



# Jacinto Benhadi Marín

DIVERSITY PATTERNS OF ARANEAE ALONG A GRADIENT OF FARMING PRACTICES IN OLIVE GROVES: LINKING LANDSCAPE PATTERN, MANAGEMENT PRACTICES, AND SPECIES INTERACTIONS

Tese no âmbito do Doutoramento em Biociências, especialização em Ecologia, orientada pelo Professor Doutor José Paulo Sousa e co-orientada pela Professora Doutora Sónia Alexandra Paiva Santos e o Professor Doutor José Alberto Pereira e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Janeiro de 2019

Departamento de Ciências da Vida

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### ABSTRACT

The olive crop (*Olea europaea*) is a significant land use type with high importance in the Mediterranean countries. The olive tree is susceptible to the attack of pests that can cause significant losses. Among the community of arthropods of the olive grove, spiders are predators occupying the highest trophic level, hunting mainly insects in all their instars. Thus, they can be useful natural control agents of olive pests; however, the intensification of farming practices can affect their abundance and effectiveness. Aspects such as the conservation of a heterogeneous agricultural landscape and the maintenance of ecological infrastructures can be seen as part of a strategy to achieve sustainability and promote conservation biological control.

The main objectives of this thesis were to study the diversity patterns of Araneae through an increasing gradient of olive grove farming practices, study the effect of ecological infrastructures as sources of non-prey foods on the survival and behavior of Araneae, study the mechanisms of foraging of Araneae, and develop tools that aim at promoting the diversity of Araneae in the olive grove agroecosystem.

The structure of the Araneae assemblages under different farming practices was studied by sampling selected olive groves in northeastern Portugal at the soil, trunk, and canopy levels, as well as the adjacent semi-natural areas (Mediterranean shrublands). The experimental design, the number of stones in the soil, the percentage of vegetation, moisture, and crop management were used to model the diversity patterns found across the olive grove and its surrounding landscape. The effect of ecological infrastructures and mechanisms of foraging of Araneae were investigated through laboratory assays using selected species of spiders as models for different functional groups. The effect of non-prey foods (glucose at 0.5 M; aphid honeydew; black scale honeydew; a mixture of glucose 0.5 M, phenylalanine 0.1 mM, proline 0.1 mM, and tryptophan 0.1 mM; honey at 10%, and pollen at 10%) on the longevity and food selection, and the functional response and prey preference were studied on immatures and adults of spiders respectively. Moreover, two simulation-based tools were developed: an R package (simaR) that simulates the functional response of a predator

# <u>Abstract</u>

and calculates its maximum attack rate, and an educational individual-based model (EcoPred) that simulates a top-down trophic cascade controlled by the pressure exerted by two model predators on a model pest within a hypothetical agricultural landscape.

Nine functional groups (ambushers, foliage runner hunters, ground hunters, orb-web builders, sheet web builders, sensing web-builders, space web builders, stalkers, and wandering sheet/tangle weavers) were found to encompass a community that changed significantly across the horizontal and vertical gradient across the olive crops. *Philodromus lividus* was the most abundant species in the canopy, Erigoninae was the most recorded group on the trunk, and the ground was dominated by *Thanatus vulgaris* whereas the adjacent shrublands were dominated by *Nomisia exornata*. The number of stones was found to significantly enhance the overall diversity of spiders, the abundance of immature individuals and abundance of ground hunters.

The overall survival of immatures of *Haplodrassus rufipes* (ground hunters) and *Synema globosum* (ambushers) fed on non-prey foods significantly increased compared to individuals fed on water. When different non-prey food items were offered together, the exploring rate was significantly higher for *H. rufipes* than for *S. globosum*. *H. rufipes* chose to feed on honey whereas *S. globosum* chose the mixture treatment. The most chosen food items corresponded with those that provided the highest longevities in both species. The most and the least efficient adult spiders against the Mediterranean fruit fly *Ceratitis capitata* in laboratory were *H. rufipes* and *S. globosum* respectively. However, opposite results were obtained when alternative preys (crickets and moths) were supplied. *Araniella cucurbitina* (orb-weavers) significantly changed its functional response with the presence of alternative preys as well. Considering the prey preference, the heavy, long-sized and highly motile preys (*C. capitata*) were preferred by the three guilds of spiders.

The effect of temperature on the attack rate of two guilds of spiders against *C. capitata* was assessed using the simaR package. It was found that the maximum attack rate of *A. cucurbitina* (orb-weavers) on *C. capitata* significantly decreased as the temperature

### Abstract

increased whereas the opposite pattern was found for *S. globosum* (ambushers). On the other hand, EcoPred reflected the changes on a fly population within a simulated olive crop according to the mortality rate caused by the predation of two guilds of spiders and energy loss, the energy gain by feeding on flowers, and the reproduction rate in olives. The model was used to teach different aspects of biological control to 26 students achieving very good results in terms of acceptance and interest in the learning method.

In conclusion, each guild of spiders may include efficient predators against pests according to its hunting strategies and the ecological exploited niches. Different aspects related to agricultural management such as the presence of adjacent shrublands, the conservation of soil microhabitats (e.g. hedgerows, stonewalls, and stones on the ground), and maintaining weed strips may allow the spillover, increase the number of shelters, and provide valuable supplementary food resources for spiders thus playing an important role on their contribution to biological control of pests in the olive grove agroecosystem.

Keywords: Functional traits; Landscape structure; Olive grove; Pest control; Spiders.

# RESUMO

A oliveira (*Olea europaea*) é uma espécie de grande importância na bacia do Mediterrâneo. No entanto, esta cultura é afetada pelo ataque de várias pragas que podem causar perdas significativas. Entre a comunidade de artrópodes do olival, as aranhas são predadores que ocupam o nível trófico mais elevado, consumindo principalmente insetos em todos os seus instares. Por esta razão, as aranhas podem ser importantes inimigos naturais de pragas da oliveira, embora a sua abundância e eficácia possa ser influenciada por vários fatores bióticos e abióticos. Assim, a manutenção de uma paisagem agrícola heterogénea pode ser parte de uma estratégia para alcançar a sustentabilidade e promover a proteção biológica por conservação.

Os principais objetivos desta tese foram estudar os padrões de diversidade do táxon Araneae ao longo de um gradiente de práticas agrícolas no olival, estudar o efeito de infraestruturas ecológicas, como fontes de recursos, na sobrevivência e no comportamento do táxon Araneae, estudar os seus mecanismos de predação, e desenvolver meios que visam promover a sua diversidade no olival.

A influência de diferentes práticas agrícolas na estrutura da comunidade do táxon Araneae foi estudada em áreas de olival no nordeste de Portugal (ao nível do solo, tronco e copa) e em áreas semi-naturais adjacentes (mato mediterrânico). O tipo de desenho experimental, o número de pedras no solo, a percentagem de vegetação, a humidade e a gestão agrícola foram utilizados na modelação dos padrões de diversidade encontrados no olival e na sua paisagem circundante.O efeito das infraestruturas ecológicas e dos mecanismos de predação do táxon Araneae foram estudados, com recurso a ensaios laboratoriais, utilizando espécies de diferentes grupos funcionais. O efeito de diferentes itens alimentares não-presa (glicose 0,5 M, melada de afídeo, melada de cochonilha-negra; uma mistura de glicose 0,5 M, fenilalanina 0,1 mM, prolina 0,1 mM e triptofano 0,1 mM; mel a 10% e pólen a 10%) na sobrevivência e escolhas alimentares, assim como a resposta funcional e preferência por distintos tipos de presa foram estudadas em aranhas juvenis e adultas respetivamente. Além

#### <u>Resumo</u>

disso, foram desenvolvidas duas ferramentas de simulação: um pacote de funções em R (simaR), que simula a resposta funcional de um predador, e um modelo baseado em indivíduos (EcoPred), que simula uma cascata trófica de uma praga modelo controlada pela população de dois tipos de predadores.

Nos olivais em estudo, foram identificados nove grupos funcionais do táxon Araneae: emboscadores, caçadores nas folhas, caçadores no solo, construtores de teia orbicular, construtores de teias laminares, construtores de teias de deteção, construtores de teias espaciais, acossadores e construtores itinerantes de teias laminares/emaranhadas. As comunidades diferiram significativamente quanto à composição ao longo dos gradientes horizontal e vertical. *Philodromus lividus* e *Thanatus vulgaris* foram as espécies mais abundantes na copa e no solo respetivamente, e a subfamília Erigoninae foi o grupo dominante no tronco. As áreas de mato adjacente foram dominadas por *Nomisia exornata*. A diversidade global de aranhas e a abundância de indivíduos juvenis e de espécimes caçadores no solo foi significativamente maior em áreas com um maior número de pedras.

A sobrevivência global de juvenis de *Haplodrassus rufipes* (caçadores no solo) e de *Synema globosum* (emboscadores), alimentados com itens não-presa, aumentou significativamente em comparação com indivíduos alimentados com água. Em ensaios alimentares de múltipla-escolha, *Haplodrassus rufipes* selecionou maioritariamente o mel e apresentou uma taxa de exploração significativamente maior do que *S. globosum*, que selecionou o tratamento de mistura. Os itens alimentares mais escolhidos corresponderam àqueles que proporcionavam maiores longevidades em ambas as espécies. Os adultos de *H. rufipes* e *S. globosum* mostraram ser, respetivamente, os mais e menos eficientes quanto à mortalidade infligida em mosca-do-Mediterrâneo, *Ceratitis capitata*. A taxa de ataque de *S. globosum* e de *Araniella cucurbitina* (construtores de teia orbicular) aumentou significativamente em ensaios de resposta funcional de múltipla-escolha quando comparada com ensaios com uma única espécie de presa. Considerando a preferência por tipologias de presas, verificou-se que as presas mais pesadas, de tamanho maior e altamente móveis (p.e., *Acheta domesticus*), eram evitadas pelos três grupos funcionais de aranhas, enquanto que as

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presas mais leves, de menor tamanho e moderadamente móveis (p.e., *C. capitata*) eram preferidas.

O efeito da temperatura na taxa de ataque de dois grupos funcionais de aranhas contra *C. capitata* foi avaliado usando o simaR. Verificou-se que a taxa máxima de ataque de *A. cucurbitina* em *C. capitata* diminuiu significativamente com o aumentou da temperatura, contrariamente ao ocorrido para *S. globosum*. O modelo EcoPred foi utilizado para ensinar, a 26 estudantes do ensino secundário, os diferentes aspetos relacionados com a proteção biológica. Depois de testarem o modelo, os estudantes mostraram muito interesse neste método de aprendizagem.

Em conclusão, cada grupo funcional de aranhas inclui espécies predadoras de pragas sendo que a sua ação varia de acordo com as estratégias de caça e os nichos ecológicos explorados. Os vários aspetos relacionados com a gestão agrícola, tais como a presença de matos adjacentes, a conservação de micro-habitats no solo e a manutenção de faixas de plantas espontâneas, poderiam promover o aumento do número de refúgios e proporcionar recursos alimentares suplementares às aranhas, desempenhando um papel importante na proteção biológica por conservação contra as pragas da oliveira.

**Palavras-chave**: Aranhas; Características funcionais; Controlo de pragas; Estrutura da paisagem; Olival.

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#### **OBJECTIVES AND THESIS OUTLINE**

Among the community of arthropods of the olive grove, Araneae are predators occupying the highest trophic level, hunting mainly insects in all their instars. For this reason, they can be useful natural control agents of olive pests, but several factors affect their abundance and effectiveness in the agroecosystem. Among those factors, the intensification of farming practices (use of pesticides, tillage and fertilization) are associated with the loss of biodiversity which is not compatible with sustainable production systems. In this sense, the main aim of this thesis is to understand the factors that influence the community composition and structure of Araneae in the olive grove by integrating variables such as olive grove management, the surrounding landscape, the local variables and species interactions. To accomplish this aim, this thesis is divided into eight chapters following five sections:

#### **1.** Review of the literature.

The objective of this section is to review the state of the art regarding the role of spiders in agroecosystems. This is addressed at different points of view, from the natural history of the order Araneae to the effects of the most common agricultural practices on the biodiversity and life-history parameters of spiders at different landscape spatial scales. Also, a review on the effects of spider venoms as bioinsecticides, and an overview on the current knowledge on spiders in the olive grove agroecosystem are provided. This section includes one chapter:

Chapter 1: General introduction: Biodiversity of spiders in agroecosystems, from community structure to conservation biological control of pests

Objectives and thesis outline

# 2. Study of the diversity patterns of Araneae communities throughout an increasing gradient of olive grove farming practices and different landscape spatial patterns.

In this section, selected olive groves and their surrounding landscape were considered to study diversity patterns of Araneae communities in Trás-os-Montes (Portugal). Organic groves and integrated production groves were selected corresponding to different farming practices and soil properties. Different variables (e.g. management, soil properties and surrounding semi-natural areas) were used to understand the Araneae community composition (e.g. abundance, species richness and functional diversity) across the farming practices and landscape spatial patterns. This section includes two chapters:

Chapter 2: Distribution of the spider community in the olive grove agroecosystem (Portugal): potential bioindicators.

Chapter 3: Stones on the ground in olive groves promote the presence of spiders (Araneae).

3. Study of the effect of ecological infrastructures (non-crop plants), as sources of supplementary resources (e.g. nectar, pollen and honeydew) on the survival and behavior of selected species of Araneae and understand how they contribute to the conservation of Araneae in the olive grove.

In this section, two sets of experiments were conceived to obtain information about the need for supplementary resources by Araneae as well as the type of resource required. Two Araneae species, *Haplodrassus rufipes* (Lucas, 1846) and *Synema globosum* (Fabricius, 1775), were selected due to their relevance in the olive grove as well as in other Mediterranean agroecosystems.

Non-choice experiments: Colonies of *H. rufipes* and *S. globosum* were fed on non-prey food items (pollen extracted from non-crop plants, honey, yeast, sucrose, glucose or fructose, and honeydews obtained from two pests, the black-scale, *Saissetia oleae* (Olivier,

1791), and the aphid, *Aphis craccae* Linnaeus, 1758. The effect of those non-prey food resources was tested on the survival of the spiders.

Multiple choice experiments: different choice tests were undertaken using both spider species and the above mentioned resources were supplied in the same arena and the preference for a particular food item was measured using video tracking software. The results obtained with the different resources were compared in order to evaluate how the maintenance of ecological infrastructures in the olive grove might alter the abundance of the spider species by retaining them in the crop and by improving their survival and development.

This section includes one chapter:

Chapter 4: Spiders actively choose and feed on nutritious non-prey food resources.

# 4. Study of the foraging mechanisms of Araneae and understand their role as potential predators of olive pests.

In this section, laboratory experiments were carried out to study the potential of selected species (*H. rufipes*, *S. globosum* and *Araniella cucurbitina* (Clerck, 1757)) belonging to different guilds of spiders as predators of the main olive pest, the olive fruit fly *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae). The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann, 1824) was used as model for the olive pests.

Two types of experiments were developed. (1) The functional response of spider species was assessed when feed on adults of *C. capitata*. In this case the functional response of the three spider species was measured using different densities of prey and the type of functional responses was determined. (2) The food preference was studied by feeding the spiders on alternative preys (the house cricket, *Acheta domesticus* (Linnaeus, 1758), the and the Mediterranean flour moth *Ephestia kuehniella* Zeller, 1879 and the food preference was

#### Objectives and thesis outline

linked to the functional traits of preys by using video tracking software and direct measures on the body parameters of preys.

This section includes one chapter:

Chapter 5: Functional responses of three guilds of spiders: comparing single and multiprey approaches.

# 5. Integration of results and development of tools aiming at promoting the diversity of Araneae in the olive grove agroecosystem.

Results obtained in the previous parts were integrated in order to promote the best management practices in the groves and oriented to the provisioning of biological control of pests by conserving or enhancing the diversity of Araneae. With this aim, knowledge transfer was approached at three target audiences:

- researchers, with one chapter (Chapter 6) providing a simulation-based method to assess the pest suppression potential of predators;

- students, with one chapter (Chapter 7) providing an Individual Based Model to explain biological control;

- general public, with the last chapter (Chapter 8) providing a conceptual overview that summarizes the results found along this thesis.

Chapter 6: A simulation-based method to compare the pest suppression potential of predators: A case study with spiders.

Chapter 7: *EcoPred: an educational Individual Based Model to explain biological control, a case study within an arable land.* 

Chapter 8: General conclusions, conceptual overview and future perspectives.

# **CHAPTER 1**

General introduction: Biodiversity of spiders in agroecosystems, from community structure to conservation biological control of pests

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# **CHAPTER 1**

General introduction: Biodiversity of spiders in agroecosystems, from community structure to conservation biological control of pests

#### Abstract

Spiders are one of the most abundant and diverse groups of predators in agroecosystems. They hunt mainly insects in all of their instars and, for this reason, together with their ubiquity and high diversity of hunting strategies, they can be successful biological control agents of pests. Although, agricultural management practices such as the use of pesticides, tillage or fertilization, landscape simplification and intensive farming may alter the diversity of spiders and their effectiveness in the agroecosystem. On the other hand, within the agroecosystem, ecological infrastructures as vegetation strips, small bushes and stonewalls can provide shelter and alternative prey that can be exploited by a range of functional groups of spiders. Aspects such as the conservation of a heterogeneous agricultural landscape as well as the maintenance of ecological infrastructures can be seen as part of a strategy to achieve sustainability and promote conservation biological control. This chapter provides an overview of the effects of the most common agricultural practices on the biodiversity and life-history parameters of spiders using complementary approaches, i.e. traditional taxonomy and functional groups, and how these pressures may change the ecological services provided by spiders. A case study regarding the community of spiders associated with the olive grove agroecosystem is described.

#### **1.1. Introduction**

Arachnids already existed in the Carboniferous, between 280 and 350 million years ago (Hubert, 1979). Currently, 45.881 species of spiders (Araneae order) distributed in 3.981 genera and 114 families are described worldwide (World Spider Catalog, 2018) and, together with Amblypygi, Holothyrida, Ixodida, Mesostigmata, Opilioacarida, Opiliones, Palpigradi, Pseudoscorpiones, Ricinulei, Scarcoptiformes, Schizomida, Scorpiones, Solifugae, Thelyphonida and Trombidiformes, they are one of the 16 arthropod orders that form the class Arachnida (Zhang, 2011). Araneae order is divided into two major suborders, Mesothelae and Opisthothelae. The suborder Mesothelae includes primitive spider families characterized by the presence of segments on the opisthosoma. The suborder Opisthothelae comprises the infraorders Mygalomorphae (commonly known as tarantulas) and Araneomorphae (the vast majority of described species including jumping, wolf, orb-weaving spiders, etc.) (Dunlop & Penney, 2011; Bond et al., 2014). The infraorder Mygalomorphae includes spiders with a morphological character that separate them from the Araneomorphae, the opening direction of the chelicerae, which are almost parallel to the cephalocaudal axis in Mygalomorphae and crossed in Araneomorphae (Figure 1.1) (Barrientos, 1988).

Spiders represent one of the most diverse groups of invertebrates and are distributed worldwide, virtually adapted to any type of habitat where other arthropods, the basic component of their diet, are present. They exist in the northernmost Arctic islands, in the hottest and driest deserts, in high altitudes, in the depths of caves, in the intertidal zone of the coasts, ponds and marshes, in arid moors, sand dunes, and floodplains. Also, spiders have invaded the aquatic environment and successfully compete in shallow water systems communities (Turnbull, 1973).

