UNIVERSIDADE Ð COIMBRA

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Native vertebrate predation over exotic eucalyptus insect pests

Dissertation in MSc in Ecology, supervised by Dr. Luís Pascoal da Silva and Prof. Dr. José Paulo Filipe Afonso de Sousa (Department of Life Sciences of the University of Coimbra) and presented to the Department of Life Sciences, Faculty of Sciences and Technology of the University of Coimbra

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Main contents

Abstract

The eucalyptus weevil, *Gonipterus platensis,* is one of the most destructive *Eucalyptus* pests worldwide*.* In Portugal it causes yearly losses of millions of euros to the paper industry. The weevil's control is done by classical biological control through the parasitoid *Anaphes nitens*. Unfortunately, this control is inefficient in many areas of Portugal and chemical control is used as a last resource. It has been proven that birds and bats are important for the pest control in several agroforestry systems and thus conservation biocontrol can be an option in eucalyptus plantations.

This thesis aims to assess the potential of native bats and birds as biocontrol agents of eucalyptus' insect pests, as well as the traits that seem to promote this service. For this, 294 faecal samples from birds and 365 samples from bats were collected in eucalyptus plantations between April and October 2019. A molecular assay was designed to detect DNA presence of four insects associated with eucalyptus (*G. platensis*, *A. nitens*, *Ctenarytaina spatulata* and *C. eucalypti)*, by designing four sets of specific primers targeting a small region of the mitochondrial COI gene of each species.

The results showed a total of 96 predation events on the target insects. The most preyed was *G. platensis* detected 40 times, followed by *C. eucalypti* (25)*, C. spatulata* (21) and at last *A. nitens* with only 10 detections. In the bat samples the detection rate was very low, with only one detection for *G. plantensis* and three for each *Ctenarytaina* species.

Despite some limitations of the study, bats appear to have low potential to control the eucalyptus pests analysed, while birds seem more promising. Birds have the potential to control *G. platensis* populations and the damage they cause to economically viable levels if conservation strategies are implemented to increase bird densities in eucalyptus plantations.

Keywords:

Biological control; Eucalyptus pests; *Gonipterus platensis*; Native vertebrates; Sustainable management

Resumo

O gorgulho-do-eucalipto, *Gonipterus platensis*, é das pestes mais destrutivas do eucalipto mundialmente. Em Portugal causa perdas anuais de milhões de euros à indústria papeleira. O controlo do gorgulho é feito por controlo biológico clássico através do parasitoide *Anaphes nitens*. Infelizmente, é ineficaz em muitas áreas de Portugal, sendo o controlo químico utilizado como último recurso. Está comprovado que aves e morcegos são importantes para controlar pragas em diversos sistemas agroflorestais, portanto, o controlo biológico por conservação pode ser uma opção em plantações de eucalipto.

Esta tese tem como objetivo avaliar o potencial de morcegos e aves nativas como agentes de controlo biológico de insetos peste do eucalipto, e as características que aparentam favorecer este serviço. Com este objetivo, foram recolhidas 294 amostras fecais de aves e 365 de morcegos em eucaliptais, entre abril e outubro de 2019. Foi desenvolvido um ensaio molecular para detetar DNA de 4 insetos associados ao eucalipto (*G. platensis, A. nitens, Ctenarytaina spatulata* e *C. eucalypti*), através da criação de quatro conjuntos de primers específicos de uma pequena região do gene mitocondrial COI de cada espécie.

Os resultados demonstraram um total de 96 eventos de predação nos insetos alvo. A peste com mais predações foi *G. platensis*, detetada 40 vezes, seguida por *C. eucalypti* (25), *C. spatulata* (21) e *A. nitens* com apenas 10 deteções. Nas amostras de morcegos, a taxa de predação foi muito baixa, com uma deteção de *G. plantensis* e três para cada espécie de *Ctenarytaina*.

Apesar de algumas limitações do estudo, os morcegos aparentam ter baixo potencial para controlar as pestes do eucalipto analisadas. As aves parecem mais promissoras, tendo potencial para controlar populações de *G. platensis* e o prejuízo que causam para níveis economicamente aceitáveis, caso estratégias de conservação sejam implementadas para aumentar a densidade de aves em plantações de eucalipto.

Palavras-chave:

Controlo biológico; Gestão sustentável; *Gonipterus platensis*; Pestes dos eucaliptos; Vertebrados nativos

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I. Introduction

Biological control

Biological control is a technique in which one organism is used to suppress the population density of another (Lenteren 2012). It is a form of ecologically based pest management, i.e. environmentally friendly, that uses one organism, a natural enemy, to reduce or mitigate pest species and their effects (Hoddle and Driesche 2000).

Biological control has been used for about two millennia and became broadly applied in pest management since the end of the nineteenth century (DeBach 1964; Lenteren and Godfray 2005; Lenteren 2012) thanks to the on-growing concerns of the use of chemical control or pesticides. Application of chemical pesticides within and outside agro-ecosystems has several secondary effects as it kills many species of non-target organisms and can also result in various side-effects, including unexpected, indirect and long-term effects on the environment and human health (Lenteren 2012; Pimentel and Burgess 2014). Another serious problem of chemical pesticides is that the indirect costs that emerge as a secondary result of their use are not accounted for the price, making them unfairly cheap (Costanza et al. 1997; Lenteren 2012; Pimentel and Burgess 2014).

Unlike most pesticides, biological controls are often specific for a given pest species. They are less dangerous for the environment (for example, they do not affect water or soil quality), representing a more friendly alternative to chemicals. They could also be used as an alternative in cases of pesticide-resistant pests (Moazami 2011). Thus, biological control is the most environmentally safe method (Lenteren 2012), having the advantage of being essentially harmless to humans and non-target organisms, while pesticides need to be actively managed for safe use to diminish the harm they can cause on humans and other non-target organisms (Hoddle and Driesche 2000).

There are 3 traditional types of biological control:

1) The inoculative biological control, also known as 'classical' biological control (DeBach 1964; Lenteren 2012), involves the use of natural enemies collected in an exploration area, usually the area of origin of the pest, and its release in areas where the pest was unintentionally introduced (Lenteren 2012). An example of a successful implementation of classical biological control use is the introduction of the highly host-specific egg parasitoid, *Gonatocerus ashmeadi*, a natural enemy of the glassy-winged sharpshooter (*Homalodisca vitripennis*), which within a few months of its release managed to reduce the pest population by over 95% (Hoddle and Driesche 2000);

2) The augmentative biological control, in which natural enemies are released in extensive numbers, being previously mass-reared in biofactories (captivity), to achieve immediate control of pests (Lenteren 2012). One of the best-known cases of augmentative biological control, which has been implemented in several countries, is the introduction of *Rodolia cardinalis,* also known as vedalia beetle, for control of the cottony cushion scale (*Icerya purchasi),* that often leads to substantial or complete control (Lenteren and Bueno 2003);

3) At last, the conservation biological control is a method of protecting and amplifying the fitness of natural enemies ensuring their effectiveness over pests through the implementation of a variety of management practices (Ehler 1998; Begg et al. 2017). For instance, habitat management can enhance natural control when diverse environmental requisites are provided, such as supplementary and complementary food sources and shelters (Whitaker 1999).

Besides these 3 traditional biological control types, recent advances in bioengineering and production of genetically modified organisms have contributed to exciting new opportunities for biocontrol (Gurr and You 2016; Karabörklü et al. 2018; McFarlane et al. 2018; Alouw et al. 2020). These recent biological control types involve the use of modified organisms, that can be obtained by artificial selection and hybridization or by more complex methodologies, selecting organisms less susceptible to pests (Romeis et al. 2008, 2019) or producing modified individuals of a pest species that can control its wild populations (Alouw et al. 2020; Shelton et al. 2020).

Native vertebrates

Nowadays, due to the over and indiscriminate use of nonspecific insecticides, several pest species have developed resistance to one or more pesticides (Ghanem and Voigt 2012). Moreover, valuable invertebrate and vertebrate predator species are being harmed or eliminated due to anthropogenic actions (Ceballos et al. 2015; Hill et al. 2020). These factors have been elevating to pest status insect species that are not normally considered pests (Kunz et al. 2011). Among vertebrates, the recognition of insectivorous birds and bats as important pest controllers has grown in the last years thanks to several studies showing their importance in pest control (e.g. Barbaro et al. 2016; Kahnonitch et al. 2018; Lindell et al. 2018). In particular, exclosure experiments with birds and bats have shown their ability to significantly constrain arthropod populations and reduce their damage to crops (Maas et al. 2016, 2020), thus providing a viable alternative to pesticides and other chemicals (Bianchi et al. 2006; Maas et al. 2016). Moreover, bats and birds are not only suppressors of insect pests but also provide many other

ecosystem services, like pollination and seed dispersion (Kunz et al. 2011), helping to maintain stable and resilient ecosystems that are essential to human welfare (Watson et al. 2019).

As flying vertebrates, bats and birds share several characteristics that allow them to be so important in providing these ecosystem services (Maas et al. 2016), such as being vast consumers of arthropods and having high metabolic rates, which consequently lead them to prey upon a large number of arthropods (Whelan et al. 2008). For example, bats were estimated to consume up to 25% of their body mass each day on invertebrates (Kunz et al. 2011). Their high mobility, ability to hunt prey on the fly, in addition to a wide range aerial drift of numerous insect pests, allows them to not be restricted to an area while feeding, moving easily when the resources are no longer sufficient which may lead to an influence on insect populations farther than their feeding range indicates (Whelan et al. 2008; Ghanem and Voigt 2012). Depending on the availability of arthropods, insectivorous birds and bats with comparatively large body sizes, high mobility, and sophisticated foraging strategies, might be able to actively switch between herbivore and predator arthropods, therefore acting as top predators and decreasing economic losses caused by pest species (Mooney et al. 2010; Maas et al. 2016).

However it also important to stress that insectivorous birds and bats, not only feed on herbivorous pests but also predatory arthropods, like spiders and ants (Mooney and Linhart 2006; Maas et al. 2016) and thus their effect on a given crop or forestry system may not always be economically advantageous (Garfinkel et al. 2020).

Eucalyptus plantations

Natural regenerating forest has been continually decreasing worldwide, while tree plantations are continuously expanding, currently constituting around 6% of the forested area in Europe, including Russia, and 7% of the total forest area worldwide (FAO 2020). In particular, *Eucalyptus* spp. is one of the most planted trees in the world (FAO 2010) and represents about 8% of the over 20 million hectares of trees planted globally (Laclau et al. 2013; Cruz et al. 2016). Its plantations have expanded globally, and according to ICNF (2019), it is now the main forest occupation in Portugal with 812 thousand ha, 26% of the country's forest area. The forest industry holds an important position in the economy of Portugal, with timber products, mostly derived from eucalyptus plantations, having been valued at over 2.68 billion euros in 2017 (CELPA 2017). Portugal is the third-largest producer of pulp in Europe (CEPI 2017), with pulp and paper industries

representing on average 2.1% of the gross domestic product and 5% of the national exportations since 2000 (ICNF, 2015).

During the last century, eucalypt trees have been planted all over the world, bringing associated numerous species of insects, some of which became serious pests as a result of a diminished repression from their natural enemies (Branco 2007). A repercussion of the large and continuous areas of monospecific eucalyptus plantations is an increasing number of pests and diseases that spread very fast and are a continuous preoccupation to the pulp industry. In Europe, several alien arthropod pests can be found disturbing eucalyptus plantations (Reis et al. 2012), and Portugal is no exception. In our country, there are eleven known Australian insect species, that feed exclusively on eucalyptus trees and that can wreak havoc on plants (CELPA 2017), hence some of them are considered pests.

The main causers of damage on eucalyptus, and therefore the most relevant species, started being detected as early as the 1980s in our country. Currently, the major cause of tree growth losses is *Gonipterus platensis.* Also known as eucalyptus weevil or eucalyptus snout beetle, this species alone has affected more than 150 000 ha and causes losses of approximately 40 million euros per year (ICNF et al. 2015).

After the detection of *G. platensis*, a biological control program to counteract *G. platensis* effects on eucalyptus plantations was established (Valente et al. 2017), using one of the main control agents applied worldwide, the egg parasitoid *Anaphes nitens* (Paine et al. 2000; Reis et al. 2012).

Contrarily to what was expected, the success observed in other countries was not attained in regions with altitudes above 400-450m, where the damage caused remained high (Valente et al. 2017). Previous studies have shown that the control of *A. nitens* on *G. platensis* is significantly lower in specific regions in the north and center of Portugal where the elevation is higher and with low temperatures during the coldest months (Reis et al. 2012), leading to a lower survival of the parasitoid and therefore a higher damage caused by the weevil in colder regions.

In the interest of finding alternative enemies to amend the inadequacy of *A. nitens* in those regions, another *Anaphes* species known to parasitize *Gonipterus spp.* in Tasmania, its native land, were surveyed and later imported (Valente et al. 2017). Amidst all of the parasitoids collected, only *Anaphes inexpectatus* was successful when Valente et al. (2017) tested it in the lab, suggesting the likelihood of its dispersal and establishment in the eucalyptus plantations. However, this possibility can only be determined after field release studies. Therefore, there is a need to search for other potential methods to mitigate the damage caused by *G. platensis* (Reis et al. 2012).

Other than the *G. platensis*, the presence of two species of psyllid pests can easily be recorded in eucalypts plantations, although the economic losses they cause are considerably much smaller in Portugal (Valente et al. 2004; Paine et al. 2011). *Ctenarytaina spatulata* is a sap-sucking psyllid that feeds on adult plants and shoots, living on the plant's surface secretions of honeydew which lead to fungal growth(Valente et al. 2004). It was recorded being predated by syphidian larvae, coccinellids, chrysopids, and especially spiders (Santana 2000), but also by minute pirate bugs (Valente et al. 2004). Another sap-feeding insect from the same genus detected in Portugal, is *Ctenarytaina eucalypti*, one of the most notable species in the gender due to its economic impact (Hodkinson 1999; Santos et al. 2008; Sharma et al. 2015). This species, contrarily to *C. spatulata,* prefers the juvenile shoots, but adults survive similarly well on both types of leaves (Brennan and Weinbaum 2001). High populations can induce juvenile shoots to dry, causing leaves to change colour, deform and fall. Additionally, the leaves' ability to perform photosynthesis can be reduced due to the presence of honeydew and wax secreted by the nymphs (Kurylo et al. 2010). Its occurrence throughout Portugal has been known for more than 40 years (Azevedo & Figo, 1979). Nevertheless, several native predators are capable of feeding on this pest including anthocorids, hoverfly larvae, lacewings, ladybirds, and spiders (Soloneski and Larramendy 2012). In Portugal, numerous species have already been listed as natural enemies of *C. eucalypti, namely Syrphoctonus abdominator, Haematopota ocelligera, Sphaerophoria scripta, Melliscaeva cinctellus, Pipizella sp., Eumerus sp.,* and *Bradysia sp.* (Azevedo & Figo, 1979).

Although various natural enemies of each species are present and known to actively feed on the eucalyptus pests, the damage caused is still high in many regions. Therefore, other methods of control are required to effectively regulate these pests and the economic losses they cause. Insect herbivory can be controlled by top-down mechanisms involving natural predators (Kambach et al. 2016), such as birds and bats, who can decrease the abundance of herbivorous arthropods, therefore reducing their negative effects (Maas et al. 2016). The potential use of native vertebrates to manage these pests is a plausible solution given the potential that birds and bats have to control *G. platensis* and other arthropods pests in eucalyptus plantations. To do so, it is necessary to know the diet of bats and birds present in eucalyptus plantations, to find out which species prey on eucalyptus pests and can act as pest controllers. Once these predators are identified, their populations can be augmented in the plantations through habitat management measures and consequentially increase the overall pest control service.

