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Coccinellid morphospecies as an alternative method for differentiating management regimes in olive orchards

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ABSTRACT

Morphospecies, also known as morphotypes, recognizable taxonomic units (RTUs) and parataxonomic units (PUs) have been used for rapid biodiversity assessment (RBA) in invertebrate diversity studies worldwide. Their utilization might lighten taxonomists' workload when rapidly evaluating the richness and diversity of arthropods for conservation or biological assessment. To validate morphospecies, as opposed to taxonomic species, ladybird beetles (Coleoptera, Coccinellidae) were chosen in order to differentiate organic and non-organic management regimes (integrated and conventional) in olive orchards in southern Spain. Ladybird beetle specimens collected over two years (1999 and 2000) from three locations were sorted by morphospecies, and then identified by Coleopteran specialists according to taxonomic species. Thus, two different datasets were created, independently analyzed and compared to measure the accuracy at the morphospecies level. The comparison of morphospecies and species datasets showed an accuracy of 62.18% (one morphospecies to one taxonomic species), with the identifying error principally made when one species was identified as two different morphospecies (32.74%). Although two Coccinellid species (*Scymnus mediterraneus* Iablokoff-Khnzorian, 1972 and *Coccinella septempunctata* Linnaeus, 1758) showed significant differences among regimes during the June–August period in spite of small errors, we suggest that the most abundant morphospecies of Coccinellidae and the June–August period could be adopted as a rapid and useful tool for evaluating the impacts of non-organic *vs.* organic management regimes in olive orchards.
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1. Introduction

The importance of olive agroecosystems in Andalusia (southern Spain) has grown considerably in recent decades despite the fact that olives have been an important crop in this region for a long time. With the present concern for the negative environmental impacts of large-scale olive monocultures, coupled with the growing demand for organic olive oil, a need to develop useful indicators of agroecosystem health in olive-growing regions has emerged. One key indicator of such health, or sustainability, is the abundance and biodiversity of invertebrates, especially arthropod fauna (Paoletti and Bressan, 1996; Van Straalen, 1997; Van Straalen and Verhoef, 1997). According to Moreno et al. (2007), invertebrates are the most commonly studied biological groups as biodiversity shortcuts. More specifically, arthropod diversity has been used to indicate the impacts of habitat modification (Andersen, 1990; Kremen, 1992; Lawton et al., 1998; Platen et al., 2001), to measure the effects

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of human disturbance (Kimberling et al., 2001), and even for conservation monitoring (Brown, 1997). Past studies of invertebrate fauna have also been used to compare organic and nonorganic farming systems (Álvarez et al., 2000; Letourneau and Goldstein, 2001; Hadjicharalampous et al., 2002; Shah et al., 2003; Ruano et al., 2004; Purtauf et al., 2005; Clough et al., 2007), reporting significant differences between agroecosystem management and faunal diversity. Since the number of arthropod species is considerably large in olive agroecosystems (Arambourg, 1986; De Andrés, 1991; Varela and González, 1999; Campos and Civantos, 2000; Ruíz and Montiel, 2000), they can be used to distinguish organic, conventional and integrated farming systems.

