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# Pest control services provided by bats in vineyard landscapes

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ABSTRACT

Faced with current health and environmental challenges, viticulture is directly concerned with the need to reduce pesticide use. Natural pest control services provided by bats have been demonstrated in other crops and is regularly mentioned as a way to reduce pesticide use. However, the trophic link between bats and grape pests as well as the effect of pest presence on bat activities remain largely unknown. To investigate the functional role of bats in vineyard landscapes, we used two independent approaches. We monitored the activities of bats and of the European grapevine moth (*Lobesia botrana*) in 23 vineyards located in the Bordeaux region (France). In parallel, we developed DNA primers to examine bat faeces from two regions, Bordeaux and Burgundy, for the presence of the three main species of grapevine moths. Our results demonstrate that bats significantly increase their hunting activity when European grapevine moths are present in vineyards. In addition, our molecular analysis of the faeces provides robust evidence that at least 10 species of bats predate the three grapevine moth species. Our results therefore suggest that bats can be natural enemies of grape pests in vineyard landscapes. Further research is now needed to investigate the consequences of predation of pests by bats on crop production as well as the effect of some management options at both the local and landscape scale to increase the level of pest control services provided by bats.

## 1. Introduction

Intensive agriculture, characterized by the homogenization of agricultural landscapes and the use of agrochemicals, has strong negative impacts on the environment and on biodiversity in particular (Tscharntke et al., 2005; Geiger et al., 2010; Tilman et al., 2011). These negative impacts strongly limit the sustainability of farming systems and highlight the need to develop more environmentally friendly agriculture. Ecological intensification, based on enhancing ecological processes to partially replace the use of agrochemicals, offers a promising way to conciliate crop productivity and a low environmental footprint (Bommarco et al., 2013; Kleijn et al., 2019).

Natural pest control is a critical ecosystem service in agricultural landscapes. Global yield losses due to pests are estimated to 30 %–40 % with the highest losses in food-deficit regions with fast-growing populations (Oerke, 2006; Savary et al., 2019). In a large majority of crops and regions across the globe, the management of pest populations is highly dependent on the use of synthetic pesticides. However, in addition to the adverse effect of pesticides on the environment, pesticide use has also promoted pest resistance and created secondary emergence of pests and diseases (Pimentel et al., 2012). In the present context, strengthening natural pest control services in agricultural landscapes therefore appears to be a promising way to reduce the level of pesticide use. Indeed, natural pest control provided by a large variety of vertebrate and invertebrate taxa, such as parasitoid wasps, predatory mites, beetles, spiders, bats or birds, can significantly contribute to agricultural production. Recent work showed that increasing the abundance or species richness of natural enemies generally increases pest control services and reduces yield losses (Letourneau et al., 2009; Dainese et al., 2019). However, several studies found that pest control services do not always benefit from greater abundance or more species-rich predator communities, suggesting negative interactions (e.g., intraguild predation) between predators that limit pest control services (Letourneau et al., 2009). Knowledge about predators' diets and behaviour is

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therefore needed to better understand the relationships between natural enemy communities and the level of pest control services.

Among all potential natural enemies contributing to limit the development of agricultural pests, bats (Mammalia, Chiroptera) are often suggested as very efficient predators that limit crop damage and yield losses due to insect pests (McCracken et al., 2012; Maas et al., 2013; Wanger et al., 2014). However, most studies examining bat activities on insect pests come from tropical ecosystems, so knowledge about trophic interactions between bats and insect pests in European agricultural landscapes remains scarce. Analysing the contribution of bats to the control of insect pest populations and pesticide use reduction in agricultural landscapes is therefore a major issue in this context (Kunz et al., 2011; Boyles et al., 2011; Maas et al., 2013; Charbonnier et al., 2014; Puig-Montserrat et al., 2015; Lee and Mccracken, 2005).

Studies focused on the relationship between bats and agricultural pests use two approaches: a direct approach based on amplification of pest DNA found in bat faeces and an indirect correlative approach based on joint analyses of bats and pest activities (Clare et al., 2009; McCracken et al., 2012; Hope et al., 2014; Puig-Montserrat et al., 2015). The first approach provides evidence about the ability of bats to consume pests (Lee and McCracken, 2005; McCracken et al., 2012; Hope et al., 2014), without demonstrating the link between bat activities and pest population dynamics. The second approach provides correlative evidence about the trophic link but does not exclude confounding effects related to the presence of other types of prey or similar responses of bats and their potential prey to environmental changes (Sentenac and Rusch, 2017). Moreover, very few studies have jointly examined the activity of bats, their diet and pest population dynamics (Puig-Montserrat et al., 2015). Here, we present a study combining both approaches to investigate predation of targeted pest species while simultaneously measuring bat activity and the local dynamics of pests in grapevine agroecosystems.

