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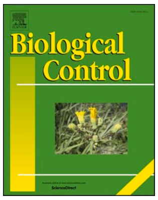

Predatory potential of *Alopecosa albofasciata* (Araneae: Lycosidae) against *Bactrocera oleae* (Diptera: Tephritidae): laboratory functional response and field predation

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HIGHLIGHTS

- *Alopecosa albofasciata* is the dominant spider in olive groves during autumn/winter.
- This period coincides with the overwintering soil stages of the olive fly *B. oleae*.
- The wolf spider *A. albofasciata* efficiently preyed on third-instar *B. oleae* larvae.
- PCR gut-content analysis confirmed field predation of *B. oleae* by *A. albofasciata*.
- Conservation strategies for *A. albofasciata* should be promoted in olive groves.

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ABSTRACT

Spiders play a crucial role in the natural suppression of pests in agroecosystems. In olive groves, the olive fruit fly, *Bactrocera oleae*, is one of the major pests, increasing the need for sustainable management strategies based on naturally occurring enemies. This study evaluated the predatory potential of a ground-dwelling spider on the overwintering generation of *B. oleae* under laboratory and field conditions. Epigeic spiders were sampled using pitfall traps in an olive grove in northeastern Portugal over two consecutive years, from October to February. The functional response of the dominant species was assessed under laboratory conditions, and field predation was evaluated using PCR-based gut-content analysis. A total of 764 spiders were collected, representing 21 families, at least 38 species, and 10 guilds. The ground-hunting *Alopecosa albofasciata* was consistently the dominant and most active species across both sampling years. Due to its abundance and temporal stability, *A. albofasciata* was selected as the model species for laboratory functional response experiments and field assays. Laboratory trials showed that *A. albofasciata* efficiently consumed *B. oleae* third-instar larvae at low prey densities and exhibited a type II functional response, whereas predation on pupae remained low across all prey densities. PCR analyses of field-collected specimens confirmed predation of *B. oleae* under natural conditions. Overall, these results indicate that *A. albofasciata* contributes to the natural mortality of *B. oleae* during the overwintering period and highlights its potential role in conservation biological control in the olive agroecosystems.

1. Introduction

The cultivation of olive trees (*Olea europaea* L.) is of great socio-economic and landscape importance in Mediterranean countries. The Mediterranean olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is one of the main pests affecting olive groves (Daane and Johnson, 2010), with negative impacts throughout entire production chain (Malheiro et al., 2015). This species is frugivorous and monophagous, depending exclusively on fruits of trees of the genus *Olea* for its development (Daane and Johnson, 2010; Campos et al., 2022). Adult females oviposit beneath the epicarp, where newly hatched larvae feed on the fruit pulp during development (Daane and Johnson, 2010; Marchini et al., 2017). Consequently, population outbreaks can cause both quantitative and qualitative damage, with estimated annual total

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production losses of up to 15% (Malheiro et al., 2015). Chemical control has been its main management strategy against *B. oleae* for several decades (Daane and Johnson, 2010). However, intensive pesticide use has led to the development of resistance in *B. oleae* populations (Kampouraki et al., 2023), as well as adverse effects on human health, environmental quality, and disturbances in biodiversity (Lykogianni et al., 2021). In this sense, sustainable and environmentally friendly approaches, such as biological control (using natural enemies to reduce pest populations), have gained increasing attention, as they diversify pest management strategies and reduce dependence on pesticides (Daane et al., 2015; Stavrianakis et al., 2025). In general, for most of the year, *B. oleae* completes its development inside the fruit, from which the adults emerge by opening an exit hole. However, during late autumn generations, third-instar larvae exit the fruit and pupate in the soil, remaining in overwintering until the following spring. During this soil-dwelling phase, larvae, pupae, and newly emerging adults become exposed to a range of natural enemies, particularly predators (Orsini et al., 2007; Dinis et al., 2016a).

Spiders are ubiquitous predators and represent one of the most abundant and diverse groups in agroecosystems (Nyffeler and Sunderland, 2003; Michalko et al., 2019a; Cardoso et al., 2025). Most species are generalists that primarily consume insects (Michalko and Pekár, 2016) and possess a wide range of hunting strategies (Cardoso et al., 2011), from passive web-building to active hunting (Michalko and Pekár, 2016). Although they exhibit generalist feeding habits, spiders are potential efficient natural enemies in conservation biological control strategies in different crops, including olive groves, by preying on various economically important pests (e.g., dipterans, moths, aphids, and leafhoppers), that cause significant agricultural losses (Monzó et al., 2010; Pekár et al., 2015; Picchi et al., 2016; Benhadi-Marín et al., 2020a; Michalko et al., 2019b; Cuff et al., 2021; Gajski et al., 2024; Dmitrović et al., 2023). However, their biocontrol effectiveness can be influenced by multiple factors, including pest traits, spider trophic ecology, management practices (e.g., agrochemicals use), landscape composition and configuration, and climate (Picchi et al., 2016; Michalko et al., 2019a; Michalko et al., 2019b; Lacava et al., 2021).

