuations of *D. kuriphilus* and its control agent, *T. sinensis*, for over a 25 years period in Japan, leading to three successive resurgences of the parasite population. Altogether, these

models and data suggest that while using *T. sinensis* as control agent does reduce *D. kuriphilus* in the first few years, as empirically shown in this study and elsewhere (see above and Avtzis et al., 2019, Borowiec et al., 2018), it is also likely to lead to the integration of a new exogen species when released in forest environments.

We then used our modelling to evaluate the efficacy of biological control at equilibrium between *D. kuriphilus* and *T. sinensis*. The agent was predicted to be most efficient in reducing the abundance of *D. kuriphilus* in chestnut-dominated environments while its effect is reduced in mixed (semi-)natural forests. The oscillatory dynamics associated with the highest frequencies of chestnut trees indeed allow for the best control. The size of the oscillations then determine the average reduction rate of the pest population, with a better biocontrol associated to the higher amplitude. Although the average rate of biocontrol remains far from parasite eradication, they typically allow maintaining infestations below the threshold of 0.3 galls per buds (associated with no substantial threat for tree production or growth, Sartor et al., 2015) for several years before the resurgence of the parasite. These modelling outcomes on the biocontrol efficiency in patches with various environmental conditions are consistent with those from Balsa et al. (2021) who predicted a local extinction of the pest in small and homogeneous orchards, while such a control was not achieved in large and/or heterogeneous forests. Overall, the biological control of *D. kuriphilus* by *T. sinensis* is then anticipated to be efficient in chestnut tree orchards and minimal in natural forests with low or mid chestnut tree frequency, raising concerns about its large scale implementation and efficacy.

Taken together, the field observation of a significant decrease in *D. kuriphilus* abundance that combined with high level of *T. sinensis* infestation, and the theoretical predictions of persistent oscillations between the two (invasive and introduced) exotic species, strongly suggest that the dynamics of their interaction in the Pyrénées-Orientales is entering a similar long term dynamic as observed in Japan with expected resurgence of the parasite.

## Material and Methods

### *Dryocosmus kuriphilus* life-history.

The worldwide invasive, *D. kuriphilus*, is an Asian wasp inducing the formation of galls on stem and leaves of sweet chestnut trees. This can cause up to 80% chestnuts production loss for infested trees (Battisti et al., 2014) and make them more vulnerable to other parasites, most notably fungus responsible for the sweet chestnut blight (Meyer et al., 2015). This hymenoptera species is univoltine, semelparous and parthenogenetic. Females emerging from galls in June-July are 1-7 days short-lived and fly to lay their asexually produced eggs in chestnut tree buds during a 2 to 3-weeks period, before to dy. Oviposition can trigger a local tree hypersensitive immune response, with the accumulation of reactive oxygen compounds, resulting in programmed cell death and leading to the death of deposited eggs (Dini et al., 2012). The efficacy of this response shows substantial variations between chestnut tree varieties, with levels of susceptibility to bud infection varying by one to two orders of magnitude (Sartor et al., 2015). Eggs escaping such a response and intrinsic developmental failure hatch within a month to develop into first instar larvae that enter an overwintering dormant stage. In spring, larvae develop into subsequent larval stages and induce the formation of galls where they eventually become pupae and adults emerging to lay the next and non-overlapping generation of eggs. Meanwhile, hymenopteran parasitoid species target the newly formed galls to lay their progeny that will ultimately feed on *D. kuriphilus* larvae, thereby downregulating the invasive population. Such hyperparasites can include native species, although their reported parasitism rates are usually low (Kos et al., 2021), and the introduced Asian control agent, *Torymus sinensis*, an univoltine and semelparous Hymenoptera of the *Torymidae* family. Galls have further been repeatedly shown to be infected by local fungus that induce an additional mortality of *D. kuriphilus* larvae. All these processes are underlying the bottom-up and top-down control that ultimately determine the fate of a local *D. kuriphilus* invasion.

### *Distribution of the Castanea sativa populations and sampling sites in the Pyrénées-Orientales.*

We investigated the spread of *Dryocosmus kuriphilus* in the *Castanea sativa* populations of the Pyrénées-Orientales, a French department located on France's Mediterranean coast along the Spanish border, in 2019 and 2020. This area is made up of highly contrasted landscapes spanning from the Vermeille Coast to the 2784 meters high Canigó Massif (Figure 2A). The

spatial distribution of the local *C. sativa* populations was characterized by combining i) cover maps of the different vegetal formations - that were derived from aerial photointerpretation of the study area (IGN, 2018), with ii) estimates of the density of chestnut trees in each of these vegetal formations - that were derived through systematic clustered sampling during national forest inventories (Ministère de l'agriculture et de la pêche, 1994). The map of the local chestnut tree populations (Figure 2A) shows that they are mostly found in the Vallespir, Aspres and Albères massifs (DREAL, Languedoc-Roussillon), where we therefore located our 24 sites in 8 stations; Prats de Mollo, Saint Laurent, Arles sur Tech and Céret (Vallespir), La Bastide and Llauro (Aspres) and Laroque and La Massane (Albères). The genomic analyses latter on showed that 3 individual chestnut trees of the third sampling site located in Llauro were almost genetically identical, while very divergent and much less infested than all other individuals included in the study (see '*Genetic structure of the chestnut tree population*' below). These outliers were then ultimately removed from the dataset and all analyses carried on the remaining 23 sites, as it appears in figures 1-2 and table 1.

#### ***Infestation of *C. sativa* populations by *D. kuriphilus* in the Pyrénées-Orientales.***

The invasion of the local chestnut tree populations by *D. kuriphilus* was assessed by two typical measures of parasitism in each sampling site. The rate of infestation, defined as the mean number of galls per leaf, was estimated on 5 geo-located chestnut tree individuals per sampling site and with a sampling effort of 250 to 500 leaves per tree. The rate of prevalence, defined as the proportion of chestnut trees infested by at least one gall, was estimated on 50 trees in each of the sampling sites. The existence of heterogeneity between sites or between stations in the rate or in the prevalence of infestation was tested by Pearson's chi-squared tests with Yates's correction for continuity. Exact confidence intervals for both measures of infestation were calculated from the binomial distribution set according to the corresponding sampling effort.

#### ***Characterization of the tree species structure in sampling sites.***

The composition of the tree species structure was characterized by sampling 1000m<sup>2</sup> in each of our study sites and by identifying the taxonomic status of each tree to the species level. Confidence intervals for the overall abundance of trees and species frequencies were drawn from Poisson and Binomial distributions, respectively.

### ***Chestnut trees genome sequencing and SNP calling.***

We randomly selected 4 out of the 5 geolocated chestnut tree individuals in each site, from each of which a 3 cm<sup>2</sup> leaf sample was collected and sent in 70° alcohol to LGC Genomics for single-end restriction-site associated (RADseq-like approach called nGBS) sequencing using the ApeK1 restriction enzyme. This allowed to obtain ~1% of the genome sequenced for each of those individuals. High-quality clean reads were mapped onto the *Castanea mollissima* reference genome (GCA\_000763605.2) using BWA-MEM (0.7.16a-r1181) with default settings. The ref\_map.pl program of the Stacks (Catchen et al., 2013) pipeline was used to call SNPs in each sample, based on assembling loci according to the alignment positions provided for each read.

### ***Genetic structure of the chestnut tree populations.***

The genetic structure of the chestnut tree populations was established from SNPs shared by 100% of the individuals, as such a stringent filter was thought to provide optimal estimates of phylogenetic distances between individuals. An individual with a very low number of sequenced loci (Wpt063 - Laroque) was removed at this stage from the analysis, which allowed to increase by a factor of 10 the number of SNPs shared by all remaining individuals. Subsequent genetic analyses made on the 449 resulting SNPs were achieved using R.4.1.1. and dedicated packages (R Core Team, 2021), but for the calculation of the Jost's differentiation index (Jost's *D*; Jost, 2008) that was done using Genodive (Meirmans, 2020). The individual genetic structure with respect to the ancestral populations was estimated using the sNMF function of the LEA package with *K* (number of ancestral populations) varying from 1 to 10 with 10 replicates for each. We then used the SNPRelate package to build phylogenetic trees from the individual genomes. The initial analyses ran on the (4\*24)-1 chestnut trees showed that 3 individuals from the third sampling site located in Llauro were genetically identical and very divergent from the rest of the individuals (See Figure S2, S3), so that they were treated as outliers and removed from our dataset. The final analyses of the genetic structure of the chestnut tree populations appearing in Figure 2 were then run on the remaining (4\*23)-1 = 91 individuals.

### ***Genetic indexes of the chestnut tree populations.***

We estimated standard genetic indexes (nucleotide diversity ( $\pi$ ), heterozygosity ( $H$ ) and inbreeding coefficient ( $F$ )) using 31736 SNPs shared by at least 80% of the 92 individual genomes sampled from the 23 sites kept in the analysis. Nucleotide diversity, heterozygosity, inbreeding coefficient and the number of private alleles were all estimated using the *populations* script of the Stacks pipeline, but the inbreeding coefficient at the individual level was not provided by Stacks and was then calculated with VCFtools (Danecek et al., 2011). The number of private alleles was estimated from the total number of SNP for each individual and site using the *populations* script of the Stacks pipeline. The frequency of private alleles was then calculated as the ratio of the number of private alleles to the total number of bases sequenced per individual or site.

### ***General Linear Mixed Models analyses of the bottom-up control of *D. kuriphilus* infestation.***

To identify the potential determinants of a bottom-up control of *D. kuriphilus* invasion, we searched if the observed levels of infestation could be explained by the set of ecological (overall density of trees, frequency of chestnut trees) and genetic (nucleotide diversity, heterozygosity, inbreeding coefficient and frequency of private alleles) variables that were measured on the 23 sampling sites and 92 individual trees. Statistical analyses were performed through 3 independent generalized linear mixed modelling (GLMM) to explain variations in prevalence of infestation between sites (i), and in the rate of infestation between sites (ii) and individual trees (iii). Each statistical model best explaining those infestation levels was identified by following the procedure recommended by Zuur et al. (2009). First, we characterized the impact of the sampling year on the infestation measures (see Figure 1) through a standard GLM analysis (Infestation : Estimate = -1.421,  $p = 3.65 \cdot 10^{-4}$ , Prevalence : Estimate = -1.7292,  $p = 7.99 \cdot 10^{-5}$ ), in order to account for such a temporal structure (by incorporating sampling year as a random factor) in our subsequent GLMM analyses (Zuur et al., (2009), p. 323-332). Second, we identified collinearity between ecological and genetic explanatory variables using the *corvif* function of the AED package, and subsequently excluded the most correlated variables with a cut-off of 3 (Zuur et al., (2009), p. 387). Once heterozygosity had been removed in such a way, all our explanatory variables appeared fairly independent one from another, with all collinearity measures ranging between 1 and 2.5. Third, the best statistical model was identified by sequentially removing non-significant predictors according to the outcomes of student tests

comparing the model fitting with and without each of the remaining explanatory variables (Zuur et al., (2009), p. 220-222). All 3 GLMM analyses were conducted in R 4.1.1 using the `glmmPQL` function from the MASS package and a quasi-binomial distribution, after the original rate of infestation (i.e. the number of galls per leaf) was converted into a proportion of leaves with one gall since we never counted more than one gall on any of the >45000 observed leaves.

### ***Genome Wide Association Studies.***

Two genome wide association studies (GWAS) were performed on the 92 genotyped chestnut trees in order to identify the genetic bases involved in the susceptibility of *Castanea sativa* to *Dryocosmus kuriphilus* infestation. The association between the 31736 SNPs shared by 80% of the individual chestnut trees, and each of the 2 individual phenotypic measures of infestation, i.e. the rate of infestation measured on individual trees during each year, were calculated using the Latent Factor Mixed Models (LFMM) package in R 4.1.1. The significant SNPs were selected by applying a cut-off equals to  $3.150996e-05$  ( $1/31736$ ). The genomic regions containing these SNPs were then identified by a sequence homology analysis using *C. mollissima* as reference genome. When SNPs were located in genes described in the annotation file of the *C. mollissima* genome, we extracted the entire gene sequence. The sequence of each of these genes was then blasted into Plaza Dicots 4.5 database, allowing for gene to be identified when the p-value of the alignment was found  $\leq 1e-40$ . When the alignment did not meet such a criterium, we used the less specific NCBI database and genes were identified when the alignment score was  $\geq 200$ , corresponding to a p-value of  $1e-45$ . When multiple alignments with a score  $> 200$  were found, we selected the alignment with the closest location to the SNPs being considered. For the remaining SNPs that were not found in genes described in the *C. mollissima* genome annotation file, we extracted 3000pb of the *C. mollissima* genome on each side of the SNPs, resulting in a 6001pb sequence. Sequence homology analysis was then performed using the NCBI database as describe above. Once all genes were identified, their functions and *Arabidopsis thaliana* homologs were determined using the Uniprot database (UniProt Consortium, 2019). Finally, a singular enrichment analysis was performed using agriGO (Tian et al., 2017) with the *Arabidopsis thaliana* TAIR9 database to identify a potential enrichment in some biological process among the candidate genes.

### ***Collection and barcoding of the invasive ACGW and its parasitoid community.***

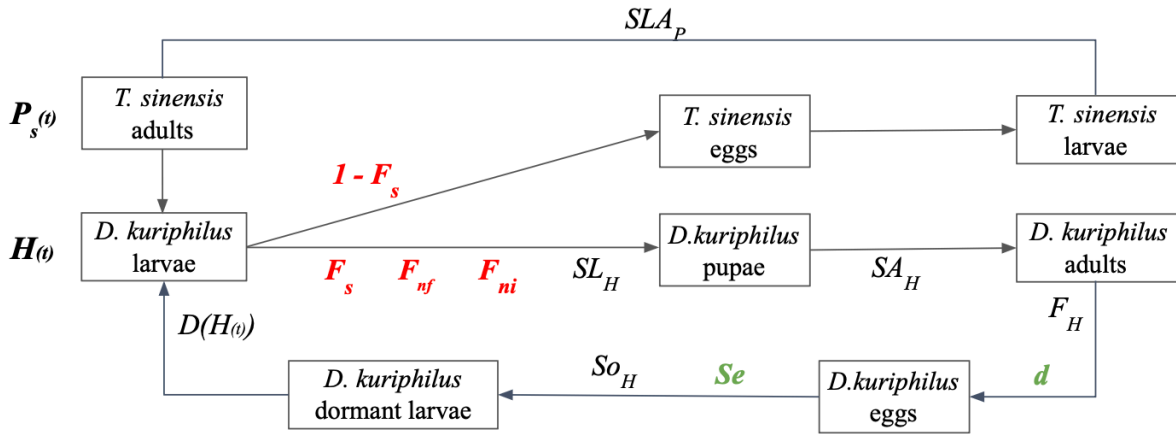
The infection of chestnut trees by *D. kuriphilus* induces the formation of galls that help protecting the larvae until they become adults and emerge. Only a fraction of the eggs laid on leaves reach the adult stage of the ACGW life-cycle because of developmental failures and hyper-parasitism. Part of the ACGW larvae can indeed be parasited by local hyperparasitic insects or fungi, and by *Torymus sinensis*, an hymenoptera of the *Torymidae* family, used as a biological control agent. To assess the importance of these potential sources of top-down regulation of ACGW populations, we combined three complementary experimental approaches. First, in order to estimate the proportions of ACGW larvae that i) failed to develop, ii) were hyperparasited by (native or introduced) insects, ii) were hyperparasited by fungi, and iii) emerged as adults, we dissected 770 galls in 2019 and 1340 galls in 2020. Those galls were collected from all sites of our 8 sampling stations, with a minimal sampling intensity of 45 galls in La Bastide in 2020. For each gall, we counted the number of lodges that were empty, occupied by an hyperparasitic insect or fungi or by their ACGW individual, so that we could estimate the above proportions in each sampled locality. Second, we used DNA barcoding to identify to which species belong the 3002 hyperparasitic insects found in all the collected galls. Individuals were pooled by sampling sites and DNA was extracted for each pool using the E.Z.N.A Tissue DNA kit (Omega BIO-TEK) extraction protocol. A Polymerase Chain Reaction (PCR) was performed to amplify the ribosomal Internal Transcribed Spacers 2 (ITS2), commonly used as DNA barcodes and phylogenetic markers in insects. Both forward et reverse primers were synthetized in 2 different versions with 1N or 3N between the adapter sequence used for sequencing and the annealing sequence (1N; forward: 5'[TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG]NTGTGAACTGCAGGACACATG 3', and reverse: 5'[GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG]NATGCTTAAATTYAG CGGGTA 3'. PCRs were performed in 35  $\mu$ L reaction volume, containing ~ 20 ng DNA template, 0.1  $\mu$ M of each dNTP, 0.04  $\mu$ M of each primer and 0.7 U Phusion High-Fidelity DNA polymerase (FINNZYMES OY, Espoo, Finland) in 7  $\mu$ L 5X manufacturer's buffer plus 21,35  $\mu$ L sterile distilled water. The thermal profile of the PCR was as follows: initial denaturation at 98°C for 30 seconds, followed by 16 to 22 cycles (depending on the sample) of denaturation at 98°C for 10 s, locus-specific annealing at 52,7°C for 30s, elongation at 72°C for 18s, and a final elongation at 72°C for 10 min. The PCR products were checked on 1% agarose gel and sequenced by using a Miseq Reagent Kit v3 (Illumina, San Diego, USA). Sequences obtained from the 44 samples (24 samples from 2019 and 20 samples from 2020) were processed with



the FROGS pipeline (Escudié et al., 2018) available on the Genotool Bioinfo galaxy server (Haddad et al., 2016). Clustering swarm step was made with aggregation distance of 1. Only OTU's with at least 50 sequences were conserved and affiliated based on the NCBI database. Third, to quantify the fraction that *T. sinensis* represent among all hyperparasite species, 45 galls were collected in each of our 8 sampling stations and brought to the laboratory for an emergence experiment. The 360 collected galls were distributed among 72 pots closed with microporous tape to allow gas exchange. Emergence of adult hyperparasites was followed from October 2019 to May 2020 by collecting emerging individuals every week and placing each of them in an individual 0.5mL Eppendorf containing 200 $\mu$ L of alcohol at 100 ° C and stored at -20 ° C. Morphologic identification then allowed to discriminate between *T. sinensis* and other insect species, and therefore to estimate the relative rates of infection by the introduced and native hyperparasites.

#### ***Dynamical modelling of the C. sativa - D. kuriphilus - hyperparasites interactions.***

We adapted the seminal host-parasitoid model proposed by Nicholson and Bailey (1935) to describe the interactions between *C. sativa*, *D. kuriphilus* and its hyperparasites. The core of such a modelling is made of formal representations of the univoltine and annual life cycles of both *D. kuriphilus* and its hyperparasitic control agent, *T. sinensis*, that were described in section ‘*Dryocosmus kuriphilus* life-history’ (Figure 7). This structure was chosen to allow predicting the yearly dynamics of the number of *T. sinensis* adults attempting to lay their eggs into *D. kuriphilus* larvae in the spring of year  $t$ , which we refer to as  $P_s(t)$  and  $H(t)$ , respectively. In our model, the population of *D. kuriphilus* larvae is then first split into individuals escaping hyperparasitism by *T. sinensis* and those who do not, according to a function  $F_s(H(t), P_s(t), p_c)$  that gives the proportion of larvae  $H(t)$  escaping parasitism from their  $P_s(t)$  hyperparasites according to the searching efficacy of *T. sinensis* in a forest with a proportion  $p_c$  of chestnut trees (see section ‘Modelling ‘top-down’ control’). These two sets of parazited and not-parazited individuals then initiate the built-up of the next year populations of *T. sinensis* and *D. kuriphilus*.



**Figure 7. Modelling of the *D. kuriphilus* life-cycle and its bottom-up and top-down control.** The lower part of the graph summarizes the different stage of *D. kuriphilus* life-cycle and the effects of the native hyperparasitic insects and fungi (top-down control, red) and of the tree species and genetic susceptibility (bottom-up control, green) on *D. kuriphilus* survival and reproduction. The upper part of the graph describe the life-cycle of the introduced control agent, *T. sinensis*. All parameters are defined in the main text and their estimates provided in SM5.

Larvae escaping the introduced hyperparasite *T. sinensis*, with probability  $F_s(H(t), P_s(t), p_c)$ , face additional challenges by native hyperparasite insects and fungi (see Figure 3: Analysis of the ‘top-down’ control of *D. kuriphilus*). Since such species typically live on their local host community, we did not explicitly modelled their population dynamics, and instead considered that they exert a constant pressure that *D. kuriphilus* larvae can escape with probabilities denoted  $F_{ni}$  and  $F_{nf}$ , respectively. Larvae not infected by any hyperparasites must develop into pupae, which they eventually do at rate  $SL_H$ , and pupae ultimately moult into adults with probability  $SA_H$ . The emerging adults of *D. kuriphilus* produce an average of  $F_H$  eggs per individual and a fraction  $d$  of those eggs are successfully deposited in chestnut tree buds. Based on the outcomes of our field study on the effect of the composition of the forest on the spread of *D. kuriphilus* (see Table 1 ‘GLMM analysis of the ‘bottom-up’ control of *D. kuriphilus*’), the fraction  $d(p_c)$  was set to increase with the frequency of chestnut trees in the forest stand (see section ‘Modelling ‘bottom-up’ control’). To develop into larvae and enter an overwintering dormant stage, the deposited eggs must then survive to a tree hypersensitive response triggered by oviposition and to intrinsic causes of mortality, which they do with probabilities denoted  $Se(g)$  and  $So_H$ , respectively. To account for the effect of individual tree genetic differentiation ( $g$ ) on the level of *D. kuriphilus* infestation (see Table 1 ‘GLMM analysis of the ‘bottom-up’ control of *D. kuriphilus*’), the survival probability  $Se(g)$  was defined with respect to the distribution of such genetic differentiation and its effect on the individual rate of infestation

(see section ‘Modelling ‘bottom-up’ control’). Finally, to complete the description of *D. kuriphilus* life-cycle, we modelled a density dependent survival of dormant larvae  $H'(t)$ ;  $D(H'(t))$ , as an increase in the number of larvae per bud is known to result in intraspecific competition for space during chambers formation and to the death of first instar larvae (see section ‘Modelling the density-dependent regulation of the *D. kuriphilus* larvae population’). The complementary part of *D. kuriphilus* larvae, i.e. the fraction  $1-F_s(H(t), P_s(t), p_c)$  not escaping parasitism by *T. sinensis*, is set to die as the hyperparasite larvae feed on them to become the next generation of adults with probability  $SL_{AP}$ . All these processes combined into the following set of non-linear difference equations describing the yearly dynamics illustrated in Figure 7 :

$$H(t + 1) = D(H'(t)) = D(H(t) \cdot F_s(H(t), P_s(t), p_c) \cdot R(p_c, g)) \quad \text{Equation 1A}$$

$$P_s(t + 1) = SL_{AP} \cdot H(t) \cdot (1 - F_s(H(t), P_s(t), p_c)) \quad \text{Equation 1B}$$

$$\text{with } R(p_c, g) = F_{ni} \cdot F_{nf} \cdot SL_H \cdot SA_H \cdot F_H \cdot d(p_c) \cdot Se(g) \cdot So_H$$

The definition of functions  $D$ ,  $d$ ,  $Se$  and  $F_s$  were completed by modelling further the biological mechanisms underlying the density-dependent survival of *D. kuriphilus* larvae and the bottom-up and top-down control of its population dynamic. This was done by accounting for the common descriptions of these mechanisms in the literature dedicated to *C. sativa* - *D. kuriphilus* and *D. kuriphilus* - *T. sinensis* interactions, and according to the ecological and genomic data collected in this study.

### ***Modelling the density-dependent regulation of the *D. kuriphilus* larvae population.***

A decrease of the within gall survival with larval population density has been observed for *D. kuriphilus* by Miyashita et al. (1965) and for other plants-gall-forming parasite interactions (Henriksen et al. 2017). Such a decrease comes from a basic form of competition for space where winning larvae get all the ‘resource’ required to make their chamber, while the rest got none and die. Such a typical “contest” competition (Varley et al., 1973, Jorgensen and Fath, 2008) can be described using a derivation of the Skellam model (Skellam, 1951) proposed by Brännström and Sumpter (2005):

$$D(H'(t)) = K(p_c) \left(1 - e^{-\frac{H'(t)}{K(p_c)}}\right) \quad \text{Equation 2}$$

where  $K(p_c)$  stands for the maximal amount of *D. kuriphilus* larvae that can be sustained, which was set to increase linearly with the proportion  $p_c$  of chestnut-trees until it reaches a maximal value estimated from field data (see SM 5) for a forest made of only chestnut trees.

### ***Modelling ‘bottom-up’ control.***

The emergence and transmission of parasites are typically up- or down-regulated by host intra- and inter-specific variations in parasite encounters and/or susceptibility to infection (Keesing et al., 2006). Such ‘bottom-up’ regulation factors are thought to naturally occur at two stages of *D. kuriphilus* life-cycle; the oviposition on chestnut bud and the development of eggs into first larvae stage (Figure 7), which was included in our modelling as described below.

#### *The effect of the forest species composition on D. kuriphilus’ oviposition rate.*

Our GLMM analyses have shown that the rates of *D. kuriphilus*’ prevalence and infestation are positively correlated with the frequency of chestnut tree in a forest patch. Such an effect had already been documented and associated with an increase of the oviposition rate of *D. kuriphilus* adult females (Fernandez-Conradi et al., 2018). The proportion of eggs deposited on buds was then modelled with respect to the chestnut tree frequency  $p_c$  and *D. kuriphilus*’ preference for its resource,  $\alpha_c$ , using to a typical ‘host-choice’ function:

$$d(p_c) = \frac{\alpha_c \cdot p_c}{\alpha_c \cdot p_c + (1-p_c)} \quad \text{Equation 3}$$

which, according to the low ability of *D. kuriphilus* females to navigate towards chestnut trees in the wild (i.e.  $\alpha_c=1$ , see SM5), ultimately led us to consider  $d$  as directly proportional to  $p_c$ .

#### *The effect of chestnut tree genetic differentiation on D. kuriphilus’s eggs development rate.*

The susceptibility of chestnut trees to *D. kuriphilus* infection has been shown to vary between individuals in wild population (Sartor et al. 2015), which we confirmed and further associated with individual genetic differentiation in the chestnut tree populations of the Pyrénées-Orientales (see Figure 2B,C). While such variations could, hypothetically, result from heterogeneities in oviposition or in *D. kuriphilus* development inside buds, it has been shown that females do not display oviposition preferences between resistant and susceptible varieties (Murakami et al., 2010). Instead, an hypersensitive reaction, a key resistance mechanism against galling insects (Fernandes and Negreiros, 2001), has been documented on resistant trees

with tissue modifications induced by egg secretions soon after oviposition (Stone et al. 2002; Nugnes et al. 2018) and the accumulation of H<sub>2</sub>O<sub>2</sub> in the inner part of infested buds (Dini et al 2012). Such early hypersensitive reactions in resistant trees are very consistent with the observed failure for eggs to develop into first instar larvae within chestnut tree buds of resistant varieties (Miyashita et al. 1965). Accordingly, the proportion of eggs developing into larvae was modelled as the weighted average of the individual tree susceptibility to *D. kuriphilus* infection defined with respect to its level of genetic differentiation,  $s(g)$ , where weights were set with respect to the distribution of such individual genetic differentiation,  $f(g)$ , which led to write:

$$Se(g) = \int_0^{\infty} f(g).s(g)dg \quad \text{Equation 4}$$

A model selection approach based on the infestation and genetic data collected in this study allowed for the identification of function  $f(g) = g^2e^{-bg}$  and  $s(g) = ae^{-cg}$ , and the partial integration of their product led to  $Se = ab^3(b+c)^{-3}$  (see SM6). Using this expression and the values of a, b and c inferred while fitting the shape of function  $s(g)$  and  $f(g)$  to our field data, we derived the local estimate of the average chestnut tree susceptibility to *D. kuriphilus* ( $Se$ ).

### ***Modelling ‘top-down’ control.***

Despite the role of galls protecting the parasitic insects that induce their formation, the latter typically suffer mortality inflicted by diverse enemies that can occur naturally or be introduced to contribute to their ‘top-down’ control (Forbes et al. 2016). Since native hyperparasitic insect species and fungi, as well as the introduced control agent, *T. sinensis*, have been shown to infect *D. kuriphilus* galls, they were all accounted for in our model, with the force of infection exerted by native enemies considered as constant and estimated from our field data (see SM5). On the contrary, the tight link between *D. kuriphilus* and its control agent, *T. sinensis*, required to dynamically model the force of infection exerted by *T. sinensis*. Such a force, given by  $1 - F_s(H(t), P_s(t), p_c)$ , was derived under the typical assumptions that encounters occurs randomly and that once a *D. kuriphilus* larvae has been parazited, additional encounters will not increase the number of deposited eggs. The proportion of *D. kuriphilus* larvae escaping parasitism, was then modelled using a Poisson distribution:

$$F_s(H(t), P_s(t), p_c) = e^{-a_s \frac{\beta_c p_c}{\beta_c p_c + (1-p_c)} P_s(t)} \quad \text{Equation 5}$$

where  $a_s$  and  $\beta_c$  represent the search area of *T. sinensis* and its preference for chestnut trees. The available data show low ability of *T. sinensis* adults to fly towards galls of *D. kuriphilus* (i.e.,  $\beta_c=1$ , see SM5), which led to consider such preference as directly proportional to  $p_c$ .

Lumping together equations 1 to 5, while accounting for the simplifications arising from the low ability of both *D. kuriphilus* and *T. sinensis* to navigate towards their hosts, we obtained the modelling framework that we used to integrate our data and assess the dynamics of *D. kuriphilus* invasion and its bottom-up and top-down control:

$$H(t + 1) = k \cdot p_c \cdot \left( 1 - e^{-\frac{H(t) \cdot e^{-a_s \cdot p_c \cdot P_S(t)} \cdot R(p_c, g)}{k \cdot p_c}} \right) \quad \text{Equation 6A}$$

$$P_S(t + 1) = SL_{AP} H(t) (1 - e^{-a_s \cdot p_c \cdot P_S(t)}) \quad \text{Equation 6B}$$

with  $R(p_c, g) = F_{ni} \cdot F_{nf} \cdot SL_H \cdot SA_H \cdot F_H \cdot p_c \cdot Se(g) \cdot S_{OH}$

### ***Model predictions about D. kuriphilus invasion dynamics and its top-down and bottom-up control.***

We first used our modelling to assess the invasion potential of *D. kuriphilus* in the Pyrénées-Orientales. We derived the expression of *D. kuriphilus* per capita annual multiplication rate,  $R_0^D$ , from equation 6A (See SM 3), and calculated its value according to the estimates of i) *D. kuriphilus* life-history parameters (summarized in the table appearing in SM 5), ii) the average frequency and genetic susceptibility of chestnut trees observed in the studied localities (i.e.  $p_c=0.60$  and  $Se=0.68$ ), and iii) the average forces of infection exerted by native hyperparasitic insects (i.e., 4.46%) and fungi (i.e., 5.44%). To further quantify the impact of the structure of the forest environment and of the native hyperparasite community on *D. kuriphilus* invasion potential, we performed a twofold sensitivity analysis of the estimate of  $R_0^D$  to changes in the values of  $p_c$ ,  $Se$ ,  $F_{ni}$  and  $F_{nf}$ . We evaluated the linear rate of variation of  $R_0^D$  in response to 1% changes in the estimates of each these parameters and the ranges of  $R_0^D$  variations that are expected from the actual changes observed between our 8 sampling stations in the estimates of each of these parameters.

We then investigated the dynamics of interaction between *D. kuriphilus* and its control agent, *T. sinensis*. We performed typical local stability analysis of the system of difference equations

6A-B to identify the conditions allowing for i) *D. kuriphilus* to be able to spread, for ii) *T. sinensis* to spread and persist in a stable equilibrium of coexistence with *D. kuriphilus*, and for iii) the spread of *T. sinensis* to lead to stable oscillations between *D. kuriphilus* and *T. sinensis*. This allowed us to assess the expected outcomes of the introduction of the control agent in different forest environments characterized by their frequency and genetic susceptibility of chestnut trees. While the conditions for *D. kuriphilus* and *T. sinensis* to spread could be established analytically, those for the introduction of *T. sinensis* to lead to either a stable coexistence or oscillations with *D. kuriphilus* were identified numerically by running simulations with R 4.1.1 (R Core Team, 2021). The model was then run for 100 generations after the introduction of 1000 *T. sinensis* adult individuals in a forest environment where *D. kuriphilus* has reached its carrying capacity.

Finally, we used such simulations to assess the potential efficacy of control interventions based on the release of *T. sinensis* according to the structure of the forest environment. Simulations were run with and without the control agent, and the effectiveness of the control intervention was measured as the percentage of reduction in the average *D. kuriphilus*. Such measurement was carried out for the range of  $p_c$  and  $Se$  values allowing for the spread of *T. sinensis* identified as explained above.

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## SUPPORTING INFORMATION

**Supplementary Material 1.** Summary table of ACGW infestation and prevalence rate, chestnut tree density, frequency and genetic susceptibility, native parasitoid impact by hymenopteran and fungi species in all sites and stations studied.

Stations	<i>D. kuriphilus</i>				Chestnut tree resource			Native parasitoid impact		
	Prevalence rate		Infestation rate		Density	Frequency (Pc)	Genetic susceptibility (Sc)	Hymenopteran (Fni)		Fungi (Fnf)
	2019	2020	2019	2020				2019	2019	2020
Prats de Mollo	0.867	0.173	0.022	0.002	1077	0.731	0.707	0.073	0.049	0.063
Site 1	0.78	0.04	0.014	0	1120	0.491	0.721	0.273	0.036	0.133
Site 2	0.94	0.26	0.029	0	870	0.908	0.639	0	0.039	0.051
Site 3	0.88	0.22	0.0236	0.005	1240	0.823	0.768	0	0.07	0.061
Saint Laurent	0.886	0.473	0.112	0.022	1277	0.671	0.671	0	0.086	0.074
Site 1	0.714	0.02	0.066	0	1030	0.418	0.535	0	0.106	0
Site 2	0.98	0.56	0.144	0.035	1230	0.854	0.731	0	0.047	0.093
Site 3	0.96	0.84	0.127	0.033	1570	0.694	0.774	0	0.103	0.05
Arles sur Tech	0.818	0.513	0.062	0.023	1173	0.58	0.526	0.051	0.081	0.031
Site 1	0.92	0.5	0.052	0.008	1070	0.869	0.427	0	0.073	0.021
Site 2	0.703	0.6	0.057	0.013	1330	0.286	0.588	0.111	0.108	0.025
Site 3	0.8	0.44	0.079	0.053	1120	0.652	0.581	0	0.064	0.046
La Bastide	0.63	0.033	0.025	0.003	1157	0.504	0.612	0.02	0.026	0.06
Site 1	0.9	0.02	0.006	0.001	1310	0.473	0.756	0	0.022	X
Site 2	1	0.08	0.063	0.008	850	0.424	0.667	0.046	0.018	0.06
Site 3	0.1	0	0.007	0	1310	0.588	0.455	0	0.037	X
Llauro	0.793	0.607	0.027	0.007	740	0.739	0.511	0.015	0.056	0.034
Site 1	0.96	0.74	0.046	0.005	1270	0.724	0.566	0	0.061	0.027
Site 2	0.64	0.44	0.033	0.005	760	0.763	0.46	0.04	0.066	0.025
Site 3	0.78	0.64	0.01	0.011	190	0.895	X	0	0.037	0.053
Céret	0.807	0.727	0.081	0.04	693	0.591	0.65	0.031	0.046	0.054
Site 1	0.9	1	0.132	0.055	740	0.581	0.686	0.1	0.047	0.088
Site 2	0.98	0.96	0.081	0.058	760	0.908	0.756	0	0.044	0.038
Site 3	0.54	0.22	0.018	0.003	580	0.19	0.53	0.035	0.047	0.033
Laroque	0.634	0.147	0.035	0.003	1073	0.562	0.575	0.18	0.054	0.037
Site 1	0.76	0.32	0.037	0.009	1170	0.735	0.581	0.133	0.044	0.041
Site 2	0.744	0.12	0.041	0.001	1150	0.583	0.702	0.167	0.05	0
Site 3	0.366	0	0.027	0	900	0.311	0.466	0.16	0.079	X
La Massane	0.492	0.11	0.027	0.001	433	0.3	0.611	0.044	0.144	0.03
Site 1	0.393	0.074	0.011	0	250	0.2	0.687	0.091	0.094	0
Site 2	0.5	0.16	0.044	0.002	670	0.418	0.644	0.046	0.12	0.083
Site 3	0.54	0.08	0.028	0	380	0.158	0.514	0	0.177	0.016

## Supplementary Material 2. List of identified loci containing significant SNPs in the GWAS analysis.

SNP p-value	Contig	Position	<i>A. thaliana</i> homolog / Name	Function	Year
1,29E-05	JRKL0200030.1	14766	AT5G10380	E3 ubiquitin-protein ligase RING1	2019/2020
8,69E-07	JRKL02000127.1	136170	At1g62640	3-oxoacyl-[acyl-carrier-protein] synthase 3 A, chloroplastic-like	2019/2020
4,45E-06	JRKL02000289.1	77000	AT3G20180	Heavy metal-associated isoprenylated plant protein 47	2019/2020
4,92E-06	JRKL02000289.1	77002			
9,66E-06	JRKL02000315.1	9112	AT5G07100	Probable WRKY transcription factor 27	2019
9,82E-07	JRKL02000819.1	146744	AT5G59190	Subtilisin-like protease SBT4.3	2019/2020
2,13E-05	JRKL02000846.1	52494	AT3G44160	Outer envelope protein 39, chloroplastic	2019/2020
6,30E-07	JRKL02001619.1	82663	AT2G02850	basic blue protein-like	2019
9,17E-07	JRKL02002197.1	57817	AT2G23770	lysM domain receptor-like kinase 4	2019/2020
7,97E-06	JRKL02002456.1	27533	AT5G36930	TMV resistance protein N	2019/2020
1,23E-05	JRKL02003778.1	12408	AT5G36930	TMV resistance protein N	2019/2020
2,22E-06	JRKL02004017.1	26247	At5g47430	E3 ubiquitin ligase PQT3-like	2019/2020
1,15E-06	JRKL02004061.1	46049	AT4G01395	Conserved oligomeric Golgi complex subunit 4	2019/2020
2,03E-06	JRKL02004333.1	33702	AT1G80150	Pentatricopeptide repeat-containing protein	2019/2020
2,39E-05	JRKL02006290.1	16586	AT1G22430	Alcohol dehydrogenase-like 1	2019/2020
2,93E-05	JRKL02012866.1	36204	AT1G49040	DENN domain and WD repeat-containing protein SCD1	2019
2,63E-05	JRKL02013037.1	13484	At1g34470	Probable magnesium transporter NIPA3	2019/2020
3,13E-05	JRKL02013037.1	13465			
1,80E-05	JRKL02000002.1	496489	AT3G57650	1-acyl-sn-glycerol-3-phosphate acyltransferase 2	2020
1,88E-06	JRKL02000008.1	352375			
9,90E-15	JRKL02000008.1	352680	AT5G63380	4-coumarate--CoA ligase-like 9	2020
2,01E-07	JRKL02000008.1	353597			
1,21E-07	JRKL02000012.1	441282	AT1G09070	protein SRC2-like	2020
1,40E-07	JRKL02000013.1	383153	EGS1	Eugenol synthase 1	2020
7,19E-06	JRKL02000017.1	90813	At3g55350	Protein ALP1-like	2020
2,26E-05	JRKL02000020.1	316194	At5g47430	E3 ubiquitin ligase PQT3-like	2020
1,22E-05	JRKL02000024.1	325924	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
1,31E-08	JRKL02000029.1	15661	At5g66560	BTB/POZ domain-containing protein	2020
3,11E-08	JRKL02000030.1	14766	AT5G10380	E3 ubiquitin-protein ligase RING1	2019/2020
4,54E-06	JRKL02000030.1	43128	At4g03230	G-type lectin S-receptor-like serine/threonine-protein kinase	2020
3,43E-12	JRKL02000031.1	153597	AT4G36480	Long chain base biosynthesis protein 1-like	2020
2,98E-05	JRKL02000054.1	214412			
1,81E-05	JRKL02000054.1	214427	9DC3	Receptor-like protein	2020
1,93E-05	JRKL02000054.1	214453			
8,21E-06	JRKL02000056.1	166556	At3g47570	Probable LRR receptor-like serine/threonine-protein kinase	2020
3,05E-06	JRKL02000062.1	34390	AT3G54010	Peptidyl-prolyl cis-trans isomerase PASTICCINO1	2020
2,01E-10	JRKL02000062.1	93594			
1,45E-08	JRKL02000062.1	93634	AT1G14550	Peroxidase 5-like	2020
9,86E-09	JRKL02000062.1	93635			
6,43E-11	JRKL02000071.1	78079	NYNRIN	protein NYNRIN-like	2020
1,54E-07	JRKL02000071.1	233481	At4g10955	GDSL esterase/lipase	2020
2,07E-07	JRKL02000071.1	233504			
1,20E-05	JRKL02000073.1	212331	AT3G01680	Protein SIEVE ELEMENT OCCLUSION B	2020
6,30E-06	JRKL02000093.1	300487	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020