Currently, vineyards are submitted to very high levels of pesticides (Muneret et al., 2018) and insect pests are mainly controlled by synthetic insecticides (Thiéry et al., 2018). The hunting techniques and the insectivorous diet of European bats make them potentially good natural enemies able to limit the dynamics of some nocturnal pests (Boyles et al., 2011). Among grapevine insect pests, three species of moths (Lepidoptera: Tortricidae) are major ones that can cause important damage and are potential prey of bats. These species (*Lobesia botrana*, *Eupoecilia ambiguella*, *Sparghanotis pilleriana*), are among the major pests of grapevine on the global scale and often cause high levels of damage to grape bunches (Thiéry, 2011). In heavily attacked vineyards, it is possible to have up to 10–30 larvae of *Lobesia botrana* per bunch leading to the complete destruction of the bunch depending on the cultivar (Fermaud et al., 2016). In addition, the perforation of the berries linked to the presence of larvae promotes the development of bunch rot, thus causing serious qualitative and quantitative damage (Delbac and Thiéry, 2016).

In this study, we combined an observational approach to monitor bats and pest activities with molecular analyses of bat faeces to investigate the trophic link between bats and the three main grapevine moths *L. botrana*, *E. ambiguella* and *S. pilleriana*. We particularly examined how bat communities respond to the presence of grape berry moths (*L. botrana*) and investigated if the three main species of grapevine moths were part of the diet of bats.

## 2. Material and methods

### 2.1. Study system for monitoring bat and grape berry moth (L. botrana) activities

We carried out our observational study in the Bordeaux area in southwestern France. We monitored bat and grape berry moth (*L. botrana*) activities in 23 vineyards distributed in wine regions that differ in terms of management, historical pest infestation levels and bat activities. The 23 vineyards plots, with an average area of 1.04 ha (SE = 0.11), were distributed over the following wine regions: Medoc, Saint-Emilion, Pessac-Léognan, Côtes de Bourg and Côtes de Bordeaux (Fig. 1).

#### 2.1.1. Bat sampling

Bat activity was measured from 11 May to 04 October 2017, which corresponds to bat seasonal peak of activity from gestation period to individual dispersion (Dietz et al., 2009). We measured bat activity when weather conditions were favourable (i.e. no rain, low wind speed (<7 m/s) and temperatures higher than 12 °C) using automatic ultrasound bat detector systems (Sound Meter SM2Bat, Wildlife Acoustics) fitted with multidirectional microphones. Timers were set up to record bat activities from 30 min before sunset and for 4 h afterwards. We repeated this sampling scheme in each of the 23 vineyards every 15 days, which represents 11 sampling times per plot. To avoid any confounding effects, bat detectors were located on a row of vines in the center of each plot and separated from any landscape elements known to affect bat activity, such as hedgerows, ponds, dwellings or lamp posts by at least 80 m (Froidevaux et al., 2017). We used the SonoChiro© software (Bas et al., 2013) to automatically classify the echolocation calls to the most accurate taxonomic level possible, and each file was then checked by trained operators with dedicated software (Batsound 4.1). In cases where the echolocation call could not be assigned to a specific species, we created groups of acoustically similar species. In this way, we grouped *Pipistrellus kuhlii/nathusii*, *Eptesicus serotinus* and *Nyctalus lesleri* in a nyctaloids group, *Plecotus auritus* and *Plecotus austriacus* in *Plecotus* spp and all *Myotis* species in *Myotis* spp. Total bat activity per plot and per night was determined by the number of bat passes. A bat pass was defined as the occurrence of three or more echolocation calls from a same bat species during a 5-second interval (Jung et al., 2012). Additionally, we assessed bat feeding activity by counting the number of characteristic echolocation call sequences (final buzz) that indicate active prey capture attempts. This foraging activity was highly correlated with total bat activity (Pearson's correlation = 0.7998; P-Val<2.2e-16) and was therefore disregarded for the analysis.

#### 2.1.2. Pest monitoring

Three grapevine moth species are usually found in the French vineyards: the European grapevine moth *L. botrana*, the grape berry moth *E. ambiguella* and the pyralid moth *S. pilleriana*. The first two species are polyvoltine and complete two to three generations per year, and the third species is univoltine. Although the larvae are polyphagous, *Vitis vinifera* is their main host in areas dominated by vineyards (Thiéry, 2008). These species mainly fly at dusk between 21:00 and 23:00, which make them potential prey for bats, which usually forage within the same time slot (Lucchi et al., 2018).

The field survey was conducted from the 10 May to 5 October 2017 corresponding to the end of the first peak until the end of the third peak of *L. botrana* emergence. We used synthetic sex pheromone trapping that catch males to estimate the phenology of *L. botrana* moths within the season (Ioriatti et al., 2011). Each trap consisted in a yellow delta-trap baited with 2 µg of the synthetic pheromone of *L. botrana* female, E7-Z9 DDA. Adult moths were trapped continuously during the study and counted every 10 days using one trap per plot located in the center of it close to the automatic recording bat-detector. Because no established statistical relationship exists between the number of males trapped by pheromonal traps and the larval pest population and because data from pheromone traps can be highly affected by surrounding landscape context (e.g., other pheromone trapping in the surrounding) or by weather conditions, we only used these data to estimate the presence or absence of *L. botrana* moths in the plots and not the absolute abundance. For each bat survey we assigned the presence of *L. botrana* based on the trap results for each 10 days period. This presence/absence per plot was then used as an explanatory variable in our models.

