# RESEARCH FOR NATIVE PARASITES AND PREDATORS OF THE BOX TREE MOTH *CYDALIMA PERSPECTALIS*, IN NATURAL BOXWOOD FOREST IN FRANCE

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## Abstract

*Since 2007, forests and parks and gardens of Europe are invaded by the pest Cydalima perspectalis (Walker, 1859) which is a moth native from Asia, and which negatively affects the boxwood (Buxus sempervirens L., 1753.). The high fecundity of the boxwood moth in addition to its high dispersal capacity allows it to colonize and settle quickly in new habitats. In order to control this pest, it is therefore important to know how the natural environment will react, and in particular the entomofauna. It is possible that some parasites will increase their host spectrum and that predators will adapt to this new prey. The objective of this project funded by Draaf Auvergne-Rhône-Alpes, is to find these native potential predators or parasites of the box tree moth. In this context, many insects and spiders were collected by beating method, in various boxwood forest in France. The collected individuals were then morphologically identified and their ecology studied. Our results revealed the presence of several potential parasites and predators of the box tree moth. In terms of parasite, the most found is a Diptera of the Tachinidae family, which represents 22% of the samples. Parasitoid hymenoptera from three different families were also collected: Braconidae, Ichneumonidae and Platygastridae. In terms of predators, beetles' individuals of the Carabidae family have been found in situ eating the moth. The two most common predator groups found on boxwood are Spiders and Hemiptera. Other insects with varied diets have also been found, in smaller quantities, and include Coleoptera, Orthoptera, Psocoptera and Hymenoptera. The perspectives of this work are to test these various parasites and potential predators, in the laboratory as a first step, to ensure their efficiency on the box tree moth; to ultimately choose the best biocontrol agent.*

**Key words:** *biological control, box tree moth, boxwood, Buxus sempervirens, collect by beating method, Cydalima perspectalis, parasites, predators*

## 1. INTRODUCTION

A species with negative ecological, economic, or health consequences is considered an invasive alien species (Mc Neeley, 2001). Biological invasions are a major threat to biodiversity and the functioning of natural ecosystems (Simberloff et al., 2013).

The box tree moth, *Cydalima perspectalis* (Walker, 1859), is one such species accidentally introduced into Europe during the box tree trade, *Buxus sempervirens* (Marumayama and Shinkaji, 1987), on which it can cause significant damage. *C. perspectalis* is a butterfly of the family Crambidae originating from East Asia. This species was first referenced in Germany in 2007 (Krüger, 2008), then continued its progression in Central Europe (Bella, 2013) with first records in Alsace in 2008, as well as in 2009 for Ile-de-France (Vidril, 2013). This species has, within a few years, colonized part of Europe (Nacambo et al., 2014), and has since largely continued its expansion.

The presence of toxic alkaloids in box tree leaves makes it a poorly consumed plant. These alkaloids, stored in the moth during its larval stage (Leuthardt et al. 2013), makes the larva unsuitable for consumption by non-adapted auxiliaries. Will parasites expand their host spectrum and predators expand their prey spectrum?

The Biocontrol laboratory, located in Antibes (France) and attached to the Villa Thuret Experimental Unit (UEVT) of INRAE PACA, develops control strategies using oophagous parasitoids, and more

recently predators. The team is currently working on several pest management projects, including the SaveBuxus project (2017-2021) to study the biology and behavior of the boxwood borer, in order to develop a method for biocontrol of populations.

It's from the hypothesis that generalist and opportunistic parasites and predators will integrate the box tree moth into their panel of prey and hosts, that the BIOPYR project (Research and inventory of potential biological regulation agents, related to the box tree moth, in natural forest environments) was born in 2017, supported by the Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt Auvergne-Rhône-Alpes (DRAAF).

The BIOPYR program (2017-2020) allows monitoring of the general state and the reaction of the different environments to the box tree moth, but also to acquire further knowledge on the boxwood ecosystem and the different species concerned by this endangered living environment.

