

Spring evaluation of three sampling methods to estimate family richness and abundance of arthropods in olive groves

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Abstract

Spring evaluation of three sampling methods to estimate family richness and abundance of arthropods in olive groves.— The intensification and expansion of agriculture is currently one of the greatest threats to biodiversity worldwide. Olive groves are one of the most extensive and diverse agroecosystems in the Mediterranean region. However, the efficiency of the methods used to sample arthropods in olive crops remains unclear. We compared the effectiveness of pan traps, sweep net and bait traps used to sample arthropods in olive groves. The pan traps collected 19 orders and 182 families, with an abundance that was 76% and 86% higher than that of sweep nets and bait traps, respectively. The composition of families differed significantly according to the method used; from a total of 234 families, 23% were sampled only by pan traps, 16% only by sweep net and 5% only by bait traps. The sampling method was the best predictor of arthropod abundance and number of families, followed by the vegetation and landscape diversity indexes. As pan trap, sweep net and bait trap methods do not obtain the same results when sampling arthropods, we recommend a combination of pan traps and a sweep net, depending on the goal of the studies and the arthropod groups targeted.

Key words: Agro-ecosystems, Arthropod surveys, Bait traps, Pan traps, Sweep net

Resumen

Evaluación en primavera de tres métodos de muestreo para estimar la riqueza de familias y la abundancia de los artrópodos en olivares.— En la actualidad, la intensificación y expansión de la agricultura es una de las mayores amenazas para la biodiversidad mundial. El cultivo de olivo es uno de los agroecosistemas más extensivo y diverso de la región mediterránea. Sin embargo, aún no está clara la eficiencia de los métodos empleados para muestrear artrópodos en cultivos de olivo. Hemos comparado la efectividad de las trampas de bandeja, la red de barrido y las trampas de cebo que se emplean para muestrear artrópodos en olivares. Con las trampas de bandeja se capturaron 19 órdenes y 182 familias, cuya abundancia fue un 76% y un 86% superior a la de los artrópodos capturados por las redes de barrido y las trampas de cebo, respectivamente. La composición de familias taxonómicas fue significativamente diferente según el método de captura empleado: de un total de 234 familias, un 23% fue capturado únicamente con las trampas de bandeja; un 16%, únicamente con las redes de barrido; y un 5%, únicamente con las trampas de cebo. Además, el método de muestreo fue el mejor factor para predecir la abundancia y el número de familias de artrópodos, seguido por los índices de vegetación y de diversidad del paisaje. Debido a que las trampas de bandeja, la red de barrido y las trampas de cebo no obtuvieron los mismos resultados en los muestreos de artrópodos, recomendamos el uso combinado de trampas de bandeja y una red de barrido, dependiendo del propósito de la investigación y del grupo de artrópodos objetivo del estudio.

Palabras clave: Agroecosistema, Muestreo de artrópodos, Trampas de cebo, Trampas de bandeja, Red de barrido

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Introduction

The intensification of agricultural practices has led to a dramatic decline in the biodiversity of agro-ecosystems (Matson et al., 1997; Tilman et al., 2001; Nentwig, 2003; Pfiffner & Luka, 2003). The survival of the arthropods in these intensive agro-ecosystems depends on the suitability of the habitat, which is in turn influenced by both agricultural management and the surrounding landscape (Jeanneret et al., 2003) in which arthropods are part of important functional groups in food webs (Gonçalves & Pereira, 2012). The diverse agricultural landscapes provide several available niches and micro-niches (canopy-ground: soil, grass, roots) in different types of management regimes, which could be used by arthropods.

Assessing the effects of these different managements and micro-niches on arthropod and plant communities is essential for the management and preservation of biological diversity (Bardgett, 2002). The evaluation, protection and management of biodiversity in agro-ecosystems have been identified as a major challenge of the future in Europe (Jerez-Valle et al., 2014). Methods to sample arthropod assemblages must be efficient, repeatable and representative because they are commonly used in environmental monitoring (Rubene et al., 2015). Monitoring and biodiversity inventories require survey methods that will permit the most efficient and comprehensive completion of study aims (Hutchens & DePerno, 2009; Popic et al., 2013). However, the effectiveness of each method may depend on a range of factors, including the location of the study plots, the type of vegetation (Pedigo & Buntin, 1993), the availability of resources (such as flowering), the sampling season, and the composition of the arthropod community (Baum & Wallen, 2011; Gollan et al., 2011). The most appropriate sampling methods will, moreover, depend on the aims and the target taxa of the study, in addition to resources and time consumption (Popic et al., 2013).

Previous studies have compared different sampling methods in different habitats (see Spafford & Lortie, 2013), such as those in Australia (Popic et al., 2013), New Zealand (Larsen et al., 2014), North America (Shapiro et al., 2014; Joshi et al., 2015), Central and North Europe (Niedobová & Fric, 2014; Rubene et al., 2015) and South America (Nemesio & Morato, 2005). However, few studies have compared their effectiveness in Mediterranean regions (Nielsen et al., 2011; Ponce et al., 2011).

One of the main crops in the Mediterranean basin is the olive tree (*Olea europaea*) (Sokos et al., 2013). The olive culture is deeply rooted in Mediterranean countries, which produce 99% of olive oil throughout the world (Lomuo & Giourga 2003). Spain occupies the first place as regards surface and olive production and its production represents 60% of the European olive production and 45% of the world olive production (MAGRAMA, 2016). The large surface area occupied by olive crops in the Iberian Peninsula, particularly in the south, means these agro-ecosystems play a crucial role in biodiversity conservation, but this role varies according to key factors such as the use of pesticides, the presence of natural

and semi-natural features (such as scrub, woodland, dry-stone walls, etc) and the age of the trees (Beaufoy, 2000). The flora present in olive crops is similar to that in a natural Mediterranean ecosystem (Margaris, 1980; Giourga et al., 1994), providing suitable conditions for arthropod communities, which are, together with the plant communities, the key factors on which mammal and bird communities depend (Beaufoy, 2000).

Olive groves have currently reached record levels in terms of area and production in the Mediterranean region. Intensive agriculture has simultaneously impoverished the arthropod fauna in the agro-ecosystem of olive orchards (Ruano et al., 2004; Allen et al., 2006; Santos et al., 2007; Castro-Caro et al., 2014; Jerez-Valle et al., 2014). However, little is as yet known about the effect of different olive orchard management regimes (organic production, conventional non-tillage, traditional farming), with different uses of agrochemicals, irrigation, tree density or cover ground, on arthropod diversity (Ruano et al., 2004; Gkisakis et al., 2015, 2016). Little is therefore known about the simultaneous effectiveness and repeatability of the different methods in this habitat.

Our objective was to compare and evaluate the three commonly used arthropod survey methods (pan traps, sweep nets and bait traps) in terms of capture rates, arthropod richness and the family composition of arthropod communities in olive groves, and to determine the influence of landscape and the diversity of herbaceous plants on the efficiency of the three sampling methods.

