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**LAND-USE EFFECT ON *CHRYSOPERLA CARNEA* AND
RELATED BIOCONTROL AGAINST *PRAYS OLEAE* IN
OLIVE GROVES**

Dissertação no âmbito do Mestrado em Ecologia orientada pelo Doutor Daniel Paredes Llanes e pelo Professor Doutor José Paulo Filipe Afonso de Sousa e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

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RESUMO

Nos decénios mais recentes, a olivicultura tem sido sujeita a produções intensivas contraproducentes que retiram complexidade e heterogeneidade a estes ecossistemas agrícolas e que negligenciam a saúde pública, através da recorrente utilização de pesticidas para controlar pragas, contaminando o ambiente e a qualidade das produções. Com o intuito de repensar a perniciosidade inerente a tais produções intensivas, técnicas de controlo biológico por meio de conservação e fomento da biodiversidade dos habitats naturais e com o foco em promover as condições necessárias para o desenvolvimento de inimigos naturais que controlem pragas em olivais têm sido devidamente examinadas. Esta obra teve a sua ação em vinte e cinco olivais da região da Beira Interior, num período compreendido entre finais de março e meados de julho no ano de 2019. As populações de traça-da-oliveira, *Prays oleae* e do seu inimigo natural, *Chrysoperla carnea* foram monitorizadas a cada duas semanas. Os níveis de infestação da azeitona por parte da praga foram igualmente quantificados. Por seu lado, a diversidade de usos de solo em redor dos olivais em estudo foi classificada e quantificada após uma análise geoespacial com 500m de raio. Os resultados obtidos corroboram a atração da *C. carnea* pela traça-da-oliveira, comprovam o potencial da *C. carnea* enquanto agente de controlo biológico em olivais, asseveram que a promoção da diversidade de usos de solo tem um efeito eloquente na redução da abundância de praga e confirmam o olival como habitat primordial para o desenvolvimento de *Prays oleae*. A presente disquisição almeja reconsiderar a formação de ecossistemas agrícolas, possibilitando a atribuição de maior valor às produções e tem

como desígnio, em detrimento da utilização de pesticidas, criar alternativas sustentáveis que não acarretem efeitos nefastos para o ambiente e para a saúde pública.

Palavras-chave: biodiversidade, *Chrysoperla carnea*, controlo biológico por conservação, análise geoespacial, olivais, paisagem, *Prays oleae*.

ABSTRACT

In recent decades, olive growing has been subjected to intensive production methods with counterproductive effects, removing complexity and heterogeneity from these agricultural landscapes and neglecting public health, through the recurrent use of pesticides to control pests, contaminating the environment and the quality of such productions. In order to rethink the perniciousness inherent to these intensive productions, biological control techniques through the conservation and promotion of natural habitats and with a focus on promoting the necessary conditions for the development of natural enemies that naturally control pests have been properly examined. This work took place in twenty-five olive groves in the Beira Interior region, in the period between late-March to mid-July of 2019. Populations of the olive moth, *Prays oleae* and its natural enemy, *Chrysoperla carnea* were monitored every two weeks. The levels of olive fruit pest infestation were also quantified. In turn, the diversity of land-uses around the selected olive groves was classified and quantified after geospatial analysis within a radii of 500m. The results obtained corroborate the attraction of *C. carnea* to the olive moth, prove the potential of *C. carnea* as a biological control agent in olive groves, assert that the promotion of the diversity of land-uses has an eloquent effect in reducing the abundance of pest and confirm the olive grove as a primordial habitat for the development of *P. oleae*. The present disquisition aims at reconsidering the formation of agricultural ecosystems, enabling the assigning of greater value to olive productions and intends, to the detriment of the use of pesticides, to create sustainable alternatives that do not have harmful effects on the environment and public health.

Key words: biodiversity, *Chrysoperla carnea*, conservation biological control, geospatial analysis, landscape, olive groves, *Prays oleae*.

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INTRODUCTION

The Global Situation of Agriculture

According to the United Nations (2015), agriculture is the largest employer in the world, providing livelihoods for 40 % of the global population. However, 80 % of the food consumed in a large part of the developing world comes from small farms worldwide, most of them still rainfed. Since the last century, when there was a considerable increase in world population, powerful market pressures led to massive production in agricultural systems through the promotion of monoculture by converting natural and semi-natural habitats into arable fields. This process invariably resulted in losses of biodiversity (UN estimates that 75 per cent of crop diversity has been lost from farmers' fields) and resilience for agricultural crops all over the planet (Sala et al., 2000; Benton et al., 2003).

In most of these mass-production agricultural systems, pest control is quite aggressive and relies on the use of external fossil and agrochemical inputs such as pesticides. As a result of these practices there is a degradation of natural resources and contamination of fundamental systems of the biosphere such as air, soil or water, seriously jeopardizing public health (Pimentel et al., 1992; Meehan et al., 2011). For this reason, the organization of the United Nations (2015) suggests that a better use of agricultural biodiversity can contribute to more nutritious diets, to support more farming communities, as well as more resilient and sustainable agricultural systems. In line with these recommendations, the European Union is trying to define agricultural policies for member states to enhance biodiversity and ultimately to protect the environment (Common Agricultural Policy 2014-2020). Based on this, the most obvious way to better exploit agricultural production in a more sustainable way is to eliminate such external inputs by replacing the use of pesticides with natural methods such as biological pest control.

Biological Control

Biological control is a pest management strategy based on the use of organisms to suppress population density or the impact of a specific pest organism, making it less abundant than what they would be if these organisms were not used (DeBach, 1964).

Biological control techniques are generally based on predation or parasitism. Despite being based on natural interactions, it often has a direct human influence and is widely used in integrated pest management (IPM) programs (Szentkirályi, 2001) along with other control strategies. IPM works as a decision support system to help farmers to select the most suitable pest control strategies taking into account their production as well as society and environmental needs. To achieve a long-term environmental well-being and food security, the management of ecosystem services has to be integrated into environmentally friendly crop production systems.

On *Suggestions for unifying the terminology in biological control*, Eilenberg et al. (2001) proposes and defines four biocontrol strategies: classical biological control, inoculation biological control, inundation biological control, and conservation biological control.

Classical biological control is a strategy that requires the introduction of not native organisms into the environment, whether they are parasitoids or predators it is intended that these agents establish themselves permanently in the targeted area for the purpose of controlling agricultural pests for the long-term. At Inoculation biological control the success of the agents' release depends not only on pest suppression but also on those organism's reproduction ability, since they do not permanently establish themselves, thus requiring new releases to support the following generations of the biocontrol agent. Inundation biological control is supposed to be a single release of parasitoids or predators

to control pests in a sufficiently high proportion, neither permanently establishing these agents nor their success depends on support for later generations. Conservation biological control depends of the preservation and creation of ecological infrastructures inside the agroecosystem or in the landscape that surrounds it to provide the biocontrol agents with resources, such as food or shelter to improve their efficiency suppressing pests.

In this document, a conservation biological control approach is studied. This strategy does not require continuous input of mass-reared natural enemies as with the inoculation and inundation biological control strategies and it also avoids problems that may be caused for introducing exotic organisms into the environment by the classical approach. In contrast it is based on a set of techniques mostly focus on habitat management (Eilenberg, 2001; Coll, 2009).

Conservation Biological Control

Conservation biological control strategies rely on the management of the non-crop habitat, both, at local (i.e., within the crop) and at landscape scale (i.e., in the surrounding area). Habitat management is based on the manipulation of the environment to enhance the survival, fecundity, longevity, and behavior of natural enemies already present in the agricultural ecosystem, consequently improving natural pest control (Barbosa, 1998; Landis et al., 2000; Bianchi et al., 2006). Those practices require the creation of a sustainable environment to balance the relationship between crop, pests and natural enemies in order to avoid production losses (Paredes et al., 2013). It can be achieved not only by limiting the use of chemical insecticides but also by providing refuges and resources to the benefit of natural enemies, which is typically accomplished by improving

the conservation or restoration of natural habitats (Van Driesche and Bellows, 1993; Lamichhane et al., 2009).

Most of the studied techniques to achieve a successful conservation biological control have been implemented on a local scale. For example, groundcover through flower strips seem to have an important effect on natural enemies of olive groves pests, since the produced pollen is important for the predator reproduction (Villa et al., 2016; Herrera et al., 2019). Despite the success that local habitat management techniques have shown on conservation biological control, ecologists, agronomists, and farmers are increasingly recognizing the critical role that surrounding landscapes can play in determining pest damage (Chaplin-Kramer et al., 2019). Landscape context determines natural enemy communities through the provision of food and shelter resources (Bianchi et al., 2006). Landscape is a mosaic of ecosystems that interact with each other under the same climate, geomorphology and disturbance, its structure is primarily a series of patches surrounded by a matrix (Forman and Godron, 1981; Forman and Godron, 1986). Simple and homogeneous landscapes are originated from mass-production agricultural systems, defined by continuously extended agricultural fields and by reduced diversity of vegetation (Margosian et al., 2009). This type of landscapes shortens the availability of refuge and resources that non-crop habitats offer to pests' natural enemies, emphasizing pest pressure over the farming systems (Chaplin-Kramer et al., 2011; Liere et al., 2015). On the other hand, complex landscapes covered by natural or semi-natural habitats, such as diverse woodlands, grasslands, scrubs, hedgerows or fallows are important elements that favor the prospection of natural enemies by providing undisturbed areas that offer shelter from crop disturbances as well as overwintering refuges, alternative hosts and prey, and additional nectar resources (Thies et al., 2003; Bianchi et al., 2006; Tscharrntke et al., 2008; Rusch et al., 2010). Consequently, the diversity and abundance of available

natural enemies to provide biological control in agricultural systems depends on the composition and structure of the surrounding landscape (Jervis et al., 1993; Landis et al., 2000; Wäckers, 2005; Bianchi et al., 2006).

