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Multifunctional areas as a tool to enhance biodiversity and promote conservation in alfalfa fields

Luis Miranda-Barroso[1] , **Oscar Aguado[2]** , **José Vicente Falcó-Garí[3]** , **David Lopez[3]** , **Michael Schade[4]** , **Vasileios Vasileiadis[4]** & **Francisco Javier Peris-Felipo[4*]**

1 *Agricultura Sostenible Syngenta España – c/. de la Ribera del Loira, 8, 10 – 28042 – Madrid – Spain.* *luis.miranda@syngenta.com*

2 *Andrena Iniciativas y Estudios Medioambientales S.L. – c/. Gabilondo 16bis – 47007 Valladolid – Spain.* *oscaraguado@lepidopteros.com*

3 *Laboratory of Entomology and Pest Control – Institute Cavanilles of Biodiversity and Evolutionary Biology – c/ Catedrático José Beltrán 2 – 46980 – Paterna – Valencia – Spain.* *jofalga@uv.es*; *david.lopez@uv.es*

4 *Syngenta Crop Protection – Rosentalstrasse 67 – 4058 – Basel – Switzerland.* *michael.schade@syngenta.com*; *vasileios.vasileiadis@syngenta.com*; *javier.peris@syngenta.com*

ABSTRACT. The present study analyses the effects of multifunctional areas (MA) for three years (2013–2015) on an intensive multi-crop farm in Portugal. The implementation of MA resulted in a wide range of enhancements in the insect community, such as significant effects as a reservoir, allowing an increase of 102.47% in the number of species and 97.64% of individuals. MA play an important role in conservation strategies and help increase the population of rare and threatened arthropod species.

Key words: agro-ecosystems, sustainability, insect conservation, flower strips, arable crops

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Introduction

Mediterranean landscapes are rich in evergreen species, frequently intersected by areas of brushwood, pasture, and farming. Near these areas, however, it is often possible to identify zones that have regained their highly diverse natural communities after the cessation of human intervention (Pungetti, 2003; Jiménez-Peydró & Peris-Felipo, 2014). This favours the proliferation of hotspots in Mediterranean ecosystems (Myers et al., 2000). Despite the huge resistance posed by Mediterranean biotopes to human pressure, isolation and fragmentation are unavoidable (Pungetti, 2003).

Agriculture is considered as one of the key drivers of biodiversity loss, although the ways in which it affects species are complex (Foley, 2011; Dudley & Alexander, 2017). Despite this loss, agriculture and biodiversity have a direct connection that provides

Corresponding author: Francisco Javier Peris-Felipo, E-mail: *javier.peris@syngenta.com*

additional values (Erisman et al., 2016). The use of hedges or field margins in the landscape create a specific habitat for insects, birds, plants and other animals (Schumacher, 1984; De Snoo, 1999; Marshall & Moonen, 2002; Jacot et al., 2007; Smith et al., 2008; Haddaway et al., 2016; Nowakowski & Pywell, 2016). This fauna have a high nature value because it can support agricultural production, e.g. through the attraction of pollinating insects or beneficials that can regulate pest populations (Brussaard et al., 2007; Smith et al., 2008; Haddaway et al., 2016; Nowakowski & Pywell, 2016).

For many years, several authors have been documenting the positive effects of multifunctional areas (MA) (hedgerows, field margins, floral margins, or flower strips) on biodiversity, enhancing birds, insects and small mammal diversity and abundance, offering resources, and reservoirs (Marshall & Nowakowski, 1995; Holland & Fahrig, 2000; Meek et al., 2002; Roy et al., 2003; New, 2005; Smith et al., 2008; Morandin & Kremen, 2013; Tschumi et al., 2015; Haddaway et al., 2016; Holland et al., 2016; Castle et al., 2019; Kremen et al., 2019; Holden et al., 2019; Albrecht et al., 2020).

In this paper, the benefit of multifunctional areas (MA) to enhance biodiversity is analysed on an intensive alfalfa field in Portugal.

