DEVELOPMENT OF A PROTOCOL BASED ON MOLECULAR TECHNIQUES IN ORDER TO IDENTIFY NATIVE PREDATORS EATING THE BOX TREE MOTH *CYDALIMA PERSPECTALIS*, IN FRANCE

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Abstract

The box tree moth, Cydalima perspectalis *(Walker, 1859), is a species of moth from Asia, belonging to the family Crambidae, that invaded Europe in 2007. Its rapid expansion and negative effects on boxwood (*Buxus sempervirens*), in the field as in parks and gardens, make it an invasive exotic species. In order to control this pest, biological control methods based on the use of* Bacillus thuringiensis var. kurstaki *are used nowadays, but this method is not specific enough and phenomena of resistance may appear. The search for alternative methods, such as the use of beneficial macro-organisms, is therefore essential. The objective of this study with the project SaveBuxus is to develop a protocol that allows to identify native predators which have eaten the box tree moth* in situ*, thanks to molecular analyses. In this context, collects of insects and spiders present in natural boxwood forest, in several sites with different environmental conditions, were carried out by beating method. The individuals collected were morphologically identified, and their digestive tracts were dissected. A molecular technique was then developed, starting with the molecular characterization of the pest for the barcode coI DNA fragment, as well as a wide variety of potential predators, in order to define strictly specific* C. perspectalis *primers for amplification. We then tested the presence of DNA of the box tree moth in the digestive tract of collected individuals. Our results demonstrate that several individuals belonging to three different species collected were tested positive for the pest's DNA. The perspectives of this work are to perfect the method, and to continue surveys and collects in the field, to verify the natural adaptation of new predators to the box tree moth* in situ*. At the same time, this would allow us to consider a biological control based on the best identified predator.*

Keywords: *biological control, box tree moth, boxwood, Cydalima perspectalis, molecular analysis, PCR, predators' macro-organisms*

1. INTRODUCTION

The box tree moth, *Cydalima perspectalis* (Walker, 1859), is a species of moth from Asia, which belongs to the family Crambidae, and which is present in several countries including China (Walker, 1859), Korea (Gu, 1970) and Japan (Hannemann, 2008). In its area of origin, the box tree moth feeds mainly on *Buxus microphylla* Siebold & Zucc. (Buxaceae). This ornamental plant is commonly used in France by individuals and professionals, especially in French gardens, historical monuments or green spaces. It is through the importation of infested ornamental boxwood that the box tree moth was accidentally introduced into Europe. It was detected for the first time in Germany in 2007 (Krüger, 2008) with the trading of *Buxus* seedlings (Billen, 2007). Its rapid expansion makes it an invasive exotic species that could invade all Europe, except the coldest areas (Nacambo et al., 2014). The importation of the box tree moth without its natural enemies as well as its negative effects on boxwood (*Buxus sempervirens* L., 1753) cause the devastation of many natural boxwood forests in the field as well as historic decorative boxwoods in parks, castles and gardens.

Box tree moth adults have a nightlife and a longevity of about two weeks (Tabone et al., 2015). Females begin to lay eggs 2 to 3 days after mating. A female can lay an average of 800 ± 300 eggs during her life under controlled conditions (25°C; 75% RH; and 14h of light / 10h of night) (Tabone et al., 2015). When the eggs hatch, the small larvae (about 2 mm) grow rapidly to reach 4 cm after their 5 to 7 stages of development. Larvae are characterized by a shiny black head and a light green body, longitudinally streaked with dark green. After about 1 month at 25°C, they turn into chrysalis (Tabone et al., 2015). 10 days after the transformation, they become a moth (**Figure 1**).

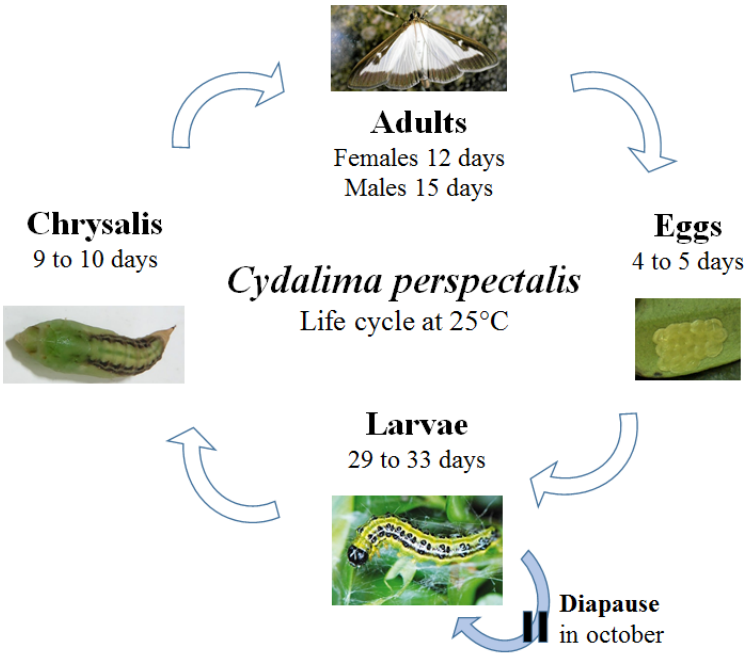


Fig. 1. Life cycle of *Cydalima perspectalis*.