The Liphistiomorpha group is restricted to the Southeast of Asia, China and Japan (World Spider Catalog, 2018). Theraphosomorpha is characteristic of tropical and subtropical areas, however, it extends to colder areas both to the north and south and considering their

extreme diversity can be expected that Araneomorpha occupy all kinds of habitats (Turnbull, 1973).



**Figure 1.1.** Opening direction of the chelicerae of spiders; (A) almost parallel to the cephalocaudal axis in Mygalomorphae and (B) crossed in Araneomorphae. Dashed lines follow the cephalocaudal axis. Illustration by J. Benhadi-Marín.

Spiders are generalist predators, which hunt mainly insects of a wide range of sizes and are considered as potential natural enemies of pests. They also exploit non-prey foods such as pollen and nectar (e.g., Nyffeler et al., 2016) and there is an increasing interest in understanding how alternative food sources affect the community of spiders in order to implement ecological infrastructures (e.g. wildflower strips or ground covers) in the agroecosystem that will provide such resources aiming at maintaining and enhancing the abundance of spiders within the crop.

In general, spider-prey interactions have been documented through direct observations of spiders hunting and feeding on prey, morphological measurements, serological and molecular gut-content analysis and studies about the feeding behavior and functional responses of spiders fed on different types of prey (e.g. Nakamura & Nakamura, 1977; Nentwig & Wissel, 1986; Sunderland et al., 1986; Samu & Bíró, 1993; Morris et al., 1999; Greenstone & Shufran, 2003; Ghavami, 2008; Harwood et al., 2009; Monzó et al., 2009;

Oelbermann & Scheu, 2009; Nyffeler et al., 2016). However, crop management such as spraying of pesticides and tillage, a common practice used to control weeds and conserve soil water (Martins et al., 2005) can influence the efficiency of spiders as biological control agents.

This chapter aims at providing an overview of the effects of the most common agricultural management practices on the biodiversity and life-history parameters of spiders using two complementary approaches: (1) how discrete species (i.e., the traditional taxonomy point of view) and (2) functional groups (focused on different groups of species using the same resource in a similar way) respond to environmental changes caused by market pressures within the agricultural landscape context at different habitat scales. Also, the emergent interest on the use of spider-venom based products as bioinsecticides is commented and a case study on the diversity of spiders and performance in olive groves is provided.

#### 1.2. Morphology of spiders

Spiders have the body divided in two parts, prosoma or carapace (anterior) and opisthosoma or abdomen (posterior) (Figure 1.2A). Prosoma and opisthosoma are joined by a pedicel corresponding to the first segment of the abdomen (Figure 1.2B) (Nieto & Mier, 1985; Barrientos, 1988; Foelix, 2011; Brusca & Brusca, 2005).

Within the prosoma two regions are distinguished, an anterior (cephalic) containing structures related to the senses and food and a posterior (thoracic) related with locomotion and housing the central nervous system (Foelix, 2011). Most of the external appendages on the spider are attached to the cephalothorax, including eight legs, six to eight eyes (exceptionally none in some troglophile spiders), chelicerae, other mouthparts, and palps. The first pair of appendages on the prosoma is formed by the chelicerae, each one divided into a basal segment and a fang (Figure 1.3). On the apical part of each fang ends the duct that connects the fang with the venom glands, which are located on both sides of the cephalic region of the prosoma.



**Figure 1.2.** External anatomy of a spider: (A) dorsal view and (B) lateral view. P: prosoma; O: opisthosoma; l: legs; pa: palps; c: cephalic area; t: thoracic area; s: spinnerets; e: eyes; q: chelicerae; b: book lungs opening, p: pedicel. Illustration by J. Benhadi-Marín.



**Figure 1.3.** Cephalic region of a spider (simplified) in ventral view. f: cheliceral fang; b: basal cheliceral segment; q: chelicerae; m: maxilla; pC: palpal coxa; pt: palpal trochanter; pf: palpal femur, l: labium. Illustration by J. Benhadi-Marín.

The second pair of appendages corresponds to the palps, made up by six segments (i.e., coxa, trochanter, femur, patella, tibia and tarsus) (Figure 1.4A). Legs have the same parts plus the metatarsus between the tibia and the tarsus (Figure 1.4B). The esophagus, as well as the stomach is also located in this division (Nieto & Mier, 1985; Barrientos, 1988; Foelix, 2011; Brusca & Brusca, 2005).



**Figure 1.4.** Detail of a spider (A) palp and (B) leg segmentation. c: coxa (not shown for the palp); t: trochanter; f: femur; p: patella; ti: tibia; m: metatarsus; ta: tarsus. Illustration by J. Benhadi-Marín.

On the anterior-ventral part of the opisthosoma are the genital openings and the book-lung spiracles openings under the epigastric furrow. The heart, testes or ovaries, midgut, hindgut and spinning glands are also housed in the opisthosoma. Normally three pairs of spinnerets are present on the body posterior end.

Finally, the hairs occurring on the body of the spider are sensory organs that response to mechanical or chemical stimulus. Most of these sensilla are movable, articulated setae,

bristles and trichobothria, which are mechanoreceptors located on the legs (Barrientos, 1988; Foelix, 2011).

#### 1.3. Development and growth of spiders

As arthropods, the development of spiders has different stages from the egg (Figure 1.5) to the adult stage. At the end of each growing period, the entire cuticle is substituted by a new one through molting (Figure 1.6). In spiders, the number of molts varies among groups. European species (depending on the size) molt between five and ten times before reaching the adult stage (Roberts, 1996). During the molting period, the spider is more susceptible to die due to dehydration, failures along the molting process, or predation. Once individuals reach the last juvenile stage, they present the appearance of an adult, but still lack the reproductive structures (Turnbull, 1973; Foelix, 2011).



**Figure 1.5.** Development of an egg of spider. (A) Early egg stage; (B) Tissues and organs development; (C) Prosoma and opisthosoma regions can be distinguished; (D) Spiderling early hatching stage; (E) Final hatching stage of the spiderling; (F) Spiderling completely hatched. In B, C and D, the arrows indicate different developmental stages of the leg. In E the arrow indicates the remains of the egg shell. Photos by: J. Benhadi-Marín.



**Figure 1.6.** Molting of a tarantula (Mygalomorphae). After removing the whole old cuticle (A), the spider stands still (B) until the new cuticle hardens (C) and the spider continues with its activity (D). The arrow in A indicates the old carapace cuticle. Photos by J. Benhadi-Marín.

In general, the life cycle of a spider is completed during one year, although it can vary from a few months to several years, depending on the species, the environmental conditions and the occupation of the habitat. Depending on when spiders reach sexual maturity, two types of cycles can be observed: (1) those spiders that reach sexual maturity in spring and reproduce during the summer, spiderlings will be in that stage during the winter, and (2) those spiders that mature during the fall and overwinter as adults (Cárdenas, 2008).

#### **1.4. Reproduction of spiders**

Spiders reproduce sexually, however, males and females can present different levels of sexual dimorphism, very pronounced in the case of Thomisidae, whose males are much smaller and graceful than females (Morse, 2007) and an extreme case can be observed in *Nephila* Leach, 1815 (Nephilidae) species (e.g., Kuntner & Coddington, 2009). Male reach maturity after the last molt, when palps appear completely modified (Figure 1.7) (Foelix, 2011). Mature male palp is a kind of hypodermic noodle that will be used to transfer, by injection, the sperm into the genital ducts of females.



**Figure 1.7.** Morphological differences between an immature male spider palp (A) and a mature palp (B). t: tarsus; ti: tibia; p: patella; f: femur; tr: trochanter; c: coxa; c: cimbium; ce: conductor and embolus (the later protected by the former); ta: tibial apophysis. Illustration by J. Benhadi-Marín.

In general, females show relatively sedentary habits and males are responsible for finding females attracted by sex pheromones (Turnbull, 1973; Foelix, 2011). This risky action can end in several ways, (1) the male dies before finding a female, (2) the male finds a female

but the latter is not mature or (3) the female does not find the male attractive. In the two last cases, the male may be seen as a prey by the female and can be attacked and consumed (Turnbull, 1973; Foelix, 2011).

Males of a great number of species can have complex courtships in order to minimize the risk of being eaten. Courtships seem to fulfill three functions: (1) to allow the male to assess if female is ready for mating from a safe distance, (2) to stimulate the female and put it into a receptive state and (3) to allow the female to discriminate different males (Platnick, 1971; Turnbull, 1973). If the male is accepted by the female, then mating occurs (Figure 1.8).



**Figure 1.8.** Mating in *Haplodrassus rufipes* (Gnaphosidae). F: female and M: male. Photo by J. Benhadi-Marín.

The level of egg care varies between groups but, in general, the female lays its eggs in a sac-like structures made of silk (Figure 1.9A). These cocoons can contain from one to hundreds of eggs and can be more or less elaborated using different types of silk, coated substrate fragments (Figure 1.9B), and in some cases transported under the abdomen fixed by the spinnerets (e.g. Lycosidae). During the life of a female, the first cocoon usually

contains the highest number of eggs that gradually decrease in the subsequent ones (Turnbull, 1973).



**Figure 1.9.** Female of *Haplodrassus rufipes* (Gnaphosidae) laying its eggs on the silken basal surface of the cocoon (A) and covering the eggs with the upper layer (B). The arrow in B indicates another cocoon covered with substrate fragments. Photos by J. Benhadi-Marín.

#### 1.5. Mobility and dispersion of spiders

When spiderlings emerge from the cocoon and are ready to search for food (Figure 1.10), the newly independent juveniles commonly use a mechanism known as *ballooning* to disperse. The spider searches for a high place and puts the abdomen pointing upwards; then it produces silk until an airstream lifts it and carries it to another place. This process is very useful and is also used by some adults, although there is a high probability of landing on inappropriate places. On the other hand, this process allows individuals to go over physical obstacles that otherwise would not be possible. Often, spiders also build silk bridges between close obstacles to move (Bristowe, 1958; Turnbull, 1973).

Silk is a resource commonly used by web building spider species or groups that are not good runner (due to their morphology), but wandering spiders have locomotor appendages adapted for running long distances and climb easily (Turnbull, 1973).



**Figure 1.10.** Spiderlings emerging from a cocoon (indicated by the arrow) of *Haplodrassus rufipes* (Gnaphosidae). Photo by J. Benhadi-Marín.

A peculiar aspect of the movement is the production of a security thread in response to a stimulus mediated by a danger. Even from great heights, an individual can jump off quickly by fixing one end of the silk fiber to the substrate and sliding down in order to escape. Moreover the spider does not cut this security thread and can climb it back to its original position (Morse, 2007).

### 1.6. Mortality of spiders

The number of eggs laid during the life of a female far exceeds the need to replace parents, so it is easy to assume that there is a high rate of mortality in juvenile stages. The magnitude of mortality is determined by factors such as predation, cannibalism or lack of a suitable place that meets the nutritional needs of spiderlings once the nest or the mother is abandoned. Even in adulthood, spiders have many natural enemies such as parasites,

specialist parasitoids and predators including birds, rodents and reptiles, but simply facing the winter can increase the mortality (Bristowe, 1958; Turnbull, 1973).

### 1.7. Spiders as predators

#### 1.7.1. Community structure

The assemblages of spiders have been seen of limited interest if regarded as a community because they represent only a node within a complex system (Wise, 1993). Moreover, biodiversity is an ambiguous concept without a universal measure or unit and cannot be considered a single attribute to assess it (Moreno, 2001). Hence the first and more intuitive aspect to be exploited is probably the distribution of the abundance of species along time and space. This can help to uncover possible trophic relationships with other species of the community but even this way may represent a hard way to go due to the high diversity of spiders. Instead, a reductionist approach in order to understand how spiders play their roles as predators is often more manageable and practical using groups of species with similar characteristics (guilds) thus helping to generalize about the role of spiders in the real whole community (Wise, 1993). The functional approach and the guild concept will be focused later in this chapter.

In general, the numerical relationships between the abundance of species in a community seems to follow a pattern, usually some species are much more abundant than the rest, a greater number of species present intermediate abundances and finally, a higher number of species are usually represented by few individuals. These three groups could be called dominant, influential and accessory species respectively (Luczak, 1960, 1963) and a similar classification can be extended to spiders inhabiting the agroecosystem, calling 'agrobiont' spiders to those that dominate the community and 'agrophile' to the other ones (Samu & Szinetár, 2002). Dominant species represent 50% of abundance in the whole community, as influential those with 15-25% of abundance and the rest as accessory. However, each community must be studied and quantified individually in order to establish the threshold between categories (Luczak, 1963). These relationships between the abundance of groups

were described and related thorough mathematical models in the Fisher logarithmic series and appear in a large number of communities (Fisher et al., 1943).

In terms of body size, the log-normal function usually represents a community considering the number of species and the body size (see Růžička, 1990). Body size is a useful parameter for investigating niche use and fitness due to its correlation with body length, a measure relatively easy to obtain. Size can be measured as wet or dry weight and Sage (1982) proposed a simple regression model to calculate the weight of spiders from a body size ranging between 3.0 and 35.4 mm:

$$\ln Y = a + bX + b^1 X^1$$

were Y is the weight (g), X is the body length (mm) and a, b and  $b^1$  are the regression coefficients, -6.504, 0.45919, -6.8653 x  $10^{-3}$  and -7.849, 0.49335 and 8.0448x $10^{-3}$  for wet and dry weight respectively. Body weight can either reflect changes in the environment (e.g. lack of food or water) or cause a different response to environmental changes or pressures. For example, Benhadi-Marín et al. (2016a), found that body size (measured as dry weight) influenced significantly the survival probability of *Araniella cucurbitina* (Clerck, 1757) (Araneidae) when exposed to kaolin.

Růžička (1990) found that three groups of spiders could be considered in terms of body size when regarding the epigeic spider community in Czechoslovakia. The three groups were formed by spiders with about 1 mm, 1.5 mm and 3 mm of cephalothorax length respectively (Figure 1.11). The first group was represented mostly by Linyphiidae and the third one by Lycosidae. Within the agroecosystem context, Birkhofer et al. (2013) proposed three different clusters of body size based on the most common species found in central European cereal fields: <4 mm (small), 2-9 mm (small to medium) and >9 mm (large), and three hunting categories based on hunting behavior, namely: (1) ground and vegetation layer using sheet webs, (2) vegetation layer using aerial web and (3) ground and vegetation layer hunting freely.

The different groups of spiders seem to appear distributed along horizontal, vertical and temporal gradients (Luczak, 1966) as a strategy to avoid competition (Tretzel, 1955). These gradients are reflected on several hunting strategies that are different among families (Wise, 1993):



**Figure 1.11.** Relationship between species richness and body size (cephalothorax length) for an epigeic spider community. Simplified from Růžička (1990).

- The Araneidae build orbicular webs with tiny sticky droplets and usually waits for prey in the web center;

- The Tetragnathidae also make orbicular webs but close to water bodies;
- The Uloboridae weave orb webs using special cribellate silk.
- The Theridiidae make irregular tangle webs;

- The Linyphiidae use sheet webs without any sticky silk;
- The Agelenidae use a funnel-like sheet web waiting in the deep end for prey;
- The Thomisidae commonly ambush their prey on flowers, leaves and trunks;
- The Lycosidae usually roam endlessly pursuing and ambushing a prey;
- The Saticidae stalk their prey using its high visual capacity;
- The Oxyopidae run on stems and leaves of plants searching for prey;
- The Clubionidae hunt during the night commonly on leaves.

Overall, such spectrum of hunting strategies results in a reduction of competition for resources allowing the coexistence of related spider groups (Turnbull, 1973; Uetz, 1977). The distribution of the different hunting strategies along time and space make the assemblage of spiders an interesting group in pest management due to the possibility of several predators can prey the same pest.

#### 1.7.2. Hunting strategies and feeding habits

All spiders produce silk, but not all spiders use it to build webs. This feature is of major relevance and the baseline when grouping spider families regarding different hunting strategies and feeding habits. In general, two groups of spiders can be separated according to their hunting strategy: (1) those spiders with a relatively sedentary life that use silk as a hunting tool and (2) those that actively seek their prey on the substrate (Turnbull, 1973; Wise, 1993). Regarding the first group, it is important to note that a sedentary lifestyle does not exclude a high plasticity when the substrate is chosen to build the web, the prey selection and even the morphology of the web itself. In addition, the web is relatively constant at family level, variable between genera and can even vary slightly between individuals of the same species (Turnbull, 1964, 1973).

The hunting place is influenced by multiple factors such as temperature, humidity, airflows, hours of sunshine and presence or absence of an appropriate structure to build the web. Consequently, the spider can consider a particular place as appropriate and, after a period without capturing prey, it can abandon the site (Jones, 1941; Tretzel, 1955; Cherrett, 1964; Turnbull, 1973).

The potential prey that can be captured by a spider is often limited by the characteristics of the prey such as body size and shape, lifestyle, ability to detect the web, presence of intimidating colors and production of unpalatable substances that allows it to avoid webs, escape them, or be discarded by the spider (Turnbull, 1973; Nentwig & Wissel, 1986).

The web acts as an extension of the sensory system of the spider and can have three functions: (1) an alarm system against hazards, (2) an enhanced system for catching prey that can extend beyond the perception range of the spider and (3) a system that allows to place the spider in a position of advantage over the prey increasing the hunting efficiency (Turnbull, 1973). In summary, the effectiveness of web-builder spiders depends mostly on the spider species, the web design and the prey characteristics.

The second group of spiders, the active hunters, shows variable levels in their itinerant habits and several factors come into play when using their hunting strategy. Depending on the development of the eyesight, length and strength of the legs, tarsal sensory organs and ability to mimicry, different ways to find and chase a prey can be observed (Turnbull, 1973). This group of spiders exploits similar food resources but using different hunting techniques. Some species ambush their prey using its ability to mimicry (e.g., *Thomisus onustus* Walckenaer, 1805 (Thomisidae)). Others take advantage of their high visual power to chase and attack their prey directly (e.g. Lycosidae, Pisauridae and Salticidae) and others take advantage of their secondary eyes (with reverse view) and are nocturnal hunters (e.g., Anyphaenidae, Clubionidae and Gnaphosidae) (Berland, 1938; Turnbull, 1973; Foelix, 2011).

Regarding the feeding habits, spiders are generalist predators and produce poison (with some exceptions, such as the Uloboridae family), which is inoculated into their prey through the chelicerae fangs as part of hunting. Notwithstanding, this is not the only function of the chelicerae as they are also used as a tool to transport objects, hold and manipulate prey and build nests (Berland, 1938). However, the spider esophagus is not able to accept solid food, therefore spiders cover the body of its prey with digestive enzymes and it will not be ingested until the tissue has been digested. Moreover, some spiders use

the maxilla to grind the food. Once the tissues are digested, the resulting mass is ingested by suction through a pharyngeal pump (Turnbull, 1973).

# 1.7.3. Spiders as natural enemies of pests

The suppression of pests by predators has been a subject of interest for agricultural managers within sustainable farming practices. Traditionally, it has been argued that the equilibrium between predators and prey populations is essential to achieve good results for biological control (Murdoch et al., 1985) especially from a reductionist point of view where a single predator or a single prey species are considered (Riechert, 1999). Also, damage thresholds are frequently exceeded despite spider predation (Bishop & Blood, 1981). This suggest that spider communities are unable to control pests, however, agroecosystems are first of all 'systems' (often complex systems) and great efforts have been made in order to model the role of spiders predation in agroecosystems (e.g. Chatterjee et al., 2009). Mathematics rarely captures the whole dynamics between all components of the network thus doing unreliable or even unpredictable the result of the interactions between its elements. In addition, biased measures of species community composition and predation rates may lead to underestimate the potential of spiders as natural enemies.