Previous studies have proposed the Coleoptera and Lepidoptera orders as possible indicators of the impacts of conventional management systems in olive orchards (Ruano et al., 2004). The criteria for selecting and testing terrestrial insects as indicators and their later application have been extensively described and defined in literature (Çilgi, 1994; McGeoch, 1998; Smith et al., 1999; Nordén and Appelqvist, 2001; Altieri and Nicholls, 2004; Moreno et al., 2007). However, the use of the Coleoptera order is complicated by having a large number of families with different life histories, biology, and environmental requirements. Different Coleopteran families have been proposed as indicators, in some cases together with other groups like Araneae (Pearce and Venier, 2006) or Isopoda (Hadjicharalampous et al., 2002). The most common groups are the epigeal beetles (Buck et al., 1992), more precisely staphylinids (Melke and Gutowski, 1995; Bohac, 1999) and carabid beetles (Michaels and McQuillan, 1995; Luka, 1996; Rainio and Niemelä, 2003; Kampichler and Platen, 2004). Coccinellidae is an abundant family in the olive canopy (Varela and González, 1999; Santos et al., 2007), the place where insecticide treatments are applied, and has been studied for its predator function in this agroecosystem (Morris et al., 1999). About 90% of the species of this family are beneficial predators (Iperti, 1999; Varela and González, 1999). Due to their beneficial role, the impact of pesticides on coccinellid species has been analyzed in olive agroecosystems (Cirio, 1997; Ruano et al., 2001; Ba M'hamed and Chemseddine, 2002; Santos et al., 2007), and synthetic pyrethroid insecticides (cypermethrin, alfa-cypermethrin) are one of the most toxic products (Ba M'hamed and Chemseddine, 2002), causing rapid mortality with a ratio that exceeded 60% after 24 h on *Scymnus mediterraneus* Iablokoff-Khnzorian, 1972 when applied in conventional olive orchards. In the same way, although with a more moderate effect, dimethoathe was also observed to cause mortality in *Sc. mediterraneus* and other coccinellids (Iperti, 1999). Furthermore, ladybirds are taxonomically one of the best known families of beetles because of their ubiquity and their presence around the world. This is a group with a vast number of species that are difficult to identify only on the basis of color pattern, which is extremely variable in most species (Iperti, 1999). However, the distinctive Coccinellid appearance facilitates the recognition of specimens at the family level (Iperti, 1999). The current scarcity of taxonomists, the difficulty in identifying separate species, especially invertebrates (Ward and Stanley, 2004), and the long experience necessary to be able to recognize the vast number of species, obligates agroecologists to appeal to other systems for evaluating diversity.

Taxonomic surrogacy is one of the four categories of rapid biodiversity assessment (RBA) proposed by Oliver and Beattie (1996a). RBA approaches have been developed to meet the short-term needs of providing scientific advice for resource managers and policy makers (Boone et al., 2005). For example, Oliver and Beattie (1993) developed RBAs for invertebrates. RBAs aim to reduce the time and effort for the identification process, greatly reducing dependence on specialist taxonomists (Wilkie et al., 2003) and allowing more ambitious sampling designs. The family Coccinellidae, identified at the morphospecies taxonomic level, can be used as a recognizable taxonomic unit (RTU). This term refers to the identification of unifying characteristics according to morphological similarities, without considering taxonomic literature or taxonomic standards (Krell, 2004). The precision of identifying morphospecies is quite variable for different arthropod groups, and therefore, it should be established based on the taxonomic morphospecies–species relationship for each group. According to Lawton et al. (1998), a large proportion of morphospecies cannot be assigned to named species, and the number of "scientist-hours" required to process samples increases dramatically for smaller-bodied taxa. Derraik et al. (2002), based on arguments in Lawton et al. (1998), suggested that the mistakes in ladybirds are made when several coccinellid species are very small. We have carried out this study in order to test the proposal that the use of coccinellid morphospecies (as a surrogate for coccinellid species) could be a possible "indicator" of the different management regimes for olive agroecosystems.

2. Materials and methods

2.1. Study zones

The study was conducted between 1999 and 2000 in three commercial olive orchards (Colomera, Arenales and Deifontes) 20 km north of Granada in southern Spain. These three sites were in an area of large olive orchards ranging between 200 and 500 ha. They were approximately 4 km apart from each other, located at similar altitudes and with similar environmental characteristics, but were under different management regimes. In one with intensive conventional management, a dimethoate spray occurred in March or April (against *Prays oleae* (Bernard, 1788)), in June there was an alpha-cypermethrin spray (against *P. oleae*), and finally, in October, a dimethoate spray (against *Bactrocera oleae* (Gmelin, 1790)) was applied. The orchard under integrated pest management (IPM) received only one treatment with dimethoate (against *P. oleae*) in June 2000. Finally, in the organic management, neither *Bacillus thuringiensis* Berliner used in other organic systems nor other permitted insecticides were applied during our study. Regarding the vegetal cover, weeds on the fields were totally eliminated in both integrated and conventional orchards; the conventional one was frequently ploughed deeply and treated with the herbicide simazine (4 L/ha of the formulation at 50%), while the same herbicide was applied twice a year in the integrated orchard. Finally, the organic olive orchard was ploughed only to a shallow depth (10 cm) from the end of May to the beginning of June.