Material and methods

Study area and sampling design

The study was conducted in Andalusia (37° 30'–37° 58' N, 4° 17'–4° 56' W; between 159–369 m a.s.l.), which is located in the south of the Iberian Peninsula (fig. 1). We selected 123 study sites in a representative geographical range of olive groves in Guadalquivir valley. All the sites were located in an olive-dominated landscape, in which agricultural intensification has eliminated most of the natural vegetation (Rey, 2011). The mean distance between study sites was 15 ± 17 km.

Sampling was conducted in the middle of May 2014. Data from three meteorological stations close to the orchards were used to obtain mean humidity, mean temperature and mean rainfall during the sampling period. The climatic conditions in the study sites were similar during the sampling period: $54.37\% \pm 0.95\%$ (mean humidity \pm SE), $20.56 \pm 0.27^\circ\text{C}$ (mean temperature \pm SE) and 12.67 ± 4.31 mm (rainfall \pm SE). The study sites were managed with similar farming system methods (conventional tillage, mineral fertilization, and planting using a traditional framework), but plant communities differed. To take the plant biodiversity on arthropod captures into account, we calculated two landscape indices and the vegetation Shannon index at each study site (see below).

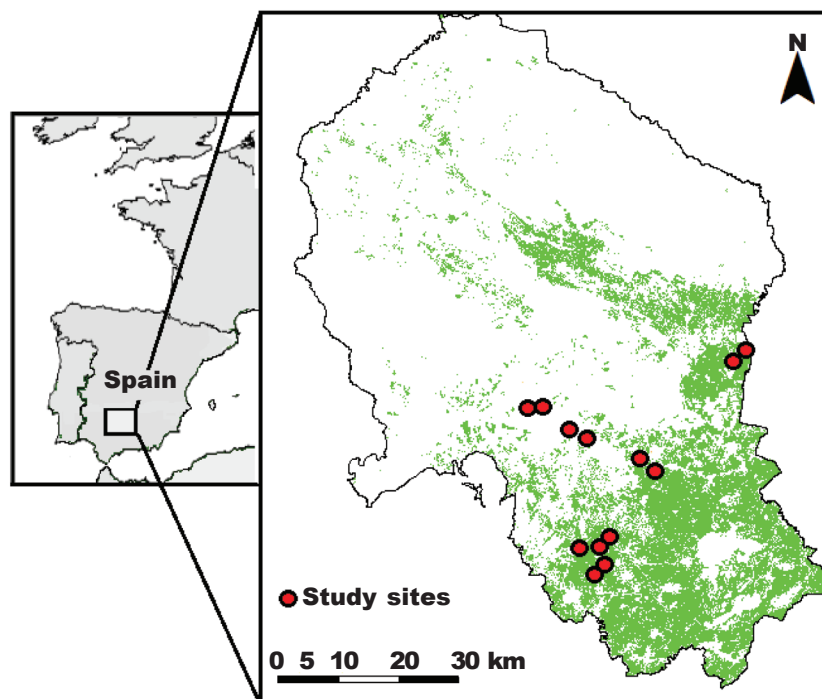


Fig. 1. Study area showing distribution of study sites (circles) and olive groves (shaded areas) in the province of Córdoba.

Fig. 1. Área de estudio en la que se muestra la distribución de los lugares de estudio (círculos) y los cultivos de olivo (zonas sombreadas) en la provincia de Córdoba.

We tested three arthropod survey methods: pan traps, sweep netting and bait traps. These sampling surveys are appropriate to sample canopy and flying arthropods, but not soil arthropods, which were not therefore included in this research. Each survey method was deployed in two transects on each study site for three consecutive days (78 transects per method). The arthropods collected on different days in the same transect were pooled to compare the arthropods captured by pan and bait traps with arthropods captured in sweep netss. To avoid the edge effect (the major vegetal complexity or simultaneous availability of one or more elements, Yahner, 1988) all the transects were surveyed at > 30 m from the nearest edge (fig. 2), and a distance of 100 m was established between the transects to ensure their independence and avoid pseudoreplication.

Arthropod sampling

Pan traps

The traps were placed in two transects (each of which was 90 m in length) with 10 traps (spaced every 10 m) per transect (fig. 2). The traps were set at each study site for three consecutive days; they were placed above the ground and between olive trees to be seen easily by arthropods. The trap-trays were made from polyethylene plastic bowls (400 ml, 110 mm in diameter, 70 mm high) and painted in UV

fluorescent yellow (Popic et al., 2013). One hundred ml of soapy water was placed in each pan (to break the superficial tension). There were a total of 260 (10 traps x 2 transects x 13 study sites) pan traps per day (780 pan traps in total, 260 x 3 days). The pans were checked and cleared of captures daily and the arthropods were transferred to plastic bottles with 70% ethanol for transportation to the laboratory. As mentioned above, the arthropods collected on different days in the same transect were pooled to allow comparison of the three methods ($n = 26$ transect data).

Sweep netting

Flower-visiting arthropods and arthropods that live or feed on vegetation were sampled along two sweep-net transects on each site. One collector (always the same person, A. J. C.) carried out the sampling of both transects for three consecutive days on each site. The sweep net transects were 90 m in length and 5 m in width (fig. 2; Popic et al., 2013) and the collector sampled arthropods from all the plant species along both transects for 1 h (each transect was sampled for 30 minutes). Sweep netting took place in morning sessions (11:00–12:00 h) in order to match the activity patterns of arthropods and to avoid the extreme midday heat (Popic et al., 2013). Sampling only took place during fine weather (days without wind or rain) so as to minimise any potential

effects of weather on captures. The arthropods were transferred to 5 ml vials for transportation. As in the case of the pan traps transects, the arthropods captured in the same transect on three different days were pooled for comparison with the pan trap and bait trap transects ($n = 26$ sweep net transects data). A total of 39 hours were spent on sweep net sampling.

Bait traps

Bait traps were set in the same way as pan traps (two transects on each site with 10 bait traps spaced every 10 meters for three consecutive days; fig. 2). The traps were made from 1.5 L plastic bottles (Allemand & Aberlenc, 1991). The top of the plastics bottles were cut off to increase the entrance opening (98 mm in diameter approximately) and were placed upside down (as funnels) to avoid arthropod escapes. The plastic bottles were filled with natural flowers from the surrounding area and 100 ml of soapy water per bottle. Many substances can be used as bait depending on the target arthropod group, but to compare this method with the pan trap and sweep netting sampling methods (non-specific sampling method), natural flowers from the surrounding area were used as bait (mainly species belonging to asteraceae, brassicaceae and fabaceae families). According to Basset et al. (1997), this method is the most appropriate for the sampling of arthropods in tree canopies. The traps were collected each day and the bait (flowers) was replaced daily. A total of 260 bait traps per day (780 in total) were used. As with pan traps and sweep netting, we pooled the numbers of arthropods captured by bait traps on the different days in the same transect ($n = 26$ transect data) to allow comparison with arthropods captured by sweep netting.