Hypotheses

In this thesis I aim to understand how the predation of eucalyptus pests varies with i) their abundance throughout the year, ii) vertebrate predators, and iii) ecological traits of the predator. I will assess both eucalyptus insects' abundance in eucalyptus plantations, as well as the predation rate by different vertebrate predators and evaluate how correlated they are. I hypothesize that as pests increase their abundance, predation rate levels will also increase. I also expect that predation levels will be different among predators, as some might be more prone to feed on eucalyptus insects due to different ecological traits like size, diet, foraging guild, and phenology. Knowing which bat and bird species prey over these pests and their functional traits is important for an efficient conservation biological control on eucalyptus plantations.

Finally, I also aim to understand whether native bat and bird species feed on *Gonipterus platensis* more often than on its parasitoid, this way positively contributing to the biocontrol of this pest in eucalyptus plantations. I expect that predation on the parasitoid will be overall low due to the very small size of the adults.

II. Materials and Methods

II.I. Sampling

The study was conducted in the center of Portugal (Figure 1), and study sites were selected in areas where the damage caused by eucalyptus weevils was easily detected. The sampling was conducted from April to October 2019, which corresponds to the period when the study pest species have their peak abundances. This period also corresponds to the usual period of bat activity in the study area.

Birds

Bird sampling was conducted in two areas: Pisão do Baeta (40.778857, -8.380713) and Boialvo (40.048145, -8.189103) in the districts of Leiria and Aveiro, respectively (Figure 1). The sampling sites were in the limits of eucalyptus plantations and where the plantations were bordered by recent abandoned agricultural fields with recovering natural forest, despite the presence of several non-native species, including non-planted eucalyptus. Eucalyptus plantations are known to have relatively low bird densities (Pina 1989), these transitions areas were chosen to maximize bird captures since birds are more abundant in native forests (Silva et al. 2012, 2019a; Calviño-cancela 2013; Goded et al. 2019).

The captures were done once a month in each site using mist-nets, only in days with no rain and no or weak wind. The nets were operated avoiding the hours of most heat and lower bird activity. Mist-nets were operated from the middle of the afternoon until nightfall, for approx. 4 hours with sunlight and again from the first light for 5 more hours in the morning.

Nets were inspected every 30 minutes and the captured individuals were placed in sterilized individual cotton bags for 30 minutes to collect their droppings. We also measured the wing (maximum chord method with a 0.5 mm precision), weight (0.1g precision), and recorded the sex and age of each individual, when possible. Droppings were stored in tubes with 98% alcohol to safely preserve DNA, and refrigerated at 4ºC until they reached the laboratory, where they were refrigerated at -20ºC until DNA was extracted.

Bats

Unlike bird sampling, only a small number of bat droppings were directly collected from individuals captured with mist-nets at Pisão and Boialvo, in the first 3 hours of each

night. The vast majority of the samples were collected at known breeding roosts or feeding perches, at two abandoned forest service's houses (at Serra da Lousã in Lousã and Castanheira de Pêra), an abandoned watermill in Porto de Carros (Murtede) and Grada (Barcouço, Figure 1). All these roosts are found in the vicinity of eucalyptus plantations, where the study pests are known to occur. To avoid possible contaminations, the bat guano was always collected with clean paper placed beforehand. Because most of the bat samples were collected from the bat roosts or feeding perches, and these can often have multiple bat species, all bat droppings' identity was confirmed genetically. If a genetic identity could not be obtained or remained inconclusive, the designation "undetermined" was given to those droppings.
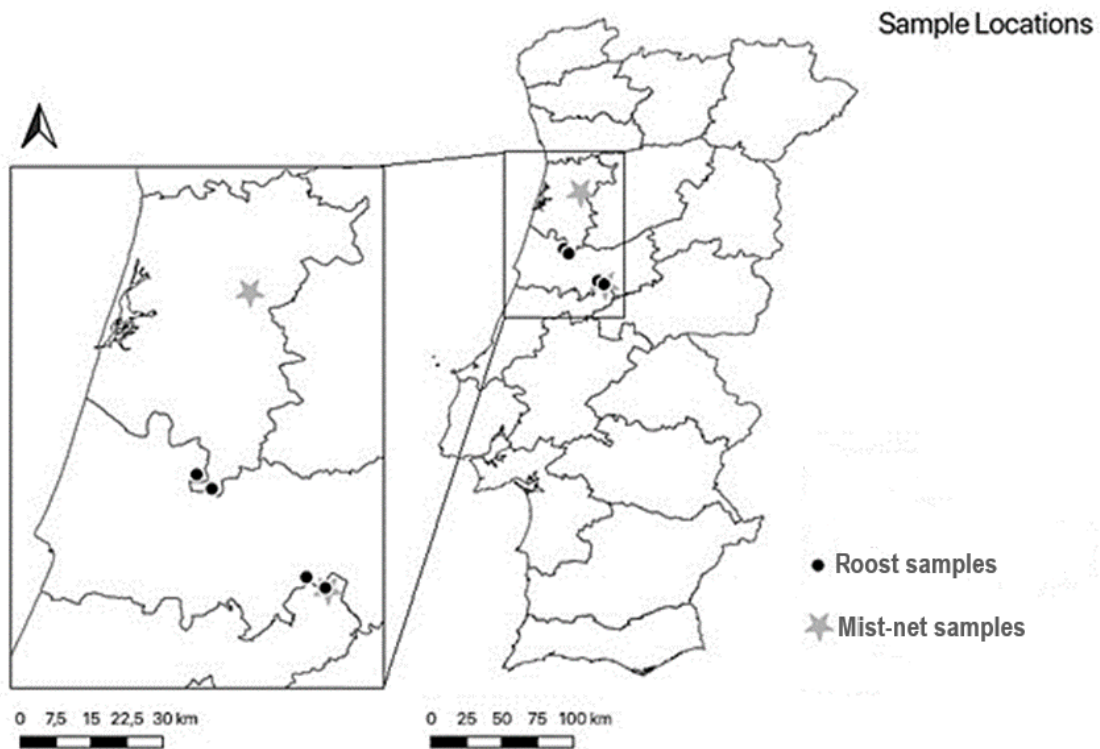
Figure 1: Sampling site locations of samples collected in northern and central Portugal

The collected droppings were stored in tubes formerly filled with silica, to dehydrate and safely preserve DNA, and refrigerated at 4ºC until they reached the laboratory where they were refrigerated at -20ºC until DNA extraction.

Pest abundance and reference collection

Local abundance of eucalyptus pests was assessed each month by counting the number of individuals of each pest species in each sampling site. The counts were conducted on ten eucalyptus trees randomly chosen on the first visit and used throughout

the sampling period. For the eucalyptus weevil, besides the number of individuals and oothecas, the development phase of each individual was also recorded (light yellow larvae measuring between 1.5 to 2.6mm – L1 – and 2.7 to 4.9mm – L2; greenish-yellow larvae with two lateral longitudinal dark stripes measuring between 5 to 7mm – L3 – and 7.5 to 12mm – L4; fully developed adult individuals – Ad). During counting, some specimens were collected from leaves or branches and stored in tubes containing 98% alcohol to create a local reference library. Additionally, some specimens were also provided by RAIZ from their lab cultures and field collections in other areas of the country.

II.II. Laboratory analysis

Design of diagnostic PCR assay

DNA was extracted from a leg of each collected pest specimen, or the entire individual in the case of *Ctenarytaina spp.* psyllids, using the EasySpin Genomic DNA Tissue Kit (Citomed, Lisbon, Portugal), following the manufacturer's protocol for columns.

Polymerase Chain Reaction (PCR) was performed using arthropod general cytochrome oxidase subunit I (COI) primers LCO1490 x HCO2198 (710bp);(Folmer et al. 1994; Shokralla et al. 2015), while in two samples we used the arthropod general cytochrome oxidase subunit I (COI) primers BF2 x BR2 (423bp; Elbrecht et al. 2017). Both reactions were carried out in volumes of 10 µl, containing 5 µl of QIAGEN Multiplex PCR Master Mix, 0.3 µl of each 10 mM primer, 3.4 µl of ultra-pure water, and 1 µl of DNA extract. The PCR cycling conditions for the first primer-pair consisted in an initial denaturing at 95°C for 15 minutes, followed by 45 cycles of denaturing at 95°C for 30 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. The second primer-pair followed the same PCR cycling conditions as the first one, except 35 cycles were done and the annealing was at 45ºC. Amplifications were done on a T100™ Bio-Rad Thermal Cycler (BioRad, Hercules, CA, USA), in independent PCR reactions, without any multiplexing, and their quality was confirmed on a 2% gel stained agarose (GelRed Biotium) (0,5% TBE) applying 3 µl of bromophenol blue to 2 µl of the PCR. DNA sequences were attained through Sanger's sequencing protocol. The sequencing of the amplified COI fragments was made on an ABI 3130xl Genetic Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA).

COI sequences from all available species of each target organism's family were additionally downloaded from GenBank (https://www.ncbi.nlm.nih.gov/) and BOLD (http://boldsystems.org/) and were aligned with the sequences gathered from our field-collected and provided specimens in Geneious v10.2.6. Specific primers were designed

for three pest species (*G. platensis, C. spatulate,* and *C. eucalypti*) and one parasitoid (*A. nittens*) in regions of high nucleotide diversity among species of each family to guarantee the specificity of the primer to each target species. Candidate regions for primer design had to be 20-35bp long, show a Tm of 50-65ºC, generate an amplicon of < 250bp in order to allow the amplification of degraded DNA obtained from droppings, and most importantly show >3bp mismatch to any other species of the family. To further validate the specificity of the primers, candidate oligos were blasted (https://blast.ncbi.nlm.nih.gov/) against the entire NCBI nucleotide database and checked for similarity to other organisms. Finally, the four developed primer pairs (Table 1) were tested on reference specimens in order to guarantee their ability to amplify each target species. PCR reactions were performed in volumes of 10 µl, containing 5 µl of NZYTaq II 2× Green Master Mix, 0.2 µl of each 10mM primer, 3.6 µl of ultra-pure water, and 1 µl of DNA extract. Cycling conditions consisted in an initial denaturing at 95°C for 15 minutes, followed by 35 cycles of denaturing at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. PCR products were tested in gel stained agarose to check for amplification success.

Diagnostic of field samples

DNA was extracted from each faecal sample using the Stool DNA Isolation Kit (Norgen Biotek Corporation) following the manufacturer's protocol. Extractions were conducted in a non-invasive extraction room to avoid contamination in sets of 23 samples in addition to a negative extraction control in which no DNA was added.

To assess the predation of the three eucalyptus pests and parasitoid, each sample was tested three times with each primer-pair in independent reactions. A negative control was included in each PCR to control for possible lab contamination issues. PCR reactions were performed as before, except cycling conditions that had to

Table 1: Sequences of the specific primers designed for the 3 pests and the parasitoid Anaphes nitens (Fs, Fragment size in base-pairs; Tm- Primer melting temperature in Celsius degrees)

Name	Sequence	Fs	Tm
A. nitens_Fwd1	5'- TTCTGGTTCATTTATTGGAAGAGA-3´	158bp	56,0
A. nitens_Rev1	5´- GCGGGAATGATATATCTGGAAC-3'		56,5
C. spatulata_*Fwd1*	5'-TCTATTTATCTACTCATTATAAGAAGACTTATTG-3'	213bp	60,0
C. spatulata_Rev1	5'- TAAAGGGTACAGTGAAGATCGT-3'		56,6
C. eucalypti_Fwd1	5´- ATAGAGGATACTCTGTAGATACAGC-3´	124bp	56,6
C. eucalypti_Rev1	5'- TCTATAGAGTACAGAGAAGATCGC-3'		56,7
G. platensis_Fwd1	5'- TCTCAACTATAATTAATATACGACCCATA-3'	158bp	56,2
G. platensis_Rev1	5' AGTGTTAATATTACGATCAGTAAGCAA-3'		57,2

be adjusted to avoid the production of primer dimers in the presence of low or no target DNA, while ensuring the successful observation of positive samples. Thus, cycling conditions consisted in an initial denaturing at 95ºC for 5 minutes, followed by 15 cycles of denaturing at 95ºC for 30 seconds, annealing at 62ºC for 30 seconds, and extension at 72ºC for 30 seconds, followed by 10 similar cycles where the annealing temperature was reduced to 59ºC, and again another 20 similar cycles with an annealing of 56ºC, and last a final extension at 72ºC for 10 minutes. Quality and amplification success were checked by visually inspecting 2 µl of each PCR product on a 2% gel stained agarose (GelRed Biotium). Each sample was only considered positive for one of the target species if at least two of the three PCR replicates were positive. All negative controls (extraction and PCR) were checked for amplification and all were scored as negative. PCR products of positive samples were sequenced by Sanger to confirm that only the target insects were amplified.

Species identification of bat samples

To assess the bat species of samples collected in roosts, and to confirm the identity of captured bat individuals, a small COI fragment was amplified using the primers SFF_145f-351r (Walker et al., 2016). PCR reactions consisted in 5uL of MyTaq Mix (Bioline), 0.3uL of each 10nM primer, 3.4uL of water and 1uL of DNA extract. Cycling conditions consisted in a 15 min period at 95ºC, 35 cycles of 30 sec denaturation at 95ºC, 30 sec annealing at 56ºC, and 30 sec extension at 72ºC, and a final extension period of 10 min at 72ºC. Finally, PCR products were sequenced in an Illumina MySeq. Sequences were then blasted on BOLD and identified if showing a 99% similarity to any portuguese bat species. When sequences of different bat species were obtained from the same sample, or in cases where no bat sequences were obtained, the identity of that sample was considered as "undetermined".

II.III. Statistical analysis

Statistical analyses were all performed in R v.3.5.2 (R Core Team 2018) and tests were considered significant if the p-value was inferior to 0,05. To understand how predator species, pest abundance, and predator traits influence the predation rate of eucalyptus insects, I built Generalized Linear Models (GLM) for each target insect (*G. platensis*, *C. spatulata*, *C. eucalypti*, and *A. nittens*) using the R-package 'lme4' (Bates et al. 2015) with a binomial distribution. The 'c-bind' function was used to define the binary dependent variable, using the number of positive vs negative samples tested for

each predator species/month/sampling location. Unfortunately, due to the extremely low number of positive samples in bats (see results below), these models were only possible to build for birds.

Pest abundance was used as an explanatory variably for the three pest insect models by using the sum of pest individuals observed in the 10 analysed trees in each month/sampling location. In the case of *G. platensis,* two independent variables were considered: the number of larvae and the number of adults. This was done as larvae have quite distinct traits (e.g. hardness and behaviour) from adult beetles, and it might be expected that they are predated by different species. Different larval stages were merged into a single category due to the better fit of the produced model. To understand how predator traits may affect the predation of a given study insect, a series of explanatory variables was also used in each model. The bird traits used as response variables were body mass, main diet, foraging guild, and phenology (Annex 1). Body mass consisted in the average weight (in g) of the birds captured in each month/sampling location, and it might be expected that smaller species (less body mass) might not feed on larger and harder pest species (like adult *G. platensis* beetles), while larger species might not feed in smaller pests (like *A. nittens*). Diet (insectivorous or other) and foraging guild (bark forager, canopy gleaner, ground gleaner, ground prober, hawker, or understory gleaner) followed the information provided by Barbaro et al. (2016) and Wilman et al. (2014). These traits may explain the predation on our target insect species as their typical foraging strategy and main food type can help explain why a given bird species is predating more or less of a certain eucalyptus insect. Finally, phenology (migratory or resident) was defined following Catry, Costa, Matias, & Rafael (2010) and was used because migratory species may have a lower co-occurrence with the study target insects and therefore explore less these novel potential preys.

To test the significance of the GLMs I used the function 'Anova' from the package 'car' (Fox and Weisberg 2011) and when a response variable was significative, I used the function 'glht' from the package multcomp (Hothorn et al. 2008) to see which groups differed. In the multiple comparisons, a single-step method was used to adjust the p-values.