In France, this multivoltine species is able to complete between 2 and 3 generations per year depending on climatic conditions (Nacambo et al., 2014; Martin et al., 2015), from March until the end of October, then the larvae start the diapause.

Chemical control methods were initially used to control this insect pest, but they are now regulated in France (Labbé law). Nowadays, several methods of biocontrol are used in order to limit the damage of the box tree moth on boxwood populations by targeting different stages of the pest. To reduce the egg stage, the releases of oophagous parasitoids of the genus *Trichogramma* are sometimes used, but it is not efficient enough (Venard et al., 2019a). To target the larvae stage, the application of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) are employed. Unfortunately, this solution has its limits: resistance phenomena may appear and the spectrum of action of the bacterium remains too broad and not specific enough, which can pose risks for the ecosystem. That's why the use of *Btk* is allowed only in parks and gardens in France. The study of some predators of the boxwood moth is also in progress (SaveBuxus project from the Biocontrôle Laboratory; Morel, 2019; Venard et al., 2019b). The installation of nesting boxes for great tits is also a method of biological control which increases the presence of the great tit *Parus major* in an area (parks and gardens or in forest), and promotes therefore the predation of the larvae of *C. perspectalis* by birds (Martin et al., 2018). To detect the beginnings of butterfly flight and to reduce adult populations, pheromone traps are also used (Martin et al., 2015). However, all these methods are not sufficient to limit the propagation of *Cydalima perspectalis* for the moment. The search for alternative methods in order to control the box tree moth in a natural and sustainable way, such as the use of beneficial macro-organisms, is therefore essential.

The team of the Biocontrol Laboratory, located in Antibes (France) and attached to the Experimental Unit of Villa Thuret (UEVT) of INRAE , is in charge of developing biological control solutions for the regulation of insect's pests with the use of several auxiliaries, mainly parasitoids and predators. These researchs are done within the framework of European, national or regional projects (Tabone et al., 2013 and Capelli et al., 2019 against the butterfly palm (*Paysandisia archon*); Tabone et al., 2016 and Venard et al., 2019a against the boxwood moth (*Cydalima perspectalis*); Capelli, 2019 and Tunca et al., 2019 against the pine processionary (*Thaumetopoea pityocampae*)).

It is in this context that the SaveBuxus project was created in 2014, in order to study the biology and the behavior of the box tree moth in France, and to develop a biocontrol method to regulate its populations. The project is coordinated by "Astredhor" and "Plante & Cité" and it receives financial support from the Val'hor inter-professional organization, ONEMA through the Ecophyto Plan and FranceAgriMer. In the same time, we worked within the project BioPyr, which was coordinated by DSF and financed by DRAAF. Among all the experiments conducted for these projects, one of them concerns the study of predators that may naturally be adapted to the box tree moth, and that are able to eat it.

The objective of this study is therefore to develop a protocol that allows us to identify native predators which have eaten the box tree moth *in situ*, thanks to molecular analyses of the digestive content of arthropods collected from infested boxwood in France.

2. MATERIALS AND METHODS

The experiment was conducted through several steps, including the collect of insects and spiders in different boxwood forests in France, the sorting and identification of collected arthropods, the dissection and conservation of potential predators, and the molecular analyses of the digestive content of collected insects.

2.1. Collect of insects and spiders in boxwood forests

Insects and spiders were collected once a month from March to September 2019, and the collects were carried out in France boxwood forests, in 5 departments from the Auvergne-Rhône-Alpes region (Ain, Ardèche, Isère, Drôme and Savoie), with at least 3 sites per department that had different environmental conditions.

The collects of insects and spiders present in natural boxwood forest were carried out by beating method with a wood stick and a Japanese umbrella, and with a mouth aspirator (**Figure 2A, B**).

