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Induction of sublethal effects for the characterization of Olive groves under different pest management systems

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Abstract- Currently, olive grove management in Spain responds to the three following clearly differentiated systems in order of decreasing area: Conventional Management (70%), Integrated Pest Management (IPM) (26%) and Ecological Management (4%) systems. These are characterized by a decreasing dependence on synthetic insecticides and by different soil tillage intensities. They are also subject to different subside regulations and application regimen by the government, so their adequate characterization represents a factor of increasing importance, which is the object of this work.During the years 2017 and 2018, olive groves corresponding to the three types of management were selected, in which two series of plots were established. In one series, Dimethoate 40® was applied, considering plots of the second series as control. After insecticide application, beneficial insects were monitored through the use of chromatic traps.The results allow elucidating two clearly different behavioral patterns in beneficial insects, depending on the use of insecticides: Conventional and IPM management, where repellency reaction is manifested, absent in the Ecological Management. Aspects about the induction of sublethal effects for the characterization of the different pest management systems are discussed.
Indexing terms: Synthetic insecticides; natural enemies; Conventional management; Integrated Pest Control (IPM); Ecological Management.

Indução de efeitos subletais para a caracterização de Olivais sob diferentes sistemas de manejo de pestes

Resumo- Atualmente, o manejo de olivais na Espanha responde a três modelos claramente diferenciados, por ordem decrescente de área: Manejo Convencional (70%), Manejo Integrado de Pragas (MIP) (26%) e Manejo Ecológico (4%). Estes são caracterizados por uma dependência decrescente de inseticidas sintéticos e por diferentes intensidades de preparo do solo e estão também sujeitos a diferentes regulamentações e regimes de subsídios por parte do governo, visto que sua adequada caracterização representa um fator de crescente importância, sendo o objeto deste trabalho.Durante os anos de 2017 e 2018, foram selecionados olivais correspondentes aos três tipos de manejo, nos quais foram implantadas duas séries de parcelas. Em uma série, foi aplicado Dimetoato 40®, considerando as parcelas da segunda série como controle. Após a aplicação do inseticida, insetos benéficos foram monitorados por meio da implantação de armadilhas cromotrópicas.Os resultados permitem elucidar dois padrões de comportamento claramente distintos em insetos benéficos, dependendo do uso de inseticidas: Manejo Convencional e MIP, onde se manifesta uma reação de repelência, ausente no manejo ecológico. Aspectos sobre a indução de efeitos subletais, na caracterização dos diferentes tipos de manejo, serão discutidos.
Termos para indexação: Inseticidas sintéticos, inimigos naturais; Manejo Convencional; Manejo Integrado de Pragas (MIP); Manejo Ecológico.

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Introduction

Traditionally, pest control has been based on the systematic use of pesticides, which has led to an environmental crisis, with fatal consequences for ecosystems, negatively affecting biodiversity (VIVES DE QUADRAS, 1988), natural resources and the health of consumers (DEL PUERTO RODRÍGUEZ et al., 2014), also causing the appearance of emerging pests and the selection of insecticide-resistant lineages (CARRERO, 1996). At the same time, the suppression of plant communities in agricultural environments is the cause of strong reduction in the entomophagous activity of natural enemies of pests, which has led to a progressively greater dependence on synthetic insecticides.

In recent years, the policy of raising awareness about the risks of the indiscriminate use of pesticides has led to a change in the behavior of farmers, who are beginning to consider the importance of natural enemies in maintaining ecological balances (ARAMBOURG, 1986; LUCKMANN; METCALF, 1990), which reflected in a growing trend towards the adoption of environmentally friendly management practices. However, the Conventional Management system currently represents the highest proportion of Spanish crops (70%) (BOLLERO et al., 2017). In these crops, herbaceous cover is excluded due to the regular application of herbicides and pests are managed through the application of synthetic insecticides.

Second, there is an area equivalent to 26% of olive groves under the Integrated Pest Management (IPM) system, (BOLLERO et al., 2017). The Conventional Management system represents a more environmentally friendly approach, including biorational criteria. In these crops, the application of synthetic insecticides is not ruled out, although it requires a more precise knowledge of the biology and ecology of pests and their natural enemies, as well as the introduction of population thresholds with the aim of suppressing unnecessary insecticide applications (EHI -EROMOSELE et al., 2013). IPM also contemplates the development of herbaceous covers, which are aimed at stimulating the proliferation of beneficial insects (CALABRESE et al., 2012; GÓMEZ et al., 2018).

In very smaller proportion, an area equivalent to 4% of olive groves present ecological certification (BOLLERO et al., 2017), which are characterized by the complete suppression of synthetic insecticides and the stimulation of populations of beneficial species, natural enemies of pests.

In the last twenty years, the granting of economic subsidies by the Ministry of Agriculture has been reflected in a notable increase in the area devoted to organic olive cultivation, in relation to the year 2001 (BOLLERO et al., 2017). Among requirements for obtaining organic certification, these crops must be open to periodic inspections, being also submitted to analysis of pesticide residues in fruits / oil. However, its reliability depends on various environmental factors, which highlights the need for applying alternative approaches. Among the different options, the diversity and relative abundance of populations of natural enemies should be revaluated by determining their adaptations to the different agroecosystem management systems, as well as by assessing and monitoring the effects that insecticide applications induce on their behavior.

Most studies on the effects of insecticides are focused on the lethality they produce in insects; however, due to their rapid degradation (light, heat, rain), large proportion of insects are affected only by sublethal doses (STARK et al., 1995), including beneficial entomofauna (LUND et al., 1979; HAYNES, 1988; DESNEUX et al., 2007), which has led to underestimating the real impact of insecticide applications (KEVAN, 1999; THOMPSON, 2003). Among the effects caused by sublethal doses, Haynes (1988), Lee (2000), Kongmee et al. (2004), Desneux et al. (2007), Correa et al. (2015), Haddi et al. (2015), França et al. (2017) indicate alterations of physiological, biological and behavioral processes in affected insects, such as hyperreflexia, irritability / repellency, and greater trend to start flight, avoiding contact with insecticide-impregnated surfaces. In these insects, adverse effects on their reproductive processes, in the search for a host, in feeding, in migration and dispersal have also been observed (LEE, 2000; SINGH; MARWAHA, 2000; MAZZI; DORN, 2012; FRANÇA et al., 2017; VÉLEZ et al., 2019). These alterations have been indicated for organochlorine compounds (KENNEDY, 1947; ROLFF; REYNOLDS, 2009), pyrethroids (VAN DAME et al., 1995; PIKE et al., 1982; QUISENBERRY et al., 1984; HAYNES et al., 1986; ROLFF; REYNOLDS, 2009), organophosphates (MOORE, 1980; ROLFF; REYNOLDS, 2009) and carbamates (ROLFF; REYNOLDS, 2009).