Finally, to compare if there are different predation rates between the main pest, *Gonipterus platensis,* and its parasitoid, *Anaphes nitens,* a Chi-Square test was performed.

III. Results

During the sampling period, a total of 659 faecal samples were collected, 294 from birds belonging to 29 different species, and the remaining 365 were bat samples collected from at least 10 different species. Given that two positive replicas were required for samples to be considered positive for a given insect, a total of 96 positive detections were observed: 40 for *Gonipterus platensis*, 10 for *A. nitens*, 21 for C. *spatulate,* and 25 for C. *eucalypti*. Most of the positive samples found were from bird samples, with 39 positives for *G. platensis found in 13 species*, 10 for *Anaphes nitens in 7 species*, 18 for C. *spatulata* in 8 species, and 22 for *C. eucalypti* in 9 species. Bats only registered 1 positive for *G. platensis*, no positives for *A.nitens*, 3 for *C. spatulata* across 2 species, and 3 in *C. eucalypti* on the same species.

III.I Effects of pest abundance

III.I.I Seasonal variation of the pest abundance

The abundance and phenology of the studied pests were considerably different in the two locations where they were counted (Boialvo, Figure 2, and Pisão, Figure 3).

In Boialvo, the number of *C. spatulata* was more consistent, only showing a reduction in the last two months. *C. eucalypti* diminished earlier having high numbers in the first 3 months and steadily reducing until September when they stopped appearing.
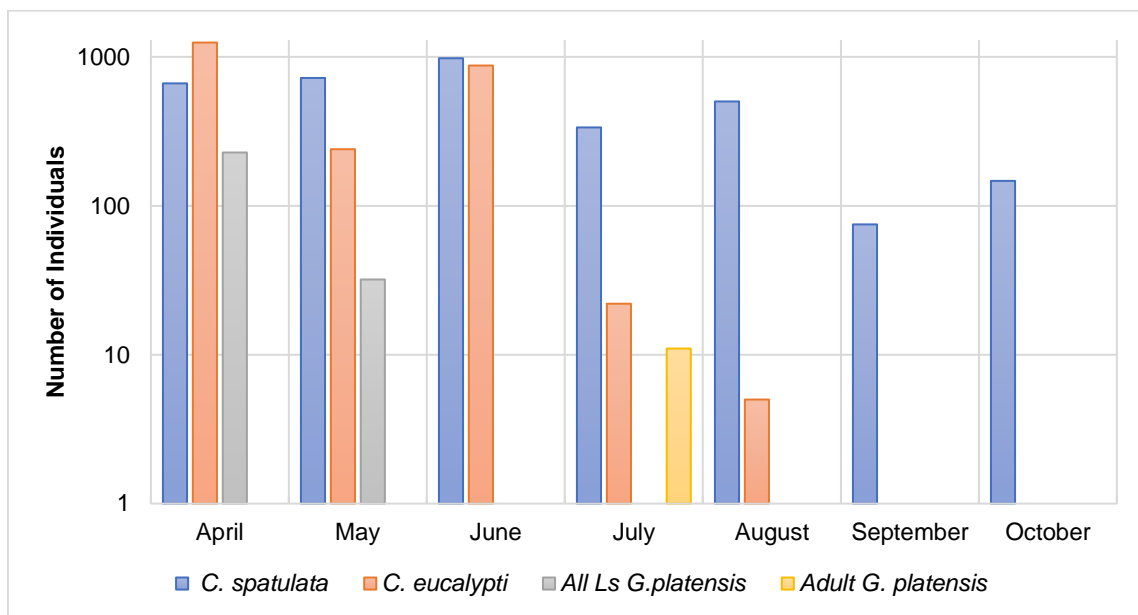
Figure 2: Pest count in Boialvo in each month of captures for each pest species (Gonipterus platensis separated in larvae and adults); logarithmic scale of 10
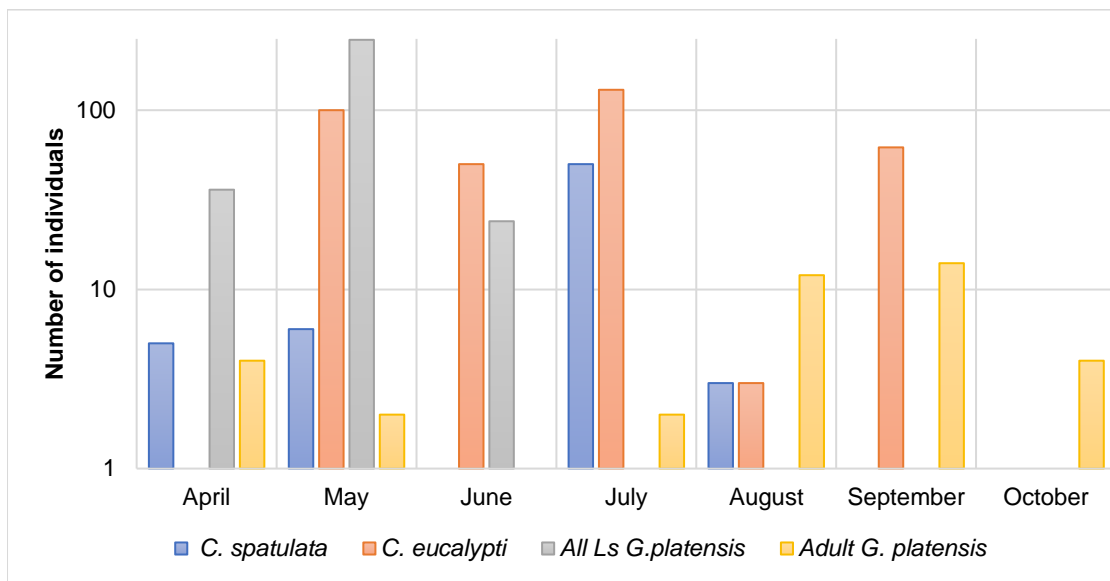
Figure 3: Pest count in Pisão in each month of captures for each pest (Gonipterus platensis separated in larvae and adults); logarithmic scale of 10.

G. platensis larvae were observed in the earlier months, having a particularly high count on the first, while adults were only found in July in this location (Figure 2).

In Pisão, except for *G. platensis,* the overall abundance of pests was lower than in Boialvo (Figure 3). Even though Boialvo showed a higher number of psilids throughout the sampling period, in the months of July to September the count of *C. eucalypti* was higher or equivalent in Pisão. It is also possible to observe that throughout the sampling period in Pisão the number of *G. platensis,* larvae and adults, was higher, being the later spotted in almost every month of sampling, while in Boialvo they were rarely found.

III.I.II Variation of predation rates with pest abundance

To understand if the variation of predation rates was influenced by the pest abundance on the field, a correlation was made within each species counted on both locations (Boialvo and Pisão).

In *G. platensis*, the Generalized Linear Model indicated that there is a significant effect of the number of *G. platensis* beetles (LR Chisq = 10.8343, Df = 1, p = 0.0009964, Figure 4) on the predation of *G. platensis* by birds. This indicates that the number of predation events of *G. platensis* is positively associated with an increase in the number
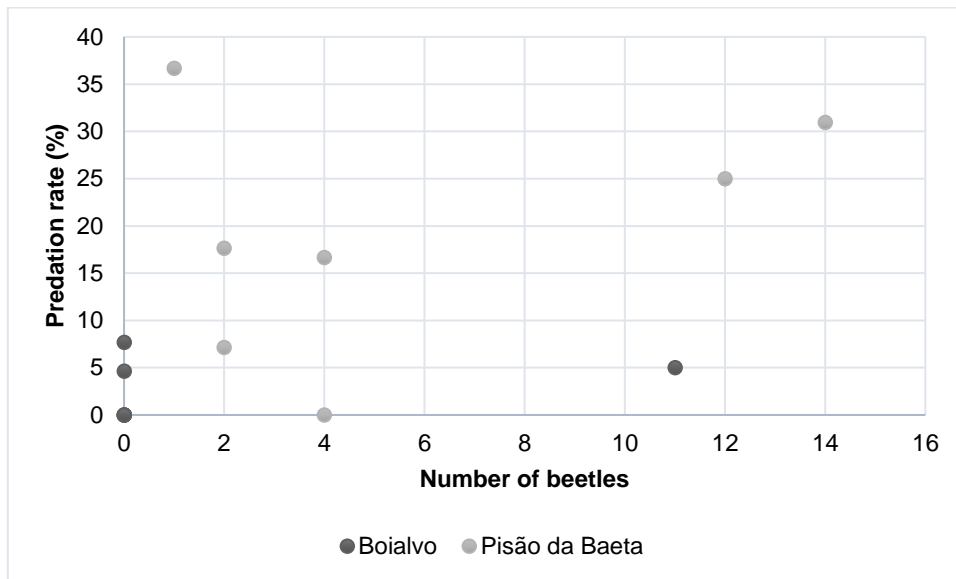
Figure 4: Relation between the number of beetles counted on the field and the predation rate in G. platensis.

of beetles (Figure 4) despite no association with its larvae (LR Chisq = 0.4293, Df = 1, p = 0.5123385, Figure 5).

In *C. eucalypti* there is no visible relation between the predation rates and the number of psillids found in a given area. In both areas the highest predation rates were registered when the pest count numbers were under 250. This was confirmed when analysed with GLM, where no significant effect of this psyllid was detected (LR Chisq= 0.0646, Df=1, p= 0.79935, Figure 6).
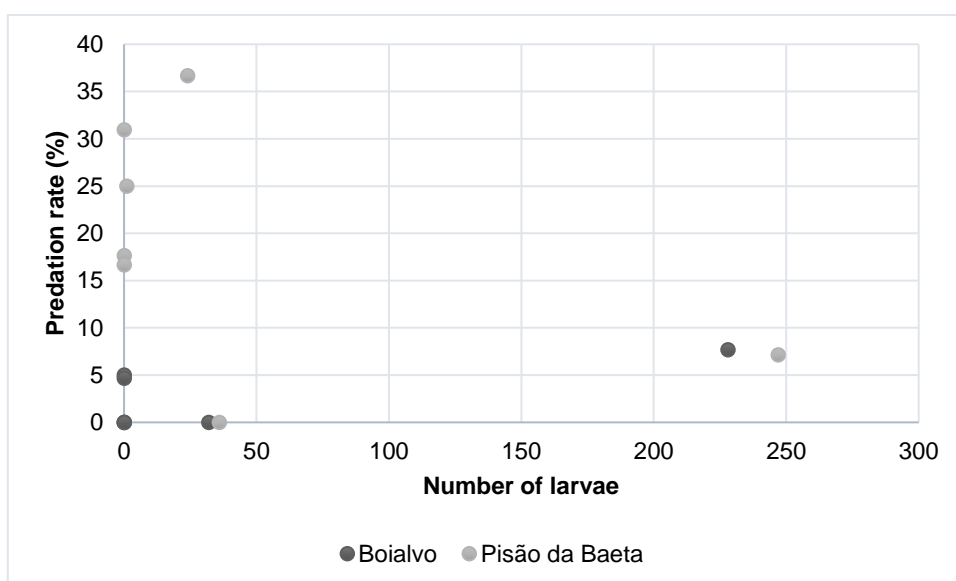
Figure 5: Relation between the number of larvae counted on the field and the predation rate in G. platensis.
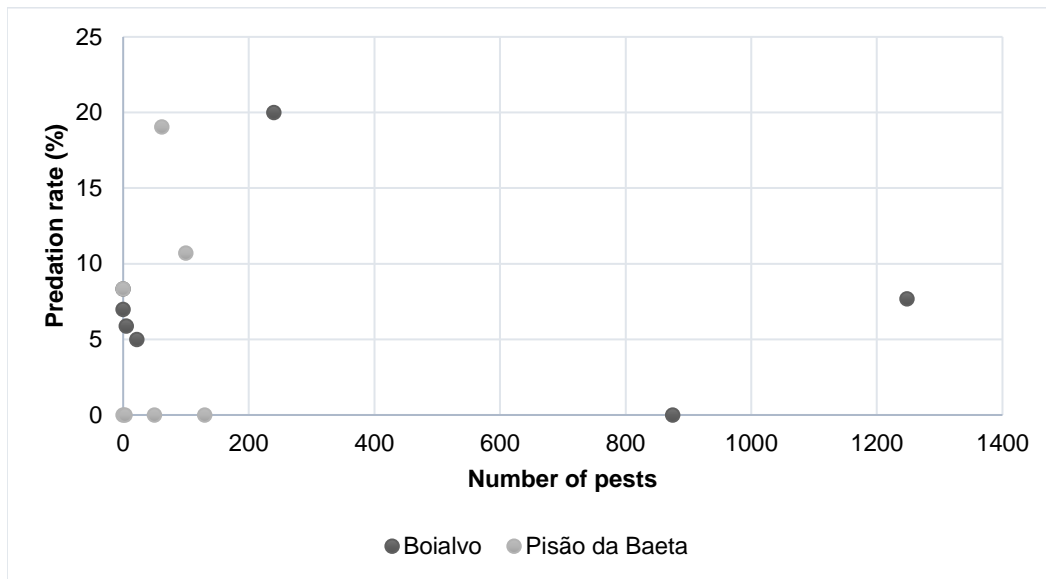
Figure 6: Relation between the pest number counted on the field and the predation rate in C. eucalypti.

In *C. spatulata*, also no relation was found, with the highest percentage of positives being recorded when number of pests was the lowest in Pisão da Baeta and in the middle of the scale in Boialvo. Once again, the model did not show any significance of *C. spatulata* abundance on its consumption (LR Chisq=0.1845, Df=1, p=0.66752, Figure 7).
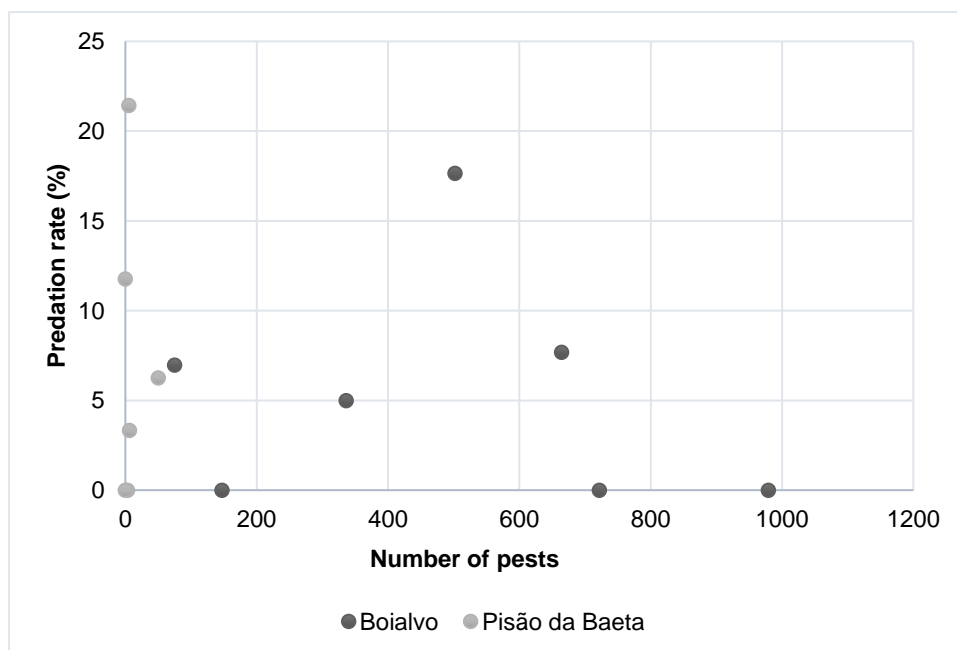
Figure 7: Relation between the pest number counted on the field and the predation rate in C. spatulata.