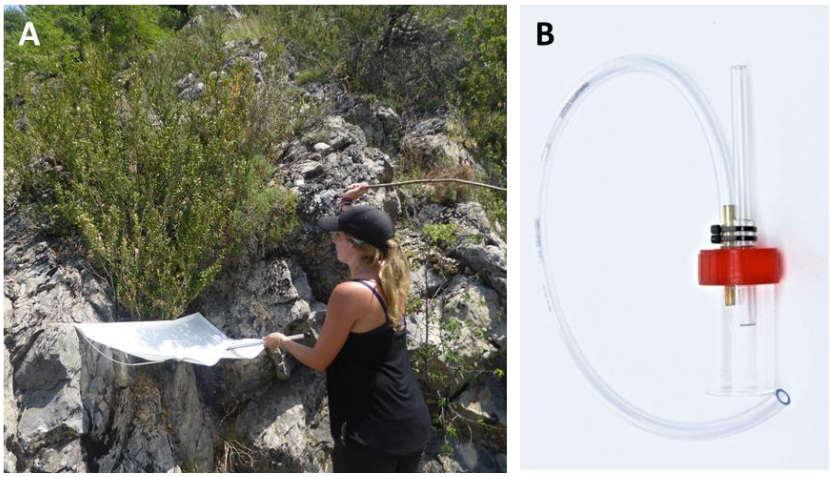


Fig. 2. Collects of insects in boxwood forests with the beating method of Japanese umbrella and wood stick (A), and mouth aspirator (B).

The Japanese umbrella is placed under the branches of a boxwood horizontally. With the wood stick, the branches are hit with a few hard hits (3 to 4) to knock down the insects. They are then sucked up with the mouth aspirator and land in the connected tube. The tube is then immediately filled with 70% alcohol using the squeeze bottle through the vacuum pipe and sealed with the tube cap. The mouth aspirator is then rinsed with water, and a new tube is put before starting the operation of collects again on another boxwood. All the collect tubes are coded with the name of the department, the name of the site, the date, and the number of the tube.

2.2. Sorting and identification of collected arthropods

The tubes with the collected arthropods are then returned to the Biocontrol Laboratory at INRAE Antibes (France). The content of a collect tube is poured into a plastic cup, and analyzed under a binocular microscope. With a clamp, the insects are sorted by order and separated from the detritus. Each individual is placed in an Eppendorf tube with 95% alcohol. On the Eppendorf tube, the order is written (coleoptera, hymenoptera, lepidoptera, etc.) followed by a number. These data are listed in an Excel table and associated with the information of the collection sites (date, department and name of the collection site, GPS coordinates, name of the collector, weather forecast, boxwood condition, moth density, etc.). The tubes are then classified by order and by number, and are sent to the Eco-entomology lab in Orléans (France), in order to morphologically identify the families, genus, and species of these insects by specialists and get information concerning the ecology of each species (phytophagus, opportunist, parasite, predator). It allowed us to separate insects according to their potential of parasitism and predation against the box tree moth.

2.3. Dissection and conservation of potential predators

The arthropod is removed from its Eppendorf tube with a dip net. The insect and its tube are rinsed under water, in order to remove all traces of DNA from species with which the individual may have been in contact. The insect is then placed back in this tube with 70% alcohol for a short period until the start of its dissection (a few minutes later maximum). The dissection is performed in cold and in the alcohol from the tube, under a binocular microscope (**Figure 3**).



Fig. 3. Dissection protocol of collected insects.

The extracting method of the digestive tract varies depending on the size and species of the arthropod. When the insect size allows it, all the paws and the head are removed with forceps. The thorax and abdomen can then be opened with a point or thoroughness and using forceps. When the arthropod is too small (less than 2-3mm) and the cuticle is too attached to internal organs, the abdomen and / or thorax can be left intact in order not to lose DNA. External parts are stored in the original tube in 95% alcohol for potential future identification. On the cap of this tube with external parts of the insects is written the letter "e" as code name for "external". The digestive tract can then be extracted and stored

in a new Eppendorf tube with absolute alcohol. On the cap of the tube, the letter "i" is written as code name for "inside". The tubes are kept in the refrigerator (3 ° C) until they are sent to the CNRS lab in Gif-sur-Yvette (France) where they are kept in a cold room.

2.4. Molecular analyses of the digestive content of collected arthropods

The development of a molecular technique was developed, in order to identify the insects that have naturally eaten the box tree moth.

The sequences of the DNA animal *coI* barcode of about 60 arthropod species were either sequenced in the lab or taken from the Genbank database in order to create our own database, composed of a wide variety of potential *C. perspectalis* predators, spanning several orders of arthropods. Laboratory *C. perspectalis* specimens were also sequenced for the *coI* barcode. This preliminary experiment allowed us to define *C. perspectalis* specific PCR primers, that could then be used to detect the pest's DNA in the digestive tubes of collected arthropods, without amplifying the DNA of the potential predator at the same time.

