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Candidate natural enemy assemblage for biological control of *Lymantria dispar* L. in oak (*Quercus* spp.) with different levels of pest infestation

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Abstract: Mediterranean *Quercus* forests have great ecological importance but face numerous threats, including pests. The spongy moth, *Lymantria dispar* L., is a major oak defoliator across its geographical range and has a natural enemy complex that may control its population dynamics. This study aimed to investigate candidate predators (Coleoptera: *Carabidae*) and parasitoids (Hymenoptera: *Encyrtidae*, *Ichneumonidae*, *Pteromalidae*, *Braconidae*, *Bethylidae*, *Ceraphronidae*, *Eulophidae*, *Eupelmidae* and *Trichogrammatidae*; and Diptera: *Tachinidae*), for the control of *L. dispar* in two areas in Andalusia (Spain). We studied 10 *Quercus* stands (*Q. suber*, *Q. ilex*, and *Q. pyrenaica*), with different *L. dispar* infestation level. Insects were collected using pitfall and cross-vane traps, during the defoliator's larval period. Four genera comprised 92.2% of all the *Carabidae* predators found: *Steropus* Dejean (34.1%), *Carabus* L. (28.4%), *Calathus* Bonelli (15.9%), and *Platyderus* Stephens (13.8%); and four Hymenoptera families comprised 93.7% of the parasitoid specimens collected: *Encyrtidae* (61%), *Ichneumonidae* (17.5%), *Pteromalidae* (10.7%), and *Braconidae* (4.5%). Both the natural enemy assemblage composition and the abundance per tree varied between geographical areas, as well as between levels of defoliator infestation. The candidate enemy complex was markedly diverse and abundant in stands not infested by *L. dispar*, where no insecticides had been applied. Our results suggest the importance of generalist predators as natural enemies of *L. dispar.*

Keywords: biocontrol; parasitoids; predators; *Quercus pyrenaica*; *Quercus suber*; spongy moth

Mediterranean *Quercus* L. forests occupy more than 7 million hectares in Spain, these forests having great economic, social, and environmental importance (Vericat et al. 2012). Over the last century, oak forests have regularly experienced episodes of decline across Europe, this affecting a wide range of *Quercus* species, from broadleaf marcescent (i.e. *Quercus pyrenaica* Willd.) to broadleaf sclerophyllous [*Quercus suber* L., *Quercus ilex* L. (Sallé et al. 2014; Gentilesca et al. 2017)]. Among

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biotic stressors contributing to oak decline, the spongy moth, *Lymantria dispar* L. (Lepidoptera: *Erebidae*), is an abundant polyphagous insect species, considered an oak pest of major economic importance in Central Europe and the entire Mediterranean Basin, with *Q. suber* and *Q. ilex* being its preferred host species in the Mediterranean regions (Tiberi et al. 2016; Boukouvala et al. 2022).

Lymantria dispar is a univoltine mid-season defoliator, which is characterised by a fluctuating population, with heavy infestations lasting about 3 years followed by latent periods, ranging from 5–6 years to 10–12 years (Hoch et al. 2006; Tiberi et al. 2016). *Lymantria dispar* is one of the most damaging oak defoliators, a single year being enough to cause almost a complete stand defoliation, subsequently inducing diebacks (Thomas 2008). Since the mid-20th century, there have been recurrent episodes of intense defoliation by *L. dispar* in Andalusia, in particular, in protected areas in Cádiz and Granada provinces, and an integrated pest management (IPM) programme has been running since 1997 (CMA 2106).

The regulation of *L. dispar* dynamics is complex, and not yet fully understood. They are affected by food quality and environmental factors, as well as the incidence of natural enemies (Boukouvala et al. 2022; Zankl et al. 2023). In addition to the damage to forests, *L. dispar* has been considered an underestimated problem in terms of the health implications for both humans and domestic animals associated with established populations of larvae (Boukouvala et al. 2022). The natural enemy complex of *L. dispar* is very diverse in its native Palearctic area, including predators, parasitoids, and pathogens (Hoch et al. 2006; Alalouni et al. 2013; Hajek et al. 2021; Holuša et al. 2021). As predators, ground beetles (Coleoptera: *Carabidae*) are frequently reported, especially the genera *Calosoma* Weber (as a specialist) and *Carabus* L. (as a generalist); and small mammals and birds may also play a role (Vanbergen et al. 2010; Tabaković-Tošić 2012). On the other hand, 125 parasitoids have been identified, 74% from three families of the order Hymenoptera: *Ichneumonidae* (33%), *Braconidae* (22%), and *Eulophidae* (19%); and 19% from the family *Tachinidae* (Diptera) (Boukouvala et al. 2022).

The role of generalist predators in pest control has been undervalued because they do not closely track pest populations (Pekár et al. 2015). In soil ecosystems, carabids contribute to ecosystem stability, acting as predators (mostly generalists) to regulate the abundance of many invertebrates, including pests such as the curculionid *Otiorhynchus sulcatus* Fabricius and the chrysomelid *Altica ampelophaga* Guérin-Méneville (Šerić Jelaska et al. 2014; Gonçalves et al. 2021). Nonetheless, carabid predation on Lepidoptera remains little explored, except in the case of *Calosoma* beetles. By analysing gut contents, Šerić Jelaska et al. (2014) found that 14 of 23 carabid species studied predated on Lepidoptera, with *Carabus violaceus* L. (43%), *Carabus convexus* Fabricius (33%), and *Nebria brevicollis* Fabricius (19.5%) being among the species that most commonly showed this feeding behaviour.

Parasitoids have been considered the most notable natural enemies of lepidopteran pests, mainly those belonging to the orders Hymenoptera and Diptera (Salim et al. 2016). *Tachinidae* is the largest and most important parasitoid family of Diptera, and it is frequently cited as the only dipteran family parasitising *L. dispar* (Alalouni et al. 2013; Zankl et al. 2023). Most parasitoids act in a density-dependent manner; e.g. the tachinids *Parasetigena silvestris* Robineau-Desvoidy and *Blepharipa pratensis* Meigen have important parasitic activity when host densities are high, while ichneumonid and braconid wasps are more relevant in periods of low host population densities (Alalouni et al. 2013).

Over the last decade, pest management organisations in South and Central Europe (Spain, France, Greece, Slovakia, Czech Republic, Germany) have employed the bacterial pathogen *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*) and a broad spectrum of insecticides, e.g. formulations based on diflubenzuron or tebufenozide, to control *L. dispar* (Golemansky et al. 2010; Boukouvala et al. 2022). The effect of these practices on non-target species remains a matter of debate. Buckner et al. (1974) reported a decline in *Carabidae* catches in pitfall traps after a *Btk* treatment, while Cameron and Reeves (1990) found no significant reduction in *Carabidae* catches in *Btk*-sprayed plots. Golemansky et al. (2010) argued that these practices, particularly the use of chemical pesticides, are insufficiently specific to not affect many other non-target insect species and aquatic organisms and, in turn, negatively affect forest biodiversity.

This study aimed to increase our knowledge of entomological diversity in *Quercus* forests affected by *L. dispar*, particularly concerning taxa that could act as natural enemies for various developmental stages of this defoliator, especially its larvae. In addition, we compared the entomological diversity (richness and evenness) of the candidate natural enemy assemblage for *L. dispar* between stands with different population levels of this lepidopteran species, these stands also having been exposed to different *L. dispar* management policies. We hypothesised that the enemy complex of *L. dispar* varies in richness and evenness between ecologically different areas (Thermo- and low Meso-Mediterranean bioclimatic stages in Cádiz, and upper Meso- and Supra-Mediterranean stages in Granada), these differences being also influenced by the level of threat and management strategy of the forest stands. Our objectives were (*i*) to assess differences in diversity of the candidate enemy complex for *L. dispar* between *Quercus* forests from two ecologically different areas (Cádiz and Granada), and (*ii*) to analyse whether the level of infestation by *L. dispar* influenced the diversity of the candidate enemy assemblage.

MATERIAL AND METHODS

Study area. This study was carried out in two protected areas: Alcornocales Natural Park (Cádiz province) and Sierra Nevada Natural Space (Granada province), in Andalusia, Spain (Figure 1).

The candidate enemy complex for *L. dispar*, comprising predators (Coleoptera: *Carabidae*) and parasitoids (Hymenoptera, and Diptera: *Tachinidae*), was studied in a total of 10 *Quercus* stands included in the IPM programme for *L. dispar* in Andalusia: six in Cádiz (Ca1–Ca6) and four in Granada (Gr1–Gr4), see Table 1 and Figures 1, 2. We studied eight stands in 2010 (four stands in each province) and two in 2011 (both in Cádiz). The mean elevation above sea level of the stands was 392.7 m a.s.l.