To assess the potential of a natural enemy in pest regulation, the functional response, defined as the relationship between prey density and the number of prey consumed within a given time, is widely used (Benhadi-Marín et al., 2019; Bruzzone et al., 2022). Holling (1959,1966) describes three types of functional responses: type I, characterised by a linear increase in prey consumption; type II, showing a decelerating, asymptotic response approaching a plateau; type III, displaying a sigmoidal pattern, where there is an initial increase followed by a decrease in the proportion of prey killed as prey density increases. Jeschke et al. (2004) defined a type IV response as dome-shaped. In general, generalist spiders typically exhibit a functional response that shifts between types II and III, depending on the composition of the prey community (Michalko et al., 2019a). Functional responses can be influenced by several ecological and environmental factors, including habitat structure, availability of alternative prey, environmental conditions, prey preference, attack rate, and handling time (Bruzzone et al., 2022). Among these, attack rate and handling time are key parameters for evaluating the efficiency of a predator as a biological control agent.

In olive groves, Picchi et al. (2016) reported a negative correlation between *B. oleae* abundance and the presence of cursorial and sheet-web spiders. However, to our knowledge, no previous studies have investigated the functional response of cursorial spiders to *B. oleae* under laboratory conditions, nor confirmed their predation under field conditions. Such information is critical for conservation biological control, which relies on maintaining and enhancing native predator populations through habitat management (Gurr et al., 2017). However, the contribution of native spider species in pest control within olive groves remain poorly understood. Molecular techniques based on polymerase chain reaction (PCR) have proven valuable for elucidating predator–prey interactions in agroecosystems (Monzó et al., 2010; Rejili et al., 2016; Albertini et al., 2018a; Rodrigues et al., 2022). Using species specific primers, prey DNA fragments can be detected in the predator's gut allowing confirmation of predation events in natural settings (Symondson, 2002; Cuff et al., 2021). This method allows the study of naturally occurring predation events and is particularly valuable for generalist predators, such as spiders, whose trophic niches are difficult to determine under field conditions based solely on direct observations. It also allows the identification of factors that drive variations in predation rates (Kobayashi et al., 2011).

Given that the intensity of predator–prey interactions is directly influenced by prey availability in the field (Marc et al., 1999; Picchi et al., 2016), we hypothesize that ground-active hunting spiders with activity periods overlapping those of *B. oleae* may prey on its overwintering larvae and pupae. Accordingly, the main objective of this study was to evaluate the predatory potential of edaphic spiders as biological control agents of *B. oleae* under both laboratory and field conditions. Specifically, we aimed to: (i) characterise the edaphic spider assemblage during the period when *B. oleae* occurs in the soil as larvae and pupae, (ii) assess the functional response of a dominant spider species to *B. oleae* in laboratory trials, and (iii) confirm field predation through PCR-based gut-content analysis of the model species.

2. Materials and methods

2.1. Study area

Fieldwork was conducted near Mirandela, in Romeu (northeastern Portugal), in a previously selected olive grove (41°32′07.1″ N 7°04′12.0″ W). According to the Köppen-Geiger classification, the region has a Csa climate, a temperate humid climate, characterized by hot and dry summers and mild, wet winters. The mean annual temperature is 14.3 °C and the mean annual rainfall is 531 mm (IPMA, 2024). The study area covers 8.33 ha and is characterised by gently undulating terrain with moderate slopes at altitude between 374 and 425 m. The olive grove has been managed according to organic production guidelines, without any application of chemicals inputs in the past ten years. The trees are planted at 10 × 10 m spacing, are non-irrigated, and natural cover vegetation maintained during the winter season.

2.2. Sampling of Araneae

To characterise the epigeic spider assemblage and identify a dominant model species for functional response experiments with *B. oleae*, spiders were sampled monthly between October and February during the 2023/2024 and 2024/2025 seasons. The grove was divided in five areas, and, in each of the established areas, eight sampling points were defined, where pitfall traps (n Total = 40) were installed. Traps were filled with 300 mL of a preservative solution consisting of ethylene glycol, water, and detergent, and were positioned with their edges level with the soil surface. Each trap was protected by a plastic lid fixed to the ground with wire, forming a cover to reduce disturbance. Throughout the sampling period, the traps remained active for seven consecutive days during each sampling month. After collection, samples were transported to the laboratory, where their contents were sorted and preserved in 70% ethanol. Spiders were identified to species using taxonomic keys (Nentwig et al., 2026) and classified into guilds according to Uetz et al. (1999) and Cardoso et al. (2011).

2.3. Origin, selection and rearing of model species

According to the dominance data obtained from field sampling, the wolf spider, *Alopecosa albofasciata* (Brullé, 1832), was selected as the model species for laboratory functional response assays. To establish a laboratory colony, 110 subadult and adult females were manually collected in October 2024 manually from the soil surface among grasses near the olive trees trunks in Romeu (41°32′6.45″ N 7°4′11.93″ W). After capture, spiders were individually placed in perforated plastic tubes and immediately transported to the laboratory. Each specimen was then transferred to a Petri dish (1.8 cm high, 7 cm in diameter) and fed *ad libitum* with *Tenebrio molitor* (Linnaeus, 1758) larvae. Water was provided using a sodium acrylate hydrogel bead placed at the base of the Petri dish. All spiders were kept in a controlled climate chamber at 24 ± 1 °C, 65 ± 10% relative humidity (RH), and 16:8 h (L:D) photoperiod.

2.4. Origin and rearing of prey

Olive fruits infested with *B. oleae* were collected in November and December 2024 from several olive groves in the Mirandela region of Portugal. In the laboratory, the fruits were stored in plastic boxes (37 × 57 × 7 cm) and kept under controlled conditions [26 ± 2 °C, 65 ± 10% RH and 16:8 h (L:D) photoperiod]. Emerged larvae from the fruits were collected daily. The larvae intended (third-instar larvae) for functional response assays were used immediately, whereas remaining larvae were transferred to Petri dishes and allowed to pupate.