Among sublethal effects, behavioral alterations in insects exposed to sublethal doses provide the first barrier of the detoxification mechanism (VAN DAME et al., 1995; GEROLD; LAARMAN, 1967; GOULD, 1984; LOCKWOOD et al., 1984; PLUTHERO; SINGH, 1984; FRANÇA et al., 2017), which has contributed to a progressive reduction in their efficacy, and to the development of resistant lineages (LEE, 2000; SINGH; MARWAHA, 2000; FRANÇA et al., 2017). These alterations are therefore secondary effects that must be taken into account for a more precise assessment of the impact of insecticides (DESNEUX et al., 2007), and especially for the improvement of integrated pest control programs (GEORGHIOU, 1972; LOCKWOOD et al., 1984; PLUTHERO; SINGH, 1984; GUEDES et al., 2009).

The possibility of verifying the occurrence or absence of repellency phenomena to insecticides in populations of beneficial insects after their application, and in this case, verifying possible behavioral alterations among the different management systems, could provide information of interest for the characterization of crops. In parallel, the negative effect of insecticide applications on the entomophagous activity of insects suggests as complementary objective, the assessment of the predatory impact in the different management systems, which will allow a much more precise analysis of their advantages / disadvantages.

Materials and methods

The study was carried out in three olive groves in the municipality of Jaén (Andalusia, southern Spain) during the spring and summer of 2017 and 2018. The selected olive groves are representatives of the Conventional Management (CM), Integrated Pest Management (IPM) and Ecological Management (ECO) systems, respectively, which have been submitted to agricultural practices from the beginning of plantation. Table 1 shows the average volume of pesticide application for the control of some of the main phytoparasites of olive groves in crops submitted to these management systems. The distance between the Ecological system and the Conventional system is approximately 2.1 km; while the distance between Ecological and IPM systems is about 4.6 km. Likewise, the distance that separates Conventional and IPM systems is about 2.5 km.

Conventional Management system (CM). Surface: 32 ha. Coordinates: 37 ° 36'18.20 '' N 3 ° 28'33.59 '' W. Olive groves are of the Picual variety, 20 to 30 years old, and are planted with density of 100 olive groves / hectare. Pre-emergence herbicides are regularly applied to keep olive groves free from spontaneous vegetation. Arthropod control is carried out using commercial organophosphate insecticides, regularly applied using Dimethoate® 40% © (400 g / l) (BASF) at concentration of 0.1% (100 cc / hl), applied during spring (May-June) and autumn (September-October), with average annual frequency of three applications.

Integrated Pest Management system (IPM). Surface: 18 ha. Coordinates: 37 ° 37'44.79 '' N 3 ° 26'32.75 '' W. Olive groves are of the Picual variety, with planting age and density similar to those of the Conventional Management system. In this case, the growth of vegetation cover located in a band of approximately 2.5 m, located in the central area between two contiguous rows of olive groves, is encouraged. In order to reduce water competition from the month of April, this cover is controlled by using brushcutter. Among species that compose this plant community associated with cultivation, the following stand out: *Lolium rigidum* Gaudin, *Senecio vulgaris* Linneo, *Poa annua* Linneo, *Silene colorata* Poiret, *Diplotaxis virgata* Candolle, *Muscari neglectum* Gussone, *Sinapis alba* Linneo, *Equisetum arvense* Linneo, *Bromus madritensis* Linneo, *Convolvulus althaeoides* Linneo, *Phalaris minor* Retzius, *Daucus carota* Linneo, *Cirsium arvense* Scopoli, and *Anacyclus clavatus* Persoon. Pest control is only carried out in compliance with the Integrated Production Regulation of the Protected Designation of Origin (PDO) Regulatory Council, and in cases in which pest population values exceed the established economic damage threshold. However, in any case, the insecticide commonly used is Dimethoate 40% ©. The Conventional Management system allows less dependence on insecticides, making 1-2 applications per year.

Table 1. Characterization of the different olive grove management systems according to chemical treatments applied to different pests / pathogens. Period of 2007–2017; Ministry of Agriculture of Spain and Spanish Committee of Ecological Agriculture.

		Conventional	**IPM**	**Ecological**
Pest/pathogen	**Pesticides Active ingredients**	**volume-weight (avg/ Ha&year)**	**volume-weight (avg/ Ha&year)**	**volume-weight (avg/ Ha&year)**
Herb vegetation	Glyphosate (20%) + Oxifluorfen (3%)	0.3 L/Ha 4 L /Ha	---	---
Fusicladium oleagineum *Colletotrichum gloesporides* *Pseudomonas savastanoi*	Copper oxychloride (20%) + Propineb (15%)	0.8 Kg /Ha	0.8 Kg /Ha	---
Aceria oleae	S (80%)	1.4 Kg/Ha	0.75 Kg/Ha	---
Prays oleae	Dimethoate 40%	0.9 L /Ha	0.3 L /Ha	---
Euzophera pinguis	Chlorpyrifos 48%	1.5 L /Ha	0.75 L /Ha	---
Phloeotribus scarabaeoides	Dimethoate 40%	0.9 L /Ha	0.45 L /Ha	---
Bactrocera oleae	Dimethoate 40%	1.8 L /Ha	0.9 L /Ha	---

Ecological Management system (ECO). Surface: 30 ha. Coordinates: 37 ° 37'24.38''N 3 ° 29'51.22 '' W. Olive groves are of the picual variety, 20 to 25 years old, with no application of synthetic chemicals, including pesticides and fertilizers. The IPM system allows the proliferation of natural vegetation cover during the autumn, winter and spring. In this system, pest control depends almost exclusively on the entomophagous activity of natural enemies. The species that compose most part of this cover are the same as in the case of the IPM system, although, in this case, vegetation characteristic of the Mediterranean forest is also included (especially in adjacent areas), among which *Quercus ilex* Linneo, *Rosmarinus officinalis* Schleid and *Stipa tenacissima* Kunth stand out.