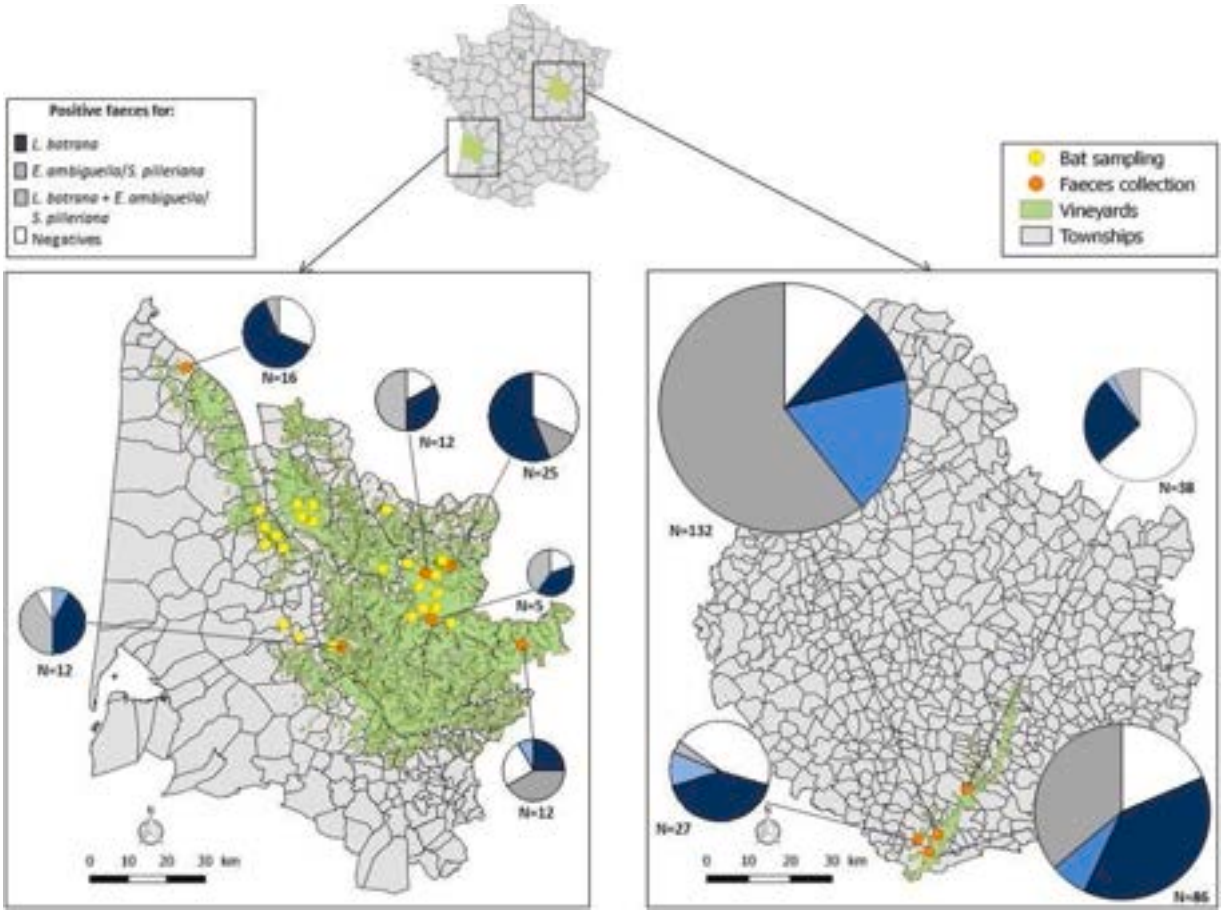


**Fig. 1.** Spatial distribution of the sampling sites for bat monitoring (southwest France) and faeces collection (southwest and northeast France). The pie charts represent the proportion of bat faeces detected positive for i) *Lobesia botrana, ii) Eupoecilia ambiguella* and/or *Sparganothis pilleriana* and iii) all three grapevine moths species (*L. botrana, E. ambiguella* and *S. pilleriana)*. N values represent the total amount of faeces tested per sampling site.

#### 2.1.3. Statistical analysis

We investigated how the presence of *L. botrana* moths affected bat activity using Generalized Linear Mixed Models (GLMM) with a Poisson distribution. We fitted a model for total bat activity (corresponding to the sum of passes per survey for each species) and then separated models for each bat species. We used the presence or absence of the *L. botrana* grape berry moths as a binary explanatory variable. In each model, we added the sampling date and plot as crossed random effects because we monitored several plots on the same date and each plot was monitored several times during our study. An observation effect was added in some models to correct for overdispersion. All analyses were performed using the R software (R Core Team, 2016) and the lme4 package was used to fit the GLMM (Bates et al., 2015). Diagnostic residual plots of all full models were confirmed using the DHARMa package (Hartig, 2019) and we explored potential spatial autocorrelation in the residuals using bubble plots and variograms and no spatial autocorrelation were detected (Zuur et al., 2009).