Two complementary mechanisms are thought to underlie landscape effects on pests and their natural enemies (Rusch et al., 2016). First, the resource concentration hypothesis posits that expansive monocultures allow specialist pest populations to rapidly build and disperse, whereas diverse landscapes mitigate population growth and spread. (Root, 1973; Risch et al., 1983; Margosian et al., 2009). Second, the natural enemy hypothesis recognizes that many natural enemies of crop pests (*i.e.*, predators and parasitoids) depend on a diversity of crops and/or natural habitats for alternate food resources, overwintering, *etc.* Thus, more diverse landscapes may facilitate better pest control (Andow, 1991; Landis et al., 2000; Langelloto and Denno, 2004). Indeed, several meta-analyses support the idea that diverse landscapes may augment natural enemy populations (Bianchi et al., 2006; Chaplin-Kramer et al., 2011) and diversity (Dainese et al., 2019), leading to higher rates of natural pest control. In addition, diversified landscapes hold most potential for the conservation of biodiversity (Bianchi et al., 2006; Tschardt et al., 2008).

The results of the application of quantitative methods in landscape ecology are grouped into landscape metrics. These indices provide interesting numerical data on the composition of the landscape - variety and abundance of patches, the proportion of each land use - and the configuration of the landscape - spatial distribution of the patches and the shape of the landscape elements. Quantitative methods in landscape ecology are applicable at a triple level (McGarigal and Marks, 1995; Vila Subirós, et al., 2006):

a) At the patch level. The calculations are applied to each fragment individually. It is the appropriate level, for example, to determine which is the fragment with the largest surface area among all those represented.

b) At the class level. The calculations are applied to each set of fragments that represent the same land use. It is the appropriate level to determine what is the area that a certain land cover occupies.

c) At the landscape level. The calculations are applied to the landscape as a whole, that is, to all the fragments and classes at the same time. The results give us information about the homogeneity or heterogeneity level of the entire area.

More knowledge is needed about which plant species and natural habitats are the best to increase the proliferation of natural enemies and avoid further pest pressure, otherwise plant diversity can be a waste of resources if it does not improve pest control (Baggen and Gurr, 1998; Winkler et al., 2009a). Although the knowledge about the use of non-crop resources is still insufficient, it is commonly recognized that due to the long years of local evolutive adaptation, the use of native plants over exotics is advantageous not only because what those plants represent to the local populations but also because they require less nutritional and hydrographic resources, which should encourage native plants conservation (Fiedler et al., 2007; Isaacs et al., 2008). Non-crop resources are sometimes enhanced to improve the pest control, but the knowledge about the effect of those resources on pests is crucial before increasing them in fields to avoid benefit pests (Lavandero et al., 2006; Winkler et al., 2009b; Saeed et al., 2015). Conserving semi-natural habitats emerges as a fundamental first step to enhance agricultural pest control services by predatory arthropods. However, these programs have a mixed record of success and the knowledge about the use of non-crop resources by most of the adult pests is still insufficient (Jonsson et al., 2010).

The Olive Crop

On *Classification, Origin, Diffusion and History of the Olive*, Bartolini and Petruccelli (2002), report the origin of olive cultivation back to ca. 5500 years ago in Chalcolithic Palestine. Since then, the olive tree (*Olea europaea* L.) has gradually established itself in the Mediterranean landscapes, spreading across North Africa and Southern Europe, where millions of households depend on olive exploitation. Inevitably, Mediterranean population are socially, culturally and economically influenced and unified by its generalized presence (Loumou and Giourga, 2003).

As a Mediterranean country, Portugal also has the olive tree as a fundamental species in its agriculture and landscape composition with its cultivation being widespread throughout the country since the 14th century. Portuguese and Spanish were responsible for taking the olive tree to the American continent (Sibbett and Ferguson, 2005). According to FAOSTAT data of 2020, Portugal is the 8th largest olive fruits producer in the world with an average of 371,818 tons produced between 1961 and 2018, with that importance being reinforced in recent years, as olive fruit production in Portugal has been increasing, with values of 740,151 tons in 2018.

Statistics Portugal, (2018) classifies Alentejo as the main olive fruits producer in Portugal, with 539,487 tons, followed by the Trás-os-Montes region with 108,821 tons and as the third largest producer, the Beira Interior region, which produced 33,785 tons of olive fruits. Contrary to what happens with intensive agricultural practices in the Alentejo region, in Beira Interior region, olive farmers generally use organic approaches, based on low impact management methods, not even using pesticides applications to control pests. This happens due to topographic configuration of this region, with a lot of relief in great part of the available agricultural lands but most importantly is due to social problems, such as the high abandonment of the rural world by the younger generations as

well as the aging of those that remain, meaning the conditions are met so that a massive production of olive fruits in these geographical areas is very unlikely. This is in line with Pienkowski and Beaufoy (2002) suggestion that the abandonment of those countryside high relief areas has social (e.g., population loss), cultural (e.g., change of traditional landscape), economic (e.g., lower local olive production) and environmental (e.g., soil erosion) implications.

In Beira Interior the most representative cultivars are Galega, Bical and Cordovil. Nevertheless, the presence of the Galega cultivar dominates the olive groves of this region, as it happens throughout the country representing about 80% of the Portuguese olive groves (Ferreira, 2002). It is considered a productive cultivar, with its fruits destined to produce olive oil and table olives. Plus, it is appreciated for its resistance to drought but it is sensitive to cold, salinity and limestone soils (Fernández-Escobar et al., 2001). Beira Interior olive groves are usually old, mostly centenary, they are non-irrigated, except when they are young. Crops usually have some vegetation cover until the summer, when they are cut to reduce the competition for water as well as to prevent fires. Farmers usually have livestock and the vegetation cover tends to be lower and the soils tend to be more fertilized.

For all these reasons, olive groves represent an important source of livelihood, being of essential economic value for those who courageously insist on remaining in these regions, even if they are often forgotten, ignored and marginalized by political decisions. It is, therefore, of great importance to preserve the socioeconomic role and the landscape value that these crops symbolize.

The Pest: *Prays oleae*

The olive moth, *Prays oleae* (Bernard) is an insect belonging to the order Lepidoptera, superfamily Yponomeutoidea, family Praydidae. It feeds exclusively on olive trees, therefore it is monophagous and one of the major pests in Southern Europe olive groves, together with the olive fly, *Bactrocera oleae* (Arambourg and Pravalorio, 1986; García, 2003; Liñán-Vicente, 1998). It extends throughout the Mediterranean basin, although the drier the climate the more residual its presence is, preferring areas of higher humidity (Arambourg and Pravalorio, 1986; García, 2003; Ruiz and Alcalde, 2011).

The adult is a moth measuring 13-15 mm in wingspan and 6 mm in length. The forewings are silver gray in color and have small black spots in the center, and a fringe of hair on the outer edge; the hind wings are uniform light gray in color and also have a fringed edge. The male is smaller than the female although the two are very similar in appearance. It has twilight activity. The eggs are round and flattened, having a diameter of ca. 0.5mm. Once laid, it is white in color, darkens and finally yellows when hatching. The larva measures between 0.7 to 8 mm. The coloration is hazelnut and presents two greenish longitudinal bands on the back and another two lighter or yellowish lateral bands. The head is brown in color and the body is covered with fine hairs of unequal length. During the pupa stage, the insect neither moves nor feeds, and undergoes a complete metamorphosis. It is reddish-brown in color and measures 5 to 6 mm in length. (Junta de Castilla y León, 2006).

Larval stages of the olive moth feed on different olive tree organs, being characterized by three well-defined generations throughout the year, synchronized with the vegetative cycle of the olive tree (Arambourg and Pravalorio, 1986; Liñán-Vicente, 1998; Junta de Castilla y León, 2006; DRAPC, s.d.). Phytophagous generation damages the leaves (Fig. 1A). Female adults of the carpophagous generation lay their eggs on the

upper side of the leaves in October and November. Newly hatched larvae overwinter inside the leaves. In February, those larvae increase their activity, forming galleries and feeding on buds at the underside of the leaves, where it predominantly pupates inside a silky cocoon. Phytophagous generation does not present economic damages in the adult trees since it only reduces its leaf surface. However, young developing trees can suffer bud destruction that can affect its future. The lifespan of adults of this generation ranges from 20 to 40 days.

Anthophagous generation damages the flowers (Fig. 1B). During April and May, adults from phytophagous generation lay their eggs mainly in the calyx of the closed flower buds. The larvae hatch after 6 or 7 days during the flowering period. They feed on different parts of the flower (corolla, ovary, style), causing desiccation and death. The butterflies that will produce the next generation emerge from chrysalids. The damages produced by this generation are difficult to assess. A larva can destroy 20 to 30 flowers, but in the olive tree, one hundred flowers means only two or three fruits are set. Plus, the tree compensates the fallen flower with a greater fruit setting. So, a significant decrease in production only occurs in the case of low flowering and high population of pest larvae. The adults of this generation lifespan range from 40 to 50 days.

Carpophagous generation damages the fruits (Fig. 1C). Between the months of May and June, the adults of the anthophagous generation lay their eggs in the olive fruit's chalices. When the larvae hatch, they directly pierce the fruit and enter the olive fruit before the core bone hardens, thus causing the first falls of the olives. To those fruits that still remain on the tree, the larva feeds on the seed throughout the summer, and in mid-September it emerges from the fruit, causing the second fall and pupating on the ground, where the adults that produce the phytophagous generation will later emerge. This is the most harmful generation. The real losses from the first fall (June) are difficult to assess

because the tree compensates it by increasing the size of the remaining olives. At the second fall, as the olive is already developed, and the tree has no time to make up for it, serious losses for farmers may be a reality. Therefore, acting on the eggs of this generation through a conservation biological control approach can be most effective.

The three generations cause damage in different stages of the olive tree, although the economic importance of these are different. In the years of low harvest is when the damage caused by this lepidopteran becomes more evident (Ramos et al., 1998). The destruction of leaves, flowers and the early fall of fruits caused by this pest can compromise annual production and even the development of olive trees in the following years (Arambourg and Pravalorio, 1986; Liñán-Vicente, 1998; Junta de Castilla y León, 2006; DRAPC, s.d.) in southern Spain as well as Bento et al. (2001) and Bento et al. (2002) in north-eastern Portugal quantified the damage that *Prays oleae* caused in the respective regions, and the results obtained suggest that the olive tree moth has a proven economic importance, justifying its fight.