Experimental design

The study was carried out during the anthophagous and carpophagous generations of the olive moth, *Prays oleae* (Bernard, 1788) (Lepidoptera: Praydidae), which occurs between mid-spring and mid-summer, a period in which beneficial insects, like phytophages, have intense activity (RAMOS and RAMOS, 1990). During this interval, insecticidal applications are frequent (Conventional Management and IPM systems) (Table 1), commonly based on 40% Dimethoate® for the control of pests such as olive moth, *P. oleae*, olive beetle *Phloeotribus scarabaeoides* (Bernard, 1788) (Coleoptera: Curculionidae: Scolytinae), or olive scale *Saissetia oleae* (Olivier, 1791) (Hemiptera: Coccidae), among others.

In each of the three selected olive groves, 6 plots of 30 x 30 m (16 olive groves) were established (Figure 1) with minimum distance between them of 150 m. In 3 of the 6 plots of each randomly selected olive grove, experimental application based on organophosphate Dimethoate 40% © was performed (400 g / l) (BASF). This commercial insecticide was used because it is the most commonly used product, which was applied at concentration of 0.1% (v / v) by means of hydraulic knapsack sprayer (MATABI Evolution 16 ©) with 16 l capacity. The proportions of olive groves experimentally treated (16 x 3 olive groves) represent 0.8%, 1.5% and 0.9% for Conventional Management, IPM and Ecological Management systems, respectively, which in the latter case does not represent an inconvenient to maintaining organic certification. On the other hand, in the remaining 3 plots considered as control, distilled water was applied on olive groves. Both in insecticide-treated plots and in control plots, it was ensured that the volume applied per grove was homogeneous, of about 2.5 l, and that during application, environmental conditions were atmospheric calm, with wind speed of about 4 or 5 km / h. Treatments were carried out on May 10 (2017) and June 2 (2018), both dates coinciding with the flowering phenological stage of olive groves (stages FI-F2).

In the 6 plots of each olive grove, the community of natural enemies was monitored through the passive sampling method based on the use of yellow chromatic traps. This type of sampling based on the movement of arthropods towards the traps (PASCUAL et al., 2014) has provided excellent results in previous studies, and has proved to be an easily replicable method (TRDAN et al., 2005; GONZÁLEZ-RUIZ; GÓMEZ-GUZMÁN, 2019).

Immediately after insecticide application, chromatic traps were placed at a rate of 1 per grove, in all olive groves of the 6 plots. Traps, measuring 20 cm x 40 cm, were placed approximately 1.5 m from the ground and in the southern sector of olive groves in order to optimize the sampling to the microclimatic preferences of natural enemies during the spring and summer months. Traps were removed and renewed every 5 days, establishing two sampling intervals in each year of study: May 10-15 and May 15-20 in 2017 and June 2-7 and June 7-12 in 2018.

To minimize the effect of pseudo-replication (HURLBERT, 1984), two plots were randomly selected in each sampling interval in each olive grove, one treated and one control. This design implies, for each olive grove and in each sampling interval, a total of 32 replicates: 16 of them corresponding to a control plot and 16 corresponding to a treated plot.

At the laboratory, traps were temporarily stored in cold chamber (4 ° C) and later examined by means of magnifying glass binoculars for the taxonomic determination and quantification of species of captured natural enemies. For the determination and taxonomic quantification, the following criteria were applied:

a. Entomophagous importance. Species associated with at least one olive pest species (ARAMBOURG, 1986; HODKINSON; HUGHES, 1993; ANDRÉS-CANTERO, 1997; GUERRERO GARCÍA, 2003; BURRACK et al., 2009).

b. Ecological adaptability. Species present in a wide range of agricultural and / or forest ecosystems.

c. Insects whose larval development period is exceptionally long, and therefore more likely of being affected by insecticide applications.

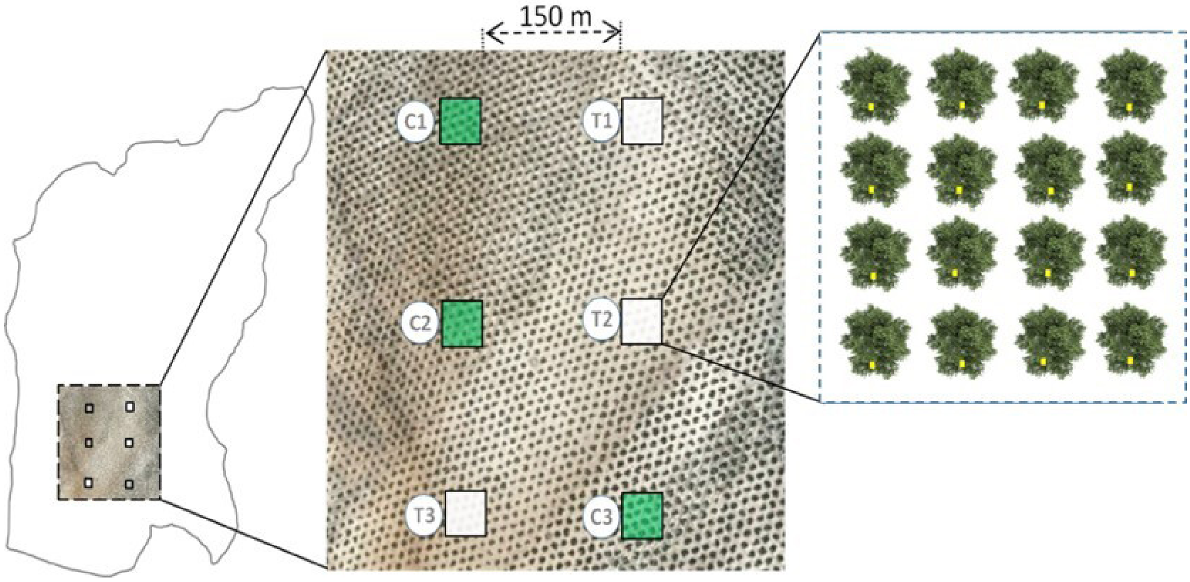


Figure 1. Distribution scheme of treated (T) and control (C) plots within the Ecological management system.