III.II Effect of predator species

Bats

Even though the number of bat samples was larger than that of birds, the number of positives was extremely low for all species analysed. The only species observed consuming any of the pests were *Rhinolophus ferrumequinum*, *Plecotus austriacus*, and *Rhinolophus hipposideros,* with only 1 positive sample for eucalyptus weevil, 3 for C. *eucalypti,* and 3 for C. *spatulata* (Figure 8). Nonetheless, it was only possible to collect more than 20 samples for 4 bat species.
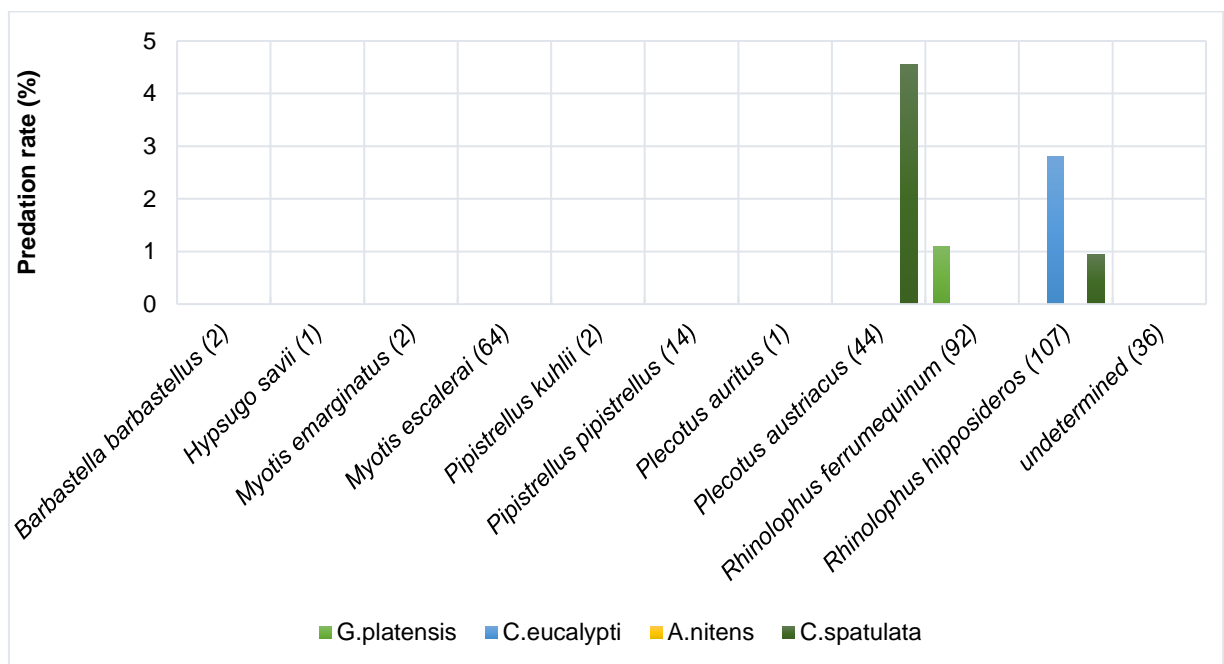
Figure 8: Predation rates (%) of the pests and weevil's parasitoid by all bat species sampled. Sample size of each species is indicated within parenthesis.

Birds

The results showed that birds prey much more often on the eucalyptus pests studied in this work than bats. In total, we detected 39 predation events of *G.platensis* and only approximately one quarter (10) on *Anaphes nites*. The psilids C. *eucalypti* and C. *spatulata* were detected on 18 and 22 bird faeces, respectively.

Predation evidence of *G. platensis* was found in 39 samples spreading throughout almost half of the species sampled (13 out of 29), three of which seem to predate only on this species (*Fringilla coelebs, Periparus ater,* and *Phoenicurus*
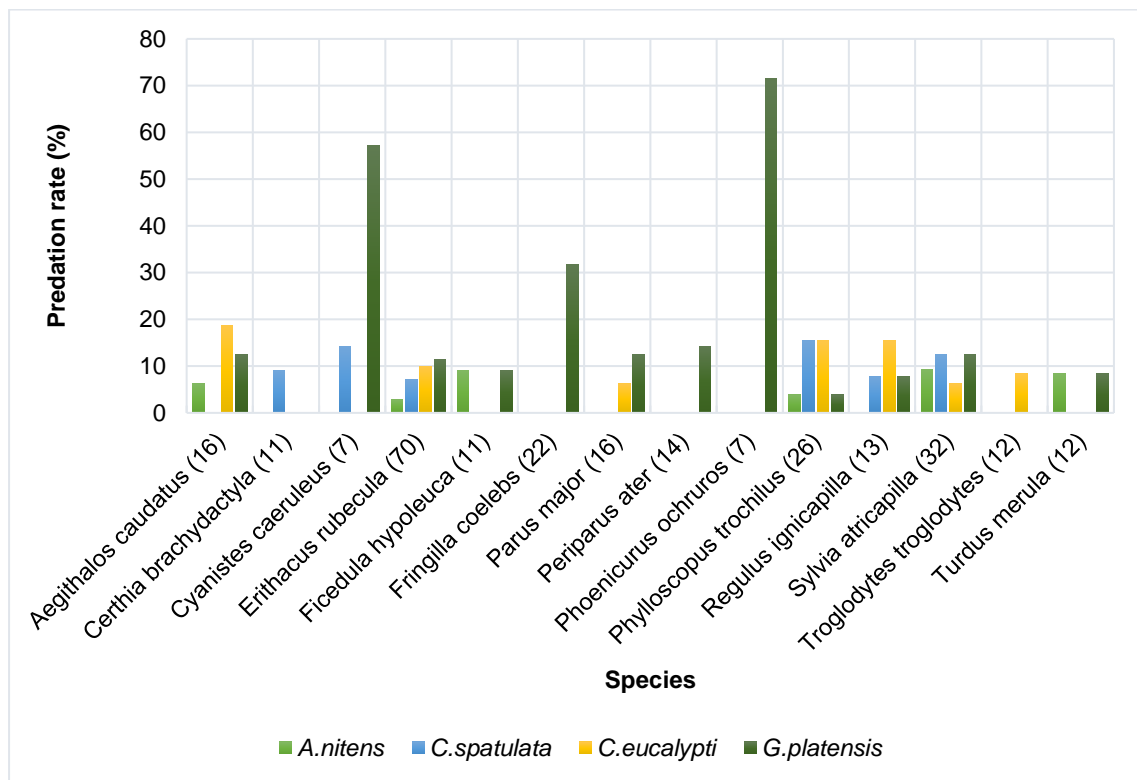
Figure 9: Predation rates (%) of the pests and weevil's parasitoid by bird species with a sample size above 5. Sample size of each group is indicated within parenthesis.

ochruros). *A. nitens* was detected in 10 samples belonging to 7 species of birds, most of them coinciding with the predation of *Gonipterus platensis* and the remaining pests.

C. eucalypti recorded 18 positive samples in 9 bird species, where two of those (*Troglodytes troglodytes*) preyed only this pest and *C. spatulata* had 22 positives across 8 species, with *Certhia brachydactyla* only showing positive amplifications for this pest.

In figure 9, we can observe that all the 3 pest species and parasitoid were preyed by the sampled bird species. Birds like *Erithacus rubecula* consumed all four, while some were only recorded to consume one or even none, but all bird species with more than 5 samples (graph with all the 29 bird species - Annex 2) showed at least one positive sample.

The highest predation rate recorded (in birds with more than 5 faecal samples) on our main pest, *G. platensis*, was registered in *P. ochruros*, being closely followed by *Cyanistes caeruleus*, with both showing predation rates above 50%. The parasitoid, *A. nitens*, obtained its highest predation rate in *Ficedula hypoleuca* and *Sylvia atricapilla*, species whose predation rate of *G. platensis* was equally high.

The psyllids showed similar predation rates with *C. eucalypti* being mostly predated by *Aegithalos caudatus*, *Phylloscopus trochilus,* and *S. atricapilla*; while *C. spatulata* was mostly consumed by *P. trochilus*, *C. caeruleus,* and *S. atricapilla*.

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III.III Effect of predator traits

Gonipterus platensis

The Generalized Linear Model for *G. plantensis* indicated that there is a significant effect of phenology (LR Chisq = 17.5022, Df = 1, p = 2.87e-05, Figure 10) and foraging guild (LR Chisq = 23.1733, Df = 5, p = 0.0003128, Figure 11) on bird's predation rates.

Resident birds prey more often on *G. platensis* than migratory birds (Figure 10).

Figure 10: Predation rate (%) of G.platensis distributed by the different categories of the trait Phenology. Sample size of each group is indicated within parenthesis. Different letters represent statistical differences between groups.

The foraging guild that preys more often on *G. platensis* is the hawker guild, that differed from the canopy, understory, and ground foragers (Figure 11).

Figure 11: Predation rate (%) of G. platensis distributed by the different categories of the trait Foraging guild. Sample size of each group is indicated within parenthesis. Different characters represent statistical differences between groups.

Figure 12: Predation rate (%) of G. platensis distributed by the different categories of the trait Diet. Sample size of each group is indicated within parenthesis.

The remaining explanatory variables were not significant (diet: LR Chisq = 1.3396, Df = 1, p = 0.2471031, Figure 12; body mass: LR Chisq = 2.7981, Df = 1, p = 0.0943741, Figure 13), and therefore there are no differences between the classes of these variables.

Figure 13: Predation rate (%) of G. platensis across 3 different categories of body mass in grams (g). Sample size of each group is indicated within parenthesis.

Anaphes nitens

The binomial GLM for the predation of *A. nitens* by birds indicated only a significant effect of the main diet type (LR Chisq= 3.9050, Df=1, p= 0.04814, Figure 14), showing that the birds that prey more often on *A. nitens* are birds without a predominant insectivore diet (category "other").

Figure 14: Predation rate (%) of A. nitens distributed by the different categories of the trait Diet. Sample size of each group is indicated within parenthesis. Different characters represent statistical differences between groups.

All the remaining variables tested did not show any significance (phenology: LR Chisq= 0.6112, Df=1, p= 0.43432, Figure 15; foraging guild: LR Chisq= 2.0840, Df=5, p= 0.83740, Figure 16; body mass: LR Chisq= 0.3515, Df=1, p= 0.55327, Figure 17).

Figure 15: Predation rate (%) of A. nitens distributed by the different categories of the trait Phenology. Sample size of each group is indicated within parenthesis.

Figure 16: Predation rate (%) of A. nitens distributed by the different categories of the trait Foraging guild. Sample size of each group is indicated within parenthesis.
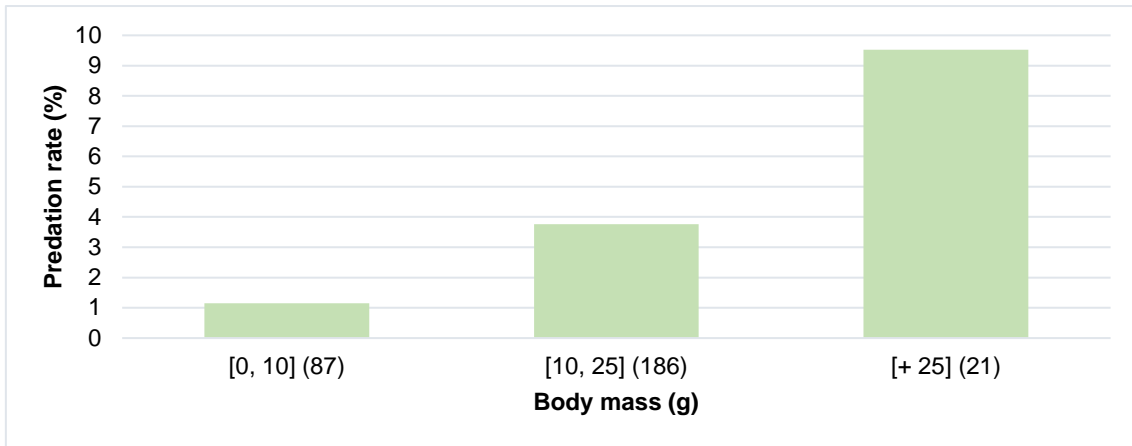
Figure 12: Predation rate (%) of A. nitens across 3 different categories of body mass in grams (g). Sample size of each group is indicated within parenthesis.