### 2.2. Laboratory bat diet experiment

#### 2.2.1. Bat faeces collection and DNA extraction

To investigate whether the three species of moths are part of the bat diet, we collected bat faeces from colonies in several locations in two winegrowing regions: Bordeaux and Burgundy. We decided to include two regions that differ in their environmental conditions and abundance of the three pest species to assess the proportion of these species in the bat diet. We collected 82 faeces samples from six locations in the Bordeaux area and 283 faeces samples from four sites in Burgundy (Fig. 1). The sampling was done between 2015 and 2019 during the flight period of the grapevine moths. Faeces were collected once a year under sterile conditions using material for collection (pliers, tubes and paper sheets). For the Bordeaux region, the sampling sites were located close to vineyards during moth activity periods. The faeces were collected 12 h after cleaning the soil surface and setting the paper sheets on the ground and immediately stored in 96 % ethanol, at −80 °C to preserve DNA prior to analysis.

#### 2.2.2. Primer design

Mitochondrial DNA-specific primers were developed to amplify short DNA fragments of the three grapevine moths (*L. botrana*, *E. ambiguella* and *S. pilleriana*) from bat faeces. First, a 658-bp fragment of cytochrome oxidase 1 (CO1 region) was targeted and amplified using the primer cocktail designed by Germain et al. (2013). Ten individuals for each of the three grapevine moth species and from four French winegrowing regions (Bordeaux, Champagne, Val de Loire and Languedoc-Roussillon) were used to establish DNA extracts. The DNA was individually extracted using leg muscle tissue to prevent any co-extraction of DNA from the gut content and/or external contaminants using the DNeasy Blood & Tissue kit (Qiagen Hilden, Germany). Sanger sequencing of purified PCR products was conducted by GENEWIZ Germany. Generated CO1 sequences were aligned using MEGA version 6 (Tamura et al., 2013) and representative sequences for the CO1 gene were submitted to GenBank: *L. botrana* NCBI accession numbers (MK693717, MK693720, MK693722, MK685349, MK685350, MK685353 and MK685354), *E. ambiguella* accession numbers (MK693715–MK693719, MK685348 and MK685352) and *S. pilleriana* accession numbers (MK693721,

MK693723, MK693724, MK693725, MK693726 and MK685351). CO1 sequences of two other tortricid apple pests available in NCBI GenBank, *Cydia pomonella* (KF491664.1; KT133122.1) and *Grapholita molesta* (MG954384.1; KC136082.1), were added to the alignment because they could also be part of the bat diet in our environment.

Primer sequences were designed for appropriate binding sites within the full 658-bp barcode CO1 region by targeting the inter-species variable zones, which were conserved within these species. The primer combinations were expected to amplify short-barcoding fragments (100–300 bp) of each of the three grapevine moth species (*L. botrana*, *E. ambiguella* and *S. pilleriana)* from the bat faeces (by using Bioedit software V7.03, Hall, 1999).

#### 2.2.3. Primer specificity and sensibility

The specificity of these three primer pairs was evaluated in PCR assay using target and non-target DNA extracts, focusing on the arthropod species commonly present in the grapevine agrosystem (Table 1).

The primer sensitivity to detect the presence of grapevine moth DNA fragments from the bat faeces was assessed by estimating the range of digestion time between the first faeces produced after ingestion of five pupae of *L. botrana* or *E. ambiguella* and those collected up to 48 h post-ingestion. These tests were carried out on injured *Pipistellus kuhlii* during their stay at the wildlife rescue center (http://lpoaquitaine.org/index.php/centre-de-soin) and before their release. Four bats were fed at one time with one or more pupae of *L. botrana* or *E. ambiguella*. Faeces were collected under sterile conditions at regular intervals after ingestion (2 h, 4 h, 6 h, 10 h, 24 h and 48 h) and were then analysed using the new developed primers.

#### 2.2.4. DNA extraction and PCR amplification

DNA was extracted from the bat faeces using the NucleoSpin 96 Plant II Kit (Macherey-Nagel) with slight modifications from the original protocol (Zarzoso-Lacoste et al., 2018). Faeces were individually homogenized using five stainless steel beads (2-mm diameter) for 30 s at 30 Hz (Tissuelyser, Retsch). The PCR amplification of the DNA fragments was performed using the three primer pairs. A total of 5 µL of diluted DNA (5–10 ng/µL) was added to the 15 µL of reaction mixture containing 1 µL 10 × buffer, 0.6 µL 25 mM MgCl2, 1 µL 10 mM dNTPs, 1 µL each of forward and reverse primer (10 mM), 0.2 U of *Taq* Silverstar DNA polymerase (Eurogentec), and 1 µL diluted DNA (20 ng/µL). Amplifications were performed on a 9700 thermocycler (PE Biosystems). After an initial denaturing step of 5 min at 94 °C, 35 cycles were performed, each consisting of 30 s at 94 °C, 30 s at the appropriate annealing temperature (Table 2) and 40 s at 72 °C. A final extension step was performed at 72 °C for 10 min. PCR products were sized in 2 % agarose gel electrophoresis.