1,03E-09	JRKL02000093.1	300490			
1,61E-08	JRKL02000105.1	144009	AT1G29330	ER lumen protein-retaining receptor A	2020
7,69E-06	JRKL02000114.1	261372	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
2,07E-09	JRKL02000127.1	136170	At1g62640	3-oxoacyl-[acyl-carrier-protein] synthase 3 A, chloroplastic-like	2019/2020
9,35E-06	JRKL02000130.1	283360	AT5G54140	IAA-amino acid hydrolase ILR1-like 3	2020
1,72E-05	JRKL02000135.1	45049	AT1G48120	Serine/threonine-protein phosphatase 7 long form homolog	2020
1,88E-08	JRKL02000135.1	224609	AT4G34215	Probable carbohydrate esterase	2020
7,46E-08	JRKL02000137.1	146840	AT1G20110	Protein FREE1	2020
2,37E-06	JRKL02000145.1	205727	ASR1	Abscisic stress-ripening protein 1	2020
2,10E-07	JRKL02000150.1	174821	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
4,50E-06	JRKL02000159.1	128330	AT2G46180	Golgin candidate 4	2020
3,53E-07	JRKL02000160.1	22000	AT5G24910	Cytochrome P450 714A1-like	2020
5,97E-06	JRKL02000161.1	79427	AT3G03720	Cationic amino acid transporter 4, vacuolar	2020
9,43E-07	JRKL02000174.1	65423			
8,31E-07	JRKL02000174.1	65443			
7,00E-07	JRKL02000174.1	65446			
9,08E-08	JRKL02000174.1	65695			
9,82E-08	JRKL02000174.1	65694			
3,23E-08	JRKL02000174.1	65689	NYNRIN	protein NYNRIN-like	2020
9,72E-08	JRKL02000174.1	65683			
3,11E-07	JRKL02000174.1	65682			
1,22E-09	JRKL02000189.1	220849	AT4G12720	Nudix hydrolase 7	2020
2,75E-05	JRKL02000206.1	181518	AT2G26440	Probable pectinesterase/pectinesterase inhibitor 12	2020
2,86E-05	JRKL02000237.1	24910	At2g14440	putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
6,47E-06	JRKL02000239.1	41222	AT5G59800	Methyl-CpG-binding domain-containing protein 7	2020
7,43E-07	JRKL02000251.1	111486	At5g47430	E3 ubiquitin ligase PQT3-like	2020
6,37E-08	JRKL02000267.1	230524	AT2G41670	Short integuments 2, mitochondrial	2020
1,89E-11	JRKL02000277.1	232266	AT2G40830	Probable E3 ubiquitin-protein ligase RHC1A	2020
5,37E-13	JRKL02000281.1	186178	At2g04865	Protein MAIN-LIKE 2	2020
4,82E-07	JRKL02000289.1	77000	AT3G20180	Heavy metal-associated isoprenylated plant protein 47	2019/2020
9,09E-07	JRKL02000289.1	77002			
1,73E-06	JRKL02000289.1	139472			
1,43E-06	JRKL02000289.1	139474			
1,86E-06	JRKL02000289.1	139500	AT4G33530	Probable potassium transporter 17	2020
2,69E-07	JRKL02000289.1	139523			
6,53E-06	JRKL02000294.1	144027	AT1G34540	Cytochrome P450 family protein	2020
7,90E-06	JRKL02000294.1	222113	AT5G63380	4-coumarate--CoA ligase-like 9	2020
1,04E-05	JRKL02000301.1	75079	AT3G21865	Peroxisome biogenesis protein 22	2020
8,02E-06	JRKL02000301.1	75109			
2,04E-06	JRKL02000338.1	216744	AT2G18660	EG45-like domain containing protein 2	2020
2,08E-05	JRKL02000346.1	107019	AT1G09070	Protein SRC2 homolog	2020
1,68E-05	JRKL02000346.1	137856	AT5G42820	Splicing factor U2af small subunit B	2020
1,08E-06	JRKL02000358.1	157107	At3g14840	Probable leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
3,82E-10	JRKL02000389.1	142755	AT4G05200	cysteine-rich RLK (RECEPTOR-like protein kinase) 25	2020
1,17E-09	JRKL02000392.1	197484	AT5G16760	Inositol-tetrakisphosphate 1-kinase 1	2020
6,68E-06	JRKL02000395.1	198684	AT2G30150	UDP-glycosyltransferase 87A1-like	2020
4,79E-10	JRKL02000395.1	198709			

1,42E-05	JRKL02000415.1	178047	AT5G10330	Histidinol-phosphate aminotransferase 1, chloroplastic	2020
6,73E-08	JRKL02000421.1	75796			
4,45E-08	JRKL02000421.1	75817	AT3G21865	Peroxisome biogenesis protein 22	2020
8,56E-08	JRKL02000421.1	75851			
3,05E-05	JRKL02000447.1	51612	AT5G36930	TMV resistance protein N	2020
8,59E-08	JRKL02000461.1	175535	AT5G02770	Protein MODIFIER OF SNC1 11	2020
1,99E-06	JRKL02000474.1	131997	AVR1	RxLR effector protein Avr1	2020
6,00E-06	JRKL02000494.1	130861	AT5G49740	Ferric reduction oxidase 7, chloroplastic	2020
3,03E-06	JRKL02000498.1	154182	NYNRIN	protein NYNRIN-like	2020
4,28E-07	JRKL02000498.1	154203			
2,15E-05	JRKL02000503.1	183738	AT5G62165	MADS-box protein AGL42	2020
5,82E-07	JRKL02000527.1	173791	AT2G32230	proteinaceous RNase P 1, chloroplastic/mitochondrial-like	2020
3,58E-07	JRKL02000536.1	33240	At3g26922	F-box/LRR-repeat protein	2020
9,15E-06	JRKL02000545.1	34990	AT2G30150	UDP-glycosyltransferase 87A1	2020
7,20E-06	JRKL02000549.1	99458	AT3G18990	B3 domain-containing transcription factor VRN1	2020
1,18E-05	JRKL02000555.1	55492	NA	(-)-isopiperitenol/(-)-carveol dehydrogenase, mitochondrial	2020
3,87E-07	JRKL02000562.1	69981			
5,29E-07	JRKL02000562.1	69972	AT4G02890	Polyubiquitin 14	2020
6,99E-07	JRKL02000562.1	69942			
1,90E-05	JRKL02000575.1	76984	AT3G56950	Probable aquaporin SIP2-1	2020
7,20E-06	JRKL02000575.1	76998			
1,24E-07	JRKL02000594.1	8470	AT4G33530	Probable potassium transporter 17	2020
7,22E-06	JRKL02000594.1	83638	AT4G02890	Polyubiquitin 14	2020
7,10E-18	JRKL02000607.1	64907	AT2G29110	Glutamate receptor 2.8	2020
1,57E-05	JRKL02000608.1	115412	AT2G34730	WPP domain-associated protein	2020
1,11E-16	JRKL02000616.1	160043			
6,40E-17	JRKL02000616.1	160040			
1,49E-16	JRKL02000616.1	160034	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
1,02E-16	JRKL02000616.1	160033			
1,79E-16	JRKL02000616.1	160019			
2,36E-06	JRKL02000645.1	15052	AT5G48570	Peptidyl-prolyl cis-trans isomerase FKBP65	2020
1,98E-05	JRKL02000653.1	39472	AT5G09900	26S proteasome non-ATPase regulatory subunit 12 homolog A	2020
3,10E-12	JRKL02000666.1	55028	At3g23950	putative F-box protein	2020
2,75E-07	JRKL02000688.1	80967	AT4G24180	THAUMATIN-LIKE PROTEIN 1	2020
3,88E-08	JRKL02000690.1	124633	AT1G66170	PHD finger protein MALE MEIOCYTE DEATH 1	2020
6,50E-06	JRKL02000726.1	136569	Avr1	RxLR effector protein	2020
2,87E-05	JRKL02000740.1	34320	AT1G01650	signal peptide peptidase-like 4	2020
2,09E-09	JRKL02000774.1	102342	AT4G34760	Auxin-responsive protein SAUR50	2020
5,92E-21	JRKL02000819.1	146744	AT5G59190	Subtilisin-like protease SBT4.3	2019/2020
7,99E-12	JRKL02000846.1	52494	AT3G44160	Outer envelope protein 39, chloroplastic	2019/2020
2,71E-05	JRKL02000846.1	83747	AT1G48120	Serine/threonine-protein phosphatase 7 long form homolog	2020
1,87E-06	JRKL02000887.1	126530	AT4G14920	Acyl-CoA N-acyltransferase with RING/FYVE/PHD-type zinc finger protein	2020
6,46E-06	JRKL02000889.1	78299	AT5G01720	F-box/LRR-repeat protein 3	2020
3,46E-07	JRKL02000908.1	39352	AT5G36930	TMV resistance protein N	2020

1,03E-05	JRKL02000908.1	123509	AT2G32810	Beta-galactosidase 9	2020
2,48E-09	JRKL02000949.1	52769	AT5G40990	GDSL esterase/lipase	2020
3,73E-07	JRKL02000949.1	52769			
2,60E-05	JRKL02000953.1	110592			
2,79E-05	JRKL02000953.1	110590	AT2G34930	receptor-like protein EIX1	2020
2,06E-05	JRKL02000953.1	110582			
1,36E-06	JRKL02001026.1	66166	At5g47430	E3 ubiquitin ligase PQT3-like	2020
1,91E-05	JRKL02001026.1	66113			
5,25E-07	JRKL02001033.1	34985			
8,38E-07	JRKL02001033.1	35005			
5,85E-06	JRKL02001033.1	35007	AT1G18570	Transcription factor MYB51	2020
2,82E-06	JRKL02001033.1	35024			
6,61E-06	JRKL02001033.1	35044			
1,84E-10	JRKL02001069.1	87308	AT1G12780	Bifunctional UDP-glucose 4-epimerase and UDP-xylose 4-epimerase 1	2020
2,14E-12	JRKL02001069.1	87278			
1,71E-05	JRKL02001078.1	109575	AT5G36930	TMV resistance protein N	2020
1,19E-06	JRKL02001112.1	102033			
2,53E-06	JRKL02001112.1	102044	AT3G21200	Glutamyl-tRNA reductase-binding protein	2020
1,99E-06	JRKL02001112.1	102050			
1,70E-08	JRKL02001194.1	54194	NA	Keratin, type I cytoskeletal 13-like	2020
3,16E-06	JRKL02001210.1	125928	AT5G46470	Disease resistance protein RPS6	2020
1,37E-05	JRKL02001210.1	125968			
7,55E-07	JRKL02001247.1	127357	AT4G01580	B3 domain-containing protein	2020
2,10E-07	JRKL02001265.1	124595	At5g47430	E3 ubiquitin ligase PQT3-like	2020
9,06E-06	JRKL02001270.1	101407			
2,80E-05	JRKL02001270.1	101436	AT3G54010	Peptidyl-prolyl cis-trans isomerase PASTICCINO1	2020
4,91E-06	JRKL02001270.1	101445			
7,54E-06	JRKL02001270.1	101455			
1,65E-06	JRKL02001337.1	11358	AT4G12010	Disease resistance-like protein DSC1	2020
3,98E-08	JRKL02001347.1	86865	AT3G22640	vicilin-like antimicrobial peptides 2-3	2020
9,34E-07	JRKL02001347.1	86879			
8,40E-12	JRKL02001352.1	48749	AT2G02040	Protein NRT1/ PTR FAMILY 8, 3	2020
1,70E-12	JRKL02001352.1	48748			
7,75E-06	JRKL02001367.1	113451	AT2G34930	Receptor-like protein	2020
1,39E-05	JRKL02001394.1	121502	At3g07870	F-box protein	2020
2,63E-05	JRKL02001411.1	3868	AT1G06490	Callose synthase 7	2020
1,74E-10	JRKL02001417.1	86661	AT2G30150	UDP-glycosyltransferase 87A1-like	2020
1,17E-06	JRKL02001462.1	30681	At5g47430	E3 ubiquitin ligase PQT3-like	2020
8,82E-06	JRKL02001462.1	30651			
8,75E-07	JRKL02001467.1	46150	AT3G26660	LOB domain-containing protein 24	2020
3,62E-08	JRKL02001467.1	46176			
2,31E-07	JRKL02001615.1	36591	AT5G36930	TMV resistance protein N	2020
3,30E-21	JRKL02001618.1	77199	AT4G33530	Probable potassium transporter 17	2020
2,18E-21	JRKL02001618.1	77181			
5,56E-08	JRKL02001624.1	1626	AT5G36930	TMV resistance protein N-like	2020
1,07E-05	JRKL02001665.1	45277	At5g25340	polyubiquitin-like isoform X1	2020
2,55E-05	JRKL02001699.1	68222	AT3G20015	Protein ASPARTIC PROTEASE IN GUARD CELL 2	2020

2,42E-05	JRKL02001706.1	86559				
1,61E-05	JRKL02001706.1	86558	AT5G57655	Xylose isomerase		2020
1,59E-05	JRKL02001706.1	86522				
3,88E-07	JRKL02001755.1	108033				
2,92E-09	JRKL02001755.1	108013	AT1G29900	Carbamoyl-phosphate synthase large chain		2020
2,35E-09	JRKL02001755.1	108005				
3,69E-09	JRKL02001755.1	107992				
8,03E-09	JRKL02001804.1	46998	At2g14440	leucine-rich repeat receptor-like serine/threonine-protein kinase		2020
2,67E-08	JRKL02001804.1	46993				
2,76E-06	JRKL02001806.1	89609	At3g47570	Probable LRR receptor-like serine/threonine-protein kinase		2020
1,86E-07	JRKL02001808.1	71628	AT1G77390	Cyclin-A1-2		2020
1,75E-07	JRKL02001808.1	71611				
1,27E-08	JRKL02001899.1	73007	AT1G06490	Callose synthase 7		2020
1,10E-07	JRKL02001899.1	73026				
2,23E-06	JRKL02001926.1	67148	AT2G43850	Integrin-linked protein kinase 1		2020
8,05E-06	JRKL02001930.1	21504	At3g47570	Probable LRR receptor-like serine/threonine-protein kinase		2020
2,34E-14	JRKL02002010.1	61610	EIX2	Receptor-like protein EIX2		2020
1,68E-10	JRKL02002010.1	61616				
1,27E-05	JRKL02002188.1	42369	AT2G02360	F-box protein PP2-B10-like		2020
3,66E-10	JRKL02002197.1	57676				
3,00E-09	JRKL02002197.1	57723	AT2G23770	lysM domain receptor-like kinase 4		2019/2020
1,22E-22	JRKL02002197.1	57817				
5,61E-08	JRKL02002303.1	83341	AT5G18820	Chaperonin 60 subunit alpha 2, chloroplastic		2020
1,28E-05	JRKL02002323.1	67944	At5g57670	receptor-like serine/threonine-protein kinase		2020
6,82E-08	JRKL02002323.1	71252	POP4	Ribonuclease P protein subunit p29		2020
1,75E-05	JRKL02002351.1	56706	At1g17630	putative pentatricopeptide repeat-containing protein		2020
3,50E-07	JRKL02002376.1	63153	AT1G68370	Chaperone protein DnaJ		2020
2,82E-13	JRKL02002395.1	37517	AT4G24180	THAUMATIN-LIKE PROTEIN 1		2020
1,66E-05	JRKL02002417.1	39873	WAG22	WAG22 antigen		2020
1,92E-11	JRKL02002454.1	62568	AT1G55150	DEAD-box ATP-dependent RNA helicase 20		2020
3,83E-08	JRKL02002456.1	27533	AT5G36930	TMV resistance protein N		2019/2020
6,53E-07	JRKL02002482.1	15232				
1,04E-08	JRKL02002482.1	15237	At1g48195	Zinc finger CCCH domain-containing protein 13		2020
7,80E-07	JRKL02002482.1	15238				
1,71E-12	JRKL02002492.1	63678	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase		2020
5,09E-13	JRKL02002492.1	63663				
4,67E-08	JRKL02002498.1	56252	TBCCD1	TBCC domain-containing protein 1		2020
5,74E-08	JRKL02002628.1	27253	At4g23050	serine/threonine-protein kinase		2020
1,41E-07	JRKL02002647.1	73373	NA	stromal 70 kDa heat shock-related protein, chloroplastic-like		2020
2,72E-05	JRKL02002705.1	33234	At1g07650	Probable LRR receptor-like serine/threonine-protein kinase		2020
7,82E-08	JRKL02002717.1	47594	AT1G22540	Protein NRT1/ PTR FAMILY 5.10		2020
1,99E-08	JRKL02002717.1	60856	AT2G18960	ATPase 1, plasma membrane-type		2020
2,64E-05	JRKL02002769.1	59563	At5g47430	E3 ubiquitin ligase PQT3-like		2020
6,89E-06	JRKL02002782.1	28443	ATMG00060	NADH-ubiquinone oxidoreductase chain 5		2020
1,67E-05	JRKL02002805.1	61219	At5g47430	E3 ubiquitin ligase PQT3-like		2020
6,21E-07	JRKL02002979.1	2464	AT4G14400	Protein ACCELERATED CELL DEATH 6		2020
2,66E-05	JRKL02003124.1	34474	AT3G20930	Organelle RRM domain-containing protein 1, chloroplastic		2020



1,15E-05	JRKL02003176.1	49537	AT1G04260	PRA1 family protein D	2020
5,72E-06	JRKL02003244.1	52268	At3g23880	F-box/kelch-repeat protein	2020
2,71E-08	JRKL02003257.1	36397	Ncapd3	Condensin-2 complex subunit D3	2020
7,55E-09	JRKL02003313.1	55345	AT2G17690	F-box protein	2020
2,37E-05	JRKL02003365.1	53908	AT2G34730	WPP domain-associated protein	2020
3,71E-11	JRKL02003416.1	50396			
4,94E-11	JRKL02003416.1	50380	At1g12700	Putative pentatricopeptide repeat-containing protein, mitochondrial	2020
2,23E-06	JRKL02003416.1	50374			
1,03E-05	JRKL02003458.1	54627	NA	nucleoplasmin-like protein NO29	2020
2,48E-05	JRKL02003526.1	41812	AT2G02010	Glutamate decarboxylase 4	2020
3,84E-06	JRKL02003610.1	18564	AT4G12010	Disease resistance-like protein DSC1	2020
4,00E-07	JRKL02003631.1	21794	AT5G60980	Nuclear transport factor 2	2020
3,27E-07	JRKL02003645.1	9415			
1,14E-06	JRKL02003645.1	9410			
1,97E-06	JRKL02003645.1	9405	At3g14840	leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
6,38E-06	JRKL02003645.1	9573			
4,46E-06	JRKL02003645.1	9564			
7,60E-06	JRKL02003649.1	42594	At3g47570	LRR receptor-like serine/threonine-protein kinase	2020
3,65E-07	JRKL02003649.1	42770			
3,07E-05	JRKL02003714.1	44349	AT3G14470	Putative disease resistance RPP13-like protein 1	2020
1,18E-06	JRKL02003714.1	44383			
1,03E-11	JRKL02003736.1	46161	AT1G67960	Protein POLLEN DEFECTIVE IN GUIDANCE 1	2020
2,34E-06	JRKL02003778.1	12408	AT5G36930	TMV resistance protein N	2019/2020
1,52E-05	JRKL02003814.1	11142	At1g27190	Probable inactive receptor kinase	2020
1,03E-08	JRKL02003814.1	11247			
8,82E-07	JRKL02003871.1	45490	AT2G34730	WPP domain-associated protein	2020
2,47E-11	JRKL02003884.1	42931			
1,94E-11	JRKL02003884.1	42937	AT3G03720	Cationic amino acid transporter 4, vacuolar	2020
2,46E-10	JRKL02003884.1	42966			
3,48E-07	JRKL02003947.1	18531	At3g47570	Probable LRR receptor-like serine/threonine-protein kinase	2020
6,26E-08	JRKL02004017.1	26247	At5g47430	E3 ubiquitin ligase PQT3-like	2019/2020
6,42E-21	JRKL02004035.1	5472	AT3G26590	Protein DETOXIFICATION 29	2020
9,72E-11	JRKL02004061.1	46049	AT4G01395	Conserved oligomeric Golgi complex subunit 4	2019/2020
1,50E-06	JRKL02004168.1	17625	AT3G14890	Polynucleotide 3'-phosphatase ZDP	2020
9,29E-06	JRKL02004168.1	41934	AT1G53320	Tubby-like F-box protein 7	2020
2,75E-06	JRKL02004168.1	47651			
2,94E-09	JRKL02004250.1	23249	AT5G66900	Probable disease resistance protein	2020
7,59E-06	JRKL02004250.1	23258			
3,83E-06	JRKL02004333.1	33702	AT1G80150	Pentatricopeptide repeat-containing protein	2019/2020
9,15E-06	JRKL02004347.1	9328	AT5G36930	TMV resistance protein N	2020
1,78E-08	JRKL02004352.1	14421			
1,32E-09	JRKL02004352.1	14411			
6,86E-10	JRKL02004352.1	14393			
7,09E-10	JRKL02004352.1	14385	AT3G05360	receptor like protein 30-like	2020
5,27E-10	JRKL02004352.1	14384			
1,31E-09	JRKL02004352.1	14382			

6,86E-08	JRKL02004352.1	14379			
2,60E-05	JRKL02004586.1	15484	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
7,83E-07	JRKL02004600.1	14208	AT4G19510	disease resistance protein RPP2B-like	2020
8,10E-06	JRKL02004706.1	44162	UGT85A23	7-deoxyloganetin glucosyltransferase	2020
3,99E-27	JRKL02004706.1	44163			
1,13E-05	JRKL02004808.1	1787			
2,24E-05	JRKL02004808.1	1945			
2,33E-05	JRKL02004808.1	1934			
1,50E-05	JRKL02004808.1	3033	AT3G05360	Receptor-like protein 30	2020
2,23E-05	JRKL02004808.1	3017			
1,89E-05	JRKL02004808.1	3016			
1,11E-07	JRKL02004814.1	20387			
1,05E-07	JRKL02004814.1	20388			
1,13E-07	JRKL02004814.1	20395	AT4G39850	peroxisomal ABC transporter 1	2020
1,01E-07	JRKL02004814.1	20418			
2,05E-06	JRKL02004814.1	20441			
2,25E-05	JRKL02004835.1	24125	AT1G48120	Serine/threonine-protein phosphatase 7 long form homolog	2020
1,86E-08	JRKL02004853.1	8937	AT5G54610	Ankyrin repeat-containing protein BDA1	2020
1,46E-06	JRKL02005034.1	14575	AT2G29940	pleiotropic drug resistance 3	2020
1,16E-12	JRKL02005058.1	15581	AT4G22120	Calcium permeable stress-gated cation channel 1	2020
4,71E-06	JRKL02005120.1	29249	AT4G27030	Fatty acid desaturase 4, chloroplastic	2020
4,56E-09	JRKL02005120.1	34273	At4g09920	F-box protein	2020
1,50E-05	JRKL02005495.1	20903	AT4G33530	Probable potassium transporter 17	2020
3,91E-07	JRKL02005711.1	33137	AT3G58040	E3 ubiquitin-protein ligase SINAT2	2020
1,86E-05	JRKL02005819.1	11968	At1g48195	zinc finger CCCH domain-containing protein 13-like	2020
7,41E-07	JRKL02005865.1	13832	AT5G40740	AUGMIN subunit 6	2020
2,09E-05	JRKL02006000.1	26103			
2,19E-07	JRKL02006000.1	26075	At2g14440	leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
8,57E-07	JRKL02006000.1	26074			
4,63E-06	JRKL02006000.1	26057			
3,88E-20	JRKL02006290.1	16586	AT1G22430	Alcohol dehydrogenase-like 1	2019/2020
2,47E-12	JRKL02006357.1	18853	AT5G36930	TMV resistance protein N	2020
1,63E-18	JRKL02006408.1	20175	AT5G36930	TMV resistance protein N	2020
1,40E-06	JRKL02006432.1	9463	At3g47570	Probable LRR receptor-like serine/threonine-protein kinase	2020
1,14E-05	JRKL02006967.1	6975	At4g27290	G-type lectin S-receptor-like serine/threonine-protein kinase	2020
1,46E-09	JRKL02007235.1	7690			
3,70E-08	JRKL02007235.1	7687	AT5G63380	4-coumarate--CoA ligase-like 9	2020
4,49E-08	JRKL02007235.1	7655			
1,35E-05	JRKL02007314.1	8572	AT4G14400	Protein ACCELERATED CELL DEATH 6	2020
6,50E-06	JRKL02007477.1	5368	At1g48195	Zinc finger CCCH domain-containing protein 13	2020
2,09E-06	JRKL02007732.1	15362	NYNRIN	protein NYNRIN-like	2020
3,44E-14	JRKL02007904.1	8938	At2g19130	G-type lectin S-receptor-like serine/threonine-protein kinase	2020
1,68E-11	JRKL02007904.1	8965			
2,13E-06	JRKL02008401.1	12886	RGA2	Disease resistance protein RGA2	2020
2,30E-06	JRKL02008401.1	12902			
8,12E-08	JRKL02009926.1	3050	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
2,89E-07	JRKL02009961.1	4059	AT2G48010	LRR receptor-like serine/threonine-protein kinase RKF3	2020
3,30E-07	JRKL02010680.1	6462	At1g12700	Putative pentatricopeptide repeat-containing protein, mitochondrial	2020
1,87E-06	JRKL02012065.1	1827	AT4G18240	Probable starch synthase 4, chloroplastic/amyloplastic	2020

3,48E-12	JRKL02012445.1	212114	At2g14440	Putative leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
1,58E-08	JRKL02012450.1	100926	At3g14840	Probable leucine-rich repeat receptor-like serine/threonine-protein kinase	2020
1,89E-08	JRKL02012450.1	100931			
1,12E-08	JRKL02012462.1	21181	AT3G02260	auxin transport protein BIG	2020
9,28E-06	JRKL02012462.1	184586	AT5G49820	Protein root UVB sensitive 6	2020
2,22E-05	JRKL02012477.1	97837	AT2G25010	Protein MAIN-LIKE 1	2020
2,63E-05	JRKL02012477.1	97825			
2,10E-05	JRKL02012530.1	107908	AT5G18240	myb-like protein K	2020
6,59E-09	JRKL02012553.1	93551			
2,85E-09	JRKL02012553.1	93515	AT3G17440	Putative plant snare 13	2020
4,20E-09	JRKL02012553.1	93508			
7,01E-08	JRKL02012554.1	15243			
1,42E-10	JRKL02012554.1	15240	AT5G24090	Acidic endochitinase	2020
5,50E-06	JRKL02012554.1	15232			
1,42E-05	JRKL02012719.1	13166	NA	2-oxoglutarate-Fe(II) type oxidoreductase hxnY	2020
5,48E-09	JRKL02012725.1	9893	AKAP7	A-kinase anchor protein 7-like	2020
1,65E-09	JRKL02012804.1	22344	AT3G08550	Glycosyltransferase-like KOBITO 1	2020
3,86E-06	JRKL02012827.1	27031	AT4G02890	Polyubiquitin 14	2020
3,35E-06	JRKL02012872.1	20226			
3,87E-06	JRKL02012872.1	20210			
3,50E-06	JRKL02012872.1	20200	At5g47430	E3 ubiquitin ligase PQT3-like	2020
3,41E-06	JRKL02012872.1	20183			
3,51E-06	JRKL02012872.1	20180			
1,34E-05	JRKL02013037.1	13463			
1,28E-05	JRKL02013037.1	13465	AS	Hydroquinone glucosyltransferase	2019/2020
9,93E-06	JRKL02013037.1	13484			
7,15E-06	JRKL02013077.1	21409			
1,04E-05	JRKL02013077.1	21392	AT5G04740	ACT domain-containing protein ACR12	2020
6,50E-06	JRKL02013077.1	21380			

**Supplementary Material 3. ACGW per capita annual multiplication rate ( $R_0^D$ ) derived from equation 6A**

Consider equation 6A expressing the abundance of ACGW in year (t+1) and taking into account the effect of density dependence on their growth rate as well as the impact of *T. sinensis*

$$H(t + 1) = k \cdot p_c \cdot \left( 1 - e^{-\frac{H(t) \cdot e^{-a_s \cdot p_c \cdot P_s(t)} \cdot R(p_c, g)}{k \cdot p_c}} \right) \quad \text{Equation 6A}$$

We assume that there is no *T. sinensis* individual at the beginning of the invasion period, so  $P_s(t) = 0$  and we have:

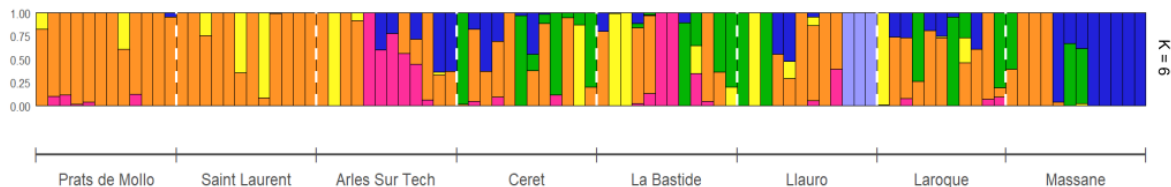
$$H(t + 1) = k \cdot p_c \cdot \left( 1 - e^{-\frac{H(t) \cdot R(p_c, g)}{k \cdot p_c}} \right)$$

In addition, the abundance of ACGW ( $H(t)$ ) is low at the start of the invasion, so  $\frac{H(t) \cdot R(p_c, g)}{k \cdot p_c}$  tends to 0 and knowing that  $\lim_{x \rightarrow 0} 1 - e^{-x} \approx x$ , we therefore have :

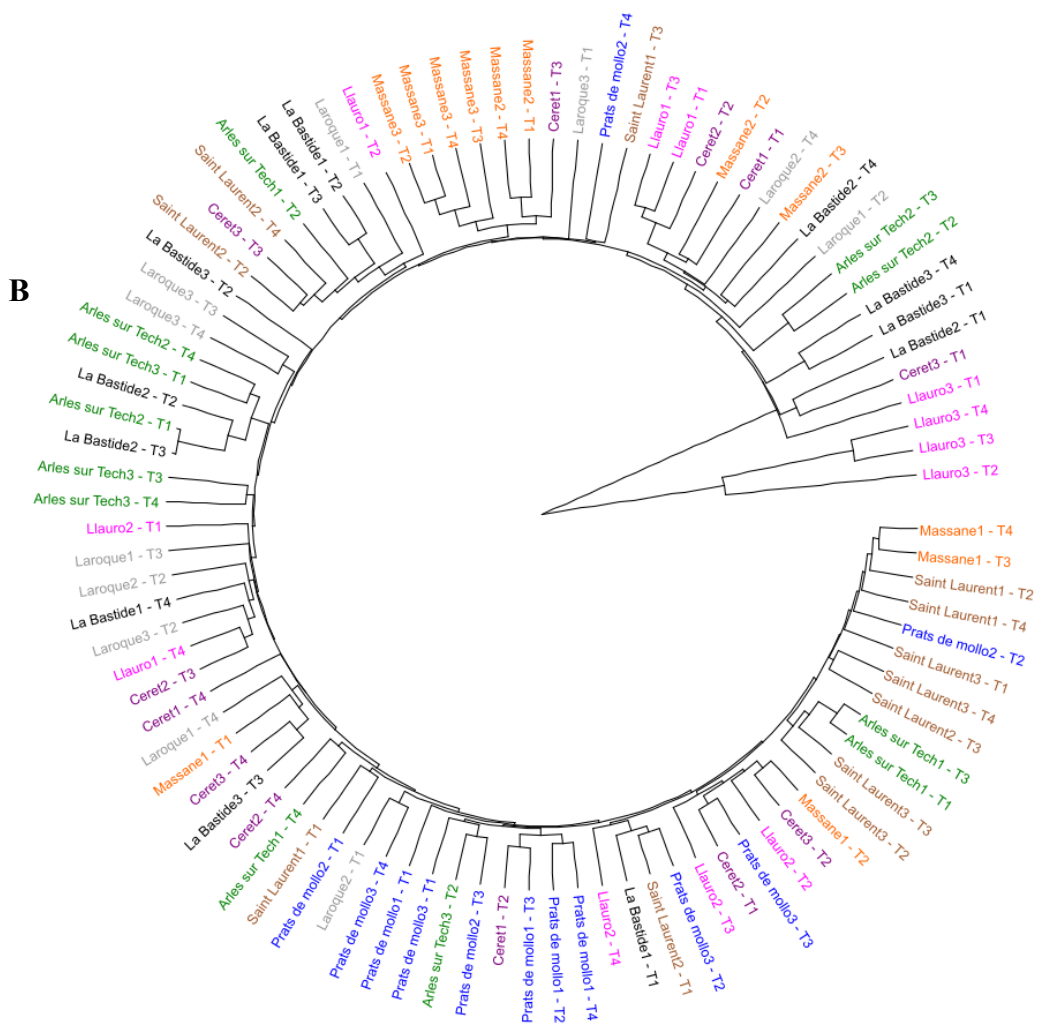
$$H(t + 1) = k \cdot p_c \cdot \frac{H(t) \cdot R(p_c, g)}{k \cdot p_c} = H(t) \cdot R(p_c, g)$$

The ACGW per capita annual multiplication rate is therefore equal to  $R(p_c, g)$  at the beginning of the invasion, when the ACGW population abundance is still low and therefore does not saturate the biotic capacity ( $k \cdot p_c$ ) of the environment (no intraspecific competition for habitat) and *T. sinensis* is not yet introduced as control agent.

**Supplementary Material 4.** Genetic structure of the chestnut tree populations in the Pyrénées-Orientales.



**A**



**B**

**Supplementary Material 5.** Definition and estimates of the parameters of the *D. kuriphilus* host - *T. sinensis* and native parasitoids dynamical model.

	Symbol	Estimate and 95% confidence interval	References and notes
<b><i>Dryocosmus kuriphilus</i></b>			
Egg survival	So	0.535 ± 0.003	Nugnes et al. (2018) <sup>(1)</sup>
Larvae survival	SL <sub>H</sub>	0.936 ± 0.010	Panzavolta et al. (2013) <sup>(2)</sup>
Adult survival	SA <sub>H</sub>	0.952 ± 0.010	Cooper and Rieske (2010) <sup>(2)</sup>
Fertility	F <sub>H</sub>	90 ± 6	This study <sup>(3)</sup>
Preference for chestnut trees	α <sub>c</sub>	1	Germinara et al. (2011) <sup>(4)</sup>
Carrying capacity per hectare	k	14 880 000	This study <sup>(5)</sup>
<b><i>Torymus sinensis</i></b>			
Larvae and adult survival	SLA <sub>P</sub>	0.913 ± 0.004	Ferracini et al. (2015)
Preference for chestnut trees	β <sub>c</sub>	1	Graziosi and Rieske (2013) <sup>(6)</sup>
Searching area	a <sub>s</sub>	4.4 x 10 <sup>-4</sup> ± 0.4 x 10 <sup>-4</sup>	This study <sup>(7)</sup>
<b><i>Native hymenoptera and fungi</i></b>			
Survival to hymenopteran infection	F <sub>ni</sub>	0.956 ± 0.02	This study <sup>(8)</sup>
Survival to fungal infection	F <sub>nf</sub>	0.946 ± 0.001	This study <sup>(8)</sup>

(1) The intrinsic rate of *D. kuriphilus* eggs' survival, SO<sub>H</sub>, accounting for all mortality causes independent of the host hypersensitive response, was calculated by averaging two separate estimates derived by Nugnes *et al.* (2018) on resistant and susceptible chestnut trees, as no statistical difference were found between them. (2) The overall rate of *D. kuriphilus* survival from larvae to adult, i.e. the product of the larval (SL<sub>H</sub>) and adult (SA<sub>H</sub>) survival, is equal to 0.871, and very similar to previous estimates of 0.857 (Cooper and Rieske, 2007) and 0.863 (Nakamura *et al.*, 1977). (3) The *D. kuriphilus* individual fertility was estimated as F<sub>H</sub>=Ne\*S/(1-S) where Ne stands for the daily number of eggs that could potentially be laid, which we estimated to be equal to 30 eggs by dividing the number of eggs that are carried by an emerging individual at the onset of its adult lifetime (Graziosi and Rieske, 2014) by the 4 days duration of this adult stage (Bosio *et al.*, 2010), and where S denotes the corresponding daily survival estimated to be equal to 0.75 from a geometric model. (4) The preference of *D. kuriphilus* for chestnut trees, α<sub>c</sub>, was set at 1, according to a Y-tube olfactometer bioassay performed by Germinara *et al.* (2011), where *Castanea sativa* twigs were found not attractive while displayed in a 30cm range from the insect. (5) The *D.kuriphilus* larvae carrying capacity, k, was calculated as the total number of lodges that can be found in chestnut galls per hectare by multiplying the maximum density of chestnut buds per hectare, estimated to be 2\*10<sup>6</sup> buds.h<sup>-1</sup> (Bounous, 2014), by the maximum number of galls per bud, observed to reach 1.24 gall.bud<sup>-1</sup> (Sartor *et al.*, 2015) and by the maximum number of lodges that we recorded by gall, i.e. 6. (6) The ability of *T. sinensis* to locate *D. kuriphilus* galls on chestnut trees in a mixed forest environment is expected to be limited as Graziosi and Rieske (2013) showed that both olfactory and visual clues are necessary for this hyperparasite to detect its resource in a typically short-range corresponding to the performed Y-tube olfactometer experiment. (7) The *T. sinensis* searching area, a<sub>s</sub>, was estimated by deriving its expression from equation 6B describing *T. sinensis* dynamics while considering a typical geometric growth soon after its release, i.e. P<sub>s</sub>(t + 1) = R<sub>0</sub><sup>T</sup>P<sub>s</sub>(t) where R<sub>0</sub><sup>T</sup> denotes *T. sinensis* intrinsic annual growth rate, so that straightforward algebraic manipulation lead to  $a_s = \frac{1}{p_c P_s(t)} \log \left( 1 - \frac{R_0^T P_s(t)}{H(t) SLA_P} \right) P_s(t)$ . The estimate of a<sub>s</sub> was then calculated using a value of R<sub>0</sub><sup>S</sup> that we derived from Muru *et al.* (2021) by fitting the annual geometric growth of *T. sinensis* parasitism of cynips galls observed over 4 to 6 years in 9 separate orchards, and by retaining the

geometric mean of the 32 resulting values as well as their standard deviation. (8) The force of infections by native species of hyperparasitic insects and fungi were estimated to reach 4.46% and 5.44%, respectively, after dissection of 2110 galls of *D. kuriphilus* and found very consistent across the different localities of our field study, which allowed to calculate the fraction of larvae escaping each of those two taxa of native hyperparasites, i.e.  $F_{ni}$  and  $F_{nf}$ , as their complementary percentages.