2.2. Collection of Coccinellidae

The canopies of olive trees were sampled by beating, five times, four branches per tree (one per compass orientation) that were chosen at random, over an insect net of 50 cm in diameter. Coccinellid specimens collected from these olive canopies were frozen and later separated from vegetal and inorganic remains. Adults and juvenile beetles were identified under a stereomicroscope (Stemi SV8, Zeiss) to the taxonomic level of order and family. The adults of coccinellids were separated from the other coleopterans in order to be identified by a parataxonomist in accordance with their morphological differences, avoiding the use of taxonomic keys as recommended by Basset et al. (2004). A taxonomist (F. Rei) examined the coccinellid morphospecies and, at the same time, trained the parataxonomist to recognize the coccinellid species. In this way, it was possible to determine the degree of ''splitting'' and ''lumping'' (Oliver and Beattie, 1996b) committed by the untrained parataxonomist. These terms mean that a taxonomic species is divided into several different morphospecies (splitting) or two or more taxonomic species are combined into a single morphospecies (lumping). The identification of ladybird species was carried out using the review of Coccinellidae from Portugal elaborated by Cardoso and Gomes (1986); however, the polymorphism and the range in length (from about 1 mm to about 10 mm depending upon species) of the family, in some cases, forced the extraction of male and/or female genitalia to differentiate between species. The scientific nomenclature was updated according to the Fauna Europaea inventory (Fauna Europaea Web Service, 2004).

2.3. Statistical analysis

The experimental sampling design was established as follows: three large olive grove zones, one per management (even orchards of different owner per zone). In each olive orchard, six blocks were sampled in 1999, each block consisting of a row of five trees separated by unsampled one, so the distance between sampled trees was 20 m, though only five blocks were considered in 2000. Each block was considered a true replication because of the large size of the studied olive zones and because the distance between blocks (a minimum of 0.5 km) ensures independence between them. Thus, the three sites with different management were sampled on sixteen occasions (monthly from March to October in 1999 and 2000), and the blocks were sampled by beating. Coccinellid family count data were analyzed by applying a principal effects quasi-Poisson (overdispersion parameters took values much greater than 1) log-linear model (Cunningham and Lindenmayer, 2005) in which the design factors are block (1–6 in 1999 and 1–5 in 2000), management (organic and non-organic) and year (1999 and 2000). The coefficients and associated p-values for the factors for each model were calculated using R-statistical software (R Development Core Team 2005). As an additional check, a Mann–Whitney U-test was used to compare both databases (species *vs.* morphospecies) monthly over the duration of the study with SPSS 14.0 for Windows.

Correspondence analysis (CA) was performed for the most abundant morphospecies and species (Hammer et al., 2001) to check the similarity graphically among regimes using PAST program.

3. Results

3.1. Splitting and lumping

From a total number of 1253 coleopteran specimens, the parataxonomist was able to identify 394 individuals by coccinellid family over the two years of study, according to the general morphological features of the family. Following the morphospecies definition, the parataxonomist sorted the individuals into 16 morphospecies. After examination by the taxonomist, the number of coccinellid specimens was 375 individuals (19 individuals less than in the morphospecies dataset), and they belonged to 12 taxonomic Coccinellidae species (Table 1). The total number of specimens was different in the two dataset, because three specimens were lost or could not be identified due to their poor state of preservation. The remaining 16 specimens belonged to phalacrid species (10 individuals) and cucujid species (6 individuals), because even though they are not in the coccinellid family, their body size, color pattern, and other features fit the morphological concept that the parataxonimst had been taught for small ladybirds. Finally, for these reasons, we consider that the total number of taxonomic species was 14 (12 coccinellids and two belonging to other families).