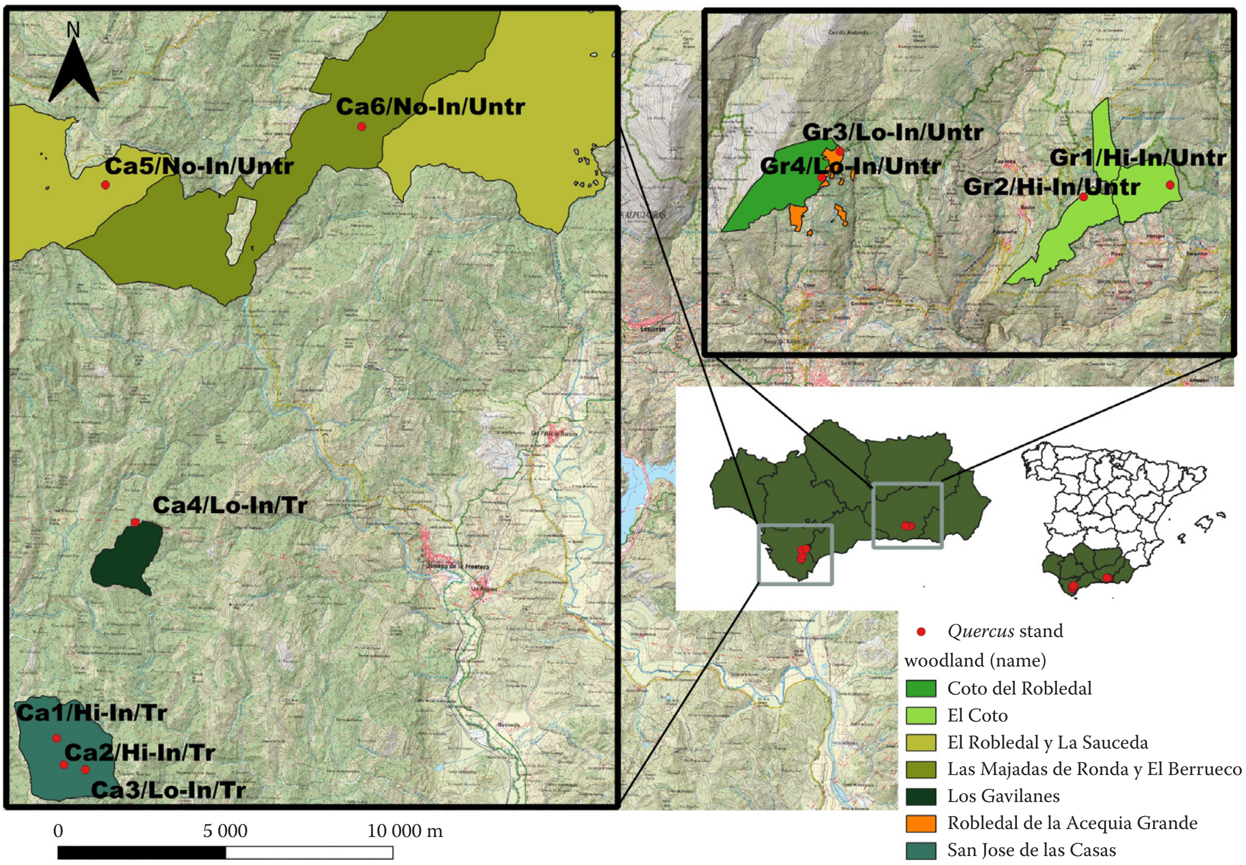

Figure 1. Location of the 10 *Quercus* stands studied to compare the diversity of the candidate enemy assemblage for *Lymantria dispar* in Cádiz (Ca) and Granada (Gr) provinces (Andalusia – dark green area)

Hi-In, Lo-In, No-In – high, low, and no infestation by *L. dispar*, respectively; Tr – treated with insecticide; Untr – untreated

Table 1. Forest stands studied to assess the candidate enemy complex for *Lymantria dispar* in Cádiz and Granada provinces (Andalusia)

Stand (code)	Woodland (name)	UTM (*X*/*Y*)	Province	Dominant tree species	Canopy coverage (%)	Elevation range (m a.s.l.)
Ca1/Hi-In/Tr	San José de las Casas	268976/4029229	Cádiz	*Quercus suber*	68	332–354
Ca2/Hi-In/Tr	San José de las Casas	269833/4028288	Cádiz	*Quercus suber*	71	347–369
Ca3/Lo-In/Tr	San José de las Casas	269191/4028439	Cádiz	*Quercus suber*	77	384–401
Ca4/Lo-In/Tr	Los Gavilanes	271321/4035715	Cádiz	*Quercus suber*	78	465–485
Ca5/No-In/Untr	El Robledal y La Sauceda	270432/4045849	Cádiz	*Quercus suber*	76	579–583
Ca6/No-In/Untr	Las Majadas de Ronda y El Berrueco	278085/4047597	Cádiz	*Quercus suber*	51	585–603
Gr1/Hi-In/Untr	El Coto	472752/4090067	Granada	*Quercus ilex*, *Quercus pyrenaica*	52	1 653–1 692
Gr2/Hi-In/Untr	El Coto	470163/4089712	Granada	*Quercus ilex*, *Quercus pyrenaica*	38	1 806–1 832
Gr3/Lo-In/Untr	Robledal de la Acequia Grande	462894/4091066	Granada	*Quercus pyrenaica*	43	1 764–1 797
Gr4/Lo-In/Untr	Coto del Robledal	462342/4090290	Granada	*Quercus pyrenaica*	25	1 761–1 774

Ca – Cádiz; Gr – Granada; Hi-In, Lo-In, No-In – high (5 to 50 egg clusters/tree), low (< 5 egg clusters/tree), and no infestation by *Lymantria dispar*, respectively, in accordance with the integrated pest management (IPM) programme in Andalusia (CMA 2016); Tr – treated with insecticide; Untr – untreated; UTM – Universal Transverse Mercator coordinates; UTM coordinates correspond to European Datum 1950, zone 30N

± 58.9 m a.s.l. in Cádiz and 1 760.5 m a.s.l. ± 60.9 m a.s.l. in Granada. All were oak stands populated by mature trees, dominated by *Q. suber* in Cádiz, and either *Q. pyrenaica* or *Q. ilex* plus *Q. pyrenaica* in Granada. In the Cádiz stands, the understory was scarce (< 20% coverage overall), attributable to the frequent shrub clearing to facilitate cork harvesting (every 9 years), with

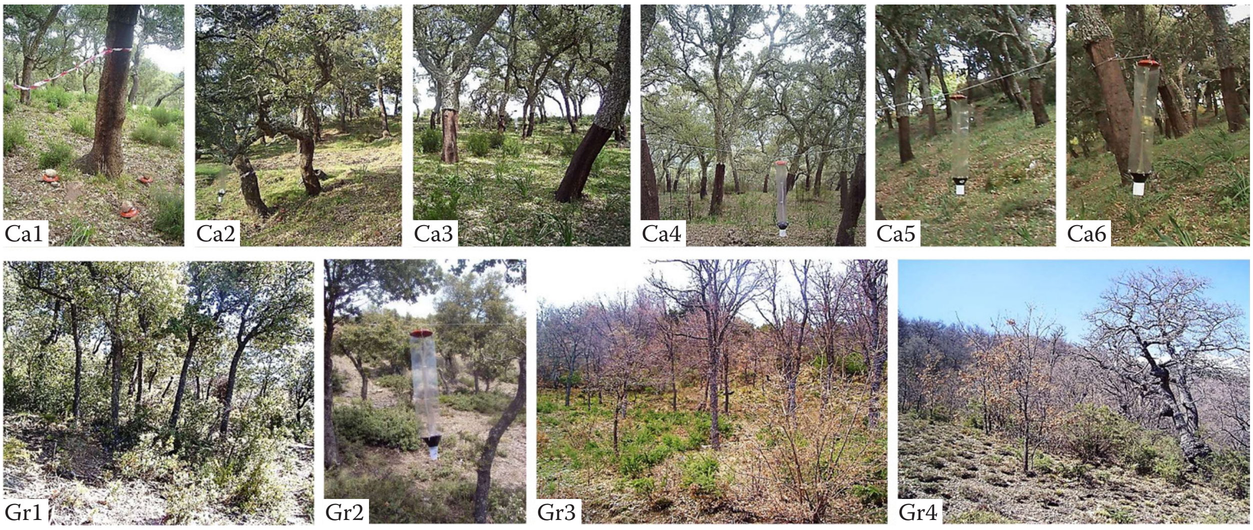

Figure 2. *Quercus* stands studied to assess the candidate enemy complex for *Lymantria dispar* in Cádiz (Ca1 to Ca6) and Granada (Gr1 to Gr4) provinces (Andalusia)

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a dominance of *Asphodelus ramosus* L. *and Erica arborea* L. The Granada stands had more dense understory coverage (40–60% overall), dominated by *Adenocarpus decorticans,* with *Thymus mastichina* L. in Gr4. Average annual temperatures ranged from 17.1 °C to 17.6 °C in 2010 and 16.8 °C to 17 °C in 2011 in Cádiz; and from 10.1 °C to 11.1 °C in Granada (Figure 3). Annual precipitation ranged from 1 877 mm to 1 904 mm in 2010 and 923 mm to 931 mm in 2011 in Cádiz; and from 1 166 mm to 1 236 mm in Granada (Figure 3).

To analyse the influence of the level of infestation by *L. dispar* on abundance (activity density) and diversity (richness and evenness) of the candidate enemy complex for this defoliator, the four stands within each protected area studied in 2010 were distributed in line with the IPM programme for *L. dispar* in Andalusia (CMA 2016): two stands located in forests highly infested by *L. dispar* and another two stands in low-infested forests (Table 1). Policies for *L. dispar* management also varied across stands. Granada stands had no aerial application of insecticides against this lepidopteran, regardless of the infestation level. In Cádiz, four stands (Ca1 to Ca4, studied in 2010) were aerially treated against first instar larvae (every 1–3 years, depending on the infestation level) with diflubenzuron 25% *w*/*w* (wettable powder formulation; mixed with water and applied at a dose of 300 g·ha^{-1}), while two stands (Ca5 and Ca6, studied in 2011) had not been treated in at least the last 10 years (Table 1).