Material and methods

Area of study

The experiment was carried out in one farm located in Alcácer do Sal in Portugal (38°22′34.22″ N, 8°31′28.92″ W) (Fig. 1A). The region is characterized by a Mediterranean-subtropical climate, with average temperatures above 17° C and an annual rainfall between 500–600 mm (IPMA, 2020). The farm is characterized by great variety of crops such as rice, alfalfa, wheat, corn, vineyards and vegetables and by activities such as stud and cattle breeding, by cork production (Fig. 1B). In the present study, the selected key crop was alfalfa where the agricultural practices such as tilling, sowing, fertilization and phytosanitary treatments remained unchanged and crop management measures were confined to the cop, trying not to interfere with the MA.
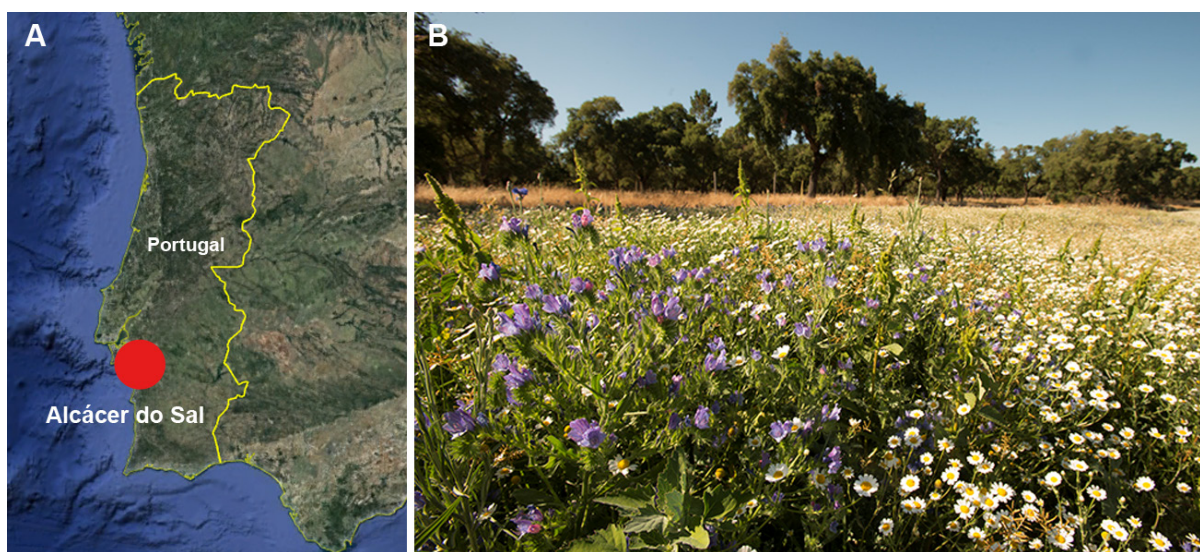

Figure 1. A. Farm location on Portugal. **B.** Multifunctional area (MA) in the alfalfa field.

MA plant selection

The MA, within an area of 50 m × 6 m (300 m²), was planted in one of the crop margins by using a herbaceous mixture of *Borago officinalis* (10% of composition), *Calendula officinalis* (22.5%), *Coriandsrum sativus* (10%), *Diplotaxis catholica* (5%), *Echium vulgare* (5%), *Melilotus officinalis* (12.5%), *Nigella damascena* (5%), *Salvia verbenaca* (10%), *Silene vulgaris* (10%), and *Vicia sativa* (10%). This mixture was planted without a specific pattern in 2013 using 13 kg of seeds per ha mowing it in autumn and then left on their own for regrowth. The plant mixture was selected following some fundamental criteria such us strict use of autochthon species, ensuring a smooth climatic adaptation; being non-weed for the crop; featuring easy maintenance and capacity for self-sowing, as well as staggered flowering phenologies; and finally, being attractive to pollinators and natural enemies but not for crop pests.

Sampling of insects

To investigate the dynamics of effects of MA on insect biodiversity, the experiment was carried out during a period of three years (2013–2015). Since the beginning (2013) the strips were sampled to determine the existing insect biodiversity. Insects were assessed by sweep net by fixed transects of 50 m × 2 m in each MA. Sampling was done four times per season and following the phenological crop stages. Collected specimens were preserved in cyanide to keep them intact and to avoid discoloration. All the specimens were identified to species level using the appropriated keys (see Aguado et al. (2016) to find the specific key for each group). Selected specimens were deposited in the entomological collection of the National Museum of Natural Sciences (Madrid, Spain; MNCN).

Data analysis

The diversity of a homogeneous community (taxa richness, abundance, and dominance values) was determined using PAST (Hammer et al., 2001). Moreover, the differences in the community of insects were tested by the analysis of variance to achieve a significance level of 0.05 on all results, using SPSS v.24 software (StatPackets statistical analysis software, SPSS Inc., Chicago, IL). The taxa richness was measured using the Margalef index, a measure of specific richness that transforms the number of species per sample in the proportion to which the species are added by expansion of the sample, establishing a functional relationship between the number of species and total number of specimens (Moreno, 2001).