Evaluation of the entomophagous activity

The evaluation of the entomophagous activity selected the main pest species in olive groves of this study, olive moth, *P. oleae*, and its main predators, species of the *Chrysoperla carnea s.l.* complex (STEPHENS, 1836) (Neuroptera: Chrysopidae), in which species *Chrysoperla agilis* (HENRY, 2003) (Neuroptera: Chrysopidae) stands out due to its greater abundance (BOZSIK et al., 2009). During the summer of 2017, between mid-July and mid-August, and coinciding with the oviposition period of *P. oleae*, olive grove samples were taken at 10-day intervals. On each sampling date, 4 olive groves were randomly selected from each of olive groves, collecting 100 fruits from each of the four cardinal directions. Harvested olives were examined in the laboratory, where the number of *P. oleae* eggs in each olive was counted. According to methodology described by Ramos and Ramos (1990), differentiation between live eggs, those that had already hatched and those that had been predated by lacewings was performed (Neuroptera: Chrysopidae), as these are those that mostly exert oophagous predation in the anthophagous generation of *P. oleae* (RAMOS; RAMOS, 1990). The following parameters were recorded:

Hatching rate: % of eggs hatched in relation to the sum of live and hatched eggs.

Predation rate: % of predated eggs in relation to the sum of live, predated and hatched eggs.

Statistical analysis

For the statistical analysis of data, the Statgraphics Centurion XVII statistical package (2016) was used. Normality of distributions has been verified using the Shapiro-Wilk normality test. If data do not have normal distribution, the Kruskal-Wallis test is applied to determine possible significant differences among the different management systems. To compare the capture values of natural enemy species between two different management systems, the Mann-Whitney U test was used. To compare the capture values of each beneficial insect in treated and control plots within the same management system, the Mann-Whitney U test was used. To determine significant differences between predation rates of lacewings in the different management systems, analysis of variance (ANOVA) was performed. Finally, to compare the predation values among the different management systems, the Tukey's HSD (honestly significant difference) test was applied.

Results and discussion

Ecology of captured beneficial insects

Among captured insects, 10 species that met the specified criteria were selected (6 predators and 4 parasitoids) for being common in olive groves of southern Spain (ARAMBOURG, 1986; VARELA-MARTÍNEZ; GONZÁLEZ-RUIZ, 1999). Among entomophagous predators, the highest capture rate corresponds to *Aeolothrips intermedius* (Bagnall, 1934) (Thysanoptera: Aeolothripidae), which is a cosmopolitan species present in a wide range of crops (DE LIÑÁN, 1998; NIKOLOVA et al., 2015). In olive groves, they feed on *Liothrips oleae* (Costa, 1857) (Thysanoptera: Phlaeotripidae), as well as on various phytophagous mites such as *Aceria oleae* (Nalepa, 1900) (Trombidiformes: Eriophyidae) and *Oxycenus maxwelli* (Keifer, 1939) (Trombidiformes: Eriophyidae).

Species of the *Ch. carnea* complex, especially *Ch. agilis*, have been frequently found. In studies carried out in southern Spain on the *carnea* complex (BOZSIK et al., 2009), four cryptic species that inhabit olive groves of southern Spain have been reported: *Chrysoperla affinis* (STEPHENS, 1836) ex *Chrysoperla kolthoffi* (Navas, 1927) (THIERRY et al., 1998); *Chrysoperla lucasina* (Lacroix, 1912) (HENRY et al., 2001); *Ch. carnea* sensu stricto (THIERRY et al., 1998) or *Chrysoperla pallida* (Henry, 2002); and *Ch. agilis* (HENRY et al., 2003). These studies revealed the presence of these cryptic species in Andalusian olive groves, with *Ch. agilis* being the dominant species (> 90%) (BOZSIK et al., 2009), which is consistent with this study. In olive groves, species of the *carnea* complex are very effective predators in the natural control of olive moth *P. oleae* (BERNARD, 1788) (BOZSIK et al., 2009), of hemipterans psyllids such as *Euphyllura olivina* (Costa, 1839) (Hemiptera: Psyllidae) and species of Coccidae and Diaspididae families.

Among predators, *Anthocoris nemoralis* (FABRICIUS, 1794) (Hemiptera: Anthocoridae) and *Orius laevigatus* (FIEBER, 1860) (Hemiptera: Anthocoridae) also stand out. These species are frequently present in a wide range of agricultural crops, where nymphs and adults feed on numerous phytophagous species. In olive groves, they feed on olive psylla *E. olivina* (ANDRÉS-CANTERO, 1997), olive thrips, *L. oleae* (ARAMBOURG, 1986; BEJARANO-ALCÁZAR et al., 2011) and olive moth, *P. oleae* (ARAMBOURG, 1986).

Ladybugs (Coleoptera: Coccinellidae), especially *Coccinella septempunctata* (Linnaeus, 1758), play an important role in the control of several olive grove pests such as *S. oleae*, *Parlatoria oleae* (Colvée, 1880) (Hemiptera: Diaspididae), *Lepidosaphes ulmi* (LINNAEUS, 1758), (Hemiptera: Diaspididae) (ARAMBOURG, 1986), and *Aspidiotus nerii* (BOUCHE, 1833) (Hemiptera: Diaspididae) (ANDRÉS-CANTERO, 1997).

Among the less abundant predators, snakefly, *Harraphidia laufferi* (NAVÁS, 1915) (Raphidioptera: Raphidiidae) stands out. Its larval development takes place in the olive grove bark, feeding on *P. scarabaeoides* larvae (GONZÁLEZ-RUIZ, 1989), as well as on *Euzophera pinguis* eggs and larvae (Haworth, 1811) (Lepidoptera: Pyralidae) (ROZAS; GONZÁLEZ-RUIZ, 2017). During its adult stage, it feeds on the nymphs of olive psylla, *E. olivina*.