Predatory and parasitic insects collection. The activity density of the insect groups was analysed by randomly establishing two trapping transects spaced 30 m apart at each stand, and randomly selecting five trees (one approximately every 30 m) along each transect. Trapping transects were located at least at 50–80 m apart from forest edges. Then, at each stand, we installed two trap types: pitfall traps (4 per tree), and custom-made cross-vane transparent intercept traps (10 per stand). Pitfall traps were installed in all 10 trees selected in each stand (a total of 40 traps), placed around the base of the trunk (50 cm away), one in each cardinal direction (100–130 cm apart from each other). Cross-vane traps were hung from ropes (80–120 cm above ground), between each tree with pitfall traps and an adjacent tree (Figure 2). The overall trapping period in 2010 and 2011 covered the previously established period of *L. dispar* larval activity

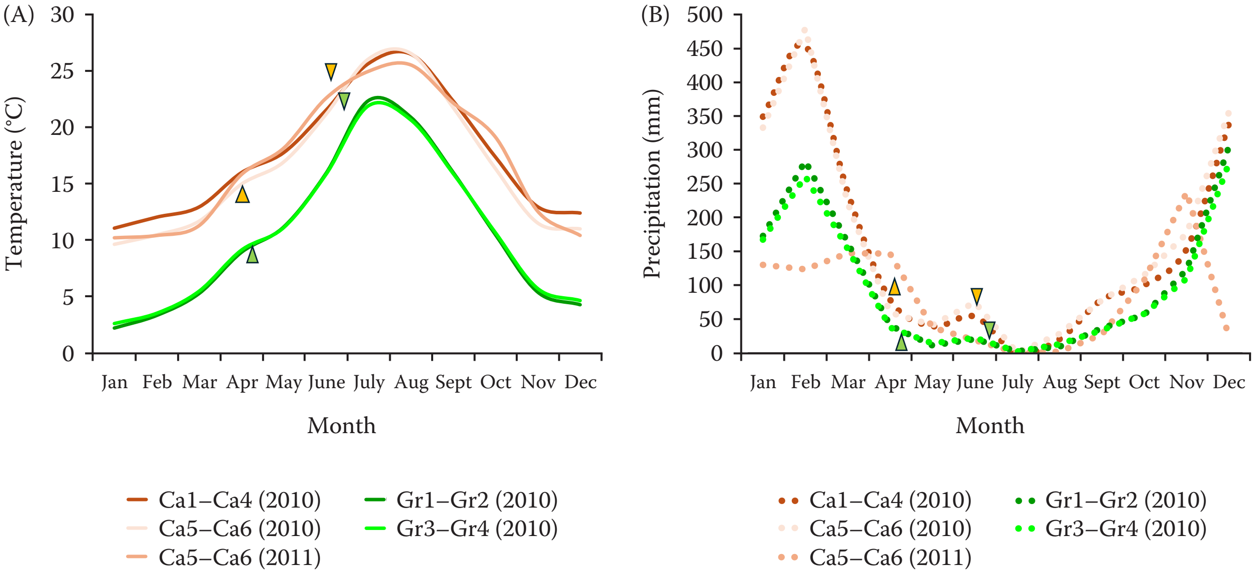

Figure 3. (A) Average temperature and (B) precipitation data in the studied stands in Cádiz (Ca) and Granada (Gr) provinces (Andalusia)

Arrows – indication of periods of *Lymantria dispar* larval activity; orange – Cádiz; green – Granada

Source: Data provided by the Environmental Information Network of Andalusia (REDIAM): Lomas de Camara station (UTM 274207/4033262; 510 m a.s.l.) for Ca1–Ca4, and El Marrufo station (UTM 270162/4048616; 422 m a.s.l.) for Ca5 and Ca6; Capileira station (UTM 468428/4093369; 1 590 m a.s.l) for Gr1 and Gr2, and Los Bancalillos-Soportújar station (UTM 464409/4088846, 1 610 m a.s.l.) for Gr3 and Gr4 in each protected area: 19 April to 20 June in Cádiz, and 27 April to 27 June in Granada (CMA 2016). In 2011, the trapping period was extended in the two untreated Cádiz stands (Ca5 and Ca6) to 18 July, to also cover most of the pupal and flight periods of this defoliator (CMA 2016).

The standard pitfall equipment consisted of cups (9.4 cm top/6.2 cm bottom diameter, 9.4 cm depth) sunk into the ground with the rim flush with the soil surface and covered with plastic lids raised 4 cm over cup tops. Cups were filled with a propylene glycol solution (50% *v*/*v*). Custom-made cross-vane traps consisted of two crossed transparent methacrylate panels (2 mm thick, 100 cm long, 20 cm wide) placed over a 20 cm diameter funnel that led catches to a 1 L cylindrical high-density polyethylene container. Catches in both trap types were collected weekly and sorted into genera (for the *Carabidae* family) and families (for Hymenoptera and Diptera orders) as appropriate taxonomic categories for statistical analysis.

Statistical analysis. Based on the literature, our study was focused on the candidate enemy assemblage for *L. dispar*, comprising *Carabidae* (as predators), and Hymenoptera and Diptera (as parasitoids). To reduce the number of variables, *Carabidae* genera comprising less than 1% of all zoophagous *Carabidae* catches, and Hymenoptera families representing less than 0.5% of all parasitoid hymenopteran catches were excluded from the analysis (33 and 15 individuals being excluded, respectively). Within the Diptera order, only the major parasitoid family *Tachinidae* was analysed (Tabaković-Tošić 2012; Salim et al. 2016; Zankl et al. 2023).

All the statistical analysis was performed in R software (Version 4.3.0, 2023), with a significance level of $\alpha = 0.05$. Silhouettes in figures were inserted with rphylopic package (Gearty, Jones 2023).

Non-metric multidimensional scaling (NMDS) was used to perform an exploratory analysis of the composition of the candidate enemy complex for *L. dispar*, across the 10 *Quercus* stands studied. Log-chord abundance-based distance matrices were computed due to their capacity to deal with double-zero cases (Legendre, Borcard 2018). Two environmental factors (*Elevation*, expressed in m a.s.l.) and canopy cover percentage (*Ccover*) were fitted to the NMDS model, to check for environmental gradients on the NMDS ordination. Differences in composition of the candidate enemy assemblage between stands (pooled datasets for each pair of stands with the same infestation level) were tested by permutational multivariate analysis of variance (PERMANOVA; log-chord distance, 999 permutations) if the multivariate dispersion was homogeneous among study groups (Vegan package – Oksanen et al. 2015); and otherwise, by robust Welch-based multivariate analysis of variance, using log-chord distances (MANOVA: W_d^* test, 999 permutations; Hamidi et al. 2019). MANOVA analyses were followed by one-versus-all post-hoc comparisons (i.e. testing each study group versus all others pooled), with false discovery rate correction for multiple testing (Hamidi et al. 2019). As classifying variables, we used *L. dispar* 'infestation' level and studied 'stand', and also the 'infestation × stand' interaction term.

To investigate the diversity of the candidate enemy assemblage for *L. dispar* in detail, we employed three diversity Hill metrics (MeanRarity package – Roswell, Dushoff 2020): species richness, Hill-Shannon and Hill-Simpson indices. The combination of these indices is considered to describe the diversity of a community more fully, as each has a different emphasis: rare taxa (richness), more common taxa (Hill-Simpson), or the evenness of taxa without focusing on either rare or common taxa [Hill-Shannon (Roswell et al. 2021)]. For this purpose, we standardised samples using the coverage method proposed by Roswell et al. (2021); this procedure mitigates sampling limitations in biodiversity assessment, by determining the expected number of studied taxa from a random subsample of individuals from the overall datasets.

To further test the effect of the stands and the level of infestation by *L. dispar* on the natural enemy complex, we analysed differences for both taxa activity density (abundance) and richness within each *Carabidae* and parasitoid assemblage dataset. As dependent variables, we computed for each tree ($N = 4$ traps per tree), for either of any carabid genera or families of the Hymenoptera and Diptera orders, the pooled abundance (i.e. the total number of insects caught) and the richness (i.e. the number of different taxa); catches in each vane trap were assigned to the nearest tree having pitfall traps. As independent variables, we used the studied 'stand' and the *L. dispar* 'infestation' level, including the interaction term 'infestation × stand'. After comparing several models with Akaike's information criterion, we fitted generalised linear models (GLMs) for count data, either using a negative binomial distribution for the *Carabidae* dataset and a Poisson distribution (log-link) for the parasitoid assemblage dataset (MASS package – Venables 2002). The dispersion and heteroscedasticity of selected models were analysed with the DHARMa package (Hartig 2022). The significance of effects was assessed using likelihood ratio tests, followed by pairwise comparisons with Tukey's method for P-value adjustment.

RESULTS

Overall catches. Overall, we obtained 2 565 specimens from the *Carabidae* family (24 genera) and 4 549 specimens from the Hymenoptera orders (14.2%, 20 families), and Diptera (85.8%, 23 families). The majority of specimens were collected in Cádiz (98.6% for *Carabidae*, 85.3% for Hymenoptera and Diptera). Nearly two-thirds (65.8%) of all *Carabidae* catches were zoophagous species (Vanbergen et al. 2010), while 60.4% of the hymenopteran catches were from families with potential parasitic activity against *Quercus* pests. The *Tachinidae* family (Diptera) accounted for 4.9% of all parasitoid dipterans collected (Table 2).