## 2. MATERIALS AND METHODS

*2.1 Study of parasitism in the natural environment (2017-2019)*

*2.1.1 Sampling method*

An average of 300 box tree moth individuals (caterpillars and chrysalis) were systematically collected at one site in each of the departments studied. The choice of departments and sites was constrained by the rate of boxwood defoliation. As box tree moth populations are collapsing in some areas, sampling sites may change during the summer. Collections were made in early April, late May, mid-July, early August, and late August.

*2.1.2 Sample Conditioning and Coding System*

During the tour, the samples were packaged in transparent plastic boxes with ventilation holes closed by a 150µm mesh on the walls. Fresh boxwood is added during transport to keep the caterpillars alive. To limit mortality due to heat, the boxes are stored in coolers with ice packs. During sampling, box tree moth eggs and suspect cases (dying caterpillars, chrysalis of unusual color, presence of pupae, parasitism, deformities, ...) are isolated in tubes closed with a thin cloth for aeration. This is to be able to recover any potential parasitoid in a targeted way.

Each tube is coded. To each box corresponds a code and a notation sheet indicating: the name of the site, the name of the operator, the date of collection, the numbers of caterpillars, chrysalis, butterflies and eggs collected, the GPS data of the collection, the number of the storage box, the numbers of the pictures taken, the observations on the state of the box tree moth, the weather of the day, the observations on the moth, the observations of the predators and all other remarks.

*2.1.3 Method of sampling the parasites in the laboratory*

In the laboratory in Antibes, samples were stored in climatic rooms (Fig. 1B) at 25±1°C, 70±10%RH, photoperiod 16L:8D. Upon receipt, the caterpillars were counted and divided into two boxes with a maximum of 150 caterpillars per box. The lid of each box is sprinkled with honey drops to attract the emerging insects and keep them alive. Fresh boxwood free of any attack is added as they are consumed.

Every day, the sides and lids of the boxes are thoroughly inspected for all types of insects and then the boxes are opened in a large transparent Plexiglas recovery cage (Fig. 1A) with a lit neon light positioned at the back.




**Figure 1.** A – Cage for the collection of butterflies and emerging insects. B - Rearing boxes for moth caterpillars

Any insect coming out of the box is recovered and put in 70% alcohol in a 1.5ml Eppendorf tube on which are written all the references of the sample. The butterflies that emerge are then recovered in the same cage. In a table are listed the code of each box, the date of the day of emergence, the number of butterflies to have an idea of the percentage of emergence.

All the parasites recovered in the boxes are photographed under a binocular microscope (VHX-2000). The photos and samples are then sent to specialists for determination using morphological or molecular criteria.

*2.1.4 Rearing*

The tachinids are reared to ensure their potential parasitism of the box tree moth. All individuals are placed as they emerge in a cage with box tree moth caterpillars of different stages. Every week, about 10 caterpillars are added and the emerging moths are counted and removed throughout the life of the tachinids. While waiting for possible emergence, individuals are kept alive at 25°C and 75%RH.

Once the insects are dead, they are packaged in tubes with alcohol and sent to specialists for determination.

*2.1.5 Data analysis*

The parasitism rates as well as the percentages of butterfly emergence according to the sampling site and the collection dates were also analyzed with Microsoft Excel ®.

*2.2 Study of predation in the natural environment*

*2.2.1 Sampling method*

The method of beating arthropods on trees or bushes was chosen. This method consists of hitting the branches with a stick over a Japanese umbrella. Arthropods that fall onto the canvas are then recovered with a mouth aspirator and preserved in tubes with a screened cap to allow the passage of air.

*2.2.2 Transfer to the laboratory for predation studies*

Individuals collected in the field are isolated in transparent plastic boxes, with boxwood and an absorbent cotton soaked in water. To evaluate their capacity to predate the box tree moth, each species of predator is put in the presence of different stages of development of the pest (egg, caterpillar and chrysalis), according to the size and biology of the selected predator.

Each day, the eggs as well as the caterpillars and/or chrysalis are counted in order to obtain the number eaten per day, per individual and per species. After counting, a sufficient number of prey of different

stages is added, (exceeding the quantity consumed per day so as not to be limiting), and this until the death or the pupation of the predators.