2.5. Functional response assays

Before the assays, spiders were fed *ad libitum* with *T. molitor* larvae until they refused additional prey and were then starved for seven days to standardise hunger levels (García et al., 2021). Each female spider was then placed individually in a glass Petri dish arena (1.8 cm high, 7 cm in diameter). Larvae and pupae of *B. oleae* were offered separately at increasing densities (1, 3, 5, 10, 15, and 20 specimens). Seven replicates were conducted for each prey developmental stage, using a total of 84 spiders. After 24 h, prey were recorded as dead if fully consumed or partially consumed. The experiment was conducted in a climate chamber at 23 ± 1 °C, 65 ± 10% RH, under direct cool white light (2130 lx).

2.6. Detection of B. oleae DNA in the gut of A. albofasciata

Adult *A. albofasciata* specimens ($n = 33$) were manually collected in the olive grove in Romeu in November 2024. Individuals were immediately placed individually in Falcon tubes (15 mL) with 99% ethanol and frozen at −19 °C. Prior to DNA extraction, the legs were removed. The prosoma and opisthosoma were used for total DNA extraction and were externally cleaned with 96% ethanol, then dried on filter paper. Each spider was then macerated in liquid nitrogen, and total DNA was extracted using the SpeedTools DNA Tissue Extraction kit (Biotools), following the manufacturer's instructions.

PCR amplification targeted two fragments (108 and 214 bp) of the mitochondrial cytochrome oxidase I (COI) gene of *B. oleae*, using primer pairs SBo1-F/SBo1-R and SBo2-F/SBo1-R (Rejili et al., 2016). Each PCR included a positive control (C+) with *B. oleae* DNA, a negative control (C−), DNA of *A. albofasciata* fed exclusively with *T. molitor*, and a no-template control. PCR products were visualised by electrophoresis on 2% agarose gels stained with GelRed™ and observed under UV light using a ChemiDoc™ XRS system (Bio-Rad).

2.7. Data analysis

All analyses were performed in the R environment (R Core Team, 2024). Data for each sampling year (2023/2024 and 2024/2025), were analysed independently. Only adult specimens were considered for statistical analyses and total spider abundance was analysed using generalized linear mixed models (GLMMs). Sampling months (October, November, December, January, and February) was included as a fixed effect, with spider abundance as the response variable, and sampling areas and sampling units were included as random effects. A negative binomial distribution with quadratic parameterisation (nbinom2) was used to account for overdispersion. Models were fitted using the *glmmTMB* function (Brooks et al., 2017). Overall differences among months were assessed using likelihood ratio test based on the chi-square distribution, implemented with the *Anova* function from the *car* package (Fox and Weisberg, 2019). When significant effects were detected, pairwise comparisons were performed using Bonferroni-adjusted post hoc tests with the *glht* function from the *multcomp* package (Hothorn et al., 2008). Model assumption and fit were validated using the *simulateResiduals* function from the *DHARMa* package (Hartig, 2023). To visualise temporal patterns in species distribution across months and years, a heat map was generated using the *pheatmap* package. Functional response type (I, II, or III) were identified using the *frair* package (Pritchard et al., 2017). This classification was based on the relationship between initial prey density and the proportion consumed, as well as on the estimation of the functional response parameters. Initially, a linear regression was fitted to the number of prey killed to assess the suitability of the type I functional response model, defined as:

$$N_e = a \times N_0 \times T \tag{1}$$

where N_e is the number of prey killed, N_0 is the initial prey density, a is the attack rate, and T is the exposure time. As the data did not fit the type I model, the *frair_test* function was used to distinguish between type II or type-III functional response. A significantly negative linear coefficient indicates a type II response, whereas a significantly positive linear coefficient followed by a negative quadratic coefficient indicates a type III response (Juliano, 2001). Because all datasets exhibited a type II functional response and prey were not replenished during the trials, Rogers' random predator equation (Rogers, 1972) was applied:

$$\mathrm{N_e} = \mathrm{N_0}\,{}_{\times}\,\{1 - \exp\,[a \times (\mathrm{N_e} \times \mathrm{T_h} - \mathrm{T})]\} \tag{2}$$

where N_e is the number of killed prey, N_0 is the initial prey density, a is the attack rate, T_h is the handling time, and T is the experiment time (24 h). Attack rate (a) and handling time (Th) were estimated by fitting the model and applying bootstrapping (999 replicates) using *frair_boot* function. This resampling procedure, based on the previously fitted models, generating 95% confidence intervals for each parameter. Differences between estimates were evaluated by comparing their confidence intervals.

3. Results

3.1. Olive grove spider community

A total of 764 spiders were collected, including 557 (72.90%) adults and 207 (27.09%) immatures during the two-years study, representing 21 families, at least 38 species, and 10 functional guilds: ambushers, foliage runners, ground hunters, orb-web builders, sensing web, sheet web builders, space web builders, specialists, stalkers and wandering sheet/tangle weavers. In 2023/2024, spiders abundance did not differ significantly among sampling months ($\chi^2 = 7.17$; df = 4; $p = 0.1267$). In contrast, in 2024/2025, abundance varied significantly among sampling periods, with the highest values observed in October ($\chi^2 = 51.78$; df = 4; $p < 0.001$) (Fig. 1).