**Supplementary Material 6.** Model selection approach for the identification of functions  $f(g)$  and  $s(g)$ .

*Distribution of chestnut tree individual genetic divergence:  $f(g)$ .*

We considered three hypothetical distribution models  $f_m(g)$  to be fitted to the observed distribution of individual genetic divergence. These models assume the density of probability of chestnut trees to carry a genetic divergence  $g$  i) to monotonically decrease either exponentially ( $m=1$ );  $f_1(g) = e^{-\alpha g}$ , or in a bell-shaped way ( $m=2$ );  $f_2(g) = e^{-\alpha g^2}$ , or ii) to increase before to decrease according to ( $m=3$ );  $f_3(g) = g^n e^{-\alpha g}$ , which lead to a standard Gamma model when  $n = 1$ . The second model was chosen to allow for a larger number of individuals with low divergences than the first, while the latter model was chosen to allow for a lower number of individuals with low divergence than the former. Under each model ( $m=1,2,3$ ), the expected probability for a given tree individual to carry a genetic divergence  $g$  then follows a Poisson distribution with mean parameter:  $\lambda = f_m(g)$ , which we used in the model selection process described below.

*Relationship between infestation (galls count) and individual genetic divergence:  $s(g)$ .*

We considered a probabilistic model of infestation assuming that each individual tree receives a random number of attempts to lay a set of eggs (forming a gall) by *D. kuriphilus*, and that the rate of success of each of those attempts depends on the tree genetic divergence  $g$ . We used the same three hypothetical modelling distributions as for the identification of  $f(g)$ . A bell-shaped model;  $s_2(g) = e^{-\alpha g^2}$ , allowed for a larger class of the chestnut tree population (with low levels of genetic divergence) to be highly susceptible to infection than under the exponential model given by  $s_1(g) = e^{-\alpha g}$ . The non-monotonous model;  $s_3(g) = g^n \cdot e^{-\alpha g}$ , allowed for individuals with intermediate levels of genetic divergence to be the most susceptible to infection. Under each model ( $m=1,2,3$ ), the expected number of galls observed on a given tree individual then follows a Poisson distribution with mean parameter:  $\lambda = \lambda \sigma_{s_m}(g)$  where  $\lambda$

stands for the per tree mean number of attempts made by *D. kuriphilus*,  $\sigma$  is the parameter adjusting the per tree mean number of ACGWs attempts, which we used in the model selection approach described below.

*Model selection.*

To choose the model fitting best with the distribution of chestnut tree individual genetic divergence, each model was then integrated from 0 to infinity since private allele rates cannot be negative. This allowed us to obtain the following equations:

$$(m=1) : f_1(g) = \int_0^{\infty} e^{-\alpha g} = \alpha e^{-\alpha g}$$

$$(m=2) : f_2(g) = \int_0^{\infty} e^{-\alpha g^2} = 2 \sqrt{\frac{\alpha}{\pi}} e^{-\alpha g^2}$$

$$(m=3 ; n=1) : f_3(g) = \int_0^{\infty} g e^{-\alpha g} = \alpha^2 g e^{-\alpha g}$$

$$(m=3 ; n=2) : f_3(g) = \int_0^{\infty} g^2 e^{-\alpha g} = \frac{\alpha^3}{2} g^2 e^{-\alpha g}$$

$$(m=3 ; n=3) : f_3(g) = \int_0^{\infty} g^3 e^{-\alpha g} = \frac{\alpha^4}{6} g^3 e^{-\alpha g}$$

$$(m=3 ; n=4) : f_3(g) = \int_0^{\infty} g^4 e^{-\alpha g} = \frac{\alpha^5}{24} g^4 e^{-\alpha g}$$

Each of our models (m=1,2,3) were fitted to the observed distribution of genetic divergences (Figure S6A) and to the observed relationship between infestation and genetic divergences (Figure S6B) by maximizing the log-likelihood (LLH<sub>m</sub>) with respect to the set of model parameters ( $\theta_m$ ). The maximum likelihood estimators of the f(g) function were identified analytically for m=1 and m=2, which allowed for direct estimation of the corresponding model parameters using the following expressions:



For (m=1) :  $f_1(g_i) = \int_0^\infty e^{-\alpha g_i} = \alpha e^{-\alpha g_i}$

$$L(\alpha) = \sum_{i=1}^n \log \alpha e^{-\alpha g_i} = n \log \alpha - \alpha \sum_{i=1}^n g_i$$

$$\frac{\partial L(\alpha)}{\partial \alpha} = -n \frac{\partial \ln \alpha}{\partial \alpha} - \sum_{i=1}^n g_i \frac{\partial \alpha^{-1}}{\partial \alpha} = -\frac{1}{\alpha^2} (n\alpha - \sum_{i=1}^n g_i)$$

Knowing that  $\frac{\partial L(\alpha)}{\partial \alpha} = 0 \longrightarrow \alpha = \frac{1}{\bar{g}}$

For (m=2) :  $f_2(g_i) = \int_0^\infty e^{-\alpha g_i^2} = 2 \sqrt{\frac{\alpha}{\pi}} e^{-\alpha g_i^2}$

$$L(\alpha) = \sum_{i=1}^n \log 2 \sqrt{\frac{\alpha}{\pi}} e^{-\alpha g_i^2} = n \log 2 \sqrt{\frac{\alpha}{\pi}} - \alpha \sum_{i=1}^n g_i^2$$

$$\frac{\partial L(\alpha)}{\partial \alpha} = n \frac{\partial \ln 2 \sqrt{\frac{\alpha}{\pi}}}{\partial \alpha} - \sum_{i=1}^n g_i^2 = \frac{n}{2\alpha} - \sum_{i=1}^n g_i^2$$

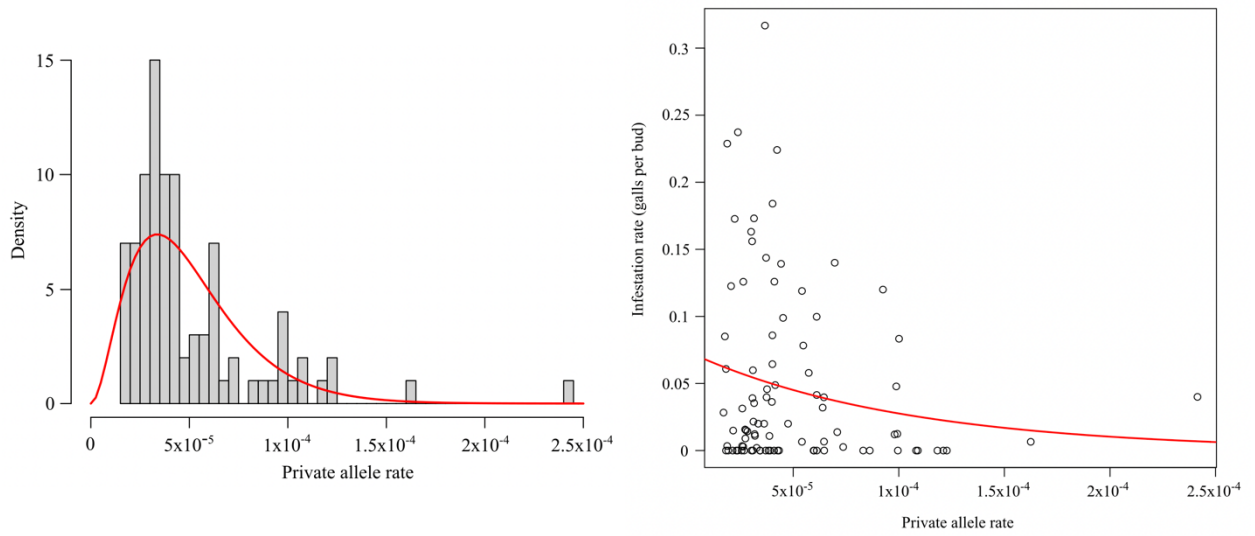
Knowing that  $\frac{\partial L(\alpha)}{\partial \alpha} = 0 \longrightarrow \alpha = \frac{1}{2\bar{g}^2}$

The maximum likelihood estimates of the parameters of model 3 for the  $f(g)$  function and of all models for the  $s(g)$  function were identified numerically using optimization function implemented in R 4.1.1 (R Core Team, 2021). For both the observed distribution of genetic divergences and the observed relationship between infestation and genetic divergences, the best (fitted) model was then selected using the standard Akaike Information Criterion, i.e.  $AIC_m = 2p - 2LLH_m$ , and we further calculated the associated weights of Akaike ( $w_m$ ) for each model.

The outcomes of the model fitting and selection are summarized in S6 - table 1 and the best fitted models are represented in S6 - figure 1.

Model	Maximum log-likelihood (LLH <sub>m</sub> )	Model Parameters estimates	Akaike Information Criterion (AIC <sub>m</sub> )	Weights of Akaike (w <sub>m</sub> )
<i>Distribution of chestnut tree individual genetic divergence: f(g).</i>				
m=1	818.1238	$\alpha = 19784.41$	-1634.248	2.103e-10
m=2	820.7526	$\alpha = 195711415$	-1639.505	2.9136e-09
m=3 (n=1)	837.5209	$\alpha = 39568.82$	-1671.042	0.0205
m=3 (n=2)	841.2873	$\alpha = 59353.2$	-1678.575	0.8878
m=3 (n=3)	839.0124	$\alpha = 79137.6$	-1674.025	0.0913
m=3 (n=4)	833.5165	$\alpha = 98922.2$	-1663.033	0.0004
<i>Relationship between infestation (galls count) and individual genetic divergence: s(g).</i>				
m=1	-1175.094	$\alpha = 9815.96$ $\sigma = 1.0769$	2354.188	>0.9999
m=2	-1192.467	$\alpha = 0.7982$ $\sigma = 48317500$	2388.934	2.851e-08
m=3 (n=1)	-1191.903	$\alpha = 63930$ $\sigma = 28810$	2389.806	1.8435e-08

**Table 1. Summary table of each model parameters estimates, their maximum log-likelihood, Akaike information criterion and weights of Akaike for the f(g) and s(g) function.**



**Figure 1.** Best fitted models to the observed distribution of chestnut tree individual genetic divergence,  $f(g) = f_3(g) = g^2 e^{-bg}$ , and to the observed relationship between infestation (galls count) and individual genetic divergence;  $s(g) = s_1(g) = a e^{-cg}$ .

## Chapter II

### **How do heterogeneity and dispersal influence the spread and biological control of the global chestnut tree pest, *Dryocosmus kuriphilus*, in the Pyrénées-Orientales ?**

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## Introduction

A biological invasion is the passage of organisms through biogeographical barriers that allows them to reach an area outside of its natural range, followed by their survival and reproduction to build-up a local invasive population (Simberloff, 2010). Such a spread of invasive species has been steadily increasing in frequency since the beginning of the 19<sup>th</sup> century, largely caused by the development of international trades (Seebens et al., 2017, 2021). While colonizing new territories, exotic species rely on typically already exploited local resources to grow their populations. Crops and exploited forests constitute favorable resources as they generally correspond to monospecific groups with low genetic diversity, predator and competing herbivores abundance (Beaumelle et al., 2021; Gepts, 2006). Damages caused by exotic pests on such valued natural resources (Pagad et al., 2018) combined with the cost of their management induce strong economic loss, with an average annual cost of US\$26.8 billion over the past few decades (Diagne et al., 2021). Beyond such economic impacts, exogenous species are one of the most important threats to ecosystem equilibrium and biodiversity (Pejchar and Mooney, 2009; Bellard et al., 2016). Invasive pests can disturb ecological networks of the local environment because of interspecific competition for resource and habitats or predation/parasitism pressures (David et al., 2017), facilitate pathogen emergence and spread (Stricker et al., 2016) and hybridize with closely related species thereby affecting the genetic structure and conservation status of native populations (Ayres et al., 2004).

By integrating local ecological networks, exotic species are subject to two main categories of regulatory forces that determine the growth of their population; the so-called ‘bottom-up’ and the ‘top-down’ regulation. The former depend on the resources used by the invasive, typically food or habitat, and is the dominant force determining the pest’s establishment and persistence (David et al., 2017). Distribution, genetic and physiological characteristics of the resources can then fine regulate the invasive population (Lotze et al., 2001; Parsche and Lattorff, 2018; Ximénez-Embún et al., 2016). The top-down forces depend on endemic or introduced natural enemies (predators, parasite and pathogens) that can down-regulate an invasive species and eventually lead its population to extinction (David et al., 2017). The introduction of natural enemies is central to biological control strategies that are increasingly intended as ‘eco-smart’ tool for pest management in sustainable agricultural practice (Jaiswal et al., 2022). Resolving the mechanisms underlying the interactions between invasive species and the bottom-up and

top-down forces, including introduced control agent, is thus essential to better understand the resilience of crops and natural ecosystems to the insertion of exotic species in their ecological networks.

Population dynamic models have been widely used to assess biological invasions (Bendor and Metcalf, 2006; Kueffer et al., 2013; Baker and Bode, 2021) as they allow to formulate predictions about the invasive species growth rate, abundance and the dynamic of interaction with control agents (Rauschert and Shea, 2017; Hawkins and Cornell, 2008; Trethowan et al., 2011; Augustinus et al., 2020). Integrating a description of the various bottom-up and top-down regulation factors into such modelling provide an efficient way to decipher the key determinants of the temporal and spatial patterns observed in the field (Goslee et al., 2006; Hunter et al., 1997; Vidal and Murphy, 2018; Zitoun et al., 2023) and to anticipate the potential efficiency of different control strategies (Büyüktaşkın et al., 2011, Baxter et al., 2008). Such models are typically implemented for a representative site, which does not allow to take explicitly into account spatial movement of the invasive species and its control agent (e.g. Huffaker et al., 1976), while there has been an increasing emphasis on landscape perspective to understand the dynamics of parasitoids and their pest hosts (Evans, 2018 and references therein). The design of well empirically informed two-sites spatial models appear as a key stage to understand the potentially complex dynamics induced by the dispersal of both the invasive species and its control agent in spatially heterogeneous environments (Engen et al. 2002).

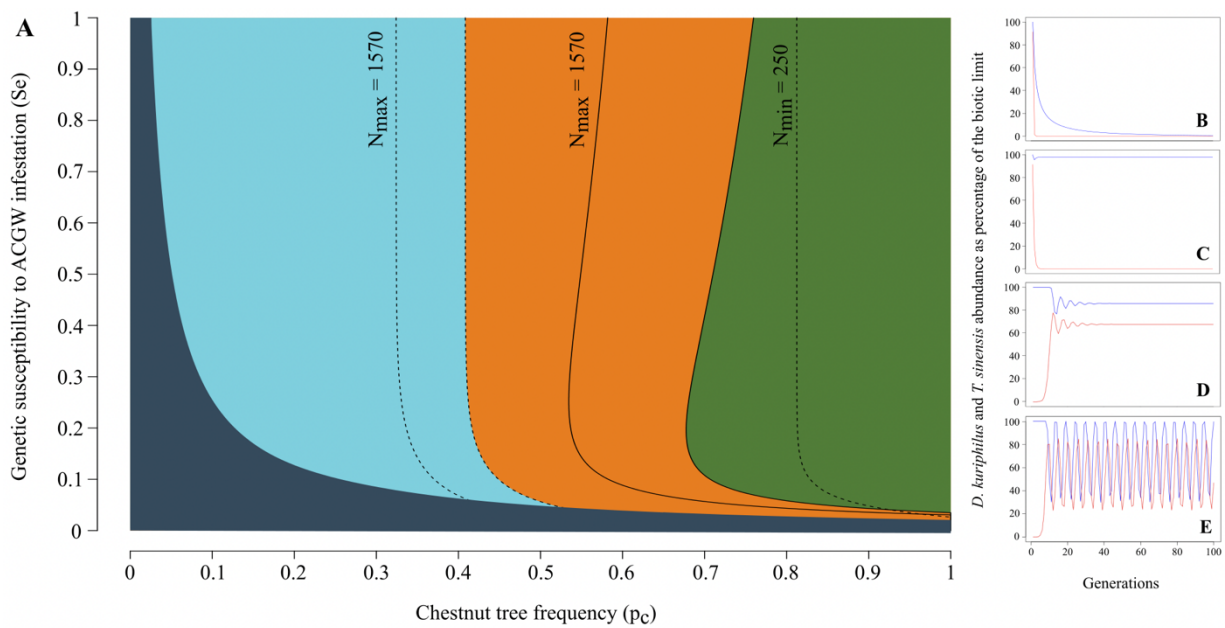
In this contribution, we investigate the worldwide invasive pest, *D. kuriphilus* (Yasumatsu, 1951), an hymenoptera species that has spread from China to infest both cultivated and wild chestnut tree populations (*Castanea sativa* Mill., 1768) in Asia (Murakami, 1981; Tamura, 1962; Ueno, 2006), the US (Payne et al., 1976) and Europe (Brussino et al. 2002; EPPO global database) within the last 50 years. We studied its invasive population in the Pyrénées-Orientales, a French area located in the south of France, where the pest has been first reported in 2013, leading to the release of its biological control agent, the parasitoid *Torymus sinensis* (Kamijo, 1982), in local chestnut tree forests in 2014 (Mansot and Castex, 2018). While the introduction of this parasitoid is typically efficient in reducing the mean *D. kuriphilus* abundance (Avtzis et al., 2019), this strategy never allowed to eliminate the invasive not to prevent its spread across natural chestnut stands in Europe (Aebi et al. 2006; EPPO global database; Martinez-Sanudo et al., 2018), Asia (Cho & Lee, 1963; Murakami et al., 1981) and

America (Payne et al. 1976; Huber and Read, 2012). The contributions of the bottom-up and top-down forces on the spread of *D. kuriphilus* across the Pyrénées-Orientales were previously investigated by integrating their field estimates into a one site dynamical model of the *C. sativa* - *D. kuriphilus* – *T. sinensis* interactions (Zitoun et al. 2023). This model allowed to assess the impact of changes in the frequency and genetic susceptibility of chestnut trees and in the abundance of endemic (insect and fungal) parasitoids, on the invasive potential of *D. kuriphilus* and on the outcomes of its biological control by *T. sinensis*. The outcomes of this empirically informed modelling confirmed the high reproductive number ( $R_0$ ) of this invasive pest, and the tendency of its interaction with *T. sinensis* to lead to the coexistence of both species rather than to their co-extinction (Zitoun et al. 2023). Here, we aim to take our understanding of the *C. sativa* - *D. kuriphilus* - *T. sinensis* interaction a step further by considering its dynamics in an heterogeneous (semi-)natural forest environment where sites characterized by different levels of bottom-up and top-down forces are connected by dispersal of the pest and its control agent. We expanded the previous modelling into a two-site model and investigate its dynamics under various levels of environmental heterogeneity characterized from a field study carried out in 23 sites located in the Pyrénées-Orientales, where we concomitantly characterized the level of chestnut tree infestation by *D. kuriphilus* and of *T. sinensis* hyperparasitism. In this way, we intend to provide new quantitative insights into the importance of *D. kuriphilus* and *T. sinensis* dispersal on the outcomes of their local and global interaction.

## Results

### *Bottom-up regulation factors as determinants of parasite-parasitoid population dynamics*

The one-site dynamical model of the *C. sativa* - *D. kuriphilus* - *T. sinensis* interaction predicted 4 possible outcomes for the populations of both the pest and its biological control agent (Zitoun et al. 2023), which are summarized and completed for the effect of tree density in Figure 1. At low frequency and/or genetic susceptibility of chestnut trees, *D. kuriphilus* is not able to invade (dark blue, B). When the availability of resource increases, the pest spreads to its carrying capacity while the control agent is still unable to establish itself as long as the frequency of chestnut trees remains lower than about 40% (light blue, C). Above such threshold, *D. kuriphilus* and *T. sinensis* coexist with stable (orange, D) or oscillatory dynamic (green, E).



**Figure 1. Local dynamics of the *C. sativa* - *D. kuriphilus* - *T. sinensis* interaction according to the characteristics of the forest environment.** (A) Influence of chestnut tree frequency, their genetic susceptibility and the tree abundance on the population dynamic of *D. kuriphilus* and its control agent, *T. sinensis*. Predicted dynamics : no invasion of *D. kuriphilus* (dark blue, B), no installation of *T. sinensis* (light blue, C) and coexistence of the two species in stable (orange, D) or oscillatory dynamic (green, E). Dotted lines split the parameter space into two areas where *T. sinensis* is not able to persist (left) and where it spreads and limit *D. kuriphilus* invasion, while continuous lines indicate when *D. kuriphilus* - *T. sinensis* coexist in a stable way (left) and through ample oscillations. The positions of those separatrices strongly depend on the density of trees, which was set to its average value of 989 trees.h<sup>-1</sup> and varied to take on the minimal ( $N_{min}=250$ ) and maximal ( $N_{max}=1570$ ) values observed across our sampling sites.

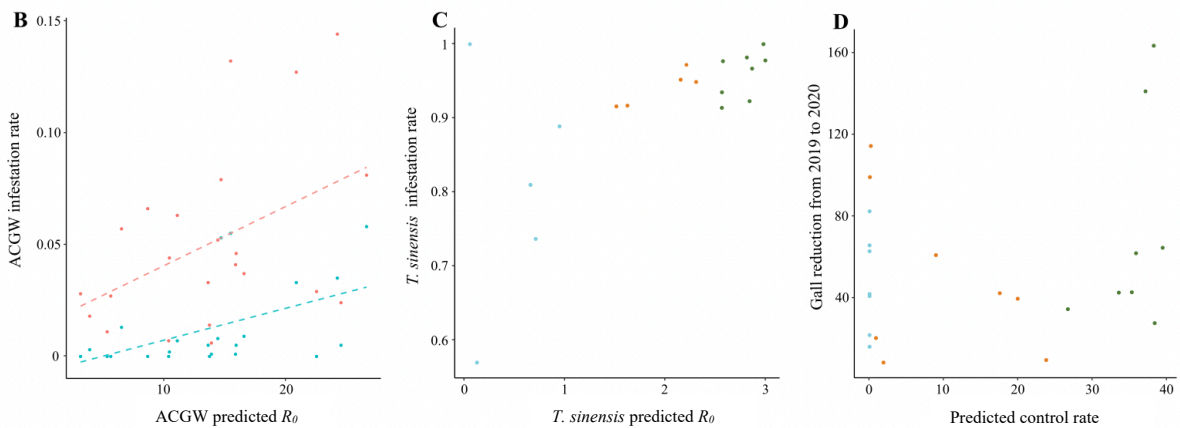


The tree density in the forest environment had a significant impact on the outcome of the interaction between *D. kuriphilus* and *T. sinensis*, although it did not lead to any alternative population dynamic regime (Figure 1). Increasing the density from its average to its maximal observed value in our sampling sites lowered the threshold frequency of chestnut trees allowing for *T. sinensis* to persist by ~10% (dotted line) and the threshold above which the densities of *D. kuriphilus* and *T. sinensis* are expected to oscillate by ~15% (continuous line). On the contrary, when considering the minimal observed density of trees, the establishment of *T. sinensis* required more than 80% of chestnut tree and could never lead to *D. kuriphilus* - *T. sinensis* coexistence with oscillations. The density of trees is thus a key parameter deciphering the outcomes of the *D. kuriphilus* - *T. sinensis* interaction through its impact on the biotic capacity of both species.

***The one-site model fails to explain the presence of T. sinensis in ‘sink’ sites with low abundance of chestnut trees***

The one-site model was further used to predict the growth rates of both *D. kuriphilus* and *T. sinensis* and the outcome of their interaction to be expected according to the abundance of trees and the chestnut tree frequency and genetic susceptibility recorded in each of our 23 sampling sites (Figure 2A). The observed rates of chestnut tree infestation (measured by the mean number of galls per bud) were significantly correlated with the *D. kuriphilus* growth rates ( $R_0$ ) predicted from the characteristics of the forest environment in both 2019 and 2020 (Figure 2B, GLMM analysis;  $\chi^2 = 0.065649$ ,  $df = 43$ ,  $p = 0.0014$ ). We also found a significant correlation between the observed rates of *D. kuriphilus* infestation by *T. sinensis* (measured by the fraction of ACGW larvae hyperparasited) estimated in 2020 and the *T. sinensis* predicted growth rates (Figure 2C, Spearman’s rank  $\rho = 0.5585958$ ,  $p = 0.01597$ ). Despite of these encouraging correlations, it is worth noting that i) *T. sinensis* was present at (lower but) significant rates in sites with a *T. sinensis*  $R_0 < 1$  (Figure 2C), which resulted in a ii) lack of correlation between the observed reduction of *D. kuriphilus* between 2019 and 2020 (at a time where galls of the invasive were heavily infected by *T. sinensis*, Figure 2A) and the predicted rates of its control (Figure 2D, Spearman’s rank  $\rho = 0.1422986$ ,  $p = 0.5172$ ).

A	Site	Tree abundance		Chestnut tree frequency		Genetic susceptibility		Model predictions			Infestation rate ( <i>D. kuriphilus</i> )		Infestation rate ( <i>T. sinensis</i> )	
		N	$p_c$	$p_e$	$S_e$	$R_0$ ( <i>D. kur.</i> )	$R_0$ ( <i>T. sin.</i> )	Predicted dynamic	$I$ ( <i>D. kur.</i> )		$I$ ( <i>T. sin.</i> )			
									2019	2020	2019	2020		
PDM1	1120	0.491	0.721	13.761	1.622	Equilibrium	0.014	0	0	0.917				
PDM2	870	0.908	0.639	22.565	2.838	Oscillations	0.029	0	0	0.923				
PDM3	1240	0.823	0.768	24.573	2.976	Oscillations	0.024	0.005	0	1				
SL1	1030	0.417	0.535	8.68	1.087	Equilibrium	0.066	0	/	/				
SL2	1230	0.854	0.731	24.274	2.995	Oscillations	0.144	0.035	0.035	0.978				
SL3	1570	0.694	0.774	20.897	2.811	Oscillations	0.127	0.033	0.033	0.982				
AR1	1070	0.869	0.427	14.444	2.573	Oscillations	0.052	0.008	0.008	0.977				
AR2	1330	0.286	0.588	6.528	0.657	No <i>T. sinensis</i> installation	0.057	0.013	0.013	0.81				
AR3	1120	0.652	0.581	14.714	2.306	Equilibrium	0.079	0.053	0.053	0.949				
CE1	740	0.581	0.686	15.497	1.512	Equilibrium	0.132	0.055	0.055	0.916				
CE2	760	0.908	0.756	26.675	2.863	Oscillations	0.081	0.058	0.058	0.967				
CE3	580	0.19	0.53	3.907	0.124	No <i>T. sinensis</i> installation	0.018	0.003	0.003	0.57				
BA1	1310	0.473	0.756	13.916	1.744	Equilibrium	0.006	0.001	0.001	/				
BA2	850	0.424	0.667	11.112	0.946	No <i>T. sinensis</i> installation	0.063	0.007	0.007	0.889				
BA3	1310	0.588	0.455	10.405	2.088	Equilibrium	0.007	0	0	/				
LL1	1270	0.724	0.566	15.95	2.563	Oscillations	0.046	0.005	0.005	0.935				
LL2	760	0.763	0.46	13.647	2.21	Equilibrium	0.033	0.005	0.005	0.972				
LA1	1170	0.735	0.581	16.601	2.562	Oscillations	0.037	0.009	0.009	0.914				
LA2	1150	0.583	0.702	15.902	2.152	Equilibrium	0.041	0.001	0.001	0.952				
LA3	900	0.311	0.466	5.642	0.526	No <i>T. sinensis</i> installation	0.027	0	0	/				
MA1	250	0.2	0.687	5.344	0.06	No <i>T. sinensis</i> installation	0.011	0	0	/				
MA2	670	0.418	0.644	10.466	0.709	No <i>T. sinensis</i> installation	0.044	0.002	0.002	0.737				
MA3	380	0.158	0.514	3.158	0.055	No <i>T. sinensis</i> installation	0.028	0	0	1				

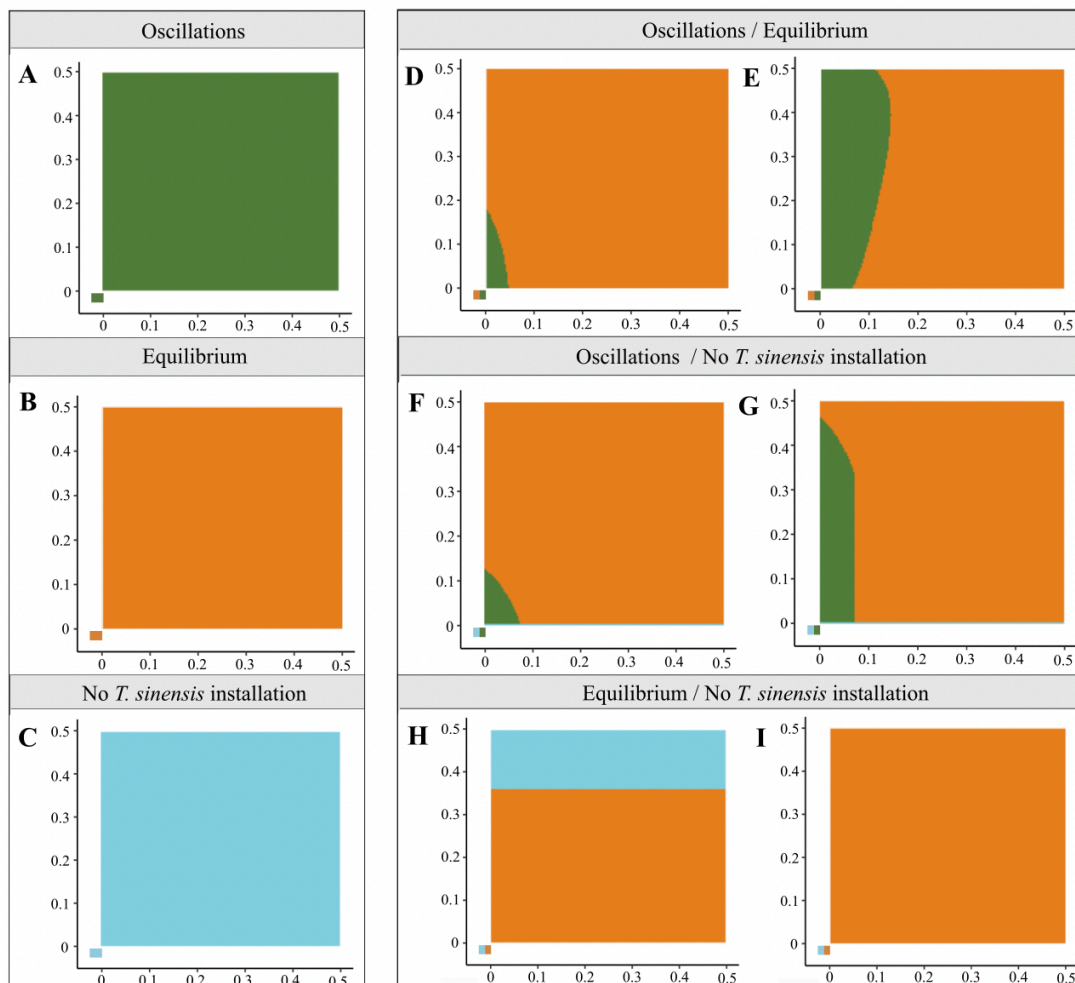


**Figure 2. Predictions of the one-site model in the 23 sampling sites and comparisons with the observed levels of *D. kuriphilus* parasitism and *T. sinensis* hyperparasitism.** (A) Summary of the characteristics of the forest environment in each of the sampling sites, the model predictions and the observed rate of *D. kuriphilus* and *T. sinensis* infestations. (B) Correlation between the predicted *D. kuriphilus* growth rates ( $R_0$ ) and the rates of (chestnut tree infestation) observed in 2019 and 2020. (C) Correlation between the predicted *T. sinensis* growth rates and the rates of (*D. kuriphilus*) infestation observed in 2020. (D) Correlation between the predicted rates of biological control and the gall reductions observed between 2019 and 2020. In C and D, each dot (site) was colored according to the expected population dynamics for *D. kuriphilus* and *T. sinensis* using the same legend as in Figure 1A.

We then hypothesize that the observation of *T. sinensis* in sites where it was not predicted to spread by our one site model could result from dispersal of the control agent from favorable ‘source’ sites, allowing for its establishment in the predicted ‘sink’ sites (Pulliam, 1988).

***The dispersal of D. kuriphilus and T. sinensis synchronize their interaction dynamics in heterogeneous habitats***

The spatial expansion of the *C. sativa* - *D. kuriphilus* - *T. sinensis* model allowed to investigate the outcomes of the interactions when two sites are connected through the dispersal of both the pest and its control agent. We coupled sites with similar (Figure 3 A-C) or dissimilar (Figure 3 D-I) population dynamics predicted at the local scale, using all combinations of forest density and frequency and genetic susceptibility of chestnut trees observed in our 23 sampling sites. The coupling of sites with the same local dynamics did not induce any qualitative changes at local scale and, as a consequence, the global dynamics was systematically similar to those predicted locally, whatever the intensity of *D. kuriphilus* and *T. sinensis* dispersal (Figure 3 A-C). More interestingly, the dispersal between sites exhibiting dissimilar population dynamics tends to synchronize the local dynamics (Figure 3 D-I) and to allow for a nearly systematic persistence of *T. sinensis* population in the predicted ‘sink’ sites when coupled to any of the observed ‘source’ sites.



**Figure 3. Global dynamics of the *C. sativa* - *D. kuriphilus* - *T. sinensis* interaction in heterogeneous environments coupled *D. kuriphilus* and *T. sinensis* dispersal. The global dynamic was predicted for sites with similar (A-C) or dissimilar (D-I) population dynamics predicted at the local scale, and while *D. kuriphilus* (x-axis) and *T. sinensis* (y-axis) rate of dispersal were varied from 0 to 50%. The local dynamics predicted for each of the two connected sites are represented in the bottom left corner of each graph. Colors indicate the nature of the predicted dynamic as in Figure 1A.**

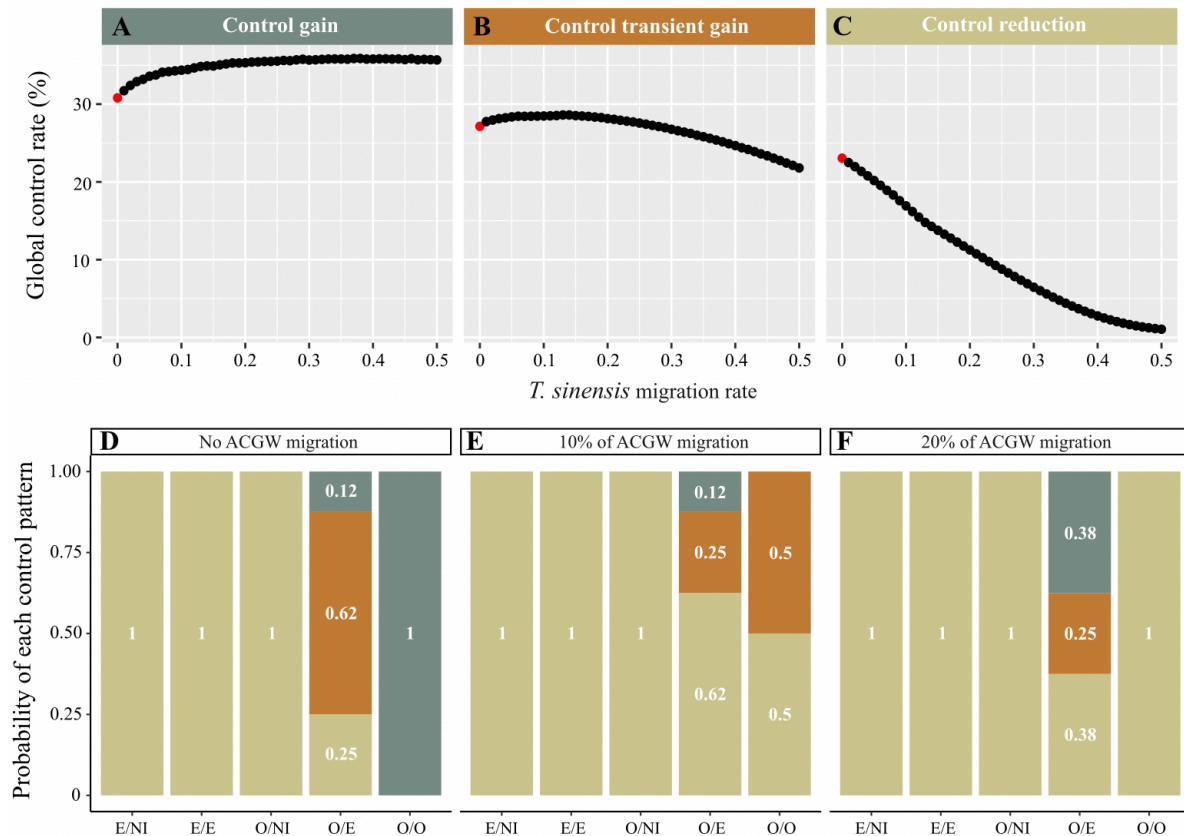
Dispersal between a site where the two species coexist with oscillations and a site where they coexist in a stable way (Figure 3 D-E) or where *T. sinensis* cannot spread (Figure 3 F-G) led to similar patterns of variations of the interaction outcome with respect to the rates of dispersal. At low rates of *D. kuriphilus* dispersal, the pest and its control agent exhibit sustained oscillations, while the two species persist stably once such rate exceeds 5-10%. In most circumstances, the local dynamics observed in each of the coupled sites are of the same nature and almost independent of what they were predicted to be in the absence of dispersal. The connection of sites where the two species coexist in a stable way and where *T. sinensis* cannot spread (Figure 3 H-I) typically led to a synchronization of the local interactions with the persistence of the two species at an equilibrium in both sites, unless the rate of *T. sinensis* dispersal was so high that the persistence of *T. sinensis* was no longer possible in the unfavorable site.