Relative catches of over 1% of the zoophagous *Carabidae* group were found for eight genera (summing to 1 655 individuals; Table 2). The four most common genera pooled comprising 92.2% of the total: *Steropus* Dejean (34.1%), *Carabus* (28.4%), *Calathus* Bonelli (15.9%), and *Platyderus* Stephens (13.8%). Most of the carabids were caught in pitfall traps, except for *Microlestes* Schmidt-Goebel, which was mainly caught in cross-vane traps (88%). Within Hymenoptera, nine families had catches that accounted for over 0.5% of all parasitic hymenopterans collected, summing to 400 individuals (Table 2). The four most common families together comprised 93.7% of the total: *Encyrtidae* (61%), *Ichneumonidae* (17.5%), *Pteromalidae* (10.7%), and *Braconidae* (4.5%). Almost all Hymenoptera and Diptera: *Tachinidae* parasitoids were caught in cross-vane traps.

The activity intensity for the four main *Carabidae* (plus *Calosoma*) and Hymenoptera families (plus Diptera: *Tachinidae*) showed different seasonal patterns between provinces (Figure 4), especially for *Carabidae*, with *Calathus* peaks being found sooner in Granada than in Cádiz; the opposite occurred to *Steropus* catches. Notably, in untreated Cádiz stands (Ca5 and Ca6, studied in 2011), the peak periods of *Steropus*, *Calathus* and, interestingly, also *Calosoma* carabids, besides that of *Encyrtidae* and *Braconidae* families, may have extended beyond the overall larval period of *L. dispar* (April to June).

Comparing meteorological data during the trapping period in 2011 with those for the same period in 2010, temperatures were similar, but precipitation patterns differed markedly in not infested Cádiz stands (Figure 3): annual average precipitation in 2011 was near the lower limit of the overall range reported for Alcornocales Natural Park (1 000 mm to > 1 500 mm), while it was exceptionally high in 2010 (1 800 mm on average).

Community composition of predatory carabids and parasitic hymenopterans and dipterans. All three NMDS models were significant (non-metric fit: $P < 0.001$ overall; Figure 5), and revealed overall differences between stands in the composition of the candidate enemy complex for *L. dispar*, considering the two faunistic groups together ($R^2 = 0.87$; Figure 5A; MANOVA: $W_d^* = 7.79$, $P = 0.001$); or separately for predators ($R^2 = 0.82$; Figure 5B) and parasitoids ($R^2 = 0.89$; Figure 5C) (MANOVA: $W_d^* < 13.01$, $P = 0.001$, for both datasets). More specifically, the composition of the carabid beetle assemblage was not significantly affected, either in Cádiz or Granada, by 'infestation level' (Hi-In – high infestation *versus* Lo-In – low infestation categories) or 'stand', or their interaction ('infestation × stand') (Figure 5B; PERMANOVA: $F < 1.56$, $P > 0.164$, overall). In contrast, the parasitoid assemblage composition in Granada differed between high and low infestation categories (PERMANOVA: $F = 2.40$, $P = 0.046$); and in Cádiz, both main factors had a marginally significant effect on the parasitoid assemblage composition, while the 'infestation × stand' interaction was significant (PERMANOVA: $F = 4.59$, $P = 0.009$). The two environmental vectors, *Elevation* and *Ccover,* had opposite gradients, with Granada stands having higher *Elevation* values (mean ± standard error) than Cádiz (1 760.5 m ± 30.5 m and 457.7 m ± 45.2 m, respectively), but lower *Ccover* (39.3% ± 5.74% and 70.1% ± 4.1%, respectively).

Diversity profiles. Stands with low infestation had higher values overall for the three Hill diversity indices than those with high infestation (Figure 6), which indicates higher diversity in stands with lower *L. dispar* infestation levels regardless of the protected area studied. The lowest diversity was found

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Table 2. Catches in pitfall and cross-vane traps of Coleoptera: *Carabidae*, Hymenoptera, and Diptera: *Tachinidae*, individuals, in 10 *Quercus* stands in Cádiz and Granada provinces (Andalusia)

Taxa	Ca1 Hi-In Tr	Ca2 Hi-In Tr	Ca3 Lo-In Tr	Ca4 Lo-In Tr	Ca5 No-In Untr	Ca6 No-In Untr	Gr1 Hi-In Untr	Gr2 Hi-In Untr	Gr3 Lo-In Untr	Gr4 Lo-In Untr	Total
Carabidae											
(*N*) individuals	29	131	120	91	367	885	5	3	12	12	1 655
(*N*) genera	6	6	7	6	7	5	1	3	5	4	–
Calosoma	–	–	–	–	10	–	–	–	8	–	18
Carabus	9	51	49	18	200	146	–	1	1	–	475
Steropus	4	24	20	21	139	360	–	1	1	1	571
Platyderus	9	18	22	12	5	179	–	–	–	–	245
Calathus	1	31	10	19	2	195	–	1	1	6	266
Pseudoophonus	5	4	5	2	–	–	–	–	–	–	14
Microlestes	–	–	3	–	4	–	5	–	–	4	16
Philorhizus	1	3	11	19	7	5	–	–	1	1	48
Hymenoptera											
(*N*) individuals	23	24	22	32	108	115	8	8	23	37	400
(*N*) families	3	4	6	5	8	5	3	2	5	6	–
Bethylidae	–	–	1	–	1	2	–	–	–	–	4
Ceraphronidae	–	–	–	–	–	–	–	–	2	1	3
Encyrtidae	19	15	10	17	65	69	5	7	15	22	244
Eulophidae	–	1	1	2	3	–	–	–	–	–	7
Eupelmidae	1	–	–	–	1	–	1	–	–	1	4
Pteromalidae	–	3	2	6	9	13	2	1	1	6	43
Trichogrammatidae	3	–	–	–	2	–	–	–	2	–	7
Braconidae	–	–	2	1	4	6	–	–	3	2	18
Ichneumonidae	–	5	6	6	23	25	–	–	–	5	70
Diptera											
(*N*) individuals	0	18	8	10	22	7	0	1	2	0	68
Tachinidae	0	18	8	10	22	7	0	1	2	0	68
Total											
(*N*) individuals	52	173	150	133	497	1 007	13	12	37	49	2 123
(*N*) taxa	9	10	13	11	15	10	4	5	10	10	97

Ca – Cádiz; Gr – Granada; Hi-In, Lo-In, No-In – high (5 to 50 egg clusters/tree), low (< 5 egg clusters/tree), and no infestation by *Lymantria dispar*, respectively, in accordance with the integrated pest management (IPM) programme in Andalusia (CMA 2016); Tr – treated with insecticide; Untr – untreated; *N* – number; stands differed by *Lymantria dispar* infestation level and exposure to insecticides used against *L. dispar*

in Granada Hi-In stands overall (Figure 6A–C, Figure 6G–I).

For the whole enemy assemblage, Cádiz Hi-In (Ca1, Ca2) and Lo-In (Ca3, Ca4) stands had higher values for both Hill-Simpson and Hill-Shannon indices than Granada stands (Gr1–Gr4; Figure 6A). In addition, Cádiz No-In (no infestation) stands (Ca5, Ca6) obtained the highest richness value (25 taxa; Table 2), but low Hill-Simpson values, which indicates a low evenness (Hill-Shannon) in these stands, and this is attributable to the dominance of a few taxa, namely, *Carabus* and *Steropus*