Successful examples involving relatively simple systems (e.g. habitat manipulation and laboratory experiments) of biological control provided by spiders are mentioned in literature. For example, considering the economic relevance of wheat, several authors studied the effect of spiders against its pests, mostly focusing on aphids. Chiverton (1986) found that linyphid spiders together with other predators such as carabids and staphilynids (Coleoptera) reduced the population of *Rhopalosiphum padi* (Linnaeus, 1758) (Hemiptera: Aphididae) in spring barley in Sweden. Kuusk et al. (2008) found that *Pardosa* C. L. Koch, 1847 spiders (Lycosidae) also fed on *R. padi* through DNA tracking using PCR and Oelbermann & Scheu (2009) also demonstrated the benefits of the presence of *Pardosa* in wheat, both in terms of aphid predation and plant performance. More recently, Chapman et al. (2013) provided a molecular framework to study the feeding habits of an epigeal spider community in a winter wheat agroecosystem.

Regarding other crops, Ghavami (2008) suggested that the salticid species *Thyene imperialis* (Rossi, 1846) could be a potential natural enemy of key cotton pests in Iran in laboratory assays. Also, Shivakumar & Kumar (2010) indicated lynx spiders (Salticidae) as potential natural enemies of *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) and Wyss et al. (1995) observed that web-building spiders, such as *Araniella* (Araneidae), contributed to aphid control in apple orchards in Switzerland. László et al. (2015) estimated that 24% of spider prey were pests in apple orchards and Monzó et al. (2010) used DNA markers of *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) to show how *Pardosa cribata* Simon, 1876 (Lycosidae) played an important role in biological control predating this harmful fruit pest in citrus orchards in Spain.

Spiders have been shown to play an important role helping to reduce pest abundance peaks, maintaining their populations at constant levels rather than reacting to pest outbreaks (Riechert & Lockley, 1984). In situations of prey population peaks, spiders show a more selective behavior choosing their food and this, added to the fact that both spiders and prey occupy different strata (vertical, horizontal and temporal) in the agricultural ecosystem, may lead to the existence of a certain degree of specialization in response to these microhabitats (Maloney et al., 2003).

Prey selection behavior is not an expected characteristic in an effective natural enemy; however, pest density seems to be an important factor driving the potential killing capabilities of spiders. In fact, spiders usually kill more prey than necessary when sufficient food is available. This behavior is known as wasteful killing or superfluous killing (Sunderland, 1999) and implies a clear advantage of the use of spiders in agroecosystems as natural enemies. Samu & Bíró (1993) described this effect using cursorial spiders (Lycosidae) as model organism and observed both partial consumption and non-feeding on killed flies at high prey densities. Also, Riechert & Maupin (1998) proved that different web-builders also exhibited this behavior suggesting greater feeding efficiency in partial feeding. Therefore, different functional groups, probably occupying different strata within the agroecosystem, are able to kill significant amounts of insects especially at high densities. Since the rate of food acquisition directly affects growth rate, molting and size at

maturity (Uetz, 1992), it is supposed that spiders will prefer a prey-rich habitat and will move to it if they can expect to capture more prey than required to survive and reproduce (Caraco & Gillespie, 1986).

Although the physiological mechanism underlying superfluous killing in spiders has not been explained yet, the consequences for prey population are clearly relevant. Several examples on how different pest populations respond to spider predation can be found on comprehensive reviews done by Greenstone (1999), Riechert (1999), Sunderland (1999) and Maloney et al. (2003). Despite the high amount of available reports on the role of spiders acting as natural enemies, pest suppression strategies based on spiders have been rarely implemented. One possible reason is the development of a complex integrated protection strategy that involves social, cultural and political interests (see Ehler, 1998) using a tool that implies high complexity itself, i.e. the assemblage of spiders.

#### 1.7.4. The functional approach: what, why and how

Beyond the role and identity of a single or a group of species in an ecosystem and its/their responses to environmental changes, groups of similar species and characteristics of individuals are currently in the spotlight within the so called functional ecology. This approach to ecosystem services, species roles and dynamics in nature is based on "functional traits".

Functional traits can be seen as key characteristics by which single species and groups of species influence ecosystem properties (de Bello et al., 2010) and are defined as a feature of an organism, which has demonstrable links to the organism's function (Lavorel et al., 1997). Consequently, "functional groups" of species (usually known as "guilds", see Root (1973)) can be defined as a assemblage of organisms with similar functional trait attributes (Harrington et al., 2010), or in other words, species using the same class of resources in a behaviorally similar way (Simberloff & Dayan, 1991).

Among animals, spiders have been suggested as good candidates for ecological studies thorough a functional approach and appropriate for all kinds of biomes and habitats (Cardoso et al., 2011). A first advantage of this framework is related to taxonomy itself. Both experienced and novice researchers have faced the hard way to deal with spider species identification. Currently, there is a lot of taxonomic literature as well as great online tools and comprehensive databases concerning spiders diversity and taxonomy. However, accuracy on species-level identification involves careful and time consuming genitalic analysis and remains impossible in the case of immature specimens. The common way to solve this situation is to turn to an expert in spider taxonomy but obviously this is not always possible (see Young & Edwards, 1990).

Nevertheless, using higher taxonomic ranks, such as family, as the baseline to construct functional groups (assuming that species perform in a similar way) can greatly facilitate the task. On the other hand, different species may play the same role in the ecosystem, overlapping in phenology, size and prey taken e.g., two big orb-web spiders *Argiope aurantia* Lucas 1833 and *Argiope trifasciata* (Forskål, 1775) (Araneidae) coexist and exploit the same resources (Enders, 1974) and, in such case, the effect of spiders on prey population should be considered as a whole, and here is why a functional approach becomes especially useful for biological control.

Defining reliable functional groups is, however, hard due to the high diversity of these arthropods and some problems need to be addressed. Firstly, the behavior of an extremely high percentage of spiders remains currently unknown and it is commonly assumed as being homogeneous among the member of the same family or genus (which may not be real). Secondly, many spider guilds are usually based on the foraging strategy. But, different strategies may target similar prey and similar strategies may be directed towards different prey (Cardoso et al., 2011); in addition, as generalist predators, knowledge about spiders prey range and preferences is scarce. Thirdly, functional traits commonly used such as body size, color and phenology can be extremely variable even within populations and follow interannual variation, thus becoming uninformative.

Hence, Cardoso et al. (2011) suggested four criteria that need to be considered in order to approach the challenging goal of defining proper functional groups according to the

hypotheses to be tested in a study: (1) the objectives of the study, (2) the spatial scale of the study, (3) the taxonomic scale and (4) data available. Summarizing, the defined guilds should reflect the natural history of its members but a certain degree of laxity has also to be assumed (Uetz et al., 1999).

The guild concept in spiders has been widely investigated, developed and used to test the effect of environmental changes on spider assemblages. Balogh & Loksa (1948) considered three groups of spiders namely, web spinners, cursorial and saltatorial spiders; however the most basic and largely used division corresponds to the two extremes of hunting strategies identified by Pianka (1966) and Schoener (1971), i.e. "sit-and-wait" and "mobile" predators. Uetz (1977) defined these two groups as 'web-builders' and 'wanderers respectively' and Post & Riechert (1977) split them into 11 groups according to the web structure and circadian activity.

Since the study of predation on insects is commonly directed toward pest suppression, several works addressed the issue of the role and composition of the guild of spiders in agroecosystems. Nyffeler (1982) considered three guilds considering the role of spiders as insect predators in abandoned grasslands, meadows, and cereal fields. Riechert & Lockley (1984) defended the potential of spiders as natural enemies on a review dealing with eight guilds mentioned along literature and Young & Edwards (1990) distributed the spiders of North America between five guilds extracted from Gertsch (1979) and Comstock (1940). This lack of homogeneity between studies was somewhat solved by Uetz et al. (1999) in a classic work where information concerning spiders commonly found in agroecosystems was hierarchically clustered and eight new functional groups (foliage runners, running spiders, ground runners, stalkers, ambushers, sheet web-builders, wandering sheet/tangle-weavers, orb-weavers and space web-builders) were described based on earlier assignments providing a framework for subsequent studies.

Unfortunately, the problem of the diversity of spiders all around the world still remained and, more recently, Cardoso et al. (2011) undertook the task at a global scale of redefining eight new guilds (sensing, sheet, space and orb web weavers and specialist, ambush, ground and other hunters) that encompassed the whole diversity of spiders at the family level. Thus, it was developed a powerful tool allowing an easy application of the guild concept thorough few functional groups that turns available the comparison between studies of different regions.

The functional approach is especially useful in agricultural research when morphological, physiological, and behavioral traits are regarded as surrogates for ecological functions. Moreover, regarding spiders as generalist predators it becomes an important tool helping to test hypotheses involving niche overlap, intra-guild competition, prey selection, functional responses, etc., and their consequences on the role and effectiveness of spiders as natural enemies.

# **1.8.** Effects of the most common agricultural practices on the biodiversity and lifehistory parameters of spiders

Spiders are one of the most abundant predators in agroecosystems. For example, in Europe, spider overall mean density have been estimated in  $\approx 80$  spiders/m<sup>2</sup> (Nyffeler & Sunderland, 2003). Basic functional groups (i.e. web-builders and active hunters) are represented in agroecosystems and in all vertical strata (Nyffeler & Sunderland, 2003). Agroecosystems seem to sustain characteristic spider communities and dynamics. Agrobiont spiders should be indicators of arable habitats and rare in other habitat types and may be able to synchronize their lifecycle with the crop-growing season. In northern temperate Europe, these key species are mostly represented by Linyphiidae whereas in USA the families Tetragnathidae, Araneidae, Linyphiidae, Theridiidae, and Dictynidae are the most representative web-builders and Oxyopidae, Salticidae, Clubionidae, Thomisidae and Lycosidae dominate within the active hunter guild (Nyffeler & Sunderland, 2003).

Land use intensification can greatly reduce species richness and ecosystem functioning (Flynn et al., 2009). Management practices such as tillage, vegetation cutting, grazing and application of herbicides, insecticides and fertilizers may alter the community of spiders and thus, decrease its effectiveness as natural enemies. On the other hand, biotic (e.g.

vegetation and landscape complexity, plant richness, coverage and density) and physic/structural characteristics (e.g. water bodies, vegetation strips, stone density and stonewalls) can benefit the spider assemblage by providing alternative food and shelter and may help to reduce intra-guild competition. Hereafter the effects of the most common agricultural practices on the population of spiders are analyzed in the scope of biological control methods.

#### 1.8.1. Soil management

The assemblage of spiders is vertically stratified in arable lands, in particular, in perennial crops. Hence an important part of the community inhabits the soil and is confined to it. Like other natural enemies, spiders rarely climb up the tree pursuing pests (see Sunderland et al., 1997). Accordingly, any agricultural practice involving soil mechanical management can affect directly the edaphic diversity of spiders by removing microhabitats provided, for example, by stones that are used by ground dwelling spiders (e.g. Gnaphosidae) as shelter or zone for hunting or reproduction (Figure 1.12).

Practices such as tilling, weed control with herbicides or cutting can result in the reduction of the population of spiders (Thorbek & Bilde, 2004) and prey availability (Butt & Sherawat, 2012, see also Dicks et al., 2013). For example, Blumberg & Crossley (1983) registered similar percentages of individuals and species represented by spiders in nontilled and old-fields whereas a substantially lower proportion was found in tilled sorghum fields; Holland & Reynolds (2003) found that the populations of Linyphiidae and Lycosidae were significantly reduced in ploughed strips in a mixed farm. Moreover, Benhadi-Marín et al. (2013) found that the reduction or absence of a suitable habitat for spiders under the trees in tilled chestnut crops might have forced spiders to migrate into the canopy and suggested that when soil cover is removed by tillage or frequently disturbed by grazing, spiders may climb the trunks and search for a new habitat or food.

Less disturbed systems favor a rich soil biota that can improve nutrient cycling and plant health (Zehnder et al., 2007) and often provide a diversification of interspersed microhabitats (Samu et al., 1999) thus, a reduction of mechanical disturbances will increase the
diversity of spiders in agroecosystems. However, conservation tillage systems may actually suffer from recurring pests, lower yields, and weed problems, but the use of novel habitat manipulation techniques such as the allocation of modular artificial shelters for natural enemies can be a successful strategy in order to mitigate the effects of tillage (Halaj et al., 2000).



**Figure 1.12.** Influence of the number and size of stones on a ground community of spiders in olive groves in north-eastern Portugal. The total number of adults is not affected (A), whereas the number of juveniles significantly increase with the number of stones. Squares correspond to smaller stones (0 - 10 cm) and triangles to bigger stones (> 10 cm). The size of stones was measured along their longer axis and the number of stones counted in 1 m<sup>2</sup>. Benhadi-Marín et al. (data not published).

Herbicides are one of the most applied pesticides in agroecosystems in order to control weeds. Hence, several studies have been carried out trying to assess the effect of these formulations on the community of natural enemies (e.g. Geiger et al., 2011). The response of different functional groups such as ground runners and web-builders to herbicides have been investigated (e.g. Michalková & Pekár 2009; Benamú et al., 2010; Evans et al., 2010;

Pékar, 2012; Wrinn et al., 2012; Leccia et al., 2016). In general, two different groups of consequences can be distinguished, indirect and direct effects.

Indirect effects involve changes in habitat structure. Spiders prefer rich and complexvegetated ecosystems (Rypstra et al., 1999), hence changes in vegetation diversity due to herbicide application may affect spiders in two ways: (1) reduction of the number of webs, shelters and overheating protection sites and (2) reduction of prey availability by decreasing the amount of food for herbivores and consequently limiting prey abundance for spiders (Pékar, 2012).

The second group (direct effects) involves both lethal and sub-lethal effects. Lethal effects are considered when mortality rate directly increases after exposure and sublethal effects are defined as physiological and/or behavioral changes in an individual that survived after exposure (Pékar, 2012). Behavioral changes may affect mobility, orientation, feeding behavior, oviposition, and learning (Desneux et al., 2007). Sublethal effects have been commonly found in laboratory tests, although it has been argued that herbicides do not have significant lethal effects on spiders (Pékar, 2012). Within a biological control framework, Korenko et al. (2016) found that *Pardosa*, a spider genus commonly found in agroecosystems, killed significantly less flies when exposed to different herbicides. Although spiders were not directly killed by herbicides, if a trait related to hunting success is affected, the global success of spiders as natural enemies is reduced as well. Thus, research on how correlated traits respond to herbicides is mandatory in order to effectively assess how pest control provided by spiders may shift from successful to unprofitable.

### 1.8.2. Insecticide application

In general, spiders are more sensitive to some pesticides than many pests, such as synthetic pyrethroids, organophosphates and carbamate (El-Wakeil et al., 2013) and a great number of studies demonstrated that, in many cases, the application of insecticides decreased both the abundance and richness of spiders (Pékar, 2012, El-Wakeil et al., 2013), therefore reducing the possibility of biological pest control provided by spiders. Fortunately, pest management based on the application of broad-spectrum synthetic insecticides has been

progressively replaced two more environmentally-friendly alternatives: (1) the integrated pest management (IPM), based on the use of synthetic insecticides with acceptable environmental impact used when required and usually only at specific times and biological insecticides, and (2) organic farming, which restricts the use of synthetic insecticides and promotes the use of predators, parasitoids and entomopathogens.

This paradigm clearly had a positive effect on the community of spiders and it has been demonstrated in tea gardens, vegetables, wheat, olive groves and fruit orchards where the diversity of spiders increased when compared to conventional management (see Pékar, 2012).

It is beyond of the scope of this chapter to provide a complete list of insecticides and its effects on different spider species; instead, a conceptual overview on how pesticides affect spiders based on the comprehensive ecotoxicological review performed by Pekár (2012) is provided (see section 1.13 Supplementary material).

### 1.8.3 Mineral particle films

Numerous negative effects resulting from the use of synthetic pesticides including risks for non-target organisms and persistence in the environment have led to the development of mineral particle films as an alternative to some pesticides used in the control of pests (Glenn et al., 1999; Glenn & Puterka, 2005). Kaolin-based products, one of the most used mineral particle films, can act as repellent or barrier for pests, affecting the recognition and attractiveness of host plants (Showler, 2002) and it has been already used in several crops such as pear, apple, olive-, walnut and almond, citrus, wine grape, pecan, cotton, tomatoes and cabbage (see Benhadi-Marín et al., 2016a). However, knowledge about its possible effects on beneficial arthropods and especially on spiders is still scarce. Sackett et al. (2007) found that kaolin application in apple orchards reduced the relative abundances of webbuilding spiders such as Araneidae, Dictynidae and Theridiidae were not affected. Also in apple orchards Markó et al. (2010) observed that *Xysticus* L. Koch, 1835 (Thomisidae) and *Theridion mystaceum* L. Koch, 1870 (Theridiidae) populations strongly decreased when

kaolin was applied. On the contrary, *Dictyna uncinata* Thorell, 1856 (Dyctinidae) and *Clubiona* Latreille, 1804 (Clubionidae) tolerated kaolin applications.

A plausible explanation about the different effects of kaolin application on the population of spiders may be related to the lifestyles of different functional groups, e.g. if kaolin is applied during the day, nocturnal spiders such as *Clubiona* can avoid direct exposure to the mineral particle (Markó et al., 2010). Notwithstanding, spiders can be exposed to kaolin directly during its application by forming a particle film on the body and/or by ingesting particles when feeding on sprayed prey or substrate surfaces. Benhadi-Marín et al. (2016a) used the web-builder *A. cucurbitina* as model species in order to assess how kaolin affects spiders in laboratory tests and found that kaolin significantly reduced the survival of *A. cucurbitina* when applications were done (1) on the substrate surface and (2) on spider and prey body together, registering a survival reduction of 48% and 56%, respectively. As in the case of synthetic pesticides, functional trait-related characteristics such as web-building ability, legs adapted to running and body size seems to be closely related to the final effect of kaolin application on the life span of spiders.

### 1.9. Landscape influence on spider populations

Spiders are not distributed randomly in ecosystems and spatial and temporal patterns have been repeatedly observed and reported in the literature. Agroecosystems are not the exception and probably, one of the most obvious patterns of the diversity of spiders is the dominance of few agrobiont species that are adapted to cyclical disturbances caused by agricultural practices. The probability of survival to regular disturbances relies on migration and recolonization skills and spiders proved to be successful travelers and colonizers by moving on the ground or by air thorough ballooning (Marc et al., 1999). Spiders also seemed to be more abundant in perennial crops than in other agroecosystems during winter (Schmidt & Tscharntke, 2005); accordingly, the existence of overwintering sites can be one determinant factor for biocontrol since it is desirable to maintain the population of spiders in crop fields during pest outbreaks and, therefore, immigration from surrounding habitats can be necessary (Pywell et al., 2005). Reproduction, survival and dispersion dynamics

involve landscape, habitat and micro-habitat levels (Samu et al., 1999) and spider fitness can be affected in different ways by the available resources provided by each level of the spatial hierarchy.

### 1.9.1. Landscape scale

The study of the effects of landscape on wildlife has been commonly studied from two different perspectives because of the difficulty of working in large areas (Samu et al., 1999). The first one consists on modeling the species distribution on the landscape based on biological knowledge using different scenarios. Topping (1999) developed individual agent based models and found that spiders abundance did not depend on the landscape spatial distribution; nevertheless, when habitat patch size increased, maintaining a constant habitat area, spider population sizes increased as well. Also, the simulations suggested that a high juvenile dispersal was the optimal dispersal strategy for spiders.