**Table 1**
Target (*) and non-target arthropod taxa used to test the specificity of the developed primers.

| Class, Order | Family | Species |
|---|---|---|
| Lepidoptera | Tortricidae | *Lobesia botrana** |
| | | *Eupoecilia ambiguella** |
| | | *Sparganothis pilleriana** |
| | | *Cydia pomonella* |
| | | *Grapholita molesta* |
| Hemiptera | Cicadellidae | *Scaphoideus titanus* |
| | | *Empoasca vitis* |
| | Phylloxeridae | *Daktulosphaira vitifoliae* |
| Collembola | not identified | |
| Hymenoptera | Ichneumonidae | *Campoplex capitator* |
| Diptera | Tachinidae | *Phytomyptera nigrina* |
| Dermaptera | Forficullidae | *Forficula auricularia* |
| Coleoptera | Carabidae | *Nebria brevicollis* |
| | | *Harpalus honestus* |
| | | *Tenebrio molitor* |
| Hemiptera | Anthocoridae | *Orius laevigatus* |
| Diptera | Drosophilidae | *Drosophila melanogaster* |
| | | *Drosophila suzukii* |
| Araneae | Salticidae | *Salticus scenicus* |
| Neuroptera | Chrysopidae | *Chrysoperla carnea* |
| Opiliones | Phalangiidae | *Phalangium opilio* |

## 3. Results

### 3.1. Bat and moth activity in vineyard landscapes

#### 3.1.1. Bat activity

We obtained 17,062 identifiable passes that we were able to attribute to 17 species. Most calls were identified at the species level, but four groups of closely-related taxa were pooled together to avoid misidentification (the four groups respectively consisted of: Myotis spp, *Pipistrellus kuhlii* and *P. nathusii*, *Plecotus austriacus* and *Plecotus auritus*, and *Eptesicus serotinus* and *Nyctalus leisleri*). Thus, 8917 passes (52.2 % of the identifiable passes) were attributed to *Pipistrellus kuhlii/nathusii,* 5332 (31.2 %) to *Pipistrellus pipistrellus*, 1678 (9.8 %) to the nyctaloids group, and 418 (2.4 %) to *Plecotus* spp. The remaining 4.4 % corresponded to *Nyctalus noctula* (364 passes), *Myotis* spp (211), *Barbastella barbastellus* (41), *Rhinolophus hipposideros* (41), *Rhinolophus ferrumequinum* (39), *Miniopterus schreibersii* (18) and *Pipistrellus pygmaeus* (3). Among all these recordings, 668 passes (3.9 %) were associated with feeding activity (buzzes).

The average activity of bats per plot remains relatively low, between 1 and 755 (mean 72.79 ± 11.05 SE) passes per night and per plot. Bat activities show strong variations in time, ranging from 227 to 5162 (mean 1554.27 ± 440.92 SE) contacts per session. During study, species richness ranged from 6 to 12 by plot and we contacted on average 9 species (mean 9 ± 0.32 SE) per session. Per night, the main specific richness was lower and included between 1 and 8 species (mean 4.19 ± 0.17 SE).

#### 3.1.2. Lobesia botrana moth activity

Pheromone traps in 11 out of 23 plots did not catch any moths during the study. Pheromone traps in the 12 other plots caught 479 moths. These captures ranged from 2 to 130 (mean 37 ± 2.08 SE) moths per trap. The catching dynamic indicated three flight periods of *Lobesia botrana* moths. Four plots had three flight periods and respectively five and three plots had two and one flight periods of *Lobesia botrana.* The first at the beginning of the study in early May (seven plots), the second, which is also the most important with moths trapped in 11 plots, around June 20. Finally, seven plots captured pest moths between 5 and 15 August.

#### 3.1.3. Responses of bat activity to the presence of L. botrana

Our data revealed that bat activity was correlated with the presence of adult male of *L. botrana* moths in the plots ($z = 3.66$; $P < 0.005$; Estimate from GLMM = 0.83 - which corresponds to an increase of exp (0.83) = 2.29 of overall bat activities when moth are present in a field compared to situation where moths are absent) (Fig. 2A). However, we

**Table 2**
Characterization of three primer pairs that specifically amplified DNA traces of the grapevine tortricid species.

| Primer pair | Primer sequence (5′-3′) | Ta (°C) | Fragment size (bp) |
|---|---|---|---|
| Lb-nb4F/Lb-nb3R | F: AGTCTTTTAATTCGAGCTG | 50 | 186 |
| | R: TGGAAAAGCTATATCAGGTG | | |
| Lb-nb5 F/R | F: CCCCCTCCATTATACTTCTA | 53 | 137 |
| | R: GGGAGAAGATAGCAAGATCTAC | | |
| Ea-nb F/R | F: TCGTGCAGAATTAGGAAGACC | 58 | 175 |
| | R: GGAAGGCTATATCTGGGGCT | | |

*Ta*, annealing temperature.