In both years, the dominant families were Lycosidae and Gnaphosidae, with ground hunters representing the dominant guild (Table 1 and Fig. 2). In 2023/2024, the ground hunter wolf spider *Alopecosa albofasciata* (Brullé, 1832) was the most abundant species, followed by the sheet-web builder *Eratigena feminea* (Simon, 1870) and the ground

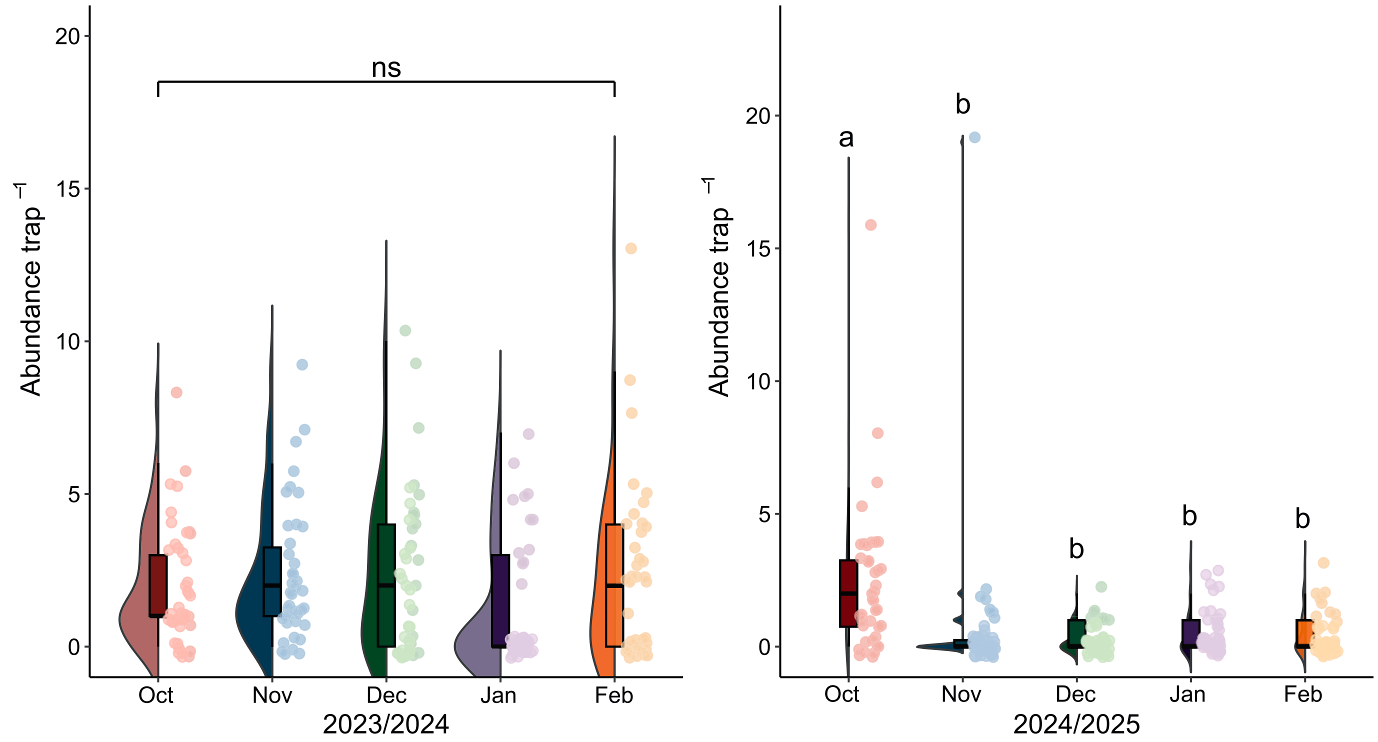

Fig. 1. Boxplot/violin plot of the total abundance of spiders sampled per pitfall trap from Oct: October; Nov: November; Dec: December; Jan: January; Feb: February in the 2023/2024 and 2024/2025 periods. Identical letters and "ns" indicate no significant difference, whereas different letters represent significant differences among months (Bonferroni-adjusted $p < 0.05$).

hunter *Zelotes egregioides* (Senglet, 2011). In 2024/2025, *A. albofasciata* and *Z. egregioides* were again the dominant species, followed by the ground hunter *Pardosa proxima* (C. L. Koch, 1847) (Table 1 and Supplementary Fig. 3).

3.2. Laboratory functional response

Logistic regression estimates revealed a significant negative linear coefficient, indicating a type II functional response to *A. albofasciata* to both larvae and pupae. For larvae, the estimated coefficient was −0.085 (SE = 0.018; Z = −4.542; $p < 0.001$), whereas for pupae it was −0.123 (SE = 0.041; Z = −2.98; $p < 0.01$). During the experiments, the mean number of larvae consumed increased with increasing prey density, whereas pupal consumption remained consistently low across all prey densities tested (Table 2 and Fig. 3).

Parameter estimates from the functional response models showed that predatory efficiency on *B. oleae* larvae was significantly higher than on pupae. When feeding on larvae, *A. albofasciata* exhibited a significantly higher attack rate (0.083) and significantly shorter handling time (2.495), resulting in a higher maximum attack rate (9.619). In contrast, predation on pupae was characterised by a lower attack rate (0.014) and a much longer handling time (43.604), leading to a substantially reduced maximum attack rate (0.550) across all prey densities (Table 3).

3.3. Molecular gut content analysis of field-collected spiders

To confirm field predation of *B. oleae*, a total of 33 adult *A. albofasciata* specimens were analysed using two different pairs of species-specific primers. Using the SBo1-F/SBo1-R primer pair, *B. oleae* mtDNA was detected in 27.27% of the spiders analysed. Detection increased to 42.42% when using the SBo2-F/SBo1-R primer pair (Supplementary Fig. 5).