Overall, the dispersal of *D. kuriphilus* and *T. sinensis* between sites that differ in their forest density and frequency and genetic susceptibility of chestnut trees, act to synchronize the local dynamics of interaction. While those dynamics first tend to align to the dynamics of the site with the highest chestnut tree frequency, they converge toward a stable coexistence at larger rates of dispersal. Importantly, this led to the systematic persistence of *T. sinensis* in ‘sink’ sites coupled with ‘source’ sites where the control agent was able to persist with its *D. kuriphilus* host either through stable (Figure 3 F-G) or oscillatory (Figure 3 H-I) dynamics.

***The global efficiency of biological control can increase and (most likely) decrease with *T. sinensis* dispersal***

We then aimed to investigate how the changes in the *D. kuriphilus* - *T. sinensis* populations dynamics induced by their dispersal impact the global efficiency of biological control of the

invasive pest. Our modelling allowed to identify 3 different patterns of variations of the efficiency of biological control with the dispersal rate of the pest and its control agent (Figure 4).



**Figure 4. Variations in biological control efficiency with respect to the dispersal of *D. kuriphilus* and *T. sinensis*.** (A,B,C) Predicted patterns of variation in the rate of biological control with the rate of dispersal of the control agent. (D, E, F) Predicted frequencies of the patterns of variations of biological control efficiency with *T. sinensis* dispersal, for combinations of sites with similar or dissimilar local populations dynamics (NI : Non-installation of *T. sinensis*; coexistence of *D. kuriphilus* and *T. sinensis* with E : Equilibrium; or O : Oscillations) and for 0% (D) , 10% (E) or 20% (F) rates of *D. kuriphilus* dispersal.

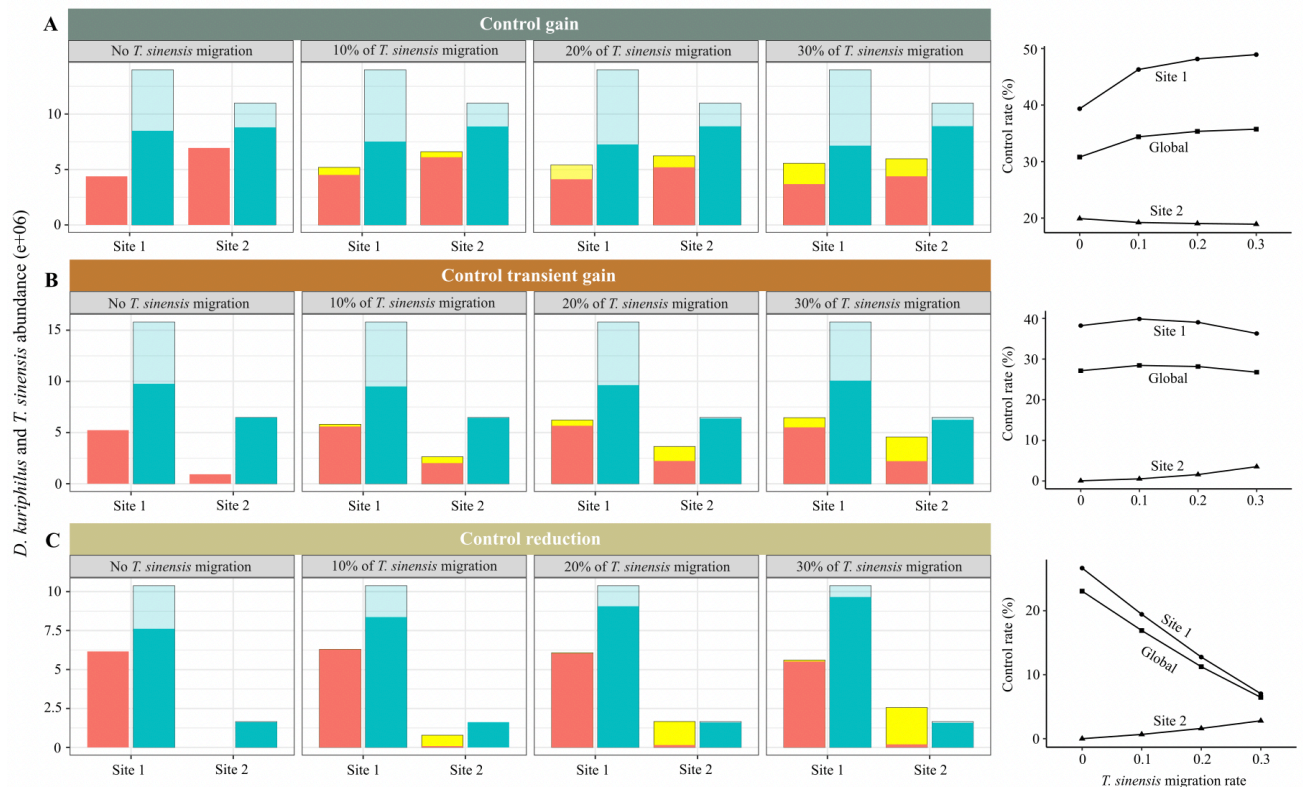
The efficiency of *D. kuriphilus* biological control was shown to increase (Figure 4A) or decrease (Figure 4C) monotonically, or to transiently improve before to slowly decrease (Figure 4B) with the *T. sinensis* dispersal. Overall, the efficiency of achieved control was higher in the first case, lower in the third, and intermediate in the case of the non-monotone relationship, which implies that *T. sinensis* dispersal is beneficial to biological control in sites where the conditions already are the most favorable for the spread of *T. sinensis* populations.

The ‘Control gain’ and ‘Control transient gain’ patterns were indeed only found when coupling two sites where *T. sinensis* is able to spread and with at least one of those exhibiting oscillations with *D. kuriphilus*, which allow for the largest growth of both parasite and parasitoid populations. In such cases, *T. sinensis* dispersal indeed allowed to rebuild more quickly its oscillating populations. As expected, the occurrence of such ‘Control gain’ and ‘Control transient gain’ also depends on *D. kuriphilus* dispersal that can allow the pest to escape the control agent, which indeed resulted in an overall decrease of the probability to observe any gain of control with the probability of dispersal of the targeted invasive pest (Figure 4 D-F).

While the characterization of the (transient) gains in the global control efficacy were predicted in specific conditions, the dispersal of the control agent and its pest are most likely to limit the global efficiency of the biological control strategy. We intended to refine the understanding of such patterns by looking at the underlying variations at the site level.

***Dispersal between heterogeneous sites tends to homogenize the local efficiencies of biological control and to amplify differences in its global rate***

We then further used our modelling to uncover the variations in abundance of *D. kuriphilus* and *T. sinensis* in the two connected sites for each of the three patterns identified at the global scale.

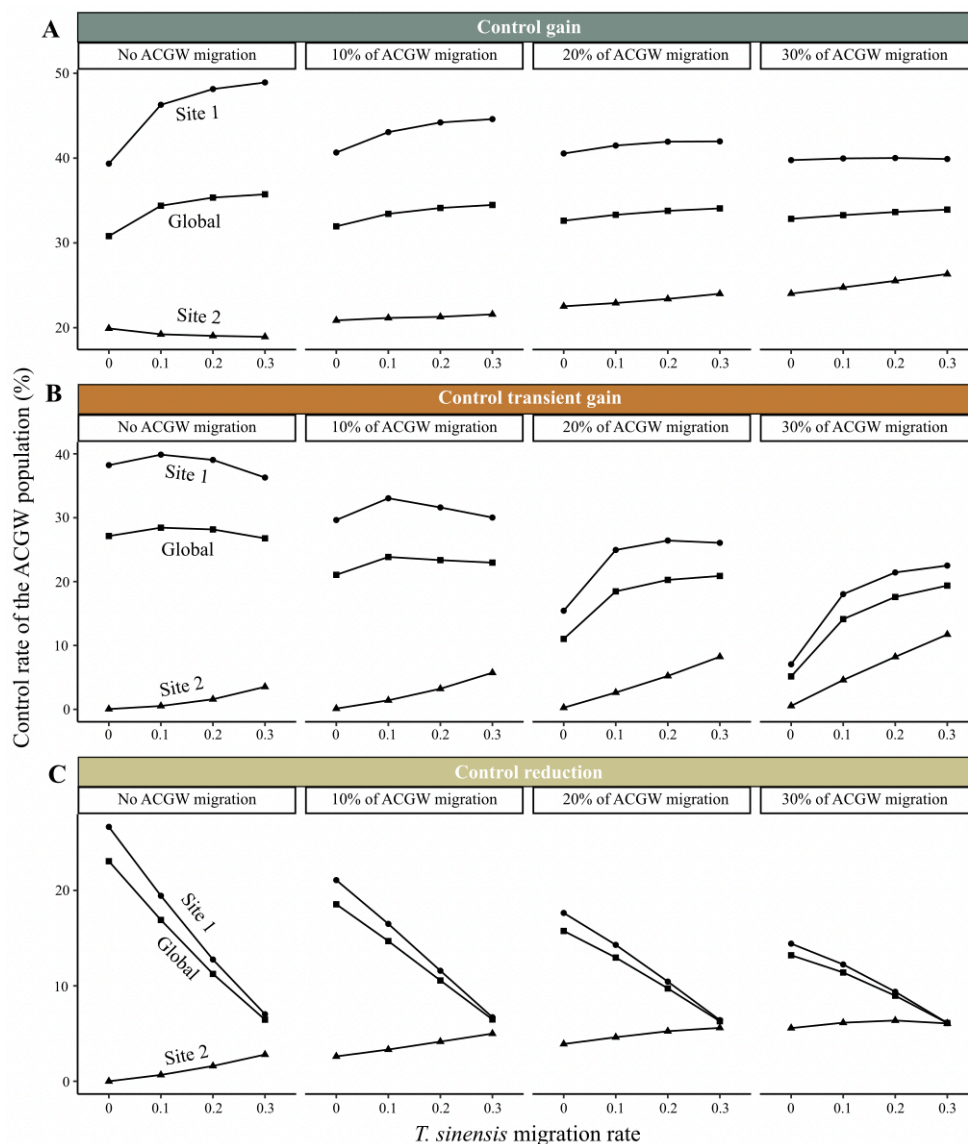


**Figure 5. Local variations of *D. kuriphilus* and *T. sinensis* abundance underlying the pattern of changes in the global control efficiency with *T. sinensis* dispersal.** *T. sinensis* local abundance (red) and individuals that have dispersed from the other site (yellow) are represented for each site, together with *D. kuriphilus* abundance (blue) and its biotic capacity in the site (light blue). Changes in all these quantities are shown in conditions leading to the three global patterns of global efficiency of the biological control observed in Figure 4, and summarized on the right panels, where changes in control efficacy is shown at the global scale and in each of the two connected sites.

The ‘Control gain’ pattern is typically observed when, in the absence of dispersal, the two sites allow for *D. kuriphilus* and *T. sinensis* to coexist with different abundances and levels of control achieved in both sites, although such differences remain limited (Figure 5A). The pattern emerges as the emigration-immigration balance resulting from dispersal typically increase the abundance of *T. sinensis* in its less populated site and reduced it in the other, and when those changes are beneficial to the control efficiency in the former while they do not significantly reduce it in the latter. The ‘Control transient gain’ occurs when, in the absence of dispersal, *D. kuriphilus* and *T. sinensis* coexist in each site, although the difference in abundance are such that the biological control achieved in one of them is virtually null (Figure 5B). Low dispersal of the control agent then benefit the control in both sites, but, when dispersal increases, it

becomes detrimental for the control level in the most populated site, which is not compensated by the benefit it brings to the less populated site. The ‘Control reduction’ pattern, was obtained when the difference in *T. sinensis* populations between the two sites are large (Figure 5C) and is simply explained; any dispersal of the control agent is beneficial for its abundance and ability to control *D. kuriphilus* in the much less favorable site, although this never compensate for the loss in the best controlled site.

While our modelling allowed to identify three patterns of variations of the global efficiency of control with *T. sinensis* dispersal, as well as the underlying co-variations of biological control in each site, we ultimately aimed at looking at the importance of *D. kuriphilus* in modulating such patterns.





**Figure 6. Variations of the control rates with *D. kuriphilus* and *T. sinensis* migration.** Impact of both parasite and parasitoid migration ranging from 0% to 30% were tested on control rates of the 3 different control patterns (A,B,C) at the site and global scale.

In conditions leading to the ‘Control gain’ pattern, the dispersal of *D. kuriphilus* had little effect on the global control levels, which remained around 33-35%, although it strongly reduces the heterogeneity between the level of control achieved in both sites (Figure 6A). Such a spatial homogenization of species abundances slightly flattened the variations of the global control rate with the control agent dispersal. On the contrary, *D. kuriphilus* dispersal significantly impacted the local and global efficiencies of biological control underlying the ‘Control transient gain’. When dispersal was higher than 10%, the pattern turned into a ‘Control gain’ shape while the global level of control had fallen by about 30%. Again, such variations were associated with a reduction of the heterogeneity of control between sites, which benefited substantially to the less controlled site. Finally, the dispersal of *D. kuriphilus* also strongly reduced the differences between sites when considering a situation corresponding to the ‘Control reduction’ pattern (Figure 6C), which again translated into a 10-30% loss of control at the global scale, with only minor benefit for the less controlled site.

The dispersal of *D. kuriphilus* tends, as expected, to make the efficiency of biological control more homogeneous between sites. While this has little effect on the global level of control when such control is high (i.e. in ‘Control gain’ conditions), we predicted 10-30% decreases when its efficiency is typically lower (i.e. in ‘Control transient gain’ and ‘Control reduction’ conditions). Overall, this suggests that *T. sinensis* dispersal shall either increase the efficiency of control when it is already effective, i.e. in ‘Control gain’ conditions, or further decrease it in conditions where it is not likely to be a highly valuable option to control *D. kuriphilus*.

## Discussion

The study of plant pest biological invasion is heavily focused on the difficult task to identify and compare the role of bottom-up and top-down forces (e.g. Hunter et al., 1997, Wilkinson and Sherratt, 2016, Vidal and Murphy 2018). While such an understanding can help identifying the key determinants of pest invasion and choosing adequate control strategies (Goslee et al., 2006, Büyüktahtakın et al., 2011, Baxter et al., 2008), it usually does not account for the additional complexity of spatial heterogeneity in such forces and local dispersal between sites that can represent ‘source’ or ‘sink’ for the invasive pest and/or its biological control agent (Evans, 2018, and references therein). By expanding a previous modelling of *D. kuriphilus* invasion and of its bottom-up and top-down regulation forces (Zitoun et al., 2023) into a two-site model, we investigated dynamics induced by the dispersal of both the invasive species and its control agent, *T. sinensis*, in the spatially heterogeneous forest environments encountered in the Pyrénées-Orientales (Engen et al., 2002).

Our modelling allowed to predict the population dynamic of *D. kuriphilus* and *T. sinensis* according to the observed characteristics of the forest environment, i.e. tree density and chestnut tree frequency and susceptibility to *D. kuriphilus* infestation, in each of the 23 sites sampled in this local area. The forest structure showed strong variations among those sites that were significantly associated with heterogeneous levels of *D. kuriphilus* infestation in 2019 ( $\chi^2 = 479.8$ ,  $df = 22$ ,  $p < 2.2 \cdot 10^{-16}$ ) and 2020 ( $\chi^2 = 428.1$ ,  $df = 22$ ,  $p < 2.2 \cdot 10^{-16}$ ). The model rightly predicted that such structure should lead to the coexistence of *D. kuriphilus* and *T. sinensis* in 16/23 sites, where both species were indeed observed in both 2019 and 2020. Such a prediction of the persistence of a control agent and its target does not fit the usual expectation whereby their interactions should lead to the extinction of the pest followed by those of its parasitoid (e.g. Hawkins and Cornell, 2008). It is however consistent with previous outcomes of the modelling of this biological system by Paparella et al. (2016) and Zitoun et al (2023) that showed their tendency to coexist by exhibiting stable equilibrium or oscillations in abundances, as well as with field observations that *T. sinensis* never led to the elimination of the pest (Gibbs et al., 2011) and instead led to three successive peaks of *D. kuriphilus* shortly followed by peaks of *T. sinensis* over 25 years of field interaction (Moriya, personal communication). Meanwhile, in the absence of dispersal, our modelling predicted that *T. sinensis* should not be able to spread in the remaining 7/23 ‘sink’ sites, where the control agent was nonetheless systematically observed.

We thus introduced dispersal of both *D. kuriphilus* and *T. sinensis* to test the hypothesis that the presence of the control agent could be explained by its dispersal from surrounding ‘source’ sites. We showed that, even at low rates of dispersal, the dynamics of the site with the lowest chestnut tree frequency tend to converge to the dynamics of the site with the highest chestnut tree frequency, before they converge toward a stable coexistence once *D. kuriphilus* dispersal rate exceed 10%. This is in accordance with a large set of theoretical studies that have previously demonstrated the strong ability of dispersal to synchronize various ecological dynamics (Ranta et al., 1995; Blasius et al. ,1999; Lande et al., 1999). An important corollary of such predictions was that *T. sinensis* population in ‘sink’ sites were systematically sustained by dispersal from ‘source’ sites whatever be the regime of coexistence of the control agent with *D. kuriphilus* in the source. This provide a truly plausible explanation to the observation of *T. sinensis* in the 7 predicted ‘sink’ sites as those were typically associated with two ‘source’ sites in the same sampling stations, namely Arles sur Tech, Céret, Bastide and Laroque. The only notable exception was in the natural reserve La Massane, where all three sites where predicted to be ‘sink’, and where *T. sinensis* may have spread from the close station Laroque, where 2 of the 3 sampling sites where predicted to be sources of *T. sinensis*.

When investigating the variation of biological control efficiency in the spatial contexts corresponding to the observed heterogeneity in the forest environment of the Pyrénées-Orientales, we identified 3 patterns of variations of the global control rate. An increase of the biological control efficiency with *T. sinensis* dispersal was shown by coupling two sites allowing for the spread of the agent and its coexistence with *D. kuriphilus*, when at least one of those exhibited oscillatory dynamics. The dispersal between sites in oscillations dynamics indeed led to a quicker reconstruction of parasitoid populations, as suggested by Mohd and Noorani (2021) for prey-predators dynamics. Interestingly, this implies that *T. sinensis* dispersal could further improve biological control efficacy when it is expected to be at its maximal level. In all other investigated cases, *T. sinensis* dispersal was shown to reduce the global efficacy of control, where it was already low. Meanwhile, *D. kuriphilus* dispersal was shown to lead to a global control reduction, mostly in the conditions where control was already low, thereby amplify the differences in the global rate of biological control between favorable and unfavorable situations.

The ecological concept “Enemies Hypothesis” theorized by Root (1973) predicts a beneficial effect of spatial heterogeneity on the biocontrol of phytophagous pests. The diversification of

plant species in the environment is thought to allow for a better biocontrol via an increase in the number of prey or alternative hosts and in the proportion of over-wintering sites or refuges against disturbances for control agents (Blitzer et al., 2012; Rusch et al., 2013, 2016). While this concept originally developed for agroecosystems has been transposed to the forest environment (Bellone et al., 2020; Stemmelen et al., 2022), it has also been pointed out that it is largely based on the assumption that predators are generalists (Staab and Schuldt, 2020). On contrary, specialist control agents, particularly parasitoids, tend to be more abundant and effective in homogeneous systems than in heterogeneous systems (Sheehan, 1986) because they depend on a single resource whose availability is impacted by the loss of favorable habitat (Tschardt et al., 2008; Legault and James, 2018). Although control agent dispersal increase is generally associated to control gain (Cacho and Hester, 2022) or transient gain (Heimpel and Asplen, 2011), we predicted that *T. sinensis* efficiency could also decrease under certain conditions. Indeed, when the control agent abundance after migration exceeds the biotic capacity in the unfavorable site, it could lead to the exclusion of a part of the parasitoids from the system, thus not being able to exercise control over the *D. kuriphilus* population.

## Materials and methods

### *Dryocosmus kuriphilus* life-history

The invasive *D. kuriphilus*, also called Asian chestnut gall wasp (ACGW), is the most virulent pest of sweet chestnut trees (*Castanea sativa*) worldwide (Fernandes et al., 2022). This hymenopteran parasite induces the formation of galls, which lead to a reduction of biomass (Marcolin et al., 2021) and chestnut production at levels up to 80% (Battisti et al., 2014). Beyond the strong corresponding economic losses, this pest also has significant environmental impacts, being responsible of the death of young chestnut trees and interacting with other infection such as the fungal chestnut blight (*C. parasitica*) (Meyer et al., 2015). *D. kuriphilus* has a high invasive potential, with an  $R_0$  estimated to reach 15.9 in the studied area (Zitoun et al., 2023), that is usually attributed to its highly effective parthenogenetic reproduction and a univoltine life-cycle well-synchronized with the chestnut tree host phenology (Avtzis et al., 2019; Bernardo et al., 2013). Semelparous females emerging from galls in June-July locate chestnut tree buds using visual and odor cues (Germinara et al., 2011) where they lay their eggs, asexually produced through thelytokous parthenogenesis, during a short 1-7 days dispersing adult life-span (Bosio et al., 2010). Oviposition has been shown to trigger a local hypersensitive immune response in chestnut trees buds and to prevent the development of deposited eggs (Dini et al., 2012; Nugnes et al., 2018; Acquadro et al., 2020). Such host resistance showed substantial variations between chestnut tree varieties, ranging from fully-resistant individuals to highly susceptible ones (Sartor et al., 2015). The *D. kuriphilus* eggs that escape such immune response, and also survive to intrinsic developmental failures, hatch within a month to develop into first instar larvae that enter in dormancy to overwinter. In spring, the development into second larval instars induces the formation of galls during the chestnut tree buds burst. *D. kuriphilus* individuals then become pupae and adults that will eventually emerge and produce the next and non-overlapping generation of eggs. In the newly invaded environment, *D. kuriphilus* galls have been shown to be infected by local fungi (Tosi et al., 2015) and hymenopteran parasitoids species. However, the typically low hyperparasitism rates are thought not to be sufficient to naturally down-regulate the invasive pest populations (e.g. Kos et al., 2021, Zitoun et al., 2023). To restrain its spread and reduce its impact on exploited chestnut tree populations, a specific natural enemy, the parasitoid *Torymus sinensis*, is

commonly used and has showed successful establishment and control rates in Japan (Aebi et al., 2006), North America (Payne, 1978) and Europe (Avtzis et al., 2019).

### ***Sampling sites and characterization of the chestnut tree resource in the Pyrénées-Orientales***

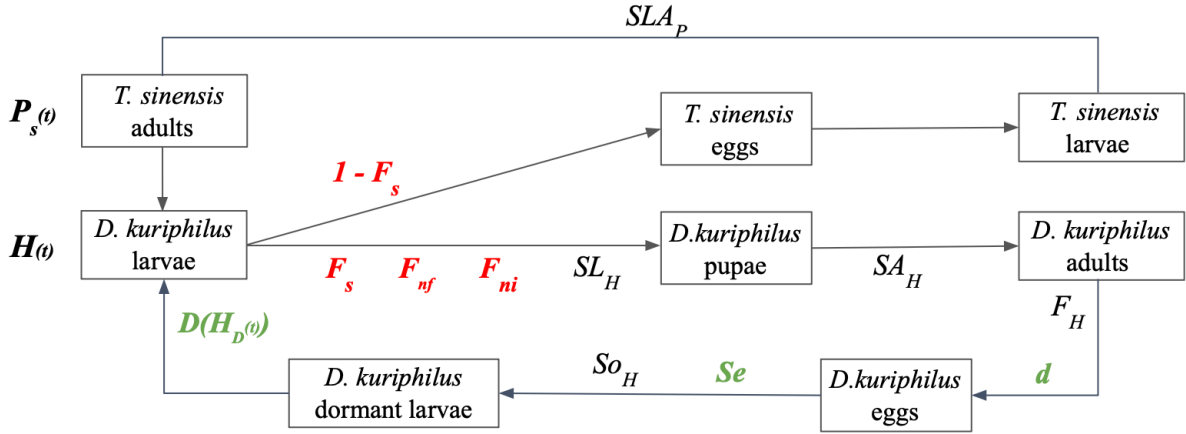
The exotic pest *D. kuriphilus* was first reported in 2013 in the French department of Pyrénées-Orientales, where we set-up a field study to concomitantly measure the levels at which it infested the local chestnut tree populations in both 2019 and 2020 (as indicated in a subsequent section) and the bottom-up factors, i.e. the forest tree community and genetic structure of *Castanea sativa* populations, regulating these levels of infestation. The density of trees in the forest environment, and the chestnut tree frequency and genetic susceptibility to *D. kuriphilus* were estimated for 23 sites located in 8 stations (Prats de Mollo, Saint Laurent, Arles sur Tech, Céret, La Bastide, Llauro, Laroque and Massane). The species composition and density of the forest environment were characterized by sampling 1000m<sup>2</sup> in each of our study sites and by identifying the taxonomic status of each tree to the species level. Confidence intervals for the overall abundance of trees and species frequencies were drawn from Poisson and Binomial distributions, respectively. The genetic susceptibility of chestnut trees to *D. kuriphilus* infestation was estimated according to the previously established relationship between the rate of private alleles carried by a chestnut tree individual and its level of infestation rate (Zitoun et al., 2023). Using RAD sequencing of a hundred chestnut trees sampled in the studied sites (Zitoun et al., 2023), we inferred the rate of private alleles for each of them and estimated the average susceptibility for each of the 23 sites. These estimates of the density of trees in the forest environment, and of the chestnut tree frequency and genetic susceptibility allowed for site specific predictions using the modelling described below. The field measures, estimates and model predictions obtained for each site are summarized in Figure 2.

### ***The one-site dynamical model of the C. sativa - D. kuriphilus - T. sinensis interactions and its predictions***

*The one-site dynamical model of the C. sativa - D. kuriphilus - T. sinensis interactions.* The model allowing to predict the dynamic of the *C. sativa* - *D. kuriphilus* - *T. sinensis* interactions in one site was initially developed in Zitoun et al. (2023). It was derived from the seminal host-parasitoid model proposed by Nicholson-Bailey (1935) and adapted to account for the effects of the bottom-up and top-down factors regulating *D. kuriphilus* invasion. The Nicholson-

Bailey framework is based on a description of the life cycles of the host and its parasitoid (Figure 7).

In the lower part of the graph representing *D. kuriphilus* life-cycle, the larvae of the host that escape their parasitoids with probability  $F_s$ , face additional challenges by native hymenopteran and fungal hyperparasites that they can escape with probabilities  $F_{ni}$  and  $F_{nf}$ , respectively. We assumed a Poisson distribution of attacks on the host to mimic the low ability of *T. sinensis* to fly towards galls of *D. kuriphilus*. The probability of escaping parasitism then reads  $F_s(P_s(t)) = e^{-a_s p_c P_s(t)}$ , where  $a_s$  and  $p_c$  stand for *T. sinensis* searching area and the frequency of chestnut trees in the forest environment, respectively. The probabilities of escaping native hyperparasites were modelled as constant forcing terms as their population dynamics primarily remain determined by their native hosts. The larvae surviving all three types of natural enemies and to intrinsic causes of mortality at rate  $SL_H$ , develop into pupae that eventually survive to become adults with probability  $SA_H$ . Dispersing adults produce on average  $F_H$  eggs, a fraction of which are laid on chestnut trees. As shown in Zitoun et al (2023), in a forest made of chestnut trees and non-host trees, and in the absence of *D. kuriphilus* ability to navigate towards hosts, the fraction  $d$  of deposited eggs corresponds to the frequency  $p_c$  of chestnut tree in the forest environment. Only a fraction  $Se$  of deposited eggs survived the immune response of the trees and a fraction  $SO_H$  of the escaping eggs further survived developmental failures to hatch into first instar larvae that enter in overwintering dormancy. The ‘contest’ competition for space during chambers formation was described using the function proposed by Brännström and Sumpter (2005):  $D(H_D(t)) = K(p_c, N)(1 - e^{-\frac{H_D(t)}{K(p_c, N)}})$ , resulting in a density dependent survival of the  $H'(t)$  larvae entering dormancy according to the maximal amount of *D. kuriphilus* larvae sustainable in a stand that we denoted  $K(p_c, N)$ . This carrying capacity was defined as the product of the abundance of trees ( $N$ ), the frequency of chestnut trees ( $p_c$ ) and of the average number of *D. kuriphilus* lodges per chestnut tree ( $k$ ), as estimated in Zitoun et al (2023). In spring, the surviving larvae induce the formation of galls, where *T. sinensis* adults can lay their eggs. The fraction  $1 - F_s(P_s(t))$  of *D. kuriphilus* larvae ending up being parasited will ultimately allow for the development of *T. sinensis* larvae and adult with probability  $SL_Ap$  which completes the description of the life cycle of the parasitoid.



**Figure 7. Schematic representation of the one-site model of the *D. kuriphilus* – *T. sinensis* interaction integrating bottom-up and top-down regulations factors.** Parameters describing the basic life cycle of each species appear in black, while represented in red and green represent the effects of top-down control (hyperparasitism by native insects and fungi) and bottom-up control (chestnut tree frequency, density and genetic susceptibility), respectively. From Zitoun et al. (2023).

The *C. sativa* - *D. kuriphilus* - *T. sinensis* interactions represented in Figure 7 led to the following set of two difference equations that predict the yearly changes in the abundance of *D. kuriphilus* larvae,  $H(t)$ , and *T. sinensis* adults,  $P_s(t)$  :

$$H(t + 1) = K \left( 1 - e^{-\frac{RH(t)e^{-\alpha'_s P_s(t)}}{K}} \right) \quad \text{Equation 1}$$

$$P_s(t + 1) = cH(t)(1 - e^{-\alpha'_s P_s(t)}) \quad \text{Equation 2}$$

where  $R = F_{nf} F_{ni} SL_H SA_H F_H d Se So_H$  and  $c = SLA_p$

*Prediction of the dynamical outcome of the *D. kuriphilus* - *T. sinensis* interaction according to features of the forest environment.* To investigate the effect of bottom-up regulation factors, i.e. the density of trees in the forest environment and the chestnut tree frequency and genetic susceptibility, on the dynamic of the *D. kuriphilus* – *T. sinensis* interaction (Figure 1), we performed a local stability analysis of the one-site model given by equations 1-2 (SM 1).

This analysis showed that conditions for the host (*D. kuriphilus*) and its parasitoid (*T. sinensis*) populations to not be able to develop are given by (3) and (4) below, respectively.



$$Se < \frac{1}{F_{nf} F_{ni} SL_H SA_H F_H So_H p_c} \quad \text{Equation 3}$$

$$Se < \frac{\ln(1 - \frac{1}{SL_{AP} K \alpha'_s} + 1)^{-1}}{F_{nf} F_{ni} SL_H SA_H F_H So_H p_c} \quad \text{Equation 4}$$

These conditions were used to represent the outcome of the interaction in the Se-P<sub>c</sub> parameter plan in Figure 1. The first allowed to delimitate the dark blue area where no species can invade, while the second allowed to draw the light blue area where only *D. kuriphilus* could develop.

The stability analysis further provided the condition where *D. kuriphilus* and *T. sinensis* were both able to invade, which lead to their coexistence at a stable equilibrium or in a regime of persisting oscillations. We showed that the equilibrium point (H\*; P\*) is stable when

$$\left| R \left(1 - \frac{H^*}{K}\right) \left(1 - \frac{P^*}{SL_{AP} H^*}\right) + SL_{AP} H^* \alpha'_s \left(1 - \frac{P^*}{SL_{AP} H^*}\right) \right| < R \left(1 - \frac{H^*}{K}\right) \left(1 - \frac{P^*}{SL_{AP} H^*}\right) SL_{AP} H^* \alpha'_s + 1 < 2 \quad \text{Equation 5}$$

This condition was evaluated numerically in order to differentiate between the orange and green areas of the Se-P<sub>c</sub> parameter plan that correspond to the conditions where the two species are expected to coexistence at stable level or through oscillations. For each set of Se, p<sub>c</sub> and N values, the condition was evaluated after the equilibrium value H\* and P\* were found by using the ‘nleqslv’ R package to numerically solve equations 1-2 and while all other parameters were set to their average estimates (see SM 3). The value of Se and p<sub>c</sub> were varied systematically from 0 to 1 to obtain a complete parameter space, and we considered 3 values of N corresponding to the minimum (250), average (989) and maximum (1570) tree density observed in our sampling stations in the Pyrénées-Orientales.

*Predictions of the intrinsic growth rates of D. kuriphilus and T. sinensis and of control rates of D. kuriphilus by T. sinensis.* The intrinsic growth rates of *D. kuriphilus* were derived from equation 1 after setting P<sub>s</sub>(t)=0, while the intrinsic growth rates of *T. sinensis* were obtained from equation 2 with its host population set at its equilibrium level. This led to the following expressions:

$$R_0^{D.kur} = R = F_{nf} F_{ni} SL_H SA_H F_H d Se So_H \quad \text{Equation 6}$$

$$R_0^{T.sin} = SLA_p H^* \alpha_s p_c \quad \text{Equation 7}$$

The value of those intrinsic growth rates were calculated for each sampling sites according to the characteristics of the local forest environment, i.e. the parameters  $p_c$ ,  $Se$  and  $N$ , and while all other parameters were set to their average estimates (see SM 3).

When both *D. kuriphilus* and *T. sinensis* were predicted to spread, the dynamical outcome of their interaction was predicted by simulations of equations 1-2 with R.4.1.1 (R Core Team, 2021). The rate of *D. kuriphilus* biological control by *T. sinensis* was then estimated as the percentage of reduction in the average abundance of *D. kuriphilus* induced by *T. sinensis* as compared to its abundance at the equilibrium state reached in the absence of the biological control agent.

The predictions of the intrinsic growth rates of *D. kuriphilus* and *T. sinensis* and of the control rates of *D. kuriphilus* made for each of the 23 sampling sites, were then compared to the observed rates of infestation in each of this sites located in the Pyrénées-Orientales.

***Observed rates of chestnut tree infestation by D. kuriphilus and of hyperparasitism by T. sinensis and their comparisons with the predictions of the one-site model***

*Chestnut tree infestation by D. kuriphilus.* The invasion of the local chestnut tree populations by *D. kuriphilus* was assessed, in 2019 and 2020, using two typical measures of parasitism in each sampling site. The rate of infestation, defined as the mean number of galls per leaf, was estimated on 5 geo-located chestnut tree individuals in each of the 23 sampling sites, and with a sampling effort of 250 to 500 leaves per tree. The rate of prevalence, defined as the proportion of chestnut trees infested by at least one gall, was estimated on 50 trees in each sites. Exact confidence intervals for both measures of infestation were calculated from the binomial distribution set according to the corresponding sampling effort.

*Hyperparasitism of D. kuriphilus by T. sinensis.* During summer 2020, we further collected 1320 galls of *D. kuriphilus* in 18 sites with a sampling abundance varying from 13 to 100 galls per site. The hyperparasitism level of these galls by the introduced control agent was assessed by dissecting them and collecting *T. sinensis* larvae and *D. kuriphilus* adults. The rate of *T. sinensis* infestation was then simply calculated as the proportion *D. kuriphilus* larvae

hyperparasited in galls of each site. Although a part of these larvae could be native parasitoids, their infestation rate is low and stable among localities, so they were not taken into account for this estimate.

*Comparisons of the observed levels of infestation with the one-site model predictions.* To test the one-site model predictions, we first looked at the correlation between the observed *D. kuriphilus* infestation rates (measured in 2019 and 2020) and its intrinsic growth rate predicted for the 23 sites. Such a correlation was tested through a generalized linear mixed modelling (GLMM) that was conducted by following the procedure recommended by Zuur et al. (2009). First, we characterized the impact of the sampling year on the infestation measure considered through a standard GLM analysis, and subsequently accounted for such a temporal structure by incorporating sampling year as a random factor in our GLMM analyses (Zuur et al. (2009), p. 323-332). This statistical analysis was conducted in R 4.1.1 using the `glmmPQL` function from the MASS package and a quasi-binomial distribution, after the original rate of infestation (i.e. the number of galls per leaf) was converted into a proportion of leaves with one gall per leaf since we never counted more than one gall on any of the >45000 observed leaves. We then looked at the correlation between the rates of *T. sinensis* infestation observed in 2020 and the control agent intrinsic growth rate predicted for each site using a Spearman's rank correlation test. Finally, we looked at the relationship between the reduction in the number of *D. kuriphilus* galls between 2019 to 2020 in each site, and the predicted rates of control using, again a Spearman's rank correlation test.

### ***The spatial model of C. sativa - D. kuriphilus - T. sinensis interactions and its analysis***

We expanded the previous modelling into a two-sites model connected by the dispersal of *D. kuriphilus* and *T. sinensis* to investigate the dynamics that could result from such coupling and the environmental heterogeneity observed across our 23 sampling sites. In each of the two sites, the local dynamics were described by two equations similar to equations 1 and 2, while accounting for the dispersal of *D. kuriphilus* and *T. sinensis* adults at rates  $M_{D.kur}$  and  $M_{T.sin}$ , respectively.