For the predator species "biting-sucking", the eggs appearing to have been drained are observed with a binocular magnifying glass in order to verify the biting by the predator.

Individuals not tested following capture were placed in alcohol, either for identification or for DNA testing (Capelli, 2021).

*2.2.3 Data analysis*

The predation rates were exploited with Microsoft Excel.

## 3. RESULTS

Following the beating collections and sorting of the recovered arthropods, the diversity of arthropods in Buxus forest was analyzed. Each of them was categorized both taxonomically and ecologically.

The table of proportions of the different orders of insects and spiders in 2019 (Fig. 2) reveals that the majority orders are Araneae, Hemiptera, Psocoptera, Coleoptera, Orthoptera and Hymenoptera. The other orders do not exceed 5%.

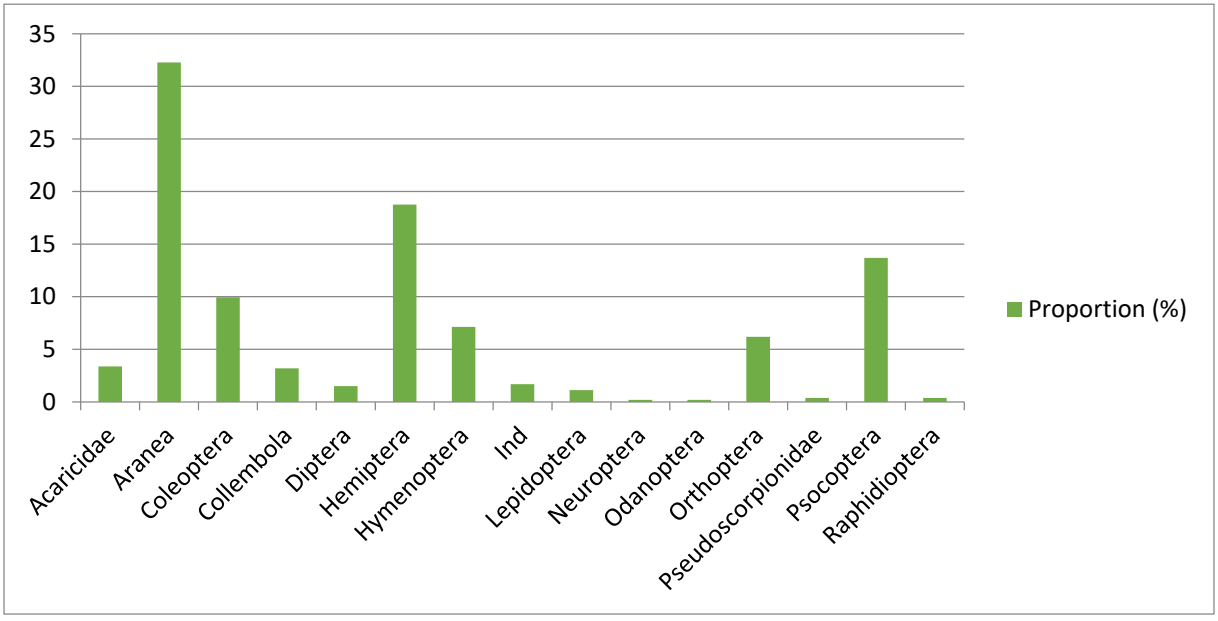



**Figure 2.** Diagram of the diversity collected during the 2019 season

The determination of ecological functions reveals three major functional groups (Fig. 3): predators, phytophagous and detritivores. A quarter of the individuals could not be classified. Most of them were in the larval stage. Taxonomic identification being difficult at this stage, the ecological functions could not be determined.

Parasites are poorly represented (1.8%), only 7% of them are potentially parasitoids of the box tree moth. Predators represent 29.23%, with 95% of the individuals potentially being predators of *C. perspectalis*.