4. Discussions

In this study, we surveyed spiders inhabiting the soil surface to evaluate the predatory potential of a soil-dwelling spider species on *B. oleae*. The characterisation of the spider community allowed the selection of a model species, the wolf spider *A. albofasciata*, for laboratory functional response assays and field predation assessment using PCR-based gut-content analysis. Among all spiders sampled over two years, the ground hunter *A. albofasciata* was consistently the most active and abundant species, particularly in November and February, and its predation on *B. oleae* was confirmed under natural field conditions. To our knowledge, this is the first study to document predatory activity of *A. albofasciata* on *B. oleae*.

The survey of soil species composition was conducted during autumn and winter, periods that have been comparatively understudied in olive grove ecosystems. Our results revealed that the spider assemblage active during this period was dominated by cursorial hunters, particularly Lycosidae, followed by Gnaphosidae. Our results are in consistent with previous studies in different crops (Benamú et al., 2017; Mashavakure et al., 2019; Pompozzi et al., 2024), including Mediterranean olive groves (Dinis et al., 2016a; Benhadi-Marín et al., 2020b; Outemzabet et al., 2025), in which the spider assemblage is primarily dominated by Lycosidae. Wolf spiders are not only abundant in agroecosystems but also exhibit high predation rates and exhibit multiple behavioral strategies, contributing to pest suppression and improved crop performance (Snyder and Wise, 2001; Birkhofer et al., 2008; Kuusk and Ekbom, 2010; Monzó et al., 2010; Rendon et al., 2016; García et al., 2021). Overall, during both sampling years, the assembly of active spiders was dominated by *A. albofasciata*. This species is the most frequently recovered member of its genus in pitfall traps and has a wide distribution throughout the Mediterranean Basin from Portugal to Central Asia (Nentwig et al., 2026). *Alopecosa albofasciata* is small- to medium-sized wolf spider, from 8 to 9 mm, in males, and 10 to 12 mm, in females (Nentwig et al., 2026), stenochronous in activity, and during the day, regularly found in open, sunny and dry habitats (Lugetti and Tongiorgi

Table 1
Guilds, families, and species of Araneae sampled in the olive grove during 2023/2024 and 2024/2025 in Romeu, Mirandela, Portugal.

Guild	Family	Species	2023/2024	2024/2025	Total abundance
Ambushers	Pisauridae	*Pisaura mirabilis* (Clerck, 1757)	1	–	1
		Immatures	–	2	2
	Sicariidae	*Loxosceles rufescens* (Dufour, 1820)	9	–	9
	Thomisidae	*Bassaniodes bliteus* (Simon, 1875)	1	2	3
		Bassaniodes bufo (Dufour, 1820)	3	9	12
		Xysticus cristatus (Clerck, 1757)	–	3	3
		Xysticus ferrugineus (Menge, 1876)	1	–	1
		Xysticus kochi (Thorell, 1872)	1	–	1
		Immatures	17	5	22
	Philodromidae	Immatures	3	–	3
Foliage runners	Cheiracanthiidae	*Cheiraignotum striolatum* (Simon, 1878)	1	–	1
	Clubionidae	Immatures	1	–	1
Ground hunters	Corinnidae	*Castianeira badia* (Simon, 1877)	11	8	19
	Dysderidae	*Dysdera machadoi* (Ferrández, 1996)	1	2	3
		Dysdera sp. 1	1	–	1
		Immatures	3	1	4
	Gnaphosidae	*Haplodrassus rufipes* (Lucas, 1846)	1	3	4
		Nomisia exornata (C. L. Koch, 1839)	1	1	2
		Zelotes egregioides (Senglet, 2011)	25	15	40
		Zelotes fulvopilosus (Simon, 1878))	2	–	2
		Zelotes thorelli (Simon, 1914)	13	10	23
		Immatures	45	21	66
	Liocranidae	Immatures	–	1	1
	Lycosidae	*Alopecosa albofasciata* (Brullé, 1832)	188	23	211
		Arctosa villica (Lucas, 1846)	6	1	7
		Hogna radiata (Latreille, 1817)	15	7	22
		Pardosa proxima (C. L. Koch, 1847)	–	10	10
		Immatures	57	10	67
	Phrurolithidae	*Phrurolithus nigrinus* (Simon, 1878)	1	–	1
	Trachelidae	*Paratrachelas ibericus* (Bosselaers, Urones, Barrientos & Alberdi, 2009)	1	1	2
	Miturgidae	*Zora manicata* (Simon, 1878)	2	–	2
Orb-web builders	Araneidae	*Mangora acalypha* (Walckenaer, 1802)	1	–	1
Sensing web	Nemesiidae	*Nemesia* sp.	11	3	11
Sheet web builders	Agelenidae	*Eratigena feminea* (Simon, 1870)	49	10	59
		Eratigena picta (Simon, 1870)	1	–	1
		Immatures	5	3	8
Space web builders	Theridiidae	*Euryopis episinoides* (Walckenaer, 1847)	4	1	5
		Immatures	3	1	4
Specialist	Zodariidae	*Selamia reticulata* (Simon, 1870)	1	2	3
		Zodarion styliferum (Simon, 1870)	9	9	18
		Immatures	3	1	4
Stalkers	Salticidae	*Euophrys gambosa* (Simon, 1868)	2	7	9
		Euophrys innotata (Simon, 1868)	10	9	19
		Phlegra bresnieri (Lucas, 1846)	1	3	4
		Immatures	8	3	11
Wandering sheet/ tangle weavers	Linyphiidae	*Agyneta* sp.	10	–	10
		Palliduphantes stygius (Simon, 1884)	2	–	2
		Pelecopsis inedita (O. Pickard-Cambridge, 1875)	–	1	1
		Sintula furcifer (Simon, 1912)	5	–	5
		Erigoninae immatures	12	–	12
		Tenuiphantes tenuis (Blackwall, 1852)	7	6	13
		Typhochrestus bogarti (Bosmans, 1990)	1	–	1
		Immatures	8	6	14
Total			**564**	**200**	**764**