Plant and landscape diversity

The study sites presented different levels of plant biodiversity. To take this difference into consideration we laid out an additional two transects in two separate rows of olive trees on each site. The transects were 90 m in length, and 10 hoops (0.5 m²) spaced 10 m apart were used as sampling points for herbaceous plants (fig. 2) (Guerrero-Casado et al., 2015). All the weed species at these sampling points were identified. The mean values of the Shannon diversity index (Shannon & Weaver, 1963) for the weed community were calculated at the site ($n = 13$). The transects for plant and arthropod surveys were sampled simultaneously on each site. The effect of the surrounding landscape was estimated by recording two environmental variables at the site level (Schweiger et al., 2005): the Shannon index of the landscape (SHDI) and the edge density of the landscape (ED). The SHDI quantified the diversity of the countryside on the basis of richness (the number of different patch types) and evenness (the proportional area distribution among patch types).

The SHDI is calculated according to the formula:

$$\text{SHDI} = \sum_{i=1}^m (P_i * \ln P_i)$$

where m is the number of patch types and P_i is the proportion of area covered by patch type (land cover class).

The ED is a measurement of the complexity of the shapes of patches and an expression of the spatial heterogeneity of a landscape mosaic. The index is calculated as:

$$\text{ED} = \frac{\sum_{k=1}^m e_{ik}}{A} (10,000)$$

where e_{ik} is the total length (in m) of edge in a landscape involving patch type (class) i , and includes the landscape boundary and background segments involving patch type i , whereas A is the total landscape area in m².

Both landscape indices were obtained using FRAGSTATS 4.1 software (McGarigal et al., 2002). The landscape diversity index and edge density were recorded in a buffer of approximately 500-m radius around the centre of the sampling site. In each buffer, different land cover classes present were recorded (urban land uses, rivers and natural streams, arable crops, olive groves, vineyard, irrigated crops, citrus and dense scrub). Information concerning land cover classes was obtained from aerial photographs (Ortofotografía digital de Andalucía).

Arthropod identification

The arthropods captured were identified at family level. Classification at species level was unnecessary because the purpose of this study was to assess the effectiveness of each sampling method as regards capturing specific arthropod families. A binocular microscope (Nikon SMZ-U) and several guides were used to identify the arthropods (Barrientos, 1988; Dindal, 1990; Chinery, 2005), but keys were used for Hymenoptera families (Goulet & Huber, 1993).

The PRIMER package, version 6 (Clarke & Gorley, 2006), was used to calculate the number of families (N_f) and Pielou's evenness index (J') for each sampling method.

Data analysis

The sampling unit used for statistical analysis was 'transect' because sweep netting has no 'trap' unit to compare with bait and pan traps. Relative abundance and total abundance of arthropod families were calculated for each sampling method. The estimation of diversity can be strongly dependent on differences in inventory completeness (Chao & Jost, 2012). We estimated the inventory completeness for each method using the sample coverage estimator recommended by Chao & Jost (2012) using the iNEXT online software (Chao et al., 2016).

Comparison of family composition obtained using each sampling method

Comparison analyses of the arthropod community were performed using the Bray-Curtis similarity index (Bray & Curtis, 1957) following square root transformation

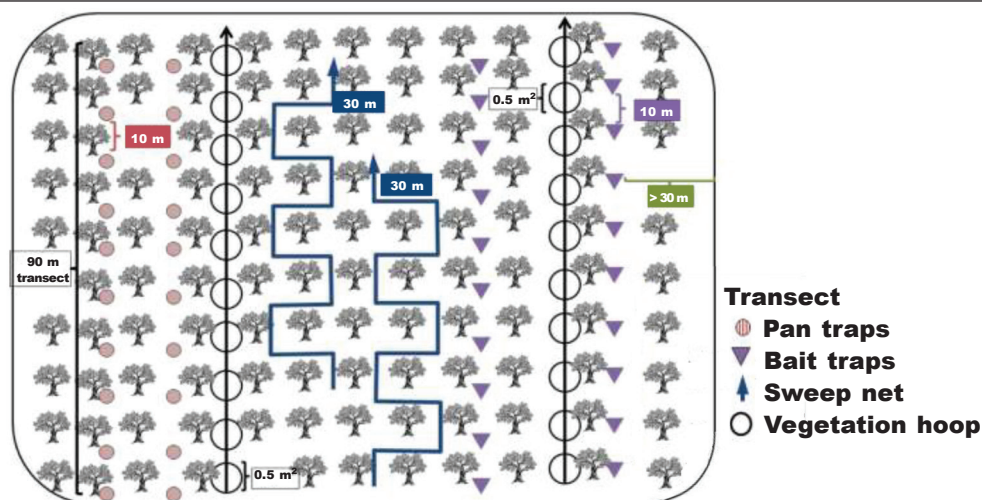


Fig. 2. Sample design and arthropod and vegetation sampling methods used at each sampling site. A total of 13 olive orchards were sampled. The distance between olive trees was less than 10 m. The vegetation hoops indicate the surface used for plant sampling.

Fig. 2. Diseño de muestreo y métodos de muestreo de artrópodos y vegetación empleados en cada lugar del estudio. En total se muestrearon 13 cultivos. La distancia entre los olivos fue inferior a 10 m. Los aros de vegetación indican la superficie empleada para el muestreo de plantas.

of transect data. Dummy values (= 1 specimen) were added to avoid a collapse in subsequent multidimensional scaling (MDS) representation resulting from empty samples. The differences in the sampling methods used in terms of arthropod composition were assessed using MDS. A permutational multivariate ANOVA (PERMANOVA) was then used to check for significant differences between the arthropod assemblages sampled using each method. The MDS and PERMANOVA were performed on the basis of the Bray–Curtis similarity index matrix. PERMANOVA constructs an F -ratio from the sums of squared distances within and between groups that are analogous to Fisher's F -ratio (Anderson, 2001). Pair-wise comparisons of the sampling methods were subsequently performed to determine which arthropoda communities differed. The PERMANOVA test was performed with 9999 permutations with the objective of increasing the power and precision of the analysis (Hope, 1968; Anderson et al., 2008). A Similarity Percentage (SIMPER, Clarke, 1993) was used to identify the arthropod families principally responsible for the dissimilarity among the sampling methods. PRIMER package, version 6 (Clarke & Gorley, 2006) was used to perform the MDS plot, PERMANOVA and SIMPER procedure.

Predictive factors of N_F and arthropod abundance

We tested the relationships between each type of sampling method, N_F and abundance using two univariate analysis of variance (UNIANOVA). In the first UNIANOVA, the number of arthropods is considered as a response variable, while in the second

UNIANOVA, N_F was used as the response variable. In both models, the method (three levels: pan trap, sweep netting, and bait trap) was used as a factor, whereas the Shannon index of vegetation, the SHDI and the ED of the landscape were included as explanatory variables (co-variables). For these analyses, we used the sum of squares type III. UNIANOVA were performed using IBM SPSS Statistics 20 software.

Results

Descriptive results

We captured a total of 19,990 arthropods belonging to 25 orders and 234 families. The pan traps captured 14,476 individuals, 22 orders and 179 families. Sweep netting captured 3,571 specimens, 15 orders and 141 families, and the bait traps captured 1,943 specimens, 20 orders and 105 families (table 1). The effectiveness of pan traps was particularly evident in the case of Diptera, Hymenoptera, Homoptera, Collembola and Thysanoptera, for which the number of individuals was greater than 1,000 (table 1). The greatest numbers of Coleoptera, Heteroptera, and Lepidoptera (with 996, 773 and 154 individuals, respectively) were collected using sweep netting (table 1). The lowest abundance values were recorded for bait traps. Some orders were present only in bait traps (e.g., Scutigera and some Hymenoptera families, see supplementary material) but in low abundance. The order with the largest number of specimens captured by bait trap was Homoptera, with 590 individuals.