The second approach is based on field experiments at a small landscape sale, usually considering patch fragments such as adjacent crops and/or wild areas and its margins. Öberg et al. (2008) found that lycosids abundance increased during pest establishment, thus these ground runners were recolonizing the crop from adjacent patches; however, abundance did not depend on the landscape heterogeneity whereas the latter was positively associated with lycosid species richness. On the other hand, dispersal based on ballooning resulted in a quick recolonization by linyphilds and this family did not show an increase in activity, density or species richness over time. Landscape may influence not only dispersal activity or behavior but also spider fitness. Large fields of annual crops originated high body condition in *Pardosa* females due to less competition for resources within homogeneous areas, whereas farming practices had no effect on either body condition or fecundity parameters (Öberg, 2009).

Landscape can also affect spiders at smaller scales, Schmidt et al. (2008) suggested that a 95 m to 3 km radius around the study fields potentially reflects dispersal distances (depending on the species) and in general, local species richness enhanced due to the presence of non-crop habitats. Even at a smaller scale, Sackett et al. (2009) found that

colonization rate and foliage-dwelling spider assemblages were affected by distances ranging from 10 to 50 m in apple orchards.

# 1.9.2. Habitat scale

Agricultural fields can typically be viewed as habitats composed by homogenous vegetation with a number of micro-habitats within a delimited area. Hence, it is supposed that farming practices alter the diversity of micro-habitats within crops, for example, topography and soil cover (e.g., plants, organic matter and mulching) may be important factors driving the community of spiders (Samu et al., 1996; Samu et al., 1999). In fact, Altieri & Schmidt (1986) caught significantly more spiders in a cover-cropped orchard than in a tilled orchard using pitfalls and addition of mulches or manures to the soil surface provides a more complex and diverse habitat giving spiders protection from natural enemies and improving microclimate conditions (Sunderland & Samu, 2000).

The number of species in weed strips and field margins is usually higher than in the adjacent fields. Richly dense structured vegetation can serve as overwintering place and accordingly, Nentwig et al. (1998) found significantly higher spider richness in older weed strips than in younger strips. In a similar way, the more diverse plant structure found in field edges can accommodate a more complex spider community regardless the agroecosystem type and management due to a reduced management intensity and higher immigration (Batáry et al., 2012). For example, field margins provide an excellent source habitat for *T. tenuis* acting also as shelter for many other beneficial invertebrates (Bell et al., 2002). On the contrary, Pfister et al. (2015) found that the abundance of the orb web builder *Mangora acalypha* (Walckenaer, 1802) (Araneidae) decreased from field centers towards the hedgerows probably due to interspecific competition avoidance and a higher pest population which may serve as prey. In general, the increasing spider densities towards field margins can be explained by the preference of spiders for seminatural habitats that provide overwintering sites, food, better microclimate conditions, and more complex vegetation structure (Pfister et al., 2015).

The presence of water bodies can also enhance the diversity of spider, especially considering species linked to watercourses or ponds such as *Tetragnatha* Latreille, 1804 (Tetragnathidae) that can walk on the water surface (Foelix, 2011). Pfister et al. (2015) found that the abundance of *Tetragnatha* cf. *montana* Simon 1874 increased towards both hedgerows and riparian margins whereas *Tetragnatha extensa* (Linnaeus 1758) increased only towards riparian margins. Interestingly, flooding episodes does not seem to affect the community of spiders (Lafage & Pétillon, 2016) probably due to their ability to survive to periods of submersion (e.g. Pétillon et al., 2009) or their capability to deal with encounters on water (Hayashi et al., 2015).

### 1.9.3. Micro-habitat scale

Spider abundance is determined by site selection, by rates of survival and reproduction related with the site and site abandonment, being in turn determined by abiotic and biotic factors (Samu et al., 1999). There is strong evidence that physical spatial structures given by vegetation complexity can be the most important abiotic factor for spiders determining the habitat quality at small scale especially for web builder functional groups. Web builders need not only appropriate attachment points but also the appropriate space arrangement to construct their webs and those are provided by a complex spatial distribution of plants. In addition, prey richness also increases with plant diversity and vegetation cover being lower at tilled sites when compared to non-tilled sites (Diehl et al., 2013). Grass and sub-shrub covers seem to be determinant for spider species richness. However, it is necessary to take into account that relationships between spider diversity and vegetation could not be direct and may depend on other abiotic factors (Schaffers et al., 2008). For example, temperature influences vegetation structure (Jiménez-Valverde & Lobo, 2007).

Biotic factors include vibratory, olfactory and visual cues related to prey, and spiders have shorter residence times in micro-habitats where prey is scarce compared with sites where food is abundant (Samu et al., 1999). In this situation, the occurrence of alternative food is relevant if it provides nutrients that can increase the lifespan of spiders. When insect preys are scarce, aerial plankton is of vital importance especially for spiderlings, and the first instars may increase the proportion of non-prey food consumed using insects as

supplementary resource (Smith & Mommsen, 1984). Non-prey food includes pollen, nectar and honeydew. Accordingly, Eggs & Sanders (2013) reported that about 25% of juvenile Araneidae orb-weaving spiders diet consisted of pollen. Microorganisms growing on the proteinaceous silk and pollen deposited on the sticky drops may be sufficient to nourish spider of the first instar (Smith & Mommsen, 1984). Moreover, adults of the orb weaver *Gasteracantha cancriformis* Linnaeus, 1758 (Araneidae) have also been observed eating pollen from the corbicula of the leg of a bee (Gregory, 1989). A different web builder guild such as Linyphiidae (sheet web builders) also consumed pollen consistently during laboratory assays (Peterson et al., 2010) revealing that ground living web builder spiders can also benefit from this resource.

Spider webs were very efficient in trapping pollen particles (Bera et al., 2002), but web builders are not the only functional group that benefit from pollen. Vogelei & Greissl (1989) fed spiderlings of the ambusher *T. onustus* in laboratory and those feed on pollen increased significantly their mean lifespan compared with starved spiderlings. Suetsugu et al. (2014) reported *Clubiona*, a foliage runner spider, removing pollen from flowers of the nectariferous orchid *Neottianthe cucullata* (L.) Schltr. 1919.

Flowers also provide nectar, an alternative source of amino acids, lipids, vitamins and minerals. Jackson et al. (2001) confirmed that 90 species of salticids fed on flowers. Suetsugu et al. (2014) suggested that *Clubiona* also consumed nectar when visiting orchids and Vogelei & Greissl (1989) found that artificial nectar (i.e., sucrose 30%) increased significantly the longevity of *T. onustus*. Taylor & Pfannenstiel (2009) collected *Cheiracanthium inclusum* (Hentz, 1847) (Miturgidae), *Hibana futilis* (Banks, 1898) and *Hibana arunda* (Platnick, 1974) (Anyphaenidae) in cotton fields and used the cold anthrone test to detect fructose in their guts and results were positive for both adults and immatures indicating consumption of extrafloral nectar. The same test was used by Chen et al. (2010) showing that *Ebrechtella tricuspidata* (Fabricius, 1775) (Thomisidae) and eight more families (Oxyopidae, Pisauridae, Salticidae, Lycosidae, Tetragnathidae, Araneidae, Nephilidae and Agelenidae) also consumed nectar. Sexual dimorphism is commonly accentuated in crab spiders and adult males rarely feed. Pollard et al. (1995) suggested that

males of *Misumenoides formosipes* (Walckenaer, 1837) drink nectar in order to replaced fluid loss through evaporation.

Benhadi-Marín et al. (data not published) tested the effect of alternative food on the ground dwelling spider *Haplodrassus rufipes* (Lucas, 1846) (Gnaphosidae) including honey 10%, glucose (0.5 M), pollen (10%) and honeydew of the black scale, *Saissetia oleae* Olivier, 1791, a secondary pest of the olive tree. Each treatment gave significantly longer longevities than control (water). Pollen provided the lowest longevity while the spiderlings that fed on the black scale honeydew almost triplicated the mean longevity reached with the negative control (Figure 1.13). Spiderlings fed on sugars probably obtained energy but lacked proteins while the opposite could happen with pollen. On the contrary, honey and honeydew probably provided both sugars and essential amino acids giving significantly longer longevity to *H. rufipes*.



**Figure 1.13.** Survival curves of *Haplodrassus rufipes* (Gnaphosidae) fed on non-prey foods. Different letters in the legend indicate significant differences between treatments.

Energetic costs and risks associated to hunting are supposed to decrease in spiders feeding on flowers; moreover, nutrients, venom and digestive enzymes necessary to capture and digest a prey are also saved (Jackson et al., 2001). Probably, each non-prey food alone is not enough to fulfill the nutritional requirements of an individual, but spontaneous plants (flowers) and honeydew may provide important nutrients to sub-adult spiders.

### 1.10. Spider venoms as bioinsecticides, a new perspective.

The interest in developing new and safer insecticides has been increasing during the last decades mostly due to legislative directives that affected chemical insecticides. Among pesticides, agrochemicals dominate the marketplace, but their spectrum of activity is still too wide with high levels of toxicity for non-pest species (Windley et al., 2012). Governments banned the use of first-generation insecticides characterized by their poor selectivity thus significantly reducing the available spectrum of insecticides (King & Hardy, 2013). In this context, natural products raised as the new paradigm. From this point of view, the potential of biocompounds could not be seen directly as a source of viable insecticides but as a source of molecules with biological activity which can be maximized by the synthesis of the analogues (Hodgson, 1984).

In recent years, research has been directed to mimic the natures' way to control pests. Several works have been focused on venomous insect predators or parasitoids searching for natural biocides naturally present in arthropods such as mites, scorpions, spiders, and wasps (Quicke & Usherwood, 1990, Windley et al., 2012). However, bioinsecticides should not be seen exclusively as products able to kill pests. Their development must minimize persistence of adverse metabolites in the environment and maximize their selectivity towards a target pest (Nentwig, 1993).

Spiders are mostly generalist predators and it would be reasonable to think that their venoms are suitable to subdue a wide range of prey (with the exception of stenophagic species such as ant-eating spiders that usually does not include agricultural pests on their diet). Moreover, spiders inject venom in their prey thorough the chelicerae fangs, a

technique than cannot be done in the field by farmers. Finally, nothing is known about the persistence of spider derived compounds in the field. These facts together may lead to consider spider venoms as bad candidates to bioinsecticides. Nevertheless there is a high interest in spider venoms nowadays because they comprise an extensive library of potent insecticidal neurotoxic peptides that are able to target novel sites in insects (Blagbrough et al., 1992; Windley et al., 2012).

Currently there are 1405 curated toxin records from 97 spider species available on ArachnoServer, a database containing information on the sequence, structure and biological activity of proteins derived from spider venoms (Herzig et al., 2011) and there is a long way to the validation of merchantable products. In addition, as summed up by Windley et al. (2012) prior to consider a spider-venom peptide as a safe novel pesticide several conditions need to be met, namely: (1) to present broad pest specificity (2) to have low toxicity in non-target organisms, (3) to remain in the environment long enough to be effective, (4) to does not persist in environment inducing resistance development, (5) to be inexpensive to produce, (6) to be easy to formulate and deliver, (7) to be able to publicly be perceived as innocuous and (8) to be accessible to stakeholders (e.g. farmers).

It is actually hard to meet all those conditions, nevertheless a continuous and increasing effort is emerging in this area and currently there are also several possible commercialization ways, such as the development of recombinant baculovirus or a transgene encoding spider toxin into an entomopathogenic fungus, orally active acaricidal and/or insecticidal agents and the design of conformational constrained non-peptide mimetics to be used as foliar sprays (Windley et al., 2012).

Pesticides can affect arthropod behavior and also exert a range of sublethal effects that may not be detectable at field scale. In the case of bees, important aspects of behavior related to foraging and survival, such as learning and memory can be affected after exposure to pesticides and should be investigated as well (Palmer et al., 2013; Decourtye et al., 2004a, 2004b). A successful spider-venom based bioinsecticide was recently tested by Nakasu et al. (2014) using the fusion protein technology for linking insecticidal peptides to a plant

lectin 'carrier' protein. The venom of the Australian funnel web spider *Hadronyche versuta* (Rainbow, 1914) (Hexathelidae) was used as peptide source and tested on honeybees (*Apis mellifera* Linnaeus, 1758) using three exposure routes: (1) injection (emulating a worst case scenario), (2) oral and (3) contact assays; no adverse effects were detected even when bees were directly injected with the fusion protein.

Finally, the Vestaron Corporation recently received the U.S. EPA approval to remove the bee toxicity warning statement from its spider-venom based biopesticide SPEAR<sup>TM</sup> label (Vestaron, 2015). In conclusion, it seems that the future of development and use of biopesticides based on spider venom is a promising research area and will occupy an important place in the market of insecticides in the future years.

### 1.11. Spiders in the olive grove agroecosystem

The olive tree (*Olea europaea* L. 1753) is a significant land use type with high importance in Mediterranean Basin where 98% of the global production of olive oil is located. In southern Member States of the EU it has a multiple importance particularly in what concerns to economic, social, cultural and landscape aspects. Since agricultural production is the result of socioeconomic pressures from society on natural ecosystems (Sevilla, 1995), the increased economic activity does not have in mind that depends on susceptible to exhaust and limited natural resources. In this scenario, current production techniques have focused on maximizing yield per unit area ignoring the various negative impacts of this model agricultural practices such as high energy costs, loss of fertility and soil erosion, problems of monoculture, pollution of natural resources, loss of natural quality of food and environmental degradation (Cárdenas, 2008; Labrador & Gilberteau, 1990).

In recent years, strong market pressures have encouraged growers to intensify production, resulting in the introduction of new olive planting systems (Estevão et al., 2012; Tous et al., 2011). These changes are of utmost concern because of their negative impacts (i.e. pollution and degradation of natural resources, loss of biodiversity, insecticide resistance and residues in olives) (Hawkes et al., 2005; Soares et al., 2004). Notwithstanding, the

European Union (EU) has supported the adoption of environmentally-friendly farming practices through the agri-environment schemes. Moreover, the EU has established an ambitious strategy to halt the loss of biodiversity and ecosystem services through the promotion of sustainable agriculture and more use of green infrastructures, by 2020 (European Commission, 2011). As an agroecosystem with high ecological potential, olive crop is a very promising cultivation for implementing environmental friendly management strategies especially where traditional olive groves are still major sources of income and employment.

In response to an increasing concern with agriculture, different tools have been developed for study and evaluation quality/sustainability of agroecosystems such as the use of bioindicators (Lawes et al., 2005, Longcore, 2003). Despite spiders are considered polyphagous and generalist predators, they have a high plasticity at the ecological level. These features fit together in this group of arthropods as potential bioindicators according to the criteria established for bioindication in Mulhauser (1990): (1) previously acquired knowledge about the organism, (2) easy field re-encounter of the group to which the organism belongs, (3) easy recognition of the organism and (4) the use as many groups as possible bioindicators in order to obtain a global perception of the ecosystem. Accordingly, the potential of Araneae within biological control schemes and bioindication resulted in an increasing research on the role of spiders in the olive grove agroecosystem. Because of that, olive growing can be model for sustainable land-use in this region where spiders are abundant predators in all compartments.

In general, is well accepted that spiders' biodiversity follows an increasing pattern from conventional to organic management. However, it is worth to be noticed the lacking of consensus concerning how land management affects separately richness and abundance of spiders (see Prieto-Benítez & Méndez, 2011). Literature mostly focuses on the differences in spider biodiversity resulting of the three classical land managements: organic, integrated and conventional regardless the internal spatial structure of each crop. However, this own operating structure of crops (e.g. vegetation and soil structure and local activities such as

grazing) may be more influent in how spider communities differ among managements than the farming system as a global point of view (Bengtsson et al., 2005).

In Spain, Cárdenas & Barrientos (2011) reported a spider richness of 32 families, 115 genera and 142 species. In terms of guilds, ground runners, stalkers, tangled web builders and space web builders were the most abundant on the ground (Cárdenas et al, 2015). In the canopy, Morris et al. (1999) cited Thomisidae and Theridiidae as the families that presented a higher species richness.

Cárdenas (2008) studied the use of spiders as bioindicators in the olive grove, suggesting that species composition does not vary between different agronomic practices, but between different landscape scales. Castro et al. (1996) reported that bare soil management reduced the abundance of spiders on the ground near olive trees. This is supported by the results obtained by Cárdenas et al. (2015) that found a negative effect on spider abundance and diversity induced by plowing, nevertheless it could be reduced by the presence of hedge vegetation. On the contrary, an intermediate disturbance level in the integrated regime may increase spider diversity (Cárdenas et al., 2006) and Cárdenas et al. (2005) found that *Thyene imperialis* Rossi, 1846 (Salticidae) and *Loxosceles rufescens* Dufour, 1820 (Sicariidae) may be favored by cereal cover crops.

In Italy, Thaler & Zapparoli (1993) reported Linyphiidae, Erigonidae, Lycosidae, Dysderidae, Gnaphosidae and Theridiidae as the dominant spider families within an assemblage of 70 species belonging to 18 families on the ground around olives and Picchi et al. (2016) demonstrated the effect of surrounding landscape, the distance towards the field edge and the agricultural management affected the olive grove spider community. The abundance and species richness of spiders as well as the abundance of sheet web spiders were lower in conventional orchards than in organic orchards and Linyphiidae density decreased with the amount of Mediterranean garigue.

In Southern Portugal, Sousa (2003) found a higher diversity of spiders in organic than in intensively managed groves both in terms of abundance and richness and reported 31

families, 104 genera and 144 species being the most abundant *Hogna radiata* Latreille, 1817 (Lycosidae), *Zodarion josefinae* Bosmans, 1994 (Zodariidae), *Pardosa proxima* (C. L. Koch, 1847) (Lycosidae), *Nomisia exornata* (C. L. Koch, 1839) (Gnaphosidae) and *Callilepis concolor* Simon, 1914. Similarly, in Northeastern Portugal groves, Benhadi-Marín et al. (data not published) found *N. exornata* (C. L. Koch, 1839) (Gnaphosidae), *Zodarion alacre* (Simon, 1870) (Zodariidae) and *Alopecosa albofasciata* (Brullé, 1832) (Lycosidae) as the most abundant species, and grouped the whole community in nine guilds namely foliage runners, ground runners, stalkers, ambushers, sheet web builders, wandering sheet/tangle weavers, orb weavers, space web builders and specialists. According to the dominant families, ground runners and specialists were the two most representative functional groups being present in all managements. In this case, foliage runners and orb weavers were exclusive of groves in which spontaneous vegetation were maintained. The remaining guilds were common to all managements except the sheet web builders that were not present in groves treated with herbicides.

In Iran, Ghavami (2006) collected 18 families, distributed in 56 genera and 18 species from olive orchards reporting *Frontinellina frutetorum* (C. L. Koch, 1834) (Linyphiidae), *T. imperialis* and *Salticus scenicus* (Clerck, 1757) (Salticidae) as the dominant species, and in general, spiders as nearly half (42.8%) of the olive grove predator community in terms of abundance, thus suggesting again a key role on pest control.

*Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae) is the main pest of olive groves and the particle film technology has been used in order to the control of the olive fruit fly. Pascual et al. (2010) referred Philodromidae as one of the most affected taxa among the natural enemy arthropod community when orchards were sprayed with kaolin in a Spanish olive grove. On the other hand, rotenone, azadirachtin and copper oxychloride are compounds utilized against *B.oleae* allowed in organic farming and weak or non effects were observed in spiders by Scalercio et al. (2009) in Italy. Regarding spiders as natural enemies, Picchi et al. (2016) found that the olive fruit fly densities were negatively correlated with the abundance of cursorial and sheet web spiders suggesting a spiders' role in *B. oleae* suppression.

In summary, the conservation of a diversified surrounding landscape as well as the ecological infrastructures growing within the grove (e.g. vegetation strips, small bushes and stonewalls) can be seen as part of a strategy to enhance spider functional biodiversity towards a biological control strategy of olive tree pests. In addition, environmental friendly practices such as organic field crop management can be a way of achieving sustainability and giving an add-value to olive products especially in regions where the landscape is typically heterogeneous, consisting of small mosaics of olive groves, other crops and natural vegetation.

### 1.12. Conclusions

Spiders are among the most abundant and widespread predators over the world. Their feeding habits have led to consider them as potential agents of biological control of pests in agroecosystems. However, alternative non-prey foods and shelter within and between crops are necessary in order to improve their presence and facilitate their permanence in the agricultural landscape. Such resources can be provided by maintaining a heterogeneous agricultural landscape and ecological infrastructures such as flower strips (providing pollen and nectar) and stones and field edges (providing shelter and overwintering sites).

In general, regardless of the cultivated species, common agricultural practices such as pesticide application and soil disturbance caused by tilling or ploughing usually affect negatively the spider assemblages in four ways: (1) by reducing the available habitat at different scales (e.g. cyclic soil disturbance and loss of vegetation complexity by herbicides), (2) by reducing the available prey populations (e.g. insecticide application), (3) by reducing sources of alternative non-prey foods (e.g. grass cutting and weed control) and by (4) direct mortality due to intoxication with synthetic compounds (e.g. pesticide applications).

Environmentally friendly practices such as organic farming can effectively improve the spider populations in arable lands thus enhancing the role of spiders as natural enemies especially when crops are integrated in a diversified landscape. In addition, novel

bioinsecticides based on spider venom proteins have appeared in market during the last years. This technology has succeeded in developing pesticides able to avoid lethal and sublethal effects on beneficial fauna and opens a door to a new generation of safer bioinsecticides allowed in organic agriculture, making increasingly possible to achieve the sustainable agriculture paradigm.

1.13. Supplementary material



Conceptual overview on how pesticides affect spiders. Arrows indicate a toxicity gradient, from low (-) to high (+), of different pesticides on spiders (left chart side) and how different life history traits are affected by them (right chart side). Based on Pekár (2012).

# CHAPTER 2

Distribution of the spider community in the olive grove agroecosystem (Portugal): potential bioindicators.

# Chapter submitted as journal article:

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# **Contribution of authors:**

J.B.M., S.A.P.S., J.A.P. and J.P.S. conceived and designed the experiment, J.B.M. and S.A.P.S. collected the field data, J.B.M. identified the spiders, J.B.M analyzed the data, and all the authors contributed to writing the paper.

### **CHAPTER 2**

Distribution of the spider community in the olive grove agroecosystem (Portugal): potential bioindicators.

# Abstract

Spiders can be successful natural enemies in different crops. Research on the role of spiders as natural enemies within agroecosystems needs to address the distribution of their communities across different spatial scales as well as the dominant species (e.g. potential biocontrol agents and indicators). In this work, the spatial distribution of the community of spiders of the olive grove agroecosystem in Northeastern Portugal studied from the center of the crop to the adjacent semi-natural patches (Mediterranean shrublands) and from the soil to the canopy strata. Also, potential species that could be used as bioindicators of agricultural management in the olive grove were investigated. Nine functional groups were found (ambushers, foliage runner hunters, ground hunters, orb-web builders, sheet web builders, sensing web-builders, space web builders, stalkers, and wandering sheet/tangle weavers) encompassing a community that changed significantly across the horizontal and vertical gradient. Philodromus lividus Simon, 1875 was the most abundant species in the canopy, Erigoninae was the most recorded group on the trunk, and the ground was dominated by the ambusher Thanatus vulgaris Simon, 1870 whereas the shrubland was dominated by the ground hunter Nomisia exornata (C. L. Koch, 1839). Thanatus vulgaris is proposed as bioindicator for the ground of the olive grove central area and Ozyptila pauxilla (Simon, 1870) for the ground of the olive grove peripheral area. The adjacent shrubland areas can play an important role in biological control of pests allowing the exchange of species and individuals with the olive crop thus mitigating the disturbances caused by agricultural management. Moreover, the role of agrobiont species as indicators of agricultural managements deserves further investigation towards the enhancement of the effectiveness of spiders within low-impact crop management in arable landscapes.

### **2.1. Introduction**

The suppression of pests by their natural enemies is a subject of interest for agricultural managers within sustainable farming practices. Agricultural landscapes are usually difficult environments for natural enemies due to high levels of disturbance caused by cyclic and frequent disturbances derived from agricultural practices (Landis et al., 2000). However, several factors can drive the spatial distribution of natural enemies enhancing their presence within crops. For example, the occurrence of ecological corridors between crops, the presence of different patches in the surroundings of the crop, and the availability of within-crop microhabitats can promote the abundance and action of natural enemies in the crop due to spillover (i.e. movement between crop and non-crop patches), existence of shelter and supplementary food (Nicholls et al., 2001; Blitzer et al., 2012; Benhadi-Marín et al., 2018b, 2019).

Also, the pest suppression exerted by natural enemies can be maximized by the existence of overlapping spatial niches (e.g. different vertical layers such as soil, trunk and canopy) that may allow their coexistence by reducing the intraguild competition (Dainese et al., 2017). Hence, crop management towards maintaining and promoting supplemental resources can improve the effectiveness of natural enemies against pests (Landis et al., 2000).

Spiders encompass a group of generalist predators that feed mostly on insects, they are well represented within the agroecosystems (Marc et al. 1999) and have been reported as successful natural enemies in different crops such as apple (Wyss et al., 1995; Isaia et al. 2008), cotton (Ghavami, 2008), wheat (Oelbermann & Scheu, 2009), and *Brassica* L., 1753 (e.g. broccoli and cauliflower) (Hooks et al. 2003).

Research on the role of spiders as natural enemies within agroecosystems needs to address not only the distribution of their communities across different spatial scales (e.g. vertical and horizontal patterns) but also the structure of the community by knowing which species are dominant within the agricultural landscape. Samu & Szinetár (2002) defined as agrobionts those species that reach high dominance in crops. These species can be indicators of arable habitats and could be used to monitor the quality and sustainability of the agroecosystem.

The olive tree cultivation (*Olea europaea* L. 1753) is an agricultural activity with high economic, social and cultural importance along the Mediterranean Basin where 95% of the global production of olive oil is located (FAOSTAT, 2018). The olive fruit fly *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae) is the key pest of the tree and their larvae develop in the fruits whereas pupae overwinter in the soil and adult flies are long-living and mobile (Civantos, 1999). *Prays oleae* (Bernard, 1788) (Lepidoptera: Yponomeutidae) is another olive tree pest that completes its life cycle in the canopy (Civantos, 1999). This species can be consumed by spiders both in the canopy as well as at the ground level (Morris et al., 1999).

Within the olive grove agroecosystem, several studies have been carried out encompassing different aspects of the biology, diversity and taxonomy of spiders as well as on the study of the influence of agricultural management and landscape on the community of spiders. In this context, Cárdenas & Barrientos (2011) provided a list of 142 spider species within the olive grove in Andalusian (southern Spain) groves; Cárdenas et al. (2015) found that organic farming favored the abundance and diversity of canopy spiders when compared with the IPM (integrated pest management) and conventional systems. Picchi et al. (2016) found a negative correlation between the abundance of the olive fruit fly and cursorial and sheet web spiders, as well as a significant effect of the adjacent semi-natural habitat type on the spider family composition in Italy. Also, Cárdenas (2008) developed preliminary studies about the use of spiders as potential bioindicators of agricultural management in the olive grove agroecosystem.

Thus, there is an increasing interest on the role of spiders as natural enemies of olive pests; however, studies about the composition of the community of spiders within the olive grove and its surrounding landscape and their potential as bioindicators of agricultural practices are still scarce. The objectives of this work were (i) to characterize the spatial distribution of the community of spiders and (ii) find agrobiont species that can be further used as

bioindicators of agricultural management in the olive grove. The working hypothesis was that the community of spiders is unequally distributed across the vertical strata (i.e., ground, trunk and canopy) and the horizontal spatial architecture (i.e., olive grove central area, peripheral area, and adjacent shrubland area) of the grove considering species and functional groups. Also, the implications of the distribution of species for the biological control focusing on the biology and bioindication potential of the species found along the olive grove agroecosystem is discussed.

# 2.2. Material and methods

### 2.2.1. Study area

The field work was conducted in May 2014, in five areas near Mirandela municipality (Trás-os-Montes - northeastern Portugal). Each area was composed by an olive grove and an adjacent semi-natural field. This field represents an important land cover type in the study region and corresponds to a typical Mediterranean habitat named "matos" (shrublands) characterized by the herbaceous stratum dominated by plants belonging to Asterceae, Poaceae and Fabaceae families, the shrub stratum dominated by plant species such as *Cistus ladanifer* L. *Cytisus* sp., *Lavandula* sp., *Rosmarinus officinalis* L., *Rubus ulmifolius* Schott, and *Crataegus monogyna* Jacq, and the tree stratum dominated by gives sites are in the Mediterranean climatic zone, *Csa*-type according to the Köppen-Geiger classification (Peel et al., 2007), that is characterized by hot and dry summers and mild and moist winters. The sampling area is located within a region with a mean annual rainfall of 776 mm and a mean annual temperature of 13.8 °C (Fick & Hijmans, 2017).

The five study sites form a polygon of 30 km<sup>2</sup> with a maximum and minimum separation between groves of 9.71 km and 0.39 km respectively. The olive groves were rain fed and two of them were conducted under the Integrated Production system (Cedães 1 and Cedães 2) and three were conducted under the organic farming system (Guribanes, Romeu, and Valbom-dos-Figos). More details of the study sites are provided in Table S1.

### 2.2.2. Sampling of Araneae

The horizontal distribution of the community of ground spiders was studied by sampling three areas: two areas were located in the olive groves (a central area and a peripheral area close to the hedgerow with the shrubland area), and a third area were located in the shrubland. The central and peripheral areas within the grove were located 150 m apart, while the peripheral and the shrubland areas were spaced 100 m apart. The ground spiders were sampled using four pitfall traps placed in each sampling area. Each trap consisted of a plastic cup (115 mm in top diameter and 130 mm in height) dug into the ground and filled with 250 ml of ethylene glycol (anti-freeze liquid) as preservative. A lid supported by iron wires was placed over each trap to exclude rain, debris and small vertebrates. Pitfall traps were placed in the south side of the canopy at 50 cm from the trunk (olive tree in the grove or shrub or tree in the shrubland) and were spaced 45-50 m apart. Traps were active for 14 days. The vertical distribution of the community of spiders was studied by collecting specimens from the soil, the trunk, and the canopy in the same central and peripheral areas in the olive groves. The canopy spiders were sampled by shaking (during five seconds) two branches per tree, one branch faced the south and another one the north. Four trees were sampled using a funnel-shaped cloth (0.42  $\text{m}^2$  in the upper opening and 0.65 m in height) that ends at its base in an opening on which a plastic bag can be placed to receive the sample. All the selected branches were between at 1.5 and 2 m in height. Spiders collected in the bag were preserved in situ with 70% ethanol to avoid predation. The spiders moving on the trunk were sampled using sticky traps (acting during 14 days). Each sticky trap was placed around the trunk (of the same trees sampled above) to capture the individuals inhabiting or using the trunk to disperse. Each sticky trap consisted in a strip of wrapping plastic transparent food film (30 cm in height) surrounding the trunk at 1 m in height and covered with glue.

All the captured spiders were transported into the laboratory, sorted, preserved in 70% ethanol, identified to the species level (when possible) using a binocular stereomicroscope, and grouped in functional groups following Nentwig et al. (2018), Cardoso et al. (2011) and Uetz et al. (1999). Since all the spiders captured in the trunk were immature, the richness of families instead of species was used in further analyses.

### 2.2.3. Data analysis

All statistical analyses and modeling were performed in R (R Core Team, 2018). The overall diversity across the vertical strata was assessed through individual-based accumulation curves instead of sample-based curves due to the different sampling methods used to sample the communities of spiders living on the ground, trunk and canopy (i.e. the considered sampling effort unit was the individuals). Since the trunk diameter was not equal between olive trees, the area of each sticky trap differed between samples, thus the number of spiders captured with sticky traps was recorded as number of individuals per m<sup>2</sup> of trap.

For ground spiders, a series of univariate and multivariate, parametric and non-parametric methods were used to investigate the spatial distribution of the different species and functional groups identified along the three sampling areas from the center of the olive grove to the adjacent shrubland area.

Firstly, the overall diversity across the horizontal strata was also assessed with individualbased accumulation curves. The total spider abundance (N), the abundance of the dominant functional groups (FGNs), the species richness (S), and the Simpson index (1-D) were calculated (Magurran, 2004). Abundances were log transformed (log (x + 1)) and the effect of the sampling area in each independent variable was investigated using linear mixed models according to Zuur et al. (2009) followed by a *posthoc* Tukey HSD test ( $\alpha = 0.05$ ). All the variables were modeled using the sampling area as explanatory variable and the locality as random factor ( $Z_i \times b_i$ ) as:

$$b_i \sim N(0,D)$$
 and  $\mathcal{E}_i \sim N(0,\Sigma_i)$ 

$$\mathbf{N} \sim \alpha + \beta_1 \times \text{Sampling area} + \mathbf{Z}_i \times \mathbf{b}_i + \mathcal{E}_i$$
[Eq. 1]

FGNs ~ 
$$\alpha + \beta_1 \times$$
 Sampling area +  $Z_i \times b_i + \mathcal{E}_i$  [Eq. 2]

- $S \sim \alpha + \beta_1 \times \text{Sampling area} + Z_i \times b_i + \mathcal{E}_i$  [Eq. 3]
- 1-D ~  $\alpha + \beta_1 \times$  Sampling area + Z<sub>i</sub> × b<sub>i</sub> +  $\mathcal{E}_i$  [Eq. 4]

Due to the low number of groves (n = 5) and samples per area (n = 4) these results were corroborated by bootstrapping the overall mean of each response (999 bootstrap replicates) and its 95% confidence interval were generated.

Both for species and functional groups of spiders, a non-metric multidimensional scaling (NMDS) was carried out using the Bray-Curtis's index (999 permutations) that consider the presence or absence of taxa as well as the abundance among samples, in order to assess the variability in the community of ground spiders along the horizontal gradient. Two permutational multivariate analyses of variance (PERMANOVA), one for the species dataset and one for the functional groups dataset, were carried out using the Bray-Curtis method (999 permutations) in order to corroborate the results of the NMDS analysis using the species and functional groups matrices as dependent variables and the sampled areas along the horizontal gradient as explanatory variable. Also, the factor "locality" was included in the model to account for the site effect.

Then, a similarity percentages (SIMPER) analysis was used (999 permutations) to uncover the species and functional groups that contributed to the within-groups dissimilarities. The average contribution of each species and functional group and the overall between-group Bray-Curtis dissimilarity was calculated for each pair of sampling areas (i.e. olive grove central area, olive grove margin area, and shrubland area).

A set of indicator species for each sampling area was proposed by calculating the positive predictive power (A), sensitivity (B), and the statistical significance (p < 0.05) of the association between species and sampling areas following de Cáceres et al. (2010). Only those species with a positive predictive power (A) higher than 0.5 were considered.

Finally, a co-inertia analysis ("cross-table" multivariate analysis) was performed in order to generate a gradient of species and functional groups along the horizontal gradient. The procedure consists in finding a co-structure between two sets of variables (the community matrix and the environmental matrix) that are linked by the same individuals, where the resulting sample scores the most covariant (Dolédec & Chessel, 1994). The co-inertia

analysis was based in a principal component analysis (PCA) carried out for each matrix. Then, the scores of the PCA were used to place each species or functional group along the x-axis and the environmental drivers along the y-axis. Finally, the correlation obtained for each "taxa-driver" pair using the co-inertia analysis was plotted altogether.

### 2.3. Results

The whole community of spiders found in the study areas included nine functional groups: ambushers, foliage runner hunters, ground hunters, orb-web builders, sensing web-builders, sheet web builders, space web builders, stalkers, and wandering sheet/tangle weavers; 28 families and 83 species (Table S2).

Considering the vertical distribution, the ambusher species *Philodromus lividus* Simon, 1875 was the most abundant species in the canopy (30 individuals) followed by *Philodromus albidus* Kulczyński, 1911 (nine individuals) and the orb-weaver *Araniella cucurbitina* (Clerck, 1757) (seven individuals) (Table S2). The most abundant group of immatures in this stratum belonged to the subfamily Erigoninae (Linyphiidae) (tiny wandering sheet/tangle weavers known as dwarf or money spiders) followed by immatures of Philodromidae (Table S2). On the trunk, Erigoninae was also the most recorded group (62.49 individuals/m<sup>2</sup>) followed by immatures of Philodromidae (3.15 individuals/m<sup>2</sup>) (Table S2).

On the ground of the olive grove, both central and peripheral areas were dominated by the ambusher *Thanatus vulgaris* Simon, 1870 (55 and 45 individuals respectively) followed by the ground hunter *Alopecosa albofasciata* (Brullé, 1832) (47 individuals in the central area) and *Zodarion styliferum* (Simon, 1870) (31 individuals in the central area) (Table S2). Immatures of *Hogna* Simon, 1885 were also abundant in the olive grove. The shrubland was dominated by the ground hunter *Nomisia exornata* (C. L. Koch, 1839) (11 individuals) followed by *A. albofasciata* (8 individuals) (Table S2). Considering the horizontal distribution, the orb-web builders were absent from the shrubland, the sensing web builders

were absent from the olive grove and the foliage runner hunters were not found in the olive grove central area (Table S2).

Across the vertical distribution, the community of spiders sampled on the ground reached the highest number of species (Fig. 2.1A) whereas horizontally, the species accumulation curves showed an increasing pattern in species richness from the olive grove central area to the shrubland area (Fig. 2.1B). However, the total spider abundance was statistically significantly lower only in the shrubland area (Fig. 2.2A). The species richness (S) was not statistically significantly different across the horizontal distribution (Table 2.1) (Fig. 2.2B). Also, the Simpson index (1-D) did not significantly vary from the olive grove central area to the shrubland area (Table 2.1) (Fig. 2.2C).

**Table 2.1**. Linear mixed models developed to test the effect of the horizontal distribution of the community of spiders across the olive grove in Trás-os-Montes (i.e. olive grove central area, olive grove peripheral area, and an adjacent shrubland area) using a varying intercept group effect (p < 0.01). <sup>†</sup>The abundance of foliage runner hunters was hereafter considered significantly different along the gradient according to the 95% confidence intervals generated by bootstrapping (see Fig. 2.2E).