Figure 1 - Representative photographs of the appearance of larvae of *Prays oleae* at the **A** - phylophagous, **B** - antophagous and **C** - carpophagous generations. www.drapc.min-agricultura.pt/base/documentos/traca_oliveira.pdf, accessed on March 19, 2020.

The Predator: *Chrysoperla carnea*

Natural enemies play an important role in suppressing agroecosystem pests (Salerno et al., 2002) and green lacewings are among the most common naturally

occurring predators in many agricultural and horticultural cropping systems and are usually integrated on IPM programs mainly by habitat manipulation to attract or conserve natural enemies' populations or by periodic release of mass-reared individuals, contributing to plant protection (Stelzl and Devetak, 1999; Tauber et al., 2000; Tilman and Mulrooney, 2000; Duelli, 2001).

Among green lacewings, *Chrysoperla carnea* (Stephens) is an insect species-complex belonging to the order Neuroptera, family Chrysopidae. It is an abundant and polyphagous chrysopid. Larvae are a generalist predator of numerous soft-bodied insects, mites, and eggs (Alrouechdi et al., 1981; Stelzl and Devetak, 1999; Porcel et al., 2013; Paredes et al., 2015). In olive groves, *C. carnea* larvae are major oophagous predators known to play a predominant role in the predation of eggs of the carpophagous generation of the olive moth, *Prays oleae* (Sacchetti, 1990; Campos, 2007; Szentkirályi 2007; Pappas et al., 2011). In contrast to larvae, *C. carnea* adults are not predaceous and mainly feed on substances of vegetal origin such as nectar, pollen, and honeydew (Principi and Canard, 1984; Bozsik, 1992; Villenave et al., 2005; Hogervorst et al., 2007).

Adults measure approximately 12-20 mm in length, are pale green and have long antennae, bright golden eyes, large transparent wings and a delicate body (Fig. 2A). They have a high dispersal capacity and an accurate searching ability (Bond, 1980). Each female may lay more than two hundred oval and pale green eggs. They are laid on the underside of the leaves at the end of long, silky stalks (Fig. 2B). After three to six days the eggs hatch and a predatory larva emerges. Lacewing larvae are tiny when emerging from the egg but grow up to 10mm. During their two to three-week developmental period, larvae actively feed on soft body insects and eggs (Fig. 2C). They are known as aphid lions since they voraciously attack aphids' eggs. After this stage, the larvae pupate by

spinning a cocoon with silken thread. Approximately five days later, adult lacewings emerge to mate and repeat the life cycle (Tauber et al., 2003).

The *C. carnea* life cycle reaches three to four weeks in summer. When temperatures are lower, the cycle can be extended to six weeks. In natural conditions it can complete up to three generations per year (Tauber et al., 2003). *C. carnea* does well under low humidity and should be used in dry regions (Tauber and Tauber, 1983). Nevertheless, it has great adaptability to adverse environmental conditions and wide thermal fluctuations.



Figure 2 - Representative photographs of the three moments in the *Chrysoperla carnea* life cycle: **A** - adult, **B** - eggs layed; **C** - larva. Fig. 2A - <http://www.diariorepublica.com/lo-mas-curioso/enigmatica-flor-que-crece-cada-3000-anos-vuelve-a-aparecer-en-la-tierra#> ; Fig. 2B - <http://hablemosdeinsectos.com/chrysopa-carnea/> ; Fig. 2C - <https://www.biologischer-pflanzenschutz.org/florfliege-chrysoperla-carnea/>, accessed on March 19, 2020.

Currently, there are several factors that can disturb and limit the biological control effectiveness of *Chrysoperla carnea* populations. Natural factors, since in some circumstances, earwigs, ants and other predaceous arthropods can attack lacewing eggs and sometimes larvae (Nyffeler et al., 1987; Rosenheim et al., 1993, 1995). However, anthropogenic factors seem to have the strongest effect on the effectiveness of *C. carnea*, since many studies have shown the sensitivity of *C. carnea* to insecticides (Grafton-Cardwell and Hoy, 1985; Vogt et al., 1992; Elzen et al., 1998; Medina et al., 2003; Moura et al., 2009; Amarasekare et al., 2019). In olive groves, pesticides treatments reportedly

compromised *C. carnea* adult development and fecundity (Corrales and Campos, 2004) as well as larvae effectiveness in plant protection from *P. oleae* attacks (Ramos et al., 1978).

Highly mobile organisms in particular are strongly affected by landscape composition because their biology, behavior, and dynamics depend on spatial distribution of resources (Kremen et al., 2007). This is the case for *Chrysoperla carnea* which the pollen produced by groundcover flower strips favored its reproductive process (Villa et al., 2016; Herrera et al., 2019). Additionally, vegetation diversity promotes higher abundance of larvae on olive groves during the olive moth egg-laying period indicating a great potential for conservation biological control approaches (McEwen and Ruiz, 1994; Porcel et al., 2017).

Since olive groves are susceptible to be attacked by pests, such as *Prays oleae*, farmers should be encouraged to adopt pest suppression methods based on the conservation of natural habitat to increase natural resources that consequently enhance the predatory efficiency of *Chrysoperla carnea*. This way, they can retain olive production while maintaining a healthy ecosystem, matching not only the environmental demands of IPM programs but also the increasing social demand for residue-free olive products (Vossen, 2007),

With this study we intend to assess the effectiveness of *Chrysoperla carnea*'s biological control on *Prays oleae* in Beira Interior olive groves, identifying the dynamics of their relationship in relation to different land-uses and with landscape diversity. We specifically aimed to answer the following questions: i) Does different types of land-uses in the surroundings of the olive groves and landscape diversity have an effect on *Chrysoperla carnea* and *Prays oleae* abundance?; ii) Does *Prays oleae* have an effect on

Chrysoperla carnea populations?; iii) Is there any effect of *Chrysoperla carnea* on *Prays oleae* olive fruit infestations?

We hypothesized that *P. oleae* would attract *C. carnea* to the olive grove. We also hypothesized that green lacewings would own conservation biological control potential by reducing olive fruit infestation. Lastly, we hypothesized that more diverse landscape composition around olive groves would negatively influence olive moth abundance.

MATERIALS & METHODS

Experimental design, study area and sampling point selection

To accomplish the objective of this study, twenty-five olive groves within the Beira Interior region in the parishes of Escalos de Cima, Lousa, Mata and Escalos de Baixo belonging to the municipality of Castelo Branco and in the parish of Ladoeiro belonging to the municipality of Idanha-a-Nova were selected (Fig. 3). To choose the olive groves that would be part of the study, a meeting with the local olive oil producers association - Associação de Produtores de Azeite da Beira Interior (APABI) took place in January 2019, before the starting of the field work. They provided us with an extensive list with the contact of all the landowners of the productions where we intended to define these twenty-five sampling points.

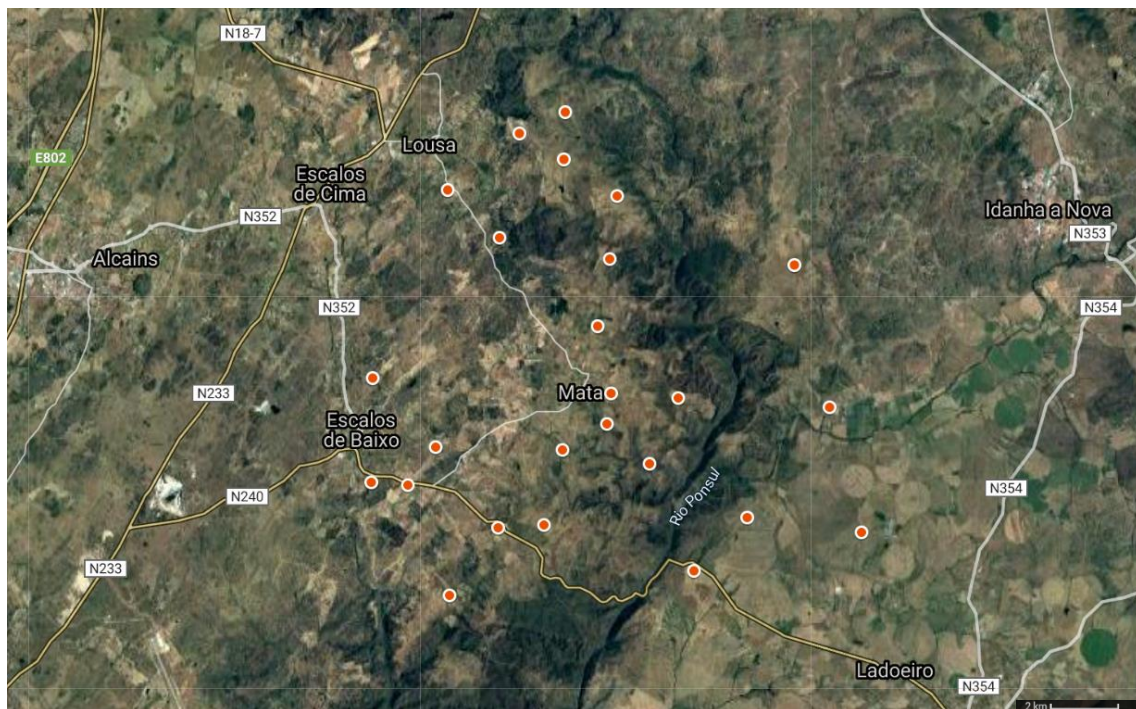


Figure 3 - Illustrative image of aerial photography of the twenty-five olive groves location where there were sampling points. Image from ©2020 Google Maps.

Olive groves selection criteria tried to keep the sampling points separated by a distance of at least one kilometer, in order to maintain spatial independence. However, this minimum distance did not always reach one kilometer, due to physical impossibilities related with the natural layout of the olive groves properties. Sampling points selection criteria also had to allow them to follow a gradient of landscape complexity measured as the Shannon diversity index. As mentioned before, Galega varieties are the most representative cultivars in this area. The olive groves are old, around 100 years of age, non-irrigated and with low groundcover vegetation due to livestock presence.