The results show that 54 arthropods families (23.07%) were collected exclusively by pan traps, 37 families (15.8%) solely by sweep netting, and 12 families (5.12%) only in bait traps. In other cases, arthropod families were collected by two of the three sampling methods (fig. 3; appendix 1).

The inventory completeness analysis indicated that all three sampling methods had high and similar values of inventory completeness (0.99, 0.98 and 0.97 for pan traps, sweep netting and bait traps, respectively). The similar values of these coverage estimators indicate that the three methods are sufficiently and similarly exhaustive to be compared.

Comparison of abundance, number of families and evenness between sampling methods

The highest arthropod abundance (mean \pm SE; 595.5 \pm 247.7) was recorded for the pan traps, followed by the sweep net (mean \pm SE; 134.9 \pm 17.2). The bait trap, meanwhile, was the method with which least arthropods were captured (mean \pm SE; 76.6 \pm 11.8). With regard to the N_F , the highest mean value was also recorded for pan traps (mean \pm SE; 30.7 \pm 3.9), followed by sweep nets (mean \pm SE; 23.1 \pm 2.5) and bait traps (mean \pm SE; 12.9 \pm 1.2). Finally, in the case of the J index, the mean values for the sweep nets and bait traps were similar (mean \pm SE; 0.80 \pm 0.02 and 0.79 \pm 0.02 respectively), while the value of this evenness index (mean \pm SE; 0.7 \pm 0.02) was lowest for pan traps.

Comparison of family composition between sampling methods

The PERMANOVA indicated that the family composition of arthropods captured using was different for the three sampling methods (Pseudo F = 6.52; p < 0.001). Sweep netting was significantly different from pan traps (Pseudo F = 2.67; p < 0.001) and bait traps (Pseudo F = 3.04; p < 0.001) in the case of arthropod family composition. The PERMANOVA also showed differences in composition of families as regards the pan traps and bait traps (Pseudo F = 1.84; p < 0.001).

The differences in arthropod family composition obtained using the different sampling methods are shown by means of an MDS ordination plot (fig. 4). The MDS plot supports the PERMANOVA results. The figure shows a differentiation between the fauna collected using the sweep nets with regard to the other two sampling methods (fig. 4), while there was no clear difference between the pan traps and bait traps in the MDS plot, although they can be grouped into subgroups (fig. 4: groups B, C, D, and E). In the case of the sweep net, most transects (less than one of them) can be grouped into a 16% similarity-level group (fig. 4: group A). There are another two subgroups in this group with a higher similarity level: 40% and 29% (fig. 4: groups A.1 and A.2).

The MDS plot did not show any distinctive grouping for pan traps and bait traps (no more than five transects, fig. 4). The pan trap transects are grouped into two groups, located at different points in the MDS

plot. These sets include transects with a similarity of 25% (one net transect is also included) and 33%, respectively (fig. 4: groups B and C). Most bait trap transects, however, are grouped in another two similarity groups (closer to each other than the pan trap groups) with a similarity of 40% and 16%, respectively (fig. 4: groups D and E).

In the SIMPER procedure, in the case of similarity between methods, families that contributed to 70% of cumulative similarity are shown, whereas in the case of dissimilarity between sampling methods, only families which contributed to more than 2% are shown owing to the high number of families needed to achieve 70% of cumulative dissimilarity. The SIMPER procedure showed that the pan trap transects had a similarity of 23.78%, the lowest similarity value. The highest contributions to similarity in the pan trap transects were made by Thripidae, Adelgidae, Formicidae and Aeolothripidae, while the sweep netting transects proved to be more similar than the pan traps and bait traps (31.53%). The most important families responsible for similarity in the sweep-netting sample family composition were Nabidae, Apidae, Pyrrhocoridae, Thripidae, Cantharidae and Mesovelidae. The similarity for bait trap transects was 27.23%, and only five families contributed to more than 5% of similarity in the case of this sampling method: Thripidae, Adelgidae, Formicidae, Aeolothripidae and Cantharidae (appendix 2).

The dissimilarity between the three methods was, in contrast, high (no less than 77%). The SIMPER indicated that Thripidae, Apidae, Formicidae, and Nabidae were the most important families as regards the dissimilarity between pan trap and sweep netting sampling (overall dissimilarity = 81.12%). Furthermore, pan traps and bait traps had a lower dissimilarity value (overall dissimilarity = 77.88%), and five families of dipterans (Mycetophilidae, Muscidae, Phoridae, Sciariidae and Chyromiidae) contributed to more than 2% of dissimilarity (appendix 3). The highest dissimilarity value was between sweep netting and bait traps (overall dissimilarity = 82.12%), and in this case, 11 families contributed to more than 2% (appendix 3), with the most numerous taxa being Apidae and Nabidae.

Predictive factors of arthropod richness and abundance

With regard to first UNIANOVA analysis (abundance as the response variable, table 2), only the sampling method and the Shannon diversity of vegetation were significantly related to abundance. The Shannon index for vegetation was positively associated with the number of arthropods, whereas the sampling method had a significant effect on the abundance of arthropods, since pan traps and sweep netting captured more arthropods than bait traps.

However, in the second UNIANOVA (N_F as the response variable, table 3), only the sampling method and the SHDI were significantly related to N_F . The SHDI was positively associated with arthropod richness, whereas the sampling method had a significant effect on the N_F value, and pan traps and the sweep captured more families than bait traps.

Table 1. Abundance (N) and number of families (N_F) of arthropods sampled using pan traps, sweep net and bait traps. Percentages are shown in brackets.

Tabla 1. Abundancia (N) y número de familias (N_F) de los artrópodos muestreados usando trampas de bandeja, red de barrido y trampas de cebo. Los porcentajes se indican entre paréntesis.