The abundance was used for valuing the faunal composition of a given area (Magurran, 1991). This was undertaken using the Shannon-Wiener index, which measures equity, indicating the degree of uniformity in species representation (in order of abundance) while considering all samples. This index measures the average degree of uncertainty that predicts which species an individual randomly picked from a sample belongs to (Magurran, 1991; Moreno, 2001; Villareal et al., 2004). The dominance value was calculated with the Simpson index, often used to measure species dominance values in each community, its negative thus representing equity. It measures the representativity of the most important species without considering the other species present. It expresses the probability that two individuals randomly picked from a sample will belong to the same species (Magurran, 1991).

Results

Species richness and abundance

During the three-year period, 3490 insects (806 in 2013, 1091 in 2014 and 1593 in 2015) were collected in Alcácer do Sal (Portugal) belonging to six orders Coleoptera (929), Diptera (879), Hemiptera (11), Hymenoptera (901), Lepidoptera (747), and Neuroptera (23) (Fig. 2). A total of 163 species were identified. Following their biological role, the species were classified in 152 as pollinators, 49 as natural enemies and seven as pest. However, some species can play different roles. For example, *Aporia crataegui* (L., 1758), *Lampides boeticus* (L., 1767), *Leptotes pirithous* (L., 1767), *Oxythyrea funesta* (Poda, 1761), *Pieris brassicae* (L., 1758), *Pieris rapae* (L., 1758) and *Tropinota squalida* (Scopoli, 1783) are considered as pests in their larval stage because the significant damage that can cause in the crop while as adults can substantially contribute to pollination. The abundance of these pest-pollinator species showed that their populations were stable during the study period without any variation produced by the implementation of MAs (Table 1). During the three years, the most abundant species were *Coccinella septempunctata* (L., 1758), *Episyrphus balteatus* (De Geer, 1776), *Eristalis arbustorum* (L., 1758), and *Syritta pipiens* (L., 1758) where the sum of these four species varied between 20–30% of abundance (Table 2).

Effects of multifunctional areas on abundance and diversity

Once the specimens had been identified, biodiversity indexes were calculated (Table 3). The Margalef analysis (D_{Mg} = 11.95–22.11), showed that species richness and biodiversity increased year after year (Table 3). The Shannon-Wiener and the Simpson dominance indexes were calculated (Table 3) to analyse the proportional abundance and results suggested a similar trend in the distribution of dominant species. Moreover, these results determine that significant differences are limited to rare species represented by a few individuals.

The analysis of species number through the years, showed a clear increase of 45.68% during the first season (2013–2014), in the second (2014–2015) was 38.98%, while the variation of species carried out in a three-year study (2013–2015) was 102.47%. On the other hand, the changes in abundance showed opposite trends, having a lower increase during the first season than the second (35.35% and 46.01%, respectively). The total increase of individuals during the study recorded was 97.64% (Fig. 3). The species composition analyses show significant differences among years. This change is clearly significant when the year compositions were compared (2013–2014: t = -5.76; df = 1785.2; p = 9.87E-09; 2014–2015: t = -6.48; df = 2326.9; p = 1.10E-10). This trend is even more significant when the communities at the beginning and at the end of the study are compared (2013–2015: t = -12.34; df = 1680.8; p = 1.41E-33).

Conservation of biodiversity

The conservation status analysis revealed that a total of 64 (out of 163 spp.) captured species are included in the Red List: 52 species as Least Concern (LC), eight as Data Deficient (DD), two species: *Colletes succinctus* (L., 1785) and *Halictus quadricinctus* (Fabricius, 1776) as Near Threatened (NT) and only one species: *Bombus muscorum* (L., 1758) is catalogued as Vulnerable (VU) (IUCN, 2020). The abundance analysis of the species classified as NT and VU showed that *C. succinctus* remained constant through the period of study with one individual captured every year (Table 4). However, individuals of *H. quadricinctus* and *B. muscorum* appeared in the field in the second year and keeping their abundance also stable during the third year (Table 4).

Table 1. Abundance of pest species per year in Alcácer do Sal.