Predator and pest population abundance monitoring

In order to determine the abundance of the populations of the pest *Prays oleae* and the predator *Chrysoperla carnea*, on March 28th (87 Julian day), just a few weeks before the adults of the phytophagous generation of *P. oleae* were expected to appear, two different traps to capture and to monitor both adult populations of *P. oleae* and *C. carnea* at each of the sampling points were placed and activated.

To collect the olive moth, one funnel trap (Fig. 4A) was located at each sampling plot. It had a closed pot shape, having a support at the top to place a specific sexual pheromone to attract the olive moth (*Z*-7-tetradecenal) which is replaced every 6 weeks. An entry hole is located next to it. Inside, it consists of approximately 150 mL of glyco-ethylene to retain and preserve captured insects.

To capture adults of the predator, one McPhail trap (Fig. 4B) per plot were also used. It had a similar shape to the pest trap, but with the entrance hole being located at the bottom. Inside, it had a liquid content consisting of a 250 mL aqueous solution with

5% diammonium phosphate and 2% borax, which is very effective in attracting *C. carnea* as well as other insects.



Figure 4 - Representative photographs of **A** – the funnel trap used to collect *Prays oleae* individuals and **B** – the McPhail trap used to collect *Chrysoperla carnea* individuals. Personal photographs.

In both traps, the collection of the organisms was done every two weeks, however their liquid contents were replaced every 4 weeks, because during this period they still maintained their characteristics. The traps were placed in a central position of each olive grove, hanging on tree branches and separated by three to four olive trees, since each trap has a radius of action of approximately fifty meters, thus avoiding influencing the predator-prey relationship.

The organism's collection lasted until the July 18th (Julian day 199). This was the moment in which we noticed that the adults of the anthophagous generation disappeared from our traps. During this period (March 28th- July 18th), they have laid the eggs of the carpophagous generation that causes serious damage to the olive production and to the future development of the olive tree. Also during this period, those eggs were susceptible to be preyed by *C. carnea*. Knowing the pest and predator abundance values allows

understanding of the dynamics of the predator populations in the olive groves during the periods of greater activity of the pest. The captured insects were stored in flasks with 70% alcohol, duly identified, and then examined in the laboratory, where the different populations of captured insects were screened and the number of individuals of both *P. oleae* and *C. carnea* were counted.

We acknowledge that since the eggs of the carpophagous generation of the pest are preyed by the larvae of *C. carnea*, then it would be more logical to collect larval individuals. To do so, on June 20th (Julian Day 171), a beating technique was applied to five olive trees selected at random from each olive grove, except for the two olive trees that contained the two traps. In the twenty-five olive groves analyzed, just one larva of *C. carnea* was found in three of them. No larval individuals were detected in twenty-two olive groves. These results were so improbable that they attest the difficulty in capturing larvae of *C. carnea* and our preference for capturing adult individuals over their larval stage. The collection of adult individuals may be a late indicator, but it is just as reliable. Because of the poor results obtained with the beating technique we decided to not include them in this work. In the future, other techniques have to be explored in order to trap a reliable number of larvae of *Chrysoperla carnea* to perform this kind of studies.

Pest infestation monitoring

To determine pest infestation levels of *Prays oleae*, twenty olive fruits were collected per tree, homogeneously captured around the tree canopy. The olives were collected after a random selection of ten olive trees per olive grove, excluding the two olive trees that contained the two traps. A total of two hundred olives per olive grove were properly bagged, identified and later analyzed in the laboratory. However, after

transportation some of them were damaged and the number of observed olive fruits varied but always enough to perform statistical analyses. The olive fruits were collected on June 20th (Julian day 171) based on the adult population monitoring. This olive fruits sampling date was determined by the moment in which the adult of the anthophagous generation population started to rise because at that point they were laying eggs, allowing us to see the infestation at that moment. This is a well-known method that is applied to monitor pest population and to take decisions about when to apply insecticide under IPM programs. It is also used to estimated potential harvest losses (Ramos et al., 1998).

At the magnifying glass, we looked for pest eggs next to the olive fruit calyx and, when present, their condition was analyzed, determining olives with Empty Eggs, Standard Eggs or Hatched Eggs.

Olive with Empty Eggs (E): Considered as a non-living form. The egg is dry and crushed, it has been preyed (Fig. 5A).

Olive with Standard Eggs (S): Considered as a living form. The whitish egg still contains the larva inside (Fig. 5B).

Olive with Hatched Eggs (H): The egg is usually yellow and there is a hole in the calyx, meaning that the larva has penetrated the fruit (Fig. 5C).



Figure 5 - Representative photographs of **A** - olive with an empty egg, **B** - olive with a standard egg and **C** - olive with a hatched egg. Personal photographs.

Through the attack of the olive moth, it was possible to record the parameter level of infestation, corresponding to the sum of olive fruits containing eggs, whether empty, standard or hatched, per olive grove.

Geospatial parameters

The abundance variables of both insect populations as well as the biological control of the predator *Chrysoperla carnea* over the pest *Prays oleae* were analyzed relating it to landscape variables. Using the QGIS software 3.10.1 version, a Geographic Information Systems (GIS) platform, we carried out a geospatial analysis of the twenty-five olive groves containing sampling points.

In a first layer, a satellite map was imported from Google, and the synchronization with QGIS was done through the Coordinate Reference System ETRS89 / UTM zone 29N. In a second layer, the exact coordinates of the twenty-five sampling points were inserted. At each sampling point, a landscape buffer was performed, that is, a geospatial delimitation of a circular area around the sampling point that encompasses both the composition of the olive grove and the composition of its adjacent ecosystems. Twenty-five buffers were made with a radius of 500 meters each. Within the buffers, polygons were generated to delimit each of the existing patches inside them (Fig. 6). These polygons were numbered and classified into one of the following categories that were chosen because they were the most typical land-uses of the area of study: olive groves, oak forests, pine forests, eucalyptus forests, grasslands, scrublands and vineyards.

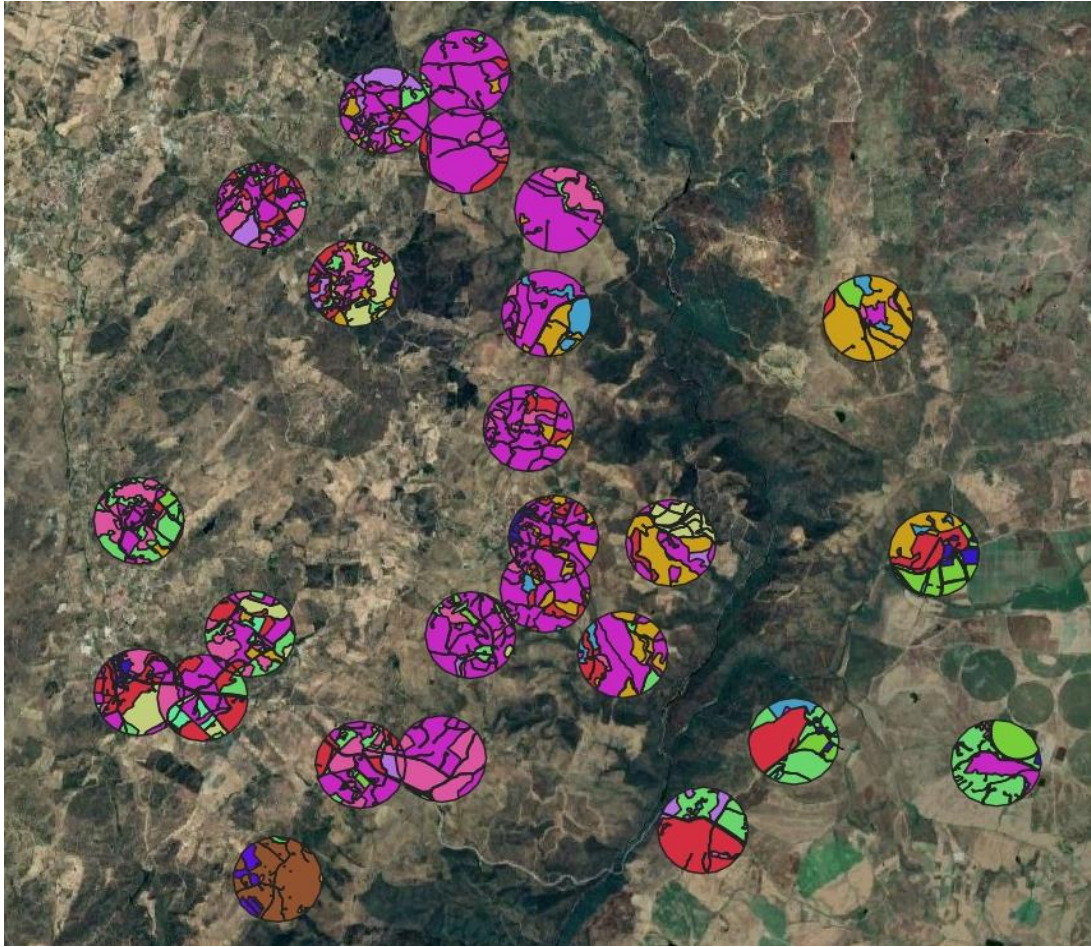


Figure 6 - Illustrative image of aerial photography of buffers and polygons layers corresponding to the twenty-five olive groves where there are sampling points. Image taken from the QGIS software.

To validate this delineation of the landscape elements, as well as adding data to the elements that cannot be identified in the aerial photographs, it was necessary to verify the existing vegetation during the periods in the field. All this geospatial information was converted into raster images and it was inserted in the Fragstats software 4.2 version. From this spatial pattern analysis program, we obtained landscape metrics at the class level, in which the total area of each patch within each of the landscape buffers was quantified.

Class level metrics measure the aggregate properties of the patches belonging to a single class or patch type and *percentage of landscape* (PLAND) is a fundamental measure of landscape composition, giving information of how much of the landscape is comprised of a particular patch type, that is quantifying the proportional abundance of each patch type in the landscape (Table 1).