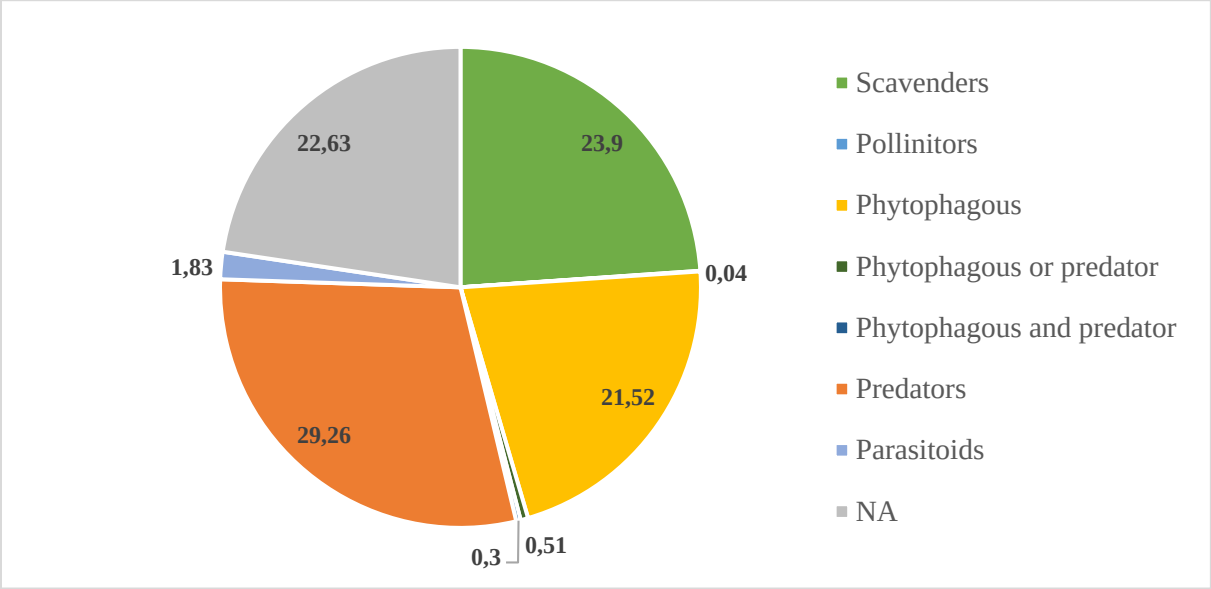



**Figure 3.** Distribution of individuals according to their ecological function

*3.1 Study of parasitism*




During the 3 years of the study, the majority of parasites found was the Tachinid *Compsilura concinnata*. The highest rate observed on a sampling station is 15%.

On average, in 2017 and 2019, the parasitism rate is 1.83%.

| Comparison 2017-2018-2019 | | |
|---|---|---|
| **2017** | **2018** | **2019** |
| 5 collections | 9 collections | 6 collections |
| 6 360 caterpillars | 10 871 caterpillars | 6 904 caterpillars |
| 120 tachinids | 19 tachinids | 124 tachinids |
| 1.9% parasitism | 0.17% parasitism | 1.8% parasitism |

**Table 1.** Average parasitism rate by year

There is no consistency from year to year. Rates are similar for 2017 and 2019 with equivalent sampling in terms of caterpillar quantities. Comparison of data over time is difficult due to frequent site changes following total defoliations of boxwood.

5 others parasitoids were also recovered and sent for identification. Unfortunately, too few individuals could be collected to do more tests.

*3.2 Predation study* - in situ *observations and laboratory monitoring*

Of the potential predators (Fig 4), 85% were spiders and ants. Three percent of the potential predators are represented by the heteropterans of the family Anthocoridae, 4% by the beetles of the family Carabidae and 1% by the Chrysopidae.