The number of *T. sinensis* adults present within site 1 after migration, denoted  $P_{S_{m1}}(t)$ , can be expressed with respect to the number of adults found in site 1 and 2 before dispersal, denoted  $P_{s1}(t)$  and  $P_{s2}(t)$ , as follows :

$$P_{Sm_1}(t) = P_{s_1}(t) (1 - M_{T.sin}) + P_{s_2}(t) M_{T.sin} \quad \text{Equation 9}$$

and the number of *T. sinensis* adults present within site 2 after migration, denoted  $P_{Sm_2}(t)$ , takes a symmetrical form :

$$P_{Sm_2}(t) = P_{s_2}(t) (1 - M_{T.sin}) + P_{s_1}(t) M_{T.sin} \quad \text{Equation 9'}$$

The number of *D. kuriphilus* adults present in site 1 after migration, denoted  $A_1(t)$ , can be expressed from the number of larvae found in galls in site 1 and site 2, i.e.  $H_1(t)$  and  $H_2(t)$ , respectively. In each of these sites, those larvae can escape *T. sinensis* with probabilities  $F_{s_1}$  and  $F_{s_2}$ , and survive to adulthood with probability  $R_H = F_{nf} F_{ni} S L_H S A_H$ . The expression of  $A_1(t)$  then reads :

$$A_1(t) = R_H (H_1(t) F_{s_1} (1 - M_{D.kur}) + H_2(t) F_{s_2} M_{D.kur}) \quad \text{Equation 10}$$

and the number of *D. kuriphilus* adults present in site 2 after migration, denoted  $A_2(t)$ , takes a symmetrical form :

$$A_2(t) = R_H (H_2(t) F_{s_2} (1 - M_{D.kur}) + H_1(t) F_{s_1} M_{D.kur}) \quad \text{Equation 10'}$$

In each of the two sites, the abundance of dormant larvae in chestnut tree buds, denoted  $H_{D1}(t)$  and  $H_{D2}(t)$  can be expressed from  $A_1(t)$  and  $A_2(t)$  with respect to the number of eggs produced per adult ( $F_H$ ), their survival probability to developmental failures ( $S_{oH}$ ), and two site dependent rates; the fraction of deposited eggs ( $d_1$ ) and survival to the chestnut tree immune response ( $S_{e1}$ ). The expression of  $H_{D1}(t)$  is given by :

$$H_{D1}(t) = A_1(t) F_H d_1 S_{e1} S_{oH} \quad \text{Equation 11}$$

and the the expression of  $H_{D2}(t)$  is

$$H_{D2}(t) = A_2(t) F_H d_2 S_{e2} S_{oH} \quad \text{Equation 11'}$$

The life-cycle of *D. kuriphilus* was then completed in each site by the survival of dormant larvae described by using the same density-dependent function as in the one site model, where the carrying capacity were defined by  $K_1 = N_1 p_{c1} k$  and  $K_2 = N_2 p_{c2} k$ , according to the densities of trees,  $N_1$  and  $N_2$ , and frequency of chestnut trees,  $p_{c1}$  and  $p_{c2}$ , in sites 1 and 2, respectively.

The extension of the one-site model to a two sites model using equations 9 to 11, provided the following sets of non-linear difference equations :

$$H_1(t + 1) = K_1 \left( 1 - e^{-\frac{(R_H(H_1(t) F_{S1}(1-M_{D.kur}) + H_2(t) F_{S2} M_{D.kur})) R_{A1}}{K_1}} \right) \quad \text{Equation 14}$$

$$P_{S_1}(t + 1) = H_1(t) \left( 1 - e^{-(\alpha_s \cdot p_c) P_{Sm_1}(t)} \right) SLA_p \quad \text{Equation 15}$$

$$H_2(t + 1) = K_2 \left( 1 - e^{-\frac{(R_H(H_2(t) F_{S2}(1-M_{D.kur}) + H_1(t) F_{S1} M_{D.kur})) R_{A2}}{K_2}} \right) \quad \text{Equation 14}$$

$$P_{S_2}(t + 1) = H_2(t) \left( 1 - e^{-(\alpha_s \cdot p_c) P_{Sm_2}(t)} \right) SLA_p \quad \text{Equation 15}$$

This model was analyzed by running simulations performed with R.4.1.1 (R Core Team, 2021). The characteristics of the forest environment (N,  $p_c$  and  $Se$ ) in each site were then adjusted to their estimates derived from our 23 sampling sites, in order to investigate levels of heterogeneity corresponding to those observed in the Pyrénées-Orientales. The rate of *D. kuriphilus* and *T. sinensis* dispersal were then varied to test the effect of a large range of coupling between the various combinations of heterogeneity between the two sites.

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## SUPPORTING INFORMATION

**Supplementary Material 1.** Local stability analysis of the one-site *D. kuriphilus* – *T. sinensis* interaction model.

Consider the equations of the non-spatialized model:

$$\begin{cases} H(t+1) = K(1 - e^{-\frac{RH(t)e^{-\alpha'_s P_s(t)}}{K}}) \\ P_s(t+1) = cH(t)(1 - e^{-\alpha'_s P_s(t)}) \end{cases}$$

where  $R = F_{nf} F_{ni} SL_H SA_H F_H d Se So_H$  and  $c = SLA_P$

The Jacobian matrix at equilibrium  $H^*$  and  $P_s^*$ , is :

$$A_{(H^*, P_s^*)} = \begin{pmatrix} \frac{\partial f}{\partial H^*} & \frac{\partial f}{\partial P_s^*} \\ \frac{\partial g}{\partial H^*} & \frac{\partial g}{\partial P_s^*} \end{pmatrix} = \begin{pmatrix} R e^{-\frac{RH^*}{K} e^{-\alpha'_s P_s^*} - \alpha'_s P_s^*} & -RH^* \alpha'_s e^{-\frac{RH^*}{K} e^{-\alpha'_s P_s^*} - \alpha'_s P_s^*} \\ c(1 - e^{-\alpha'_s P_s^*}) & cH^* \alpha'_s e^{-\alpha'_s P_s^*} \end{pmatrix}$$

where  $H^* = K \left(1 - e^{-\frac{RH^*}{K} e^{-\alpha'_s P_s^*}}\right)$ , so that  $e^{-\frac{RH^*}{K} e^{-\alpha'_s P_s^*}} = 1 - \frac{H^*}{K}$  leading to :

$$A_{(H^*, P_s^*)} = \begin{pmatrix} R(1 - \frac{H^*}{K})e^{-\alpha'_s P_s^*} & -RH^* \alpha'_s (1 - \frac{H^*}{K})e^{-\alpha'_s P_s^*} \\ c(1 - e^{-\alpha'_s P_s^*}) & cH^* \alpha'_s e^{-\alpha'_s P_s^*} \end{pmatrix}$$

From this matrix, the stability of any equilibrium points can be found using the stability condition that is equivalent to the absolute value of both eigenvalues of A being lower than 1 (Edelstein-Keshet, 2005, page 57) :  $|tr(A)| < \det(A) + 1 < 2$ .

1) We first considered the stability of the trivial equilibrium ( $H^* = 0, P_s^* = 0$ ).

The stability condition then simplifies to  $R < 1$  as  $tr(A)=R$  and  $\det(A)=0$ . This leads to

$$F_{nf} F_{ni} SL_H SA_H F_H d Se So_H < 1$$

which we expressed as

$$Se < \frac{1}{F_{nf} F_{ni} SL_H SA_H F_H d So_H}$$

to ease its representation in Figure 1, where it defines the upper limit of the dark blue area.

2) We second considered the stability of the equilibrium where only *D. kuriphilus* persists, i.e.  $(H^*, 0)$ .

The Jacobian matrix then becomes

$$A_{(H^*, 0)} = \begin{pmatrix} R\left(1 - \frac{H^*}{K}\right) & -RH^* \alpha'_s \left(1 - \frac{H^*}{K}\right) \\ 0 & cH^* \alpha'_s \end{pmatrix}$$

and the stability condition reads:

$$\left| R\left(1 - \frac{H^*}{K}\right) + cH^* \alpha'_s \right| < R\left(1 - \frac{H^*}{K}\right) cH^* \alpha'_s + 1 < 2$$

In the absence of the control agent *T. sinensis*, i.e.  $P_s^* = 0$ , *D. kuriphilus* tends to saturate the environment :  $H^* = K \left(1 - e^{-\frac{RH^*}{K}}\right) \rightarrow K(1 - e^{-R}) \approx K$  (as R is typically in the range of 10-20, e.g. Zitoun et al 2023), and the condition simplifies as

$$cH^* \alpha'_s < 1$$

which can be re-written

$$cK(1 - e^{-R}) \alpha'_s < 1$$

and, since  $R = F_{nf} F_{ni} SL_H SA_H F_H d So_H Se$ , this leads to

$$Se < \frac{\ln\left(1 - \frac{1}{cK\alpha'_s}\right)^{-1}}{F_{nf} F_{ni} SL_H SA_H F_H d So_H}$$

which allowed to draw the upper limit of the light blue area in Figure 1.

The last two dynamics to discriminate were when both species would coexist at a stable equilibrium  $(H^*, P^*)$  and when such point would be unstable and the two species would instead coexist through stable oscillations. The stability condition of the equilibrium point  $(H^*, P^*)$  read:

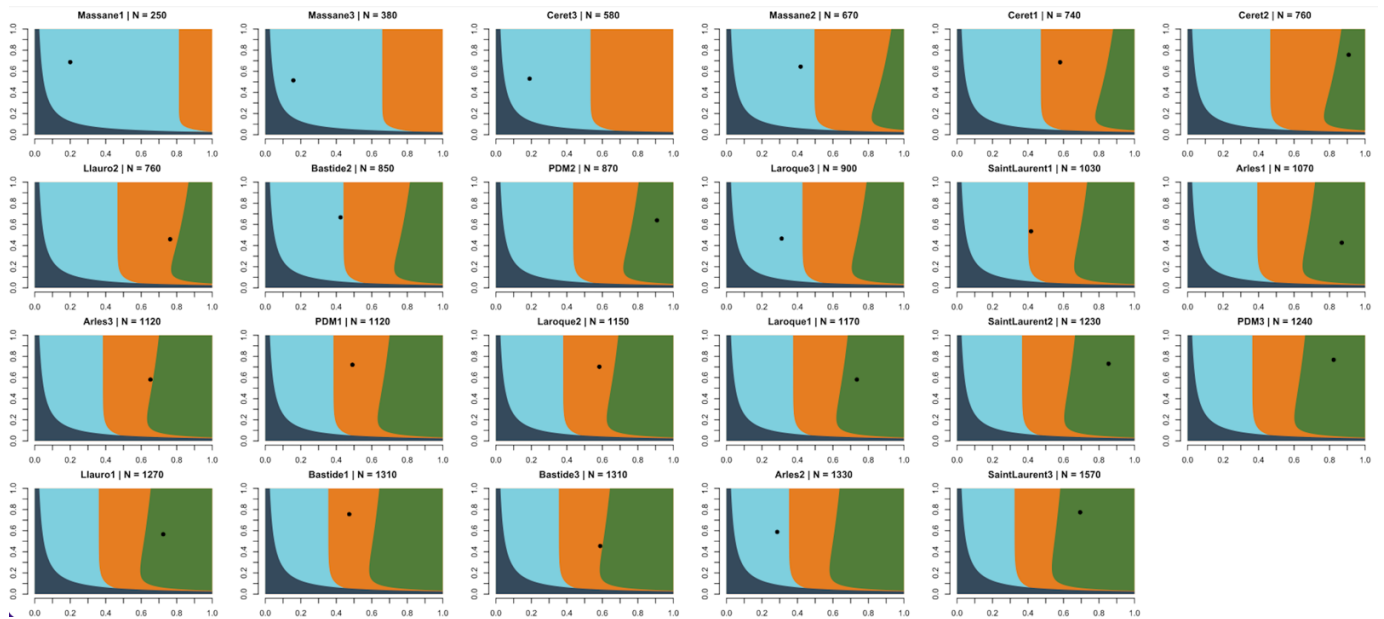
$$|R A B + SLA_p H^* \alpha'_s B| < R A B SLA_p H^* \alpha'_s + 1 < 2$$

where  $A = R\left(1 - \frac{H^*}{K}\right)$  and  $B = cH^* \alpha'_s$ .

Those conditions were evaluated numerically, which allowed to draw the line between the orange and green areas in Figure 1.



**Supplementary Material 2.** Predicted population dynamic outcomes of the *D. kuriphilus* – *T. sinensis* model for each of the 23 studied sites according to their chestnut tree density, frequency and genetic susceptibility. The dot in each panel corresponds to the observed frequency (x-axis) and average genetic susceptibility (y axis) of chestnut trees, and the limits between the different areas are set with respect to the observed tree density in the forest environment.



**Supplementary Material 3.** Definition and estimates of the parameters of the *D. kuriphilus* host - *T. sinensis* and native parasitoids dynamical model.

	Symbol	Estimate and 95% confidence interval	References
<b><i>Dryocosmus kuriphilus</i></b>			
Egg survival	So	$0.535 \pm 0.003$	Nugnes et al. (2018)
Larvae survival	SL <sub>H</sub>	$0.936 \pm 0.01$	Panzavolta et al. (2013)
Adult survival	SA <sub>H</sub>	$0.952 \pm 0.01$	Cooper and Rieske (2010)
Fertility	F <sub>H</sub>	$90 \pm 6$	Zitoun et al. (2023)
Carrying capacity per hectare	k	14 880 000	Zitoun et al. (2023)
<b><i>Torymus sinensis</i></b>			
Larvae and adult survival	SL <sub>Ap</sub>	$0.913 \pm 0.004$	Ferracini et al. (2015)
Searching area	a <sub>s</sub>	$4.4 \times 10^{-4} \pm 0.4 \times 10^{-4}$	Zitoun et al. (2023)
<b><i>Native parasitoids fungi</i></b>			
Survival to fungal infection	F <sub>nf</sub>	$0.946 \pm 0.001$	Zitoun et al. (2023)
Survival to hymenopteran infection	F <sub>ni</sub>	$0.956 \pm 0.02$	Zitoun et al. (2023)

## Chapter III

### **An integrative modelling of key species interference with invasive species and biological control strategies : the case of the global chestnut tree pest, *Dryocosmus kuriphilus*, in the natural forests of the Pyrénées-Orientales.**

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## Introduction

Invasive species are considered as a global threat associated with the rise of international trade (Seebens et al., 2017) as they have strong environmental and economic impacts (Keller et al., 2011; Marbuah et al., 2014). They represent a major risk factor for biodiversity in natural ecosystems (Winter et al., 2009) being involved in 74% of animal extinctions (Clavero and García-Berthou, 2005) and in hybridization with native species (Ayres et al., 2004), spreading pathogens worldwide (Stricker et al., 2016) and perturbing trophic networks (Hulme, 2017). Damages caused by alien pests on ‘natural valued’ biological resources and attempts to limit them stand as one of the main causes of economic losses (Pagad et al., 2018), with an annual cost reaching US\$26.8 billion worldwide (Diagne et al., 2021). When integrating a local ecosystem, any invasive pest faces natural bottom-up and top-down regulation factors that contribute to define its population growth and invasion success (e.g. Wilkinson and Sherratt 2016; Vidal and Murphy 2018; Strange et al., 2019; Despland and Santacruz, 2020). Resolving the mechanisms underlying these interactions and their impact on invasion dynamics then represents one of the major challenges to anticipate the risk of pest invasions and to set-up efficient control strategies once they occur.

Bottom-up regulation factors are associated with the resources used by an invasive species to grow its population and typically related to its feeding requirements. The distribution, genetic and physiological characteristics of such resources can indeed impact the establishment and spreading success of a pest population (Parsche and Lattorff, 2018; Ximénez-Embún et al., 2016). On the other hand, top-down regulation factors correspond to the predators, parasite and pathogens that can directly affect the survival or reproductive rates of an invasive species and therefore reduce its local growth (Twining et al., 2022; Prider et al., 2009; Lacey et al., 2015). Most control strategies implemented to prevent the spread or eradicate alien pests are actually based on either lowering the access of the invasive species to its resources by environmental and/or genetic modifications (Raman, 2017; Drechsler et al., 2016) or on reinforcing its top-down control through the ecological engineering of its natural enemies (Josephraj Kumar et al., 2022) or the introduction of biological agents (Bale et al., 2008).

Although feeding resources and natural enemies typically are the main local determinants of an invasion through their direct impact with the alien species (e.g. Vidal and Murphy 2018), other species, more distant in the trophic network, can also have significant impacts. According

to the host (plant) visual apparency and semiochemical redundancy hypotheses (Castagneyrol et al., 2013; Beyaert and Hilker, 2014; Randlkofer et al., 2010), the presence of species phylogenetically close to the resource used by the invader could induce a so-called ‘dilution effect’ on the pest population by lowering its ability to detect its resource (Salazar et al., 2016 ; Coley and Kursar, 2014). Beyond the effect of plants diversity in complex environments, some ‘key’ species could thus determine the level of interference in host location and contribute modulating the spread of invasive pests. Moreover, biological control strategies can fail or be enhanced due to biotic interference with native key species. Indeed, predation pressure on the control agent (Goeden et Louda, 1976), inter-specific competition for the resource (Crowe and Bouchier, 2006) or alternative host/refuge (Roschewitz et al., 2005) can alter the efficacy of biological control. Although key species have no direct bottom-up or top-down effects on the invasive species, they can influence its ability to spread in the natural environment and/or the efficacy of introduced biological control agents (Pschorn-Walcher, 1977; Dahlsten and Mills, 1999).

Despite their potential impacts, the intrinsically indirect contributions of such key species remain broadly overlooked as invasion ecology is usually focused on the already delicate partitioning of the role of bottom-up or top-down factors (Hunter et al., 1997, Frost et al., 2019, Wilkinson and Sherratt, 2016, Vidal and Murphy, 2018). The integration of empirical knowledge of the direct (bottom-up and top-down) determinants of invasion into dynamic models provides an efficient approach to assess their relative impacts on the spread of exogenous species in newly invaded environments (e.g. Bendor and Metcalf, 2006, Kueffer et al., 2013 , Zitoun et al., 2023). The analysis of such modelling, further accounting for the indirect effect of ‘key’ species, would allow for quantitative predictions about their contributions to typical features of the invasion and its control, such as the rate of spread of the invasive in its natural environment (Prasad et al., 2010; Kueffer et al., 2013; Baker and Bode, 2021) and the dynamical outcomes of the interaction with its control agents (Zalucki and Van Klinken, 2006; Trethowan et al., 2011; Augustinus et al., 2020).

We intended to develop such an approach to improve our understanding of the determinants of the spread of the Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus*, a worldwide invasive species that represents the most dangerous insect pest to orchards and forests worldwide (Fernandes et al., 2022). The chestnut tree (*Castanea sativa*) is an exploited resource of great economic interest for both chestnut and wood production. It has incurred very

significant losses since *D. kuriphilus*, a cynipid originated from China, has spread across Asia (Murakami et al., 1981; Cho & Lee, 1963), North-America (Payne et al., 1976; Huber and Read, 2012) and Europe (Brussino et al., 2002; Aebi et al., 2006; EPPO global database). The galling activity of the pest inhibits the development of the shoots and ultimately lead to reduction of up to 80% of fruit production (Battisti et al., 2014) and of up to 60% of the annual tree-ring increment (Marcolin et al., 2021). The impact of bottom-up and top-down regulation factors on the spread of *D. kuriphilus* in the natural chestnut tree forests of the Pyrénées-Orientales, an area at the French-Spanish border, have recently been investigated using an ‘ecogenomic’ modelling (Zitoun et al., 2023). While this integrative approach has shown that the chestnut tree frequency and genetic susceptibility are the main natural determinants of *D. kuriphilus* invasion potential and that the outcome of releasing its control agent, *Torymus sinensis*, primarily depends on the frequency of chestnut trees, the impacts of other species present in the natural chestnut tree forests, namely *Quercus pubescens*, *Fagus sylvatica* and *Cryphonectria parasitica*, the causal agent of the chestnut blight, were not assessed in this study.

In this contribution, we aim at producing quantitative insights into four main hypothetical mechanisms by which those ‘key’ species could have an impact on the spread of *D. kuriphilus* and its control agent, *T. sinensis*. First, *Quercus pubescens*, the major host of most cynipid parasites, could lower the ability of *D. kuriphilus* to detect its chestnut tree hosts through the perturbation of odor clues (Salazar et al., 2016; Coley and Kursar, 2014). Second, local trees attacked by natives gall-forming parasites, i.e. *Q. pubescens* and *F. sylvatica*, could increase the native parasitoid production (Ferracini et al., 2018) or, third, constitute a new ecological niche for the control agent *T. sinensis* (Ferracini et al., 2017). Fourth, *C. parasitica*, could alter the detection of chestnut trees by *D. kuriphilus* by modifying the physiological characteristics of the host (Ximénez-Embún et al., 2016). Infected trees are indeed expected to release more phenolics compounds due to the damages induced by the chestnut blight (Germinara et al., 2011). To assess the impact of those four potential effects of key species, we set-up a three years ecological field study to estimate the level of *D. kuriphilus* and *C. parasitica* infestation in 24 chestnut tree populations located in the Pyrénées-Orientales. We concomitantly identified the parasitoids, both native species and the control agent *T. sinensis*, infesting *D. kuriphilus* galls found on chestnut trees, and galls from other parasites that were collected on oaks and beeches within the sampled forests. These data allowed to test the impact of the four above mechanisms and were integrated into a host-parasite-hyperparasite model to quantify the

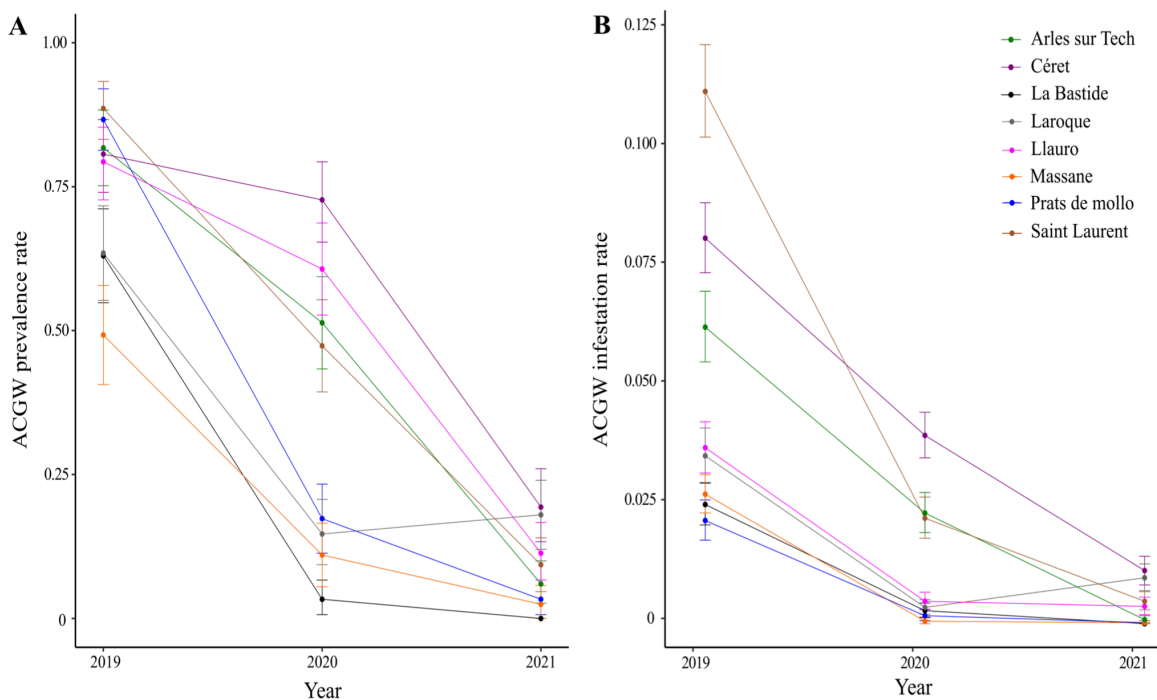
dynamical implications of the putative key species on the spread of *D. kuriphilus* and its control by *T. sinensis*.

## Results

### Significant and uneven decreases of the spatially heterogeneous infestation of chestnut trees by *D. kuriphilus* between 2019 and 2021

The chestnut tree populations located in the 24 sampling sites were all found infested by *D. kuriphilus* in 2019, and the infestation level decreased across the entire study area between 2019 and 2021, with heterogeneous reduction rates among sites.

In 2019, 75% of individual chestnut trees were infested with an average of 0.05 galls per leaf, representing a parasitic burden of 1 gall every 20 leaves. The overall proportion of infested trees dropped to 35% with an average of 0.01 galls per leaf in 2020, and then to 9% with an average of 0.004 galls per leaf in 2021 (Figure 1, SM1). The prevalence of infestation (proportion of infested trees) showed spatial heterogeneity between sites ( $\chi^2 = 271.45$ ,  $df = 71$ ,  $p < 2.2 \cdot 10^{-16}$ ) and between stations ( $\chi^2 = 160.96$ ,  $df = 23$ ,  $p < 2.2 \cdot 10^{-16}$ ), and the rate of infestation (average number of galls per leaf) was also found to be heterogeneous between sites ( $\chi^2 = 1056.6$ ,  $df = 71$ ,  $p < 2.2 \cdot 10^{-16}$ ) and stations ( $\chi^2 = 682.3$ ,  $df = 23$ ,  $p < 2.2 \cdot 10^{-16}$ ).



**Figure 1. Infestation of the chestnut tree populations by *D. kuriphilus* in the Pyrénées-Orientales.** Measures of the prevalence (A) and rate (B) of infestation made in 2019, 2020 and 2021 in 8 stations appear with their 95% confidence intervals. Each dot represents the mean prevalence/infestation rate estimated from 3 sites per station and per year.

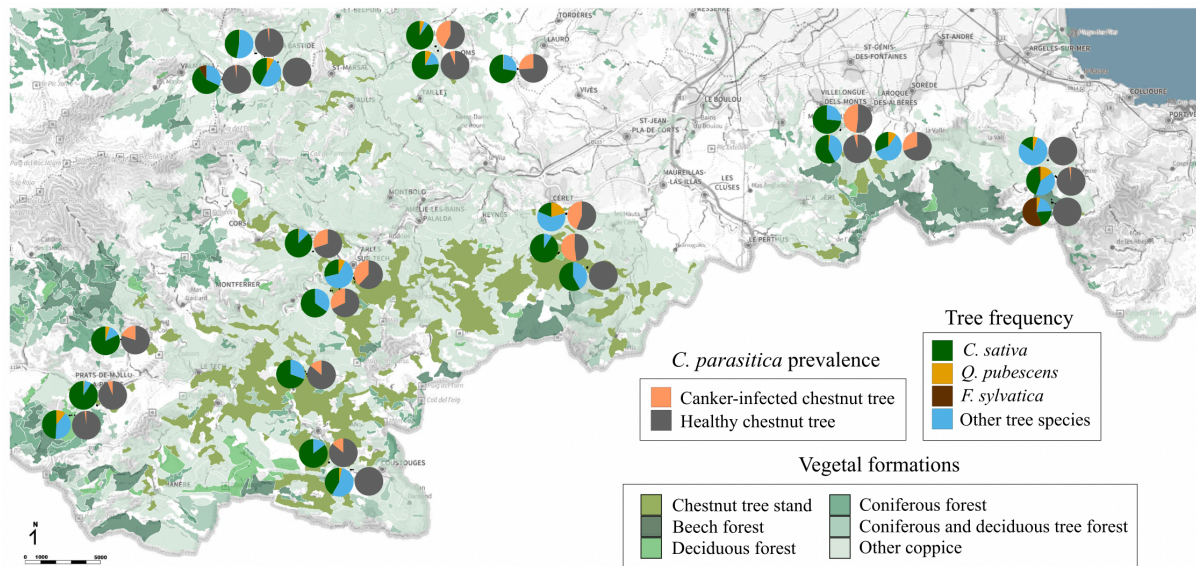
The decrease in prevalence and infestation rate observed in Figure 1 between 2019 and 2020 represents an average reduction of 53% and 73% in these two infestation measures, while drops of 75% and 71% were observed for the 2020-2021 period, leading to mean reductions of 88% and 92% over two years. Although a decrease in both prevalence and infestation rate was observed in almost all sampling sites between 2019 and 2021, significant variations in the amplitude of changes were observed in prevalence between stations ( $\chi^2 = 16.815$ ,  $df = 7$ ,  $p = 0.01863$ ) and sites ( $\chi^2 = 53.618$ ,  $df = 23$ ,  $p = 0.000302$ ) and in the rate of infestation ( $\chi^2 = 261.46$ ,  $df = 7$ ,  $p < 2.2 \cdot 10^{-16}$ ,  $\chi^2 = 466.29$ ,  $df = 23$ ,  $p < 2.2 \cdot 10^{-16}$ ). Notably, 6 sampling sites, 3 of which corresponding to Laroque station, even showed an increase in infestation between 2020 and 2021.

Overall, *D. kuriphilus* was widely spread across the chestnut tree populations of the Pyrénées-Orientales with a strong spatial heterogeneity in prevalence and infestation rates observed at station and site scales, and with both indexes of infestation pointing toward a decrease in *D. kuriphilus* abundance in almost all stations sampled between 2019 and 2021. In the following, we intend to look for the contribution of key species to the observed spatial heterogeneity in the infestation of chestnut tree populations, and to the overall decrease of *D. kuriphilus* abundance, which required to estimate their distribution across all sampling sites.

### ***D. kuriphilus* spread across admixtures of pubescent oaks, beeches and chestnut trees with heterogeneous chestnut blight infestation levels**

The tree community structure of the study area was characterized by assessing, in the 24 sampling sites, the proportions of chestnut trees, pubescent oaks and beeches (Figure 2). Chestnut trees were found predominant in 19 of the 24 sampling sites where their overall frequency ranges from 15.8% to 90.8% (SM 1). Pubescent oak was present in 17 sites with frequency varying between 0.8% and 20.7%, while beech was recorded only in 3 sites with frequency ranging from 3% to 56%. The local estimates of the species structure in sampling sites were consistent with the largest scale description of vegetal formations found in national

inventories, as sites located ‘Chestnut tree stand’ typically showed higher chestnut tree frequencies as compared to those located in ‘deciduous’ and ‘deciduous and coniferous forests’ ( $0.51 \pm 0.01$ ) (Figure 2).



**Figure 2.** Map of the vegetal formations in Pyrénées-Orientales with key species frequencies recorded in the 24 sampling sites. Measures of the frequency of *Quercus pubescens*, *Fagus sylvatica*, *Castanea sativa* and *Cryphonectria parasitica* prevalence made in 2020 in the 24 sampling sites are represented with pie charts. Different vegetal formations are represented on the map according to the legend.

Chestnut blight symptoms were observed on chestnut trees in 19 sites located in all 8 stations (Figure 2, SM 1) with significant heterogeneity in the infestation levels by the parasitic fungus ( $\chi^2 = 115.32$ ,  $df = 23$ ,  $p = 2.871 \cdot 10^{-14}$ ). Overall, an average of 17.6% (207/1177) of chestnut trees were found to be infected by *C. parasitica*, with such prevalence rate varying between 2% and 52% between sites.

To look at the impact of the proposed key species, we looked at the correlation between the observed *D. kuriphilus* prevalence and rate of infestation, and the frequencies of chestnut trees, pubescent oaks, beeches and chestnut blight measured at the individual and/or site level.

**The *D. kuriphilus* infestation of chestnut trees is prevented by pubescent oaks and facilitated by chestnut blight infections.**



The generalized linear mixed model (GLMM) regression analysis revealed that the frequency of chestnut tree among the local forest stand strongly favours its infestation by *D. kuriphilus*. Significant positive correlations were observed between the frequency of chestnut trees and both site prevalence and rate of infestation (Table 1A-B). However, the best model explaining *D. kuriphilus* infestation rate variations accounted for both pubescent oaks and chestnut trees frequencies, with the former lowering the level of infestation. Meanwhile, the prevalence of *D. kuriphilus* infestation was also positively correlated with the chestnut blight prevalence estimated in each site. The correlation with canker infections was further found to be significant at the individual tree level ( $\chi^2 = 57.972$ ,  $df = 1$ ,  $p = 2.7 \cdot 10^{-14}$ ), with a higher number of chestnut trees infected by both species than expected under the assumption of independent parasitism (Table 1C).

Fixed effects	Estimate	Standard error	df	P
<b>A</b> Response variable : Prevalence rate				
Intercept	-2.506713	0.9800511	67	0.128
Chestnut tree frequency	2.629348	0.6340386	67	0.0001
Canker prevalence	1.698257	0.8205657	67	0.0423
<b>B</b> Response variable : Infestation rate				
Intercept	-4.875602	0.775379	66	2.2e-16
Chestnut tree frequency	1.471378	0.644681	66	0.0257
Pubescent oak frequency	4.993287	5.494050	66	0.3667
Chestnut tree * Pubescent oak frequency	-30.591761	12.240434	66	0.0149
<b>C</b>				
	ACGW (-)	ACGW (+)		
<i>C. parasitica</i> (-)	676 (627.99)	294 (342.01)		970
<i>C. parasitica</i> (+)	86 (134.01)	121 (72.99)		207
	762	415		

**Table 1. Statistical analysis results for key species frequency impact on ACGW infestation levels.** (A) GLMM results for the correlation between chestnut tree, pubescent oak frequency, chestnut blight prevalence with prevalence and (B) infestation rate at the site level. (C) Contingency table of the number of chestnut tree showing ACGW galls and/or chestnut blight symptoms.

The facilitative effect of *C. parasitica* infection on the prevalence of *D. kuriphilus* on chestnut trees supports the hypothesis that the detection of chestnut blight infected trees is eased by the physiological characteristics of the hosts (Germinara et al. 2011), while the negative contribution of *Q. pubescens*, to explain *D. kuriphilus* rate of infestation is consistent with the hypothesis that the release of odor clues by this alternative tree species can perturbate dispersing insects and create a dilution effect through the oviposition on oak buds (Salazar et al., 2016; Coley and Kursar, 2014; Huang et al., 2016). The presence of gall-forming tree species, *Q. pubescens* and *F. sylvatica*, was also proposed to have an impact on the *D. kuriphilus* populations by allowing for the production of native parasitoids (Ferracini et al., 2018) and/or providing an additional ecological niche for the control agent, *T. sinensis* (Ferracini et al., 2017). We thus characterized the parasitoid communities associated with galls formed on each of these two tree species to identify their possible indirect impacts on the invasive pest.

**The galls found on *Quercus pubescens* contain both the control agent, *T. sinensis*, and native parasitoids able to infest *D. kuriphilus***

We dissected 471 galls collected on *Q. pubescens* (208) and *F. sylvatica* (263) in order to identify the different species of native or introduced parasitoids able to hyperparasite the larvae of the local hymenoptera, *Andricus kollari* and *Andricus dentimitratus*, and diptera, *Mikiola fagi*, that induce the formation of those galls on oaks and beeches, respectively.

*A. kollari* galls were found on pubescent oaks in 21 of our sampling sites, with 34% of individual oak trees being infested at an average rate of 0.004 galls per leaf. *A. dentimitratus* galls were observed in 18 sites, with 18% of pubescent oaks infested at an average rate of 0.011 galls per leaf. *M. fagi* galls were observed on beeches in all 3 stations where the tree species was present. All individual trees were then found infested with an average rate of 0.102 galls per leaf. From the 471 galls formed on oaks and beeches by the three local hymenoptera and diptera parasite species, we obtained 400 larvae that were pooled according to their geographic origin for molecular taxonomic identification. The parasite and parasitoid species identified inside the galls of each local parasite species and in the different geographic locations are presented in Table 2.

Species	AKAD-1 n = 39	AKAD-2 n = 33	AKAD-MA n = 66	AD-MA n = 45	AK-MA n = 16	MF-BA n = 97	MF-MA n = 104
<i>Andricus caputmedusae</i>	X	X	X	X			
<i>Andricus kollari</i>	X	X	X				
<i>Andricus quercustozae</i>	X	X		X			
<i>Andricus sp.</i>	X	X	X				
<i>Eulophidae sp.</i>						X	X
<i>Eupelmus sp.</i>						X	X
<i>Eurytoma rosae</i>		X		X			
<i>Eurytoma sp.</i>	X		X				
<i>Megastigmus dorsalis</i>			X				
<i>Megastigmus stigmatizans</i>	X	X	X	X	X		
<i>Nasiona sp.</i>	X						
<i>Synergus sp.</i>		X		X			
<i>Torymus auratus</i>		X					
<i>Torymus flavipes</i>			X				
<i>Torymus sinensis</i>	X	X	X		X		
<i>Torymus sp.</i>						X	

**Table 2. Parasite and parasitoid species observed in galls found on *Q. pubescens* and *F. sylvatica*.** Results of the molecular taxonomic identification of the larvae contained in galls of *A. kollari* (AK), *A. dentrimitratus* (AD) and *M. fagi* (MF) and found in the geographic pools denoted (1) = Céret, Saint-Laurent and Laroque; (2) = Prats de mollo, Arles sur Tech and Llauro; (MA) = Massane, and (BA) = La Bastide. n stands for the number of larvae in each subset.

The molecular taxonomic identification of larvae found in galls (of *A. kollari* and *A. dentimitratus*) that were collected on pubescent oaks revealed the presence of at least 3 hymenopteran species known to infest *D. kuriphilus* larvae (*Torymus sinensis*, *Torymus auratus*, *Torymus flavipes*). Although native parasitoids (*T. auratus*, *T. flavipes*) were expected to be observed on their endemic hosts, the finding of the *T. sinensis* control agent in 4 out of 5 DNA pools originated from galls collected on *Q. pubescens* sharply contrasts with its description as a specialist parasitoid of *D. kuriphilus*. While the DNA of *Torymus sp.*, *Eulophidae sp.* and *Eupelmus sp.* were found in galls (of *M. fagi*) collected on beeches, the taxonomic resolution remained insufficient to establish that the corresponding species could potentially infest *D. kuriphilus* larvae.

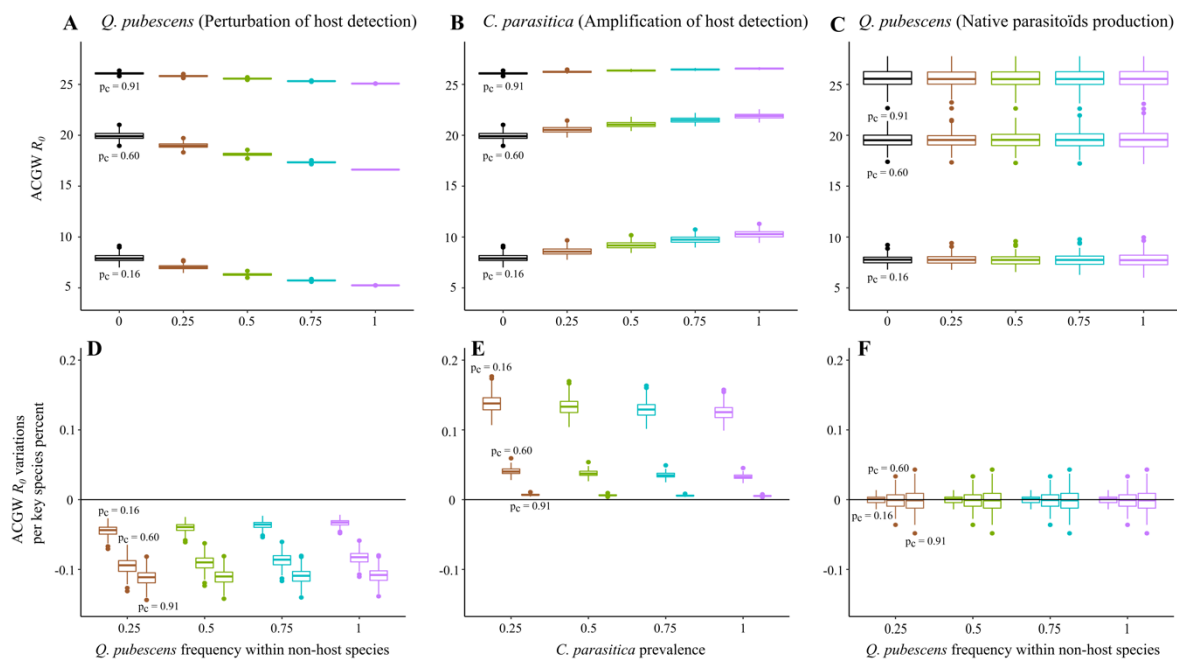
This analysis of the parasitoid community associated with *Q. pubescens* and *F. sylvatica* provided evidence that galls found on pubescent oaks harbour native and introduced parasitoids able to infect *D. kuriphilus*. This supports the hypothesis that, beyond its dilution effect on oviposition, *Q. pubescens* presence could contribute to the regulation of *D. kuriphilus* invasion

by increasing native parasitoid production (Ferracini et al., 2018) and/or providing a new ecological niche for the control agent *T. sinensis* (Ferracini et al., 2017).