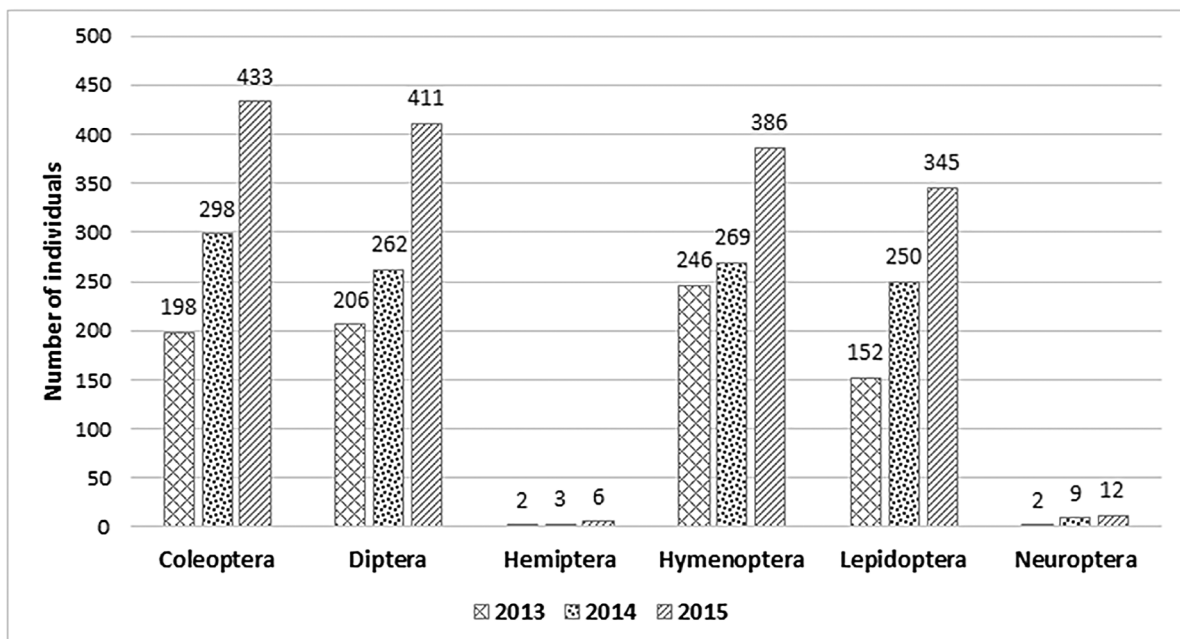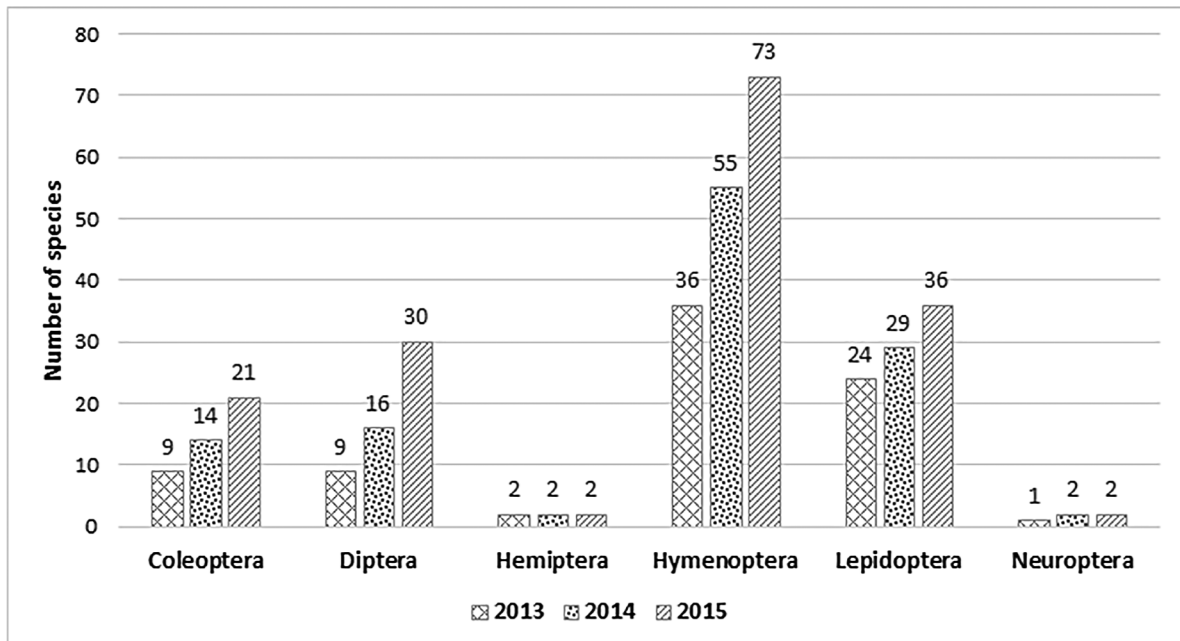| Order	Family	Species	2013	2014	2015
Coleoptera	Cetoniidae	*Oxythyrea funesta*	30	34	29
	Scarabaeidae	*Tropinota squalida*	12	12	15
	Pieridae	*Aporia crataegui*	3	3	3
	Pieridae	*Pieris brassicae*	6	7	8
Lepidoptera	Pieridae	*Pieris rapae*	2	1	3
	Lycaenidae	*Lampides boeticus*	14	17	15
	Lycaenidae	*Leptotes pirithous*	12	19	19