Dependent variable	$\chi^2$	Df	Р
Total spider abundance (N)	13.724	2	< 0.01*
Species richness (S)	2.183	2	0.336
Simpson index (1-D)	0.454	2	0.797
Abundance of ambushers	40.556	2	< 0.01*
Abundance of foliage runner hunters	8.968	2	$0.011^{\dagger}$
Abundance of ground hunters	10.207	2	< 0.01*
Abundance of sheet web builders	1.564	2	0.458
Abundance of stalkers	6.015	2	0.049
Abundance of wandering sheet/tangle weavers	2.862	2	0.239





**Figure 2.1.** Richness of spider species found across a vertical gradient (i.e., ground, trunk and canopy) and a horizontal gradient (i.e., olive grove and an adjacent shrubland area) in Trás-os-Montes (Portugal). A: Species accumulation curves for the vertical, and B: horizontal spatial gradient. OGC: olive grove central area; OGP: olive grove peripheral area, and S: shrubland area. Vertical bars around lines represent the standard error of the iterations.



**Figure 2.2**. Composition and structure of the community of spiders across a horizontal spatial gradient (olive grove central area, olive grove peripheral area, and the surrounding shrubland) in Trás-os-Montes. Dots represent the mean and bars the 95% confidence interval obtained by bootstrapping (999 replicates). Different letters above bars represent significant differences between sampling areas according to a linear mixed effects model developed for each variable (p < 0.05). OGC: olive grove central area; OGP: olive grove peripheral area, and S: adjacent shrubland area. A: Total spider abundance; B: total species richness (S); C: Simpson index (1-D); D: abundance of ambushers; E: abundance of foliage runner hunters; F: abundance of ground hunters; G: abundance of sheet web builders; H: abundance of stalkers, and I: abundance of wandering sheet/tangle weavers.

In terms of functional groups, the abundance of ambushers found in the shrubland area was statistically significantly lower than in the olive grove (Table 2.1) (Fig. 2.2D). The abundance of foliage runner hunters significantly increased (considering the 95% confidence interval) from the olive grove central area to the shrubland area (Fig. 2.2E), whereas the abundance of ground hunters found in the olive grove peripheral area was significantly higher than in the shrubland area (Table 2.1) (Fig. 2.2F). On the contrary, the abundance of sheet web builders, stalkers, and wandering sheet/tangle weavers did not significantly differ among the three areas (Table 2.1) (Figs. 2.2G, 2.2H & 2.2I).

The NMDS showed a significant change of the community of ground spiders across the horizontal gradient both in term of species (stress = 0.095) and functional groups (stress = 0.151). In the case of species, there was a gradually and significant change from the olive grove central area to the shrubland area (PERMANOVA: df = 2; F=2.2826; P < 0.01) (Fig. 2.3A), and in terms of functional groups, the olive grove community was significantly separated from the shrubland community (PERMANOVA: df = 2; F = 3.6725; P < 0.01) (Fig. 2.3B).

According to the SIMPER analysis, 10 species were responsible for 70% of the differences found between the communities of spiders sampled in the olive grove central and the peripheral area (Table S3); 15 species were responsible for the differences found between the olive grove peripheral area and the adjacent shrubland, and 14 species were responsible for the differences found between the olive grove central area and the shrubland area (Table S3). The highest differences were found between the olive grove central area and the shrubland area and the shrubland area (Table S3). In terms of functional groups, the ambushers and ground hunters were the most influential groups in all the cases, and the shrubland area (Table S4).

*Thanatus vulgaris* can be a potential bioindicator for the olive ground central area, *Ozyptila pauxilla* (Simon, 1870) for the olive ground peripheral area and *Eratigena feminea* (Simon, 1870) and *Scytodes velutina* Heineken & Lowe, 1832 for the shrubland area (Table 2.2). These results were consistent with the co-inertia analysis for the species dataset (RV =

0.172) that positioned *T. vulgaris* and *S. velutina* on the extreme of a gradient of species distribution across the horizontal spatial gradient (Fig. 2.4A). The co-inertia analysis for functional groups (RV = 0.147) also supported the results of the NMDS by associating the foliage runner hunters, sheet web builders, sensing web builders and stalkers to the shrubland area, and the remaining functional groups to the olive grove (Fig. 2.4B).

**Table 2.2**. Ground spider species that could be bioindicators of agricultural practices along a horizontal spatial gradient in Trás-os-Montes (Portugal) (i.e. olive grove central area, olive grove peripheral area, and shrubland). A: Positive predictive power; B: sensitivity; OGC: olive grove central area; OGP: olive grove peripheral area, and S: shrubland area. Positive predictive power values > 0.5 are indicated in bold. Tv: *Thanatus vulgaris* (Ambushers); Op: *Ozyptila pauxilla* (Ambushers); Ef: *Eratigena feminea* (Sheet web builders); Sv: *Scytodes velutina* (Stalkers).

Species	Α			В			Indicator for	D
	OGC	OGP	S	OGC	OGP	S	Indicator for	r
Tv	0.610	0.390	0.000	0.450	0.400	0.000	OGC	0.010
Op	0.387	0.563	0.050	0.450	0.300	0.100	OGP	0.020
Ef	0.240	0.000	0.760	0.050	0.000	0.250	S	0.025
Sv	0.000	0.000	1.000	0.000	0.000	0.200	S	0.030



**Figure 2.3**. NMDS analysis for (A) species, and (B) functional groups of the community of ground spiders along the different strata of a horizontal gradient in the olive grove agroecosystem in Trás-os-Montes (Portugal). OGC: olive grove central area, OGP: olive grove peripheral area, and S: adjacent shrubland area. Samples are indicated by the letters C (olive grove peripheral area), L (olive grove central area) and S (shrubland area) followed by a number. Species (in red) correspond to the codes indicated in Table S2. Functional groups (in red) correspond to the codes indicated in Table S2 preceded by "X". Each sample (in black) is connected to its group centroid, and each centroid is surrounded by its 95% confidence limit. In A, two samples (one on the left side and one on the right side) were left out of the plot area in order to facilitate the visualization.

Chapter 2





# 2.4. Discussion

In this work, the composition and structure of the community of spiders inhabiting the olive grove showed vertical and horizontal distributions that differed, respectively, from the ground to the olive tree canopy and from the olive grove to the adjacent shrubland. Considering the vertical distribution, P. lividus and P. albidus were the most abundant species in the canopy of olive trees. The genus *Philodromus* includes small and medium sized wandering ambusher spiders, i.e., the body length of P. lividus ranges from 4.19 - 5.04 mm in males, and 4.39 - 6.9 mm in females (Nentwig et al., 2018) that are able to run fast on plant surfaces (Wolff & Gorb, 2012). The occurrence of immature Philodromidae and the dominance of the adults among the community of spiders in the canopy and in the trunk together with the absence of *Philodromus* on the ground suggest that some species of the genus may complete their life cycle in the canopy. Therefore, further attention should be given to specimens of *Philodromus* inhabiting the canopy since they could be useful natural enemies of olive tree pests. Namely, the reduction of the use of selective insecticides should be an important aspect, since their application decreased the predation rate of Philodromus cespitum (Walckenaer, 1802) on Drosophila melanogaster Meigen, 1830 (Diptera: Drosophilidae) without significantly increasing its mortality rate as Řezač et al. (2010) showed in laboratory experiments.

Orb-weavers such as *A. cucurbitina* and other functional groups of web builders may also play an important role in the canopy as natural enemies. In fact, Picchi et al. (2017) found that the abundance of the olive fruit fly, *B. oleae* was negatively correlated with the abundance of sheet web spiders in the canopy.

In terms of total spider abundance in the olive tree canopy, Picchi et al. (2016) found a decreasing pattern from the center toward the olive grove edge. In this work, the high number of immature Erigoninae (Linyphiidae) both in the canopy and trunk suggests a high level of ballooning (i.e. aerial dispersion especially at immature stages). These results agree with those found by Simonneau et al. (2016) that reported the family Linyphiidae as the most "ballooner" among the recorded spiders.
Benhadi-Marín et al. (2016a) demonstrated that the lifespan of *A. cucurbitina*, the third most recorded species in the olive tree canopy, was negatively affected by the application of kaolin, a white aluminosilicate mineral clay that forms a particle film and is commonly sprayed as alternative to pesticides. Thus, aerial dispersion could be seen as a strategy to escape from a seasonally disturbed habitat such as an agroecosystem (Simonneau et al., 2016) thus generating spurious diversity patterns.

On the ground, the community of spiders was dominated by *T. vulgaris*. All *Thanatus* species are of small and medium size (body length of *T. vulgaris* ranges from 3.7 - 5.8 mm in males and 6.1 - 9.1 mm in females) (Nentwig et al., 2018; Szita & Samu, 2000). The genus includes epigeic diurnal hunting spiders that do not use a web for hunting or shelter, they are less agile than the plant dwellers of the genera *Philodromus* and *Tibellus* and they are found on open ground or on logs and stones (Dondale & Redner, 1978b; Szita & Samu, 2000). Females lay the eggs under stones and cavities of rocks, and take care of them until hatching (Szita & Samu, 2000). *T. vulgaris* is an holarctic species that has been described as synanthropic (i.e. a species that lives and benefit from artificial habitats created by humans) and has been found in dry steppes in Europe (Nentwig et al., 2018) which could justify its presence in a rain fed agroecosystem such as was the case of the olive groves studied.

Alopecosa albofasciata was the second most abundant species in the central area of the olive grove. This agrees with the ecology of this stenochronous (i.e. short-lived species with rapid growth and maturation) circum-Mediterranean species which lives in dry, open and sunny places (Lugetti & Tongiorgi, 1969; Nentwig et al., 2018). Alopecosa species are also small and medium size spiders (body length of *A. albofasciata* ranges from 8 - 9 mm in males and 10 - 12 mm in females) (Lugetti & Tongiorgi, 1969; Nentwig et al., 2018). *A. albofasciata* has been reported from meadows, in the Mediterranean scrub, in the open areas of the forest, in the pine forests and in the olive groves (Lugetti & Tongiorgi, 1969). In the peripheral area, *Z. styliferum* was also the second most recorded species. The genus *Zodarion* includes small mirmecomorphic ground anteating spiders (male and female body length ranging from 2.2 - 2.9 mm and 2.9 - 4.8 mm

respectively in *Z. styliferum*) (Nentwig et al., 2018; Pekár, 2004). Accordingly, the abundance of *Zodarion* species within the olive grove agroecosystem is strongly linked to the soil fauna that is numerically dominated by Formicidae (Santos et al., 2007).

Significant changes on the community of spiders were observed from the central area of the olive grove toward the adjacent shrubland area. In terms of functional groups, the abundance of ambushers was significantly lower in the shrubland adjacent area. This is probably due to dominance of *T. vulgaris* in the grove. Moreover, the species richness in the shrubland was not significantly different from those recorded in the olive grove. On the contrary, the abundance of foliage runner hunters significantly increased toward the shrubland area. This increase was probably due to the higher vegetation complexity within the shrubland area which could provide a higher number of habitats than the vegetation cover of the olive grove ground thus reducing the intraguild competition (Finke & Denno, 2002).

The agroecosystem margin usually present physical structures such as stone walls, trails, roads, and different types of edges that may affect the local community composition. In this work, *Civizelotes dentatidens* (Simon, 1914), *Drassodes lapidosus* (Walckenaer, 1802), *Gnaphosa alacris* Simon, 1878, *Haplodrassus dalmatensis* (L. Koch, 1866), *Heser hispanus* Senglet, 2012, *Setaphis carmeli* (O. P.-Cambridge, 1872) represent a pool of species belonging to the family Gnaphosidae which is composed mainly by nocturnal spiders commonly found sheltering under stones that were found exclusively in the peripheral area of the grove or linked to it. These species probably depend on physical structures such as logs and stones that can be used as refuges (Lecq et al., 2017).

Although no significant differences were found between the total species richness in the shrubland and the olive grove, the accumulation curves indicate higher spider diversity in the shrubland area if the sampling effort would have increased to the same number of individuals captured in the grove. In terms of diversity patterns, Picchi et al. (2016) found that densities of Linyphiidae within the grove decreased with the proportion of shrubland in the surrounding landscape. The foliage runner hunters, sensing web builders, sheet web builders and stalkers were

found to be linked to the adjacent shrubland area. These results agree with Langellotto & Denno (2004) who showed that different natural enemy guilds such as hunting spiders (foliage runner hunters and stalkers in this work) and web-building spiders (sensing web builders and sheet web builders in this work) strongly responded to higher habitat complexity.

The adjacent shrubland areas can also play an important role in biological control of pests, Picchi et al. (2017) found an increasing predation pressure on exposed pupae of *B. oleae* in groves and suggested that the vegetation structure and physical characteristics of the Mediterranean shrubland such as dry and warm microclimates and rocky habitats might influence the ground dwelling community. Also, the adjacent shrubland area explained the differences found in the carabid community structure among olive groves at the landscape level (da Silva et al., 2017).

According to Samu & Szinetár (2002), agrobionts are indicators of arable habitats. Nevertheless, in terms of species, field studies are commonly context dependent, for instance, these conclusions should be constrained to the region of Trás-os-Montes, however, the extension of the interpretations to a wider geographical range can be approached from the functional point of view. In this sense, functional counterparts can be found between different regions. For example, Samu & Szinetár (2002) provided a list of agrobiont spiders in alfalfa and cereal crops including the ambusher *Tibellus oblongus* (Walckenaer, 1802) a Philodromidae (ambusher) found amongst grass in wet and dry sunny places (Nentwig et al., 2018) that could be functionally analogous to *T. vulgaris* in this region and crop, and *A. pulverulenta* (Clerck, 1757) a Lycosidae (ground hunter) that could be functionally analogous to *A. albofasciata* in this work. Considering the second indicator of the arable land in this work, *Ozyptila pauxilla* (Simon, 1870) has been also found in the citrus agroecosystem (Mestre et al., 2012) which supports the proposal as agrobiont species.

#### 2.5. Conclusions

The olive grove and its surrounding semi-natural areas provide multiple habitats that support a diverse community of spiders in terms of functional groups and species. Some of these species

are ecologically linked to each area being representative of them which allows their use as bioindicators. Further research on agrobiont and indicator species among spiders within agroecosystems is mandatory to uncover the biotic and abiotic factors that could drive the changes on the community of spiders and how these changes are related to management practices. Disentangling these management-habitat-community relationships is essential to enhance the effectiveness of potential natural enemies such as spiders through the implementation of low-impact agricultural management practices in arable landscapes.

### 2.6. Supplementary material

**Table S1.** Main characteristics of the selected olive groves and their corresponding adjacent shrublands. OGT: olive grove topography; SAT: shrubland area topography; OGA: olive grove altitude; SAL: shrubland altitude; OGA: Olive grove area; SAA: shrubland area; ADR: average distance between rows; ADIR: average distance between inter-rows.

Site name & location (GPS coordinates)	OGT	SAT	OGA (m)	SAL (m)	OGA (ha)	SAA (ha)	ADR (m)	ADIR (m)
Guribanes	Plane/Flat	Sloped	234	265	2.10	2.63	9	9
(41°34'8.97"N, 7°10'1.08"O)								
Valbom-dos-Figos	Sloped	Sloped	355	371	4 00	2.23	9	9
(41°32'59.77"N, 7°8'39.15"O)	Stoped	Stoped	555	571		2.20		
Cedães 1	Sloped	Sland	252	217	8 00	5 22	7	7
(41°29'15.63"N, 7°7'33.58"O)	Sloped	Sloped	555	347	8.90	3.22	1	1
Cedães 2	Dlana/Elat	Claure d	240	226	4.01	2 10	7	7
(41°29'16.86"N, 7°7'51.50"O)	Plane/Flat	Sloped	342	330	4.01	3.18	/	/
Romeu (41°31'47.89"N, 7°4'19.22"O)	Sloped	Plane/Flat	411	398	10.40	2.71	10	10

**Table S2.** Total number of Araneae (N), richness (S) and spider guilds identified in all the samples collected at each strata (vertical and horizontal) in the five study areas in 2014. The code of each species corresponds to the codes used in the speciesbased NMDS analysis and co-inertia plot (Fig. 2.3A & 2.4A). The number (shown in parentheses) of each spider guild corresponds to the number used in the guild-based NMDS analysis and co-inertia plot (Figs. 2.3B & 2.4B). The abundance of the spiders collected in the trunk is expressed as number of individuals/m<sup>2</sup> of sticky trap. OGC: Olive grove central area; OGP: olive grove peripheral area, and S: shrubland area. \*Richness of families.

Cuild/Family	Species	Cada	Conony	C	Fround	Trumb	
Gunu/Fanniy	Species	eciesCodeCanopyGround $0GC$ $0GP$ $S$ $r$ ński, 1911Pa9001875Pl3000 $Simon, 1870$ )Pb1001 $S70$ Tv055450 $370$ Tv055450 $757$ )Pm0113 $r, 1820$ )Lr0020 $S70$ Op010151 $s, 1775$ )Sg1000 $1802$ )Tp4000 $K$ 06621266	1 Fullk				
Ambushers (1)							
Philodromidae	Philodromus albidus Kulczyński, 1911	Pa	9	0	0	0	0.00
	Philodromus lividus Simon, 1875	Pl	30	0	0	0	0.00
72	Pulchellodromus bistigma (Simon, 1870)	Pb1	0	0	0	1	0.00
	Thanatus vulgaris Simon, 1870	Tv	0	55	45	0	0.00
	Philodromidae immatures		34	1	1	0	4.32
Pisauridae	Pisaura mirabilis (Clerck, 1757)	Pm	0	1	1	3	0.00
Sicariidae	Loxosceles rufescens (Dufour, 1820)	Lr	0	0	2	0	0.00
Thomisidae	Ozyptila pauxilla (Simon, 1870)	Op	0	10	15	1	0.00
	Synema globosum (Fabricius, 1775)	Sg	1	0	0	0	0.00
	Tmarus piger (Walckenaer, 1802)	Тр	4	0	0	0	0.00
	Xysticus kochi Thorell, 1872	Xk	0	6	6	2	0.00
	Thomisidae immatures		12	6	6	1	3.15

**Foliage runner hunters (2)** 

Anyphaenidae	Anyphaenidae immatures		0	0	0	0	0.11
Clubionidae	Clubiona spp. (immatures)	Cs	1	0	1	5	0.35
Eutichuridae	Cheiracanthium striolatum Simon, 1878	Cs1	0	0	0	1	0.00
Sparassidae	Olios argelasius (Walckenaer, 1806)	Oa	0	0	0	1	0.00
Ground hunters (3)							
Dysderidae	Dysdera falciformis Barrientos & Ferrandez, 1982	Df	0	0	1	0	0.00
	Dysdera machadoi Ferrandez, 1996	Dm	0	0	0	1	0.00
	Dysdera sp. (immatures)	Ds	0	1	0	0	0.00
	Harpactea sp. 1	Hs	0	0	0	1	0.00
Gnaphosidae	Aphantaulax trifasciata (O. PCambridge, 1872)	At	0	0	0	2	0.00
	Callilepis concolor Simon, 1914	Cc	0	0	1	2	0.00
	Civizelotes civicus (Simon, 1878)	Cc	0	1	0	0	0.00
	Civizelotes dentatidens (Simon, 1914)	Cd	0	0	2	0	0.00
	Civizelotes ibericus Senglet, 2012	Ci	0	6	0	0	0.00
	Drassodes aff. inermis	Da	0	1	0	0	0.00
	Drassodes lapidosus (Walckenaer, 1802)	Dl	0	2	6	2	0.00
	Gnaphosa alacris Simon, 1878	Ga	0	0	3	0	0.00
	Haplodrassus dalmatensis (L. Koch, 1866)	Hd	0	1	4	0	0.00
	Haplodrassus invalidus (O. PCambridge, 1872)	Hi	0	1	0	0	0.00
	Haplodrassus signifer (C. L. Koch, 1839)	Hs1	0	5	0	0	0.00
	Heser hispanus Senglet, 2012	Hh	0	0	2	0	0.00
	Micaria guttulata (C. L. Koch, 1839)	Mg	0	2	0	0	0.00