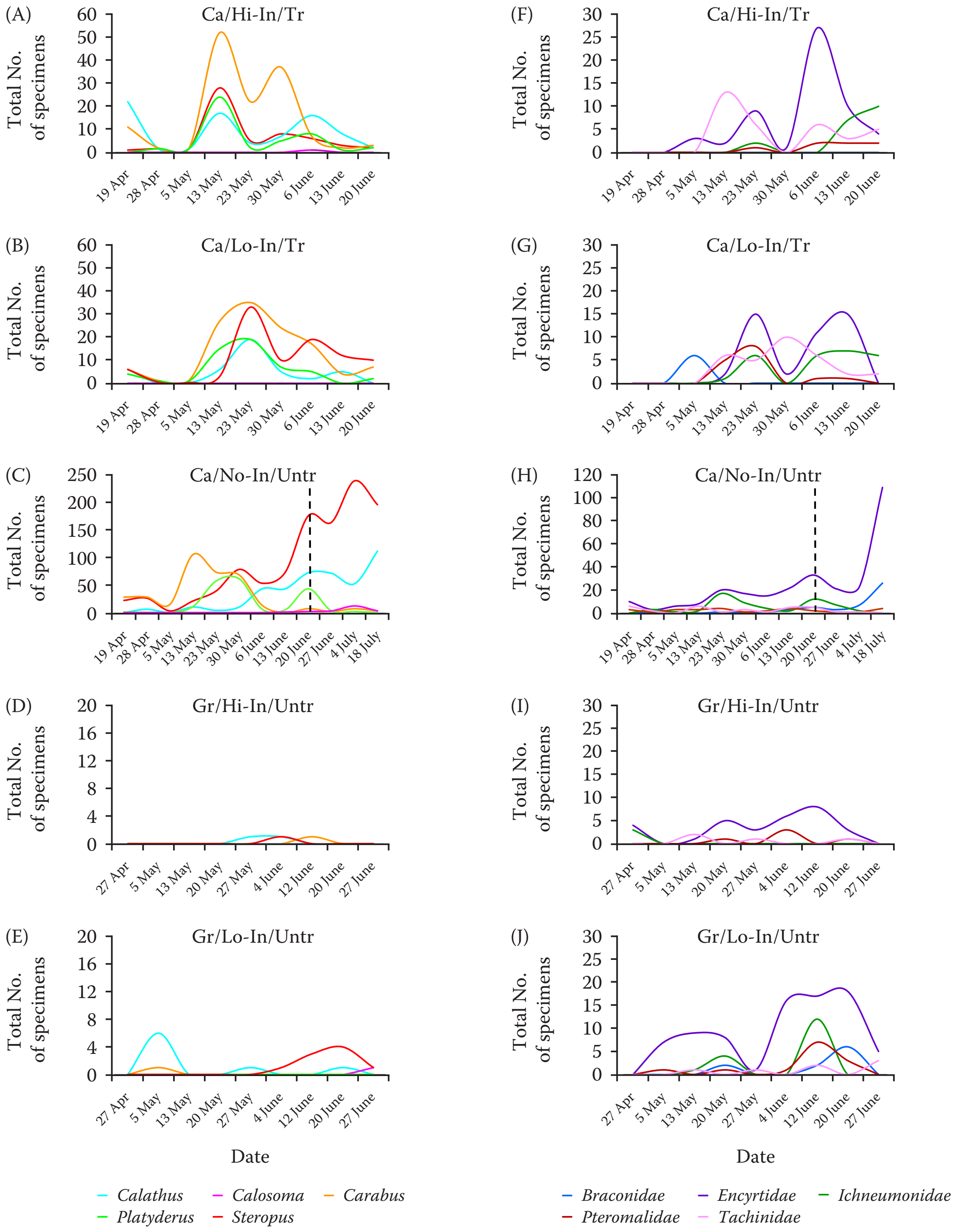

Figure 4. Seasonal activity of (A–E) the most abundant *Carabidae* genera (*Steropus*, *Carabus*, *Calathus*, and *Platyderus*) and the *Calosoma* genus; and (F–J) the most abundant hymenopteran families (*Encyrtidae*, *Ichneumonidae*, *Pteromalidae*, and *Braconidae*) and the dipteran family (*Tachinidae*); collected in pitfall and cross-vane traps in *Quercus* stands in (A–C and F–H) Cádiz and (D–E and I–J) Granada provinces (Andalusia)

Dashed lines – the end of the larval period in Cádiz not infested stands (C, H); Ca – Cádiz; Gr – Granada; Hi-In, Lo-In, No-In – high, low, and no infestation by *L. dispar*, respectively; Tr – treated with insecticide; Untr – untreated; catches are pooled for each pair of stands with the same infestation level by *Lymantria dispar*

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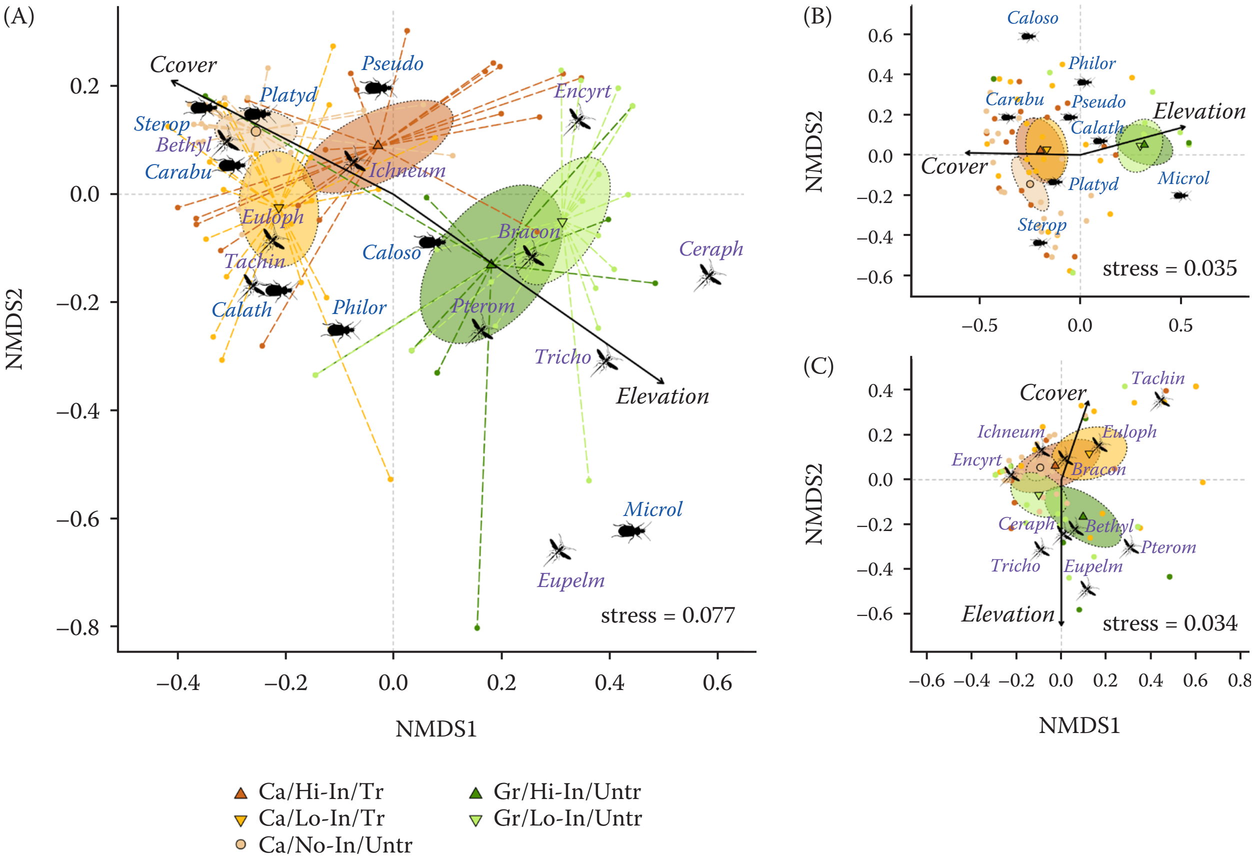

Figure 5. Non-metric multidimensional scaling (NMDS) ordination plots (log-chord abundance-based distances), showing variation in (A) the composition of the candidate enemy complex for *Lymantria dispar*, including predators (*Carabidae*, blue font) and parasitoids (Hymenoptera, and Diptera: *Tachinidae*, purple font); (B) the *Carabidae* composition; and (C) the Hymenoptera and Diptera (*Tachinidae*) composition across 10 *Quercus* stands in Cádiz and Granada provinces (Andalusia)

Ca – Cádiz; Gr – Granada; Hi-In, Lo-In, No-In – high, low and no infestation by *L. dispar*, respectively; Tr – treated with insecticide; Untr – untreated; *Bethyl – Bethylidae; Bracon – Braconidae; Calath – Calathus; Caloso – Calosoma; Carabu – Carabus; Ceraph – Ceraphronidae; Encyrt – Encyrtidae; Euloph – Eulophidae; Eupelm – Eupelmidae; Ichneu – Ichneumonidae; Microl – Microlestes; Philor – Philorhizus; Platyd – Platyderus; Pseudo – Pseudoophonus; Pterom – Pteromalidae; Sterop – Steropus; Tachin – Tachinidae; Tricho – Trichogrammatidae*; to improve clarity, data were pooled for each pair of stands in each category of infestation by *Lymantria dispar*

(*Carabidae*) and the family *Encyrtidae* (Hymenoptera; Figure 6D–F; Table 2). The nearly horizontal profiles found for the *Carabidae* community in some Granada stands (Figure 6H) are a consequence of the markedly low values for both richness and abundance (< 5 taxa and < 13 individuals, overall; Table 2).