Table 1 – Description, units and range of Percentage of landscape metrics.

$\text{PLAND} = P_i = \frac{\sum_{j=1}^n a_{ij}}{A} (100)$	<p>P_i = proportion of the landscape occupied by patch type (class) i.</p> <p>a_{ij} = area (m²) of patch ij.</p> <p>A = total landscape area (m²).</p>
Description	<p>PLAND equals the sum of the areas (m²) of all patches of the corresponding patch type, divided by total landscape area (m²), multiplied by 100 (to convert to a percentage); in other words, PLAND equals the percentage the landscape comprised of the corresponding patch type.</p> <p>Note, total landscape area (A) includes any internal background present.</p>
Units	Percent.
Range	<p>$0 < \text{PLAND} \leq 100$</p> <p>PLAND approaches 0 when the corresponding patch type (class) becomes increasingly rare in the landscape. PLAND = 100 when the entire landscape consists of a single patch type; that is, when the entire image is comprised of a single patch.</p>

From Fragstats, we also obtained landscape metrics at the landscape level in which Shannon's diversity index (SHDI) values were quantified. SHDI is based on information theory (Shannon and Weaver, 1949). The value of this index represents the amount of "information" per patch, independently of their spatial configuration. It is used as a relative index for comparing different landscapes or the same landscape at different times (Table 2).

Table 2 – Description, units and range of Shannon's Diversity Index metrics.

$\text{SHDI} = -\sum_{i=1}^m (P_i \cdot \ln P_i)$	P_i = proportion of the landscape occupied by patch type (class) i .
Description	SHDI equals minus the sum, across all patch types, of the proportional abundance of each patch type multiplied by that proportion. Note, P_i is based on total landscape area (A) excluding any internal background present.
Units	Information.
Range	<p>SHDI ≥ 0, without limit.</p> <p>SHDI = 0 when the landscape contains only 1 patch (i.e., no diversity).</p> <p>SHDI increases as the number of different patch types (i.e., patch richness, PR) increases and/or the proportional distribution of area among patch types becomes more equitable.</p>

Statistical analysis

All statistical treatment of the data was performed using R programming language version 3.6.3 for statistical computing through RStudio software version 1.2.5042 which operates the R language to develop statistical programs, allowing users to edit those programs in R by supporting a large number of statistical packages as well as higher quality graphics (R Development Core Team, 2012).

To perform the analysis we generated two different datasets, one containing the abundance values of the *C. carnea* and the pest *P. oleae* recorded over the trial period ($N = 225$). And another one where we aggregated those values of abundance by averaging them ($n = 25$). This aggregated data was merged with the data from the fruit infestation (Appendix 1) by the olive moth carpophagous generation. Finally, both datasets were joined by the data obtained through geospatial analysis (Appendix 2) that resulted in landscape metrics at the class level (percentage of different land-uses) and landscape level (Shannon's diversity index).

An initial exploration of data was done through exploratory work on distributions and correlations using Generalized Pairs Plot (GGPAIRS), package “*GGally*” (Schloerke et al., 2012) which offers a range of displays of paired combinations of categorical and quantitative variables, allowing the selection of the variables that may be used in a same model (Emerson et al., 2012). We decided to set our collinearity criteria at a level of $r = 0.5$. After carrying out exploratory work, the factors that have the most potential to be used as reference variables were besides *C. carnea* and *P. oleae* abundance, the Shannon diversity index, and the percentage of oak forests, pine forests, eucalyptus forests, grasslands, scrublands and vineyards by olive grove (Fig. 7). However, a high correlation between surrounding olive groves and Shannon Diversity index ($r = -0.77$; Fig. 7) was found so we decided not to include them in the model to avoid problems with collinearity.

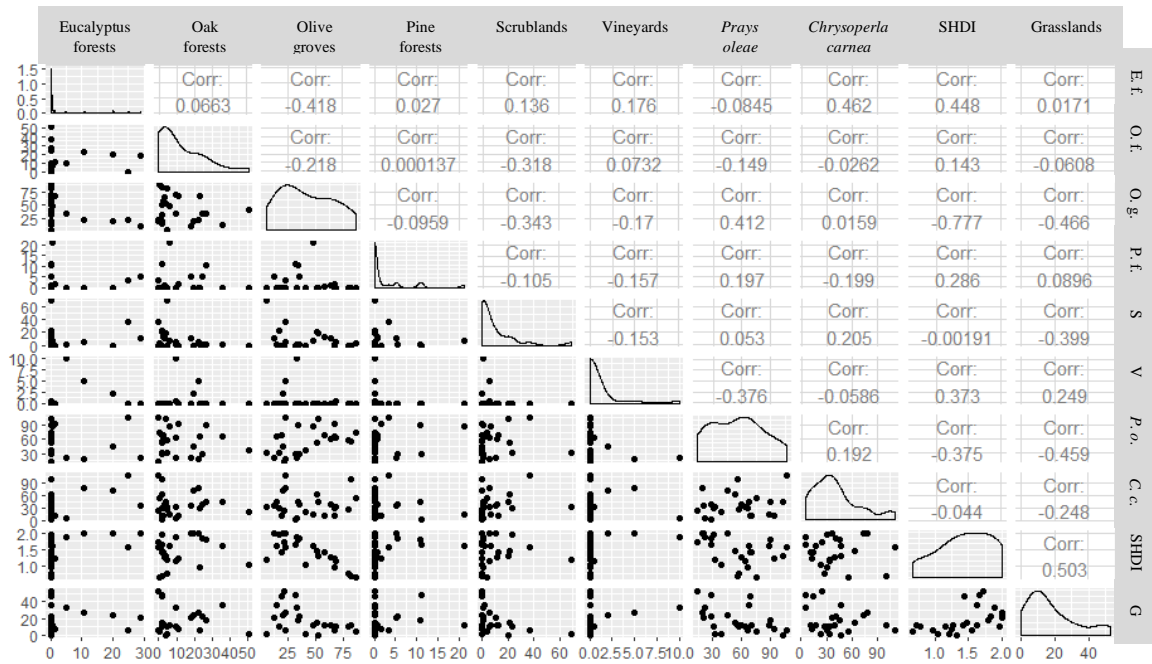


Figure 7 – Correlations of the different predictor variables included in the models.

To achieve the objective presented in this work we used different approaches. First, to account for the factors that determinate the presence of *Chrysoperla carnea* adults in olive groves, an inferential Generalized Additive Mixed Model (GAMM), package “*mgcv*” (Wood, 2011) was created in which we included as response variable the abundance of *C. carnea* adults and as predictors the Julian day in which these data were recorded, so we could have an idea of the evolution of the abundance of the predator along time. The abundance of adults of *Prays oleae* was also included as we believe that *C. carnea* can feel an attraction for this pest as reported in bibliography (Sacchetti, 1990; Campos, 2007; Porcel et al., 2017). To account for the effect of the different land-uses and the overall diversity surrounding the sampling points we included, as predictors and by olive grove, the different proportions of the land-uses (oak forests, pine forests, eucalyptus forests, grasslands, scrublands and vineyards) as well as the Shannon’s diversity index. A Poisson error distribution was used. Finally, as different samples were

collected in the same location, the olive grove identity was used as a random factor. To perform this model, the dataset that was not aggregated was used. From the previous model, the variables that showed a significant effect on the predator abundance were used as predictable variables to graphically demonstrate the effects of its different levels.

A similar approach was used for the abundance of *Prays oleae* in which we included as response variable the abundance of *P. oleae* and as predictor the Julian day for the complete sampling period. However, in this model *Chrysoperla carnea* abundance was not included as predictor. The reason for not including it was that a reverse causality effect was noticed, that is, the effect of the pest on the predator was equal in sign and magnitude as the effect of the predator on the pest. In this kind of studies is normal to find this kind of circular effect in which it is to know if the pest is influencing the abundance of the predator or if the predator is influencing the abundance of the pest. As the pest is in the olive trees before the predator due to it being a specific pest of the olive groves, we decided not to include the predator in the model of the pest. We also included the different proportions of the land-uses (oak forests, pine forests, eucalyptus forests, grasslands, scrublands and vineyards) as well as the Shannon's diversity index as predictor. As we mentioned before the surrounding olive groves variable was very correlated with the variable Shannon Diversity index so was not advisable to include in this model. However, as this work is based on the resource concentration hypothesis, surrounding olive groves should be included in order to test if a concentration of optimal resources for the pest (i.e. olives) would have an effect on it. For that reason, we decided to create another generalized additive mixed model in which we only included surrounding olive groves as a predictor of the abundance of *Prays oleae*. Similarly to the *C. carnea* model, a Poisson error distribution was used as the error distribution. To account for heteroskedasticity we plotted the residuals versus the fitted values finding no pattern (Zurr et al., 2009). We also

checked for overdispersion by testing it with the package “AER” (Kleiber and Zeileis, 2008) finding no over or under-dispersion.

To account for the effect of *Chrysoperla carnea* and different surrounding land-uses on *Prays oleae* infestation we opted for a model selection approach through Generalized Linear Models (GLM), package “lme4” (Bates et al., 2015). To do so, the Akaike Information Criteria corrected for small sample size (AICc) was used. This parameter estimates the quality of each model relative to each of the other models under comparison. The model selected for further consideration is that with the lowest AICc of all the models proposed with a difference of two units with the next one. To perform these models, we first aggregated *Chrysoperla carnea* abundance thus having a single value per plot. Such models were performed for a time lag between the day the study started (Julian day 88) and the day when the peak of the *Prays oleae* population was reached (Julian day 154). That was done because the effect of the abundance of *C. carnea* on the infestation rate of *P. oleae* can account from the date when infestation data was collected. Along with the aggregate abundance of *C. carnea*, each one of the land-uses were separately included as an addition and as an interaction. Each land-use proportion alone, the abundance of *Chrysoperla carnea* alone and a null model were also included in the model set. The effect of this model predictors on the infestation rate of *P. oleae* was assessed. As the number of collected olive fruits varied, we opted for a response variable as proportion of counts with a binomial error distribution. We also checked the best model for heteroskedasticity using the same method mentioned above finding no problem with the procedure (Zuur et al., 2009).