	Pan traps		Sweep nets		Bait traps		Total	
	N	N_F	N	N_F	N	N_F	N	N_F
Actinedida	0 (0)	0 (0)	0 (0)	0 (0)	1 (< 1)	1 (< 1)	1 (<1)	1 (< 1)
Araneae	125 (< 1)	14 (7.8)	122 (3.4)	18 (12.7)	23 (1.2)	11 (10.4)	270 (1.4)	22 (9.4)
Coleoptera	396 (2.7)	32 (17.8)	996 (27.9)	24 (17)	130 (6.7)	18 (17.1)	1,522 (7.6)	37 (15.8)
Collembola	5,272 (36.4)	5 (2.7)	2 (< 1)	1 (< 1)	97 (5)	3 (2.8)	5,371 (26.9)	5 (2.1)
Dermaptera	5 (< 1)	1 (< 1)	2 (< 1)	1 (< 1)	2 (< 1)	1 (< 1)	9 (< 1)	2 (< 1)
Diptera	2,902 (20)	42 (23.4)	238 (6.7)	31 (21.9)	319 (16.4)	20 (19)	3,459 (17.3)	50 (21.3)
Dyctioptera	1 (< 1)	1 (< 1)	0 (0)	0 (0)	1 (< 1)	1 (< 1)	2 (< 1)	1 (< 1)
Embioptera	5 (< 1)	2 (1.1)	0 (0)	0 (0)	2 (< 1)	2 (1.9)	7 (< 1)	2 (1.2)
Ephemeroptera	0 (0)	0 (0)	1 (< 1)	1 (< 1)	0 (0)	0 (0)	1 (< 1)	1 (< 1)
Heteroptera	58 (< 1)	6 (3.3)	763 (22.1)	15 (10.6)	16 (< 1)	5 (4.7)	847 (4.2)	17 (7.2)
Homoptera	2,571 (17.8)	13 (7.2)	167 (4.7)	7 (4.9)	590 (30.4)	9 (8.5)	3,328 (16.6)	13 (5.5)
Hymenoptera	1,225 (8.5)	35 (19.5)	834 (23.4)	15 (10.6)	311 (16)	19 (18)	2,370 (11.9)	38 (16.2)
Isopoda	3 (< 1)	2 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	3 (< 1)	2 (< 1)
Ixodida	1 (< 1)	1 (< 1)	2 (< 1)	2 (1.4)	1 (< 1)	1 (< 1)	4 (< 1)	2 (< 1)
Lepidoptera	47 (< 1)	11 (6.1)	154 (4.3)	17 (12.7)	8 (< 1)	6 (5.7)	209 (1)	21 (8.9)
Mesostigmata	13 (< 1)	1 (< 1)	0 (0)	0 (0)	1 (< 1)	1 (< 1)	14 (< 1)	1 (< 1)
Neuroptera	1 (< 1)	1 (< 1)	5 (< 1)	2 (1.4)	1 (< 1)	1 (< 1)	7 (< 1)	3 (1.2)
Orthoptera	10 (< 1)	2 (1.1)	21 (< 1)	3 (2.1)	1 (< 1)	1 (< 1)	32 (< 1)	3 (< 1)
Pseudoescorpionida	3 (< 1)	2 (1.1)	0 (0)	0 (0)	1 (< 1)	1 (< 1)	4 (< 1)	2 (< 1)
Psocoptera	4 (< 1)	3 (1.6)	0 (0)	0 (0)	3 (< 1)	1 (< 1)	7 (< 1)	4 (1.7)
Raphidioptera	8 (< 1)	1 (< 1)	6 (< 1)	1 (< 1)	0 (0)	0 (0)	14 (< 1)	1 (< 1)
Sarcoptiformes	5 (< 1)	1 (< 1)	0 (0)	0 (0)	0 (0)	0 (0)	5 (<1)	1 (< 1)
Scutigromorpha	0 (0)	0 (0)	0 (0)	0 (0)	1 (< 1)	1 (< 1)	1 (< 1)	1 (< 1)
Thysanoptera	1,820 (12.6)	2 (1.1)	248 (6.9)	3 (2.1)	434 (22.6)	3 (2.8)	2,502 (12.5)	3 (1.2)
Zygentoma	1 (< 1)	1 (< 1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (< 1)	1 (< 1)
Total	14,476	179	3571	141	1,943	105	19,990	234

Discussion

In this study, climatic conditions (humidity, temperature and rainfall) and management practices (such as tillage or fertilizer) during the sampling period were similar in all study plots. These factors were considered to avoid introducing noise into the models or influencing captures rates.

Our results show that the three methods are strongly biased towards certain taxa, highlighting the importance of combining various sampling methods if the aim of the study is to monitor the biodiversity

or complete community of superior arthropod taxa. We found that pan traps were more effective than bait traps and sweep netting as regards detecting arthropods (for abundance and N_F), although this may depend on the taxon.

Other studies have also found that pan traps are highly effective when sampling arthropod species richness (Nielsen et al., 2011; Spafford & Lortie, 2013) and that they are an unbiased method (Westphal et al., 2008). However, although passive sampling methods such as pan traps and bait traps avoid collector bias (present in the sweep net), they are

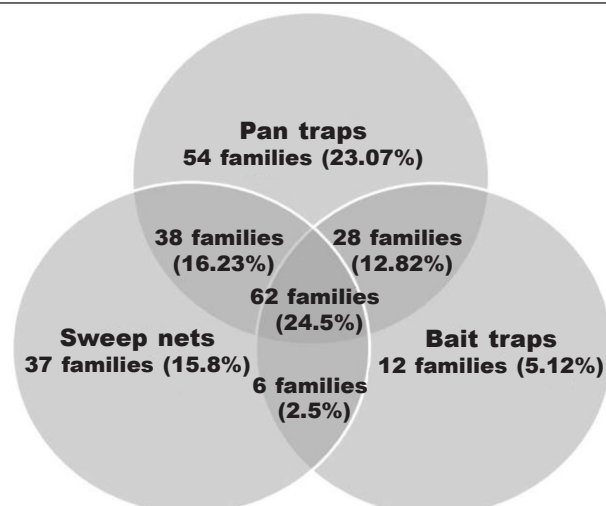


Fig. 3. Venn diagram representing the number and percentage (in brackets), of families captured using the sampling methods.

Fig. 3. Diagrama de Venn que representa el número y el porcentaje (entre paréntesis), de las familias capturadas por los métodos de muestreo.

associated with other biases, as they capture species with an unequal probability owing to specific visual or olfactory attractors (Cane et al., 2000; Roulston et al., 2007). Nevertheless, our findings contrast with those of other studies in which sweep netting has been found to capture a greater species richness and abundance of arthropods (Popic et al., 2013), although this observation depended on the taxonomic group. For example, we found that pan traps sampled a greater abundance of Diptera, Hymenoptera, Colembola, Homoptera or Thysanoptera, while sweep netting collected a higher abundance of Coleoptera, Heteroptera or Lepidoptera, and bait traps captured a mixture of both, with a greater abundance of Homoptera and Thysanoptera. The richness of the families captured using each sampling method shows that the group from which the most families were captured was Diptera, followed by Hymenoptera (in pan traps and bait traps) and Coleoptera (in sweep nets). The poor abundance of flowers and vegetation in olive groves may contribute to the superiority of pan traps when compared to the other two methods (Roulston et al., 2007).

Although pan traps captured the highest number of families—followed by sweep netting and bait traps—the combination of pan traps and the sweep netting proved to be more effective, capturing 95% of total families. This further emphasizes the importance of including more than one method when conducting arthropod species richness inventories. The various methods have advantages and disadvantages. Pan traps and bait traps (static methods) may not reveal the spatial variation in arthropod assemblages between sites and communities (Nielsen et al., 2011).