To obtain the sequences of the specimens for the database, DNA was extracted with the NucleoSpin® Tissue kit from Macherey-Nagel, following the manufacturer's protocol. The universal primers LCOI490 (5'-GGTCAACAAATCATAAAGATATTGG-3 ') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Vrijenhoek et al., 1994) (abbreviated respectively "LCO" and "HCO" in this article) were used to amplify the *coI* barcode fragment. One microliter of DNA was mixed with final concentrations of 1X Buffer, 3mM MgCl2, 0.4mM dNTP, 0.4µM of each primer, 1U of GoTaq Flexi DNA Polymerase (Promega), completed to 25µL with water. The tubes were then placed at 95°C for 5 minutes, followed by 40 cycles of 1 minute at 95°C, 1 minute at 50°C, 1 minute at 72°C, and then at 72°C for a final elongation of 5 minutes.

The PCR solutions were then purified in order to remove the dNTPs and residual primers: 2.5µL of PCR was mixed with 1µL of Illustra™ ExoProStar™, and put for 15 minutes at 37 ° C then 15 minutes at 85 ° C. One microliter of each PCR product was sequenced with BigDye™ Terminator v1.1 Cycle Sequencing Kit (ThermoFisher Scientific), following the manufacturer's protocol.

The sequences were manually corrected with the software CodonCode Aligner and aligned with the Mesquite software. From this alignment, specific *C. perspectalis* were defined, using the website Primer3 (Untergasser et al. 2012): Pyr68F (5'-GAATTAGGTAATCCAGGT-3 ') and Pyr314R (5'-CATTTTCAACAATTCTTCTTGAG-3'), amplifying a 247bp fragment.

In order to test the collected arthropods for the presence of *C. perspectalis* DNA with the newly designed primers, the DNA of digestive system samples was extracted with the NucleoSpin Tissue Kit (Macherey Nagel) for large samples, and the NucleoSpin Tissue XS Kit for small ones, following the protocol recommended by the manufacturer. Samples were considered large when one of their dimensions visibly exceeded 3mm.

Two amplifications were performed on each sample, one with the barcode *coI* universal primers LCO and HCO, and one with the *C. perspectalis* specific primers Pyr68F and Pyr314R. For each PCR, the protocol was the same as previously described, except that the Tm used for the Pyr68F/ Pyr314R pair was 60°C.

The PCR with universal primers did not work for several samples, which lead to the use of alternative *coI* primers for these samples: BF1: 5'-ACWGGWTGRACWGTNTAYCC-3 ' (Elbrecht and Leese, 2017)/ BR2: 5'-TCDGGRTGNCCRAARAAYCA -3 '(Elbrecht and Leese, 2017) and LCOmod: 5'-GGTCAACWAAYCAYAARGAYATTGG-3 ' (designed in our lab)/ BF1R: 5'-GGRTANACWGTYCAWCCWGT-3 '(reverse complement of BF1). The protocol for amplification was the same as for the universal primers.

The PCR products obtained with the generic *coI* primers were purified and sequenced as described previously. After their manual correction with CodonCode Aligner, a BLAST was performed for each sequence on the BOLD database (http://www.boldsystems.org/), in order to identify the species corresponding to the specimen. If there was more than 97% sequence identity with a species in the

database, the individual was considered to belong to that species. This allowed to produce a rich database with a good representation of the arthropod diversity found on boxwood.

The PCR products obtained with the *C. perspectalis* specific primers were run on a 2% agarose gel to check if they were negative or positive for the pest's DNA. When samples were found positive, another amplification was performed on the initial DNA to confirm the result, and the PCR products were sequenced to confirm they corresponded to *C. perspectalis*.

3. RESULTS

3.1. Diversity of collected arthropods

226 insects and 97 spiders were collected, belonging to 33 families, 48 genus, and 54 species.

Aranea and Hemiptera are the two most represented groups, with 34% and 21% of all individuals, respectively (**Figure 4**). The next fourth consists of Coleoptera (10%), Psocoptera (9%), Hymenoptera (8%) and Orthoptera (6%) Finally, other orders are present but rarer (< 4%).