	Nomisia exornata (C. L. Koch, 1839)	Ne	0	22	26	11	0.00
	Nomisia spp. (immatures)	Ns	0	2	0	0	0.00
	Setaphis carmeli (O. PCambridge, 1872)	Sc	0	0	1	0	0.00
	Synaphosus sauvage Ovtsharenko, Levy & Platnick, 1994	Ss	0	0	0	1	0.00
	Trachyzelotes holosericeus (Simon, 1878)	Th	0	0	2	0	0.00
	Zelotes fulvopilosus (Simon, 1878)	Zf	0	1	0	1	0.00
	Zelotes thorelli Simon, 1914	Zt	0	0	5	3	0.00
	Gnaphosidae immatures		0	19	22	28	3.13
Liocranidae	Liocranidae sp. 1	Ls	0	0	0	1	0.00
	Mesiotelus grancanariensis Wunderlich, 1992	Mg1	0	0	2	1	0.00
Lycosidae	Alopecosa albofasciata (Brullé, 1832)	Aa	0	47	8	8	0.00
	Hogna spp. (immatures)	Hs	0	33	25	0	0.00
	Pardosa hortensis (Thorell, 1872)	Ph	0	0	1	0	0.00
	Pardosa proxima (C. L. Koch, 1847)	Рр	0	0	7	0	0.00
	Lycosidae immatures		0	22	44	20	0.84
Miturgidae	Zora manicata Simon, 1878	Zm	0	0	1	4	0.00
Phrurolithidae	Phrurolinillus lisboensis Wunderlich, 1995	Pl	0	0	0	2	0.00
	Phrurolithus nigrinus (Simon, 1878)	Pn	0	0	3	5	0.00
Zodariidae	Selamia reticulata (Simon, 1870)	Sr1	0	0	4	2	0.00
	Zodarion alacre (Simon, 1870)	Za	0	22	11	7	0.00
	Zodarion duriense Cardoso, 2003	Zd	0	1	1	2	0.00
	Zodarion styliferum (Simon, 1870)	Zs	0	31	29	7	0.00

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	Zodarion spp. (immatures)	Zs	0	2	1	1	0.14
Orb-web builders (4)							
Araneidae	Araniella cucurbitina (Clerck, 1757)	Ac	7	0	0	0	0.00
	Cyclosa algerica Simon, 1885	Ca	1	0	0	0	0.00
	Mangora acalypha (Walckenaer, 1802)	Ma	2	0	1	0	0.00
	Araneidae immatures		6	0	0	0	0.26
Tetragnathidae	Tetragnathidae immatures		3	0	0	0	0.00
Uloboridae	Uroctea durandi (Latreille, 1809)	Ud	0	1	0	0	0.00
Sensing web builders (5)	•						
Oecobidae	Oecobius spp. (immatures)	Os	0	0	0	2	0.00
Sheet web builders (6)							
3 Agelenidae	Eratigena bucculenta (L. Koch, 1868)	Eb	0	0	1	0	0.00
	Eratigena feminea (Simon, 1870)	Ef	0	2	0	6	0.00
	Eratigena picta (Simon, 1870)	Ep	0	0	0	3	0.00
	Eratigena spp.	Es	0	2	3	0	0.00
	Malthonica lusitanica Simon, 1898	Ml	0	0	4	4	0.00
	Agelenidae immatures		0	1	1	0	0.27
Eresidae	Eresus kollari Rossi, 1846	Ek	0	0	0	1	0.00
Space web builders (7)							
Dictynidae	Brigittea civica (Lucas, 1850)	Bc	2	0	0	0	0.00
	Nigma puella (Simon, 1870)	Np	0	0	1	0	0.00
	Dictynidae immatures		6	0	0	0	0.37

Theridiidae	Asagena phalerata (Panzer, 1801)	Ap	0	2	2	1	0.00
	Phylloneta impressa (L. Koch, 1881)	Pi	1	0	0	0	0.00
	Theridion mystaceum L. Koch, 1870	Tm	2	0	0	0	0.00
	Theridiidae immatures		2	0	0	0	1.89
Titanoecidae	Titanoeca praefica (Simon, 1870)	Тр	0	1	0	0	0.00
Stalkers (8)							
Oxyopidae	Oxyopes nigripalpis Kulczyński, 1891	On	1	1	1	0	0.00
	Oxyopes sp. (immatures)	Os	1	0	0	0	0.11
Salticidae	Aelurillus luctuosus (Lucas, 1846)	Al	0	0	1	3	0.00
	Ballus variegatus Simon, 1876	Bv	3	0	0	0	0.00
	Chalcoscirtus infimus (Simon, 1868)	Cil	0	1	1	0	0.00
	Euophrys gambosa (Simon, 1868)	Eg	0	1	1	1	0.00
	Euophrys herbigrada (Simon, 1871)	Eh	0	0	1	3	0.00
	Icius hamatus (C. L. Koch, 1846)	Ih	0	1	0	0	0.00
	Leptorchestes peresi (Simon, 1868)	Lp	0	0	0	2	0.00
	Pellenes brevis (Simon, 1868)	Pb2	0	0	1	1	0.00
	Pellenes geniculatus (Simon, 1868)	Pg	0	0	1	1	0.00
	Salticidae immatures		6	2	1	0	2.76
Scytodidae	Scytodes spp. (immatures)	Ss	0	0	0	2	0.00
	Scytodes velutina Heineken & Lowe, 1832	Sv	0	0	0	5	0.00

Wandering sheet/

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tangle weavers (9)

Linyphiidae	Agyneta fuscipalpa (C. L. Koch, 1836)	Af	0	3	0	0	0.00
	Agyneta rurestris (C. L. Koch, 1836)	Ar	0	2	0	0	0.00
	Agyneta sp. 1	As	0	5	2	0	0.00
	Diplocephalus marijae Bosmans, 2010	Dm1	0	5	0	0	0.00
	Diplocephalus permixtus (O. PCambridge, 1871)	Dp	0	0	0	1	0.00
	Neriene furtiva (O. PCambridge, 1871)	Nf	0	0	0	1	0.00
	Pelecopsis inedita (O. PCambridge, 1875)	Pi	0	4	3	0	0.00
	Pelecopsis bucephala (O. PCambridge, 1875)	Pb	0	4	8	8	0.00
	Styloctetor romanus (O. PCambridge, 1873)	Sr	0	1	0	0	0.00
	Tenuiphantes tenuis (Blackwall, 1852)	Tt	2	0	0	0	0.00
	Walckenaeria dalmasi (Simon, 1914)	Wd	0	0	0	1	0.00
	Erigoninae immatures		99	8	11	7	62.49
	Linyphiidae immatures		6	0	0	1	0.00
Araneae immatures			11	0	0	1	1.19
Total species richness (S)			15	35	43	43	14*
Total abundance (N)			252	348	334	182	81.38

**Table S3.** Results of the similarity percentages (SIMPER) analysis for the spider species collected along a horizontal gradient in the olive grove agroecosystem in Trás-os-Montes. Species presenting a superscript correspond to the pool of species that contributed at least to 70 % of the differences between a pair of sampling areas, 1: OGC-OGP, 2: OGP-S, and 3: OGC-S respectively. OGC: Olive grove central area; OGP: olive grove peripheral area, and S: shrubland area. Average: average contribution to overall Bray-Curtis dissimilarity; sd: standard deviation of contribution, and P: permutation p-value (i.e. the probability of getting a larger or equal average contribution in random permutation of the group factor) (p < 0.01).

Spacing	OG	C vs. O(	<b>JP</b>	0	GP vs. S	5	0	GC vs. S	5
Species	Average	sd	Р	Average	sd	Р	Average	sd	Р
Aelurillus luctuosus <sup>2,3</sup>	0.002	0.008	0.993	0.017	0.047	0.143	0.015	0.046	0.367
Agyneta fuscipalpa <sup>3</sup>	0.012	0.058	0.629	-	-	-	0.016	0.074	0.001*
Agyneta rurestris	0.007	0.024	0.387	-	-	-	0.010	0.031	0.001*
Agyneta sp. 1	0.013	0.032	0.170	0.004	0.019	0.882	0.012	0.034	0.357
Alopecosa albofasciata <sup>1,2,3</sup>	0.074	0.127	0.344	0.045	0.083	0.920	0.081	0.137	0.168
Aphantaulax trifasciata	-	-	-	0.006	0.029	0.574	0.006	0.029	0.658
Asagena phalerata	0.009	0.021	0.554	0.009	0.025	0.513	0.008	0.023	0.590
Callilepis concolor	0.004	0.021	0.883	0.013	0.045	0.213	0.008	0.038	0.634
Chalcoscirtus infimus	0.005	0.016	0.448	0.003	0.013	0.667	0.004	0.016	0.448
Cheiracanthium striolatum	-	-	-	0.003	0.015	0.574	0.003	0.014	0.658
Civizelotes civicus <sup>1,3</sup>	0.002	0.007	0.351	-	-	-	0.002	0.008	0.002*
Civizelotes dentatidens	0.003	0.015	0.646	0.004	0.017	0.002*	-	-	-
Civizelotes ibericus	0.017	0.047	0.228	-	-	-	0.021	0.057	0.001*

Diplocephalus marijae	0.008	0.027	0.413	-	-	-	0.009	0.030	0.002*
Diplocephalus permixtus	-	-	-	0.003	0.015	0.552	0.003	0.014	0.662
Drassodes aff. inermis	0.002	0.007	0.606	-	-	-	0.002	0.008	0.002*
Drassodes lapidosus <sup>1,2</sup>	0.020	0.047	0.534	0.026	0.058	0.084	0.012	0.030	0.890
Dysdera falciformis	0.002	0.010	0.668	0.003	0.013	0.002*	-	-	-
Dysdera machadoi	-	-	-	0.004	0.019	0.558	0.004	0.019	0.635
Eratigena bucculenta	0.002	0.010	0.668	0.003	0.013	0.002*	-	-	-
Eratigena feminea <sup>2,3</sup>	0.006	0.029	0.982	0.025	0.056	0.321	0.030	0.059	0.068
Eratigena picta	-	-	-	0.009	0.024	0.180	0.009	0.024	0.224
Eresus kollari	-	-	-	0.004	0.014	0.367	0.004	0.014	0.434
Euophrys gambosa	0.005	0.016	0.756	0.007	0.021	0.320	0.005	0.018	0.594
Euophrys herbigrada	0.004	0.019	0.968	0.016	0.039	0.138	0.012	0.034	0.477
Gnaphosa alacris	0.006	0.019	0.427	0.007	0.023	0.001*	-	-	-
Haplodrassus dalmatensis	0.011	0.028	0.284	0.010	0.031	0.275	0.004	0.016	0.835
Haplodrassus invalidus	0.002	0.010	0.606	-	-	-	0.003	0.012	0.003*
Haplodrassus signifer <sup>3</sup>	0.013	0.039	0.235	-	-	-	0.017	0.049	0.001*
Harpactea sp. 1	-	-	-	0.004	0.019	0.558	0.004	0.019	0.635
Heser hispanicus	0.005	0.015	0.417	0.006	0.018	0.001*	-	-	-
Icius hamatus	0.002	0.007	0.606	-	-	-	0.002	0.008	0.002*
Leptorchestes peresi	-	-	-	0.006	0.019	0.338	0.006	0.018	0.403
<i>Liocranidae</i> sp. 1	-	-	-	0.003	0.015	0.552	0.003	0.014	0.662
Loxosceles rufescens	0.004	0.013	0.399	0.005	0.016	0.001*	-	-	-

Malthonica lusitanica <sup>2</sup>	0.007	0.031	0.887	0.021	0.055	0.204	0.013	0.045	0.591
Mangora acalypha	0.002	0.007	0.646	0.002	0.009	0.002*	-	-	-
Mesiotelus grancanariensis	0.003	0.015	0.873	0.008	0.025	0.234	0.004	0.019	0.677
Micaria guttulata	0.003	0.010	0.354	-	-	-	0.003	0.012	0.001*
Neriene furtiva	-	-	-	0.003	0.013	0.561	0.003	0.013	0.624
Nigma puella	0.002	0.008	0.639	0.002	0.009	0.002*	-	-	-
Nomisia exornata <sup>1,2,3</sup>	0.075	0.079	0.750	0.099	0.118	0.054	0.082	0.103	0.526
Olios argelasius	-	-	-	0.003	0.015	0.574	0.003	0.014	0.658
Oxyopes nigripalpis	0.006	0.021	0.415	0.004	0.016	0.661	0.005	0.022	0.428
Ozyptila pauxilla <sup>1,2,3</sup>	0.045	0.064	0.208	0.040	0.070	0.449	0.038	0.069	0.539
Pardosa hortensis	0.002	0.008	0.671	0.002	0.009	0.002*	-	-	-
Pardosa proxima	0.012	0.053	0.639	0.014	0.062	0.002*	-	-	-
Pelecopsis bucephala <sup>1,2,3</sup>	0.025	0.050	0.904	0.045	0.081	0.128	0.033	0.068	0.651
Pelecopsis inedita	0.012	0.025	0.143	0.008	0.024	0.714	0.009	0.020	0.613
Pellenes brevis	0.002	0.010	0.877	0.007	0.024	0.284	0.004	0.021	0.648
Pellenes geniculatus	0.002	0.009	0.890	0.005	0.017	0.237	0.003	0.014	0.679
Phrurolinillus lisboensis	-	-	-	0.007	0.032	0.616	0.007	0.031	0.596
Phrurolithus nigrinus <sup>2</sup>	0.005	0.016	0.949	0.019	0.041	0.081	0.014	0.039	0.499
Pisaura mirabilis	0.003	0.010	0.966	0.011	0.024	0.205	0.010	0.024	0.267
Pulchellodromus bistigma	-	-	-	0.004	0.019	0.558	0.004	0.019	0.635
Scytodes velutina <sup>2,3</sup>	-	-	-	0.017	0.039	0.132	0.017	0.039	0.171
Selamia reticulata <sup>2</sup>	0.010	0.027	0.762	0.019	0.038	0.033*	0.008	0.026	0.879

Setaphis carmeli	0.002	0.008	0.627	0.002	0.010	0.002*	-	-	-
Styloctetor romanus	0.002	0.008	0.359	-	-	-	0.003	0.009	0.002*
Synaphosus sauvage	-	-	-	0.004	0.014	0.367	0.004	0.014	0.434
Thanatus vulgaris <sup>1,2,3</sup>	0.183	0.211	0.036*	0.112	0.172	0.913	0.163	0.246	0.208
Titanoeca praefica	0.003	0.016	0.582	-	-	-	0.004	0.020	0.002*
Trachyzelotes holosericeus	0.006	0.029	0.649	0.008	0.036	0.002*	-	-	-
Uroctea durandi	0.005	0.025	0.554	-	-	-	0.007	0.034	0.002*
Walckenaeria dalmasi	-	-	-	0.004	0.017	0.594	0.004	0.017	0.622
Xysticus kochi <sup>1,3</sup>	0.020	0.036	0.478	0.015	0.034	0.802	0.023	0.044	0.237
Zelotes fulvopilosus	0.003	0.015	0.847	0.004	0.014	0.739	0.007	0.022	0.128
Zelotes thorelli <sup>2</sup>	0.010	0.027	0.724	0.019	0.045	0.025*	0.008	0.035	0.842
Zodarion alacre <sup>1,2,3</sup>	0.055	0.070	0.384	0.050	0.074	0.654	0.055	0.069	0.399
Zodarion duriense	0.005	0.020	0.864	0.012	0.033	0.245	0.009	0.027	0.568
Zodarion styliferum <sup>1,2,3</sup>	0.087	0.105	0.360	0.093	0.122	0.170	0.067	0.099	0.871
Zora manicata	0.002	0.008	0.991	0.013	0.033	0.138	0.012	0.033	0.369
Overall between-group Bray-Curtis dissimilarity		0.840			0.922			0.937	

**Table S4.** Results of the similarity percentages (SIMPER) analysis for the functional groups of spiders collected along a horizontal gradient in the olive grove agroecosystem in Trás-os-Montes. Functional groups presenting a superscript correspond to the pool of functional groups that contributed at least to 70 % of the differences between a pair of sampling areas, 1: OGC-OGP, 2: OGP-S, and 3: OGC-S respectively. OGC: Olive grove central area; OGP: olive grove peripheral area, and S: shrubland area. Average: average contribution to overall Bray-Curtis dissimilarity; sd: Standard deviation of contribution, and P: permutation p-value (i.e. the probability of getting a larger or equal average contribution in random permutation of the group factor) (p < 0.01).

Functional group	OGC	vs. OC	SP	0	GP vs. S	5	00	OGC vs. S			
r uncuonar group	Average	sd	Р	Average	sd	Р	Average	sd	Р		
Ambushers <sup>1,2,3</sup>	0.145	0.150	0.227	0.128	0.132	0.622	0.159	0.185	0.034		
Foliage runner hunters	0.001	0.006	0.999	0.016	0.031	0.060	0.015	0.030	0.135		
Ground hunters <sup>1,2,3</sup>	0.248	0.180	0.651	0.260	0.185	0.400	0.266	0.212	0.263		
Orb web builder	0.004	0.015	0.417	0.001	0.007	0.648	0.004	0.021	0.327		
Sensing web builders	0.000	0.000	1.000	0.004	0.018	0.655	0.004	0.017	0.610		
Sheet web builders	0.017	0.029	0.974	0.038	0.070	0.234	0.035	0.069	0.418		
Space web builders	0.008	0.017	0.425	0.007	0.015	0.761	0.009	0.021	0.312		
Stalkers	0.016	0.021	1.000	0.038	0.042	0.022*	0.036	0.039	0.082		
Wandering sheet/tangle weavers	0.051	0.052	0.919	0.055	0.056	0.763	0.067	0.071	0.067		
Overall between-group		102			0 5 4 9			0.505			
Bray-Curtis dissimilarity	0.492				0.348			1.373			

# CHAPTER 3

Stones on the ground in olive groves promotes the presence of spiders (Araneae)

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### **Contribution of authors:**

J.B.M., S.A.P.S., J.A.P. and J.P.S. conceived and designed the experiment, J.B.M. and S.A.P.S. collected the field data, J.B.M and J.A.B. identified the spiders, J.B.M analyzed the data, and all the authors contributed to writing the paper.

#### **CHAPTER 3**

#### Stones on the ground in olive groves promotes the presence of spiders (Araneae)

#### Abstract

Spiders are generalist predators that contribute to the control of pests in agroecosystems. Land use management determines habitats including refuges for hibernation and aestivation. The availability of shelters on the ground can be crucial for maintaining populations of spider within crops. The effect of the number of stones on the surface of the soil on the spider community was studied in selected olive groves in Trás-os-Montes (northeastern Portugal). The number of stones significantly influenced the overall diversity of spiders, abundance of immature individuals and abundance of ground hunters. Agricultural management practices aimed at the conservation of soil microhabitats such as hedgerows, stonewalls and stones on the ground should be promoted in order to maintain or increase the number of shelters for potential natural enemies of pests.