Ctenarytaina eucalypti

The model showed no significance for the traits tested in *C. eucalypti*, although there was a weak signal for the effect of bird's Foraging guild but without statistical significance (LR Chisq= 10.5505, Df=5, p= 0.06106, Figure 18), with gleaner guilds predating more often on this pest than the other ones. The remaining variables (phenology: LR Chisq= 1.7893, Df=1, p= 0.18101, Figure 19; diet: LR Chisq= 0.1430, Df=1, p= 0.70529, Figure 20; body mass: LR Chisq= 1.5805, Df=1, p= 0.20869, Figure 21) showed no effect on predation rates.
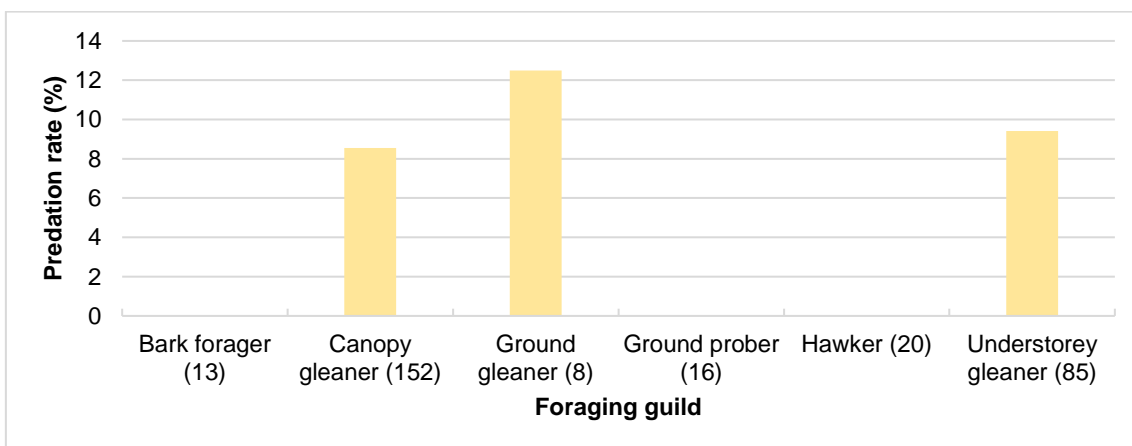
Figure 13: Predation rate (%) of C. eucalypti distributed by the different categories of the trait Foraging guild. Sample size of each group is indicated within parenthesis.
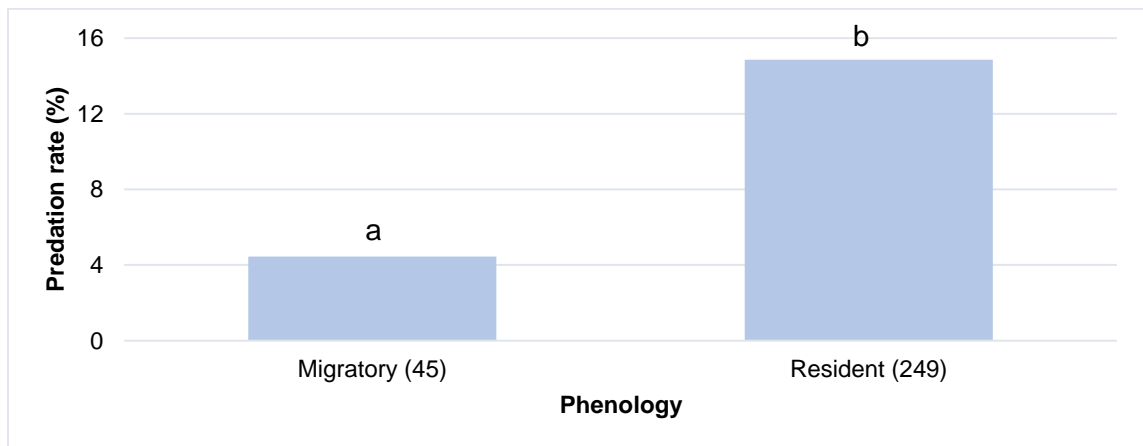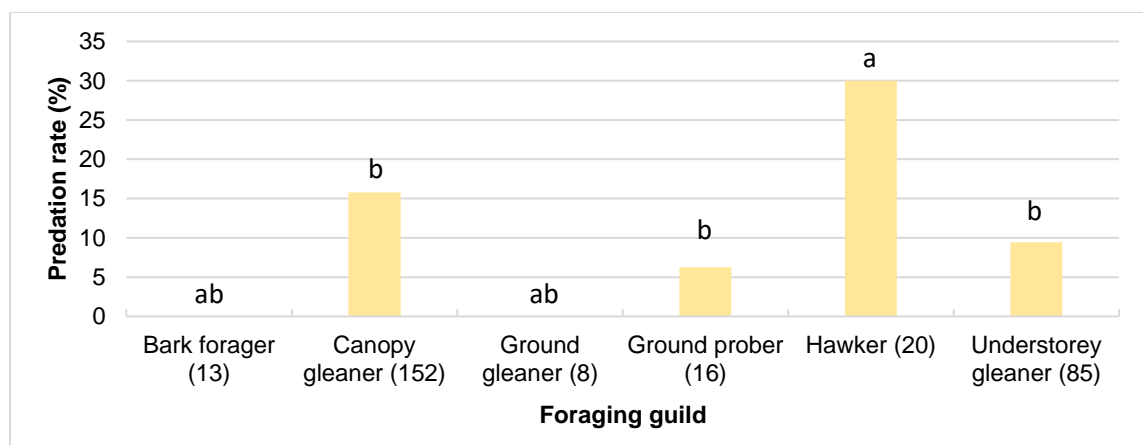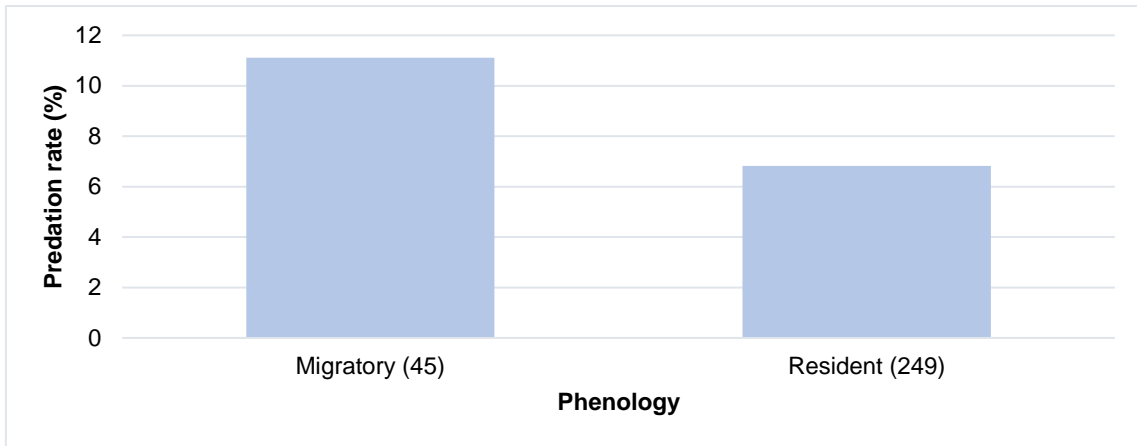
Figure 14: Predation rate (%) of C. eucalypti distributed by the different categories of the trait Phenology. Sample size of each group is indicated within parenthesis.

Figure 15: Predation rate (%) of C. eucalypti distributed by the different categories of the trait Diet. Sample size of each group is indicated within parenthesis.
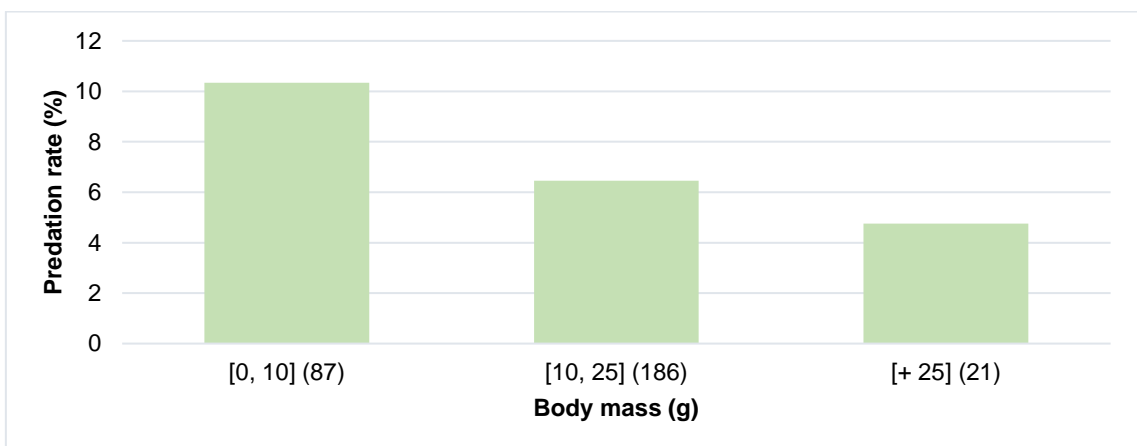
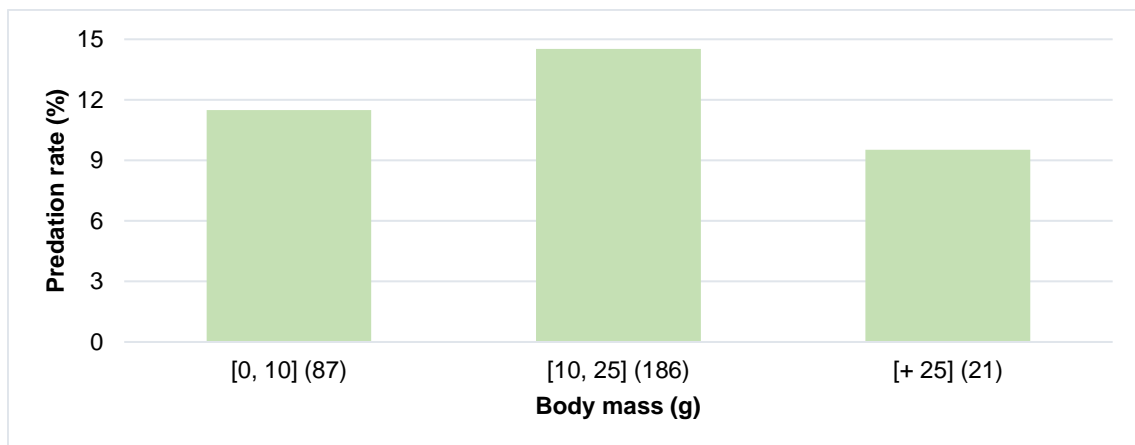*Figure 16: Predation rate (%) of C. eucalypti across 3 categories of body mass in grams (g). Sample size of each group is indicated within parenthesis.*

Ctenarytaina spatulata

In *C. spatulata* the model indicated a significant effect of Phenology (LR Chisq= 6.2800, Df=1, p= 0.01221, Figure 22), with migratory birds preying more often on *C. spatulata* than resident birds.
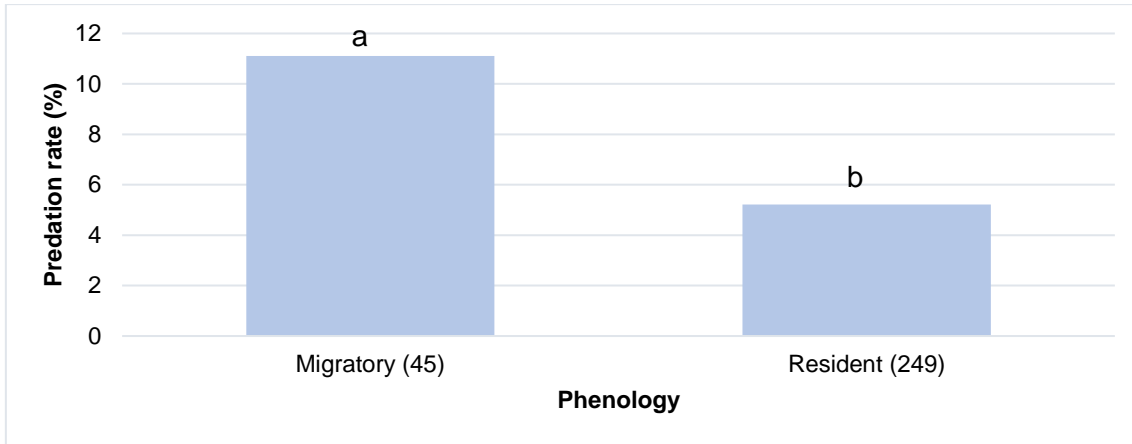
Figure 17: Predation rate (%) of C. spatulata distributed by the different categories of the Phenology. Sample size of each group is indicated within parenthesis. Different characters represent statistical differences between groups.

Foraging guild (LR Chisq=11.8716, Df=5, p= 0.03659, Figure 23) was also significant in the model, but multiple comparisons did not find any statistical differences between groups after the p-value adjustment for multiple testing (Annex 3).
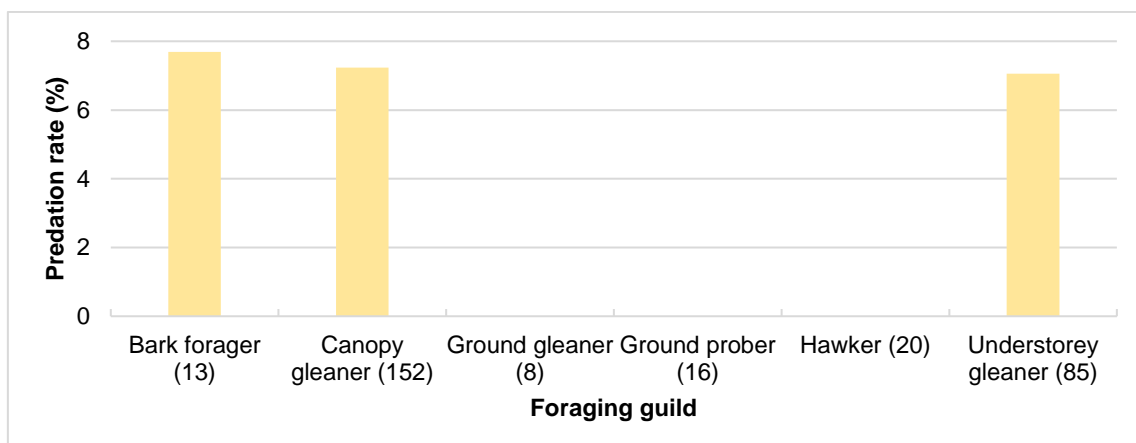
Figure 18: Predation rate (%) of C. spatulata distributed by the different categories of the trait Foraging guild. Sample size of each group is indicated within parenthesis.

The remaining traits tested did not show any significant effect on the predation rate of *C. spatulata* (diet: LR Chisq=3.2441, Df=1, p=0.07168, Figure 24; body mass: LR Chisq=0.8499, Df=1, p=0.35657, Figure 25).
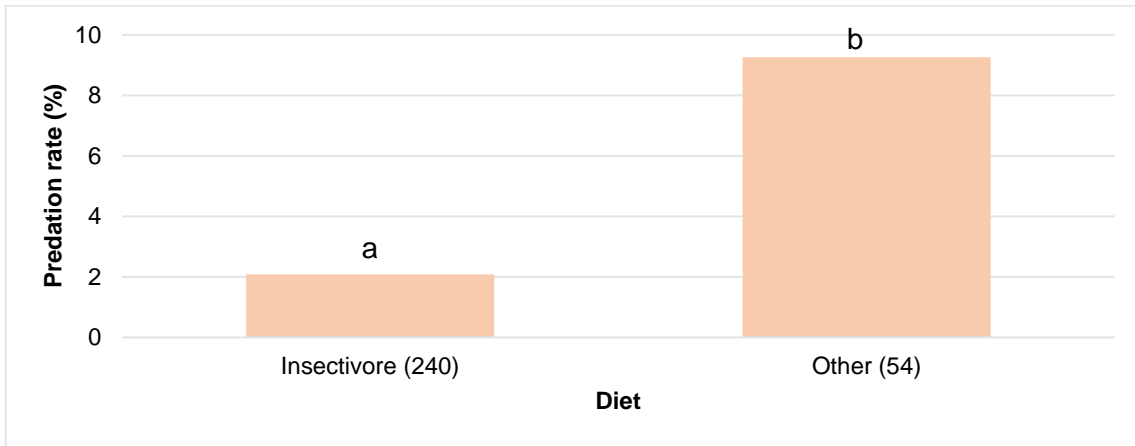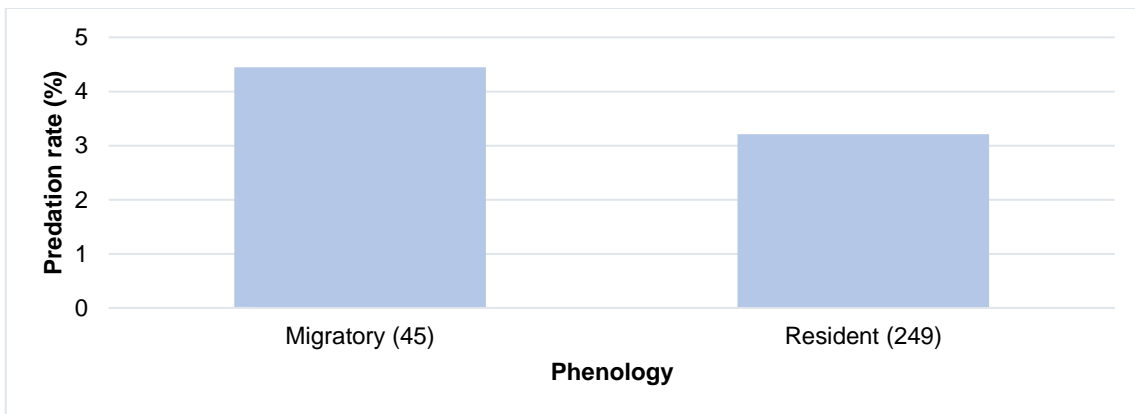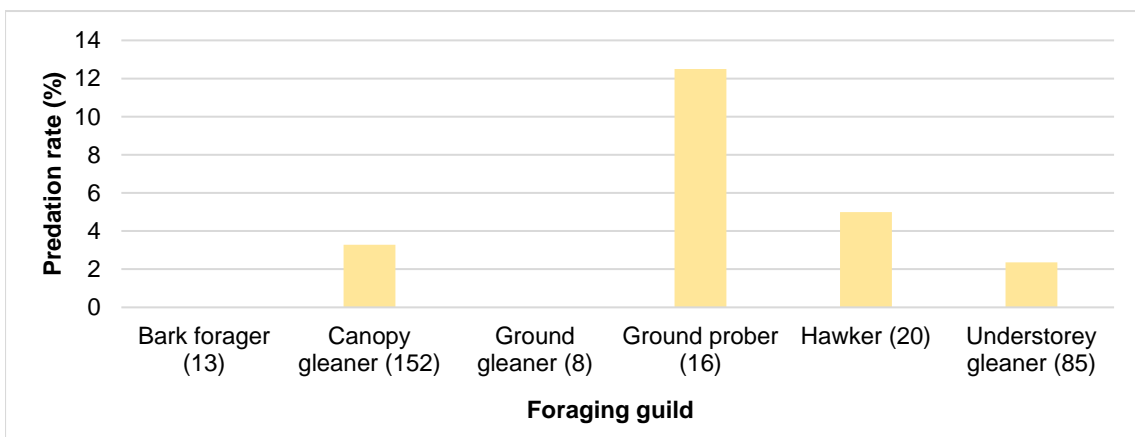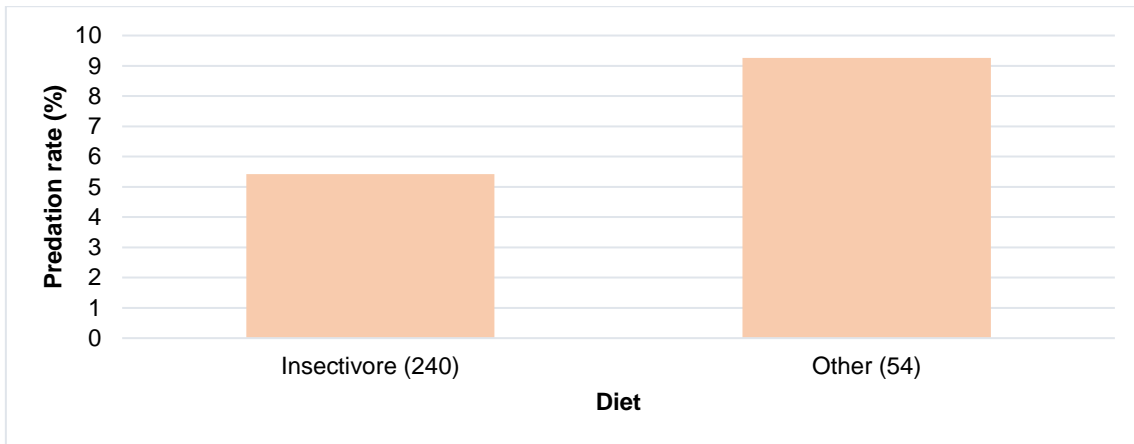