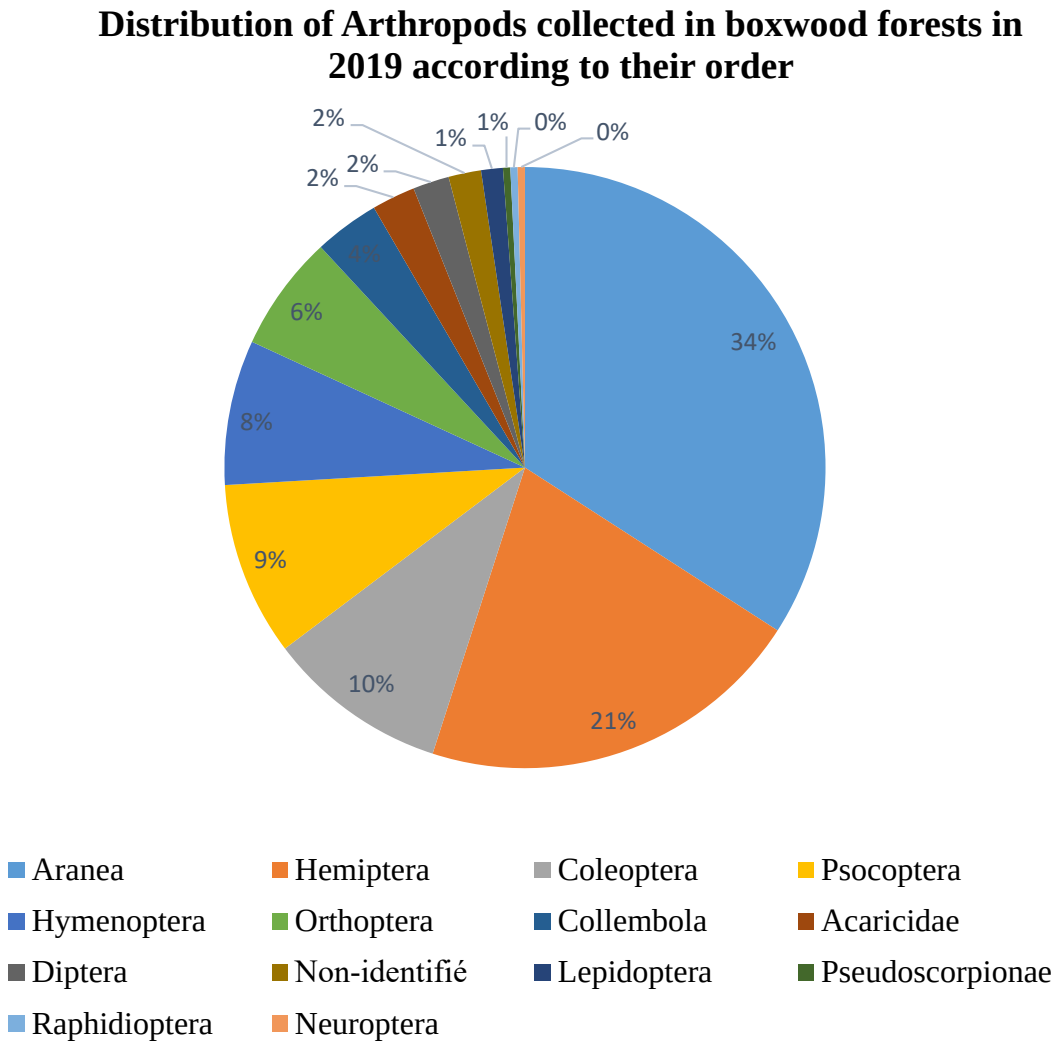


Fig. 4. Distribution of Arthropods collected in boxwood forests between May and August 2019.

For spiders, diversity is not dominated by one particular species. However, the Salticidae family represents just over a quarter of individuals. For Hemiptera, it is an insect of the Coreidae family which predominates, representing 50% of the individuals collected. It is important to note that the threshing collection method has a bias: flying insects are not captured.

Among the 226 insects collected and sequenced with molecular analysis, the ecology of 78 of them is known. Concerning the ecological role of these 78 individuals, 53% of them are phytophagous (**Figure 5**). Predators and parasitoids represent 12% and 1% of the total studied individuals respectively.