To provide further quantitative insights into the role of key species in the spread of *D. kuriphilus* and its control agent, *T. sinensis*, we integrated the effects that were empirically supported into a host-parasite-hyperparasite dynamical model. The modelling developed by Zitoun et al (2023) was expanded to account for the i) perturbation of *D. kuriphilus* oviposition by *Q. pubescens*, the ii) facilitative impact of *C. parasitica* on *D. kuriphilus* host detection, and the iii) production of native parasitoids and iv) introduced *T. sinensis* in galls formed on *Q. pubescens*. We then used this modelling to assess the overall effect of *C. parasitica* and *Q. pubescens* on the spread of *D. kuriphilus* and the efficacy of its biological control by *T. sinensis*.

### The $R_0$ of *D. kuriphilus* is under contrasted effects of pubescent oaks and chestnut blight

To understand the impact of the above key species on the invasive potential of *D. kuriphilus*, we investigated their effects (i to iii) on the growth rate ( $R_0$ ) of the pest population, which we estimated by varying their frequencies in our modelling (Figure 3). We overall showed that the effects of pubescent oaks and chestnut blight on *D. kuriphilus* host detection and oviposition could induce significant variations of the  $R_0$  (Figure 3 A, B, D, E), while the production of native parasitoids allowed by *Q. pubescens* had almost no effect (Figure 3 C, F).



**Figure 3. Variations of *D. kuriphilus*  $R_0$  with the effects of *Q. pubescens* or *C. parasitica* in forests with low, medium and high abundances of chestnut trees.** The variations of  $R_0$  are shown with respect to the effect of *Q. pubescens* (A) and *C. parasitica* (B) on *D. kuriphilus* detection of chestnut trees and oviposition, and according to the impact of *Q. pubescens* on the production of native parasitoids (C). The absolute variations of  $R_0$  predicted in A-C, were then transformed into a variation of  $R_0$  per percent of change in the frequencies of key species (D,E,F). In each specific condition, the variability in the estimate of  $R_0$  (calculated from the confidence intervals around the mean value of all its defining parameters, see ‘Material and Method’) was represented by a boxplot, and found limited as compared to changes induced by the key species.

The reduction of the oviposition rate of *D. kuriphilus* associated with the presence of *Q. pubescens* led to a dilution effect (Fernandez-Conradi et al., 2018; Huang et al., 2016) in all three chestnut tree forests considered, as measured by a systematic decrease of  $R_0$  proportional to the frequency of *Q. pubescens* (Figure 3A). In a forest made of 60% of chestnut trees, the average value observed across our sampling stations, this effect reduced the  $R_0$  value from 19.9 to 16.6 (-16%) when the frequency of pubescent oaks (among the remaining trees) was risen from 0 to 100%. As expected, the intensity of this dilution of *D. kuriphilus* invasion was accentuated when considering the lowest frequency of chestnut trees measured in our sampling sites, i.e. 16%, as the frequency of *Q. pubescens* in the stand was allowed to reach higher values up to 84%. The *D. kuriphilus*  $R_0$  then decreased by up to 35%, falling from 6.8 to 4.4. Accordingly, a lower dilution was observed when considering the maximal frequency of chestnut trees observed in our study, i.e. 91%. The value of  $R_0$  then varied from 26.1 to 25.1, representing a 3.8% reduction. Noteworthy, the calculation of those reductions of the invasive potential of *D. kuriphilus* per percent of change in the frequency of *Q. pubescens* (Figure 3 D-E), confirmed that its effects are mostly cumulative as adding a percent of oaks had the same impact whatever its current occurrence in the stand.

The facilitation of *D. kuriphilus* host detection and oviposition associated with the presence of *C. parasitica* contributed to amplify the pest population growth in the modelled chestnut tree forests, as measured by the increases of  $R_0$  proportional to the prevalence of *C. parasitica* (Figure 3B). In forest with an intermediate frequency of chestnut trees, this amplification effect increased the value of  $R_0$  from 19.9 to 21.9, i.e. by up to 10%. As for the dilution effect induced by *Q. pubescens*, the impact of chestnut blight was greater in a forest where the chestnut tree frequency was set at its lowest value, where it rose the value of  $R_0$  from 6.8 to 9, i.e. by up to 24%. Accordingly, the effect of *C. parasitica* was minimal when the frequency of chestnut

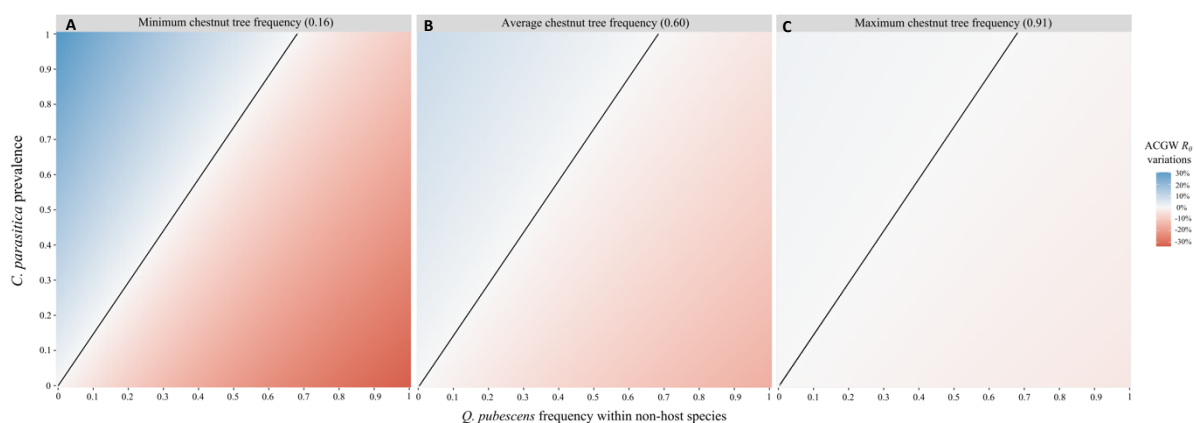
trees was at its highest. The value of  $R_0$  was then increased by a maximum of 1.9%, from 26.1 to 26.6. The expression of the changes in the invasive potential of *D. kuriphilus* per percent of change in the prevalence of *C. parasitica* (Figure 3E) showed that its amplification effect also appears to be a merely cumulative process, as any additional percent of canker infected chestnut trees had the same effect on whatever the current level of infection.

While the impacts of *Q. pubescens* and *C. parasitica* on *D. kuriphilus* host detection and oviposition lead to significant effects on the invasion potential of the pest, the production of native parasitoids in galls formed on *Q. pubescens* had almost no effect on *D. kuriphilus*  $R_0$ . (Figure 3C and F). Even when the chestnut trees were only mixed with *Q. pubescens* the reduction of the value of  $R_0$  was indeed limited to 0.1%.

The above analyses of the dependency of *D. kuriphilus*  $R_0$  on the presence of *Q. pubescens* and *C. parasitica* have shown that, they can dilute and amplify the invasive potential of the pest in roughly similar proportion when considered independently. We further used our modelling to investigate how these effects combine when considered jointly.

### The dilution effect of pubescent oaks tend to dominate the facilitative effect of chestnut blight on the invasive potential of *D. kuriphilus*

The variations of the  $R_0$  of *D. kuriphilus* with the frequency of pubescent oaks and the prevalence of chestnut blight showed that the dilution effect of *Q. pubescens* tends to have a stronger impact than the amplification effect due to *C. parasitica* (Figure 4).



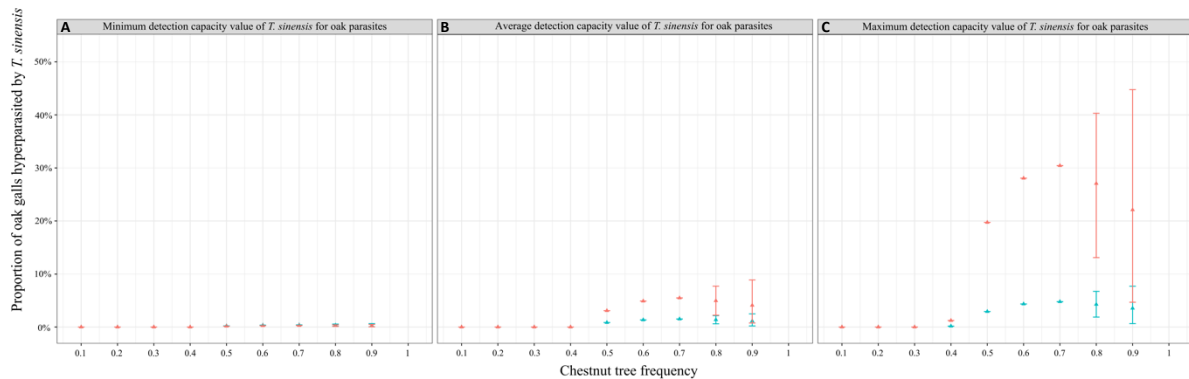
**Figure 4. Variations of *D. kuriphilus*  $R_0$  induced by the concomitant presence of *Q. pubescens* and *C. parasitica* in chestnut tree forests.** The value of  $R_0$  were estimated according to the frequency of *Q. pubescens* (among non-chestnut trees) and the prevalence of *C. parasitica*, for the same 3 frequencies of chestnut trees as in figure 3; A = 16%, B = 60% and C = 91%. In all three panels, the conditions allowing for  $R_0$  to be positive, null and negative appear in blue, white and red, respectively, with intensity revealing the percentage of variations in  $R_0$ .

The percentage of change in *D. kuriphilus*  $R_0$  associated with the simultaneous variations of *Q. pubescens* and *C. parasitica* were predicted to decrease with the frequency of chestnut trees in the forest, in line with the results shown in figure 3A-B. At intermediate frequency of chestnut trees, *D. kuriphilus*  $R_0$  varied from -17% (at high *Q. pubescens* frequency and low prevalence of *C. parasitica*) to +10% (at low *Q. pubescens* frequency and high prevalence of *C. parasitica*). This range of variations significantly changed when considering the lowest and highest frequencies of chestnut trees observed in our study area. In such cases, the  $R_0$  of *D. kuriphilus* varied from -35% to +33% and -4% to +2%, respectively. Importantly, for all three frequencies of chestnut trees in the forest environment, the set of *Q. pubescens* frequencies and *C. parasitica* prevalences leading to an overall reduction of the invasive potential of *D. kuriphilus* was broader than the set of conditions leading to a faster spread of the pest, suggesting that the dilution effect of pubescent oaks has a robust tendency to dominate the amplification effect of chestnut blight. According to our modelling outcomes, whatever be the frequency of chestnut trees, the effects of *C. parasitica* could still cancel out those of *Q. pubescens*, but only if its prevalence was about 150% larger than the frequency of pubescent oaks.

Overall, our model analysis showed that *Q. pubescens* and, to a lower extent, *C. parasitica* can actually be key species generating dilution and amplification effects on *D. kuriphilus* invasion through the modulation of its ability to detect chestnut trees to oviposit. While, on the contrary, our modelling predicted that the production of native parasitoids in galls formed on *Q. pubescens* should have no impact on *D. kuriphilus*, it remains to be used to test if the spread of the control agent, *T. sinensis*, can be eased by the hyperparasitism of those galls formed by native parasites of *Q. pubescens*.

## A significant proportion of *T. sinensis* population is able to colonize oak galls

Our modelling allowed to predict the rate of infection of the galls formed by *A. kollari* and *A. dentimitratus* on oaks by *T. sinensis*, according to the searching efficiency of the control agent and the proportion of oaks and chestnut tree in the forest.



**Figure 5. Predicted rates of hyperparasitism of oak gall forming parasites, *A. kollari* and *A. dentimitratus*, by *T. sinensis*.** The proportions of galls of *A. kollari* (blue) and *A. dentimitratus* (red) infested by *T. sinensis* were calculated for the minimum (A), average (B) and maximum value (C) of the 95% confidence interval of the searching area of the control agent (SM 3). Simulations were run for chestnut tree frequency ranging from 0 to 1, and while considering all other trees as pubescent oaks.

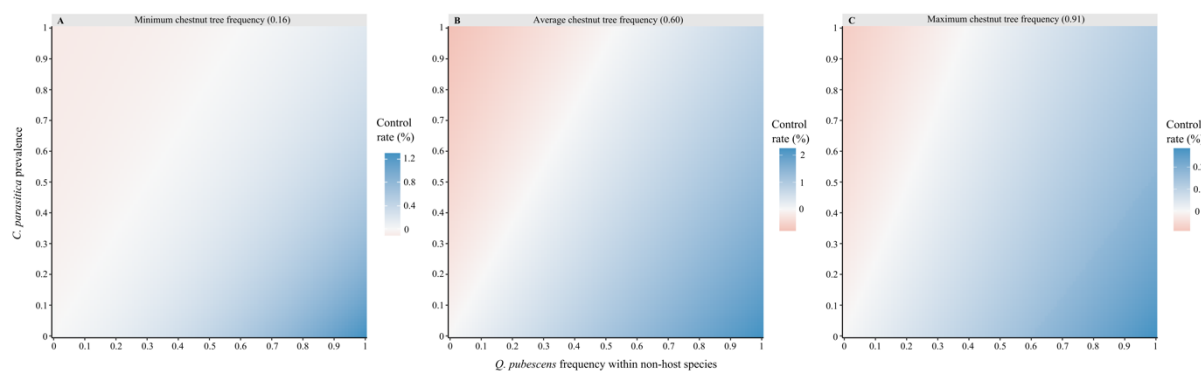
The mean rates of infection of *A. kollari* and *A. dentimitratus* by *T. sinensis* were shown to reach up to 1.51% and 5.49%, respectively, when considering the average estimate of *T. sinensis* searching area for each of the two parasites (Figure 5B). These predicted rates of parasitism varied substantially when considering lower and larger values of *T. sinensis* searching areas with variations ranging from 0.39% to 4.79% for *A. kollari* and from 0.29% to 30.5% for *A. dentimitratus* (Figure 5 A and C). Interestingly, behind these significant changes in the rates of hyperparasitism according to the search ability of the control agent, the remaining variations with respect to the proportion of chestnut trees showed a similar trend. The rate of oak galls infested by *T. sinensis* is typically null at chestnut tree frequencies lower than 40%, it increases to reach its highest level for chestnut tree frequency of 50-60%, and then decreases in stands dominated by chestnut trees, where strong oscillations between *D. kuriphilus* and *T. sinensis* are typically predicted.



Those model analyses strongly suggest that *T. sinensis* is indeed able to infect the galls of native parasite of pubescent oak, which could then be used as refuge during large oscillations known to result from the still relatively specific *D. kuriphilus* - *T. sinensis* interaction. We then proceed to the test of the impact of such a refuge on the biological control efficacy.

### Key species have a minor impact on the efficacy of *D. kuriphilus* biological control by *T. sinensis*

The presence of *Q. pubescens* and *C. parasitica* had little impact on the control of *D. kuriphilus* abundance by *T. sinensis*. The refuge provided to *T. sinensis* by pubescent oak galls thus only allowed for a minor increase of the biological control.



**Figure 6. Variations of the efficacy of *D. kuriphilus* biological control induced by the presence of key species.** The impacts of *Q. pubescens* frequency (x-axis) and *C. parasitica* prevalence (y-axis) on the limitation of *D. kuriphilus* abundance by *T. sinensis* were tested for the minimum (A), average (B) and maximum (C) chestnut tree frequency observed in the study area. The increases and decreases in the level of control induced by *Q. pubescens* and *C. parasitica* were expressed as a percentage of variations of the efficacy of control in their absence. Positive and negative variations of the control rate of the pest population appear in blue and red, respectively.

The variations of the control efficacy of *T. sinensis* induced by *Q. pubescens* and *C. parasitica* were qualitatively similar with the effect of those key species on *D. kuriphilus*  $R_0$  (Figure 4), with a gain in control at high frequency of *Q. pubescens* and a reduction of control at high prevalence of *C. parasitica*. The amplitude of those changes remained however lower than 2.2%, whatever be the frequency of chestnut tree or key species. The most important effects of key species on *D. kuriphilus* abundance were found at intermediate chestnut tree frequency (Figure 6B), with changes spanning from -0.8% to +2.2%.

## Discussion

The spread of *D. kuriphilus* observed across the 8 studied stations was very significant in 2019, with 50-90% of infested trees and rates of infestation that, in 3 stations, exceeded the threshold of 0.6 galls per bud that is thought to lead to drastic decrease of productivity (Sartor et al. 2015). A strong decrease of *D. kuriphilus* infestation was observed in the two following years, resulting in less than 25% of infested trees with rates of infestation below the value of 0.3 galls per bud, where tree yield loss are no longer expected (Sartor et al. 2015). This pattern was very consistent across stations and is likely explained by the introduction of the control agent, *T. sinensis*, in 2014. The dissection of galls collected in 2020 sites indeed showed that about 90% of *D. kuriphilus* larvae were hyperparasited by *T. sinensis*, a rate of control that was found highly consistent across stations. This wide spread and fast growth of the control agent in the Pyrénées-Orientales was very similar to the rate at which *T. sinensis* was previously shown to spread to reach >90% rates of *D. kuriphilus* infestation within 5-7 years in Italy (Bosio et al. 2013).

While the decrease in infestation was observed in all our sampling stations, we also observed significant spatial heterogeneity in the initial infestation level as well as in its reduction over the two years of our field study. As illustrated in the introduction, such variations can typically result from heterogeneity in bottom-up and top-down effects affecting the spread of the invasive species, as well as from the presence of key species whose indirect effects are often overlooked.

The level of chestnut tree infestation by *D. kuriphilus* was indeed shown to be positively correlated with the frequency of its hosts in forest environments, confirming previously studies in the Pyrénées-Orientales (Zitoun et al., 2023) and in other European places (Fernandez-conradi et al., 2018; Castagneyrol et al., 2014). Such a correlation is consistent with the resource concentration hypothesis (Root, 1973) which states that more dense stands of a plant shall recruit more herbivores per plant unit, with a stronger effect for specialist herbivores. On the contrary, the rates of *D. kuriphilus* infestation by native parasitoids and fungi were previously found to be both low, i.e. less than 4.5 and 6.7% respectively, and highly similar between stations, so that such top-down factors could not explain observed variations in infestation (Zitoun et al 2023) confirming previous findings in Occitanie (Mansot and Castex, 2018), Europe (Kos et al. 2015; Szabó et al., 2014; Tosi et al. 2015) and the US (Cooper and

Rieske 2010). In addition to the predominant bottom-up effects shown to regulate *D. kuriphilus* invasion in the Pyrénées-Orientales, we further observed that two key species, *Q. pubescens* and *C. parasitica*, have significant effects on its prevalence and rate of infestation of chestnut trees.

The *D. kuriphilus* rate of infestation was negatively correlated to the frequency of pubescent oaks, as observed by Fernandez-Conradi et al. (2018) who recorded a lower number of galls per buds in chestnut tree stands mixed with *Q. pubescens*. Such an effect of the presence of alternative tree species in the environment is likely explained by the waste of time spent by dispersing *D. kuriphilus* on trees that are not suitable for oviposition (Salazar et al., 2016; Coley and Kursar, 2014), leading to a typical dilution effect (Huang et al., 2016) of the spread of the invasive pest. Meanwhile, *D. kuriphilus* infestation was higher on chestnut trees infected by *C. parasitica* than on healthy hosts, which was previously linked to the facilitation of *D. kuriphilus* host detection by chestnut blight symptoms (Germinara et al., 2011). The authors observed, in a Y-tube olfactometer experiment, a higher detection of host twigs presenting old damages as those were associated with changes in chemical volatile compounds. Altogether, this experimental study and our field data suggest that canker infection could amplify the spread of *D. kuriphilus* by facilitating host detection and oviposition, which is consistent with the invasional meltdown hypothesis (Simberloff and Von Holle, 1999, Braga et al. 2018) that states that introduced species could promote the spread of pathogen or other invasive species if they enhance their infection success (Hoyer et al. 2017).

Another anticipated indirect effect associated with the presence of *Q. pubescens* was to provide an alternative source of native parasitoids and introduced *T. sinensis* that would emerge from galls formed on this alternative niche. While the native parasitoids *Eurytoma setigera*, *Torymus auratus*, *Torymus flavipes* and *Eupelmus sp.* found in *D. kuriphilus* galls collected on chestnut trees (Zitoun et al., 2023) are known to be able to infest galls formed on pubescent oaks (Avtzis et al. 2019), only 2 of them (*T. auratus*, *T. flavipes*) were observed in the 208 oaks galls used in our barcoding analysis. The pubescent oaks found in our sampling stations were significantly infested by *A. kollari* with a prevalence of 34%, similar to the 35.7% observed by Banach and Lenowiecki (2011), and by *A. dentimitratus* with a prevalence of 18%. The barcoding analysis of the content of the galls formed by those two species showed that they were both infested by the control agent *T. sinensis*, as previously observed by Ferracini et al. (2017). These authors recorded *T. sinensis* emergence from 15 different gall-forming parasite species of pubescent

oaks, evidencing the ability of the pest to switch to closely related host species. On the contrary, they did not record any switch to galls formed on *F. sylvatica* by *M. fagi*, which we confirmed as no *T. sinensis* was found among the 263 galls of this parasite species that we collected on beeches. The repeated observation of a plasticity of *T. sinensis* in its host choice sharply contrasts with the expected specificity of biological control agent towards their target. In addition, the inter-specific competition occurring between native and introduced parasitoids for *D. kuriphilus* and oak parasites galls may induced a loss of 14% of native parasitoids species and 32% of their individuals hyperparasiting chestnut tree galls (Ferracini et al., 2018; Cooper and Rieske, 2007; Loru et al., 2021; Kos et al. 2021). However, no long-term assessment of this introduced species impact on native parasitoids species richness or abundance has been performed in oaks galls. Taken together, these observations should encourage to further evaluate the potential impact of this biological control agent on non-target endemic species.

The integration of the above indirect effects of *Q. pubescens* and *C. parasitica* into a host-parasite-hyperparasite dynamical model provided further quantitative insights into the role of these key species in the spread of *D. kuriphilus* and its control agent, *T. sinensis*.

Model analysis allowed quantifying the balance between the dilution and amplification impacts that *Q. pubescens* and *C. parasitica* can have on the invasive potential of *D. kuriphilus*. The effects induced by *Q. pubescens* were shown to be stronger than those due to *C. parasitica*, for most of the range of values tested for their frequency and prevalence in the forest environment. Such predominant effect of *Q. pubescens* was especially strong when considering the observed prevalence of blight chestnut tree, i.e. 17.5% (95% CI : 15% - 20%), where the overall effect of the two key species was detrimental as soon as the oak prevalence was larger than about 10% whatever be the frequencies of oaks and chestnut trees in the forest. The magnitude of this overall dilution effect reached up to 15% in a typical stand made of 60% of chestnut trees, and could potentially increase further, up to 25%, when such frequency was given its lowest observed value across all our sampling stations. On the contrary, the very limited effect predicted at high frequency suggest that, as expected, those key species shall have little effect in chestnut tree coppices.

Our modelling also showed that, although *Q. pubescens* constitute an ecological niche for native parasitoids species, those are likely to have almost no impact on the rate of spread of *D. kuriphilus*. Such predictions are consistent with the field observations that the rate of *D.*

*kuriphilus* hyperparasitism by native insects is typically lower than 5% (Kos et al., 2015; Szabó et al., 2014) and that the abundance of such parasitoids is only slightly impacted by the density of pubescent oaks in the stand (Ferracini et al. 2018), which overall confirm the general understanding that parasitoids naturally occurring in the invaded forest are insufficient to regulate the *D. kuriphilus* populations (Gil-Tapedo et al., 2018; Kos et al., 2021).

While the presence of oaks was predicted not to increase the abundance of native parasitoids, our modelling showed that *Q. pubescens* could serve as a refuge for *T. sinensis* with 5-10% of oak galls predicted to be infected by *T. sinensis* when considering its average detection capacity and the average frequency of chestnut trees in the forest environment, which provides a quantitative support to the idea that alternative hosts can increase the building of biological control agent population (Roschewitz et al. 2005). The rate of oak galls infestation was shown to decrease at high chestnut tree frequency, where *D. kuriphilus* - *T. sinensis* typically enter oscillatory dynamics (Zitoun et al., 2023), although such infestation then showed large variations with pubescent oaks helping the persistence of the control agent at low abundance of *D. kuriphilus*. This suggests that the presence of *Q. pubescens* in chestnut tree dominated forests could still contribute to constrain the re-emergence of the invasive population that is expected to happen as a result of *D. kuriphilus* - *T. sinensis* oscillations. Despite of these evidence for galls found on oaks to contribute to the production and persistence of *T. sinensis*, the effect of *Q. pubescens* on the efficacy of *D. kuriphilus* biological control was predicted to remain lower than 2%.

To conclude, we provided empirical and modelling insights into the role of two key species, *Q. pubescens* and *C. parasitica*, on the invasion and biological control of *D. kuriphilus* invasion in the Pyrénées-Orientales. The dilution effect exerted by *Q. pubescens* on the invasive potential of *D. kuriphilus* was shown to typically dominate the amplification effects induced by *C. parasitica* infection. Although *Q. pubescens* was shown to provides alternative niches for both native parasitoids able to infest *D. kuriphilus* and *T. sinensis*, our modelling predicted that the presence of this key species has only low indirect consequences on the efficiency of *D. kuriphilus* biological control. The ecological engineering of these key species may then contribute reducing *D. kuriphilus* intrinsic invasive potential by up to 10-20%, but not to improve the efficacy of its biological control.

## Material and Methods

### *Dryocosmus kuriphilus* life-history and its control

The invasive Asian wasp, *D. kuriphilus*, is the most virulent pest of sweet chestnut trees (*Castanea sativa*) worldwide. The galling activity of the pest inhibits the development of the shoots resulting in abnormal branch structures (Maltoni et al 2012), reduction of leaf area and gradual loss of photosynthetic biomass (Kato and Hijii 1997), which influence plant allocation patterns and ultimately lead to reduction of up to 80% of nut production (Battisti et al. 2014) and up to 60% of the annual average of the basal area increment (Marcolin et al 2021). Its rapid and successful invasion history is attributed to its effective parthenogenetic reproduction, the lack of natural enemies in the invaded ranges and its long range dispersal assisted by human-mediated transport of infested plant material (Avtzis et al. 2019). Univoltine and semelparous females emerge from galls in June-July and fly to lay their asexually produced eggs in chestnut tree buds during their 1-7 days short.life-span (Bosio et al., 2010). Oviposition could then trigger a local immune response in chestnut trees buds and prevent the development of the deposited eggs (Dini et al. 2012). This host resistance mechanism has been shown to exhibit substantial variations between chestnut tree strains, ranging from totally-resistant individuals to highly susceptible ones (Sartor et al. 2015). Eggs that survive the host response and intrinsic developmental failure, hatch within a month and develop into first instar larvae that stay in dormant stage to overwinter. In spring, they emerge from dormancy and develop into subsequent larval stage to induce the formation of galls during the chestnut tree buds burst, where they eventually become pupae and adults that will produce the next and non-overlapping generation of eggs. Beyond their native ranges, newly formed galls have been shown to be targeted and infected by local fungus (Tosi et al. 2015) and hymenopteran parasitoids, although the additional mortality they induce on *D. kuriphilus* larvae is generally too low to allow for a natural regulation of the invasion (Gil-Tapetado et al 2018, Kos et al. 2021). The control agent, *Torymus sinensis*, was then introduced worldwide to restrain the spread of *D. kuriphilus* and reduce its impact on both cultivated and (semi-)natural chestnut tree populations. This univoltine and semelparous Hymenoptera of the *Torymidae* family typically shows successful establishment and effective control rates in Japan, North America and Europe (Avtzis et al. 2019).

### ***Distribution of the *Castanea sativa* populations and sampling sites in the Pyrénées-Orientales.***

The monitoring of *Dryocosmus kuriphilus* infestation levels was conducted in *Castanea sativa* populations of the Pyrénées-Orientales between 2019 and 2021. This French department located on France's Mediterranean coast is composed of various vegetal formations with different tree community structure spanning from the Vermeille Coast to the 2784 meters high Canigó Massif. The spatial distribution of the *C. sativa* populations was characterized using maps of the different vegetal formations that were derived from aerial photointerpretation of the study area (IGN, 2018). 24 sampling sites distributed equally in 8 stations (Prats de mollo, Saint-Laurent de Cerdans, Arles-sur-Tech, La Bastide, Llauro, Céret, Laroque des Albères, Massane) were positioned in chestnut tree patches belonging to different vegetal formations. The tree community structure was further characterized by field assessment in each of the site (see below).

### ***Estimating the frequency of *C. sativa*, *Q. pubescens*, and *F. sylvatica* in the tree community, and the infestation of chestnut trees by *D. kuriphilus* and *Cryphonectria parasitica****

The proportions of the gall-forming tree species (*C. sativa*, *Q. pubescens*, *F. sylvatica*) among all trees were estimated in each of the 24 site by sampling 1000m<sup>2</sup> of forest and identifying the taxonomic status of each tree to the species level. Confidence intervals for the overall species frequencies were drawn from the corresponding Binomial distributions.

The infestation levels of the local chestnut tree populations by *D. kuriphilus* were estimated using two typical measures of parasitism. First, the infestation rate, defined as the mean number of galls per leaf, was estimated on 5 geo-located chestnut trees per sampling site and with a sampling effort of 250 to 500 leaves per tree. The prevalence rate, defined as the proportion of chestnut trees infested by at least one gall, was estimated on 50 chestnut trees per site. The prevalence of chestnut blight (*Cryphonectria parasitica*), defined as the proportion of chestnut trees presenting chestnut blight symptoms, was estimated on the same 50 chestnut trees per site used to assess the prevalence of *D. kuriphilus* infestation. While the measures of *D. kuriphilus* were repeated each of the three years of our field study, the long-lasting infestation by *C. parasitica* was assessed only once in 2020. The existence of heterogeneity between sites or

stations in the prevalences and rates of infestation was tested by Pearson's chi-squared tests with Yates's correction for continuity. Exact confidence intervals for both measures of infestation were calculated from the binomial distributions set according to the corresponding sampling efforts.

### ***General Linear Mixed Models Analyses (GLMMs) and statistical tests.***

The frequencies of chestnut trees and pubescent oaks and the chestnut blight prevalences were estimated at site level and tested as continuous fixed-effects to explain the prevalence and rate of infestation of chestnut trees by *D. kuriphilus*. Statistical analyses were performed by generalized linear mixed modelling (GLMMs) following the procedure recommended by Zuur et al. (2009). Sampling year having a significant impact on both infestation indexes (Infestation : Estimate = -1.3674,  $p = 3.77 \cdot 10^{-8}$ , Prevalence : Estimate = -1.7243,  $p = 9.05 \cdot 10^{-11}$ ), we accounted for a temporal structure with year of sampling as a random factor. Collinearity of the variables was tested using the `corvif` function of the AED package. The significance of any parameter was assessed using  $\chi^2$  tests by comparing models with and without the term to be tested. We applied model simplification by starting with the highest order interaction and sequentially removing non-significant predictors. All analyses were conducted in R 4.1.1 (R Core Team, 2021) using the `glmmPQL` function from the MASS package. For both GLMMs, implemented to predict either the prevalence or the rate of *D. kuriphilus* infestation, a quasi-binomial distribution was used as it is suitable for proportional data and allowed to deal with over-dispersion. At the individual level, the observed numbers of chestnut trees infected by none, ACGW, chestnut blight or both species were compared to expected values with Pearson's chi-squared tests with Yates's correction for continuity.

### ***Collection and barcoding of the parasitoids found in galls of *Q. pubescens* and *F. sylvatica****

After infection by *D. kuriphilus*, chestnut trees develop galls which can be hyperparasited by native parasitoid species and/or *Torymus sinensis*, the introduced biological control agent. *Q. pubescens* and *F. sylvatica* are also typically infested by endemic gall-forming insect species. We collected galls formed by *Andricus kollari* and *Andricus dentimitratus* on *Q. pubescens*, and by *Mikiola fagi* on *F. sylvatica*, to identify which (native or introduced) parasitoid species could develop in the galls associated with each tree species.



### *Infestation levels and collection of galls on Q. pubescens and F. sylvatica.*

The prevalences and rates of infestation of *Q. pubescens* (by *Andricus kollari*, *Andricus dentimitratus*) and of *F. sylvatica* (by *Mikiola fagi*) were estimated as for the infestation of chestnut trees by *D. kuriphilus* (see above and SM1). We simultaneously collected 56 *A. kollari* and 152 *A. dentimitratus* galls on pubescent oaks from the different sites where the tree species was present, and dissected them in the lab. The 199 larvae collected from these galls (50 from *A. kollari* galls and 149 from *A. dentimitratus* galls) were divided into 5 pools; 3 pools corresponding to the 'Massane', where 52% of the galls (and 64% of the larvae) were found, and 2 for the 6 stations (Céret, Saint-Laurent, Laroque and Prats de mollo, Arles sur Tech, Llauro) where the remaining galls collected on oaks came from. Finally, 263 galls of *Mikiola fagi* were collected in the 3 sites where *F. sylvatica* was found. Their dissection allowed for the collection of 201 larvae that were pooled according to the stations where they had been collected, i.e. la Bastide and Massane.

### *DNA extraction and barcoding analyses*

DNA was extracted for each pool or individual using the E.Z.N.A Tissue DNA kit (Omega BIO-TEK) extraction protocol. A Polymerase Chain Reaction (PCR) was performed to amplify the ribosomal Internal Transcribed Spacers 2 (ITS2), commonly used as DNA barcodes and phylogenetic markers in insects. Both forward and reverse primers were synthesized in 2 different versions with 1N or 3N between the adapter sequence [Adapter] used for sequencing and the annealing sequence. The 1N version was as it follows :

Forward : 5'[TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG]NTGTGAACTGCAGGACACATG 3'

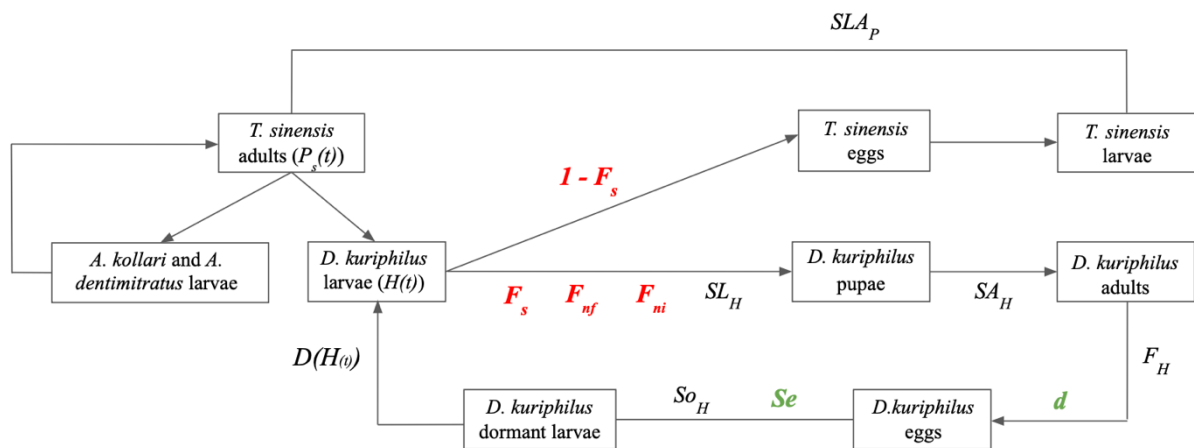
Reverse : 5'[GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG]NATGCTTAAATTYAGCGGGTA 3'

PCRs were performed in 35 µL reaction volume, containing ~ 20 ng DNA template, 0.1 µM of each dNTP, 0.04 µM of each primer and 0.7 U Phusion High-Fidelity DNA polymerase (FINNZYMES OY, Espoo, Finland) in 7 µL 5X manufacturer's buffer plus 21,35 µL sterile distilled water. The thermal profile of the PCR was as follows: initial denaturation at 98°C for 30 seconds, followed by 16 to 22 cycles (depending on the sample) of denaturation at 98°C for 10 seconds, locus-specific annealing at 52,7°C for 30 seconds, elongation at 72°C for 18s, and a final elongation at 72°C for 10 minutes. The PCR products were checked on 1% agarose gel and sequenced using a Miseq Reagent Kit v3 (Illumina, San Diego, USA). Sequences obtained from the 7 samples were processed with the FROGS pipeline (Escudié et al. 2018) available on the Genotool Bioinfo galaxy server (Haddad et al. 2016). Clustering swarm step was made

with aggregation distance of 1. Only OTU's with at least 50 sequences were conserved and affiliated based on the NCBI database.

### ***Integration of key species effects into a *D. kuriphilus* – *T. sinensis* dynamic model***

To investigate the role of key species in the spread of *D. kuriphilus* and *T. sinensis*, we integrated their empirically supported effects into the dynamical model developed by Zitoun et al (2023). This modelling was based on the seminal Nicholson-Bailey model (1935) that is widely used in theoretical ecology to describe host-parasitoids relationships and was tailored to account for the effect of top-down and bottom-up on the pest population. Specifically, the initial framework was expanded to account for the i) perturbation of *D. kuriphilus* oviposition by *Q. pubescens*, the ii) facilitative impact of *C. parasitica* on *D. kuriphilus* host detection, and the iii) production of native parasitoids and iv) introduced *T. sinensis* in galls formed on *Q. pubescens*. The effects of *Q. pubescens* and *C. parasitica* and the impacts of the bottom-up and top-down factors (described as in Zitoun et al. 2023) on the dynamic of the *D. kuriphilus* - *T. sinensis* interaction are summarized in Figure 7 and described below.



**Figure 7. Dynamical model of the *D. kuriphilus* – *T. sinensis* interaction accounting for the indirect effects of the key species *Q. pubescens* and *C. parasitica*.** The lower part of the graph depicts the different stage of *D. kuriphilus* life-cycle and the effects of the native hyperparasitic insects and fungi (red) and of the tree species and genetic diversity (green) on *D. kuriphilus* survival and reproduction. The upper part of the graph describe the life-cycle of the introduced control agent, *T. sinensis*, with its hyperparasitism of *D. kuriphilus* larvae (in galls formed on chestnut trees) and of larvae of *Andricus kollari* and *Andricus dentimitratus* whose galls are formed on *Q. pubescens*. All parameters are defined in the main text and their estimates provided in SM 2 to 4.