Among parasitoids, the most frequent species is *Pnigalio mediterraneus* (FERRIÈRRE; DELUCCHI, 1957) (Hymenoptera: Eulophidae), an ectoparasitoid of second- and third-instar larvae of the olive fly *Bactrocera oleae* (ROSSI, 1790) (Diptera: Tephritidae), as well as larvae of several miner microlepidopterans, including *P. oleae*. *Pnigalio mediterraneus* does not present diapause, and remains active throughout the winter, causing high parasitism rates, which makes it a very relevant species within the complex associated with olive fly (ARAMBOURG, 1986).

Chelonus elaeaphilus (Silvestri, 1908) (Hymenoptera: Braconidae) and *Ageniaspis fuscicollis* (Dalman, 1820) (Hymenoptera: Encyrtidae) are parasitoids specifically associated with olive moth *P. oleae* in the Mediterranean area (CAMPOS, 1981; ARAMBOURG, 1986; CARRERO, 1996; SÁNCHEZ; LÓPEZ-VILLALTA, 1993; KATSOYANNOS, 1992) and in Portugal, reaching high parasitism rates (NAVE et al., 2017). *Chelonus elaeaphilus* attacks larvae and during its development, it has two endophageal stages and a third ectophagous stage, while *A. fuscicollis* attacks eggs, and adults emerge a few days later than *P. oleae* (ARAMBOURG, 1986). *Tetrastichus cesirae* (Russo, 1938) (Hymenoptera: Eulophidae) is a relatively polyphagous species associated to olive beetle *P. scarabaeoides*, olive scale *S. oleae*, olive leaf gall midge *Dasineura oleae* (LOW, 1885) (Diptera: Cecidomyiidae), olive gall midge, *Lasioptera berlesiana* (PAOLI, 1907) (Diptera: Cecidomyiidae), red olive scale *Chrysomphalus dictyospermi* (MORGAN, 1889) (Hemiptera: Diaspididae) (ANDRÉS-CANTERO, 1997), and olive thrips *L. oleae* (ARAMBOURG, 1986).

3.2. Abundance of beneficial insects according to the different agricultural management system (control plots)

The normality test indicates that the capture distributions of selected species do not follow a parametric distribution (Shapiro-Wilk: $p < 0.001$), which is frequent in this type of sampling (GONZÁLEZ-RUIZ; GÓMEZ-GUZMÁN, 2019; GÓMEZ-GUZMÁN et al., 2017). Data from control plots during the years 2017 and 2018 indicate the existence of statistically significant differences between capture values in the different pest management systems (Figure 2). The maximum capture values correspond to the Ecological Management system, minimum values to the Conventional Management system, and intermediate values to the IPM system, although in this case, values are more similar to the Conventional system. The absence of synthetic insecticides in the Ecological Management system would explain the higher capture frequency, with values up to 10 times higher in most species in relation to Conventional and IPM management systems. Likewise, the distinctive presence of species such as *H. laufferi*, absent in the Conventional system and practically absent in the IPM system, stands out in the Ecological system. Unlike the other species, this one has an exceptionally long life cycle, requiring larvae a period even longer than two years for their full development (ASPÖCK, 2002; PANTALEONI, 2007), which represents a more than remarkable increase in the frequency of exposure to the toxin, and the risk of suffering the lethal effects of insecticide applications, which explains its practical absence in Conventional and IPM systems.

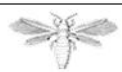
	Order	Family	Species			Kruskal-Wallis Chi²	Kruskal-Wallis p	NT	CM NCM	CM (%)	CM M-W	IPM NIPM	IPM (%)	IPM M-W	ECO NECO	ECO (%)	ECO M-W
Predators	Coleoptera	Coccinellidae	*Coccinella septempunctata*		2017	6,42	0,0403	79	11	(13,92%)	(b)	13	(16,46%)	(b)	55	(69,62%)	(a)
					2018	9,09	0,011	139	25	(17,99%)	(b)	32	(23,02%)	(b)	82	(58,99%)	(a)
	Hemiptera	Anthocoridae	*Anthocoris nemoralis*		2017	20,74	<0,001	34	0	(0%)	(b)	2	(5,88%)	(b)	32	(94,12%)	(a)
					2018	30,96	<0,001	57	3	(5,26%)	(b)	6	(10,53%)	(b)	48	(84,21%)	(a)
			Orius laevigatus		2017	2,66	0,265	171	43	(25,15%)	(a)	40	(23,39%)	(a)	88	(51,46%)	(a)
					2018	9,11	0,01	175	31	(17,71%)	(b)	35	(20%)	(b)	109	(62,29%)	(a)
	Neuroptera	Chrysopidae	*Chrysoperla agilis*		2017	16,39	<0,001	147	20	(13,61%)	(b)	23	(15,65%)	(b)	104	(70,75%)	(a)
					2018	9,75	0,007	131	13	(9,92%)	(b)	21	(16,03%)	(b)	97	(74,05%)	(a)
	Raphidioptera	Raphidiidae	*Harraphidia laufferi*		2017	32,78	<0,001	20	0	(0%)	(b)	0	(0%)	(b)	20	(100%)	(a)
					2018	56,33	<0,001	35	0	(0%)	(b)	1	(2,86%)	(b)	34	(97,14%)	(a)
	Thysanoptera	Aeolothripidae	*Aeolotrhips intermedius*		2017	18,75	<0,001	2540	448	(17,64%)	(b)	445	(17,52)%	(b)	1607	(63,27%)	(a)
					2018	27,25	<0,001	2304	372	(16,15%)	(b)	416	(18,06%)	(b)	1516	(65,8%)	(a)
Parasitoids	Hymenoptera	Braconidae	*Chelonus eleaphilus*		2017	21,94	<0,001	182	33	(18,13%)	(b)	33	(18,13%)	(b)	116	(63,74%)	(a)
					2018	23,25	<0,001	231	38	(16,45%)	(b)	51	(22,08%)	(b)	142	(61,47%)	(a)
	Hymenoptera	Encyrtidae	*Ageniaspis fucicollis*		2017	18,48	<0,001	274	25	(9,12%)	(b)	90	(32,85%)	(a)	159	(58,03%)	(a)
					2018	14,25	<0,001	281	33	(11,74%)	(b)	62	(22,06%)	(b)	186	(66,19%)	(a)
		Eulophidae	*Pnigalio mediterraneus*		2017	20,62	<0,001	471	46	(9,77%)	(b)	194	(41,19%)	(a)	231	(49,04%)	(a)
					2018	24,37	<0,001	365	39	(10,68%)	(b)	147	(40,27%)	(a)	179	(49,04%)	(a)
			Tetrastichus cesirae		2017	9,76	0,007	240	19	(7,92%)	(c)	52	(21,67%)	(b)	169	(70,42%)	(a)
					2018	29,62	<0,001	325	27	(8,31%)	(c)	61	(18,77%)	(b)	237	(72,92%)	(a)