Figure 19: Predation rate (%) of C. spatulata distributed by the different categories of the trait Diet trait. Sample size of each group is indicated within parenthesis.
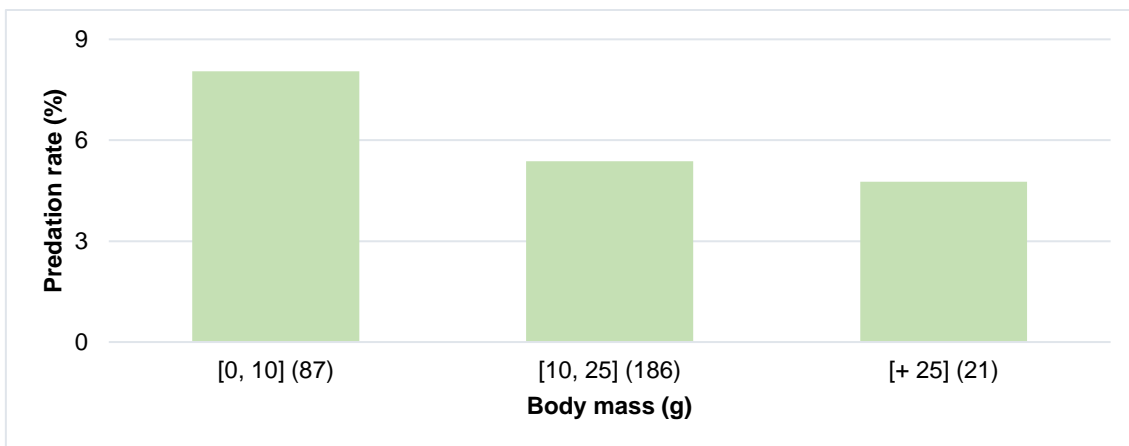
Figure 20: Predation rate (%) of C. spatulata across 3 categories of body mass in grams (g). Sample size of each group is indicated within parenthesis.

III.IV Eucalyptus weevil biocontrol potential by native vertebrates

The overall predation by native vertebrates on *Gonipterus platensis* was higher than on its parasitoid *Anaphes nitens* (χ^2_2 = 8.710, p-value < 0.001)*. G. platensis* showed 40 positives registered throughout 14 vertebrate species, while *A. nitens* only showed 10 positives across 7 species. Most of the vertebrate species predated both the pest and the parasitoid, with no species having a particularly higher predation rate of the pest or parasitoid*.

IV. Discussion

Overall, our study demonstrates that the eucalyptus pests and the parasitoid *Anaphes nitens* are preyed on by native bird and bat species. In particular, *Gonipterus platensis*, our main pest, is preyed four times more often than its parasitoid and twice more than *Ctenarytaina spp.* psyllids. Our results have crucial repercussions as we show that, not only do several bird species prey on the weevil but also unveil their predominant functional traits. This study reveals which bird species should be promoted in eucalyptus plantations to help manage the damage caused by eucalyptus pests, mainly *G. plantensis*.

IV.I Effect of pest abundance

IV.I.I Seasonal variation of pest abundance

The number and distribution of the pests throughout the sampling period were distinct in both locations. Although both psyllids were present in both sites, the prevailing one and the concentration of each one was different. While in Boialvo in the earlier months the number of psyllids was all near a thousand and *C. spatulata* was mostly the predominant pest, in Pisão da Baeta the highest number did not even reach 150 individuals and the main psyllid was *C. eucalypti*. *G. platensis* larvae had similar numbers and were recorded during the same period, only being observed in the earlier months, whilst beetles had an overall higher number in Pisão being observed in almost every month. The phenology of G. platensis found coincides with the literature, where larvae are mostly present between March and May, and October, which occurred in our study in the earlier months but not in October in either location (Santolamazza-Carbone et al. 2009; Valente et al. 2017). Beetles normally have peaks in April, July, and November although found throughout the year if the temperatures allow it, coinciding with the peak found in Boialvo, and the increase in individuals from July until September recorded in Pisão (Santolamazza-Carbone et al. 2009; Valente et al. 2017).

The psyllids normally have similar phenology being observed during all year, but increase in response to shoot growth starting in December and peaking in July to September (Brennan et al. 2001), noted in one location (Pisão da Baeta), but not so visibly in the other.

Although the number of psyllids found was notably higher than that of *G. platensis*, this species is still our main concern as there is a high number of known species that prey on the psyllids (Santana 2000; Soloneski and Larramendy 2012)

compared with the number that feeds on *G. platensis*. Being most of them imported parasitoids, that as referred are not always successful at containing the damage, because of its limiting factors, such as winter temperatures (Valente et al. 2017). Therefore, there is a possibility that the natural predators of the psyllid can maintain a stable and economically viable damage level in Portugal, unlike the ones preying on *G. platensis*.

IV.I.II Variation of predation rates with pest abundance

When analysing the predation rates against the pest count in *G. platensis* an association between the number of beetles and predation events was shown and contrarily to what was expected the results showed no association between the predation events and the larvae number. This was an unexpected result as many birds e.g. *Fringilla coelebs* and tit species (*Cyanistes caeruleus, Lophophanes cristatus, Parus major, and Periparus ater*) are known to actively feed on several larvae (Snow et al. 1998).

In the remaining species observed, the psillids *C. eucalypti* and *C. spatulata,* no significance was found in the number of pests counted against the predation rate on both locations, leading us to infer that the number of pests in the area is not associated with an increase in predation by the species actively feeding on the species. Perhaps because allied with the peaks of *Ctenarytaina* spp. other insect species, that are preferred due to either their size, palatability, or previous encounters, also have their peaks at the same time. Birds could thus be targeting other species and consequently reducing their predation on *Ctenarytaina* spp. during that time frame and selecting them only when there is a shortage of other favoured prey. This supposition could be tested by analysing the total diet of birds and comparing it to overall insect availability.

IV.II Effect of predator species

Bats

Contrarily to what was hypothesised and even though the number of bat samples was higher than that of birds, the predation of the studied species by bats was limited, amounting to an extremely low number of positives.

This number can be due to various non-exclusive reasons:

1) The different home ranges between bats and birds. Generally, bats have wider and more heterogenous home ranges compared with small passerines, especially in the breeding period, leading them to travel further in search of food, foraging covering up to

a few thousand square kilometres (Marques et al. 2004; Rainho and Palmeirim 2011; Charbonnier et al. 2016). Which can lead us to infer that during the sampling period, because of their wider foraging territory, the food available outside eucalyptus plantations was sufficient for them not to feed in those areas. While some birds had to prey on these pests as traveling further was out of their home range and was energetically unsustainable.

2) Activity period. Its commonly know that bats and birds have different periods of activity, birds feeding during the day and bats during the night, but little is known about the pest's activity period. In a recent study, performed in *Eucalyptus* sp. predominant forests in Australia, the abundance of nocturnal beetles was analysed and the family of our main pest (Curculionidae) was caught less than 10 times, which suggests that their activity period is mostly diurnal (Forbes et al. 2019). This would explain why birds, which would have a coinciding activity period, are preying more *G. platensis* compared to bats.

3) The samples being insufficient. Only 4 of the 10 bat species sampled, recorded more than 20 samples collected (where the positives in bats were found), which shows that some species are able to feed on the pests, but seemingly always in low quantities.

Bats have been identified as vertebrate predators that provide ecological and economical valuable services, such as pollination (Aguilar-Rodríguez et al. 2019; Tremlett et al. 2020), seed dispersal (Kunz et al. 2011; Abedi-lartey et al. 2016), and pest-control (Taylor et al. 2018). The pest control services provided by bats are known to have a positive effect in several systems as rice fields, cornfields, cotton crops, vineyards, and so on (Cleveland et al. 2006; Wanger et al. 2014; Maine and Boyles 2015; Rodríguez-San Pedro et al. 2020).

Hence, in a future analysis, having a higher number of bat species, including species such as *Eptesicus* sp., *Myotis myotis,* and *Pipistrellus pygmaeus*, which are known to occur in the area (Rainho et al. 2013) and a higher number of samples collected could display different results from the ones obtained. But it should be stressed that even if these changes are made, the results might not be particularly different as the activity of bats in eucalyptus plantations is known to be low when compared with other forest types (Rainho 2009; Cruz et al. 2016; da Silva et al. 2019a).

Nonetheless, the results of this thesis indicate that the predation of eucalyptus pests by bats in the center of Portugal is marginal, and therefore, it is not expected that bats can act as important suppressors of eucalyptus pests, at least in comparison with birds.

Birds

From birds, the number of positives samples was much higher than from bats, with all eucalyptus insects tested being recorded, including the parasitoid *A. nitens*.

More than half of the species (18 out of 29), were proven to feed on one or more of the eucalyptus insects, which suggests a high functional diversity of pest predators, as the species represented have a broad range of different traits. There was also a high disparity in the number of samples per species with only 4 species having more than 20 samples in total.

The highest predation rates were recorded on *G. platensis* by *Phoenicurus ochruros* and *Cyanistes caeruleus,* two medium-sized and resident birds that prey on the higher levels of eucalyptus trees. Due to their body size, this predation was most likely on larvae, particularly *C. caeruleus,* which was also recorded to feed on *C. spatulata,* a smaller prey.

Besides *C. caeruleus*, there were other species observed preying on many of the studied species, like *Erithacus rubecula*, *Phylloscopus trochilus*, *Regulus ignicapilla,* and *Sylvia atricapilla*. On the smallest of the 4 (*P. trochilus and R. ignicapilla*) the prevailing prey were the psyllids while on the other two bigger species *G. platensis* was the main prey, suggesting that smaller species might be more effective in the predation of psyllids and larger ones on weevils.

Sylvia atricapilla also registered a higher than expected predation rate on *A. nitens,* predation that was also observed in *Turdus merula*, both birds with body sizes too big to actively predate on *A. nitens*.

IV.III Effect of predator traits

For *G. platensis*, we observed that resident birds preyed more often on it than migratory birds. This might be due to their lower temporal co-occurrence, leading to a lower predation rate on this novel prey. Migratory species might prefer to feed on previously known sources of food, while some may not even occur simultaneously with the target species, making it impossible for them to prey on it, leading to this discrepancy between the two traits. When analysing the effect of foraging guild, hawkers were the most prominent species feeding on *G. platensis*. This probably means that the beetles are being caught in mid-flight rather than when standing on the eucalyptus leaves where they feed and where larvae are also present.

Regarding *A. nitens*, although the predation rate on this species was low, we could observe that the diet category that more often preyed on it was birds without a

predominant insectivore diet. This might lead us to speculate that the predation of this insect was mostly involuntary, as the majority of adults obtain sugar solutions from floral or extrafloral nectar and homopteran honeydew (Lee et al. 2004; Santolamazza-Carbone et al. 2009). These feeding sources are also used by birds with a non-predominant insectivore diet. As the parasitoids reduced size and solitary behaviour do not make them ideal for active predation (Santolamazza-Carbone et al. 2004), these shared feeding strategies might lead birds to unintentionally prey on this small insect as they are feeding on plants.

For *C. eucalypti* none of our studied bird traits significantly influenced predation rates, although there was a weak signal for the effect of foraging guild. Although divided into 3 different categories, gleaning birds seemed to prey more often on this pest species than aerial hawking species, or bark or ground foragers. This result was not statistically significant, but this lack of statistical significance could be a consequence of the low number of positives since birds that glean insects from vegetation are more likely to pick up *Ctenarytaina spp.* individuals from eucalyptus leaves.

Contrarily to what was observed for *G. platensis, C. spatulata* was preyed more often by migratory bird species than resident ones. Resident birds have defined territories, unlike migratory, who only spend part of their time in each location, having to compete for space and resources (Wittwer et al. 2015; Morganti et al. 2017). This might lead migratory birds to feed on other, smaller prey (in this case *C. spatulata)*, as other sources are being monopolized by resident species. These, as seen before, seem to prefer to feed on larger prey like *G. platensis* beetles rather than psyllids, which end up being preyed by the migratory species.

The observed relatively low number of predation events of psyllids was unexpected. Small birds, like warblers (eg. small *Sylvia* sp. and *Phylloscopus* sp.), that usually forage in the canopy and bushes, and that have been previously recorded to prey on psyllids (Garcia 1981; Mostafa 2017), although having been the ones that more often predated of psyllids, were expected to feed more frequently on these pests, but in this study, the predation rates were not as high as anticipated.

The detection of *Ctenarytaina spp.* DNA in relatively big passerines, as *Garrulus glandarius*, was not expected because it is unlikely that they are actively predating such small insects. The same is true for *Turdus* spp. preying *A. nitens*. This may indicate that some of the positives can be the result of secondary detections (Sheppard et al. 2005; da Silva et al. 2019b). Despite this limitation, and others of DNA based methods in analysing diets (Nielsen et al. 2018), except for the previously mentioned predation events, all others are likely true predation events, even if some may not be real, those cases should be very few.

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Although several positives were attained during this study, a large number of the vertebrate species had an extremely low number of samples collected, often just one or two. This translates into a low confidence level on the predation rates observed on those species, as it is not possible to properly estimate predation rates with small sample sizes.

IV.IV Eucalyptus weevil biocontrol potential of native vertebrates

As expected, the predation of *G. platensis* was significantly higher than that of *A. nitens*. That means that although birds feed on *A. nitens*, which could be counterproductive for the control of this pest, the predation was 4 times less frequent than on *G. platensis.* Proving that even if actively preyed on, the predation is low, and as previously discussed, could even be the result of secondary detection. Moreover, the major economic problems caused by *G. platensis* happen in areas where the parasitoid is very scarce or absent due to its limitation in dealing with low winter temperatures (Reis et al. 2012), so birds are unlikely to have a significant role in regulating *A. nitens* populations in the areas where the damage caused by *G. plantensis* are more serious.