Furthermore, in the case of pan traps, different colours may significantly affect the capture rate for different arthropod taxa (Yi et al., 2012). For example, yellow pans are used in studies of diverse groups of pollinators (Kitching et al., 2001; Popic et al., 2013), while blue pan traps are more effective as regards catching Stephanidae (Aguiar & Sharkov, 1997) and red pans are attractive to Amphicomma beetles (Dafni et al., 1990). This should be taken into consideration during general surveys. It should also be kept in mind that a large number of families were not collected by bait traps. These traps are effective sampling methods for live catches of arthropods. However, the selection of the food source is vitally important, and a basic knowledge of the feeding habits is therefore a prerequisite when using this method (Yi et al., 2012). Sweep netting offers several advantages. It is not only a highly cost-effective and fairly non-intrusive method (Yi et al., 2012), but is also particularly useful when comparing relative species abundance and richness of arthropods in different areas with similar vegetation types (Siemann et al., 1997), as is the case of olive groves. However, the capture rate of sweep netting, depends to a great extent on the collector's skills and the method is relatively time-consuming. Furthermore, it is mainly suitable for open habitat types such as grassland or agriculture land and not easy to standardise in forest environments with a high vegetation density (Yi et al., 2012).

The different assemblages captured by the survey methods suggest the need for complementary sampling methods if the objective is to describe the invertebrate community (Spafford & Lortie, 2013). Our findings suggest a combination of sweep netting

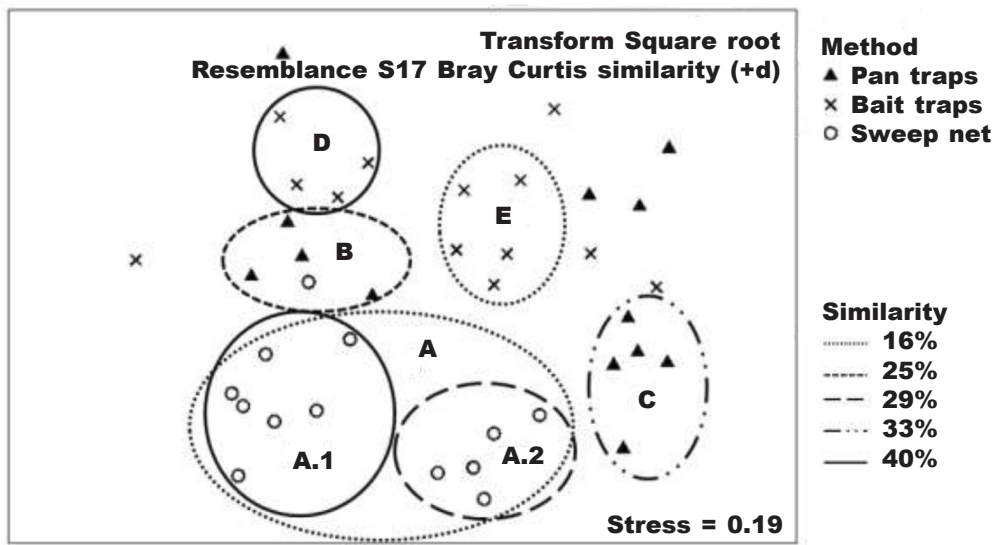


Fig. 4. MDS plot for arthropod community captured in the pan trap, sweep net and bait trap transects. Arthropod abundance data for the same study site and captured by using the sampling method were pooled. Groups are delineated according to the results of the cluster analysis.

Fig. 4. Gráfico MDS para la comunidad de artrópodos capturados en las trampas de bandeja, la red de barrido y las trampas de cebo. Se agrupan los datos relativos a la abundancia de los artrópodos capturados con el mismo lugar del estudio y con el mismo método de muestreo. Los grupos se definieron según los resultados del análisis de conglomerados.

and pan traps could be an appropriate approach to determine arthropod diversity. The low similarity in family composition within the pan trap transect is

evidence of the effectiveness of this method when used to sample diverse arthropod taxa. A single sampling method should be selected to sample a

Table 2. UNIANOVA results considering the abundance of arthropods as a response variable and showing the degree of freedom (df), type III sum of square (SS), mean square (MS), Fisher statistic (F) and p-values: ^a R² = 0.136 (adjusted R² = 0.076).

Tabla 2. Resultados de UNIANOVA que considera la abundancia de los artrópodos como variable de respuesta y de los valores de los grados de libertad (df), la suma de cuadrados tipo III (SS), el cuadrado medio (MS), el parámetro de Fisher (F) y los valores de p: ^a R² = 0,136 (R² ajustado = 0,076).

	df	SS	MS	F	p
Corrected model	5	5,186,594.5 ^a	1,037,318.91	2.26	0.057
Intercept	1	797,265.1	797,265.10	1.74	0.192
SHDI	1	127,849.4	127,849.39	0.28	0.599
ED	1	823,540.3	823,540.27	1.79	0.185
Vegetation Shannon index	1	1,285,260.2	1,285,260.20	1.93	0.049
Sampling method	2	3,564,859.5	1,782,429.78	3.88	0.025
Error	72	33,049,909.3	459,026.52		
Total	78	43,240,344			
Corrected total	77	38,236,503.8			

Table 3. UNIANOVA results considering the number of families (N_F) as a response variable and showing the degree of freedom (df), type III sum of square (SS), mean square (MS), Fisher statistic (F) and p -values: ^a $R^2 = 0.348$ (adjusted $R^2 = 0.303$).

Tabla 3. Resultados de UNIANOVA que considera el número de familias (N_F) como variable de respuesta y los grados de libertad (df), la suma de cuadrados tipo III (SS), el cuadrado medio (MS), el parámetro de Fisher (F) y los valores de p : ^a $R^2 = 0,348$ (R^2 ajustado = 0,303).

	df	SS	MS
Corrected model	5	6,142.9 ^a	1,228.59
Intercept	1	18,543.5	18,543.59
SHDI	1	837.9	837.99
ED	1	134.1	134.19
Vegetation			
Shannon index	1	166.8	166.82
Sampling method	2	3,853.4	1,926.70
Error	72	11,505.4	159.80
Total	78	54,569.0	
Corrected total	77	17,648.4	

specific arthropod group. Some examples of this might be pan traps for Hymenoptera (Westphal et al., 2008), pit fall traps for ants (Wang et al., 2001), baiting techniques for wireworms (Coleoptera, Elateridae, Parker, 1996) or live-bait traps for *Rhodnius* (Hemiptera, Reduviidae) (Abad-Franch et al., 2000). The table of supplementary material presented in this study can be considered as a guide when choosing an effective sampling method for specific families.

The UNIANOVA results indicate the importance of vegetation and landscape diversity as regards abundance and number of families, respectively. However, the sampling method had a great influence for arthropod abundance and the number of families. This result highlights the importance of appropriately selecting sampling methods to describe arthropod communities, and the scope of any research could be limited by the sampling method chosen (Marshall et al., 1994).

Conclusion

Our results showed that the pan traps were the most effective method for sampling a large abundance of arthropod families in olive groves. However, the high number of families not found in pan traps suggests

that a combination of methods is recommended. As sweep netting caught different family compositions to those obtained in bait traps and pan traps, a combination of sweep netting and pan traps may be a more effective approach for arthropod community monitoring in olive orchards. However, the selection of the sampling method depends greatly on the target taxa. The limitation of the sampling period made this research a first approximation to survey method effectiveness. Our conclusions should be evaluated in olive orchards with other management systems and climatic and seasonal variations should be considered. Further research including environmental variations is clearly needed.