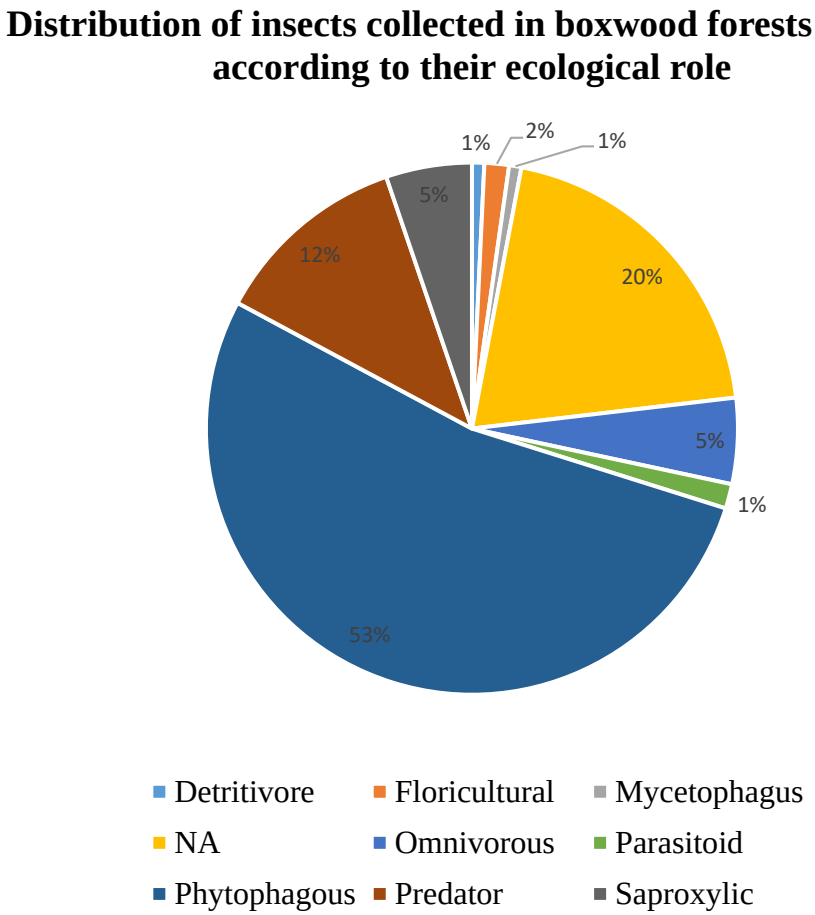


Fig. 5. Distribution of the 78 insects collected in boxwood forests in 2019 whose ecology was known according to their ecological role.

To these are added 97 spiders which are at least known to be predators; without knowing whether some belong to families that are also phytophagous. The proportion of predators goes from 12% considering only insects, to 67% when spiders are added.

3.2. Consumption of the box tree moth by arthropods

Most of the individuals tested are negative for eating the box tree moth (**Figure 6**).

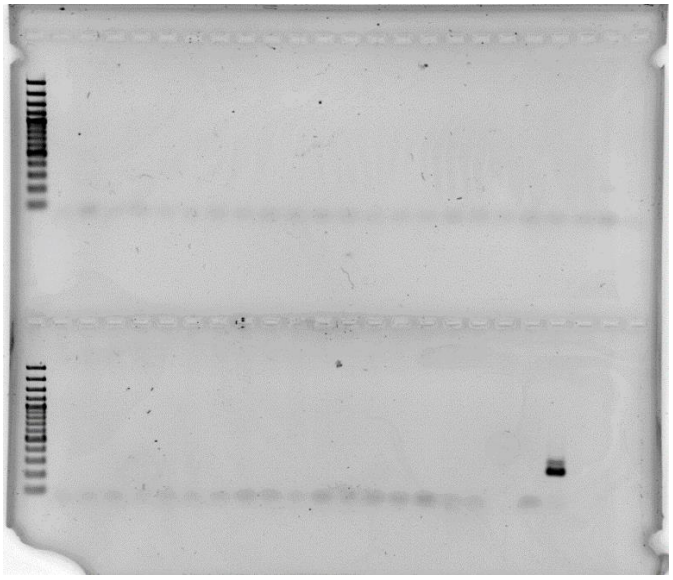


Fig. 6. Example of negative results for the presence of box tree moth on 40 individuals (the last two products correspond respectively to the negative control and to the positive control for the box tree moth).

Five individuals on 323 individuals collected are tested to be positive to the specific PCR of *Cydalima perspectalis*. All the five positive individuals are Hemiptera (**Figure 7**).

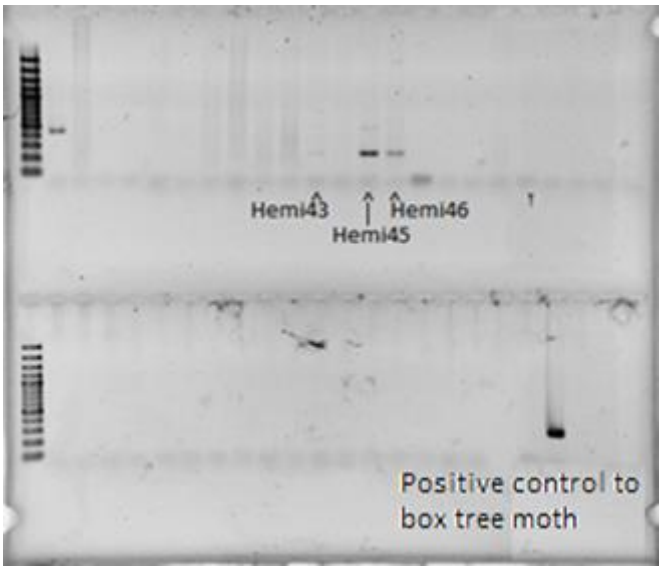


Fig. 7. Example of three positive results (individuals Hemi 45 and Hemi 46) in the presence of box tree moth (the last product corresponds to the positive control for the box tree moth).