On one hand, following the formula proposed by Oliver and Beattie (1993) to calculate the relation between identified morphospecies and verified taxonomic species, we obtained a 14.2% error rate (error % = 100 $\times$ [No. of taxonomic species (14) $-$ No. of morphospecies (16)]/No. of taxonomic species (14)).

On the other one hand, from the 394 individuals of the morphospecies dataset, the proportion of correctly assigned morphospecies to taxonomic species was 62.18% (245 $\times$ 100/394). However, during the identification, a 32.74% lumping (129 $\times$ 100/394) and a 5.08% splitting (20 $\times$ 100/394) error occurred. *Sc. mediterraneus* one of the species causing splitting errors and assigned to msp. 3, was the most abundant species (294 individuals), but a total of 89 individuals were assigned to msp. 4 (Table 1). However, this species was the smallest of the captured ladybirds, and the extraction of genital apparatus was necessary to identify it correctly. Another splitting error occurred when *Hippodamia variegata* (Goeze, 1777) was identified as two different morphospecies, but verified by the taxonomist as two varieties of one species (*H. variegata carpini* and *H. variegata constellata*). Regarding the non-coccinellids in the sample, msp. 1 and msp. 8 were identified by the taxonomist as one unique phalacrid species; for that reason, a splitting error was committed. On the other hand, only one lumping case occurred with *Scymnus subvillosus* (Goeze, 1777), of which only two specimens were found and included as *Sc. mediterraneus*, due to the small size of its body length (Table 1). Mistakes were not made with the other species, since each taxonomic species was identified as a morphospecies: *Coccinella septempunctata* Linnaeus, 1758, the second most abundant species, *Adalia decempunctata* (Linnaeus, 1758), *Adalia bipunctata* (Linnaeus, 1758), and *Myrrha octodecimguttata* (Linnaeus, 1758) could be identified on the basis of their particular

Table 1 – Coccinellid morphospecies and species dataset and total abundance in integrated (I), conventional (C) and organic (O) management regime over the two years, with type of identification error and general body size of species

Morphospecies dataset	I	C	O	Species dataset	I	C	O	Correspondence sp. msp.	Error	Body length (mm)
msp. 1	1	0	3	Family Phalacridae	–	–	–	1:2	Splitting	–
msp. 2	1	4	2	*R. chrysomeloides*	3	10	1	1:1	Correct	–
msp. 3	73	10	36	*Sc. mediterraneus*	73	48	174	1:2	Splitting	1–1.5
msp. 4	3	44	140						Splitting	1–1.5
				Sc. subvillosus	0	0	2	2:1	Lumping	1.9–2.5
msp. 5	4	10	21	*C. septempunctata*	4	10	22	1:1	Correct	5.5–8
msp. 6	0	1	1	*H. variegata var. carpini*	0	1	1	1:2	Splitting	3–5.5
msp. 7	0	0	8	*A. decempunctata*	0	0	8	1:1	Correct	3–5.5
msp. 8	0	0	8	Family Phalacridae	–	–	–	1:2	Splitting	–
msp. 9	0	0	5	*Sc. apetzi*	1	0	4	1:1	Correct	2–3
msp. 10	0	0	3	*A. bipunctata*	0	0	3	1:1	Correct	3.5–5
msp. 11	2	4	0	Family Cucujidae	–	–	–	1:1	Correct	–
msp. 12	1	0	0	H. variegata var. constellate	1	0	0	1:2	Splitting	3–5.5
msp. 13	0	2	4	*Sc. punctillum*	0	2	4	1:1	Correct	1.2–1.5
msp. 14	0	0	1	*H. reppensis*	0	0	1	1:1	Correct	2.5–4.5
msp. 15	0	0	1	*M. octodecimguttata*	0	0	1	1:1	Correct	3.5–5.5
msp. 16	0	0	1	*P. luteorubra*	0	1	0	1:1	Correct	2.5–3.5
Total	85	75	234	Total	82	72	221			
Richness of msp.	7	7	14	Richness of sp.	5	6	11			

coloration. There was no error in identification of *Rhyzobius chrysomeloides* (Herbst, 1792), *Stethorus punctillum* Weise, 1891, *Scymnus apetzi* Mulsant, 1846, *Hyperaspis reppensis* (Herbst, 1783) and *Platynaspis luteorubra* (Goeze, 1777).