In this framework, the *D. kuriphilus* (host) larvae ( $H(t)$ ) are split into those used by the *T. sinensis* adults ( $P_s(t)$ ) to lay their eggs, and those escaping the control agent. The fraction of *D. kuriphilus* larvae escaping the parasitoid is modelled assuming a random dispersal of *T. sinensis* within its searching area ( $a_s$ ), which fits its low ability to fly towards galls of *D. kuriphilus*, so that  $F_s(H(t), P_s(t), p_c) = e^{-a_s p_c P_s(t)}$ , where  $p_c$  stands for the frequency of chestnut trees in the forest environment.

The larvae of *D. kuriphilus* escaping *T. sinensis* face additional challenges by native hyperparasite insects and fungi that exert a constant infective pressure, which larvae can escape with probabilities  $F_{ni}$  and  $F_{nf}$ , respectively. The production of native parasitoids in galls formed on *Q. pubescens* (effect *iii*) was thus introduced through a variation in parameter  $F_{ni}$  with the frequency of oaks, as further specified below. Larvae infected by none of those hyperparasites develop into pupae at rate  $SL_H$  and moult into adults with probability  $SA_H$ . After emergence, the *D. kuriphilus* females produce an average of  $F_H$  eggs per individual, and a fraction  $d$  of those eggs are deposited in chestnut tree buds. The effects of *Q. pubescens* and *C. parasitica* on *D. kuriphilus* oviposition (effects *i* and *ii*) were accounted for by using a typical host-choice function to model  $d$  with respect to the frequencies of healthy chestnut trees, blight infected chestnut trees, oaks, and the preferences of *D. kuriphilus* for each of these trees, as further specified below. To develop into larvae and enter in dormancy to overwinter, the deposited eggs must then survive to a tree hypersensitive response and intrinsic causes of mortality during development, which they do with probabilities  $Se$  and  $So_H$ , with the former depending on the observed distribution of *C. sativa* susceptibility, as estimated in Zitoun et al (2023). The description of *D. kuriphilus* life-cycle, was completed by modelling the density dependent survival of dormant larvae associated with the competition for space during chambers formation. Such a typical ‘contest’ competition was described using a function proposed by Brännström and Sumpter (2005):  $D(H'(t)) = K(p_c)(1 - e^{-\frac{H'(t)}{K(p_c)}})$ , where  $H'(t)$  stands for the number of larvae entering dormancy and  $K(p_c)$  for the maximal amount of *D. kuriphilus* larvae that can be sustained in a stand, which was set to increase linearly with the proportion  $p_c$  of chestnut-trees, as estimated in Zitoun et al (2023).

The complementary part of *D. kuriphilus* larvae, i.e. the fraction  $1-F_s$  not escaping parasitism by *T. sinensis*, is set to die as the hyperparasite larvae feed on them to become, with probability  $SLAP$ , the next generation of adults that will intend to lay their eggs into *D. kuriphilus* larvae. To account for the production of *T. sinensis* associated with the oviposition in larvae of

*Andricus kollari* and *Andricus dentimitratus* whose galls are formed on *Q. pubescens* (effect *iv*), we assumed a constant number of larvae available in such galls, and described their infection by *T. sinensis* according to specific searching efficiency for those hosts, as further specified below.

#### *Impact of Q. pubescens and C. parasitica on D. kuriphilus host detection and oviposition*

To integrate the effects of the pubescent oaks and chestnut blight on *D. kuriphilus* host detection and oviposition (effects *i* and *ii*), the fraction of eggs deposited on chestnut tree (*d*) was formally linked, through a typical host-choice function, with the frequencies of healthy chestnut trees, blight infected chestnut trees, oaks, and the preferences of *D. kuriphilus*' for each of these trees.

We first looked at the detrimental effect induced by pubescent oaks (effect *i*) by setting *d* as :

$$d = \frac{a_c p_c}{a_c p_c + a_g p_g + a_n (1 - p_c - p_g)} \quad \text{Equation 1}$$

where  $p_c$  et  $p_g$  represent the frequencies of chestnut trees and pubescent oaks, and  $a_c$ ,  $a_g$ , and  $a_n$  stand for the ability of *D. kuriphilus* to detect chestnut trees, pubescent oaks and non-host tree species.

We then considered the positive effect that chestnut blight infection has on the detection of chestnut tree (effect *ii*) by subdividing the chestnut trees into healthy and infected trees according to the prevalence of *C. parasitica*. The ability of *D. kuriphilus* to detect the latter was then assumed to be higher than its ability to detect the former. The proportion of *D. kuriphilus* eggs laid on (healthy and infected) chestnut trees was then given by:

$$d = \frac{a_{hc} p_{hc} + a_{ic} p_{ic}}{a_{hc} p_{hc} + a_{ic} p_{ic} + (1 - p_{hc} - p_{ic})} \quad \text{Equation 2}$$

where  $p_{hc}$  et  $p_{ic}$  represents the frequency of healthy and *C. parasitica* infected chestnut trees, and  $a_{hc}$  and  $a_{ic}$  stand for the ability of *D. kuriphilus* to detect each of the two chestnut tree categories.

Finally, to consider simultaneously the effects of *Q. pubescens* and *C. parasitica* on the detection of chestnut tree (effects *i* and *ii*), we defined *d* as

$$d = \frac{a_{hc} p_{hc} + a_{ic} p_{ic}}{a_{hc} p_{hc} + a_{ic} p_{ic} + a_g p_g + a_n(1 - p_{hc} - p_{ic} - p_g)} \quad \text{Equation 3}$$

The ability of *D. kuriphilus* to detect healthy and symptoms (of *C. parasitica* infection) carrying chestnut trees and other non-host tree species were estimated from preferences for each of these tree categories observed by Germinara et al. (2011). Detection capacity estimates (shown in SM 2) were derived by assimilating the absence of preference to a detection capacity of 1, and considering the capacity of *D. kuriphilus* to detect one tree category as proportional to the observed preference for this category. We further assumed that the *D. kuriphilus* ability to detect *Q. pubescens* was similar to its ability to detect healthy chestnut trees as both species are closely related.

#### *Impact of Q. pubescens on the production of native parasitoids.*

To account for the production of *D. kuriphilus* native parasitoid from galls found on oaks (effect *iii*), we intended to find a quantitative relationship between *Q. pubescens* density and chestnut tree galls infestation by the different native parasitoid species that we identified in our barcoding experiment. To do so, we took advantage of data provided by Ferracini et al. (2018) on the emergence rate of native parasitoids (*Torymus flavipes*, *Torymus auratus*, *Eupelmus sp.*) in galls found on chestnut tree in stands with different oak densities. We derived the expression of the expected rate of such hyperparasitism from the y-intercept and slope of the linear relationship obtained between the rate of emergence of each parasitoid species and the density of pubescent oaks (SM 3), and by reducing their rate of hyperparasitism according to the competition imposed by *T. sinensis* for the *D. kuriphilus* larvae. The expression of the probability to escape such parasitism is given by

$$F_{ni} = 1 - (O_F + O_A + O_E + D_{pub} (P_F + P_A + P_E)) * \left( 1 - \frac{P_s(t)}{H(t)_{max}} \right) \quad \text{Equation 4}$$

where  $O_i$  and  $P_i$  corresponds to the y-intercept and slope of the linear relationship observed between the emergence rate of each parasitoid species with  $D_{pub}$ , the density of pubescent oaks,

and with  $P_s(t)$  standing for the number of adult *T. sinensis* competing for the *D. kuriphilus* larvae resource denoted  $H(t)_{max}$ .

*Integration of the new ecological niche provided by Q. pubescens to T. sinensis*

To account for the production of *T. sinensis* associated with its hyperparasitism of larvae of *Andricus kollari* and *Andricus dentimitratus* whose galls are formed on *Q. pubescens* (effect iv), we considered that AK and AD larvae were available in galls formed by each of the parasite species. We then modelled their hyperparasitism by *T. sinensis* under the same assumption of random dispersal within the parasitoid searching area that is made to model the hyperparasitism of *D. kuriphilus* larvae present in galls formed by the invasive species on chestnut trees, and by considering specific searching efficiency of *T. sinensis* for those alternative hosts. The amount of *T. sinensis* adults produced on pubescent oaks was then given by

$$P_{so} = AK(1 - e^{-\mu_s \cdot P_s(t)}) + AD(1 - e^{-\lambda_s \cdot P_s(t)}) \quad \text{Equation 5}$$

where  $\mu_s$  and  $\lambda_s$  represent the *T. sinensis* searching efficiency for *A. kollari* and *A. dentimitratus*.

In order to estimate those searching efficiency, we used the only available data on the infestation rates of these oak parasites by *T. sinensis*. Ferracini et al. (2017) observed 3 *T. sinensis* emergence from 707 *A. kollari* galls, and 1 adult emerging from 64 *A. dentimitratus* galls. We assume that the corresponding rates of parasitism resulted from the equilibrium dynamic of our modelling, which provided the relationships;

$$\mu_s = \frac{-\ln(\text{Inf}_{AK}+1)}{P^*} \quad \text{Equation 6}$$

$$\lambda_s = \frac{-\ln(\text{Inf}_{AD}+1)}{P^*} \quad \text{Equation 7}$$

where  $\text{Inf}_{AK}$  and  $\text{Inf}_{AD}$  represent the estimated rates of infestation for *A. kollari* and *A. dentimitratus* by the *T. sinensis*, whose abundance at equilibrium point is denoted  $P^*$ . The number of *T. sinensis* at equilibrium was estimated from our modelling while considering a mixed forest with 45% percent of chestnut trees, to mimic the stand where infestation rates

have been estimated. The searching efficiency values of *T. sinensis* for both oak parasite species and their 95% confidence interval are provided in SM 4.

The yearly dynamics of *D. kuriphilus* – *T. sinensis* interactions incorporating all the effects described above was then described by the following set of non-linear difference equations:

$$H(t + 1) = K \left( 1 - e^{-\frac{H(t) \cdot F_s(H(t), P_s(t), p_c) \cdot F_{nf} \cdot F_{ni} \cdot SL_H \cdot SA_H \cdot F_H \cdot d \cdot Se \cdot So_H}{K}} \right) \quad \text{Equation 8}$$

$$P_s(t + 1) = H(t)(1 - F_s) * SLA_p + P_{so} \quad \text{Equation 9}$$

$$\text{With } F_s(H(t), P_s(t), p_c) = e^{-a_s p_c P_s(t)}$$

To provide further quantitative insights into the role of key species in the spread of *D. kuriphilus* and its control agent, *T. sinensis*, we integrated the effects that were empirically supported into a host-parasite-hyperparasite dynamical model. The modelling developed by Zitoun et al (2023) was expanded to account for the i) perturbation of *D. kuriphilus* oviposition by *Q. pubescens*, the ii) facilitative impact of *C. parasitica* on *D. kuriphilus* host detection, and the iii) production of native parasitoids and iv) introduced *T. sinensis* in galls formed on *Q. pubescens*. We then used this modelling to assess the overall effect of *C. parasitica* and *Q. pubescens* on the spread of *D. kuriphilus* and the efficacy of its biological control by *T. sinensis*.

### ***Modelling analysis of the effects of key species on D. kuriphilus invasion potential (R<sub>0</sub>) and its control***

All analysis were made after implementation of our dynamical model on R 4.1.1 (R Core Team, 2021).

We first looked at the variations of *D. kuriphilus* invasion potential ( $R_0$ ) induced by the i) perturbation of *D. kuriphilus* oviposition by *Q. pubescens*, the ii) facilitative impact of *C. parasitica* on *D. kuriphilus* host detection, and the iii) production of native parasitoids in galls formed on *Q. pubescens*, when considered independently. To do so, we accounted for one of

these three effects (i to iii) at a time, and estimated *D. kuriphilus*  $R_0$  in 15 forest structures by setting the frequency of chestnut trees to its minimal (0.16), average (0.60) or maximal (0.91) values estimated in our sampling sites, and by letting the frequency of *Q. pubescens* (among non-chestnut trees) or the prevalence of *C. parasitica* infection take on five different values (0; 0.25; 0.5; 0.75; 1). In each of these 15 conditions, we estimated  $R_0$  from the average estimates of *D. kuriphilus* life history parameters and hyperparasitism by native fungi species (SM4) and by accounting for the variability in our estimates of the *D. kuriphilus* preferences (for *C. parasitica* infected and non-infected chestnut trees and for pubescent oaks) and of (the intercept and slope of) the linear relationship between *Q. pubescens* densities and chestnut tree galls infestations for each native parasitoid species (SM 3). To do so, we randomly sampled 200 combinations of values using the confidence intervals described in SM 3, which provided a distribution of  $R_0$  values represented by each boxplot in Figure 3. These variations were shown in figure 3A-C, and further expressed per additional percent of key species (frequency of *Q. pubescens* or prevalence of *C. parasitica*) introduced in the modelled chestnut tree forest in figure 3D-F.

Second, we investigated the joint effects of the presence of *Q. pubescens* and *C. parasitica* (i-iii) on the rate of invasion of *D. kuriphilus*. We then estimated  $R_0$  from all average estimates of *D. kuriphilus* life history parameters and tree host preferences (SM 4), and from the average estimates of the intercept and slope of the linear relationship between *Q. pubescens* densities and chestnut tree galls infestations for each native parasitoid species (SM 3). Again, we set the frequency of chestnut trees to its minimal (0.16), average (0.60) or maximal (0.91) observed values and we varied systematically the frequency of *Q. pubescens* (among non-chestnut trees) and the prevalence of *C. parasitica* infection between 0 and 1.

Third, we aimed to assess if the spread of the control agent, *T. sinensis*, can be eased by its hyperparasitism of the galls formed by the two native parasites of *Q. pubescens* (effect iv). We then used our modelling to predict the proportions of *A. kollari* and *A. dentimitratus* oak galls that were infested by *T. sinensis*. Such predictions were made by running simulations for the minimum, average and maximum value of the 95% confidence interval of the searching areas of *T. sinensis* for *A. kollari* and *A. dentimitratus* (SM 4) and for different values of frequency of chestnut tree regularly spread between from 0 to 1. In each of these cases, the average rates of *A. kollari* and *A. dentimitratus* oak galls infestation by *T. sinensis* were recorded. When the dynamic of the *D. kuriphilus* – *T. sinensis* interactions lead to sustained oscillation, which



typically occurs when the frequency of chestnut trees is large (Zitoun et al. 2023), we further recorded the minimal and maximal rates of infestation, which provided the intervals around the mean appearing in Figure 5.

Finally, we predicted the impact of the presence of the two key species, *Q. pubescens* and *C. parasitica*, (effect i-iv) on the level of control achieved by *T. sinensis*. We run simulations in all conditions used to investigate the joint effects of the presence of *Q. pubescens* and *C. parasitica*. Those simulations were run with and without the control agent, so that the level of biological control could be estimated as the ratio between the abundance of *D. kuriphilus* found in each circumstances. We then expressed the increase or decrease in the levels of control induced by each considered frequency of *Q. pubescens* and prevalence of *C. parasitica* as a percentage of the efficacy of control in their absence.

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## SUPPORTING INFORMATION

**Supplementary Material 1.** Summary table of *D. kuriphilus*, *A. kollari* and *A. dentimitratus* infestation and prevalence rates and frequencies of *C. sativa*, *Q. pubescens* and *F. sylvatica* recorded in the 24 sites.

Stations	<i>D. kuriphilus</i>						<i>A. kollari</i>		<i>A. dentimitratus</i>	
	Prevalence rate			Infestation rate			Prevalence rate	Infestation rate	Prevalence rate	Infestation rate
	2019	2020	2021	2019	2020	2021	2020	2020	2020	2020
Prats de Mollo	0.867	0.173	0.033	0.022	0.002	<1e10-3	0.373	0.007	0.255	0.014
Site 1	0.78	0.04	0	0.014	0	0	0.462	0.017	0	0
Site 2	0.94	0.26	0.06	0.029	0	0.001	0.524	0	0.143	0.029
Site 3	0.88	0.22	0.04	0.024	0.005	0	0.118	0.004	0.588	0.012
Saint Laurent	0.886	0.473	0.093	0.112	0.022	0.005	0.22	0.001	0.22	0.019
Site 1	0.714	0.02	0	0.066	0	0	0.143	0.001	0.286	0.005
Site 2	0.98	0.56	0.12	0.144	0.035	0.009	0.375	0.002	0.063	0.017
Site 3	0.96	0.84	0.16	0.127	0.033	0.005	0.091	0.002	0.364	0.035
Arlés sur Tech	0.818	0.513	0.06	0.062	0.023	0.001	0.32	0.003	0.02	0
Site 1	0.92	0.5	0.04	0.052	0.008	0.001	0.79	0.008	0.053	0.001
Site 2	0.703	0.6	0.04	0.057	0.013	0	0	0	0	0
Site 3	0.8	0.44	0.1	0.079	0.053	0.002	0.091	0.001	0	0
La Bastide	0.63	0.033	0	0.025	0.003	0	0.396	<1e10-3	0.076	0.04
Site 1	0.9	0.02	0	0.006	0.001	0	0.389	0	0.167	0.05
Site 2	1	0.08	0	0.063	0.008	0	0.4	0.001	0.029	0.03
Site 3	0.1	0	0	0.007	0	0	0	0	0	0
Llauro	0.793	0.607	0.113	0.027	0.007	0.004	0.471	0.002	0.098	0.008
Site 1	0.96	0.74	0.06	0.046	0.005	0	0.727	0.001	0.091	0.01
Site 2	0.64	0.44	0.08	0.033	0.005	0.006	0.5	0.002	0.05	0.006
Site 3	0.78	0.64	0.2	0.01	0.011	0.005	0.3	0.002	0.15	0.008
Céret	0.807	0.727	0.193	0.081	0.04	0.011	0.25	0.004	0.039	0.001
Site 1	0.9	1	0.16	0.132	0.055	0.014	0.136	0.001	0.046	0.002
Site 2	0.98	0.96	0.28	0.081	0.058	0.018	0.273	0.004	0.091	0.002
Site 3	0.54	0.22	0.14	0.018	0.003	0.001	0.368	0.005	0	0
Laroque	0.634	0.147	0.18	0.035	0.003	0.01	0.257	0.003	0.057	0.001
Site 1	0.76	0.32	0.26	0.037	0.009	0.015	0	0	0	0
Site 2	0.744	0.12	0.24	0.041	0.001	0.004	0.214	0.002	0.071	0.002
Site 3	0.366	0	0.04	0.027	0	0.01	0.286	0.005	0.048	0.001
La Massane	0.492	0.11	0.025	0.027	0.001	<1e10-3	0.377	0.011	0.557	0.012
Site 1	0.393	0.074	0.046	0.011	0	0	0.364	0.01	0.591	0.014
Site 2	0.5	0.16	0	0.044	0.002	0	0.435	0.021	0.565	0.014
Site 3	0.54	0.08	0.04	0.028	0	0.001	0.313	0.003	0.5	0.009

Stations	<i>C. parasitica</i>	Tree species frequencies		
	Prevalence rate 2020	<i>C. sativa</i>	<i>Q. pubescens</i>	<i>F. sylvatica</i>
Prats de Mollo	0.093	0.731	0.059	0
Site 1	0.02	0.491	0.107	0
Site 2	0.06	0.908	0.012	0
Site 3	0.2	0.823	0.048	0
Saint Laurent	0.093	0.671	0.008	0
Site 1	0	0.418	0.029	0
Site 2	0.14	0.854	0	0
Site 3	0.14	0.694	0	0
Arlés sur Tech	0.333	0.58	0.031	0
Site 1	0.3	0.869	0.009	0
Site 2	0.38	0.286	0.075	0
Site 3	0.32	0.652	0	0
La Bastide	0.013	0.504	0.023	0.035
Site 1	0.02	0.473	0.008	0
Site 2	0	0.424	0.082	0
Site 3	0.02	0.588	0	0.092
Llauro	0.247	0.739	0.036	0
Site 1	0.26	0.724	0.008	0
Site 2	0.06	0.763	0.079	0
Site 3	0.42	0.895	0.053	0
Céret	0.32	0.591	0.058	0
Site 1	0	0.581	0	0
Site 2	0.52	0.908	0	0
Site 3	0.44	0.19	0.207	0
Laroque	0.273	0.562	0.034	0
Site 1	0.48	0.735	0	0
Site 2	0.04	0.583	0.017	0
Site 3	0.3	0.311	0.1	0
La Massane	0.008	0.3	0.1	0.123
Site 1	0	0.2	0.04	0.56
Site 2	0.02	0.418	0.149	0.03
Site 3	0	0.158	0.053	0

**Supplementary Material 2.** Detection capacities of *D. kuriphilus* and correlations between *Q. pubescens* densities and chestnut tree galls infestations by the native parasitoids. The estimates of the detection capacities of *D. kuriphilus* for healthy ( $a_{hc}$ ) and chestnut blight infected chestnut trees ( $a_{ic}$ ), pubescent oaks ( $a_g$ ) and other non-host tree species ( $a_n$ ) are given with the upper and lower bounds of their 95% confidence interval. The y-intercept ( $O_F, O_A, O_E$ ) and slope ( $P_F, P_A, P_E$ ) of the linear relationship between *Q. pubescens* densities and chestnut tree galls infestations for each native parasitoids species (*Torymus flavipes*, *Torymus auratus*, *Eupelmus sp.*) are given with the upper and lower bounds of their 95% confidence interval.

Parameters	Low CI (95%)	Fit	Up CI (95%)
$a_{hc} / a_g$	0.7	1.12	1.5
$a_n$	0.32	0.66	1.1
$a_{ic}$	1.22	1.6	1.84
$O_F$	5.345e-03	1.658e-02	2.781e-02
$P_F$	-2.184e-05	8.539e-07	2.355e-05
$O_A$	1.391e-04	7.475e-04	1.356e-03
$P_A$	-1.332e-06	-1.032e-07	1.126e-06
$O_E$	2.974e-03	5.061e-03	7.148e-03
$P_E$	1.864e-06	6.080e-06	1.03e-05

**Supplementary Material 3.** Infestation rates and estimates of searching efficiencies values of *T. sinensis* for *A. kollari* and *A. dentimitratus*, and the lower and upper bounds of their 95% confidence interval.

Parasite species	Parameters	Low CI (95%)	Fit	Up CI (95%)
<i>A. kollari</i>	$Inf_{AK}$	1.22	1.6	1.84
	$\mu_s$	5.433129e-10	2.105671e-09	6.699653e-09
<i>A. dentimitratus</i>	$Inf_{AD}$	5.345e-03	1.658e-02	2.781e-02
	$\lambda_s$	4.04344e-10	7.798834e-09	4.965897e-08

**Supplementary Material 4.** Definition and estimates of the parameters of the *D. kuriphilus* host - *T. sinensis* and native parasitoids dynamical model.

	Symbol	Estimate and 95% confidence interval	References
<b><i>Castanea sativa</i></b>			
Genetic susceptibility	Se	0.68	Zitoun et al. (2023)
<b><i>Dryocosmus kuriphilus</i></b>			
Egg survival	So	$0.535 \pm 0.003$	Nugnes et al. (2018)
Larvae survival	SL <sub>H</sub>	$0.936 \pm 0.01$	Panzavolta et al. (2013)
Adult survival	SA <sub>H</sub>	$0.952 \pm 0.01$	Cooper and Rieske (2010)
Fertility	F <sub>H</sub>	$90 \pm 6$	Zitoun et al. (2023)
Carrying capacity per hectare	k	14 880 000	Zitoun et al. (2023)
<b><i>Torymus sinensis</i></b>			
Larvae and adult survival	SLA <sub>P</sub>	$0.913 \pm 0.004$	Ferracini et al. (2015)
Searching area	a <sub>s</sub>	$4.4 \times 10^{-4} \pm 0.4 \times 10^{-4}$	Zitoun et al. (2023)
<b><i>Native parasitoids fungi</i></b>			
Survival to fungal infection	F <sub>nf</sub>	$0.946 \pm 0.001$	Zitoun et al. (2023)

## **General Discussion**

## 1) Towards integrative approaches for biological invasions

When an invasive species colonizes a new environment, it becomes part of the ecological network made of native species. As presented in the introduction of this document, bottom-up and top-down regulatory forces can then impact the growth rate of the alien population, which can be further affected by the control strategies implemented to eliminate the invader and/or limit its damages (e.g. David et al. 2017). To properly understand the natural forces underlying the success of an invasion and their potential interactions requires quantitative integrative approaches, which shall further help anticipating the success of human interventions (Kueffer et al. 2013; Baker and Bode 2021; Januchowski-Hartley et al. 2018). This is the approach that I intended during my PhD by combining the outcomes of an ecological field study and genomic analyses into ‘eco-genomic’ models that allowed to assess i) the different factors regulating the invasive species *D. kuriphilus* parasiting the natural *C. sativa* populations in the French department of Pyrénées-Orientales, as well as ii) the level of control that the biological agent, *T. sinensis*, can have on this local invasion. I will now discuss a bit further the main outcomes of my PhD work and the general interest of integrative approaches to better understand and control biological invasions.

### *1.1) What are the main determinants of a biological invasion success?*

In the first two chapters of this thesis I have shown how the heterogeneity of the bottom-up and top-down factors can explain the spatial variations of the levels of *D. kuriphilus* infestation, and help disentangling the relative importance of their respective contributions to the regulation of its invasion. While to estimate such respective contributions remain a longstanding difficulty in both terrestrial (Hunter et al., 1997) and aquatic (Li et al., 2020) plant-parasite communities, this is an obvious step in designing efficient control strategies to limit the impact of plant pest biological invasions.

#### **Bottom-up factors : distribution and genetic resistance of the resource**

A key outcome of my first manuscript is that the frequency of chestnut trees in the stand and their genetic resistance to the parasite are the main determinants of the growth of *D. kuriphilus* pest populations (Article 1, Figure 4). The increase in pest prevalence with the frequency of its



host resource is consistent with the ‘resource-enemy release’ hypothesis proposed by Blumenthal (2006) and the evidence that, outside of its original area, *D. kuriphilus* has escaped most of its natural enemies, which favors its spread and make the remaining bottom-up control the key regulation force. The low *D. kuriphilus*’ capacity to detect its host (Germinara et al. 2011) is likely to reinforce such a control and to make the frequency of chestnut tree an even stronger determinant of the infection success and population growth of the invasive pest. Overall, the importance of the resource distribution in explaining the abundance of *D. kuriphilus* is consistent with the general understanding that such a distribution is the predominant structuring force in both terrestrial and marine ecosystems (Vucetich and Peterson, 2004 ; Lotze et al. 2001). Meanwhile, Parsche and Lattorff (2018) highlighted the greater importance of host genetic diversity, as compared to the host distribution, in explaining the prevalence of infection. Such host diversity can limit the spread of its parasites through resistance mechanisms, as it has been shown for the *C. sativa* - *D. kuriphilus* interaction (Dini et al. 2012), as well as for others parasites (Carton et al. 2005). This diversity could indeed allow the host to exit the genetic compatibility filter with the parasite, leading to slower spread in genetically diverse host populations (Altermatt and Ebert, 2008). Interestingly, Sartor et al. (2015) showed a high variability of susceptibility of infection between different varieties of chestnut trees. The natural population studied in the Pyrénées-Orientales was to shown to consist of 5 ancestral populations (Article 1, Figure 2), which correspond to endemic strains and strains introduced for the repopulation of forest areas and ornamental purposes. I have shown significant variations in susceptibility to infestation between individual chestnut trees that was correlated to their level of genetic differentiation measured by the rate of private alleles (Article 1, Table 1). Such resistance of the most genetically divergent individuals is consistent with the well-established theory of genetic compatibility filter of host-parasite relationships (Ebert, 2008 ; Parsche and Lattorff, 2018).

The evidenced effects of the distribution and genetic resistance of chestnut trees on *D. kuriphilus* are thus consistent with existing ecological theories, as expected for a specialist species like the Asian chestnut gall wasp that depends on only one resource with little or no diet plasticity (Anderson et al. 2019). While we have shown that such bottom-up factors are critical for the pest population growth, I have also investigated the role of its native parasitoids as top-down regulators.

### **Top-down factors : native parasitoids species**

The second key outcome of my first manuscript is that hyperparasitism by hymenopteran insects and fungi are the two main top-down regulation factors impacting the *D. kuriphilus* population (Article 1, Figure 3). These observations are consistent with the results of different studies made across European, Asian and American countries. The native insect parasitoid species we identified through molecular taxonomic determination in chestnut tree galls (*Eupelmus* sp., *Eurytoma setigera*, *Torymus auratus*, *Torymus flavipes*) have indeed all already been reported as infecting *D. kuriphilus* in Italy and France (Ferracini et al. 2018 ; Muru et al. 2021) while different or more diverse parasitoids cohorts have been observed in USA and Japan (Cooper and Rieske 2007; Murakami 1980). These parasitoids are typically found in galls induced by parasites of oak, a tree species often associated with chestnut in the forest environment. It is therefore not surprising to find these same species in chestnut tree galls even in ecosystems that are geographically distant. Our morphological observations of parasitic fungal species are similar to those by Tosi et al. (2015) who isolated the entomopathogenic fungi *Fusarium proliferatum* from dead *D. kuriphilus* found in chestnut tree galls. As for hymenopteran parasitoids, parasitism by fungi has been reported in many studies worldwide with values ranging from 0.91% to 11.65% (Cooper and Rieske, 2010 ; Morales-Rodriguez et al., 2019 ; Tosi et al., 2015 ; Otake, 1980), which correspond to our own estimates of 4.6% to 6.7%. Such low level of infestation is adding to the general understanding that native parasitoids pressures are not sufficient to fail local invasion of the Asian chestnut gall wasp because of its high reproductive potential. As a matter of fact, since its introduction in Italy in 2002, this parasite has spread to most of the *C. sativa* geographical range in Europe through heterogeneous habitats in terms of abundance and species richness of parasitoids (Aebi et al. 2006, Knapič et al. 2009, Csóka et al. 2009 ; Forster et al. 2009 ; Matošević et al. 2010). Such a slight impact of endemic species is in agreement with the ‘enemy release hypothesis’ (ERH) which specify that native enemies in the area of introduction have lower impact on invasive than on endogenous species (Cappuccino and Carpenter, 2005). Interestingly, according to the ‘Evolution of Increased Competitive Ability’ (EICA) hypothesis proposed by Blossey and Notzold (1995), such a low impact of native parasitoids is likely to increase the invasive ability of *D. kuriphilus* by reallocation of its resources from defense into growth and development.

While assessing the field impacts of the different bottom up and top down determinants of the local spread of an invasive species is an essential step, this, on its own, cannot allow predicting

how such determinants and their interplay shape the temporal dynamics of the interactions between the invader, its resource and parasitoids.

### **Integrative modeling approach for biological invasions**

In all three chapters of my PhD, I have intended to integrate the outcomes of my field and genomic study of the natural regulatory factors impacting *D. kuriphilus* populations into specifically tailored population dynamic models. My objective was to provide quantitative predictions about the potential interactions between these different factors and their impacts on the temporal dynamic of invasion in the different study sites of the Pyrénées-Orientales.

Similar predictive dynamic models had already been developed for the invasion of *D. kuriphilus* and its control agent *T. sinensis* (Paparella et al., 2016 ; Balsa et al. 2021). While those two models allowed to follow the time evolution of the pest and control agent populations in a spatially explicit framework, they are limited to homogeneous chestnut tree orchards where only the initial insect distributions and their overwintering mortality rates were varied. Although interesting, those two papers did not take explicitly into account the bottom-up and top-down regulatory factors associated to the resource and endemic species in the invaded environment. Integrating both genetic and ecological data into dynamical model is essential to investigate the role of community interactions on biological invasions (Lawson et al. 2011). Based on the seminal model of Nicholson-Bailey (1935) that is typically used to study host-parasitoid interactions (e.g. Mills and Getz 1996, Books), we developed a *D. kuriphilus* - *T. sinensis* model integrating ecological and genomic variables describing the naturally occurring bottom-up and top-down regulation factors. This allowed to identify the potential range of effect depending of the heterogeneity of each of these factors as well as the consequences for the development and persistence of the invasive population (Article 1, Figure 4). Such a modelling represents an innovative step in the study of *D. kuriphilus* invasion modelling, and in the field of populations dynamics studies where most modelling remain based on demographic and ecological data (Fletcher et al. 2016 ; Brown and Collopy, 2013). Indeed, the simultaneous description of demographic and genomic features into dynamical models remain rare even if they are starting to be implemented, for example, to assess the population dynamics of great whales by developing a Bayesian population dynamics model integrating abundance data, the strong anthropic impact from hunting and the genetic constraints from the subsequent bottleneck (Jackson et al. 2016). Integrative models considering ecological and evolutionary

genetic aspects could be of great interest for epidemiologic models, especially to integrate processes such as host-parasite co-evolution or virulence evolution (Schmid-Hempel, 2021).

With the increasingly easier access to -omics data (Gomez-Cabrero et al. 2014), even on non-model species (Ellegren, 2014), the use of integrative approaches based on the parameterization of dynamic models from ecological and -omics data, will be increasingly needed to help using such data to gain a better understanding of the evolution of invasive populations, which could further help refining our general knowledge of host-parasite, here plant-pathogen, interaction dynamics. Indeed, as it has been studied for epidemiological models (Moury et al. 2006 ; Tibayrenc, 2007), this could help to understand how i) the genetic diversity of the resource can influence the dynamics of invasive populations and ii) how the genetic plasticity of the host and the parasite can generate diversity and lead to complex co-evolutionary patterns.

### ***1.2) How to assess the efficiency of a biological control strategy ?***

In the introduction part of this thesis I have shown that biological invasions come at various direct and indirect costs to human society and local economies, which lead to different local, national and international control initiatives to limit their impacts. Amongst the set of typical control strategies, the use of biological control agents is being increasingly encouraged, e.g. by the European Green Deal, to limit chemical entrances into our soils, protect biodiversity and grow healthy food for all (Tataridas et al. 2022). To assess the efficiency of a potential biological control strategy is however not obvious, and two key features are mainly studied. First, the growth rate of the control agent population and therefore its ability to establish and to both develop large populations and spread to control away from the release sites (Baars, 2003; Bean et al., 2014). Secondly, the control agent ability to actually downregulate the invasive population (Sheppard et al., 2001; Dhileepan, 2003; Story et al., 2006). While essential for the success of the biological control strategy these two features do not necessarily come together naturally, and several studies have indeed shown a significant population growth of the biological agent without an effective control of the invasive species (Milan et al. 2006; Pearson and Callaway, 2003). This clearly raises the question of how to properly assess the overall ability of a control agent?

#### **The growth rate of the control agent population.**

The classical measure used to quantify the growth potential of a population is the well-known net reproductive rate,  $R_0$ , which gives the mean number of offspring that a female produces during her lifetime and that has to be larger than 1 for the population, here of control agents, to grow. The regulatory forces imposed by the environment can influence such growth potential and therefore the introduction success of an agent by impacting on its ability to access its resource and/or to encounter its natural enemies and therefore its basic survival and reproductive rates (Harms et al. 2020). Accordingly, field studies in heterogeneous environments can allow for a better understanding of spatial variability in control agent establishment success and to the identification of the most favorable conditions for its population growth (Van Klinken et al. 2003). This approach has already been used in recent invasion biology studies (Pyšek and Richardson, 2006; Cronin et al. 2015) as well as for the monitoring of biological control strategies (Swirepik and Smyth, 2003; Denoth and Myers, 2005).

In the second chapter of this PhD, I have used such an approach and demonstrated that the main determinant of the installation success of *T. sinensis* is the chestnut tree frequency, where its host *D. kuriphilus* is located. Such a result makes entire sense since, as for its host *D. kuriphilus*, this parasitoid i) is highly dependent of its (host) resource distribution because of the specificity of their parasitic relationship, and it ii) has a poor ability to navigate toward its resource in field conditions (Graziosi and Rieske, 2013). Such a result is highly consistent with a review that have also shown that the success of biological control strategy can strongly rely on biotic differences between introduction sites (Harms et al. 2020). Furthermore, studies have shown that the geographic distribution of the resource, its genetic combability with the agent (Boughton and Pemberton, 2011) and its quality (Van Hezewijk et al. 2008) influence the biocontrol efficiency.

Our estimates of the resulting *T. sinensis*'s  $R_0$  ranged from 0.055 to 2.995 (Article 2, Figure 2) and it was larger than 1 in most studied sites (16 over 23), allowing for the broad establishment of the control agent in natural chestnut tree forests at the scale of the Pyrénées-Orientales, as previously evidenced in Chapter 1. Such a large spread of the agent confirms and extend previous studies assessing *T. sinensis* ability to grow a population when introduced. It is indeed consistent with repeatedly observed successes of introduction in orchards where the frequency of chestnut trees is obviously high, and it demonstrated that the agent can also spread in natural or semi-natural forests as long as the frequency of chestnut trees is larger than 41%.

Fieldwork studies also provide opportunities to determine how inoculum size and composition influences colonization success of the introduced agent (Shea and Possingham, 2000). Those have been showed as major determinants of the biocontrol success for numerous biological invasions (Hopper and Roush, 1993; Memmott et al. 1998). Interestingly, Borowiec et al. (2018) demonstrated a limited effect of the propagule pressure on *T. sinensis* post-introduction dynamics, as they showed that largest introductions lead to a small demographic advantage, but that the fractionation of this introduction effort into multiples releases had no influence on the parasitoid density after 4 years. The slight impact of inoculum conditions could be explained by the quick and exponential growth of both invasive and control agent populations (Article 2, Figure 2). The sex-ratio in the set of control agent individuals could also represents a critical variable for its establishment and success. Indeed, an uneven balance between males and females may lead to lower reproductive success and slow their population growth rate. This was observed following the introduction of *T. sinensis* in Southwestern Japan where the low female sex ratio lead to no increase of its population abundance during the first 6 years (Murakami and Gyoutoku, 1995).

Overall, *T. sinensis* has a strong potential to quickly build-up large populations in both chestnut tree orchards and forest. However, it does not seem to fully achieve its ultimate role as a control agent optimal. A biological control strategy indeed aim at reducing the invasive population until its extinction, which in return shall lead to the collapse of the introduced agent. This does not seem to happen with *T. sinensis* that typically persist in the environment, as evidenced in Japan where it remained for 25 years following its initial introduction (Moriya, personal communication) and as reported in chapters 1 and 2 of this PhD. In those contexts, it is critical to evaluate the rate of control exerted on *D. kuriphilus*.

### **The control rate exerted on the pest population**

The evaluation of the control rate exerted by a control agent is typically twofold. One shall assess the decrease in abundance of the invasive species following the introduction of the control agent and, concomitantly, the prevalence of infestation of the invasive species by the introduced agent, so that the former can effectively be associated with the biological control agent.