RESULTS

During the entire experimental period between Julian days 88 and 199, a total of 1004 individuals of *Chrysoperla carnea* and 1394 individuals of *Prays oleae* were captured. The presence of the olive moth in the olive groves was, therefore, 38.84% higher than the abundance of its predator. *Chrysoperla carnea* abundance registered its peak at Julian day 143 (Fig. 8). While *Prays oleae* has an initial peak registered between the Julian days 101 and 115 corresponding to the anthophagous generation preceding a drastic decrease on registered individuals due to the transition from the antophagous to the carpophagous generations of the pest, detected between the collection of Julian days 129 and 143, having its second and most representative peak at Julian day 154, corresponding to the carpophagous generation (Fig. 9).

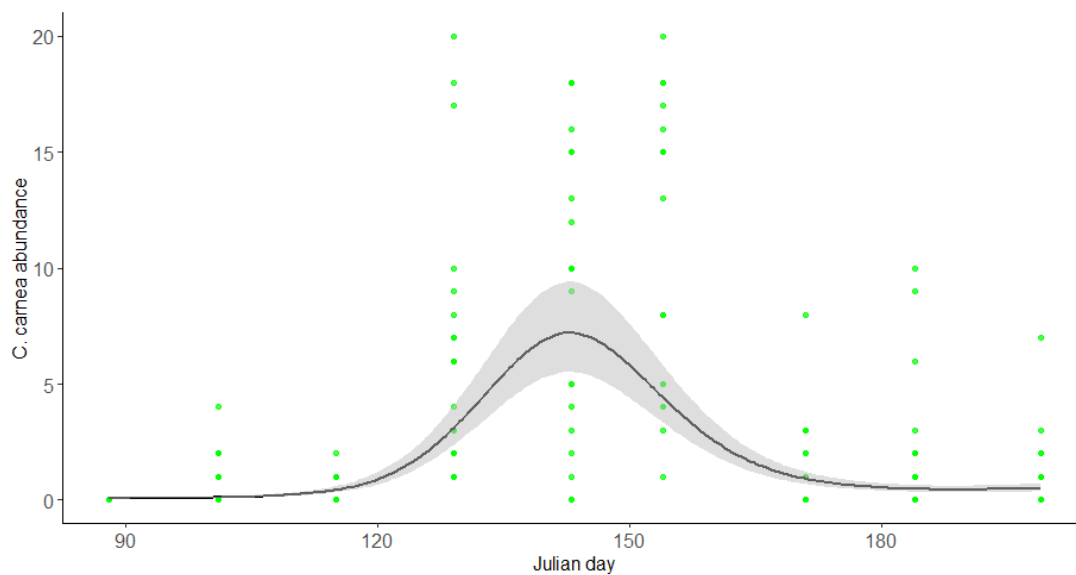


Figure 8 – *Chrysoperla carnea* abundance records during the study period (in Julian days).

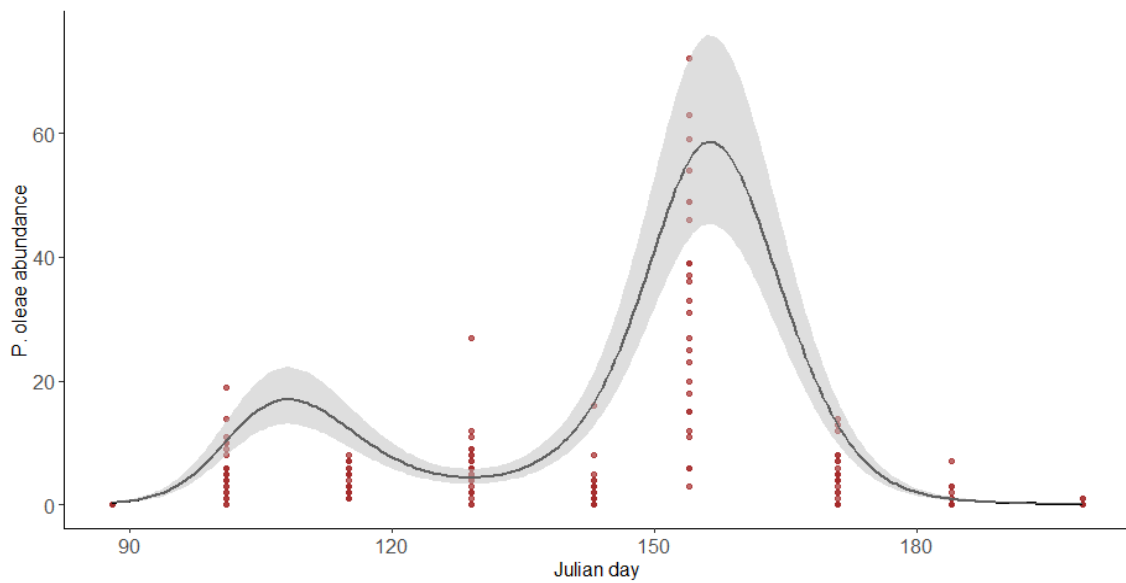


Figure 9 – *Prays oleae* abundance records during the study period (in Julian days).

The effect of different land-uses on *Chrysoperla carnea* abundance

Prays oleae abundance ($P < 0.001$) was the only factor that significantly affected the presence of adults of *C. carnea* in olive groves (Table 3). *Chrysoperla carnea* abundance almost doubled its abundance when there was a higher abundance of the pest. However, when the abundance of the pest was medium or low, the abundance of adults of *Chrysoperla carnea* was almost the same (Fig. 10).

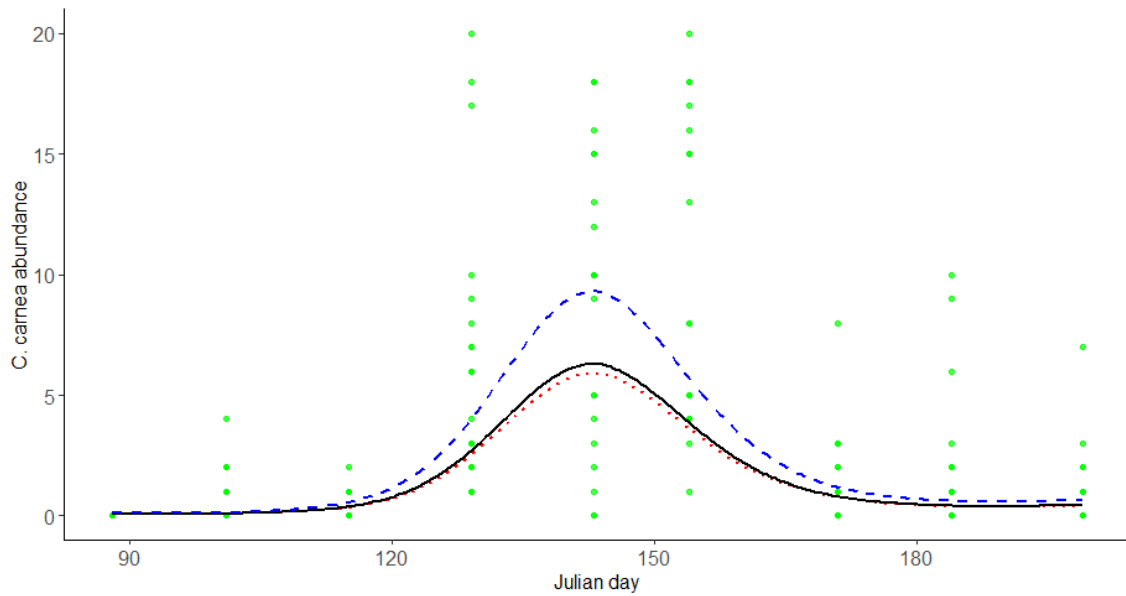


Figure 10 – Abundance of *Chrysoperla carnea* along time under the influence of different *Prays oleae* abundance levels. Black solid line represents the estimated abundance of *Chrysoperla carnea* under the influence of a *Prays oleae* abundance of a 50% of the observed data. Red dotted line represents the the estimated abundance of *Chrysoperla carnea* under the influence of a *Prays oleae* abundance of a 10% of the observed data. Blue dashed line represents the estimated abundance of *Chrysoperla carnea* under the influence of a *Prays oleae* abundance of a 90% of the observed data.

Table 3 - Chi-square test, p-value, coefficient of determination and deviance explained of the prediction variables of the GAMM model for the *C. carnea* abundance along time.

	<i>Chi.sq</i>	<i>p-value</i>
<i>Prays oleae</i>	35.243	2.458e-08
<i>Oak forests</i>	0.851	0.356
<i>Pine forests</i>	0.575	0.448
<i>Eucalyptus forests</i>	0.492	0.484
<i>Grasslands</i>	0.009	0.922
<i>Scrublands</i>	1.811	0.285
<i>Vineyards</i>	2.013	0.291
<i>Shannon's Diversity Index</i>	3.778	0.153
<i>R</i>²		0.761
<i>Deviance explained</i>		79%

The effect of landscape metrics on the level of infestation in olive groves

The interaction amidst the percentage of surrounding olive groves and the abundance of *C. carnea* for the time lag between Julian days 88 and 154 (AICc = 449.70) was the most significant model based on the Akaike information criterion corrected (AICc) comparison (Table 4).

Table 4 – AICc models comparison for the studied variables of the *P. oleae* carpophagous generation eggs infestation on olive fruits during the complete study period and until the time lag settled at the 154 Julian day.