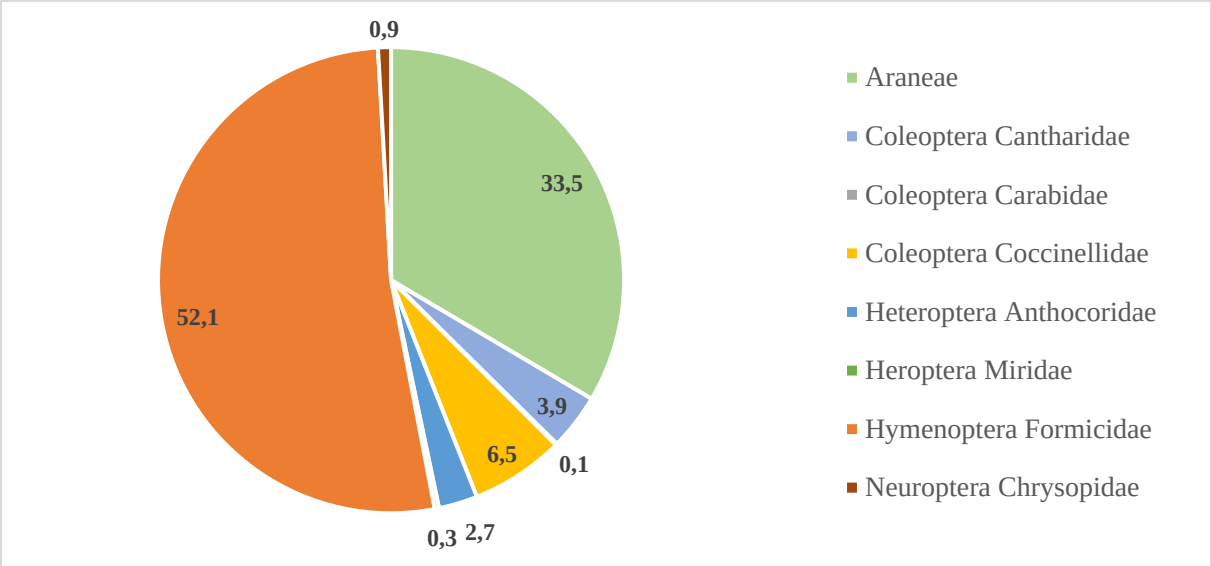



**Figure 4.** Representation of potential predators by taxonomic family

Among the predators collected, several species showed particular interest either by our observation of box tree moth predation *in situ*, or after a study of their digestive tract that gave a positive response after DNA tests (Capelli, 2021).

*3.2.1 Calosoma sycophanta*

*Calososma sycophanta* (L., 1758) (Coleoptera: Carabidae) is listed as a predator of *Lymantria dispar* and of pine and oak processionaries. Its habitat is strictly forest (oaks and pines), associated with the herbaceous stratum. However, it was clearly observed in situ feeding on box tree moth larvae (Fig. 5A). Indeed, 4 individuals of this species were found in May 2019 on boxwood heavily attacked, in an oak undergrowth.




**Figure 5.** A - C. sycophanta predating box tree moth caterpillars in the wild; B - C. sycophanta predating box tree moth chrysalis in the laboratory

In the laboratory, the 4 *C. sycophanta* recovered in the field were fed for 1 month with caterpillars and/or chrysalis of box tree moth. They went into aestivation at the end of June. In the bibliography, this carabid can consume up to 5 caterpillars of *L. dispar* per day (in Wikipedia). In our trials, an average of 1 box tree moth caterpillar of L4/L5 stage per day was consumed (Table 2).

| | **Calosoma 1** | **Calosoma 2** | **Calosoma 3** | **Calosoma 4** |
|---|---|---|---|---|
| Total number of larvae eaten | 32 | 25 | 11 | 47 |
| Number of feeding days | 32 | 35 | 31 | 32 |
| Average number of larvae eaten/day | 1 | 0,71 | 0,35 | 1,47 |

**Table 2.** Predation of Calosoma sycophanta

*3.2.2 Barbitistes serricauda*

*Barbitistes serricauda* (Fabricius, 1794) (Orthoptera: Tettigoniidae) is a nocturnal orthopteran of forest environments occurring in sunny edges with hardwood forests. It is considered to be mainly a phytophagous of soft plants but not only. It is present from June to October in the natural environment.