Figure 2. Number of species and individuals by insect order per year.

Table 2. List of the most abundant species (number of specimens and average) in Alcácer do Sal.

Order	Family	Species	2013	2014	2015
Coleoptera	Coccinellidae	*Coccinella septempunctata*	94 (11.66%)	102 (9.34%)	78 (4.89%)
Diptera	Syrphidae	*Episyrphus balteatus*	44 (5.45%)	52 (4.76%)	68 (4.26%)
Diptera	Syrphidae	*Eristalis arbustorum*	39 (4.83%)	26 (2.38%)	82 (5.14%)
Diptera	Syrphidae	*Syritta pipiens*	67 (8.31%)	71 (6.50%)	90 (5.64%)
		Total	30.25%	22.98%	19.89%

Table 3. Diversity and abundance values of the collected species in Alcácer do Sal.

Biodiversity analysis	2013	2014	2015
Species	80	117	163
Specimens	806	1091	1593
Dominance	0.038	0.029	0.021
Simpson	0.962	0.970	0.979
Shannon	3.751	4.039	4.33
Margalef	11.95	16.73	22.11

Table 4. Abundance of Near Threatened (NT) and Vulnerable (VU) species per year in Alcácer do Sal.

Order	Family	Species	2013	2014	2015	Conservation status
Hymenoptera	Colletidae	*Colletes succinctus*	1	1	1	NT
Hymenoptera	Halictidae	*Halictus quadricinctus*	0	5	4	NT
Hymenopera	Apidae	*Bombus muscorum*	0	1	1	VU
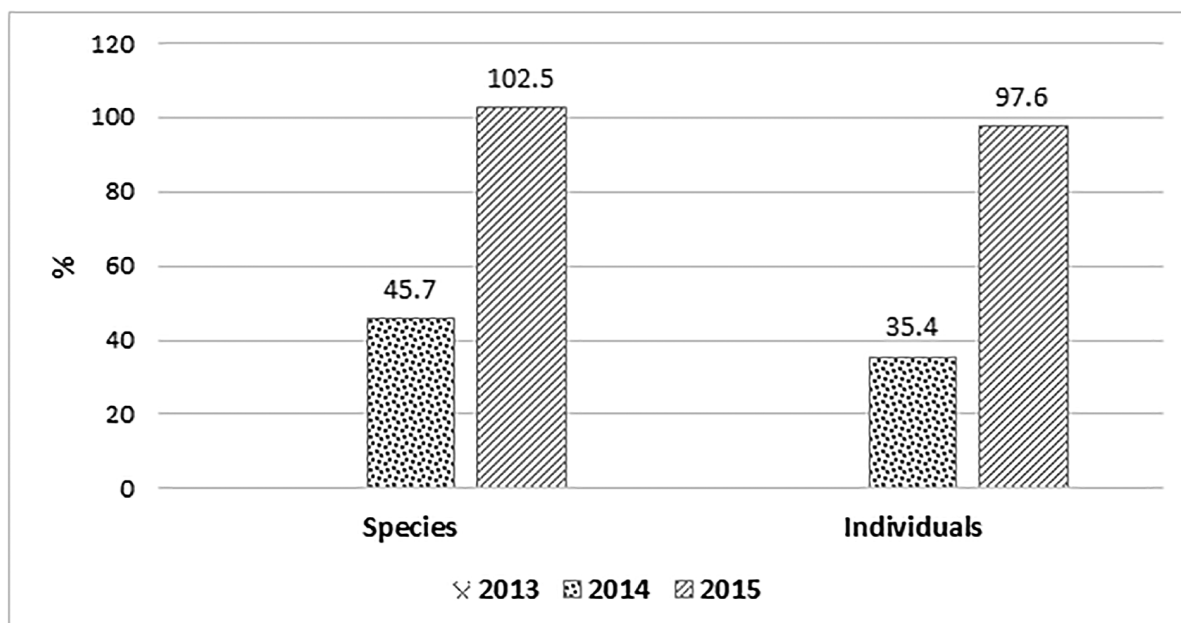
Figure 3. Average of the number of species and individuals from the first year (2013 values were considered as 0 because it was the first year of the experiment).