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Appendix 1. List of all arthropod families found in the study and sampling method: PT. Pan traps; SN. Sweep net; BT. Bait traps.

Apéndice 1. Lista de todas las familias de artrópodos encontradas en el estudio y el método de muestreo: PT. Trampas de bandeja; SN. Red de barrido; BT. Trampas de cebo.

Order	Family	Sampling method	Order	Family	Sampling method
Actinedida			Cleridae		PT
Stigmaeidae		BT	Coccinellidae		PT, SN, BT
Araneae			Curculionidae		PT, SN, BT
Agelenidae		PT, SN	Dascillidae		PT
Araneidae		PT, SN	Dasytidae		PT, SN, BT
Atypidae		SN	Dermestidae		PT, SN
Clubionidae		SN	Elateridae		PT, SN
Ctenizidae		PT, SN	Staphylinidae		PT, SN, BT
Gnaphosidae		PT, BT	Histeridae		PT, BT
Linyphiidae		PT, SN, BT	Hydrophilidae		PT
Lycosidae		PT, SN	Lycidae		PT
Lyniohiidae		SN	Malachiidae		PT
Mimetidae		SN, BT	Meloidae		PT, SN, BT
Miturgidae		PT, SN, BT	Melyridae		PT, SN, BT
Oecobiidae		PT, BT	Mordellidae		PT, SN, BT
Oxyopidae		SN, BT	Nitidulidae		SN, BT
Pisauridae		PT, SN, BT	Oedemeridae		PT, SN, BT
Salticidae		PT, SN, BT	Pselaphidae		PT
Selenopidae		BT	Ptiliidae		PT, SN
Theraphosidae		SN	Scarabaeidae		PT, SN, BT
Theridiidae		PT, SN, BT	Scolytidae		SN
Thomisidae		PT, SN, BT	Silvanidae		PT
Thretagnatidae		SN	Collembola		
Zodaridae		PT, SN	Entomobryidae		PT, SN, BT
Coleoptera			Isotomidae		PT, BT
Aegialiidae		PT	Onychiuridae		PT
Aesalidae		PT, BT	Poduridae		PT
Anaspididae		PT	Tomoceridae		PT, BT
Anobiidae		SN	Dermaptera		
Anthicidae		PT, SN, BT	Forficulidae		SN
Anthribidae		SN	Labiidae		PT, BT
Attelabidae		PT, SN, BT	Diptera		
Bostrychidae		PT	Agromyzidae		PT, SN, BT
Bruchidae		PT	Anisopodidae		PT, SN, BT
Buprestidae		PT, SN, BT	Anthomyiidae		PT
Byrrhidae		SN	Asilidae		PT
Cantharidae		PT, SN, BT	Bombyliidae		PT, SN
Carabidae		PT, SN, BT	Calliphoridae		PT, SN, BT
Cerambycidae		PT, SN	Camillidae		PT
Chrysomelidae		PT, SN, BT	Cecidomyiidae		PT, SN, BT

Appendix 1. (Cont.)

Order	Family	Sampling method
Diptera		
	Chamaemyiidae	PT
	Chloropidae	PT, SN, BT
	Chyromiidae	PT, SN, BT
	Conopidae	SN
	Dolichopodidae	PT, SN, BT
	Drosophilidae	PT, SN
	Dryomyzidae	PT, SN
	Empididae	PT, SN, BT
	Heleomyzidae	PT, SN
	Hybotidae	PT, SN, BT
	Keroplastidae	PT
	Lauxaniidae	PT, BT
	Lonchoceridae	PT
	Lycoriidae	BT
	Micropezidae	PT
	Milichiidae	PT
	Muscidae	PT, SN, BT
	Mycetophilidae	PT, SN, BT
	Mydidae	PT
	Oestridae	PT, BT
	Oscinellidae	PT
	Otitidae	SN, BT
	Phoridae	PT, SN, BT
	Pipunculidae	SN
	Platypodidae	SN
	Psychodidae	PT, BT
	Rhagionidae	BT
	Sarcophagidae	PT
	Scatopsidae	PT
	Sciaridae	PT, SN, BT
	Sciomyzidae	PT, SN
	Sepsidae	PT, SN
	Simuliidae	PT
	Sphaeroceridae	PT
	Stratiomyidae	PT, SN
	Syrphidae	PT, SN
	Tachinidae	PT, SN
	Tephritidae	PT, SN
	Therevidae	SN
	Tipulidae	PT, SN, BT
	Trichoceridae	PT, SN
	Trypetidae	SN

Order	Family	Sampling method
Dyctioptera		
	Blattodea	PT, BT
Embioptera		
	Embiidae	PT, BT
	Oligotomidae	PT, BT
Ephemeroptera		
	Oligoneuriidae	SN
Heteroptera		
	Alydidae	SN
	Anthocoridae	SN
	Berytidae	SN
	Cimicidae	PT, SN
	Coreidae	SN
	Cydnidae	SN
	Dipsocoridae	PT, BT
	Lygaeidae	PT, SN, BT
	Mesovelidae	SN
	Microphysidae	BT
	Miridae	PT, SN, BT
	Nabidae	SN
	Pentatomidae	PT, SN
	Pyrrhocoridae	SN
	Reduviidae	PT, SN, BT
	Rhopalidae	SN
	Saldidae	SN
Homoptera		
	Adelgidae	PT
	Aleyrodidae	PT, SN, BT
	Aphididae	PT, SN, BT
	Cercopidae	PT, SN, BT
	Cicadellidae	PT, SN, BT
	Cicadidae	PT, BT
	Cixiidae	PT
Homoptera		
	Coccidae	PT, SN
	Delphacidae	PT, BT
	Ledridae	PT
	Membracidae	PT
	Pemphigidae	PT, SN, BT
	Psyllidae	PT, SN, BT
Hymenoptera		
	Agamoidae	PT
	Anthophoridae	BT

Appendix 1. (Cont.)

Order	Family	Sampling method
Hymenoptera		
	Apidae	PT, SN, BT
	Bethylidae	PT, SN, BT
	Braconidae	PT, BT
	Cephidae	PT, SN
	Ceraphronidae	PT, BT
	Chalcididae	PT, SN
	Chrysididae	PT, SN
	Cleptidae	PT
	Cynipidae	PT, SN, BT
	Dryinidae	PT
	Encyrtidae	PT
	Eulophidae	PT
	Eurytomidae	PT
	Evaniidae	BT
	Formicidae	PT, SN, BT
	Gasteruptiidae	PT
	Halictidae	PT, SN, BT
	Ichneumonidae	PT, SN, BT
	Leucospidae	PT
	Megaspilidae	PT
	Melittidae	PT
	Mutillidae	PT
	Mymaridae	PT, BT
	Orussidae	PT, BT
	Platygasteridae	PT, BT
	Proctotrupidae	PT
	Pteromalidae	PT, BT
	Scelionidae	BT
	Scoliidae	PT, SN, BT
	Sphecidae	PT, SN, BT
	Stephanidae	PT, BT
	Thenthredinidae	PT, SN
	Torymidae	PT, SN
	Trichogrammatidae	PT, SN
	Trigonalidae	PT, BT
	Vespididae	PT, SN
Isopoda		
	Anthuridea	PT
	Armadillidae	PT
Ixodida		
	Argasidae	PT, SN
	Ixodidae	SN, BT