A 2nd PCR from DNA samples is performed to ensure that there has been no contamination. The result is validated. Two of the five individuals tested positive to the box tree moth DNA belong to the species *Gonocerus acuteangulatus* (Goeze, 1778) (Hemiptera: Coreidae). Concerning the three other individuals, the verifications also confirm the consumption of *Cydalima perspectalis*. Unfortunately, the obtained sequences for these individuals do not correspond to any known taxon in the BOLD database, making their identification impossible on this basis. However, two of them are 99.3%

identical to each other, which means they belong to the same species. Morphological analyses allow us to identify these two individuals as *Picromerus nigridens* (Fabricius, 1803) (Hemiptera: Pentatomidae). The last one was also a Hemiptera, but the genus and the species could not be identified.

4. DISCUSSION

Our results have enabled to develop this molecular analysis technique for detecting the presence of the box tree moth in contents of the digestive tract of several insects, in a significant way. Indeed, after wondering about the time of detection of the box tree moth in the digestive tract of individuals after they eat it, complementary experiments were carried out (not shown in this article). It emerges that the method allows the detection of box tree moth DNA in the digestive tract of individuals even if it is not the only source of food. It also makes it possible to detect the consumption of box tree moth up to at least three days after consumption followed by fasting. These results tell us that, a priori, the individuals tested *in situ* with a negative result had not eaten any moth at least in the past three days. We are thus able to demonstrate that several individuals collected, belonging to different species, were tested positive by PCR specific to the moth. Indeed, the molecular analyses of the digestive tract of the 323 collected insects reveal five individuals that were positive to the box tree moth DNA, which represent 1.5% of collected insects. This result means that five individuals eat the box tree moth *in situ*, and they all belong to the order of Hemiptera (**Figure 7**). On the 53 individuals of *Gonocerus acuteangulatus* collected, belonging to the family of Coreidae, two of them only are tested positive to the box tree moth DNA. Though this species is rather known to be phytophagous in fruit orchards, we tested this species in laboratory with some food choice experiments, in order to verify if it eats box tree moth frequently, or if it prefers plants when it had the choice (Morel et al., 2021). Our results showed that *G. acuteangulatus* eat only leave or fruits of boxwood. The positive result on this species cannot be due only to a contact with the box tree moth because individuals were rinced and only the digestive tract was dissected. It is likely that *G. acuteangulatus* ingest eggs of box tree moth while they consume leaves. The two other individuals tested positive to the box tree moth DNA and identified are *Picromerus nigridens*, belonging to the family of Pentatomidae. In addition to the fact that this species is absent from the database, few information on the biology and ecology of this species is available, potentially due to the low number of presence data of this species in France. However, the only two individuals of *P. nigridens* collected *in situ* were positive to the box tree moth DNA. It could therefore be a potential candidate for the biocontrol of *Cydalima perspectalis.* It would be interesting to find more individual of *P. nigridens in situ* in order to test their predation capacity in laboratory against the box tree moth. It is important to notice that the 5 individuals tested positive to the box tree moth DNA were Hemiptera. This result can tell us either Hemiptera is an order which is well adapted to this toxic insect, or it is important to improve some element of this methodology (finer dissection, different method to collect also smaller insects). It is therefore important to continue this study of detection of box tree moth DNA in indigenous insects in order to, on the one hand, find other species that could adapt to the box tree moth and eat it, and on the other hand, improve the method to detect in a better way which insects are able to eat the box tree moth. This could allow us to find an indigenous predator that could potentially regulate the box tree moth, in field populations as well as in parks and gardens.

It is known that other arthropods and animals can also consume the box tree moth. From the bibliography available in Europe and Asia, several biocontrol programs are conceivable. In Asia, the original region of *Cydalima perspectalis*, there are several parasites and predators of the box tree moth; this is partly why it is not considered to be a pest over there. In China, spiders and a genus of thrips (*Aeolothrips sp.*) are cited as predators of the box tree moth (Wan, 2014). Some species of *Aeolothrips* occur naturally in Western Europe and are auxiliary predators of crops, for example *Aeolothrips intermedius* (Ephytia, 2015). It comes from Europe and shows results in biocontrol against thrips (FAO, 2009). We could then imagine using this species in a biocontrol program against box tree moth. The adults are floricultural and the attack larvae. However, we haven't collected them in boxwood in a forest environment. On the other hand, in gardens or green spaces, it could be possible