Figure 2. Capture values of the different species of beneficial insects in the control plots of Conventional, IPM and Ecological systems. The first two columns correspond to the application of the analysis of variance by ranges of capture values in control plots (Kruskal-Wallis test: Chi-square and p-value). Significant differences, obtained from the paired comparison among management systems (years 2017 and 2018), are indicated with different letters (Mann-Whitney test, $p < 0.05$).

The similarity between Conventional and IPM systems (Figure 2, Mann-Whitney) is evident when considering the abundance of most beneficial insects, with few exceptions, such as *P. mediterraneus*, *A. fuscicollis* and *T. cesirae*, whose presence is superior in the IPM system. In these crops, insecticide Dimethoate 40% is frequently (IPM) or constantly used (Conventional) (MORENO; SORIANO, 2010), which could explain the similarities found between them. This fact is very striking, given that IPM incorporates differential elements compared to the Conventional system, such as the presence in the cultivation of herbaceous cover (CALABRESE et al., 2012) and the establishment of decision thresholds whose purpose is to limit the frequency of insecticide application. Therefore, data suggest the need to readjust IPM crop management protocols, most likely giving priority to insecticides such as those of natural origin, or those of biological origin, as they are less aggressive and more favorable alternatives for beneficial insects (NIKOLOVA et al., 2015).

3.3. Evaluation of the experimental insecticide application (treated plots)

Data obtained from treated plots show a very similar pattern in Conventional and IPM systems for most of the beneficial species (Figures 3 and 4), with significantly higher capture values in treated plots compared to control ones. Unlike previous ones, in the Ecological management system, captures of most cases are significantly higher in control plots compared to treated plots, which reveals two clearly different behavioral patterns in the populations of beneficial insects, according to the presence or absence of insecticides in the crop management:

In Conventional and IPM systems, the increase of captures in treated plots could be explained by the fact that the beneficial insects in these systems would very probably have developed resistance mechanisms, since the application of experimental insecticide induces a repellency reaction, which may explain the greater trend of insects to land on insecticide-free surfaces, such as chromatic traps. Regarding this phenomenon, Lee (2000), Singh and Marwaha (2000) indicate that the regular use of insecticides in agroecosystems triggers the selection of lineages that are progressively better adapted to elude the action of the insecticide, unlike wild lineages, typical of agroecosystems in which synthetic insecticides are not applied. The stimulation of the locomotor behavior in insecticide-affected individuals has also been reported by Haynes (1988) and França et al. (2017), who point out that the regular application of insecticides causes uncoordinated and convulsive reactions in insects, which allow them to increase their chances of survival (LEVINSON, 1975). Among anomalies induced in insecticide-affected insects, alterations in the search for the host, dispersal flight, feeding, reduction in longevity, in development rate, in fertility, and changes in the sexual ratio have been reported (BEEMAN; MATSUMURA, 1978; HAYNES, 1988; LEE, 2000; SINGH; MARWAHA, 2000; FRANÇA et al., 2017). Although these effects do not cause short-term mortality, they are very negative for the protection of the community from natural enemies, which requires improving the compatibility between chemical control and biological control (AIL-CATZIM et al., 2015; FRANÇA et al., 2017).

The repellency reaction in treated plots (IPM, Conventional) may explain that species whose presence goes almost unnoticed in control plots, such as *A. nemoralis*, present relatively high capture values in areas where the insecticide has been applied.

Unlike Conventional and IPM systems, in the Ecological system, the wild populations of beneficial insects have not developed resistant lineages, since their greater susceptibility to the insecticide would explain higher capture values in control plots, as observed (Figures 3 and 4).

The regular use of synthetic insecticides, or their suppression, allows establishing a clear correspondence with the two behavioral patterns described in entomophagous species. Consequently, the possibility of using the technique based on the experimental application of insecticides on a small scale, and the subsequent monitoring of the populations of beneficial insects, can represent a complementary tool of great interest for the determination of Ecological crops, allowing their identification with respect to management based on the use of synthetic insecticides.

3.4. Influence of the management system on the predatory activity

Data about the impact of predation on the carpophagous generation of the olive moth coincide in pointing almost exclusively to *Ch. carnea* s. lato as the main group of associated species, based on their prevalence (CANARD, 1979; ALROUECHDI, 1980; CAMPOS; RAMOS, 1985). In southern Spain, the predatory activity of larvae controls large part of the egg population of the carpophagous generation of *P. oleae*, having indicated *Ch. agilis* as the most abundant within the *Ch. carnea* complex, reaching predation rates greater than 90% - 95% (GONZÁLEZ-RUIZ et al., 2008).

In biotopes studied here, oviposition corresponding to the carpophagous generation of *P. oleae*, and the hatching of eggs took place in the months of July and August (2017), observing that predation data adequately fit to a parametric distribution (Shapiro-Wilk: $W = 0.873$; $p > 0.05$). The hatching rates of olive moth eggs during this period, and predation rates are shown in Table 2.