	AICc
Null Model	554.02
<i>Chrysoperla carnea</i>	550.58
Eucalyptus forests	549.72
Eucalyptus forests + <i>Chrysoperla carnea</i>	549.82
Eucalyptus forests * <i>Chrysoperla carnea</i>	538.85
Grasslands	542.97
Grasslands + <i>Chrysoperla carnea</i>	532.63
Grasslands * <i>Chrysoperla carnea</i>	500.81
Oak forests	555.71
Oak forests + <i>Chrysoperla carnea</i>	552.59
Oak forests * <i>Chrysoperla carnea</i>	551.89
Scrublands	552.34
Scrublands + <i>Chrysoperla carnea</i>	546.73
Scrublands * <i>Chrysoperla carnea</i>	548.72
Vineyards	513.40
Vineyards + <i>Chrysoperla carnea</i>	514.09
Vineyards * <i>Chrysoperla carnea</i>	506.92
Olive Groves	537.42
Olive Groves + <i>Chrysoperla carnea</i>	530.84
Olive Groves * <i>Chrysoperla carnea</i>	449.71
Pine Forests	545.28
Pine Forests + <i>Chrysoperla carnea</i>	538.09
Pine Forests * <i>Chrysoperla carnea</i>	538.72
Shannon's diversity index	543.25
Shannon's diversity index + <i>Chrysoperla carnea</i>	536.85
Shannon's diversity index * <i>Chrysoperla carnea</i>	505.53

When the abundance of *Chrysoperla carnea* was low or medium, the infestation of *Prays oleae* tended to increase along a gradient of surrounding olive groves. However, when the abundance of *C. carnea* was high the infestation of the pest tended to notably decrease from a level of around 20% to a level of 10 % (Fig. 11).

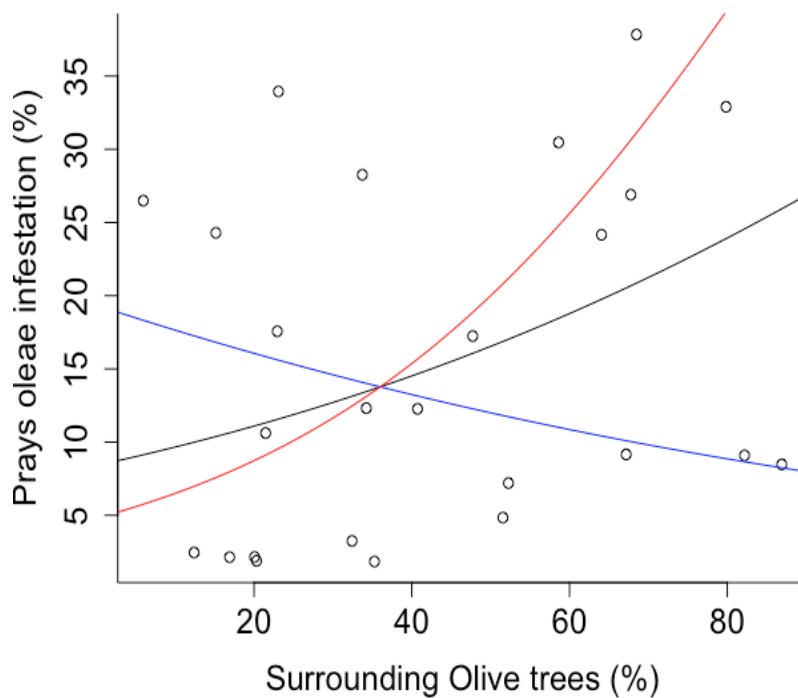


Figure 11 – Infestation of *Prays oleae* along a gradient of surrounding olive groves and different levels of *Chrysoperla carnea* abundance. Red line represents the estimated effect of surrounding olive groves under a low abundance of *Chrysoperla carnea* abundance (10% of observed data). Black line represents the estimated effect of surrounding olive groves under a medium abundance of *Chrysoperla carnea* abundance (50% of observed data). Blue line represents the estimated effect of surrounding olive groves under a high abundance of *Chrysoperla carnea* abundance (90% of observed data).

The effect of different land-uses on *Prays oleae* population

From the Generalized Additive Mixed Models, the presence of *P. oleae* in olive groves was significantly affected by Shannon's diversity index ($P = 0.022$; Table 5). Only

high values of this landscape metric can decrease the abundance of *Prays oleae* in olive groves (>1.2). Lower values induce an increase of olive moth values of abundance (Figure 12).

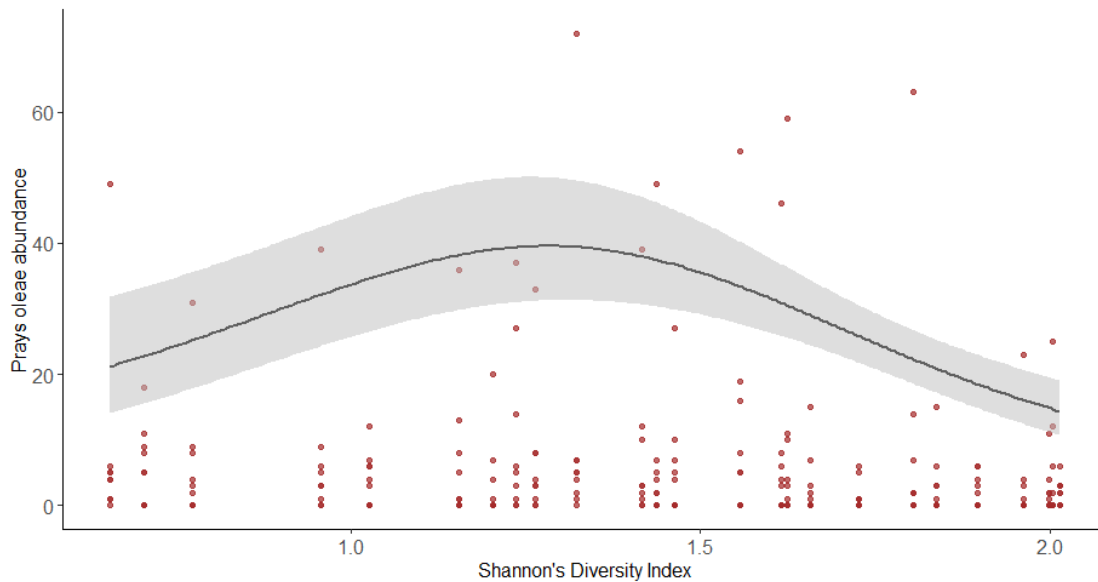


Figure 12 - **Effects of surrounding diversity measured through Shannon's diversity index on *Prays oleae* abundance.** Black solid line represents the abundance of *Prays oleae*. Shaded areas represents the interval of confident at 95%.

In the same line, the abundance of *P. oleae* increases for higher percentages of surrounding olive groves (Figure 13).

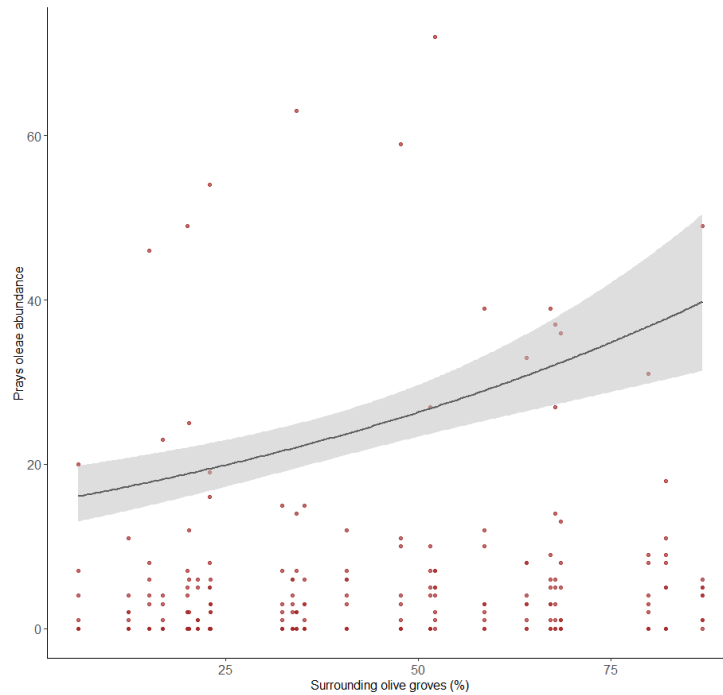


Figure 13 – **Effects of surrounding olive groves percentages on *Prays oleae* abundance.** Black solid line represents the abundance of *Prays oleae*. Shaded areas represents the interval of confident at 95%.

Table 5 - Chi-square test, p-value, coefficient of determination and deviance explained of the prediction variables of the GAMM model for the *P. oleae* abundance along time.

	<i>Chi.sq</i>	<i>p-value</i>
<i>Oak forests</i>	1.109	0.292
<i>Pine forests</i>	0.863	0.091
<i>Eucalyptus forests</i>	1.129	0.414
<i>Grasslands</i>	2.637	0.112
<i>Scrublands</i>	2.863	0.091
<i>Vineyards</i>	0.787	0.375
<i>Shannon's Diversity Index</i>	7.572	0.022
<i>R</i>²		0.792
<i>Deviance explained</i>		75.9%.

DISCUSSION

In our study we found that the abundance of both the adult predator *Chrysoperla carnea* and the pest *Prays oleae* were almost synchronized, with the abundance of *C. carnea* increasing with the greater presence of *P. oleae* in olive groves and hitting its peak just a few days before *P. oleae* highest peak. It may indicate that the predator is directly attracted by the presence of this pest, which is an assumption in line with what has already been described (Bento, 1999, Porcel et al., 2017). *C. carnea* larvae are major oophagous predators known to play a predominant role in the predation of eggs from the carpophagous generation of the olive moth (Sacchetti, 1990; Campos, 2007). Although, and as the *C. carnea* adult captures may be a late indicator it is possible that it really has reached its peak at the transition from the antophagous to the carpophagous generations of the pest, detected between the collections of May, which precedes *P. oleae* maximum peak corresponding to the carpophagous generation registered at the beginning of June, occurring here the main desynchrony between both populations, since the increase in the abundance of *C. carnea* is not affected by the decrease in *P. oleae* corresponding to the transition of generations, this is explained by the possibility of this predator feeding on the eggs and larvae of the antophagous generation. However, the different land-uses do not seem to have a direct attraction effect on the *C. carnea* population and the predator levels of abundance are more related to the olive moth presence than to the different land-uses.