Effect of stand and *Lymantria dispar* infestation level on mean abundance and richness of natural enemies per tree. The average number of candidate enemy individuals collected per stand was markedly higher in Cádiz than in Granada: 33.8 times for *Carabidae*, 2.8 times for Hymenoptera, and 14.4 times for *Tachinidae* (Diptera; Table 2). Differences in abundance and richness per tree between stands with high and low infestation were not significant both in Cádiz and Granada, for either predatory carabids or parasitoids assemblage (Table 3). In contrast, 'stand' had a significant effect on both *Carabidae* and the parasitoid assemblage abundance per tree, and this affected the influence of 'infestation level' on the number of carabid beetles caught into pitfall traps (i.e. there was a significant 'infestation × stand' interaction – Table 3, Figure 7); the 'infestation × stand' interaction was also significant for

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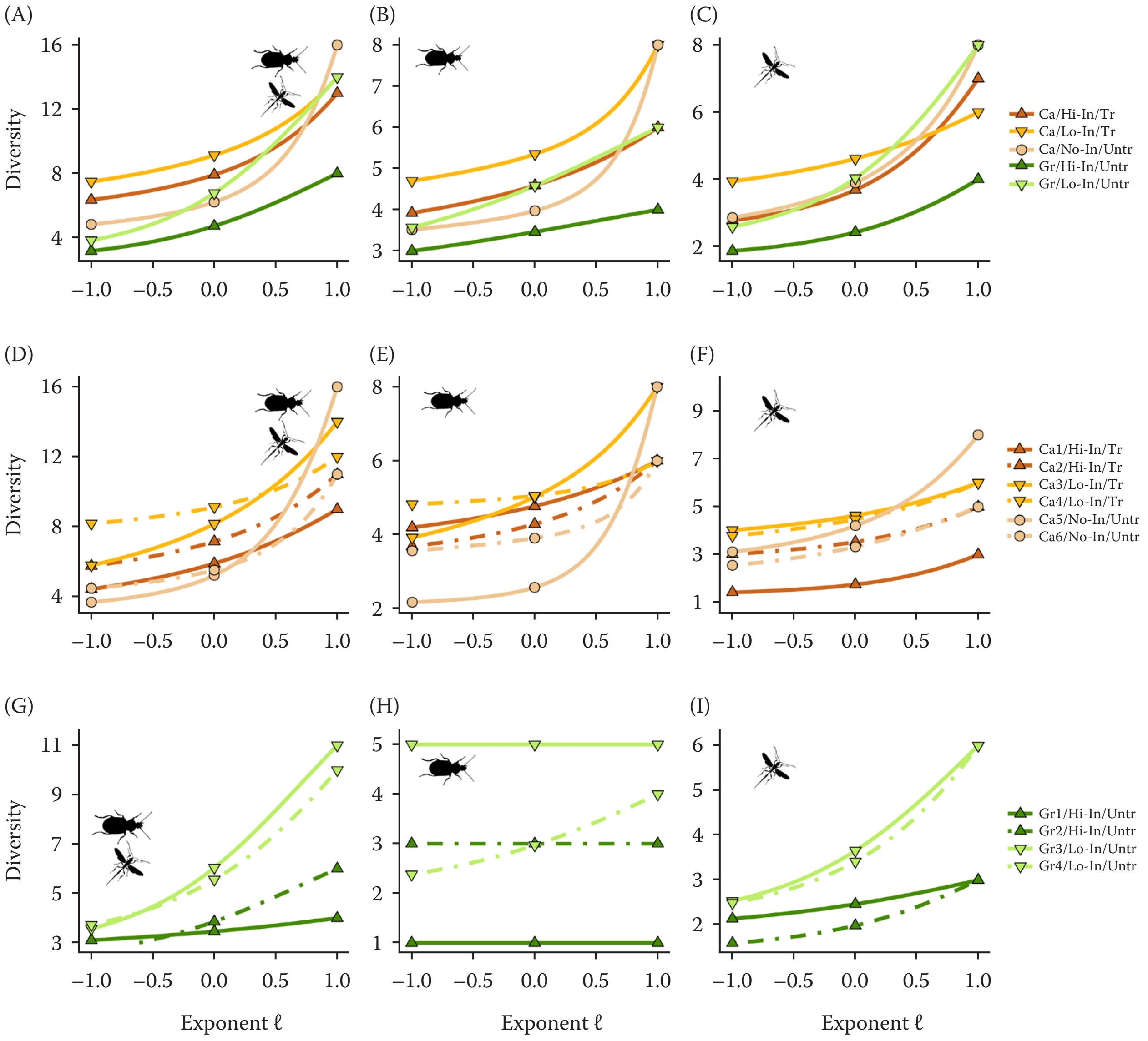

Figure 6. Sample diversities for the candidate enemy complex for *Lymantria dispar* (*Carabidae*, Hymenoptera, and Diptera: *Tachinidae*) in 10 *Quercus* stands in Cádiz and Granada provinces (Andalusia)

Ca – Cádiz; Gr – Granada; Hi-In, Lo-In, No-In – high, low and no infestation by *L. dispar*, respectively; Tr – treated with insecticide; Untr – untreated; the y-axis is the value for each diversity index, as calculated from raw samples; on the x-axis, exponent ℓ = 1 represents the taxa richness index (i.e. the number of rare taxa), ℓ = –1 is the Hill-Simpson index (i.e. the number of common taxa), and ℓ = 0 is the Hill–Shannon index (i.e. taxa evenness)

Carabidae richness per tree. In Granada, only the effect of 'stand' on the parasitoid assemblage was significant, this affecting both abundance and richness per tree (Table 3).

The highest abundance for the whole enemy complex was found in untreated No-In stands in Cádiz (Figure 7), where the pooled abundance in these two stands (Ca5 and Ca6) surpassed that obtained for the sum of the four treated stands (Ca1 to Ca4): 3.5 times for *Carabidae*, and 1.8 times for the parasitoid assemblage. Significant differences between stands were also found in the abundance per tree for each of the four most relevant *Carabidae* genera (*Steropus*, *Carabus*, *Calathus*, and *Platyderus*) and Hymenoptera families (*Encyrtidae*, *Ichneumonidae*, *Pteromalidae*, and *Braconidae*), analysed separately (negative binomial GLM: deviance > 11.38; $P < 0.001$ overall). No significant between-stand differences were found for Diptera: *Tachinidae* (Figure 7).

Table 3. Effects of stand studied and *Lymantria dispar* infestation level (high/low; *N* = 4 stands/province) on abundance and richness per tree (*N* = 10) of the candidate enemy complex for the defoliator, comprising predators (Coleoptera: *Carabidae*), and a parasitoid assemblage (Hymenoptera plus Diptera: *Tachinidae*)

Area and model term	Abundance				Richness			
	Carabidae		parasitoids		*Carabidae*		parasitoids	
	deviance (NB)	*P*-value	deviance (NB)	*P*-value	deviance (Poisson)	*P*-value	deviance (Poisson)	*P*-value
Cádiz								
Stand	10.82	**0.001**	6.99	**0.008**	2.53	0.111	3.17	0.074
Infestation	0.66	0.420	0.44	0.510	0.44	0.509	1.18	0.275
Infestation × stand	26.33	**< 0.001**	0.57	0.451	5.44	**0.019**	0.05	0.824
Granada								
Stand	3.29	0.069	19.45	**< 0.001**	3.48	0.062	7.48	**0.006**
Infestation	0.00	0.995	1.05	0.305	0.02	0.885	1.19	0.274
Infestation × stand	0.35	0.552	0.19	0.662	0.01	0.949	0.20	0.652

N – number; NB – negative binomial generalised linear models; Poisson – Poisson (log-link) generalised linear models; individuals were collected in pitfall (*N* = 4 per tree, catches pooled) and cross-vane (*N* = 10) traps, in each *Quercus* stand in Cádiz and Granada provinces (Andalusia); bold – significant differences

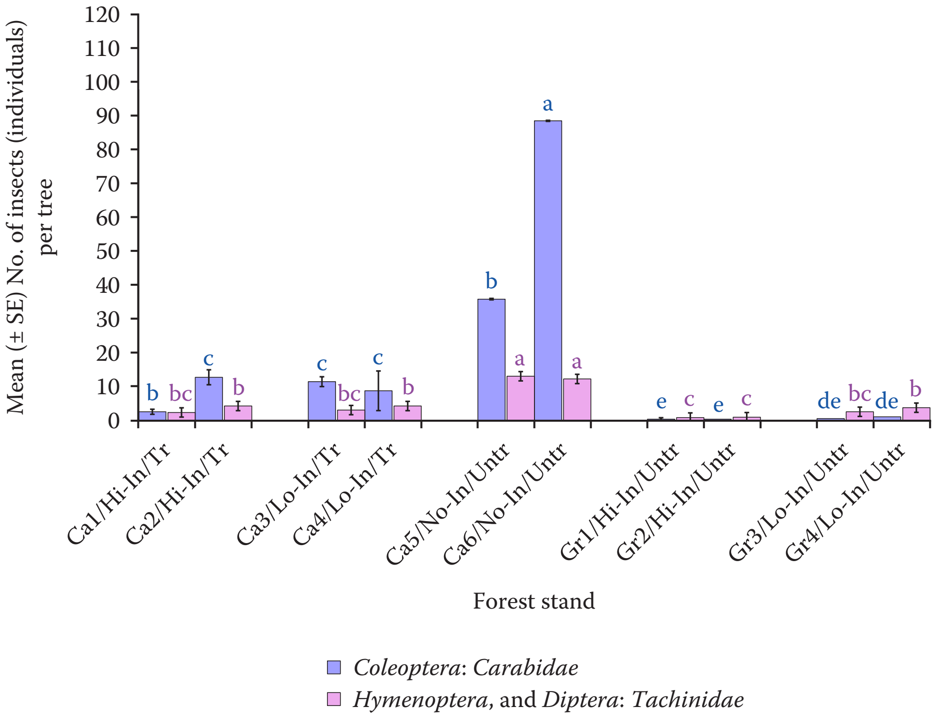

Figure 7. Mean abundance per tree (± standard error; *N* = 10 per stand) of the candidate enemy complex for *Lymantria dispar* (*Carabidae*, Hymenoptera, and Diptera: *Tachinidae*)

a–e – indication of significant differences (analysis of deviance for negative binomial general linear models, followed by pairwise comparisons with Tukey's adjustment; $P < 0.033$ for *Carabidae*, and $P < 0.040$ for the parasitoid assemblage, overall); Ca – Cádiz; Gr – Granada; Hi-In, Lo-In, No-In – high, low, and no infestation, respectively; *N* – number; SE – standard error; Tr – treated with insecticide; Untr – untreated; catches obtained in pitfall and cross-vane traps in 10 *Quercus* stands in Cádiz and Granada provinces (Andalusia)

DISCUSSION

Lymantria dispar is one of the most challenging forest pests, both across its natural range of distribution and in non-native areas. Currently, the management of *L. dispar* is mainly based on biopesticides, and biocontrol agents such as entomopathogenic microorganisms (Golemansky et al. 2010; Hajek et al. 2021; Holuša et al. 2021; Leroy et al. 2021; Boukovala et al. 2022). On the other hand, *L. dispar* has a wide complex of natural enemies, which have a potential role in IPM; however, their use, especially parasitoids, has not often been proposed in IPM programmes for this defoliator in Europe (Alalouni et al. 2013; Boukovala et al. 2022). In our study, we found several *Carabidae* genera and parasitoid families (mainly Hymenoptera) that are known to include natural enemies for *L. dispar*, with both the composition and diversity of this candidate enemy complex, and the activity density per tree, varying between ecologically different geographical areas as well as between forest stands with different levels of infestation by this lepidopteran.