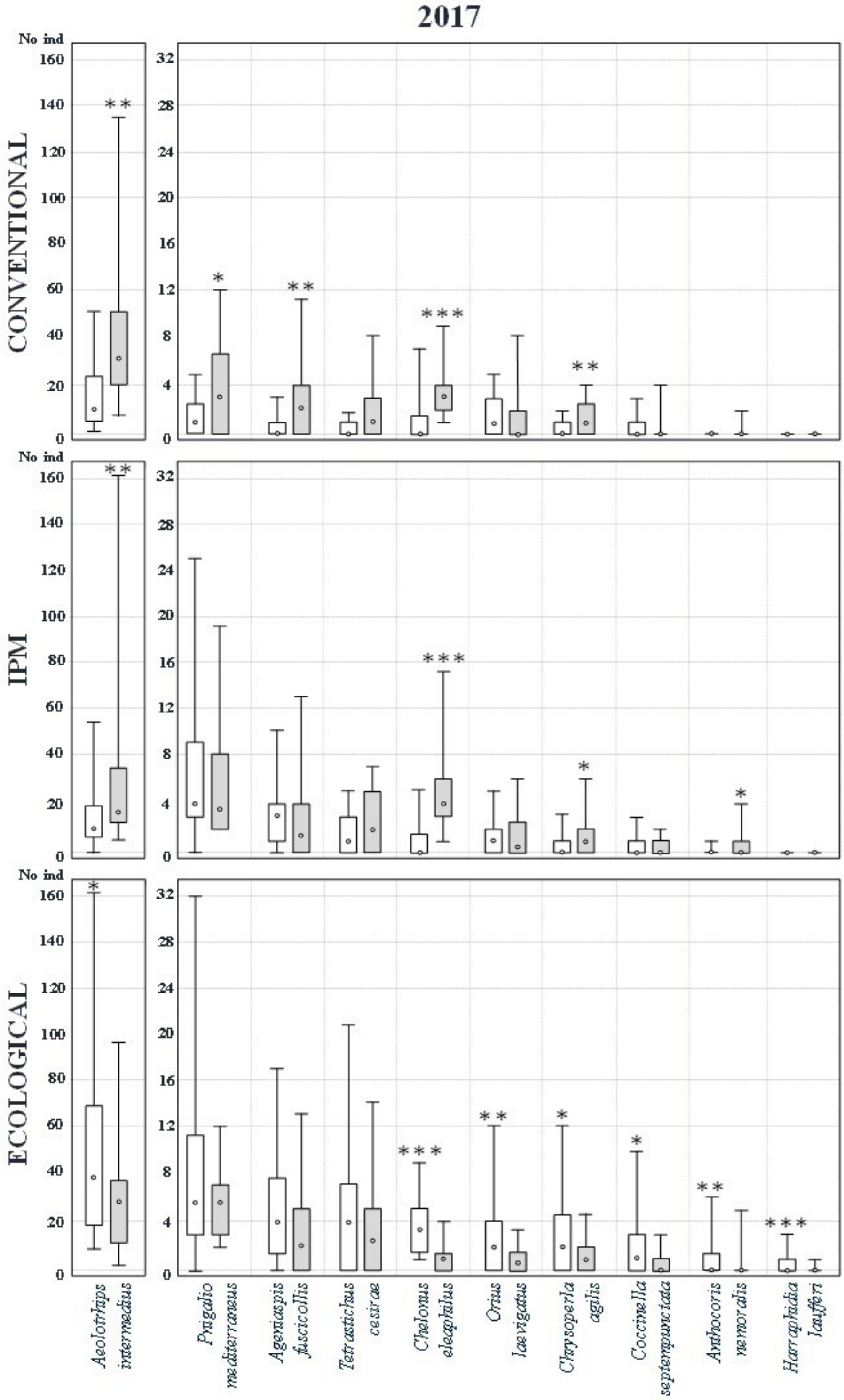


Figure 3. Statistical parameters (median, minimum, maximum and Q25-Q75 interval) corresponding to the year 2017 of beneficial insects captured in control (white) and treated (gray) plots in Conventional, IPM and Ecological management systems. Asterisks indicate significant differences [Mann-Whitney test; p <0.05 (*); P <0.01 (**), p <0.001 (***)].