IV.V Final remarks

In 2018 alone, more than 2.9 billion euros were produced by the pulp and paper industry, where eucalyptus is the main source (CELPA 2017), making eucalyptus plantations a source of great economic importance in Portugal. Without eucalyptus pest control the paper industry would have a loss of approximately 40 million euros per year (ICNF 2019).

Evidence for potential pest control by native birds was found in this study, with many species being reported to be active predators of the studied eucalyptus pests. Previous studies can attest that bird species can regulate densities such as three-toed woodpeckers (*Picoides tridactylus*) in bark beetles of spruces (Fayt et al. 2005) and even eradicate psyllids from eucalyptus forest patches (Loyn et al. 1983).

In spite of birds being recorded to feed on *A. nitens*, which could be disadvantageous for the regulation of G. platensis, the predation rate on the pest was 4 times higher than on the parasitoid. And combined with low rates of parasitoids found in the main economic problem areas caused by *G. platensis*, birds are improbable to play a significant role in controlling *A. nitens* populations. On the other hand, bats appear to

have much less potential to serve as controllers of eucalyptus pests, possibly due to a divergent activity period with the study species.

As a concluding remark, I suggest the management of eucalyptus plantations to promote higher abundances of native birds, as these are natural enemies of several eucalyptus pests. Conservational measures like the application of semi-natural/natural woodland margins and islands should also be implemented (Begg et al. 2017), as they provide beneficial habitats around the cropped areas, alternative food supplies in the absence of pest species, shelter, and assist the natural enemy movement (Perovic et al. 2010; Rusch et al. 2010; Begg et al. 2017). These measures can potentially improve the natural control of the pests, leading to a decline in the density of the pests that cause havoc on the eucalyptus plantations, and consequently reduce the damage inflicted on the plants, keeping it within an acceptable economic threshold.

Bibliography

Abedi-lartey M, Dechmann DKN, Wikelski M (2016) Long-distance seed dispersal by straw-coloured fruit bats varies by season and landscape. Glob Ecol Conserv 7:12–24. doi: 10.1016/j.gecco.2016.03.005

Aguilar-Rodríguez PA, Krömer T, Tschapka M, et al (2019) Bat pollination in *Bromeliaceae*. Plant Ecol Divers 12:1–19. doi: 10.1080/17550874.2019.1566409

Alouw JC, Hosang MLA, Nguyen Q (2020) Biotechnology Contributing to Integrated Pest Management: The Example of Two Major Coconut Pests, *Oryctes rhinoceros* and *Brontispa longissima*. In: Coconut Biotechnology : Towards the Sustainability of the ' Tree of Life .' pp 151–168

Azevedo F, Figo M (1979) *Ctenarytaina eucalypti* Mask (Homoptera, Psyllidae). Boletín Sanid Veg Plagas 5:41–46

Barbaro L, Rusch A, Muiruri EW, et al (2017) Avian pest control in vineyards is driven by interactions between bird functional diversity and landscape heterogeneity. J Appl Ecol 54:500–508. doi: 10.1111/1365-2664.12740

Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:. doi: 10.18637/jss.v067.i01

Begg GS, Cook SM, Dye R, et al (2017) A functional overview of conservation biological control. Crop Prot 97:. doi: 10.1016/j.cropro.2016.11.008

Bianchi FJJA, Booij CJH, Tscharntke T (2006) Sustainable pest regulation in agricultural landscapes : a review on landscape composition , biodiversity and natural pest control. Proc R Soc B Biol Sci 273:1715–1727. doi: 10.1098/rspb.2006.3530

Branco M (2007) Agentes bióticos do eucalipto em Portugal. In: O Eucaliptal em Portugal–Impactes Ambientais e Investigação Científica. pp 255–282

Brennan EB, Weinbaum SA (2001) Stylet penetration and survival of three psyllid species on adult leaves and "waxy" and "de-waxed" juvenile leaves of *Eucalyptus globulus*. Entomol Exp Appl. doi: 10.1023/A:1019201412579

Brennan EB, Weinbaum SA, Rosenheim JA, Karban R (2001) Heteroblasty in *Eucalyptus globulus* (Myricales: Myricaceae) affects ovipositonal and settling preferences of *Ctenarytaina eucalypti* and *C. spatulata* (homoptera: Psyllidae). Environ Entomol 30:1144–1149. doi: 10.1603/0046-225X-30.6.1144

Calviño-cancela M (2013) Forest Ecology and Management Effectiveness of eucalypt

plantations as a surrogate habitat for birds. For Ecol Manage 310:692–699. doi: 10.1016/j.foreco.2013.09.014

Catry P, Costa H, Matias G, Rafael E (2010) Aves de Portugal: Ornitologia do território continental. Assírio & Alvim, Lisboa. Pp., 944

Ceballos G, Ehrlich PR, Barnosky AD, et al (2015) Accelerated modern human-induced species losses: Entering the sixth mass extinction. Sci Adv 1:9–13. doi: 10.1126/sciadv.1400253

CELPA (2017) Boletim estatistico 2017

CEPI (2017) Key Statistics European Pulp and Paper Industry

Charbonnier Y, Gaüzère P, van Halder I, et al (2016) Deciduous trees increase bat diversity at stand and landscape scales in mosaic pine plantations. Landsc Ecol 31(2):291–300. doi: 10.1007/s10980-015-0242-0

Cleveland CJ, Betke M, Federico P, et al (2006) Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. Front Ecol Environ 4(5):238–243

Costanza R, J RA, Groot R De, et al (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260

Cruz J, Sarmento P, Rydevik G, et al (2016) Bats like vintage: Managing exotic eucalypt plantations for bat conservation in a Mediterranean landscape. Anim Conserv 19:53–64. doi: 10.1111/acv.12216

da Silva LP, Heleno RH, Costa JM, et al (2019a) Natural woodlands hold more diverse, abundant, and unique biota than novel anthropogenic forests: a multi-group assessment. Eur J For Res 138:461–472. doi: 10.1007/s10342-019-01183-5

da Silva LP, Mata VA, Lopes PB, et al (2019b) Advancing the integration of multi-marker metabarcoding data in dietary analysis of trophic generalists. Mol Ecol Resour 19:1420–1432. doi: 10.1111/1755-0998.13060

DeBach P (1964) Biological control of insect pests and weeds. Chapman & Hall Ltd., London

Ehler LE (1998) Conservation biological control: past, present, and future. In: Press A (ed) Conservation biological control. pp 1–8

Elbrecht V, Leese F, Nichols SJ (2017) Validation and Development of COI

Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment. Front Environ Sci 5:1–11. doi: 10.3389/fenvs.2017.00011

FAO (2010) Planted forests in sustainable forest management: a statement of principles. Rome

FAO (2020) Global Forest Resources Assessment 2020 - Key findings. Rome

Fayt P, Machmer MM, Steeger C (2005) Regulation of spruce bark beetles by woodpeckers — a literature review. For Ecol Manage 206:1–14. doi: 10.1016/j.foreco.2004.10.054

Folmer O, Black M, Wr H, et al (1994) DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3(5), 294-:

Forbes RJ, Watson SJ, Connor EO, et al (2019) Diversity and abundance of Lepidoptera and Coleoptera in multiple-species reforestation plantings to offset emissions of carbon dioxide. Aust For 82:89–106. doi: 10.1080/00049158.2019.1622833

Fox J, Weisberg S (2011) An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Garcia EF (1981) An experimental and observational study of interspecific territoriality between the Blackcap *Sylvia atricapilla* (Linnaeus) and the Garden Warbler *Sylvia borin* (Boddaert)

Garfinkel MB, Minor ES, Whelan CJ (2020) Birds suppress pests in corn but release them in soybean crops within a mixed prairie / agriculture system. Condor 122:1–12. doi: 10.1093/condor/duaa009

Ghanem SJ, Voigt CC (2012) Increasing Awareness of Ecosystem Services Provided by Bats, 1st edn. Elsevier Inc.

Goded S, Ekroos J, Domínguez J, et al (2019) Effects of eucalyptus plantations on avian and herb species richness and composition in North-West Spain. Glob Ecol Conserv 19:. doi: 10.1016/j.gecco.2019.e00690

Gurr GM, You M (2016) Conservation biological control of pests in the molecular era: New opportunities to address old constraints. Front Plant Sci 6:1–9. doi: 10.3389/fpls.2015.01255

Hill JE, DeVault TL, Wang G, Belant JL (2020) Anthropogenic mortality in mammals increases with the human footprint. Front Ecol Environ 18:13–18. doi: 10.1002/fee.2127

Hoddle MS, Driesche RG Van (2000) Biological Control of Insect Pests, Second Edi. Elsevier Inc.

Hodkinson ID (1999) A Biocontrol of eucalyptus psyllid *Ctenarytaina eucalypti* by the Australian parasitoid *Psyllaephagus pilosus*: a review of current programmes and their success. Biocontrol News Inf 20:129–134

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346–363. doi: 10.1002/bimj.200810425

ICNF (2019) 6.º inventário florestal nacional. ICNF Lisbon

ICNF IP, DGAV, CELPA, INIAV IP (2015) Manual de boas práticas Gorgulho-do-eucalipto (*Gonipterus platensis*)

Kahnonitch I, Lubin Y, Korine C (2018) Insectivorous bats in semi-arid agroecosystems − effects on foraging activity and implications for insect pest control. Agric Ecosyst Environ 261:80–92. doi: 10.1016/j.agee.2017.11.003

Kambach S, Ku I, Castagneyrol B, Bruelheide H (2016) The Impact of Tree Diversity on Different Aspects of Insect Herbivory along a Global Temperature Gradient - A Meta-Analysis. PLoS One 11:1–14. doi: 10.1371/journal.pone.0165815

Karabörklü S, Azizoglu U, Azizoglu ZB (2018) Recombinant entomopathogenic agents: a review of biotechnological approaches to pest insect control. World J Microbiol Biotechnol 34:1–12. doi: 10.1007/s11274-017-2397-0

Kunz TH, de Torrez EB, Bauer D, et al (2011) Ecosystem services provided by bats. Europe, 31, 32. doi: 10.1111/j.1749-6632.2011.06004.x

Kurylo CL, Garcia MS, Costa VA, et al (2010) Ocorrência de *Ctenarytaina eucalypti* (Maskell) (Hemiptera: Psyllidae) e seu Inimigo Natural *Psyllaephagus pilosus* Noyes (Hymenoptera: Encyrtidae) em *Eucalyptus globulus* no Rio Grande do Sul. Neotrop Entomol 39:671–673. doi: 10.1590/S1519-566X2010000400031

Laclau JP, Gonçalves JL de M, Stape JL (2013) Perspectives for the management of eucalypt plantations under biotic and abiotic stresses. For Ecol Manage 301:1–5. doi: 10.1016/j.foreco.2013.03.007

Lee JC, Heimpel GE, Leibee GL (2004) Comparing floral nectar and aphid honeydew

diets on the longevity and nutrient levels of a parasitoid wasp. Entomol Exp Appl 111:189–199. doi: 10.1111/j.0013-8703.2004.00165.x

Lenteren JC Van (2012) The state of commercial augmentative biological control : plenty of natural enemies , but a frustrating lack of uptake. BioControl 57:1–20. doi: 10.1007/s10526-011-9395-1

Lenteren JC Van, Bueno VHP (2003) Augmentative biological control of arthropods in Latin America. BioControl 48:123–139

Lenteren JC Van, Godfray HCJ (2005) European science in the Enlightenment and the discovery of the insect parasitoid life cycle in The Netherlands and Great Britain. Biol Control 32(1):12–24. doi: 10.1016/j.biocontrol.2004.08.009

Lindell C, Eaton RA, Howard PH, et al (2018) Enhancing agricultural landscapes to increase crop pest reduction by vertebrates. Agric Ecosyst Environ 257:1–11. doi: 10.1016/j.agee.2018.01.028

Loyn RH, Runnalls RG, Forward GY, Tyers J (1983) Territorial bell miners and other birds affecting populations of insect prey. Science (80-) 221(4618):1411–1413

Maas B, Heath S, Grass I, et al (2020) Methodological insights , potential improvements , and cost-benefit trade- Experimental fi eld exclosure of birds and bats in agricultural systems — Methodological insights , potential improvements , and cost-bene fi t trade-offs. Basic Appl Ecol 35:1–12. doi: 10.1016/j.baae.2018.12.002

Maas B, Karp DS, Bumrungsri S, et al (2016) Bird and bat predation services in tropical forests and agroforestry landscapes. Biol Rev 43:1081–1101. doi: 10.1111/brv.12211

Maine JJ, Boyles JG (2015) Bats initiate vital agroecological interactions in corn. Proc Natl Acad Sci U S A 112:12438–12443. doi: 10.1073/pnas.1505413112

Marques JT, Rainho A, Carapuço M, et al (2004) Foraging Behaviour and Habitat use by the European Free-Tailed Bat *Tadarida teniotis*. Acta Chiropterologica 6:99–110. doi: 10.3161/001.006.0108

McFarlane GR, Whitelaw CBA, Lillico SG (2018) CRISPR-Based Gene Drives for Pest Control. Trends Biotechnol 36:130–133. doi: 10.1016/j.tibtech.2017.10.001

Moazami N (2011) Biological Control. In: Comprehensive Biotechnology (Second Edition). Elsevier B.V., pp 731–739

Mooney KA, Gruner DS, Barber NA, et al (2010) Interactions among predators and the

cascading effects of vertebrate insectivores on arthropod communities and plants. Proc Natl Acad Sci 107:7335–7340. doi: 10.1073/pnas.1001934107

Mooney KA, Linhart YANB (2006) Contrasting cascades : insectivorous birds increase pine but not parasitic mistletoe growth. J Anim Ecol 75:350–357. doi: 10.1111/j.1365-2656.2006.01054.x

Morganti M, Assandri G, Aguirre JI, et al (2017) How residents behave : home range fl exibility and dominance over migrants in a Mediterranean passerine. Anim Behav 123:293–304. doi: 10.1016/j.anbehav.2016.10.021

Mostafa DAA (2017) Space use strategies of two Palaearctic migrants on the wintering grounds

Nielsen JM, Clare EL, Hayden B, et al (2018) Diet tracing in ecology: Method comparison and selection. Methods Ecol Evol 9:278–291. doi: 10.1111/2041-210X.12869

Paine TD, Dahlsten DL, Millar JG, et al (2000) UC scientists apply IPM techniques to new eucalyptus pests. Calif Agric. doi: 10.3733/ca.v054n06p8

Paine TD, Steinbauer MJ, Lawson SA (2011) Native and Exotic Pests of Eucalyptus : A Worldwide Perspective. Annu Rev Entomol 56:181-201:. doi: 10.1146/annurev-ento-120709-144817

Perovic DJ, Gurr GM, Raman A, Nicol HI (2010) Effect of landscape composition and arrangement on biological control agents in a simplified agricultural system : A cost – distance approach. Biol Control 52:263–270. doi: 10.1016/j.biocontrol.2009.09.014

Pimentel D (2005) Environmental and Economic Costs of the Application of Pesticides Primarily in the United States. Environ Dev Sustain 7:229–252. doi: 10.1007/978-94-007-7796-5

Pimentel, D., & Burgess, M. (2014). Environmental and economic costs of the application of pesticides primarily in the United States. In *Integrated pest management* (pp. 47-71). Springer, Dordrecht.

Pina JP (1989) Breeding bird assemblages in eucalyptus plantations in Portugal. Ann Zool Fennici 26:287–290

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. In: A Lang. Environ. Stat. Comput. Found. Stat. Comput. Vienna, Austria. https://www.r-project.org/.