Ground beetles (Coleoptera: *Carabidae*) play an important role as regulators of prey abundance in many ecosystems, with adults of some species being able to climb into shrubs and trees in search of prey (Lövei, Sunderland 1996; Šerić Jelaska et al. 2014; Gonçalves et al. 2021). Ecological processes governing the diversity of zoophagous carabids have been found to operate only at habitat scale (Vanbergen et al. 2010). Our results are compatible with this habitat-scale influence, in that we found differences in both carabids diversity and activity density between stands spaced < 900 m apart (i.e. Ca1 and Ca2) within the same infestation category. In our study, the *Carabidae* genera *Carabus*, *Steropus*, *Calathus*, and *Platyderus*, together with the *Encyrtidae* and *Ichneumonidae* (Hymenoptera) parasitoid families, were responsible for the most prominent compositional differences in the enemy assemblage for *L. dispar*. *Carabus* and *Calosoma* are well known as active predators of *L. dispar* (Hoch et al. 2006), while *Pterostichus* (*Steropus* genus formerly classified as *Pterostichus*) and *Calathus,* above all the former, have been considered to be among the main natural enemies of *L. dispar* in the USA (MacLean, Usis 1992). Further, the specialised tachinids (Diptera) *P. silvestris* and *B. pratensis* have been cited as the most important parasitoids for *L. dispar* (parasitising larvae, and larvae and pupae, respectively), particularly in periods of high infestation, while ichneumonid wasps are important in periods of low population densities (Alalouni et al. 2013; Zankl et al. 2023). The *Encyrtidae* genus *Ooencyrtus* is considered among the most important egg parasitoid species in Central Europe (Alalouni et al. 2013).

Our results showed a higher diversity of the enemy complex overall in stands with a low level of *L. dispar* infestation than in highly infested stands. While pathogens are known to cause high mortality rates in *L. dispar* under dense outbreak populations (Alalouni et al. 2013), with a dominant role of the fungus *Entomophaga maimaiga* Humber, Shimazu et Soper (Hajek et al. 2021; Holuša et al. 2021), the activity of parasitic and predatory natural enemies has been considered more important when spongy moth populations are sparse (McManus et al. 1989). Carabids differ in their degree of polyphagy and specialisation (Vanbergen et al. 2010; Šerić Jelaska et al. 2014), and there is a complex relationship between their abundance and that of their prey (Guillemain et al. 1997). In relation to parasitoids, some generalist species, such as *Compsilura concinnata* Meigen (Diptera: *Tachinidae*), have shown relevant activity against larvae of *L. dispar* in its invasive range but did not within its native range (Alalouni et al. 2013; Zankl et al. 2023). The activity of both carabid and parasitoid enemies, not only their prey, may vary due to environmental conditions (Hoch et al. 2006). Dry and warm conditions were associated with decreased parasitic activity of *P. sylvestris* (Hoch et al. 2006); consistent with this, the fecundity of *Carabus abbreviatus* Brullé was higher in periods of high rainfall. One of the genera most often caught in Granada stands was the high-altitude carabid *Microlestes*; however, *Carabus*, *Steropus*, and *Calosoma* have been reported over 1 200 m a.s.l, *Calathus* over 1 400 m a.s.l. and *Platyderus* over 1 800 m a.s.l. (Ortuño et al. 2023). Thus, differences in *Carabidae* catches between the two geographical areas we studied cannot be solely attributed to abiotic limits (i.e. elevation a.s.l.). On the other hand, it has been proposed that litter thickness strongly influences the composition and structure of forest carabid communities (Guillemain et al. 1997), and *Carabidae* activity is also known to be affected by the presence of competitors and vegetation cover, as well as weather conditions (Lövei, Sunderland 1996; Guillemain et al. 1997).

Spongy moth larvae are only available as a food source for a few weeks a year, and hence, they may not be a favoured option for predators (Petrovskii, McKay 2010). Nonetheless, polyphagous species, due to their wide trophic niche and predatory period, show high resilience to reductions in food supply, and good persistence in stochastic environments (Pekár et al. 2015; Gonçalves et al. 2021). Interestingly, the seasonal pattern in activity density for some key taxa in our study, especially the generalist carabids *Steropus* and *Calathus*, and the specialist *Calosoma*, as well as *Encyrtidae* and *Braconidae* hymenopterans, revealed that their peak period in Cádiz stands may occur at the end of July or even later, which is beyond the larval period of *L. dispar.* In contrast, the seasonal pattern for the *Tachinidae* family matched the larval period of *L. dispar.* Though it has been suggested that egg parasitoids (e.g. some *Braconidae* and *Ichneumonidae* wasps) play a subordinate role, specialist larval parasitoids, such as the tachinid *P. sylvestris*, cause significant mortality in *L. dispar* (Hoch et al. 2006; Zankl et al. 2023). These considerations, together with our results, suggest that generalist predators (i.e. *Carabus*, *Calathus* and *Steropus* genera) could play a more important role than specialists (in the *Calosoma* genus) in the control of *L. dispar* populations in our areas, contrasting with the highly host-specific activity of parasitoids (Salim et al. 2016; Zankl et al. 2023).

In our study, the candidate enemy assemblage was markedly diverse and abundant in stands classified as not infested by *L. dispar* and not treated with chemicals or biopesticides. Carabid beetles are sensitive to changes caused by management practices, with physico-chemical environmental variations inducing modifications in the composition of carabid assemblages (Guillemain et al. 1997; Makwela et al. 2023). Indirect effects of pesticides on natural enemy assemblages depend on the type of natural enemy, life stages exposed to pesticides, age, and sex (Abdel-Raheem 2021), with specific effects on predatory arthropods remaining poorly known, compared with the effects on predatory birds and rodents (Leroy et al. 2021). It has been suggested that the chitin synthesis inhibitor diflubenzuron has less impact on natural enemies than other insect growth regulators; however, diflubenzuron has shown to have negative effects on both generalist predators such as *Podisus nigrispinus* Say (*Heteroptera*: *Pentatomidae*) and *L. dispar* parasitoids of the *Tachinidae* and *Braconidae* families (Madrid, Stewart 1981; Castro et al. 2012). In contrast, US-APHIS (2019) reported numerous examples of non-significant overall environmental repercussions of the use of diflubenzuron.

An accurate description of biodiversity gradients requires extended spatial and temporal research; nonetheless, some differences in biodiversity can arise in time-constrained research when study areas show remarkable ecological differences. Our results suggest that, in those areas having a potential natural enemy complex for *L. dispar* (as Cádiz stands), management practices should avoid negatively affecting this potential biocontrol; in contrast, in areas with an enemy complex less represented (as Granada stands), where no treatments against *L. dispar* are applied, management practices should focus on the early detection of new outbreaks. Our results contribute to the debate about the environmental effect of pesticides within an IPM context. We agree with Castro et al. (2012) in that there is a need for further assessment of the impact of diflubenzuron on beneficial insects, focusing on specific taxa and sensitive ecological areas, to guide IPM programmes for *L. dispar*.

CONCLUSION

Both differences in composition and diversity of the candidate enemy assemblage for *L. dispar*, and the activity density per tree, varied between geographical areas as well as between *Quercus* stands with different levels of infestation by the lepidopteran. In particular, stands not infested by *L. dispar* and not chemically treated against this defoliator had the most diverse and abundant enemy complex. Understanding the effect of this enemy complex in *L. dispar* population dynamics could help predict spatial and temporal patterns of pest population outbreaks, thus facilitating pest management (Alalouni et al. 2013). More research is needed to determine the main *Carabidae* predators involved, exploring in more detail the roles of generalist predators, as well as the specialist larval-pupal parasitoids in the areas studied. Finally, predation by other arthropods, such as spiders and the *Formicidae* (Hymenoptera) family, as well as vertebrates (in particular, rodents and birds) should be also investigated (Hoch et al. 2006; Petrovskii, McKay 2010).

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REFERENCES

Abdel-Raheem M. (2021): Effect of insecticides on natural-enemies. In: Rebolledo R.E. (ed.): Insecticides – Impact and Benefits of Its Use for Humanity. London, IntechOpen Limited: 2–3.

Alalouni U., Schädler M., Brandl R. (2013): Natural enemies and environmental factors affecting the population dynamics of the gypsy moth. Journal of Applied Entomology, 137: 721–738.