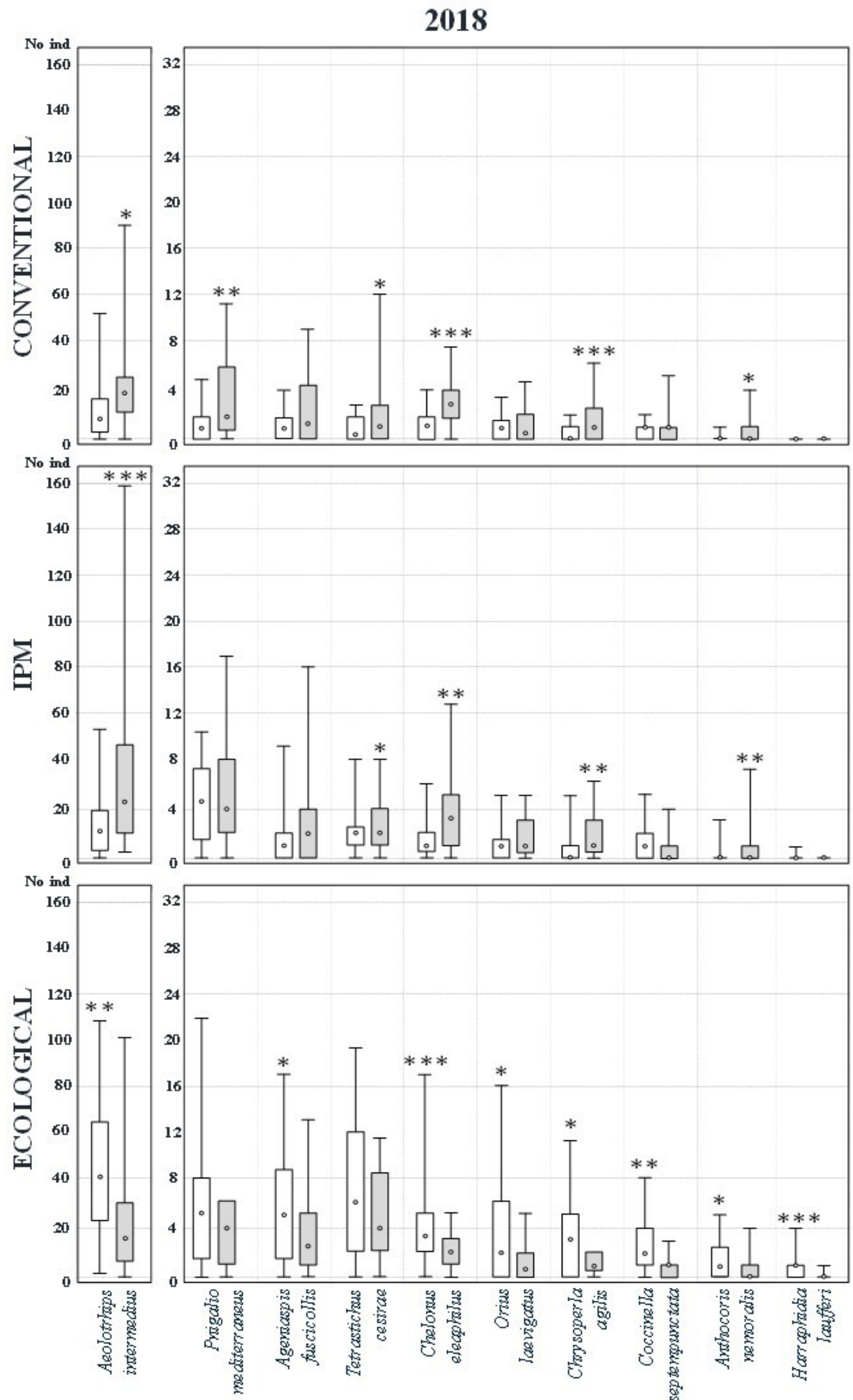


Figure 4. Statistical parameters (median, minimum, maximum and Q25-Q75 interval) corresponding to the year 2018 of beneficial insects captured in control (white) and treated (gray) plots in Conventional, IPM and Ecological management systems. Asterisks indicate significant differences [Mann-Whitney test; p <0.05 (*); P <0.01 (**), p <0.001 (***)].

Table 2. Predation rates (X: average, SE: standard error) on the *P. oleae* egg population by lacewings in the control plots of Conventional (CM), IPM and Ecological (ECO) management systems during the oviposition period (early July to mid-August). The average percentages of olive moth hatched eggs during the sampling period are indicated. Significant differences are indicated with different letters (Tukey's HSD test, p <0.05).

		egg predation (%)				
sampling date	**egg hatching (%)**	**CM**	**IPM**	**ECO**	**ANOVA**	
		X ± SE	**X ± SE**	**X ± SE**	**F**	***p***
July 17th	**12,8**	85,8 ± 1,99 **(b)**	82,54 ± 0,27 **(b)**	92,23 ± 0,37 **(a)**	17,36	0,003
July 27th	**25**	65,86 ± 0,74 **(c)**	78,80 ± 2,11 **(b)**	91,37 ± 0,59 **(a)**	91,55	<0,001
6th August	**74,1**	76,11 ± 3,05 **(b)**	85,64 ± 7,23 **(ab)**	96,00 ± 0,63 **(a)**	4,78	0,047
August 16th	**96,1**	57,95 ± 4,68 **(c)**	77,04 ± 2,33 **(b)**	90,49 ± 0,18 **(a)**	29,3	<0,001
General Average	**--**	71,43 ± 3,41 **(c)**	81,01 ± 1,96 **(b)**	92,52 ± 0,66 **(a)**	20,99	<0,001

Predation values were relatively high comparing the three management systems, ranging from 58% to 96%. Throughout this period, the number of eggs laid on fruits increases gradually, which explains the reductions in predation rates, especially between the third and fourth samplings. Percentages corresponding to August 16th are those that most accurately report the final predation values, as they correspond to the completion of the oviposition curve.

The maximum values corresponded to the Ecological management system (general average of 92.52%); in the IPM system, values were significantly lower in three of the four samplings, with general average of 81.00%. The lowest predation rates corresponded to the Conventional management system, where the general average was 71.43%. A very similar value (74.7%) has been reported by Ramos and Ramos (1990) who, in their 20-year study of conventional management system in southern Spain, indicate average values of 71% in most (60 %) of the years.

The results of this study show an inverse relationship between frequency of pesticide application and the predatory efficacy of lacewings, which is most likely due to the higher mortality of chrysopid adults and larvae in Conventional and IPM systems. From these data, it is clear that insecticidal applications for the control of *P. oleae* during the carpophagous generation would not only be unnecessary, but even more counterproductive, in view of the high predation rates in the Ecological management system. However, it is a striking fact to find surprisingly high predation rates in Conventional and IPM systems, which is undoubtedly related to the relatively high capacity of lacewings to develop tolerance to insecticides (BOZSIK, 2008; KHAN-PATHAN et al., 2008). This fact, together with their relative easy breeding under controlled conditions, and their relatively short development cycle, has made these natural enemies excellent agents for use in IPM programs (BOZSIK et al., 2009).

Conclusions

The relative abundance values of the different beneficial species were much higher in the Ecological management system compared to Conventional and IPM systems.

The results allow differentiating two behavioral patterns in the populations of most natural enemies, clearly different from each other, depending on the application or not of synthetic insecticides in the usual crop management:

On the one hand, in olive groves where synthetic insecticides (Conventional, IPM) are applied, results are consistent with the existence of resistant lineages.

On the other hand, in the Ecological management system, results are consistent with the absence of resistant lineages.

The possibility of discriminating Ecological crops by means of small-scale insecticide applications in the pilot areas of crops can be a tool of great interest, allowing them to be identified with respect to any other type of management that includes the use of synthetic insecticides.

The greater dependence on insecticidal applications for pest control in Conventional and IPM systems negatively affects the predatory efficacy of lacewing larvae. The maximum predation values correspond to the Ecological system, which justifies the highest capture rate of *Ch. agilis* in this management system.

Finally, as a complementary conclusion, agricultural technicians should be warned about the very probable possibility of not considering the repellency factor of insects in areas submitted to insecticide applications, which could suggest erroneous interpretations of capture values in chromatic traps (commonly used in olive growing). This error consists in considering exclusively the chromatic attraction and extrapolating the number of captured individuals as a representative or proportional index of their population size in the crop.

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