1969). The dominance of *A. albofasciata* observed in our study agrees with observed by other authors in olive groves (Benhadi-Marín et al., 2020b; Picchi, 2020; Dmitrović et al., 2023; Outemzabet et al., 2025), as well as in other ecosystems such pastures, Mediterranean shrublands, and open forest areas (Lugetti and Tongiorgi, 1969).

Other abundant species showed marked interannual variation. *Eratigena feminea* was the second most abundant species in the first year, whereas *Z. egregioides* was observed in both years of the study. However,

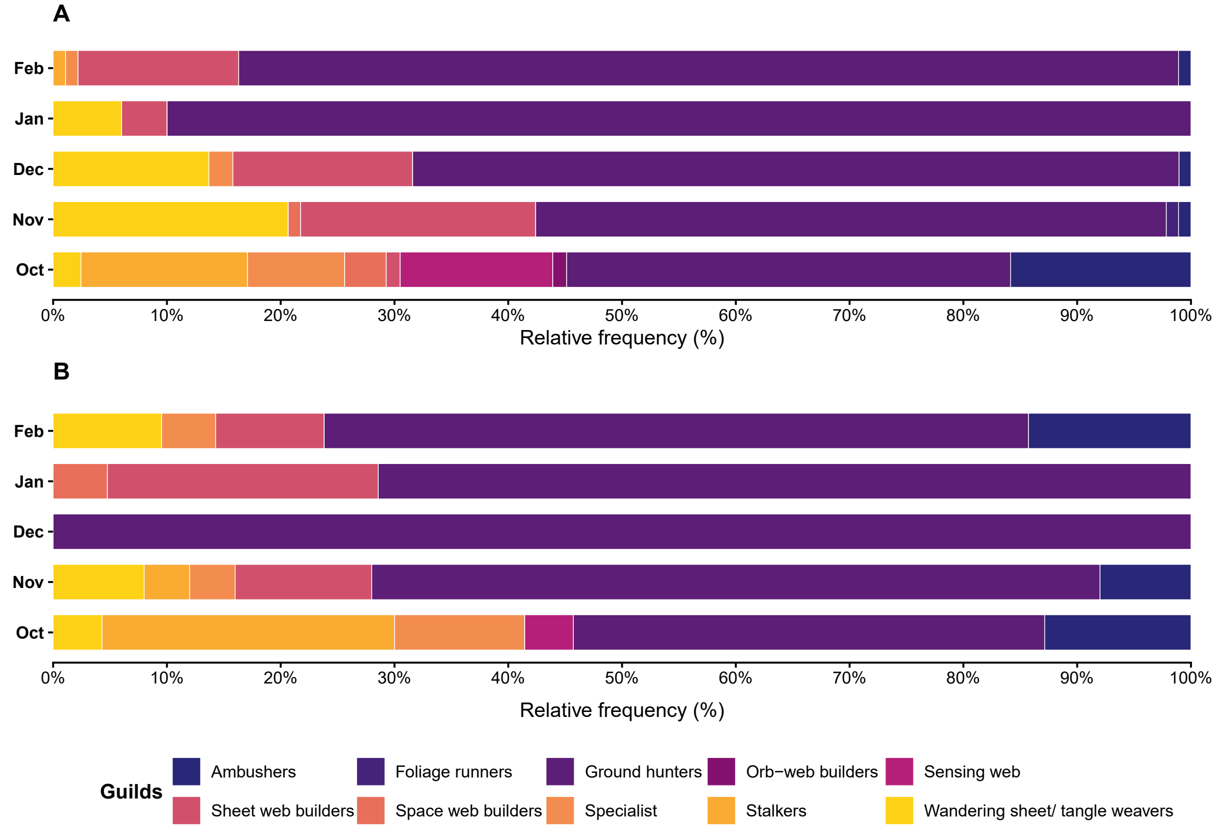

Fig. 2. Relative frequence (%) of spiders guilds sampled from Oct: October; Nov: November; Dec: December; Jan: January; Feb: February in the A: 2023/2024 and B: 2024/2025 periods.

Table 2
Mean number of consumed prey (*B. oleae*) at different densities by *A. albofasciata* in laboratory assays.