3.2. *Coccinellid abundance and species–morphospecies richness*

The total numbers of individuals assigned to coccinellid morphospecies over the two years of study in the integrated, conventional and organic orchards were 85, 75 and 234, respectively, giving a total of 394 individuals (Table 1). The mean abundance of ladybird specimens found in organic, integrated and conventional olive orchards did not show a similar pattern over the two years, but the greatest number of individuals was achieved between June to September in 1999 and June and July 2000. In the non-organic orchards, a repeating pattern was not observed during the two years. The integrated orchard showed low values from July to October in 1999; however, the mean abundance increased in March 2000, but decreased again from April to June, and dimethoate was applied in July 2000, which is perhaps the cause of absence of ladybirds until October 2000. The mean abundance in the conventional orchard also had an irregular pattern, where March and April 1999 showed a low abundance, and there were no captures until October, when the mean number of caught individuals was highest in the conventional orchard. From March to June 2000, the mean abundance decreased and no individuals were captured until October, probably corresponding to the three applications of chemical products (alpha-cypermethrin and dimethoate). The total number of individuals identified as coccinellid

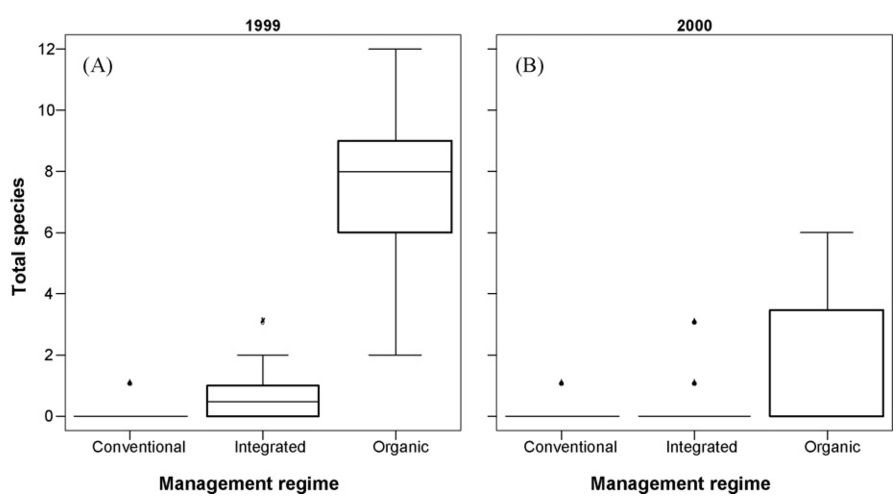

Fig. 1 – Box and Whisker diagram of the abundances of coccinellid species according to management regime in 1999 (A) and 2000 (B).

Table 2 – *t*-student coefficients and associated significance *p*-values for the adjusted models of species and morphospecies, considering block, year and management regime factors

Dependent variable	Year (t-value)	Management regime (*t*-value)	Block (t-value)	Overdispersion parameter
Total species	-4.811^{***}	8.186^{***}	−0.637	2.040
Most abundant species	-5.271^{***}	8.029^{***}	−0.601	1.875
Total morphospecies	-4.750^{***}	8.304^{***}	−0.413	2.029
Most abundant morphospecies	-5.348^{***}	8.138^{***}	−0.243	1.806