to set up a method of biocontrol with auxiliary thrips. The consumption of the box tree moth by these species should be evaluated by laboratory consumption tests. Consumption of the box tree moth by birds has also been observed in several European countries. Surveys describe the predation of the European box tree moth: in Italy in 2016, by the species *Turdus merula* and *Parus major*, and in the Netherlands in 2017 by *Haematopus ostralegus*, *Parus major*, *Cyanistes caeruleus*, *Pica pica*, *Sturnus vulgaris*, *Passer domesticus* (Mostini, 2018). Moreover, the European sand martin (*Riparia riparia*) have been seen hunting moths in flight in France. Otherwise, bats are another type of known predator of European moths and can be favored by nesting boxes in the same way (Carré, 2018). In France, one of the SaveBuxus projects aimed to monitor great tits nest boxes. It has been shown that great tit feed on moths and that consumption was not toxic to chicks at the doses consumed (Astredhor, 2018). It was then concluded that "the nesting of tits could be a strategy of biological regulation to be combined with the other methods". The other methods evoked, for example, the trapping of adults with pheromon trap, or and parasitism by oophagous parasitoids such as *Trichogramma*. However, in this study we show that parasitoids represent only 1% of collected insects (**Figure 5**), compared to 12% for the predators. The probability that a predator adapt to the box tree moth seems more likely than for a parasitoid. That's why the study of other predators of the boxwood moth is in progress, *in situ* (Morel et al., 2021) as in laboratory (Morel, 2019). Assembling different methods of nesting boxes, traps, parasitism and predation, could make it possible to target the three stages of the box tree moth and potentially gain in efficiency.

The importance and speed of the expansion of the box tree moth and its destruction on boxwood can partly explain the very few predators and parasites found, which don't have the time to adapt to the moth. This non-consumption and the failure of the parasitism can also be explained by a potential toxicity of the box tree moth. In fact, box tree moths sequester some of the boxwood alkaloids and some of them have proven toxicity. Cyclobuxin D, for example, has an LD 50 of 300 mg / kg in mice (NCBI, 2005a) and cyclovirobuxin D of 293 mg / kg (NCBI, 2005b). It should be noted, however, that the alkaloid composition in the box tree moth differs from that of boxwood. Larvae have been shown to store dibasic alkaloids, sometimes at concentrations 20 times greater than that of boxwood, while monobasic were metabolized and excreted (Lethardt, 2013). Cyclovirobuxin D is just one of these dibasic alkaloids. In the case of nestling, non-toxicity may be explained by consumption that is too low to be lethal. In addition, alkaloids protect moths by their toxicity but also by their role in the food choices of predators. Indeed, a study carried out on the Lepidoptera species *Utetheisa ornatrix* showed that the predatory ants *Leptothorax longispinosus* avoided these eggs containing alkaloids up to a certain level of hunger. These alkaloids are transmitted to eggs from parents (Hare and Eisner, 1993). We showed that *C. perspectalis* is also capable of transmitting alkaloids to its eggs and larvae (data unpublished), and that it seems to be repellent to potential predators; that's probably why the box tree moth itself is very little consumed.

5. CONCLUSIONS

In our study, we have succeeded in developing a reliable technique to identify which arthropods eat the box tree moth *in* situ. It is a really important work because it could be adapted to others pests in the future. We also identify 5 individuals which eat the box tree moth: these confirmed predators of the box tree moth are Hemiptera of the family of Coreidae or of Pentatomidae, and they represent 1.5% of collected arthropods. Arthropod sequencing has also made it possible to identify many species present on boxwood. All the other insects identified negatively to the consumption of box tree are probably not major consumers of this one. Some other tests show that the DNA of the box tree moth can be detected at least three days after consumption and with other food sources. The negative individuals had therefore not consumed the box tree moth in the past few days. It is therefore reasonable to eliminate these species as a candidate as a biological control agent against *Cydalima perspectalis*. The perspectives of this work are to perfect the method, and to continue surveys and collects in the field, to verify the natural adaptation of new predators to the box tree moth *in situ*.

At the same time, this would allow us to consider a biological control based on the best identified predator. Indeed, others insects could be used to fight against the box tree moth. The contribution of flying arthropods should be investigated, especially considering that wasps have been seen to consume moth caterpillars. Still in biological control, we know that individuals of the orders of Neuroptera and of Hemiptera can consume the box tree moth; it would be interesting to assess the efficiency and impacts of the use of these species on boxwood forests *in situ* and in parks and gardens (work in progress). It would also be possible to find a specific parasite or predator of the moth in its area of origin in Asia and introduce it by acclimatization. On the other hand, *Aeolothrips* are another still unexplored path, which could be applied in gardens and green spaces.

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