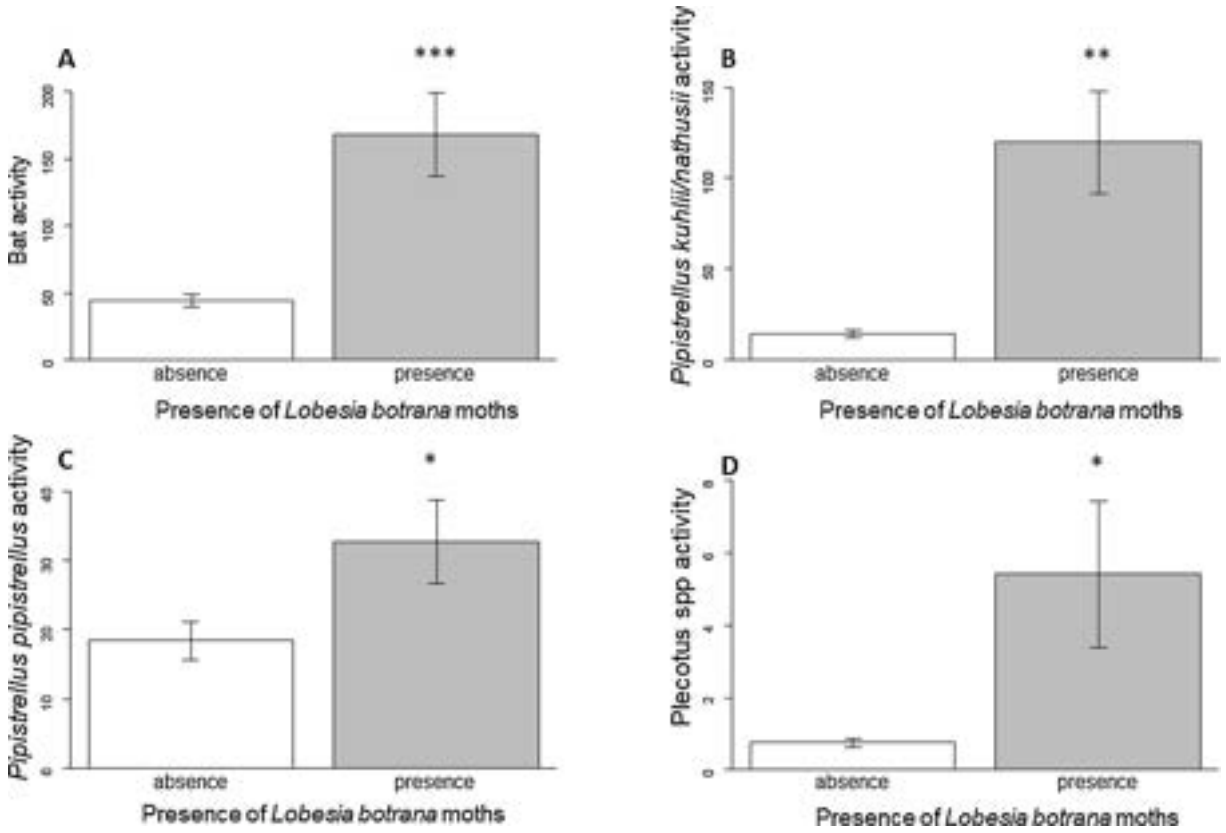


**Fig. 2.** Comparison of the average activity of bats (number of bat passes) according to the presence of the grape berry moth (*Lobesia botrana*) in the focal vineyard. White bars show the average activity of bats in fields where no moths were captured; the grey bar shows the average activity of bats in fields where moths were captured. (A) Activity of all bat species, (B) activity of *Pipistrellus Kuhlii/Nathusi*, (C) activity of *Pipistrellus pipistrellus* and (D) activity of *Plecotus* spp. $0.05 < * < 0.01$; $0.01 < ** < 0.001$; $*** < 0.001$.

found that bat species richness was not affected by the presence of *L. botrana* moth in the plots ($z = 1.77$; $P = 0.075$). As with the global bat activity, the activity of *Pipistrellus kuhlii/nathusii* ($z = 3.01$; $P < 0.005$) (Fig. 2B), *Plecotus spp* ($z = 2.26$; $P = 0.02$) (Fig. 2D) and *Pipistrellus pipistrellus* ($z = 2.10$; $P = 0.03$) (Fig. 2C) was significantly higher in plots with flying moths compared with plots without moths (Fig. 2). The change in activity level is respectively stronger for *Pipistrellus pipistrellus* (Estimate: 0.54), *Plecotus spp* (Estimate: 1.04), and *Pipistrellus kuhlii/nathusii* (Estimate: 0.97). We found that the nyctaloïds species did not modify their activity with the presence of moths ($z = 0.68$; $P = 0.49$) (Table 3).

### 3.2. Molecular analyses of bat diet

#### 3.2.1. Performance of molecular markers

Our laboratory assessment using fed *Pipistrellu kuhlii* revealed good performance of the designed markers. Two primer pairs, Lb-nb4F/Lb-nb3R and L-5nb5F/R, specifically amplified short digested DNA fragments of *L. botrana* (186 bp and 137 bp, respectively) until 48 h post-ingestion (Fig. 3). Ninety percent of the faeces collected were positive for *L. botrana* up to 2 h after ingestion (SE = 14.4), and this high level of detectability was maintained until 6 h after ingestion. After this time, the percentage of detection decreased, but it was still around 70 % (SE = 3.53) at 48 h after ingestion. One primer pair, Ea-nbF/R, amplified a short DNA fragment (175 bp) of *E. ambiguella* and *S. pilleriana* (Table 2). Seventy-three percent of the faeces collected (SE = 13.77) were positive 6 h after ingestion. The *E. ambiguella* CO1 fragment shows a high sequence similarity to *S. pilleriana* in the primer region, which explains why the developed marker, Ea-nbF/R, cannot discriminate between these two species.