Discussion

The selection of the mixture plants plays a decisive role in the successful attraction of insects. This significant association between plants and insects can be observed by the increased number of insect species and individuals during the three experiment years (2013= 80 species and 806 individuals; 2014 = 117 and 1091; 2015 = 163 and 1593) reflecting the positive impact of MA enhancing biodiversity of pollinators and natural enemies. Paoletti et al. (1997), Tschumi et al. (2015) and Amy et al. (2018) observed similar results in arable crops in their one-season experiment.

Moreover, numerous studies carried out in Europe have summarized the role of the MA in conservation (Marshall & Moonen, 2002; Hannon & Sisk, 2009; Van Rijn & Wächers, 2016; Bauer et al. 2017; Amy et al. 2018). Brown & Paxton (2009) were the first ones to suggest that future conservation strategies need to primarily aim at minimising habitat loss or making an agricultural habitat 'bee-friendly'. The present work reinforces these conclusions because 64 of 163 species collected are included in the IUCN Red List Categories and Criteria which intended to easily classify the species at high risk of global extinction (IUCN, 2020).

These results emphasise the importance of MA to enhance biodiversity, support the conservation of species at risk of extinction because MA creates a perfect reservoir area with shelter and food (pollen, nectar, alternative preys) that allow them to maintain and extend their populations. Despite all these studies carried out to enhance biodiversity in agricultural ecosystems, very few studies analyse the effectiveness of MA on the crop. Further studies are therefore necessary to understand the full benefits of MA.

Acknowledgments

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

ORCID

Luis Miranda-Barroso: https://orcid.org/0000-0002-3457-7532
Oscar Aguado: https://orcid.org/0000-0002-7895-6016
José Vicente Falcó-Gari: https://orcid.org/0000-0002-8685-2292
David Lopez: https://orcid.org/0000-0003-2249-979X
Michael Schade: https://orcid.org/0000-0002-5487-5975
Vasileios Vasileiadis: https://orcid.org/0000-0002-5697-7078
Francisco Javier Peris-Felipo: https://orcid.org/0000-0001-9929-3277

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مناطق چند منظوره به عنوان ابزاری برای افزایش تنوع زیستی و ارتقای حفاظت در مزرعه یونجه

لوئیس میراندا-باروسو[1]، اسکار آگوادو[2]، خوزه ویسنته فالکو-گاری[3]، دیوید لـوپز[3]، مایکـل شــاد[4]، واسـیلیوس واسیلیادیس[4]، فرانسیسکو ژاویر پریس-فلیپو[4]*

۱ بخش کشاورزی پایدار، شرکت سینجنتا، اسپانیا

۲ شرکت ابتکارات و مطالعات زیست‌محیطی آندرنا، اسپانیا

۳ آزمایشگاه حشره‌شناسی و کنترل آفات، موسسه مطالعات زیست‌شناسی فرگشتی و تنوع گونه‌ای، والنسیا، اسپانیا

۴ بخش گیاه‌پزشکی، شرکت سینجنتا، بازل، سوئیس

* پست الکترونیکی نویسنده مسئول مکـاتبه: *javier.peris@syngenta.com*

چکیـده: مطالعه حاضر، به تجزیه و تحلیل اثر نواحی چندمنظوره در یک مزرعه با سیستم چندکشتی وسیع در کشور پرتغال در یک دوره زمانی سه ساله (۲۰۱۵-۲۰۱۳) می‌پردازد. اجرای طرح نواحی چندمنظوره منجر به طیف گسترده‌ای از تغییرات افزایشی در جمعیت گونه‌های مختلف حشرات شد. این اثرات شامل تقویت ذخیره‌گاه‌ها، افزایش ۱۰۲،۴۷٪ در تعداد گونه‌ها و ۶۴،۹۷٪ در تعداد افراد گونه‌های مختلف بود. نواحی چندمنظوره نقش مهمی در راهبردهای حفاظتی داشته و به افزایش جمعیت بندپایان نادر و در معرض خطر انقراض نیز کمک می‌کند.

واژگــان کلیدی: اکوسیستم‌های زراعی، پایداری، حفاظت از حشرات، ردیف گیاهان گلدار، محصولات زراعی