Boukouvala M.C., Kavallieratos N.G., Skourti A., Pons X., Alonso C.L., Eizaguirre M., Fernandez E.B., Solera E.D., Fita S., Bohinc T., Trdan S., Agrafioti P., Athanassiou C.G. (2022): *Lymantria dispar* (L.) (Lepidoptera: *Erebidae*): Current status of biology, ecology, and management in Europe with notes from North America. Insects, 13: 854.

Buckner C.H., Kingsbury P.D., McLeod B.B., Mortensen K.L., Ray D.G.H. (1974): Impact of aerial treatment on non-target organisms. In: Evaluation of Commercial Preparations of *Bacillus thuringiensis* With and Without Chitinase Against Spruce Budworm. Information Report CC-X-97. Ottawa, Chemical Control Research Institute: 1–72.

Cameron E.A., Reeves R.M. (1990): *Carabidae* (Coleoptera) associated with gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: *Lymantriidae*), populations subjected to *Bacillus thuringiensis* Berliner treatments in Pennsylvania. The Canadian Entomologist, 122: 123–129.

Castro A.A., Lacerda M.C., Zanuncio T.V., de S. Ramalho F., Polanczyk R.A., Serrão J.E., Zanuncio J.C. (2012): Effect of the insect growth regulator diflubenzuron on the predator *Podisus nigrispinus* (*Heteroptera*: *Pentatomidae*). Ecotoxicology, 21: 96–103.

CMA (2016): Plan de Lucha Integrada contra *Lymantria dispar*. Sevilla, Consejería de Medio Ambiente y Ordenación del Territorio, Junta de Andalucía: 46. (in Spanish)

Gearty W., Jones L.A. (2023): Rphylopic: An R package for fetching, transforming, and visualising PhyloPic silhouettes. Methods in Ecology and Evolution, 14: 2700–2708.

Gentilesca T., Camarero J.J., Colangelo M., Nolè A., Ripullone F. (2017): Drought-induced oak decline in the western Mediterranean region: An overview on current evidences, mechanisms and management options to improve forest resilience. iForest, 10: 796–806.

Golemansky V., Pilarska D., Georgiev G., Takov D., Todorov M., Pilarski P. (2010): Protozoan parasites and pathogens of forest pest arthropods. Silva Balcanica, 11: 67–72.

Gonçalves F., Carlos C., Crespo L., Zina V., Oliveira A., Salvaçao J., Pereira J.A., Torres L. (2021): Soil arthropods in the Douro demarcated region vineyards: General characteristics and ecosystem services provided. Sustainability, 13: 7837.

Guillemain M., Loreau M., Daufresne T. (1997): Relationships beetween the regional distribution of carabid beetles (Coleoptera, *Carabidae*) and the abundance of their potential prey. Acta Oecologica, 18: 465–483.

Hajek A.E., Diss-Torrance A.L., Siegert N.W., Liebhold A.M. (2021): Inoculative releases and natural spread of the fungal pathogen *Entomophaga maimaiga* (*Entomophthorales*: *Entomophthoraceae*) into U.S. populations of gypsy moth, *Lymantria dispar* (Lepidoptera: *Erebidae*). Environmental Entomology, 50: 1007–1015.

Hamidi B., Wallace K., Vasu C., Alekseyenko A.V. (2019): W_d^*-test: Robust distance-based multivariate analysis of variance. Microbiome, 7: 51.

Hartig F. (2022): DHARMa: Residual Diagnostics for Hierarchical (Multi-level/Mixed) Regression Models. R Package Version 0.4.6. Available at: https://CRAN.R-project.org/package=DHARMa

Hoch G., Kalbacher G., Schopf A. (2006): Gypsy moth revisited: Studies on the natural enemy complex of *Lymantria dispar* L. (*Lep*., *Lymantriidae*) during an outbreak in a well known gypsy moth area. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie, 15: 201–204.

Holuša J., Zúbrik M., Resnerová K., Vanická H., Liška J., Mertelík J., Takov D., Trombik J., Hajek A.E., Pilarska D. (2021): Further spread of the gypsy moth fungal pathogen, *Entomophaga maimaiga*, to the west and north in Central Europe. Journal of Plant Diseases and Protection, 128: 323–331.

Legendre P., Borcard D. (2018): Box-Cox-chord transformations for community composition data prior to beta diversity analysis. Ecography, 41: 1820–1824.

Leroy B.M.L., Lemme H., Braumiller P., Hilmers T., Jacobs M., Hochrein S., Kienlein S., Müller J., Pretzsch H., Stimm K., Seibold S., Jaworek J., Hahn W.A., Müller-Kroehling S., Weisser W.W. (2021): Relative impacts of gypsy moth outbreaks and insecticide treatments on forest resources and ecosystems: An experimental approach. Ecological Solutions and Evidence, 2: e12045.

Lövei G.L., Sunderland K.D. (1996): Ecology and behavior of ground beetles (Coleoptera: *Carabidae*). Annual Review of Entomology, 41: 231–256.

MacLean D.B., Usis J.D. (1992): Ground beetles (Coleoptera: *Carabidae*) of Eastern Ohio forests threatened by the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: *Lymantriidae*). Ohio Journal of Sciences, 92: 46–50.

Madrid F.J., Stewart R.K. (1981): Impact of diflubenzuron spray on gypsy moth parasitoids in the field. Journal of Economic Entomology, 74: 1–2.

Makwela M.M., Slotow R., Munyai T.C. (2023): Carabid beetles (Coleoptera) as indicators of sustainability in agroecosystems: A systematic review. Sustainability, 15: 3936.

McManus M., Schneeberger N., Reardon R., Mason G. (1989): Gypsy Moth. Forest Insect & Disease Leaflet 162. Washington, D.C., U.S. Department of Agriculture Forest Service: 14.

Oksanen J., Simpson G.L., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Solymos P., Stevens M.H.H., Szoecs E., Wagner H., Barbour M., Bedward M., Bolker B., Borcard D., Carvalho G. et al. (2015): Vegan: Community Ecology Package. R Package Version 2.2-1. Available at: http://CRAN.R-project.org/package=vegan

Ortuño V.M, Arribas Ó, Andrés E. (2023): The *Carabidae* (Insecta: Coleoptera) of the upper Salientes Valley (León, northern Spain): Fauna, chorology, and taxonomic notes. Graellsia, 79: e189.

Pekár S., Michalko R., Loverre P., Líznarová E., Černecká L. (2015): Biological control in winter: Novel evidence for the importance of generalist predators. Journal of Applied Ecology, 52: 270–279.

Petrovskii S.V., McKay K.A. (2010): Biological invasion and biological control: A case study of the gypsy moth spread. Aspects of Applied Biology, 104: 37–48.

Roswell M., Dushoff J. (2020): MeanRarity: Hill Diversity Estimation and Visualisation. CRAN. Available at: https://github.com/mikeroswell/MeanRarity/

Roswell M., Dushoff J., Winfree R. (2021): A conceptual guide to measuring species diversity. Oikos, 130: 321–338.

Salim M., Gökçe A., Naqqash M.N., Bakhsh A. (2016): An overview of biological control of economically important lepidopteron pests with parasitoids. Journal of Entomology and Zoology Studies, 4: 354–362.

Sallé A., Nageleisen L.M., Lieutier F. (2014): Bark and wood boring insects involved in oak declines in Europe: Current knowledge and future prospects in a context of climate change. Forest Ecology and Management, 328: 79–93.

Šerić Jelaska L., Franjević D., Jelaska S.D., Symondson W.O.C. (2014): Prey detection in carabid beetles (Coleoptera: *Carabidae*) in woodland ecosystems by PCR analysis of gut contents. European Journal of Entomology, 111: 631–638.

Tabaković-Tošić M. (2012): Gypsy moth, *Lymantria dispar* (L.), and its natural enemies in the forests of Central Serbia. Sustainable Forestry Collection, 65/66: 133–147.

Thomas F. (2008): Recent advances in cause-effect research on oak decline in Europe. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3: 1–12.

Tiberi R., Branco M., Bracalini M., Croci F., Panzavolta T. (2016): Cork oak pests: A review of insect damage and management. Annals of Forest Science, 73: 219–232.

US-APHIS (2019): Final Human Health and Ecological Risk Assessment for Diflubenzuron Rangeland Grasshopper and Mormon Cricket Suppression Applications. Riverdale, United States Department of Agriculture, Animal and Plant Health Inspection Service: 53.

Vanbergen A.J, Woodcock B.A., Koivula M., Niemela J., Kotze D.J., Bolger T., Golden V., Dubs F., Boulanger G., Serrano J., Lencina J.L., Serrano A., Aguiar C., Grandchamp A.C., Stofer S., Szel G., Ivits E., Adler P., Markus J., Watt A.D. (2010): Trophic level modulates carabid beetle responses to habitat and landscape structure: A pan-European study. Ecological Entomology, 35: 226–235.

Venables W.N., Ripley B.D. (2002): Modern Applied Statistics with S. 4th Ed. New York, Springer: 497.

Vericat P., Piqué M., Serrada R. (2012): Gestión adaptativa al cambio global en masas de *Quercus* mediterráneos. Solsona, Centre Tecnològic Forestal de Catalunya: 172. (in Spanish)

Zankl T., Schafellner C., Hoch G. (2023): Parasitoids and pathogens in a collapsing *Lymantria dispar* (Lepidoptera: *Erebidae*) population in Lower Austria. Journal of Applied Entomology, 147: 676–687.

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