No cross-amplifications with the non-target DNA were observed when this primer pairs (Lb-nb4F/Lb-nb3R, L-5nb5F/R and Ea-nbF/R) were tested against the 21 arthropod species commonly present in vineyards (Table 1)

#### 3.2.2. Prey detection from bat faeces

The faeces collected from the Bordeaux wine-growing region between 2015 and 2019 was identified as belonging to 10 bat species:

**Table 3**
Effect of *Lobesia botrana* moth on bat activity according to different species groups.

| Species or groups tested in models | Pooled species | pass number | Estimate | Std Error | *Z* | *P* |
|---|---|---|---|---|---|---|
| Total bat actvity | All species | 17,062 | 0.8321 | 0.2271 | 3.663 | < 0.001 |
| *Pipistrellus Kuhlii/ nathusii* | *Pipistrellus Kulhii*<br>*Pipistrellus nathusii* | 8917 | 0.9721 | 0.3229 | 3.01 | 0.00261 |
| *Pipistrellus pipistrellus* | *Pipistrellus pipistrellus* | 5332 | 0.5471 | 0.2597 | 2.106 | 0.0352 |
| *Plecotus* spp | *Plecotus auritus*<br>*Plecotus austriacus* | 418 | 1.0434 | 0.4616 | 2.26 | 0.0238 |
| Nyctaloïds | *Eptesicus serotinus*<br>*Nyctalus lesleri* | 1678 | 0.3458 | 0.5038 | 0.686 | 0.4925 |

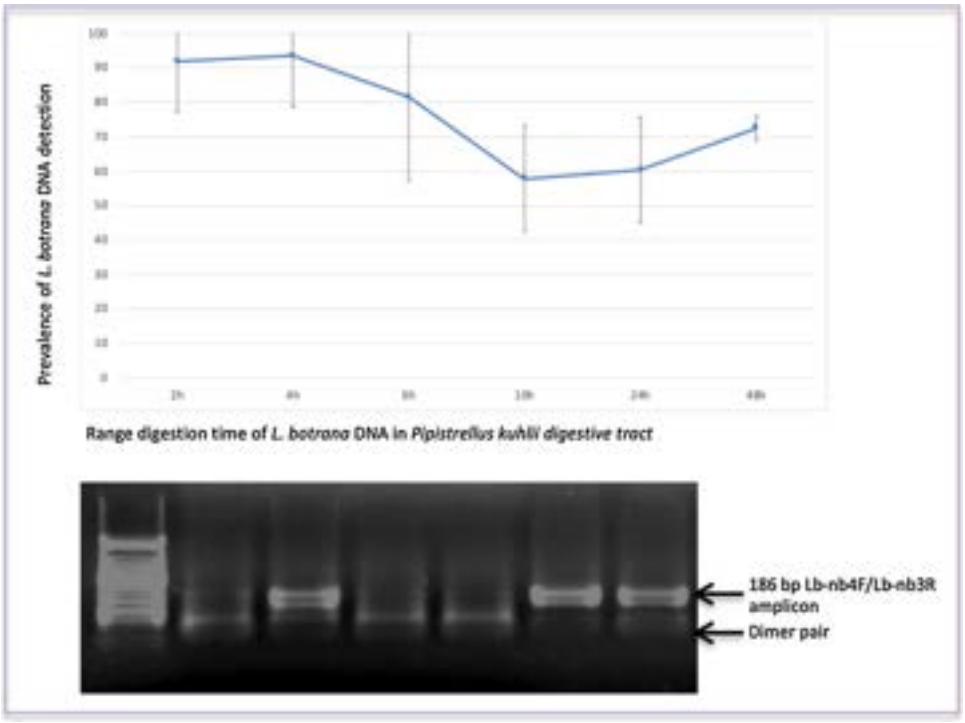


**Fig. 3.** Prevalence of *L. botrana* DNA fragment (186 bp) amplified by using the Lb-nb4/Lb-nb3 primer pair, from the *P. kuhlii* faeces collected at regular post-ingestion intervals between 2 and 48 h.

*Pipistrellus pipistrellus*, *Pipistrellus kuhlii, Plecotus austriacus, Plecotus auritus, Rhinolophus hipposideros, Rhinolophus ferrumequinum, Eptesicus serotinus, Myotis bechsteinii, Myotis daubentonii* and *Nyctalus leisleri*. In Burgundy, the analysed faeces belonged to three bat species: *Rhinolophus hipposideros, Pipistrellus pipistrellus, and Pipistrellus kuhlii.*

High levels of faeces were found positive for moths in both regions; in Bordeaux, the average detection ranged from 68 % to 91.7 % (mean ± SE: 77.8 % ± 9.1 %) and in Burgundy, the average ranged from 36.8 %–88.6 % (mean 65.6 % ± 20.6 %). All three grapevine moth species (*L. botrana*, *E. ambiguella* and *S. pilleriana*) were detected in most of the sample sites, but *L. botrana* was the most frequently detected in the two wine-growing regions (Fig. 1). In Bordeaux, 61.3 % (SE = 4.6 %) of the positive faeces amplified *L. botrana*, 35.5 % (SE = 1.9 %) amplified both *L. botrana* and *E. ambiguella* (or *S. pilleriana*), and only 3.2 % (SE = 0.5 %) amplified *E. ambiguella* (or *S. pilleriana*). In Burgundy, 31.1 % (SE = 10.5 %) of the positive faeces amplified *L. botrana*, 53.2 % (SE = 36.8 %) amplified both *L. botrana* and *E. ambiguella* (or *S. pilleriana*), and 15.7 % (SE = 10.5 %) amplified *E. ambiguella* (or *S. pilleriana*) (Fig. 1).