A significant outcome of my field study and of the third chapter of this thesis is to provide evidence that following the introduction of *T. sinensis*, that was first reported in 2014, there was a significant drop of the infestation levels by *D. kuriphilus* between 2019 and 2021 in most of the studied sites (Article 3, Figure 1). The observed time-delay between introduction of *T. sinensis* and an apparent control of *D. kuriphilus* populations is consistent with results from Borowiec et al. (2018) who reported a decrease in pest infestation 4-5 years after the first releases of the control agent. We simultaneously estimated the prevalence of infection in chestnut tree galls collected in 2020 to be on average 92.8%, which was similar to the 91.3% to 95.5% observed rates of parasitism previously recorded 5-7 years after release (Bosio et al. 2013). The concomitant reduction in *D. kuriphilus* infestation and high prevalence of hyperparasitism by *T. sinensis* provided strong evidences of the actual impact of *T. sinensis* on *D. kuriphilus* populations in the Pyrénées-Orientales. While we confirmed that the biological control strategy with *T. sinensis* can lead to a strong and rapid reduction of the invasive population, we also showed that *D. kuriphilus* populations were still persisting in the environment 8 years after the introduction of the control agent. This could either reflect a transitory stage in the dynamic of co-extinction between the invasive species and its control agent or, alternatively, lead to the persistence of both introduced species in the natural environment. Such an outcome was indeed shown by Moriya and his colleagues who monitored the control agent over 25 years following its introduction in Japan in the late 1970s, and showed three successive resurgences of *D. kuriphilus* population (Moriya, personal communication).

To conclude, even if a biological control strategy seems to be effective during the first few years of its implementation, only a long-term monitoring of both the invasive species and the agent populations can provide definite evidences. In a review, McFadyen (1998) suggest that, considering that agent establishment and control usually takes 10 or more years (Hoffmann, 1995), at least 15 years are necessary to allow confirming the success of a biological control program. Although the actual amount of time required for such a diagnostic is likely to vary according to the life-histories of the invading species and the control agent, there is no doubt that long term monitoring will require significant human and economic resources. In such a context, expanding the integrative modelling discussed in section 11 can be of great interest to predict the ability of an agent to control the invading population and/or their long-term persistence in the environment.

### **Integrative modeling for the biological control of invading species.**

Pre-release modeling is a promising approach to evaluate the potential effectiveness of a biological control strategy (Morin et al., 2006, Raghu et al. 2007). Using data collected during exploratory surveys, environmental niche modeling could allow to predict not only the distribution but also the abundances of the control agent within the introduced range, which is a key information as a high abundance agents is usually critical to achieve an efficient control (Augustinus et al., 2020). Spatial variability of biological control efficiency could then be anticipated using model predictions about the spatial variability in establishment success (Zalucki and van Klinken, 2006). The underlying working assumptions of such approach is that pest and control agent distributions, and subsequent variations in the magnitude of control, are readily explained by environmental gradients that induced variations of key regulation factors of both populations (Augustinus et al., 2020). Such population growth modelling approach could allow to predict pest and control agent occurrence along these gradients (Gotelli and Kelley, 1993) and to make reliable long-term predictions. Nevertheless, such deterministic models do not take into account random natural events, that could have significant effects when population densities are low (Black and McKane, 2012).

During this PhD, I have chosen to develop population dynamic models that explicitly represent the mechanisms of *D. kuriphilus* - *T. sinensis* interactions, to make predictions about the ability of *T. sinensis* to control *D. kuriphilus* in different environment encountered in the Pyrénées-Orientales. The analysis of the one-site modelling in Chapter 1 lead to predict 4 potential dynamics of interaction according to the frequency of chestnut trees and their genetic susceptibility to *D. kuriphilus*. Interestingly, none of those dynamics ended-up in the typical co-extinction of the invasive and its control agent, and instead both introduced species persisted in the environment where they could experiment either stable or oscillating coexistence as soon as the frequency of the host chestnut tree exceeded 41%. Similar modeling predictions were made by Balsa et al. (2021) who show that the two species can coexist around an unstable fixed point, leading to a population dynamic that clearly corresponds to stable host-parasitoid cycles.

Interestingly, the analysis of the two-sites model in Chapter 2 strongly confirmed such predictions as even low levels of dispersal between a site where *D. kuriphilus* and *T. sinensis* co-exist and a site where *T. sinensis* fail to persist, allowed for the persistence of both species in both sites. Such a predicted long-term persistence of the *D. kuriphilus* - *T. sinensis* interaction, with or without the help of dispersal from local sources of *T. sinensis*, is consistent



with the observation made by Moriya and its colleagues' in Japan and suggests that we might expect similar resurgences of *D. kuriphilus* in the chestnut tree forests of the Pyrénées-Orientales.

Those predictions made from the development of invasive host-parasitoid population dynamic models illustrate how this type of modelling provide the opportunity to investigate quantitatively the trade-off between the ability to grow and to control of the biological agents and the resulting impact on the invasive population. In the case studied, although the installation of *T. sinensis* is successful and leads to a strong reduction of *D. kuriphilus* abundance in the first years, its low ability to navigate towards the targeted pest individuals does not subsequently allow to eliminate the invasive from the environment. The type of dynamical models that we have developed can typically be used to further compare several implementations of the intended biological control strategies in order to choose the most efficient one (Büyüktaktın et al. 2011, Baxter et al. 2008), which could be especially useful to anticipate the relationship between the amount and timing of annual releases and the control efficacy.

Dynamical modelling used to estimate the spread and efficiency of biocontrol strategies are generally designed and parameterized for cultivated areas, such as orchards in the biological system considered in this study. Nevertheless, dispersal of control agents from crops to the natural environment is common (Simberloff and Stiling, 1996; Strong and Pemberton, 2000) and could lead to different scenarios of control as well as trophic perturbations within the surrounding natural ecosystems (Tscharntke et al., 2005). To transpose such modelling to natural and semi-natural areas, as exemplified in this PhD, is thus necessary and it can unravel interesting variations in the importance of regulatory factors and/or the effectiveness of the biological control strategy as compared to crops.

## **2) Biological control in complex orchards, semi-natural and natural interconnected environments**

Exploited and (semi-)natural environments typically show differences in community and genetic structures, which is likely to lead to variations of the regulation pressures between those areas. The efficiency of biological control is then expected to vary with the complexity of the

local environment (Jonsson et al. 2015) and management strategies must then be adapted to the different ecological and biological conditions encountered in such environments and according to the interconnections between them, in order to be most efficient at controlling the spread of the invasive while limiting the risks associated with the introduction of a control agent for the local ecosystems, e.g. the perturbation of trophic webs through interaction with native and non-target species (Delfosse, 2005).

## ***2.1) Differences in resource distribution, genetic structure and issues between exploited and natural environments***

### **Biological invasions and control strategies in highly susceptible agricultural monoculture**

In order to increase their productivity, crops generally are monoculture associated to a low genetic diversity (Bourke et al., 2021; Bhandari et al., 2017). This cultivation method has negative environmental effects such as a loss of biodiversity and a stronger susceptibility to infection by invasive and pathogenic agents (Potapov et al., 2021). The decrease in species richness will indeed lead to a reduction in the abundance and richness of predators, thereby lowering the natural shield against biological invasions (Harwood et al. 2009), and to lower levels of interspecific competition for the resource used by the invasive species. Exploited areas thus provide optimal conditions for the growth of invasive populations because they correspond to large amount of easily accessible and unprotected resources that, in addition, typically have a restricted genetic potential for adaptation. Such facilitative effects have been demonstrated by several studies that showed a “monoculture effect” resulting in an increase susceptibility of agricultural areas to invasive species (Ebert, 1998; Baer and Schmid-Hempel, 1999). This fits into the broader picture that the invasibility of a habitat is enhanced by traits such as low richness, low diversity and empty ecological niches (Ziller, 2000; Elton, 1958; MacArthur, 1970, 1972). Our results provide additional evidences that monospecific and genetically homogeneous stands are more vulnerable since i) the higher infestation levels by *D. kuriphilus* were recorded in homogeneous chestnut tree orchards (Article 2, Figure 2), as observed by Fernandez-Conradi et al. (2018), and ii) individual chestnut trees that are most genetically different from the rest of the population are less susceptible to ACGW infection (Article 1, Table 1).

Contemporary agricultural environments are thus prone to invasion and, as presented in the introduction of this PhD, there are different management strategies to limit the large associated economic losses. Biological control is an highly advisable strategy not only because it is relatively cheap, does not require much human resources, has proven to be effective in many cases (Obrycki et al. 2009 ; Seastedt, 2015), but also because it does not carry the environmental and human health burden that the use of pesticides (Rani et al. 2021). In the case of agroforestry, biological control is often the only strategy to control an invasion in the short term, and the invasion of cultivated chestnut tree orchards by *D. kuriphilus* typically fall in this category. Evaluation of damages caused by this pest showed that infestation levels above 0.6 galls per bud induced a drastic decrease of productivity, while no significant loss were observed below 0.3 galls per bud (Sartor et al. 2015). According to our field study in the Pyrénées-Orientales, while some of the stations exhibited rates of infestation ranging between 0.2 and 1.1 galls per bud in 2019, they all decreased below the threshold which does not lead to a loss of chestnut production under the control of *T. sinensis*. Such a control strategy is adapted to agricultural areas because it leads to a strong and rapid drop of the AGCW abundance below the acceptability threshold for chestnut production. However, we showed that, while allowing to reduce the abundance of *D. kuriphilus* in semi-natural and natural sites studied in the Pyrénées-Orientales, *T. sinensis* did not allowed to remove the invasive pest, leading to the long term persistence of both species in the natural an semi-natural environments.

### **Biological invasions and control strategies in naturally resistant complex environments**

Agroforestry systems and natural forests are generally more complex than crops as they are associated to a greater species and genetic diversity (Wiersum, 1997; Ratnam et al., 2014; Finkeldey and Ziehe, 2004). Natural ecological network includes more species, which accentuates the phenomena of interference between introduced and native species (Abram and Moffat, 2018). The pressures exerted by the bottom-up and top-down regulatory factors are then stronger, which lead to a greater resistance to biological invasions and, although these ecological interactions rarely impede the invasive population settlement, they are expected to constrain its abundance. This has led to the biotic resistance hypothesis which state that communities with strong diversity may resist invasion through biotic processes such as predation, herbivory, competition or disease (Levine et al. 2002, 2004). As shown in chapter

3, such a pattern hold for *D. kuriphilus* with the lower levels of infestation recorded in the natural reserve of the Massane, a 336 hectares protected area exhibiting an exceptional species richness with over 9500 identified species. This strong species richness and intraspecific diversity are likely to act as an environmental filter against invasive species (Ramos et al., 2015). In such natural areas, the preservation of the native biodiversity is the major challenge set by a biological invasion (Margules and Pressey, 2000). One shall typically still aim to remove the alien species from the invaded environment, which fits the biological control purpose, but one is typically reluctant to the introduction of a control agent that can potentially results in the further persisting addition of a species into the protected ecosystem. Such an outcome was indeed suggested in chapter 2 of this PhD where our integrative modelling predicted the long-term coexistence of *D. kuriphilus* and its control agent in most studied sites, including La Massane. In most other studied sites that were semi-natural exploited or natural (but not protected) environments, we came to the same prediction of a long-term coexistence between the invasive and its control agent, which might come at the expense of alternative environmental risks posed to non-targeted species (Van Lenteren et al. 2006) whose evaluation fall outside of the scope of this PhD.

A significant consequence of the large range of expected outcomes of *D. kuriphilus* - *T. sinensis* interactions (from typical co-extinction in orchards, to stable or unstable coexistence in (semi-)natural environment) is that the effectiveness of *D. kuriphilus* biological control is likely to depend on the local complexity and heterogeneity of the environment and on the dispersal ability of both species.

## ***2.2) The efficiency of biological control in complex environments***

As presented in the review of Pluess et al. (2012), eradication of alien species were more likely to succeed in man-made agricultural habitats than in (semi-)natural environments. While the greater complexity of natural forests indeed accentuates potential interactions between the control agent and native species (Jonsson et al. 2015), these ecological interference can also be beneficial to the development of the introduced agent by providing it with refuges and/or additional food sources (Roschewitz et al. 2005). This rises the interesting questions of the relationship between the effectiveness of biological control and the local complexity of the environment.

### **Specialist and generalist control agents in simple and complex environments**

When the local complexity of the environment increases, especially its species richness, it is important to make a difference between generalist and specialist control agents as the former are expected to interact with more native species. Such agents can indeed have several nutrient sources, which favors their establishment. According to the "enemies" hypothesis formulated by Root (1973) this is especially relevant in heterogeneous environments that provide greater amount of prey/hosts, wintering sites and refuge available for generalist species. Stiling and Cornelissen (2005) conducted a quantitative meta-analysis on data from 145 biocontrol studies and showed that biocontrol efficiency tend to be higher when agents are generalists in complex environments. This greater downregulation of the pest abundance would be explained by the generalists ability to subsist on non-targeted species and therefore to not decline in number and efficacy when they significantly reduce the abundance of the targeted species. Interestingly, while being more efficient, generalist are obviously associated to higher risks for the local environment as they can have larger impact on non-targeted native species as a result of their larger host range (Loomans, 2021), which actually is a reason why specialist agents are commonly favored for biological control. Meanwhile, in an homogeneous environment, the habitat fragmentation hypothesis (Murdoch et al. 2013) suggests that a better biocontrol could be reached using specialist agents. Natural enemies that aggregates in sites with higher host density are indeed more susceptible to develop large populations and reduce the pest abundance because of their greater encounter rate with the host. The efficacy of the highly specialist *T. sinensis* to control *D. kuriphilus* in chestnut tree monospecific stands that was predicted from our modelling (Article 1, Figure 6) and from the model recently developed by Balsa et al. (2021), are truly consistent with the above hypothesis. However, the expected variations of the effectiveness of *T. sinensis* control with the local species tree diversity and with the chestnut trees susceptibility led us to start investigating how dispersal within heterogeneous environments could influences the overall rate of control of *D. kuriphilus* by *T. sinensis*.

### **Connection of heterogeneous areas via dispersal**

Local population growth rates and dispersal abilities of the pest and control agent are the main determinants of biological control success (Fagan et al. 2002). The rate at which control agents disperse from release sites is crucial to maximize its probability of establishment and has important implications for a successful management. Spatial models allow to explore how

habitat-specific demographic rates and patch connectivity can explain spatial structure and persistence in heterogeneous landscapes (Reilly and Elder, 2014 ; Petrovskii et al. 2005 ; Dauphinais et al., 2018).

By implementing a two-sites model, we have shown that *T. sinensis* dispersal between heterogeneous areas leads to a synchronization of their population dynamics that typically match the dynamic of the most favorable area for the control agent (Article 2, Figure 3). However, because of their different initial control agent abundance, the number of emigrants individuals is unequal between the two sites. It results in an increase of *T. sinensis* abundance in the less favorable site which could then facilitate the establishment success of the control agent, while a dilution of the initial abundance is expected in the site with the higher resource abundance, potentially impacting its efficiency to control the pest population. According to our predictions, the dispersal of both *D. kuriphilus* and *T. sinensis* between heterogeneous area with low or mid chestnut tree frequency induces a negative effect on the level of control while, on the contrary, biocontrol efficiency is enhanced between homogeneous chestnut tree orchards (Article 2, Figure 4,6). Such phenomena are typical of source-sink dynamics, that describe how habitats varying in quality influence the overall population viability (Thomson, 2007). According to such dynamics, the maintenance of an invader population in marginal habitats may depend on inputs of individuals from adjacent favorable ‘source’ areas, as observed for the invasive carp (Dauphinais et al., 2018). Furthermore, such ‘sink’ invader populations are expected to lower the global control rate through the dispersion and dilution of control agents from “source” populations. The outcomes of our modelling observed in Figure 5 from Article 2 detailed how *D. kuriphilus* and *T. sinensis* populations expected in the different natural and semi-natural sites studied in the Pyrénées-Orientales could exhibit such source-sink dynamics when connected by either or both species dispersal. These results highlighted that site connectivity and habitat quality could increase the control agent population size and favor a successful colonization of the invaded sites, as actually observed for *T. sinensis* (Borowiec et al. 2018) and for other biological control strategies (Roschewitz et al. 2005 ; Perry et al. 2017 ; Tschardt and Brandl, 2004). Such two-site models could now be further extended to help understanding and optimizing the control agent release strategy by identifying the sites most favorable to the agent population local growth and spread to establish themselves beyond the introduction site. In addition, such spatial modeling could allow to better understand the control efficacy of *T. sinensis* in chestnut tree orchards, while considering dispersal to and from the

surrounding (semi-)natural forest environment, which could potentially help identifying the condition for (observed) reemergence.

Two-patch models could also be extended to spatially explicit models in order to study the spatial the spread of the pest in a given biogeographic framework. Such models have been used to construct risk maps predicting the invasion front speed and quantifying its potential impact in areas with various susceptibility to invasion (Prasad et al. 2009; Venette et al. 2010). The quantification of this risk could then allow to adapt management strategies in order to reduce the invasive species spread and impact on its resource. Balsa et al. (2021) developed a space-dependent model for the *D. kuriphilus* – *T. sinensis* interaction and predicted a cyclic density wave pattern, resulting in the continuous alternation of local extinctions and recolonizations of the pest. This failure to eradicate *D. kuriphilus* from large or heterogeneous areas would be due to its higher diffusivity as compared to the dispersal ability of *T. sinensis*. The authors then suggested to perform additional releases of the parasitoid in years when the ACGW abundance is high in order to reach a higher efficacy of biocontrol. Such an adaptive strategy could indeed allow to reduce the number of years during which *D. kuriphilus* is present at its maximum population density by accelerating the reconstitution of the parasitoid population, although it does not prevent its persistence in the long term. A spatially explicit model of the *D. kuriphilus* spread in the Pyrénées-Orientales integrating the main results of this thesis is currently being implemented in the team, and it should soon allow to address such questions.

To conclude, complex community structure is expected to globally reduce the efficiency of specialist control agents at the local scale by reducing its resource availability and increasing potential ecological interference (cf. Section 1.2). Such control reduction further tend to be accentuated at the landscape scale by dispersal events between these complex habitats (cf. Section 2.2). Although interactions between specialist agents and endemic species are generally deleterious for the biocontrol efficiency, the complexity of the natural biodiversity could lead to additional effects on biocontrol and therefore need to be investigated. The study presented in the third chapter of this thesis then allowed to evaluate how such interference with the native biodiversity could influence the *D. kuriphilus* abundance, *T. sinensis* efficiency and ecosystem balance.

### ***2.3) Ecological interference and potential impact on native biodiversity***

Despite their specificity for their resource, specialist agents could experience variation of their control efficiency due to biotic interference with the native biodiversity (Goeden and Louda, 1976; Chacón et al. 2008). These landscape dependent processes generally act through direct and/or indirect relationships, such as predation (Jones et al. 2020), interspecific competition (Howarth, 1990) or disruption of host detection (Castagneyrol et al. 2013 ; Beyaert and Hilker, 2014 ; Randlkofer et al. 2010). While direct interactions are commonly observed for generalist agents, they are less frequent for specialists, although they remain possible if those are to show unexpected plasticity of their diet or reproduction needs. These potential ecological interferences could then lead to positive or negative variations of control agent population growth rate but also impact the specific richness and abundance of native biodiversity (Howarth, 1990; Li et al. 2021)

#### **Indirect interactions with native species**

The predation on biological control agents have been shown to impact their population growth rate and therefore their establishment success (Pearson and Callaway, 2003 ; Müller et al. 1990 ; Dray et al. 2001). Several studies indeed report attacks by native predators of introduced agents, leading to a slower colonization of the invaded environment (Jones et al. 2020). Although there is still no report of predation of *T. sinensis*, Nakamura and Nakamura (1977) showed that spiders exhibit strong predation rates on its host *D. kuriphilus* during its adult stage. Hypothetically, flying parasitoid could then also be consumed by arachnids, even if such a predation should remain of low impact on both the pest and its control agent considering the low population density of these predators.

Interspecific competition has also been shown to slow down the population growth of control agents, leading to a potential reduction of the biocontrol efficacy resulting from competitive exclusion (Crowe and Bouchier, 2006). The resource provided by the invasive population can indeed present an interesting opportunity for native predators, which are commonly observed preying upon introduced species (Parker and Hay, 2005; Pio et al., 2019; King et al., 2006). In our field study, we observed an interspecific competition for the access to *D. kuriphilus* larvae between *T. sinensis* and endemic species of insects parasitoids and parasitic fungi that generally infest oak gall-forming parasites (Avtzis et al. 2019). However, the rates of parasitism of *D.*



*kuriphilus* by these species remain typically low, i.e. lower than 4.7% and 14% respectively, as reported in the first chapter of this study and in the literature (Kos et al. 2015 ; Szabó et al., 2014 ; Tosi et al. 2015 ; Cooper and Rieske 2010), so that they should not significantly impact the control agent population growth or the control level of *D. kuriphilus*.

The alteration of the detection capacity of control agents for their resource could also be a factor limiting their population growth. Parasitoids typically locate their host using odors and visual clues (Benelli and Canale, 2012; Wajnberg and Colazza, 2013). These stimuli could be scrambled in the natural environment by chemical signals associated to other species and potentially lead to a reduction of their host detection. Graziosi and Rieske (2013) showed that both visual and olfactory clues are indeed required to enable *T. sinensis* to successfully find suitable host. Nevertheless, Y-tube olfactometer experiments with these stimuli do not lead to an optimal detection of its host against blank control. We can then question how efficient the experimentally demonstrated capacities could be in a complex natural environment composed of various species. Ferracini et al. (2015) repeated this experiment, replacing the blank control with galls of several oak parasite species, and observed that 17% to 37% of *T. sinensis* individuals are actually attracted to the gall of the non-host species. Given the very low oviposition rate and emergence success of *T. sinensis* on those species (Ferracini et al., 2017), one can make the hypothesis that the observed reduction in the detection success of the control agent linked to the presence of oak can well be deleterious for the growth of its population.

Although few studies are devoted to these ecological interferences, they could potentially reduce the effectiveness of the *T. sinensis* biocontrol. In addition, this phenomenon could be accentuated if the control agent, although classified as a specialist parasite, experiences plasticity in its diet and reproduction in contact with native species, as it have been observed for others specific introduced agents (Cock, 2013 ; Havill et al., 2012).

### **Diet and reproduction plasticity of specialist agents**

Hybridization and consumption of non-target species could enhance the efficacy of biocontrol by an introduced control agent through beneficial effects such as a potential larger host range for hybrids or the possibility to subsist on non-pest prey (Depotter et al, 2016 ; Prior and Stroyan 1960 ; Stiling and Cornelissen, 2005). Hybridization events involving *T. sinensis* and the phylogenetically close *Torymus beneficus* have been recorded by Yara et al. (2010). Since

this *Torymidæ* native from China has a wider host range than *T. sinensis* (Matsuo et al., 2021), their hybrids could present a greater plasticity or a shift in host choice, as compared to the control agent, as observed in other insects (Hoffmann et al. 2002 ; Mathenge et al. 2010 ; Bitume et al. 2017). However, *T. beneficus* ecological range itself has been shown to be plastic and restricted in Japan (Matsuo et al., 2021), which could limit the impact of hybridization that, in any case, is unlikely to happen in the Pyrénées-Orientales where we have not been able to observe *T. beneficus* during our field study. On the contrary, we have shown diet plasticity of the introduced control agents in *Andricus kollari* and *Andricus dentimitratus* galls by molecular taxonomic identification (Article 3, Table 2). Such a finding is consistent with other studies that observed *T. sinensis* emergence from these oak gall-forming parasite (Ferracini et al., 2017). This new ecological niche could then constitute an alternative host for the control agent and ease its population persistence, especially when its interactions with *D. kuriphilus* lead to oscillations dynamics with period of lower abundance of its primary host. Such a refuge effect has actually been previously observed for invasive species under biological control (Hawkins et al., 1993) and could then be extended to specific parasitoids such as *T. sinensis*.

### **Impacts of introduced species on native biodiversity**

Direct and indirect interactions with native species discussed above could have either positive or negative impact on the abundance of endemic species. Indeed, introduction of alien species has been shown to have positive effect on biodiversity (Schlaepfer et al, 2011), for example when the new species is used as a resource by native species. Residents may change their diet to exploit them, resulting in direct positive consequences at higher trophic levels that can impact their population dynamics and subsistence. This phenomena has been observed in the Lake Erie, where an endemic threatened water snake draw large benefits from the introduction of gobies, which make up now more than 90% of their diet (King et al., 2006). Although *D. kuriphilus* is used as a food resource by endemic oaks parasitoids, their attacks remain opportunist and results in low parasitism rate (4.5%) which is unlikely to favor the population growth of these endemic parasitoids (Ferracini et al. 2017). As a matter of fact, biological invasions and their management strategies most often have detrimental effects on endemic ecological network. Interactions between endogenous and exogenous species such as interspecific competition (Gurnell et al. 2004), parasitism/predation (Doherty et al. 2016) or hybridization (Bleeker et al. 2007) have indeed been shown to decrease the native biodiversity. Given the very low parasitism exerted by *T. sinensis* on non-target oaks parasites that range

around 0.8%, Ferracini et al. (2017) concluded that these occasional feeding have no significant impacts on the distribution and abundance of both oak parasites and parasitoids. Finally, introduced species could promote the spread of pathogen or other invasive species if they act as vectors (Halstead, 2008) or enhance their infection success (Hoyer et al. 2017), as stated by the invasion meltdown hypothesis (Simberloff and Von Holle, 1999, Braga et al. 2018). According to the results presented in chapter 3 of this PhD thesis and to previous studies, the simultaneous infection of chestnut trees by *D. kuriphilus* and the chestnut blight can result in a synergic effect favoring the prevalence of infection by each pest. Indeed, Meyer et al. (2015) assumed that *D. kuriphilus* galls could be entry points for the fungi, and our statistical analysis (Article 3, Table 1) and previous observations by Germinara et al. (2011) suggest that chestnut blight infected chestnut tree are more easily detected by *D. kuriphilus* for oviposition.

I have highlighted that differences in community structure and stakes are observed between the exploited and (semi-)natural environments during biological invasions, and that such heterogeneities can influence both biological control efficiency and its impacts on the native biodiversity. One shall therefore adapt the management of invasive species to the specificities of the invaded area if one is to attempt to make it optimal while considering their ecological, economic and social implications.

## **General conclusion**

During this 3-year research study, I assessed *D. kuriphilus* invasion in the natural forest of the Pyrénées-Orientales and the bottom-up and top-down regulation factors underlying the spatial heterogeneity of its infestation levels. The data I collected were integrated into a Nicholson-Bailey dynamical modelling for host-parasitoid in order to anticipate the dynamic of the interaction between the invasive pest and its control agent, *T. sinensis*. This ‘Eco-genomic’ model predicted a major impact of the chestnut tree frequency and of their genetic susceptibility on the ACGW population growth rate, while the top-down pressure induced by native parasitoids appears minimal.

The results of this PhD thesis, combined with the empirical and (restricted) theoretical literature in this field, show that the establishment success of the control agent *T. sinensis* is primarily

determined by the distribution of its resource (the galls formed by *D. kuriphilus*) and the site connectivity. Furthermore, the integrative modelling approach that I developed allowed to conclude that while the biological control strategy is efficient in quickly downregulating ACGW population in natural and semi-natural chestnut tree stands, the typical co-extinction of both species ca not be reached. Indeed, I the implementation of such management method was predicted to lead to the permanent coexistence of the two non-native species in either a stable or oscillating populations dynamics. The two species are then expected to enter source-sink dynamics through the heterogeneous environment, which was shown to facilitate their spread and establishment at a wide spatial scale beyond the introduction sites.

I also provided evidences that the integration of these species in endemic ecological networks can induced perturbations of the ecological balance through biotic interference with native species and subsequently affect the *D. kuriphilus* invasion success as well as its control efficiency by the biological control agent. Indeed, I showed indirect interactions such as the perturbations of host detection, invasion meltdown with *C. parasitica* and alternative niche for *T. sinensis* in endogenous oak parasites. Finally, I concluded that the impact of the biological control strategy on the native biodiversity appears minimal although its long-term impact on the ecosystem remains uncertain and should then be investigated through long-term monitoring and complementary studies.

My research also provided information about the potential optimization of management methods of *D. kuriphilus* to face the specific ecological, technical, and socio-economic challenges set by the different environments to be protected. Indeed, while most of the studies on the current ACGW invasion and its biological control has been focused on exploited orchards, we aimed at extend these research to the natural environment. Such a complex system of exploited orchards, semi-natural and natural interconnected environments is predicted to result in source-sink dynamics for both introduced species beyond the biological control implemented in crops. Such dynamics could then favor the re-emergence of the parasite in orchards due to disperser coming from the naturel environment and raises concerns about the efficiency of biological control strategies in such complex interconnected heterogeneous environments.

By integrating demographic, ecological and genetic data, our dynamic model of the *D. kuriphilus* – *T. sinensis* interaction is designed to test different perspectives of adaptation of

control strategies focus on the resource, i.e. its distribution and its genetic resistance, and/or on the survival of the invasive against endemic parasitoids or the biological control agent. Such management methods could then be tested independently but also combined in order to optimize synergistically their efficiency. Mixed host resistance and biological control would then be the most efficient strategy to minimize the ACGW damages and subsequent economic loss in exploited crops. Indeed, their combination could strengthen the broad biocontrol, improve crop protection and allow to reach a quick reduction of the *D. kuriphilus* population under the threshold allowing no significant yield loss. While replacing the entire chestnut stand with a more resistant strain does not seem feasible for financial reason, the implantation of a limited number of resistant individuals could lead to their hybridization with native varieties and allow to reduce the genetic susceptibility of the population. Although such a combination is a promising method to enhance the effectiveness of both control strategies, that it is not necessarily the most appropriate management strategy for natural reserves. Indeed, the preservation of the intraspecific diversity of the resource and the maintenance of the ecological network balance are the priorities in such a conservation context. Nevertheless, the greatest complexity of such protected areas could naturally restrain the pest population growth and spread through biotic resistance mechanisms.

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## Scientific productions

### First author articles:

**Zitoun, J.L., Rousseau, R., Bertrand, J., Toulza, E., Rosalie, M., Gourbière, S. (2023).** (2023). Assessing the invasion of the global chestnut tree pest, *Dryocosmus kuriphilus*, and its bottom-up and top-down control: an integrative eco-genomic modelling study in the natural forests of the Pyrénées-Orientales. (in prep).

**Zitoun, J.L., Rousseau, R., Rosalie, M., Gourbière, S. (2023).** How do heterogeneity and dispersal influence the spread and biological control of the global chestnut tree pest, *Dryocosmus kuriphilus*, in the Pyrénées-Orientales ? (in prep).

**Zitoun, J.L., Rousseau, R., Gourbière, S. (2023).** An integrative modelling of key species interference with invasive species and biological control strategies : the case of the global chestnut tree pest, *Dryocosmus kuriphilus*, in the natural forests of the Pyrénées-Orientales. (in prep).

**Zitoun, J. L., Gourbière, S. (2023).** Study of the bottom-up regulation factors limiting the chestnut blight (*Cryphonectria parasitica*) spread in natural forests of the Pyrénées-Orientales. (in prep).

### As collaborator :

**Rosalie, M., Casteigts, A., Zitoun, J.L., Gourbière, S. (2023).** Modelling parasite invasion with graph theory: *Dryocosmus kuriphilus* a threat for *Castanea Sativa*. (in prep)



## **Activities in the lab (LGDP) and at the University of Perpignan (UPVD)**

### **Intern's supervision**

**2022 – Raphaël Rousseau, Master 2 Internship:** Modélisation du contrôle « Top-down » et « Bottom-up » de l'invasion des peuplements de châtaigniers des Albères et du Vallespir par un insecte phytophage invasif, le cynips du châtaignier.

**2021 - Raphaël Rousseau, Master 1 Internship:** Modélisation de l'effet de l'hétérogénéité spatiale sur l'invasion des peuplements de châtaigniers des Albères et du Vallespir par un insecte phytophage invasif, le cynips du châtaignier.

**2020 - Camille Florescu, technician** recruited for the 2020 fieldwork.

### **Teaching**

**2020-2021:** 40h Modelling of biological systems

23h Ecotoxicology

**2021-2022:** 38h Descriptive statistics

## Résumé

Les fréquences des invasions biologiques n'ont cessé d'augmenter au cours des deux derniers siècles et sont en étroite association avec l'essor des activités humaines. Les espèces envahissantes pouvant entraîner de fortes pertes économiques sont aujourd'hui reconnues comme une menace majeure pour la biodiversité. Il est donc essentiel d'identifier les déterminants qui entravent ou favorisent l'implantation et la propagation locale de ces espèces envahissantes afin d'améliorer l'évaluation du risque, d'anticiper son évolution et de quantifier l'efficacité des mesures de protection potentielles. L'hyménoptère *Dryocosmus kuriphilus* est le ravageur le plus nocif des châtaigniers (*Castanea spp.*) affectant la production de châtaignes et de bois, ainsi que les insectes natifs dans les vergers et les forêts du monde entier. Cet insecte envahissant, originaire de Chine, s'est propagé à l'échelle mondiale jusqu'à atteindre et envahir la région Occitanie en 2007, entraînant par la suite l'introduction de l'agent de contrôle *T. sinensis*. Au cours des trois dernières années, j'ai mis en place des études écologiques et génomiques dans 24 sites du département afin de permettre d'identifier les apports des facteurs de régulation 'Bottom-up', i.e. l'abondance, la fréquence et la sensibilité génétique des châtaigniers, et 'top-down', i.e. le taux de parasitisme de l'envahissant *D. kuriphilus* par des espèces parasitoïdes endémiques, et d'expliquer ainsi l'hétérogénéité spatiale du niveau d'infestation des châtaigniers par le ravageur invasif. Ces données écologiques et génomiques ont toutes été analysées et intégrées dans des modèles dynamiques « éco-génomiques » spécifiquement conçus pour permettre d'identifier i) les principaux déterminants de la diffusion de *D. kuriphilus* dans les châtaigneraies des Pyrénées-Orientales, ii) la capacité de l'agent de lutte biologique à limiter l'invasion locale et iii) à fournir une compréhension quantitative de l'interaction *D. kuriphilus* – *T. sinensis* dans ces forêts.

## Abstract

Biological invasions have been steadily increasing in frequency over the last two centuries in tight association with the rise of human activities. These invasive species could lead to strong economic losses are now fully recognized as a major threat to biodiversity. It is then essential to identify the determinants that impede or promote the establishment and local spread of invasive species in order to improve risk assessment, anticipate its evolution, and quantify the efficacy of potential protection measures. The Asian chestnut gall wasp, *Dryocosmus kuriphilus*, is the most dangerous pest of chestnut trees (*Castanea spp.*) affecting chestnut production and native communities in orchards and forests worldwide. This invasive insect, native to China, has spread globally until it has reached and invaded the Occitanie region in 2007, subsequently leading to the introduction of the control agent *T. sinensis*. Over the last three years, I set-up ecological and genomic studies in 24 sites within the department in order to allow for the identification of the contributions of 'Bottom-up', i.e. the abundance, frequency and genetic susceptibility of chestnut trees, and 'Top-down' regulation factors, i.e. the rate of parasitism of the invasive *D. kuriphilus* by endemic parasitoid species, in explaining the spatial heterogeneity in infestation level of chestnut trees by the invasive pest. These ecological and genomic data were all analyzed and integrated into 'eco-genomic' dynamic models that were specifically designed to allow identifying i) the main determinants of *D. kuriphilus* spread in the (semi-)natural chestnut tree population of the Pyrénées-Orientales, ii) the ability of the biocontrol agent to limit the local invasion and to provide a quantitative understanding of the *D. kuriphilus* – *T. sinensis* interaction in (semi-)natural tree forests.

## Résumé détaillé

Les fréquences d'invasions biologiques n'ont cessé d'augmenter au cours des deux derniers siècles et sont en étroite association avec l'essor des activités humaines. Les espèces envahissantes sont désormais pleinement reconnues comme une menace majeure pour la biodiversité pouvant provoquer l'extinction d'espèces, l'homogénéisation de la faune et de la flore et des changements dans la biogéographie. Leurs impacts sur le fonctionnement et/ou la productivité de tous les (agro-)écosystèmes altèrent les services rendus aux sociétés humaines, avec 40 % des coûts globaux estimés étant associés aux dommages causés par les insectes envahissants à la foresterie et à l'agriculture. Dans de tels contextes écologiques et économiques, il est essentiel d'identifier les déterminants qui entravent ou favorisent l'implantation et la propagation locale des espèces envahissantes afin d'améliorer l'évaluation des risques, d'anticiper son évolution et de quantifier l'efficacité des mesures de protection potentielles.

L'hyménoptère asiatique, *Dryocosmus kuriphilus*, est l'insecte ravageur le plus dangereux pour les châtaigniers (*Castanea spp.*) affectant la production de châtaigniers et les communautés indigènes dans les vergers et les forêts du monde entier. Originaire de Chine, il s'est répandu au Japon et dans d'autres pays asiatiques dans les années 1950, avant d'être introduit en Amérique du Nord en 1974, et en Europe en 2002, où il a envahi un large éventail de région depuis l'Italie et a atteint la région Occitanie en 2007. Bien que les institutions locales en charge des cultures et de la santé des plantes aient signalé la présence du ravageur invasif *D. kuriphilus* et de l'agent de lutte *T. sinensis* en plusieurs endroits situés dans les Pyrénées-Orientales, aucune évaluation quantitative de leur abondance et de leur répartition n'était disponible en 2019, lorsque j'ai commencé mon projet de doctorat.

J'ai donc mis en place des études écologiques et génomiques pour combler cette lacune et concevoir des modèles intégratifs de dynamique de population permettant d'identifier i) les principaux déterminants de la propagation de *D. kuriphilus* dans le peuplement (semi-)naturel de châtaigniers des Pyrénées-Orientales qui sont typiquement gérées pour la production de bois, et ii) la capacité de l'agent de lutte biologique à limiter l'invasion locale, qui ont été financées par la mission interdisciplinaire du CNRS dans son appel « Modélisation du Vivant ». Au cours des trois dernières années, j'ai mené une étude de terrain dans 24 sites situés dans

différentes formations végétales du département afin de permettre l'identification des apports du "Bottom-up", c'est-à-dire l'abondance, la fréquence et la susceptibilité génétique du châtaignier, et des facteurs de régulation « Top-down », c'est-à-dire le taux de parasitisme de l'envahissant *D. kuriphilus* par des espèces parasitoïdes endémiques, pour expliquer l'hétérogénéité spatiale du niveau d'infestation des châtaigniers par le ravageur invasif.

J'ai en outre collecté des échantillons biologiques afin de mettre en œuvre le séquençage RAD des châtaigniers et des analyses GWAS pour identifier les locus associés à leur sensibilité à l'infestation par *D. kuriphilus*, ainsi que des analyses « barcoding » pour identifier les espèces endémiques d'hyménoptères qui parasitent réellement *D. kuriphilus*. Ces données écologiques et génomiques ont toutes été analysées et intégrées dans des modèles dynamiques « éco-génomiques » spécifiquement conçus pour répondre aux points i) et ii), et pour fournir une compréhension quantitative de l'interaction *D. kuriphilus* – *T. sinensis* dans les forêts (semi-)naturels, alors que la plupart des connaissances actuelles se concentrent sur ces dynamiques d'interaction dans les vergers.