Prey density	Mean number of consumed prey (Mean ± SE)	
	Larvae	Pupae
1	1 ± 0	0 ± 0
3	2.28 ± 0.28	0.71 ± 0.28
5	3.57 ± 0.42	0.57 ± 0.29
10	5.14 ± 0.91	0.28 ± 0.18
15	6.42 ± 1.50	0.28 ± 0.18
20	8.14 ± 1.62	0.57 ± 0.20

P. proxima only occurred in the second year of sampling. All these species present small to medium-sized bodies and frequent in open spaces, herbaceous and epiedaphic environments (Senglet, 2011; Nentwig et al., 2026). In general, the dominant species observed in this study are commonly associated with herbaceous habitats. The presence of herbaceous ground cover in the studied olive grove during winter likely played a key role in shaping the assemblage. Plant diversity may have contributed to increased structural heterogeneity at ground level, providing suitable microhabitats for shelter and resource (Bowers et al., 2021). Ground cover during periods of reduced activity can help maintain active predators throughout winter, sustaining trophic interactions during a period often overlooked in agroecosystems. Furthermore, the interannual differences observed among species may be related to climatic conditions and associated changes in prey availability, as spider populations and trophic interactions are highly sensitive to these factors (Cuff et al., 2023). Variations in temperature, precipitation, soil moisture, and wind can modify not only the structure of the prey community but also the activity and foraging behaviour, including adjustments in food selectivity or trophic niche expansion (Cuff et al., 2023). Despite interannual variability among several species, *A. albofasciata* maintained high abundance and temporal consistency in the olive grove among the years, supporting its selection as a model species. In this context, the stability of *A. albofasciata* suggests a strong ecological association with olive grove environments, supporting the hypothesis that winter-active agrobiont spiders can help sustain predation pressure when other predatory arthropods are less diverse or active, potentially impacting the overwintering stages of *B. oleae*.

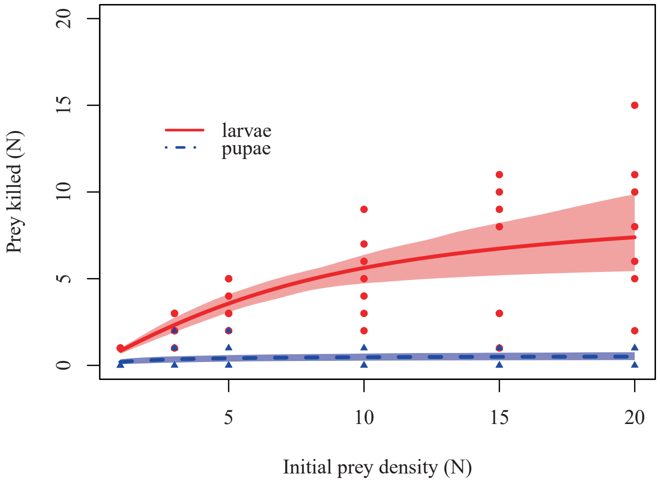

Fig. 3. Functional response models obtained for *Alopecosa albofasciata* fed with different densities of *Bactrocera oleae* larvae and pupae (1, 3, 5, 10, 15, and 20) over a 24 h period. Thick lines represent the fitted model values, and shaded areas indicate the 95% confidence interval limits of the curves.

Table 3
Estimated parameters from the model for the functional response of *A. albofasciata* fed on larvae and pupae of *B. oleae*. *a*: coefficient of attack rate; h: estimated handling time; T/h: maximum number of prey that can be consumed during the experimental period T; CI: 95% confidence interval. Significant differences in the estimates are indicated by lowercase and capital letters for the attack rate (*a*) and estimated handling time (h), respectively.

	Parameter	Estimate	SE	Z-Value	*p*	95%CI
Larvae	*a*	0.083a	0.019	4.19	$<$0.001	[0.049; 0.160]
	h	2.495A	0.405	6.150	$<$0.001	[1.305; 3.939]
	T/h	9.619				[6.092; 18.385]
Pupae	*a*	0.014b	0.013	1.08	0.279	[0.004; 0.057]
	h	43.604B	14.859	2.93	$<$0.001	[24.509; 78.970]
	T/ h	0.550				[0.303; 0.980]

Laboratory functional response assays demonstrated that *A. albofasciata* efficiently preyed on third-instar larvae of *B. oleae*, particularly at low prey densities, and exhibited a clear type II functional response, characterized by a plateau in consumption at approximately eight larvae as prey density increases. In contrast, predation on pupae remained consistently low across all prey densities. Overall, our results suggest higher predation pressure at low prey densities. However, as prey density increases, despite the presence of spider, their consumption tends to saturate and the predatory impact decreases (Michalko et al., 2019a). Type II functional response are commonly observed for efficient biological control agents (Fathipour et al., 2020), and are typical observed for generalist predators and parasitoids (Li et al., 2025; Sánchez-Ramos and González-Núñez, 2023). Similar responses have been observed in carabid beetles [*Calathus granatensis* (Vuillefroy) and *Pterostichus globosus* (Fabricius)] preying on *B. oleae* pupae (Dinis et al., 2016b), or *Pseudophonus rufipes* De Geer (Coleoptera: Carabidae) feeding on *Ceratitis capitata* (Wiedemann) (Monzó et al., 2011).

Type II functional responses are also frequently observed for spiders (Benhadi-Marín et al., 2019; Li et al., 2025). For example, in citrus orchards in Spain, *Pardosa cribata* Simon (1883) (Araneae: Lycosidae) has been shown to be an efficient predator of *C. capitata* and *Myzus persicae* (Sulzer), exhibiting a type II functional response (Monzó et al., 2009). Similarly, *Araniella cucurbitina* (Clerck, 1757) and *Synema globosum* (Fabricius, 1775) display type II functional response when feeding on of *C. capitata* adults (Benhadi-Marín et al., 2019). The characteristic plateau effect observed may have been influenced by satiation and digestion time, factors that likely limited its consumption even when prey density increases. Additionally, the presence of alternative prey may influence predator–prey interactions (Rendon et al., 2019) and potentially affect the functional response. Although interactions with alternative prey were not evaluated in the present study, future research should investigate how the availability of other common prey during the active periods of *A. albofasciata* may influence its predatory behaviour and functional response.