## 4. Discussion

Pest control services provided by bats, which have nocturnal insectivorous diets, have been demonstrated on other similar pest species and in other crops, such as the codling moth *Cydia pomonella* (Jay et al., 2012), corn earworm moth *Helicoverpa zea* (Lee and McCraken, 2005) or rice borer moth *Chilo supressalis* (Puig-Montserrat et al., 2015). However, although the role of bats as predators in vineyard landscapes has very often been put forward (Froidevaux et al., 2017; Sentenac and Rusch, 2017; Rodríguez-San Pedro et al., 2019), no study had yet demonstrated it. Our study which relies on measures of bat and pest activity with molecular analyses of bat faeces on two different experimental designs, provides robust evidence that grape berry moths are part of the bat diet and that bat activity is affected by the presence of *Lobesia botrana*. Our results therefore suggest that bats are natural enemies of grape pests and can potentially help in reducing the level of insecticide use in vineyard landscapes.

### 4.1. Temporal differences in bat activity

In our study, species richness was relatively constant both between the different plots and between sampling sessions. However, our data reveal that bat activity showed significant variations in space and time and that, except for *E. serotinus* and *N. leisleri*, these variations were correlated with the presence of moths. The temporal variation in bat activity is generally related to changes in the availability of food resources and the quality of habitats (Lehnen, 2008). For instance, previous studies have shown that insectivorous bat activities are strongly correlated with arthropod abundance, suggesting that bats actively search for areas of concentrated prey resources (Müller et al., 2012; Put et al., 2018). Our data therefore suggest that temporal variability in bat activity is partly explained by the presence of moths in the vineyard because we found a significant effect of moth presence on bat activity. However, we also know from previous studies that bat activities are affected by local and landscape factors such as farming practices (Rodriguez-San Pedro et al., 2018) or landscape structures (Froideveaux et al., 2017; Sentenac and Rusch, 2017; Rodríguez-San Pedro et al., 2019). Similar responses of bats and moths to changes in environmental conditions could therefore contribute to explaining the spatial variations in bat activity observed in our study. However, the molecular evidence that we provide about the trophic link between bats and grape moths strongly support the hypothesis that bat activity is affected by the presence of grape moths in vineyard landscapes. Our results indicate that at least three species, or species groups, of bats significantly increase their hunting activity within vineyards when adult *Lobesia botrana* moths are active. This is consistent with previous studies showing the capacity of insectivorous bats, through a significant numerical response, to adjust their predatory activity to pest availability (McCracken et al., 2012; Charbonnier et al., 2014).

The activity of nyctaloïds (*E. serotinus* and *N. leisleri),* the largest species, were not affected by the presence of the *Lobesia botrana* moth, suggesting that this insect is not their preferential prey in this habitat. This moth species may be too small to constitute interesting prey in view of the optimal foraging theory (Fossette et al., 2012). By contrast, the

smallest bat species showed a significant increase of their activity when pest moths were present in vineyards. We found that *Plecotus* spp. exhibited the most important change of activity rate in relation to the presence of moths. This result is consistent with the ecology of the species because its diet is specialized for moths and it can adapt its hunting area in relation to emergence of pests (Ashrafi et al., 2011). The generalist bat species *Pipistrellus kuhlii/P. nathusii* and *P. pipistrellus* (Dietz et al., 2009) were the most active species in our study. Generalist predators have a greater impact on prey populations at low density whereas specialized predators are more effective at high prey density (Symondson et al., 2002). In our study, it is therefore possible that moth density was too low to cause specialist bat species to be the most active. It is also possible that resources, such as alternative prey, are not sufficient at the landscape scale to maintain sustainable populations of more restricted ecological niche species.

### 4.2. Grapevine moth species are part of the bat diet

First, we showed that the detection of grapevine moth DNA from bat faeces is now possible using the molecular markers with good sensitivity and specificity developed for this study. Second, our work represents the first demonstration of the consumption of grapevine moths by bats. This approach is a promising method to specifically detect soft-body prey, such as grapevine moths, which is usually very difficult to identify by other approaches, from bat faeces (Kunz and Parsons, 2009). Moreover, in addition to providing evidence about the trophic link between bats and grapevine berry moths, our study highlights the key predatory role of some bat species in vineyard landscapes, such as *P. pipistrellus, P. kuhlii, P. austriacus and R. hipposideros*. The information provided by molecular analyses reinforces our hypothesis that the increased activity of bats is probably due to the presence of grape moths at this time of the year and not only to the emergence of other potential prey, even if other prey in these environments can be part of the bat diet. For the most common and active bat species in vineyards our results indicate that European grapevine moths are part of their diet.

### 4.3. Conclusions

Our study formally provides the first evidence that bats consume the three pest moth species and suggests that bats adapt their hunting activity according to the presence of one of them: *Lobesia botrana*. We now have to assess the consequences of pest predation by bats on crop damage and to better understand the effect of environmental changes, such as changes in farming practices or landscape structure, on bat communities and pest control services in vineyard landscapes.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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