Rainho A (2009) Summer foraging habitats in a Mediterranean region of the Iberian Peninsula. Acta Chiropterologica 9:171–181. doi: 10.3161/1733-5329(2007)9[171:SFHOBI]2.0.CO;2

Rainho A, Alves P, Amorim F, et al (2013) Atlas dos Morcegos de Portugal Continental

Rainho A, Palmeirim JM (2011) The Importance of Distance to Resources in the Spatial Modelling of Bat The Importance of Distance to Resources in the Spatial Modelling of Bat Foraging Habitat. PLoS One 6(4):e1922: doi: 10.1371/journal.pone.0019227

Reis AR, Ferreira L, Tomé M, et al (2012) Efficiency of biological control of *Gonipterus platensis* (Coleoptera: Curculionidae) by *Anaphes nitens* (Hymenoptera: Mymaridae) in cold areas of the Iberian Peninsula: Implications for defoliation and wood production in *Eucalyptus globulus*. For Ecol Manage. doi: 10.1016/j.foreco.2012.01.038

Rodríguez-San Pedro A, Allendes JL, Beltrán CA, et al (2020) Quantifying ecological and economic value of pest control services provided by bats in a vineyard landscape of central Chile. Agric Ecosyst Environ 302:107063. doi: 10.1016/j.agee.2020.107063

Romeis J, Driesche RG Van, Barratt BIP, Bigler F (2008) Insect-resistant transgenic crops and biological control. Springer, Dordrecht, 2008

Romeis J, Naranjo SE, Meissle M, Shelton AM (2019) Genetically engineered crops help support conservation biological control. Biol Control 130:136–154. doi: 10.1016/j.biocontrol.2018.10.001

Rusch A, Valantin-morison M, Sarthou J, Roger-estrade J (2010) Biological Control of Insect Pests in Agroecosystems : Effects of Crop Management , Farming Systems , and Seminatural Habitats at the Landscape Scale : A Review. Elsevier Ltd

Santana DL de Q (2000) Flutuação populacional de *Ctenarytaina spatulata* em Eucalyptus grandis no Município de Colombo, PR. Embrapa Florestas

Santolamazza-Carbone S, Pestaña Nieto M, Pérez Otero R, et al (2009) Winter and spring ecology of *Anaphes nitens*, a solitary egg-parasitoid of the Eucalyptus snout-beetle *Gonipterus scutellatus*. BioControl 54:195–209. doi: 10.1007/s10526-008-9184-7

Santolamazza-Carbone S, Rodríguez-Illamola A, Rivera AC (2004) Host finding and host discrimination ability in *Anaphes nitens* Girault, an egg parasitoid of the Eucalyptus snout-beetle *Gonipterus scutellatus* Gyllenhal. Biol Control 29:24–33. doi:

10.1016/S1049-9644(03)00134-8

Santos GP, Zanuncio JC, Zanuncio TV, Pires EM (2008) Pragas do eucalipto. Inf Agropecuário, Belo Horiz 29:47–70

Sharma A, Raman A, Taylor GS, et al (2015) Bionomics and feeding impact of *Ctenarytaina eucalypti* (Hemiptera: Psylloidea: Aphalaridae) on *Eucalyptus globulus* (Myrtaceae) in the central tablelands of New South Wales. Austral Entomol 54:159–171. doi: 10.1111/aen.12103

Shelton AM, Long SJ, Walker AS, et al (2020) First Field Release of a Genetically Engineered, Self-Limiting Agricultural Pest Insect: Evaluating Its Potential for Future Crop Protection. Front Bioeng Biotechnol 7:1–15. doi: 10.3389/fbioe.2019.00482

Sheppard SK, Bell J, Sunderland KD, et al (2005) Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Mol Ecol 14:4461–4468. doi: 10.1111/j.1365-294X.2005.02742.x

Shokralla S, Porter TM, Gibson JF, et al (2015) Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. Sci Rep 5: 9687: doi: 10.1038/srep09687

Silva LP da, Alves J, Alves A, et al (2012) Variation in the Abundance and Reproductive Characteristics of Great Tits Parus major in Forest and Monoculture Plantations Variation in the abundance and reproductive characteristics of Great Tits Parus major in forest and monoculture plantations. Acta Ornithol 47:146–154. doi: 10.3161/000164512X662250

Silva LP, Heleno RH, Costa JM, et al (2019) Natural woodlands hold more diverse , abundant , and unique biota than novel anthropogenic forests : a multi - group assessment. Eur J For Res 138:461–472. doi: 10.1007/s10342-019-01183-5

Snow DW, Perrins CM, Hillcoat B, et al (1998) The Birds of the Western Palearctic. Press, Oxford University

Soloneski S, Larramendy M (2012) Integrated Pest Management and Pest Control: Current and Future Tactics., (Eds.). BoD-Books on Demand

Taylor PJ, Grass I, Alberts AJ, et al (2018) Economic value of bat predation services – A review and new estimates from macadamia orchards. Ecosyst Serv 30:372–381. doi: 10.1016/j.ecoser.2017.11.015

Tremlett CJ, Moore M, Chapman MA, et al (2020) Pollination by bats enhances both

quality and yield of a major cash crop in Mexico. J Appl Ecol 57:450–459. doi: 10.1111/1365-2664.13545

Valente C, Gonçalves CI, Reis A, Branco M (2017) Pre-selection and biological potential of the egg parasitoid *Anaphes inexpectatus* for the control of the Eucalyptus snout beetle , *Gonipterus platensis*. J Pest Sci (2004) 90:911–923. doi: 10.1007/s10340-017-0839-y

Valente C, Manta A, Vaz A (2004) First record of the Australian psyllid *Ctenarytaina spatulata* Taylor (Homoptera : Psyllidae) in Europe. J Appl Entomol 128:369–370. doi: 10.1111/j.1439-0418.2004.00860.369

Walker FM, Williamson CHD, Sanchez DE, Sobek CJ, Chambers CL (2016) Species From Feces: Order-Wide Identification of Chiroptera From Guano and Other Non-Invasive Genetic Samples. PLOS ONE 11(9): e0162342. https://doi.org/10.1371/journal.pone.0162342

Wanger TC, Darras K, Bumrungsri S, et al (2014) Bat pest control contributes to food security in Thailand. Biol Conserv 171:220–223. doi: 10.1016/j.biocon.2014.01.030

Watson R, Baste I, Larigauderie A, et al (2019) Summary for policymakers of the global assessment report on biodiversity and ecosystem services–unedited advance version

Whelan CJ, Wenny DG, Marquis RJ, Louis S (2008) Ecosystem Services Provided by Birds. Ann N Y Acad Sci 1134(1):25–60. doi: 10.1196/annals.1439.003

Whitaker P (1999) Enhancing biological control: habitat management to promote enemies of crop pests. Am J Altern Agric 14:94–95. doi: 10.1017/S0889189300008158

Wilman H, J. B, J. S, et al (2014) EltonTraits 1 . 0 : Species-level foraging attributes of the world ' s birds and mammals. Ecology 95:2027

Wittwer T, Hara RBO, Caplat P, et al (2015) Long-term population dynamics of a migrant bird suggests interaction of climate change and competition with resident species. Oikos 124(9):1151–1159. doi: 10.1111/oik.01559

Annexes

Annex 1: Table of each captured bird species with the respective phenology, diet, foraging guild, and body mass (g; presented in range since the weight during the different months varies).

Species	Phenology	Diet	Foraging Guild	Body mass
Aegithalos caudatus	resident	insectivore	canopy gleaner	6,9 to 8
Anthus trivialis	migratory	insectivore	ground gleaner	19,4 to 22,4
Caprimulgus europaeus	migratory	insectivore	hawker	62,3
Certhia brachydactyla	resident	insectivore	bark forager	7,5 to 9,2
Cyanistes caeruleus	resident	insectivore	canopy gleaner	8,8 to 10,7
Dendrocopos major	resident	insectivore	bark forager	69,6
Emberiza cia	resident	insectivore	ground gleaner	20,5 to 22,3
Emberiza cirlus	resident	insectivore	ground gleaner	22,9
Erithacus rubecula	resident	insectivore	understorey gleaner	13,8 to 19,5
Ficedula hypoleuca	migratory	insectivore	hawker	11,1 to 16,4
Fringilla coelebs	resident	insectivore	canopy gleaner	16,7 to 22,1
Garrulus glandarius	resident	other	canopy gleaner	162,8 to 180
Hippolais polyglotta	migratory	insectivore	understorey gleaner	12,8
Locustella naevia	migratory	insectivore	understorey gleaner	18,2
Lophophanes cristatus	resident	insectivore	canopy gleaner	9,4 to 10,9
Muscicapa striata	migratory	insectivore	hawker	16,9
Parus major	resident	insectivore	canopy gleaner	14,4 to 20,3
Periparus ater	resident	insectivore	canopy gleaner	7,1 to 8,3
Phoenicurus ochruros	resident	insectivore	hawker	14,7 to 17
Phylloscopus trochilus	migratory	insectivore	canopy gleaner	6,4 to 12,2
Regulus ignicapilla	resident	insectivore	canopy gleaner	4,7 to 6
Serinus serinus	resident	other	ground gleaner	10,4 to 11
Sitta europaea	resident	insectivore	bark forager	19
Sylvia atricapilla	resident	other	canopy gleaner	13,7 to 18,2
Sylvia communis	migratory	other	understorey gleaner	20, 3
Troglodytes troglodytes	resident	insectivore	understorey gleaner	7,5 to 10,8
Turdus merula	resident	other	ground prober	71,6 to 101,6
Turdus philomelos	resident	other	ground prober	69
Turdus viscivorus	resident	other	ground prober	105,1 to 112

Annex 2: Predation rates (%) of the pests and weevil's parasitoid by all bird species sampled. Sample size of each group is indicated within parenthesis.
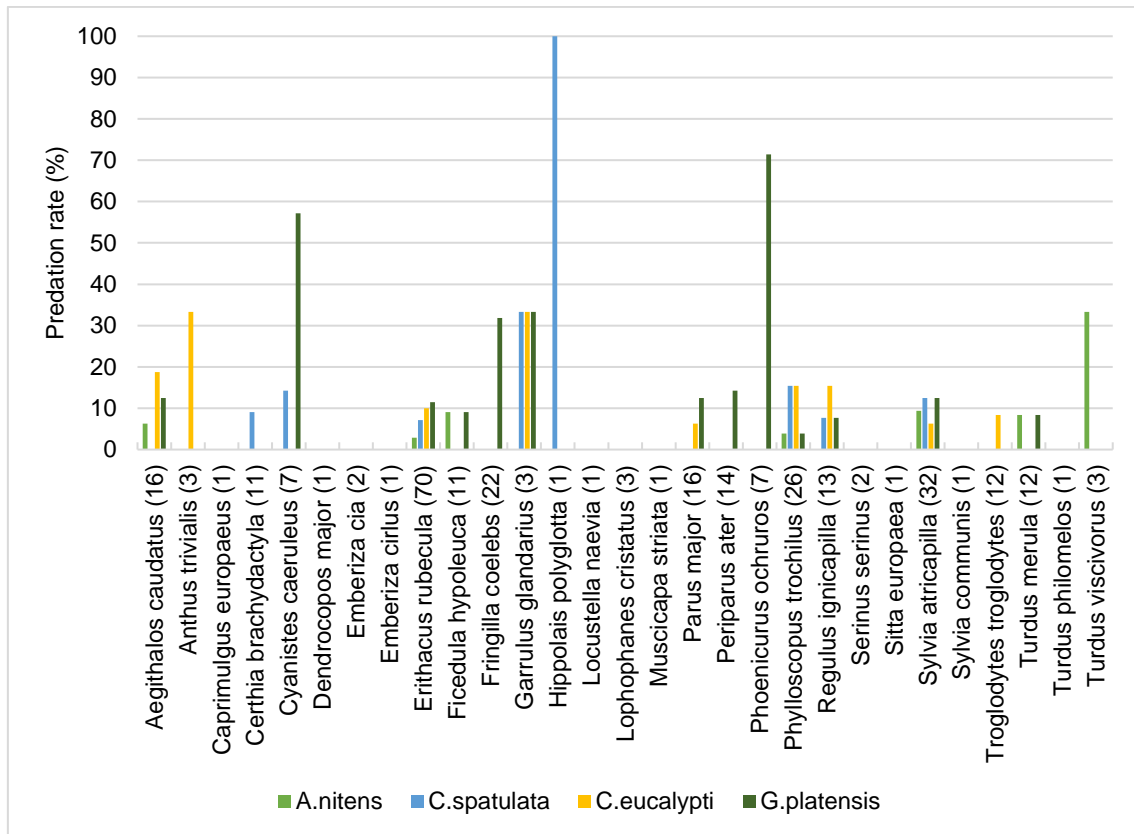
__Annex 3:__ Table of the adjusted p-values using the single-step method for multiple comparisons for the statistical significant variables from the Generalized Linear Models of the predation of the study pest species.

Pest species	Variable	Interaction	Estimate Std.	Error t	z value	Pr(>|z|)
A. nitens	Diet	Other - Insectivore	1.8876	0.9201	2.051	*0.0402*
C.spatulata	Phenology	Resident - Migratory	-1.741	0.666	-2.614	**0.00895**
C.spatulata	Foraging guild	Canopy gleaner - Bark forager	-0.9543	1.1673	-0.818	0.947
		Ground gleaner - Bark forager	-18.2831	3846.9435	-0.005	1.00000
		Ground prober - Bark forager	-19.3261	3092.4639	-0.006	1.00000
		Hawker - Bark forager	-18.9007	2928.3604	-0.006	1.00000
		Understorey gleaner - Bark forager	-0.2501	1.1373	-0.220	1.00000
		Ground gleaner - Canopy gleaner	-17.3287	3846.9433	-0.005	1.00000
		Ground prober - Canopy gleaner	-18.3718	3092.4637	-0.006	1.00000
		Hawker - Canopy gleaner	-17.9464	2928.3602	-0.006	1.00000
		Understorey gleaner - Canopy gleaner	0.7042	0.6332	1.112	0.828
		Ground prober - Ground gleaner	-1.0431	4935.8185	0.000	1.00000
		Hawker - Ground gleaner	-0.6176	4834.6940	0.000	1.00000
		Understorey gleaner - Ground gleaner	18.0330	3846.9434	0.005	1.00000
		Hawker - Ground prober	0.4254	4258.9465	0.000	1.00000
		Understorey gleaner - Ground prober	19.0760	3092.4637	0.006	1.00000
		Understorey gleaner - Hawker	18.6506	2928.3602	0.006	1.00000
G.platensis	Phenology	Resident - Migratory	3.282	1.013	3.239	**0.0012**
G.platensis	Foraging guild	Canopy gleaner - Bark forager	17.4246	1991.2512	0.009	1.00000
		Ground gleaner - Bark forager	0.5466	3014.8223	0.000	1.00000
		Ground prober - Bark forager	15.4913	1991.2516	0.008	1.00000
		Hawker - Bark forager	19.6530	1991.2513	0.010	1.00000
		Understorey gleaner - Bark forager	16.7360	1991.2512	0.008	1.00000
		Ground gleaner - Canopy gleaner	-16.8780	2263.6414	-0.007	1.00000
		Ground prober - Canopy gleaner	-1.9333	1.2712	-1.521	0.55756
		Hawker - Canopy gleaner	2.2284	0.7899	2.821	**0.03338**
		Understorey gleaner - Canopy gleaner	-0.6887	0.4732	-1.455	0.60409
		Ground prober - Ground gleaner	14.9447	2263.6417	0.007	1.00000
		Hawker - Ground gleaner	19.1064	2263.6415	0.008	1.00000
		Understorey gleaner - Ground gleaner	16.1893	2263.6414	0.007	1.00000
		Hawker - Ground prober	4.1617	1.4898	2.793	**0.03625**
		Understorey gleaner - Ground prober	1.2447	1.3501	0.922	0.91239
		Understorey gleaner - Hawker	-2.9170	0.8304	-3.513	**0.00337**