As many individuals were collected from boxwood attacked (22 specimens in 2019), they were kept alive until the laboratory. Feeding tests on box tree moth eggs and young caterpillars were performed for 1 month. Cannibalism was observed but no box tree moth predation. However, the boxwood leaves made available were consumed (Fig 6B).




**Figure 6.** A - Barbitistes serricauda in natural environment; B - Boxwood leaves eaten by B. serricauda in laboratory

*3.2.3 Gonocerus acuteangulatus*




*G. acuteangulatus* represents 50% of the Hemiptera collected. It is a pest bug of hazel trees (Tavella et al., 2003) known to be phytophagous. Because its host plant species is boxwood, a large number of individuals were collected and it tested positive in the DNA test (Capelli, 2021), its potential as a biological control agent was verified on box tree moth eggs and young caterpillars.

53 individuals were collected in 2019 at a first site (DNA test), followed by 16 individuals at another site.

The individuals collected did not show a conclusive response following laboratory predation tests on box tree moth eggs and young caterpillars.

*3.2.4 Picromerus nigridens*

*Picromerus nigridens* (Fabricius, 1803) (Hemiptera: Pentatomidae) lives preferably in bushes of juniper, bramble, ivy or holm oak. It is a voracious predator of insect larvae with slow movements and a soft cuticle, such as lepidopteran and beetle larvae.

2 individuals were collected in 2019 for DNA testing (Capelli, 2021), following possible predation of the box tree moth. Given its high predation potential, it will be interesting to test during 2021.

*3.2.5 Ephippiger diurnus*




*Ephippiger diurnus* (Dufour, 1841) (Orthoptera: Tettigoniidae) is listed as a phytophagous (vine, oak, bramble or dandelion leaves) but can also be carnivorous (flies, caterpillars, insect eggs). It occurs naturally as an adult from June to October in warm, dry places, wasteland and bushes.

5 individuals were collected in 2019 on boxwood attacked. They were all sent for identification. It will be interesting to test this during 2021.

*3.3 Predation not quantified but observed*




Other types of predation were observed but not quantified. These include caterpillar and butterfly predatory birds. Consumption of box tree moth caterpillars by various birds, such as chickadees has been observed in the SaveBuxus project in France (Astredhor, 2018). The UEVT Biocontrol team was able to observe box tree moths being chased by rock swallows (Ptyonoprogne rupestris) in 2019.

Among vertebrates, bats are also box tree moth predators. At least 9 of 34 French species actually consume the box tree moth, including Miniopterus schreibersii, Nyctalus leisleri, and Pipistrellus pipistrellus (Carré, 2018).

## 4. DISCUSSION

*4.1 Parasitism study:* C. concinnata, *native parasitoid of* C. perspectalis

In 2017, 120 individuals of the tachinid were recovered from the 6 360 caterpillars collected, for an overall parasitism rate of 1.9%. In 2018, even though caterpillar numbers were doubled, only 15 individuals were isolated, for an overall parasitism rate of 0.13%. The parasitism rate is highly variable between sites, but also between sampling dates. The weeks with the highest parasitism rates are the weeks when the box tree moth cycle corresponds to the largest larval or chrysalis stage. The tachinid *C. concinnata* thus appears to parasitize *C. perspectalis* at later stages. The information on its biology allows us to confirm this. We were able to isolate chrysalis and observe the emergence of pupae. Collecting *C. perspectalis* at advanced stages increases the probability of observing parasitism by *C. concinnata*.

Other parasitoids were collected, but in very low numbers. Most of the hymenopterans collected are parasites of other boxwood pests such as leafminers and phytophagous beetles.

The difficulty of parasitoids to settle on this new pest may be due to the rapidity of boxwood defoliation or to the presence of alkaloids in the eggs and larvae of the box tree moth (collaboration with University of Nice).

*4.2 Study of potential predators*

*C. sycophanta* could be a biological control agent against the box tree moth during the period of exit from diapause. Its potential as a predator of the box tree moth is no longer to be verified, however its development cycle (obligatory diapause) remains difficult for a massive production for biological control.

*G. acuteangulatus* and *B. serricauda* did not show conclusive results in the predation of the box tree moth. However, there are still other potential species to be tested in the coming year, such as *Picromerus* or *Epphipiger*.