Analysis of functional response parameters further highlighted the greater predatory efficiency of *A. albofasciata* on *B. oleae* larvae compared with pupae. In general, *A. albofasciata* required a longer handling time for *B. oleae* pupae than for larvae, and its attack rate was approximately six times higher for larvae than estimated for pupae. Handling time was substantially longer and attack rate markedly lower when spiders were offered pupae, resulting in a much lower maximum attack rate. The maximum attack rate also suggests greater predatory capacity for *B. oleae* larvae. The maximum attack rate estimate obtained was 9.619 individuals in 24 h, while for pupae it was approximately 0.55 pupae. This result suggests that, under natural conditions, predation pressure may be concentrated on the larval stages of *B. oleae*, potentially reducing the number of individuals reaching the overwintering stage. Wolf spiders are known to consume larvae efficiently (Monzó et al., 2009; Rendon et al., 2016), although pupae may also be preyed upon, albeit in smaller numbers (Urbaneja et al., 2006). Indeed, wolf spiders are opportunistic generalist predators that exhibit little prey selectivity and tend to feed according to availability (Rendon et al., 2019), often choosing prey that maximises energy intake due to their high energetic demands (Jensen et al., 2011; Rendon et al., 2016). The low predation observed on *B. oleae* pupae by *A. albofasciata* in our study may be explained by the immobility of the pupae. Because wolf spiders depend on vibrations emitted to detect prey (Wrinn and Uetz, 2008), immobility *B. oleae* pupae are less likely to be detected and attacked. In addition, the sclerotized body of pupae may make them less attractive, as spiders feed more efficiently on soft-bodied insects such as larvae.

While laboratory experiments provide valuable insights, they cannot fully reflect predator–prey interactions in the field. In agroecosystems, spiders interact with various predators and prey in a complex food web (Dmitrović et al., 2023; Gajski et al., 2024) that include alternative prey, intraguild predation, and competition with other predators, all of which can alter predation pressure on a focal pest species (Pekár et al., 2015; Senior et al., 2016; Dmitrović et al., 2023; Gajski et al., 2024). Therefore, confirmation of predation under natural conditions is essential. PCR-based gut-content analysis confirmed that *A. albofasciata* preys on *B. oleae* in the field, with detection rates ranging from 27.27% to 42.42% depending on the used primer pair. Our results corroborate previous molecular studies that analysed the gut content of spiders, with positive rates between 5% and 26%, and confirm its contribution to agricultural pest control (Kuusk et al., 2008; Kuusk and Ekbom, 2010; Monzó et al., 2010; Li et al., 2025). Although both pairs of primers used showed high efficiency and specificity for *B. oleae*, the difference observed between primer pairs reflects variation in target DNA fragment length, reinforcing the advantage of using multiple primers when analysing field-collected predators (Albertini et al., 2018b).

Considering the laboratory results, detection rates probably involves third instar larvae of *B. oleae*, which leaving the fruit to the soil to pupate and thus become exposed to edaphic predators (Orsini et al., 2007). In October, field observations showed high activity and abundance of *A. albofasciata* in the herbaceous vegetation surrounding olive tree trunks, that coinciding with larval drop to the soil. This temporal and spatial overlap suggests a behaviour associated with the availability of food resources, namely third-instar larvae of *B. oleae*, that constitutes an important food resource during the adverse periods of reduced prey diversity in autumn and winter (Birkhofer et al., 2022). This pattern is consistent with the characterisation of the species as a functional agrobiont analogue in olive groves in Trás-os-Montes (Benhadi-Marín et al., 2020b).

Although there is an overlapping temporal dynamic between *A. albofasciata* and *B. oleae*, it is important to consider that the probability of amplification also depends on the digestion time, which can vary widely among spider species, ranging from a few hours to several days (Kuusk et al., 2008; Monzó et al., 2010; Kuusk and Ekbom, 2010; Kobayashi et al., 2011; Rodrigues et al., 2022). In this context, future studies should focus on determining the post-feeding detection half-life of *A. albofasciata* to refine estimates of predation rates, expand dietary analyses using metabarcoding to better characterize its trophic niche within in olive agroecosystem. Furthermore, spiders are most effective as biological control agents when their populations are abundant (Sunderland and Samu, 2000). Thus, conservation strategies to enhance *A. albofasciata* populations, such as habitat diversification, maintenance of ground vegetation, provision of refuges, and selective pesticide use, are fundamental to increase the biological control of *B. oleae* in olive agroecosystems.

5. Conclusion

This study demonstrated that *A. albofasciata* is the most abundant spider species in the ground of olive groves during autumn and winter, coinciding with the soil-dwelling stages of the most important olive pest, the olive fly *B. oleae*.

Laboratory assays showed that *A. albofasciata* efficiently preyed on third-instar larvae of *B. oleae* and exhibited a type II functional response, characterised by a high attack rate and short handling time. In contrast, predation on pupae was consistently low, indicating that larval stages are more vulnerable to predation by this species. PCR-based gut-content analysis confirmed laboratorial observation that *A. albofasciata* is a potential biological control agent of immature stages of *B. oleae* on natural condition in the olive grove.

Overall, our results indicated the importance of *A. albofasciata* as a promising candidate for conservation biological control of *B. oleae*. Management practices that promote the conservation and activity of this species, such as maintaining ground vegetation, reducing soil disturbance, and minimising non-selective insecticide use, may enhance natural pest regulation in olive agroecosystems.

CRediT authorship contribution statement

Ketrin Kubiak: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Paula Baptista:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization. **José Alberto Pereira:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2026.106009.

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