Order	Family	Sampling method
Lepidoptera		
	Arctiidae	BT
	Eriocraniidae	PT, BT
	Gelechiidae	PT, SN
	Geometridae	PT, SN, BT
	Hesperidae	SN
	Incurvariidae	SN
	Micropterigidae	PT
	Nepticulidae	PT, SN, BT
	Noctuidae	PT, SN
	Notodontidae	PT, SN
	Nymphalidae	SN
	Papilionidae	PT, SN
	Pieridae	PT, SN, BT
	Pterophoridae	PT
	Pyralidae	SN
	Riodinidae	SN
	Satyrinae	SN
	Sesiidae	PT
	Tineidae	PT, SN, BT
	Tortricidae	SN
	Zygenoidea	SN
Mesostigmata		
	Phytoseiidae	PT, BT
Neuroptera		
	Chrysopidae	PT, SN
	Sialidae	BT
	Sisyridae	SN
Orthoptera		
	Acrididae	PT, SN
	Pyrgomorphidae	SN
	Tettigoniidae	PT, SN, BT
Pseudoescorpionida		
	Garypidae	PT
	Neobisidae	PT, BT
Psocoptera		
	Epipsocidae	PT
	Lachesillidae	BT
	Psyllipsocidae	PT
	Trogiidae	PT
Raphidioptera		
	Raphidiidae	PT, SN

Appendix 1. (Cont.)

Order	Family	Sampling method	Order	Family	Sampling method
Sarcoptiformes	Oribatidae	PT	Thysanoptera	Aeolothripidae	PT, SN, BT
Scutigeromorpha	Scutigeridae	BT		Phlaeothripidae	PT, SN, BT
				Thripidae	SN, BT
			Zygentoma	Lepismatidae	PT

N° families captured only in pan traps	54
N° families captured only in sweep net	37
N° families captured only in bait traps	12
N° families captured by pan traps and sweep net	38
N° families captured by pan traps and bait traps	28
N° families captured by sweep net and bait traps	6
N° families captured by pan traps, sweep net and bait traps	60

Appendix 2. Arthropod families that contributed to 70% of the cumulative similarity within the three sampling methods (pan traps, sweep net and bait traps): C. Contribution (%)

Apéndice 2. Familias de artrópodos que contribuyeron al 70% de la similitud acumulada entre los tres métodos de muestreo (trampas de bandeja, red de barrido y trampas de cebo): C. Contribución (%).

Order	Family	C(%)	Order	Family	C(%)
Pan traps similarity: 23.78%			Sweep net similarity: 31.53%		
Thysanoptera	Thripidae	11.81	Heteroptera	Nabidae	11.75
Homoptera	Adelgidae	9.68	Hymenoptera	Apidae	8.99
Hymenoptera	Formicidae	8.20	Heteroptera	Pyrrhocoridae	7.12
Thysanoptera	Aeolothripidae	7.66	Thysanoptera	Thripidae	7.11
Hymenoptera	Apidae	4.52	Coleoptera	Cantharidae	6.30
Diptera	Phoridae	4.07	Heteroptera	Mesovelidae	5.36
Diptera	Mycetophilidae	3.95	Coleoptera	Curculionidae	5.01
Diptera	Muscidae	3.89	Coleoptera	Melyridae	4.43
Diptera	Chironomidae	3.02	Lepidoptera	Pterophoridae	4.19
Hymenoptera	Ichneumonidae	2.87	Coleoptera	Chrysomelidae	4.07
Hymenoptera	Halictidae	2.87	Coleoptera	Coccinellidae	3.72
Coleoptera	Cantharidae	2.37	Homoptera	Adelgidae	2.71
Coleoptera	Curculionidae	2.22	Bait traps similarity: 27.23%		
Diptera	Dolichopodidae	2.13	Thysanoptera	Thripidae	19.05
Diptera	Sciaridae	1.77	Homoptera	Adelgidae	16.25
			Hymenoptera	Formicidae	15.38
			Thysanoptera	Aeolothripidae	13.51
			Coleoptera	Cantharidae	5.85

Appendix 3. Arthropod families that contributed (C in %) to more than 2% of dissimilarity between the three sampling methods: PT. Pan traps; SN. Sweep net; and BT. Bait traps.

Apéndice 3. Familias de artrópodos que contribuyeron (C en %) con más de un 2% a la diferencia existente entre los tres métodos de muestreo: PT. Trampas de bandeja; SN. Red de barrido; BT. Trampas de cebo.

Order	Family	Average of abundance		
		PT	SN	C(%)
Pan traps vs. sweep net dissimilarity: 81.12%				
Thysanoptera	Thripidae	6.66	2.97	4.43
Hymenoptera	Apidae	2.18	3.58	3.23
Hymenoptera	Formicidae	4.93	0.90	3.08
Heteroptera	Nabidae	0.47	4.43	3.03
Homoptera	Adelgidae	5.17	1.71	2.85
Collembola	Isotomidae	6.41	0.15	2.67
Hymenoptera	Halictidae	2.68	3.07	2.45
Thysanoptera	Aeolothripidae	3.22	1.50	2.38
Heteroptera	Pyrrhocoridae	0.66	2.99	2.29
Diptera	Mycetophilidae	4.14	0.25	2.28
Coleoptera	Melyridae	0.35	2.24	2.03
Sweep net vs. bait traps dissimilarity: 82.12%				
Hymenoptera	Apidae	3.58	0.15	5.01
Heteroptera	Nabidae	4.43	0.15	4.72
Heteroptera	Pyrrhocoridae	2.99	0.00	3.59
Thysanoptera	Thripidae	2.97	3.43	3.54
Hymenoptera	Formicidae	0.90	3.51	3.39
Coleoptera	Melyridae	2.24	0.19	3.17
Homoptera	Adelgidae	1.71	3.18	3.16
Thysanoptera	Aeolothripidae	1.50	2.71	2.98
Hymenoptera	Halictidae	3.07	0.45	2.77
Heteroptera	Mesovelidae	2.01	0.29	2.13
Homoptera	Aphididae	0.00	1.85	2.01
Pan traps vs. bait traps dissimilarity: 77.88%				
Thysanoptera	Thripidae	6.66	3.43	5.97
Hymenoptera	Formicidae	4.93	3.51	4.08
Homoptera	Aphididae	3.04	1.85	3.63
Collembola	Isotomidae	6.41	0.90	3.61
Hymenoptera	Apidae	2.18	0.15	3.47
Homoptera	Adelgidae	5.17	3.18	3.43
Thysanoptera	Aeolothripidae	3.22	2.71	3.26
Diptera	Mycetophilidae	4.14	1.88	3.26
Diptera	Muscidae	3.22	0.71	2.59
Diptera	Phoridae	3.68	1.12	2.56
Diptera	Sciaridae	2.43	0.67	2.08
Diptera	Chyromiidae	1.82	0.83	2.01