Prays oleae infestation and abundance increased with surrounding olive groves which can be explained by the resource concentration hypothesis. This hypothesis states that expansive monocultures allow specialist pest populations to rapidly build and disperse, whereas diverse landscapes mitigate population growth and spread (Root, 1973; Risch et al., 1983; Margosian et al., 2009). However, this effect was counteracted when there was a high abundance of *C. carnea* on olive groves. This is even more explicit when

the abundance of *Chrysoperla carnea* was medium or low as in these situations the infestation levels of *Prays oleae* continued to increase when more olive groves were surrounding the sampling points.

Hereupon, it is possible to realize that olive groves as a land-use have both the ability to increase the abundance of *Prays oleae* and, on the contrary, when associated with high values of *Chrysoperla carnea* it has the ability to decrease infestation and, consequently, to decrease the abundance of the following generations of olive moth. Agricultural management practices, competition between natural enemies and their preference for different food resources other than *P. oleae* can be a set of factors that explain the abundance of *C. carnea* being more directly related to the presence of *P. oleae* inside the olive crops than to the different land-uses. The effects of agricultural management practices such as the application of pesticides or land ploughing are known to have a negative effect on the abundance of natural enemies of pests within agroecosystems (Tscharrntke et al., 2005; Meehan et al., 2011). During this study, agricultural managers did not apply pesticides and did not use land ploughing methods in any of the twenty-five olive groves where sampling points were located. Therefore, as there was no interference from these factors, it explains the predictable significant effect of *Prays oleae* on the attraction of its natural enemy, *C. carnea*. This findings allow us to confirm the biological control potential of *C. carnea* as it can control the infestation of this pest as well as it feels attraction for it (Sacchetti, 1990; McEwen and Ruiz, 1994; Campos, 2007; Szentkirályi, 2007; Pappas et al., 2011, Porcel et al., 2017).

Using class-level landscape metrics such as Shannon's diversity index, it was observed that greater diversity in the vicinities of the crop tends to decrease the abundance of *Prays oleae*. The most conceivable explanation is based on the natural enemy hypothesis, which recognizes that many natural enemies of crop pests (*i.e.*, predators and

parasitoids) depend on a diversity of crops and/or natural habitats for alternate food resources, overwintering, *etc.* Thus, more diverse landscapes may facilitate better pest control (Andow, 1991; Landis et al., 2000; Langelloto and Denno, 2004). In the same line, Villa et al., (2020) related the landscape diversity and configuration at larger scales with a decrease of *P. oleae* abundance. Some authors have suggested, that complex landscapes covered by natural or semi-natural habitats are important elements that favor the prospection of natural enemies of olive pests by providing undisturbed areas that offer shelter from crop disturbances as well as overwintering refuges, alternative hosts and prey, and additional food resources (Bianchi et al., 2006; Tschardtke et al., 2007; Paredes et al., 2017). The assemblage of natural enemies of *Prays oleae* not only includes *C. carnea* but also other common predators of the olive moth such as ants, Coleoptera, Hemiptera and spiders (Lozano et al., 2000). As described by Paredes et al. (2015), effective assemblages of natural enemies are better suppressing a Lepidopteran pest, such as *P. oleae* than a species of natural enemies acting alone. For a Lepidopteran pest with a complex life cycle, the single best predator taxon was markedly poorer at suppression than the most effective assemblage.

As an example, *Anthocoris nemoralis* biological control effectiveness on *P. oleae* was strongly related with its abundance being positively influenced by natural habitat (Paredes et al., 2019). Such decrease in *P. oleae* abundance indicate that diverse land-uses surrounding olive groves own the most potential to reduce pest harm in such agroecosystems because they are able to provide undisturbed areas that offer shelter from crop disturbances as well as overwintering refuges, alternative hosts and prey, and additional nectar resources (Thies et al., 2003; Bianchi et al., 2006; Tschardtke et al., 2008; Rusch et al., 2010). Although such diversity could be enhanced in order to establish conservation biological control strategies, in-depth knowledge is needed about which

plants and natural habitats are best ecological infrastructures to increase the proliferation of natural enemies and avoid further pest pressure, otherwise plant diversity can be a waste of resources if it does not improve pest control (Winkler et al., 2009a; 2009b; Saeed et al., 2015).

Green lacewings preference for different food resources other than olive moth could influence the distribution and recorded abundance of this generalist predator. However, as a significant attraction of *C. carnea* individuals was observed in agroecosystems with a greater abundance of *P. oleae*, it is highly unlikely that different food resources other than the pest could have played a disruptive role in the results obtained.

Conclusions

This study identifies chain relationships that confirm the attraction of the predator, *Chrysoperla carnea*, to the olive moth, *Prays oleae*. It confirms the biological control potential of *C. carnea* in olive groves. And it identifies the potential to reduce harmful effects of *P. oleae* in olive groves. While olive groves themselves have a direct contribution to the increase in the abundance of olive moth, our study highlights that promoting landscape diversity through conserving semi-natural habitats in the vicinities of olive groves directly affects pest abundances by decreasing it. Although in-depth knowledge is needed about which plants and semi-natural habitats are best ecological infrastructures to increase the proliferation of natural enemies and avoid further pest pressure, the prospects are that the enhancement of land-uses diversity can help olive growers to improve and make their production healthier by doing their integrated pest management through conservation biological control strategies.

This work seeks to rethink the formation of agroecosystems, in this case olive groves, with a view to assigning greater value to products obtained from olive production and to create sustainable alternatives to the use of pesticides without causing damage to the environment, instead, promoting public health. The conceptions originated from this study are intended not only to complement the existing literature on conservation biological control methods but also to create a robust knowledge foundation that provides both olive growers and policy makers with relevant information that they can apply in order to improve and attribute added value to their production economy and to meet the increasingly demanding and necessary environmental standards through alternatives to the use of pesticides that compromise and threaten community health.

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APPENDICES

Appendix 1 – Values of olive fruits with standard, hatched and empty eggs, total of olives samples and infestation by olive grove. The numbering of each olive grove corresponds to a personal method of identification and does not intend to be disclosed.

<i>Olive Grove</i>	<i>Standard eggs</i>	<i>Hatched eggs</i>	<i>Empty eggs</i>	<i>Total of olives sampled</i>	<i>Infestation</i>
55	1	3	5	125	9
56	1	10	2	142	13
58	2	39	10	155	51
59	0	13	2	177	15
60	0	21	4	145	25
62	3	43	6	184	52
63	1	46	8	162	55
64	1	22	2	203	25
65	0	41	2	177	43
67	1	17	8	148	26
68	0	7	2	186	9
69	0	5	0	154	5
70	0	16	3	179	19
71	2	17	1	163	20
72	0	2	1	163	3
73	0	13	4	187	17
74	0	4	0	187	4
75	4	31	5	151	40
76	0	3	1	186	4
77	0	33	6	145	39
78	3	40	13	148	56
79	1	28	7	149	36
81	3	22	14	128	39
82	0	3	0	159	3
84	1	3	0	163	4

Appendix 2 – Values of landscape metrics at the class level (percentage of surrounding olive groves, oak forests, pine forests, eucalyptus forests, grasslands, scrublands and vineyards) and landscape level (Shannon's diversity index). The missing proportions of the patches covered by the 500m radii buffer correspond to unclassified geospatial parameters. The numbering of each olive grove corresponds to a personal method of identification and does not intend to be disclosed.

<i>Olive Grove</i>	<i>SHDI</i>	<i>Surrounding olive groves</i>	<i>Oak forests</i>	<i>Pine forests</i>	<i>Eucalyptus forest</i>	<i>Grasslands</i>	<i>Scrublands</i>	<i>Vineyards</i>
55	1.3223	52.2578	3.5780	0.0000	0.0000	0.0520	19.9065	0.0000
56	0.9565	67.1771	23.1828	0.0000	0.0000	0.0000	1.2344	0.0000
58	0.7725	79.8431	4.8721	0.0000	0.0000	10.7676	0.0000	0.0000
59	0.6552	86.9087	0.6654	0.0000	0.0000	2.0990	3.6457	0.0000
60	1.6250	47.7248	5.9283	21.0243	0.5379	3.4988	7.1625	0.0000
62	1.8967	33.7305	9.3007	0.0000	4.7137	25.9448	0.4940	10.0060
63	2.0143	23.0948	22.5804	0.0000	10.6839	13.0420	5.7645	5.0264
64	1.8037	34.2320	26.8400	10.7799	0.0000	13.7215	0.9268	0.0000
65	1.6153	15.1647	36.0720	0.0000	0.0000	2.6894	2.1700	0.0000
67	1.5559	22.9495	0.0000	3.3746	25.0680	6.2708	36.5458	0.0000
68	1.4625	51.5544	1.8286	0.0000	0.0000	10.0176	21.5598	0.0000
69	1.6578	32.4259	2.1029	10.9845	0.0000	21.1961	0.0000	0.0000
70	1.7273	21.4725	0.0000	0.0000	0.0000	15.1313	0.0000	0.0000
71	1.0258	40.7316	50.5708	0.0000	0.0000	0.0000	0.0000	0.0000
72	1.8382	35.2671	24.6698	5.3683	0.0000	12.2457	0.8679	0.0000
73	0.7029	82.1884	1.7516	0.0000	0.0000	11.6142	0.0000	0.0000
74	1.9626	16.9057	1.5031	0.0000	0.0000	8.6288	23.5046	0.0000
75	1.2036	5.9626	4.5854	0.0000	0.0000	2.0482	69.2783	0.0000
76	1.4366	20.0692	1.8113	0.0000	0.0000	0.0000	0.0000	0.0000
77	1.2350	67.7764	11.1514	1.5652	1.0698	0.3199	0.0000	0.0000
78	1.1547	68.4890	9.4947	0.0000	0.0000	10.1769	5.3175	0.5062
79	1.2638	64.0526	3.4855	0.7444	0.0000	11.5980	11.9334	0.0000
81	1.4162	58.6117	2.4485	0.6337	0.0000	12.0152	12.4760	0.0000
82	2.0033	20.3158	19.8344	0.0000	20.1337	20.2129	0.1181	2.0639
84	1.9989	12.4040	17.8763	5.0685	28.9729	12.7399	10.1236	0.0000