*** $p < 0.001$.

morphospecies was greater than the number identified as coccinellid species, corresponding 82, 72 and 221 in the integrated, conventional and organic orchards, respectively, with 375 individuals altogether over the two years of study (Table 1). The patterns of mean abundance of ladybird species did not differ statistically from the patterns of morphospecies over the time. Both species and morphospecies datasets showed significant differences in March 1999 (Mann–Whitney test: $p < 0.05$). However, the mean abundance was very low for this month. In the morphospecies dataset, a total of 16 morphospecies were identified, while in the species dataset, 12 species were identified. The correspondence among species and morphospecies is represented in Table 1. The numbers of morphospecies for the two years were 7, 7 and 14 on the integrated, conventional and organic orchards, respectively while the numbers of species were 5, 6 and 11. This indicates that the richness and abundance of ladybirds were higher in the organic than the non-organic orchards, with respect to morphospecies as well as species.

3.3. *Comparison among management regimes*

In the canopies of the olive trees in the organic orchard, the months with the highest number of individuals were June, July and August, and for this reason this period over the two years was chosen for analyzing the statistical differences between organic and non-organic farming systems. In spite of the significant differences observed among the years for this period (Table 2), the Box-and-Whisker diagram (Fig. 1A and B) shows that the abundance of coccinellids was highest in organic orchards, such as in 1999 as well as in 2000. Several quasi-Poisson log-linear models without interactions between factors were considered to analyze and compare both the morphospecies and species datasets. The respective coefficients of the adjusted models show that we can leave out the factor block (Table 2). Results for the morphospecies dataset showed that there were significant differences among regimes, and this was also true for the species datasets (Table 2). This indicates that organic farming can be differentiated from non-organic farming (integrated and conventional) from June to August, using either morphospecies or species. For the same period, the proposed models were applied for the most abundant morphospecies and species. The abundances of morphospecies 3, 4 and 5 and for *Sc. mediterraneus* and *C. septempunctata* were significantly different among management regimes (Table 2). These results lead us to conclude that the most abundant species or morphospecies could be used to distinguish between organic and non-organic systems at first glance. Furthermore, two correspondence analysis maps were elaborated for the visualization of the data for the four most abundant ladybird morphospecies: msp. 3, msp. 4, msp. 5 and msp. 7 (Fig. 2), and for the three most abundant species: *Sc. mediterraneus*, *C. septempunctata* and *A. decempunctata* (Fig. 3) during the June–August period. The CA plots display organic farming data in 1999 as more grouped and closer to msp. 3 and msp. 4, or *Sc. mediterraneus*, than integrated sites, while the non-organic data in 2000 were

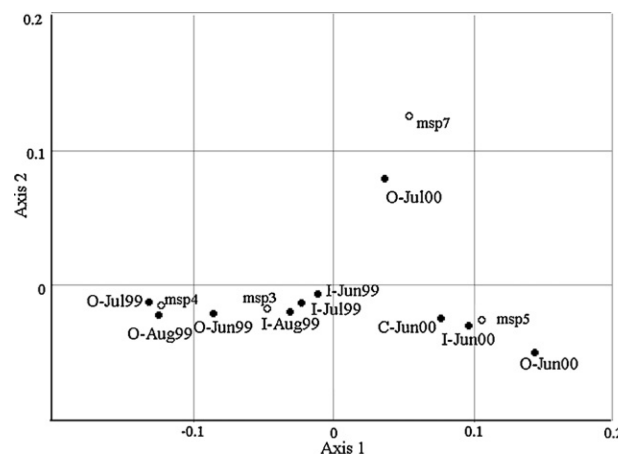

Fig. 2 – Correspondence analysis map for the organic (O), integrated (I) and conventional (C) olive orchards from June till August period in 1999 and 2000, using the most abundant morphospecies.