*4.3 General discussion and perspectives*

Although we found few natural auxiliaries on the sites studied, the methodology implemented in this study remains adequate and adaptable to different types of ecological and invasive pest control studies.

In our study, we were able to demonstrate the real involvement of the tachinid *C. concinnata* in the regulation of *C. perspectalis*, with low parasitism rates however. Native to Europe, its introduction to the United States in 1906 to control invasive populations of *L. dispar* and *Euproctis chrysorrhoea* was an ecological disaster (Elkinton and Boettner, 2012). Although the regulation of these species is considered successful by scientists a century later, *C. concinna* also had a strong impact on other non-target species and remains an example of a generalist biological control agent to avoid in biological control (Elkinton and Boettner, 2012).

Likewise, the low rate of parasitism observed *in situ* and the non-target effects of *C. concinnata* are not in its favor. Indeed, the low number of individuals collected also agrees with the work of Nacambo (2012) where he essentially isolated about 30 individuals of the tachinid *Pseudopericha nigrolineata* in the Swiss region of Basel during the box tree moth introduction.

Kenis et al. (2017) hypothesized that invasive insects are likely to be adopted by natural enemies if they are usually attacked by generalists and if there are closely related insects. In the Asian literature, *C. perspectalis* is regulated mainly by polyphagous natural enemies, which would be promising, except that no species of its kind is present in Europe. Thus, it is difficult to predict the adaptive potential of native entomofauna for this pest.

According to Cornell and Hawkins (1997), parasitism of invasive insects by native parasitoids increases weakly over time. Trends may be different with predators and pathogens, so they offer other interesting avenues to explore. For this reason, we also looked at predators.

The adaptation of birds to this invasive pest is not negligible either. The naive behavior of the predators requires some time at first for them to become tame to this new invasive. Recent observations of predation by chickadees *Parus caeruleus* and *Parus major* are therefore encouraging (Guerin and Robert, 2018).

Work has shown that the configuration and spatialization of boxwood settlements have a key role in the dynamics of *C. perspectalis* (Ledru, 2021). Urban outbreaks are also sources of infestation in the natural environment and must be considered in the evolution of the dynamics. The monitoring networks conducted by the DSF and the ONF are thus very important in understanding the dynamics of this pest and of boxwood settlements.

Even if few species have been found in the natural environment so far, the aim of the search for parasitoids and indigenous predators is to release these auxiliaries in a localized manner, with the aim of their permanent establishment with survival, multiplication and dispersion.

## 5. CONCLUSION

The parasitism of *C. perspectalis* is currently very low, and the natural predator community is limited for the moment; at least in the areas studied. Biological balances, which will potentially appear in the future, have not yet had time to be established in Europe to limit the spread of the box tree moth, probably because of its recent arrival.

The invasiveness of this pest is due to its rapid development and movement, lack of competition from phytophagous on boxwood and its ability to resist to alkaloids. The dynamics of the box tree moth, with lightning defoliations followed by a population collapse, also makes it difficult for parasites or predators to appropriate a new host or prey. A stabilization of box tree moth populations linked to the availability of food resources would have been more favorable to the installation of predators and parasites.

The BIOPYR project has allowed to refine the inventory methodology which could be extended to the whole France. Temporal monitoring also remains essential to detect changes in box tree moth-natural enemy relationships. Campaigns conducted every five years could be interesting in order to keep this monitoring constant while leaving enough time between campaigns to observe the emergence of new relationships.

The importance of boxwood in the French JEVI heritage and at the ecological level in forests makes the threat of box tree moths particularly worrying. The implementation of the control of this pest requires the search for innovative biocontrol methods, whether macro-organisms or micro-organisms, pheromones or natural repellent or attractive substances, the observation of resistant boxwoods or the search for substitute plants. Research projects such as BIOPYR are therefore essential to consider a control of *C. perspectalis* on a global scale, at an acceptable level of balance and of course with a reasonable cost, in order to bring efficient and sustainable solutions to this problem.

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