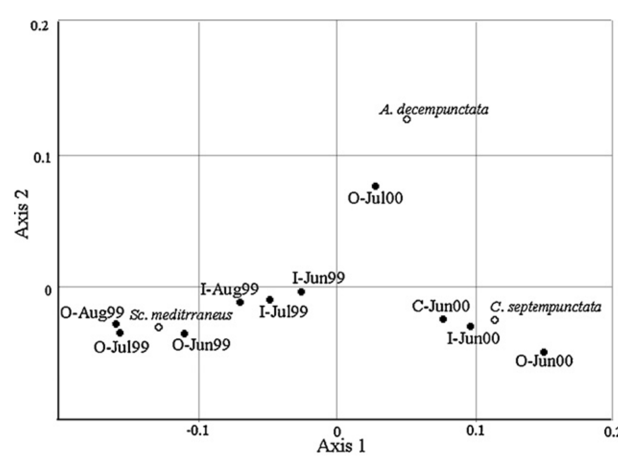

Fig. 3 – Correspondence analysis map for the organic (O), integrated (I) and conventional (C) olive orchards from June till August period in 1999 and 2000, using the most abundant species.

closer to msp. 5, or *C. septempunctata*. Organic orchards were closer to msp. 5 (*C. septempunctata*) and msp. 7 (*A. decempunctata*) in June and July 2000, respectively. In some summer months, none of the most abundant species or morphospecies were caught, and for this reason they do not appear on the CA maps (Figs. 2 and 3).

4. Discussion

Coccinellids were chosen because they are the most abundant family of coleopteran in the olive canopy and are abundantly present in the olive agroecosystem (Cirio, 1997; Varela and González, 1999; Ruano et al., 2001; Ba M'hamed and Chemseddine, 2002; Santos et al., 2007). Iperti (1999) suggested that the ladybeetle species found in different geographic areas of the world can be utilized as bioindicator insects due to their climatic and trophic characteristics. Regarding their sampling, the method seems to be most efficient during the June–August period because the average number of captured individuals is greatest in organic orchards (sampling surrogacy) (Ward and Larivière, 2004). Species identification presents relative difficulty when based only on color patterns and is only possible by looking carefully at morphological characteristics, such as ventral size or by using genital apparatus extraction, especially for the small species (Derraik et al., 2002) as *Sc. mediterraneus*. Because of this, it has been suggested that the number of scientist-hours to process samples is inversely related to each group's geometric mean body length (Lawton et al., 1998). Even though the parataxonomist made a splitting error (one species to two morphospecies), this error was worthwhile, because an enormous amount of time was saved, which is very valuable if rapid biodiversity is being assessed. Ladybirds met some of the requirements to be a good indicator group, since their predator condition, their biologies and their life cycles have been widely described in biological control (Iperti, 1999). Ladybird migration has been associated with short photoperiod, unfavorable temperatures, and changes in food availability and quality in ecosystems (Iperti, 1999). In southern Spain, olive landscapes cover large, continuous areas, and ladybirds can fly to nearby olive orchards under such uniform conditions while perturbation is occurring, and then after the perturbation they can return. It would be necessary to study adjacent lands to understand if the insects take shelter from perturbation there. As another requirement of an indicator group, Coccinellids in olive agroecosystems also suffer the effects of chemical products, and dimethoathe and alpha-cypermemethrin were applied in non-organic orchards, which might have caused the rapid mortality of *Sc. mediterraneus*, the most abundant species of sampled ladybirds in the organic orchard. Ladybirds fulfill all the requirements for a useful bioindicator, because they: (1) are a widely distributed and abundant species; (2) are relatively easy to sample and identify; (3) have well-known biologies and life cycles; (4) are relatively immobile; and finally (5) seem to be more abundant in organic farming from June to September, which has been shown in our study comparing organic and non-organic practices (i.e. use of agrochemical products).

From this study we conclude that coccinellid species could be used as bioindicators for organic *vs.* non-organic management systems in olive orchards, while coccinellid morphospecies could be also used as a RBA method when necessary. Moreover, statistical analysis has shown that the June–August period is optimal for sampling Coccinellids. It is also not necessary to take into account all morphospecies, only the most abundant, and despite some lumping errors, enough information can be provided.

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