#### **3.1. Introduction**

The cultivation of olive trees (*Olea europaea* L., 1753) is a common agricultural activity in the Mediterranean region, where it is of high economic and cultural importance (Breton et al., 2009; Benhadi-Marín et al., 2016b). Olive trees are susceptible to attack by different species of pests such as the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) and olive moth *Prays oleae* Bernard (Lepidoptera: Yponomeutidae), which cause important quantitative and qualitative losses in the main olive crop areas every year (Ramos et al., 1998; González-Núñez et al., 2008).

Olive groves can be managed in one of three main ways: the conventional system (based on chemical pest control), the integrated production system (based on a narrow spectrum tools), and the organic farming system, which avoids the use of synthetic chemicals (Cárdenas et al., 2015).

Among the arthropods inhabiting olive groves, spiders are one of the dominant groups (Cárdenas et al., 2015). Since spiders are euryphagous predators (i.e., they are non-selective and can consume a great variety of species of prey) they can contribute to the reduction of important pests. There are several examples of biological control provided by spiders in different crops such as wheat (Chiverton, 1986; Kuusk et al. 2008; Oelbermann & Scheu, 2009), cotton (Ghavami, 2008), apple (László et al., 2015) and citrus (Monzó et al., 2010). Also, within the olive grove agroecosystem spiders can control the olive fruit fly (Picchi et al., 2016).

However, different management practices within integrated production and organic farming systems, such as superficial tillage and plowing, could affect the community of spiders in different ways, for instance, by reducing the habitat complexity and destroying shelters. Several habitats (e.g. pounds, dry stone walls and hedges) are often used as ecological infrastructures by animals including small mammals, birds, reptiles, amphibians, molluscs, grasshoppers, ground beetles, dragonflies, butterflies and bees (see Boller et al., 2004). Nonetheless, the influence of potential ground shelters, such as stones on the surface of the

ground, on animal biodiversity in agroecosystems has been poorly investigated. The objective of this work was to study the effect of the number of stones occurring on soil, on the abundance, richness and guilds of the ground spider community in selected olive groves in Trás-os-Montes (Portugal).

#### **3.2. Material and methods**

Field sampling sites were located in eight olive groves near Mirandela (northeastern Portugal), Avantos Bio (41°33'34.39"N, -7°05'37.17"W), Avantos Prodi (41°32'17.31"N, -7°05'43.89"W), Cedães (41°29'16.86"N, -7°07'34.02"W), Guribanes (41°34'12.25"N, -7°09'59.01"W), São Pedro Bio (41°25'44.91"N, -7°12'20.71"W), São Pedro Prodi (41°26'38.09"N, -7°13'28.69"W), Suçães (41°29'30.02"N, -7°15'28.72"W) and Valbom-dos-Figos (41°33'00.58"N, -7°08'39.92"W). Organic farming was carried out at Avantos Bio, Guribanes, São Pedro Bio and Valbom-dos-Figos and integrated pest management at Avantos Prodi, Cedães, São Pedro Prodi and Suçães.

Sampling took place in the spring of 2011 using pitfall traps. A total of 16 pitfall traps distributed in the form of a regular  $4 \times 4$  square grid, spaced between 45–50 m from one another and located in the center of each of the olive groves studied. Each trap consisted of a plastic cup (115 mm in diameter at the top and 130 mm in height) dug into the ground and filled with 250 ml of ethylene glycol as a preservative. Traps were operated over a period of seven nights. In the laboratory, all the spiders were preserved in 70% ethanol, sorted and identified to species (when possible) using a binocular stereomicroscope and following Nentwig et al. (2018).

Since stones are located on the ground in a non-random way 12 areas of  $1 \text{ m}^2$  were randomly selected in each olive grove independently to the position of the pitfall traps. In each area, the number of stones was counted and scaled to the mean number of stones/m<sup>2</sup> in each grove. Considering the importance of herbaceous vegetation and moisture on the arthropod communities within agroecosystems (Stamps & Linit, 1998), the percentage of vegetation cover and relative humidity at the location of each pitfall trap were included as

explanatory variables. Also, the factor "management type" (organic vs. integrated) and the random effect of grove were included in the models.

The dependent variables considered were: (1) total abundance of spiders, (2) total species richness, (3) abundance of adults, (4) abundance of immatures and (5) abundance of functional groups (i.e., six guilds) according to Cardoso et al. (2011). The guild corresponding to orb-weavers was excluded from the analysis because it was represented by a singleton. The effect of the number of stones on each dependent variable was evaluated using generalized mixed linear models following Zuur et al. (2009). Since all the dependent variables were count data, they were modeled using a random intercept and slope model with negative binomial distribution (to deal with over dispersion) and logarithmic link. For each dependent variable, a full model was firstly fitted of the generic form:

$$Yi \sim NB(\mu_i, k)$$

$$E(Y_i) = \mu_i \text{ and } \operatorname{var}(Y_i) = \mu_i + \mu^2 i/k$$

$$\log(\mu_i) = \eta(X_{i1}, \dots, X_{iq}) = \alpha + \beta_1 \times X_{i1} + \dots + \beta_q \times X_{iq} + Z_i \times b_i$$
[Eq. 1]

were  $\alpha$  denotes the intercept,  $\beta$  the parameters to be estimated, *X* each explanatory variable, and the term  $Z_i \times b_i$  the random effect of the grove. Then backward model selection based on the Akaike information criterion (AIC) was used to obtain the optimal model for each response variable (Zuur et al., 2009). In the cases where the effect of management was statistically significant, an independent model was built for each management and the model selection performed as described above. The random effect term was systematically kept in all models. All statistical analyses and modeling were performed in R (R Core Team, 2018).

#### 3.3. Results and discussion

A total of 1621 spiders, 1105 adults (9.13  $\pm$  0.44) (mean number per trap  $\pm$  SE) and 516 immatures (4.26  $\pm$  1.19) (mean number per trap  $\pm$  SE) were captured. Among the collected

material there were 19 families, 60 species (Table S5) and seven functional groups. The guild of ground hunters  $(8.32 \pm 0.76)$  (mean number per trap  $\pm$  SE) was the most abundant functional group, followed by specialists  $(2.12 \pm 0.19)$  (mean number per trap  $\pm$  SE), sheet web builders  $(1.12 \pm 0.10)$ , ambushers  $(0.87 \pm 0.08)$ , other hunters  $(0.69 \pm 0.06)$ , space web builders  $(0.22 \pm 0.02)$  and orb weavers (one individual).

Among the dependent variables considered, the number of stones had a statistically significant effect on total spider abundance, species richness, abundance of immatures (Fig. 3.1), abundance of sheet web builders under integrated pest management, and abundance of ground hunters (Fig. 3.2; Table S6). Also, the percentage of vegetation cover significantly affected the abundance of space web builders, whereas a significant effect of management was found only for the abundance of sheet web builders (Table S6). In all cases, the effect of the number of stones was found to be positive when statistically significant whereas the effect of the percentage of vegetation cover negatively influenced the abundance of space web builders (Table S7).

The significantly negative effect of a higher percentage of vegetation recorded for space web builders could be due to a numerical artifact because of zero-inflation. However, the positive effect of the abundance of stones on the abundance of ground hunters was consistent and can be explained in terms of spider growth and their need to hide from predators. Birds and other spiders are common predators of spiders. While large spiders ( $\geq$  2.5 mm) seem to be more vulnerable to bird predation, intraguild predation may have a greater effect on small individuals ( $\leq$  2.5 mm), especially during winter (Gunnarsson, 1983). However, there is still a lack of knowledge on the effect of predators on spider richness (Gunnarsson, 2007). In fact, intraguild predation is an important community driver for predator population regulation. Within structurally-simple habitats such as most agricultural landscapes, intraguild predation between predators can reduce the pressure of predators on the herbivore community (Finke & Denno, 2006). Hence, sites with a high availability of stones for refuge may help both immature and adult spiders to cope with

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**Figure 3.1.** Total abundance of spiders (A), species richness (B), abundance of adult spiders (C) and immatures (D) relative to the number of stones. Each line indicates the relationship between spider abundance and the number of stones per square meter.



**Figure 3.2.** Total abundance of different guilds of spiders: space web weavers (A), ground hunters (B) and sheet web weavers under organic (C), and integrated management (D) relative to the number of stones. Each line indicates the relationship between spider abundance and the number of stones per square meter.

different selective forces by providing a spatial refuge from predators and reducing intraguild predation (i.e. allowing coexistence) that could enhance pest suppression.

Finally, environmental factors such as light and temperature affect the embryogenesis and post-embryogenesis of spiders (Napiórkowska et al., 2018). Thus, the survival of spiders, especially nocturnal species (e.g. Agelenidae and Gnaphosidae) may depend on the existence of dark and warm (in winter) or fresh (in summer) breeding sites (i.e. under stones) in which immature development is accelerated and mortality reduced.

This study focuses for the first time on the effect of the number of stones on the surface of the soil on the spider community. In this context, roots, logs and stones can provide refuges for many species and provide a complex structure of microhabitats (Lecq et al., 2017). In this study, the diversity of spiders, both in terms of abundance and species diversity are positively associated with the number of stones on the surface of the soil, most likely due to their need for shelter in the early stages of development in the case of immature spiders, and as places where food and shelter can be found as well as places for reproduction in the case of adult ground hunters. The results are similar to those reported by Lecq et al. (2017) on the availability of shelter in agricultural hedgerows. For example, they report that the trend in the relative abundance of the morph species, *Tegenaria* sp. (Agelenidae), a species of spider that depends on ground refuges for shelter, was opposite to that of species inhabiting open habitats such as *Microtus* sp. (Cricetidae). As da Silva et al. (2017) suggest, conservation and management strategies within agricultural landscapes need to consider small-scale changes in landscape architecture.

The "habitat heterogeneity hypothesis" assumes that complex habitats provide a greater diversity of niches and ways of exploiting environmental resources, which results in an increase in species diversity (Bazzaz, 1975; Tews et al., 2004). Moreover, species can be closely linked to 'keystone structures' (i.e. distinct spatial structures that provide resources, shelter or 'goods and services' that may determine biological diversity) whose detection is important for conservation and biodiversity management (Tews et al., 2004). Land management determines the number of habitats, abundance of food and refuges, and

hibernation, and estivation shelters (Duru et al., 2015) and an understanding the resources needed by natural enemies can help to identify the key factors determining their diversity. In terms of shelter, providing habitats for overwintering and reducing the use of pesticides (Landis et al., 2000) may enhance the overall action of natural enemies. This conforms with the results found in this work, especially in the case of the sheet web builders' guild, which was clearly favored by a higher number of stones in groves under integrated production management, which indicates that maintaining keystone structures such as shelter is especially important in non-organic farming.

Regarding spiders in general, increasing the number of stones within crops could be a promising area in biological control. In this context, further research on how small-scale shelters (e.g. stones, ground holes, roots, and logs) and other potentially collinear local variables such as ground density, soil pliability and vegetation structure influence the community of natural enemies is needed.

In conclusion, low-cost activities for the farmer such as building dry stone walls, and maintaining hedgerows can provide abundant ground refuges (Lecq et al., 2017; Le Viol et al., 2008). The manipulation of habitat structure with the objective of increasing its complexity can improve the biocontrol service provided by spiders (Michalko et al., 2017). In addition, it is recommended reducing aggressive agrarian practices that affect the structure of the soil (e.g. deep plowing) which alters its physical characteristics. Instead, soil scarification is a method traditionally used for natural regeneration (Jäärats et al., 2012) that can also help in controlling weeds in agricultural landscapes and is more respectful of the ground refuges of potential natural enemies, such as spiders.

## **3.4. Supplementary material**

**Table S5**. Total number of Araneae and spider guilds identified in all the samples collected on the ground in the olive groves at the eight sites studied in 2011. (1) Ambush hunters, (2) ground hunters, (3) orb web weavers, (4) other hunters, (5) sheet web weavers, (6) space web weavers, and (7) specialists.

Family (guild) / Species	Total
Agelenidae (5)	
Eratigena picta (Simon, 1870)	1
Eratigena feminea (Simon, 1870)	13
Agelenidae unid. immatures	1
Subtotal Agelenidae	15
Araneidae (3)	
Hypsosinga albovittata (Westring, 1851)	1
Subtotal Araneidae	1
Dictynidae (6)	
Dictynidae sp. 1	1
Subtotal Dictynidae	1
Dysderidae (7)	
Dysdera fuscipes Simon, 1882	1
Subtotal Dysderidae	1
Gnaphosidae (2)	
Drassodes lapidosus (Walckenaer, 1802)	13
Drassodes sp. 1	2
Haplodrassus dalmatensis (L. Koch, 1866)	71
Haplodrassus invalidus (O. PCambridge, 1872)	3
Haplodrassus rufipes (Lucas, 1846)	15
Haplodrassus signifer (C. L. Koch, 1839)	11
Micaria pallipes (Lucas, 1846)	1
Nomisia exornata (C. L. Koch, 1839)	258
Setaphis carmeli (O. PCambridge, 1872)	19
Zelotes thorelli Simon, 1914	18

Zelotes sp. 1	13	
Gnaphosidae unid. immatures	64	
Subtotal Gnaphosidae	488	
Linyphiidae (4)		
Erigone promiscua (O. PCambridge, 1873)	16	
Agyneta fuscipalpa (C. L. Koch, 1836)	32	
Agyneta pseudorurestris Wunderlich, 1980	1	
Agyneta rurestris (C. L. Koch, 1836)	10	
Oedothorax fuscus (Blackwall, 1834)	2	
Ostearius melanopygius (O. PCambridge, 1879)	1	
Ouedia rufithorax (Simon, 1881)	1	
Pelecopsis bicornuta Hillyard, 1980	3	
Pelecopsis inedita (O. PCambridge, 1875)	5	
Pelecopsis sp.	1	
Prinerigone vagans (Audouin, 1826)	11	
Trichopterna cucurbitina (Simon, 1881)	2	
Typhochrestus bogarti Bosmans, 1990	7	
Walckenaeria dalmasi (Simon, 1914)	1	
Linyphiidae immatures	19	
Subtotal Linyphiidae	112	
Liocranidae (2)		
Mesiotelus mauritanicus Simon, 1909	7	
Subtotal Liocranidae	7	
Lycosidae (2)		
Alopecosa albofasciata (Brullé, 1832)	79	
Arctosa perita (Latreille, 1799)	3	
Arctosa villica (Lucas, 1846)	8	
Hogna radiata (Latreille, 1817)	11	
Pardosa proxima (C. L. Koch, 1847)	41	
Lycosidae unid. immatures	367	
Subtotal Lycosidae		
Miturgidae (2)		
Zora spinimana (Sundevall, 1833)	1	

Subtotal Miturgidae		
Oxyopidae (4)		
Oxyopes heterophthalmus (Latreille, 1804)	2	
Subtotal Oxyopidae	2	
Philodromidae (4)		
Philodromus sp.	17	
Subtotal Philodromidae	17	
Phrurolithidae (2)		
Phrurolithus nigrinus (Simon, 1878)	1	
Phrurolithus sp.	1	
Subtotal Phrurolithidae	2	
Pisauridae (5)		
Pisaura mirabilis (Clerck, 1757)	8	
Subtotal Pisauridae	8	
Salticidae (4)		
Aelurillus luctuosus (Lucas, 1846)	3	
Chalcoscirtus infimus (Simon, 1868)	4	
Euophrys gambosa (Simon, 1868)	2	
Euophrys herbigrada (Simon, 1871)	3	
Evarcha jucunda (Lucas, 1846)	2	
Neaetha membrosa (Simon, 1868)	9	
Pellenes brevis (Simon, 1868)	21	
Pellenes geniculatus (Simon, 1868)	4	
Phlegra bresnieri (Lucas, 1846)	13	
Salticidae unid. immatures	2	
Subtotal Salticidae	63	
Sparassidae (4)		
Micrommata ligurina (C. L. Koch, 1845)	1	
Subtotal Sparassidae	1	
Theridiidae (6)		
Asagena phalerata (Panzer, 1801)	12	
Euryopis episinoides (Walckenaer, 1847)		
Steatoda albomaculata (De Geer, 1778)	12	

Total	1621
Araneae unid. immatures	7
Subtotal Zodariidae	255
Zodarion sp.	10
Zodarion styliferum (Simon, 1870)	51
Zodarion duriense Cardoso, 2003	25
Zodarion alacre (Simon, 1870)	168
Selamia reticulata (Simon, 1870)	1
Zodariidae (7)	
Subtotal Titanoecidae	1
Titanoeca monticola (Simon, 1870)	1
Titanoecidae (6)	
Subtotal Thomisidae	105
Thomisidae unid. immatures	12
<i>Xysticus</i> sp.	1
Xysticus kochi Thorell, 1872	72
Xysticus ferrugineus Menge, 1876	3
Xysticus bliteus (Simon, 1875)	2
Ozyptila pauxilla (Simon, 1870)	15
Thomisidae (1)	
Subtotal Theridiidae	25

**Table S6**. Results of the generalized linear mixed model selection of the different components of spiders diversity considered (Response) against different crop variables in olive groves at Trás-os-Montes. The full and the optimal model (in bold) after model selection is presented for each diversity component. Stones: number of stones /  $m^2$ ; Vegetation: percentage of vegetation cover; Management: integrated vs. organic; Moisture: percentage of relative humidity; Stones|Grove: random effect component for the olive grove. Df: degrees of freedom; AIC: Akaike information criterion; Organic: Organic management; IPM: Integrated Pest Management. An asterisk indicates statistical significance at  $\alpha < 0.05$ .

Response	Model			$\chi^2$	Р	AIC
Total spider abundance (TSA)			1	128.267	< 0.001*	
		Vegetation	1	0.1242	0.725	850.044
	$1SA \sim \alpha + \beta_1 \times Stones + \beta_2 \times Vegetation + \beta_3 \times Management + \beta_4 \times Moisture + \beta_5 \times (Stones Grove)$		1	15.684	0.210	830.044
			1	27.519	0.097	
	$TSA \sim \alpha + \beta_1 \times Stones + \beta_2 \times (Stones   Grove)$	Stones	1	83.038	0.004*	845.658
		Stones	1	60.074	0.014*	
a	$S \sim \alpha + \beta_1 \times Stones + \beta_2 \times Vegetation + \beta_3 \times Management + \beta_4 \times Moisture + \beta_5 \times (Stones Grove)$	Vegetation	1	0.4818	0.488	547.030
Species richness		Management	1	11.675	0.280	
(8)		Moisture	1	0.0374	0.847	
	$\mathbf{S} \sim \alpha + \beta_1 \times \mathbf{Stones} + \beta_2 \times (\mathbf{Stones}   \mathbf{Grove})$	Stones	1	53.364	0.021*	542.516
	A ~ $\alpha + \beta_1 \times \text{Stones} + \beta_2 \times \text{Vegetation} + \beta_3 \times \text{Management} + \beta_4 \times \text{Moisture} + \beta_5 \times (\text{Stones} \text{Grove})$	Stones	1	38.414	0.050	722.114
		Vegetation	1	0.9124	0.340	
Abundance of adults		Management	1	11.883	0.276	
(A)		Moisture	1	22.204	0.136	
	$\mathbf{A} \sim \boldsymbol{\alpha} + \boldsymbol{\beta}_1 \times \mathbf{Stones} + \boldsymbol{\beta}_2 \times (\mathbf{Stones}   \mathbf{Grove})$		1	35.851	0.058	719.050
	I ~ $\alpha + \beta_1 \times \text{Stones} + \beta_2 \times \text{Vegetation} + \beta_3 \times \text{Management} + \beta_4 \times \text{Moisture} + \beta_5 \times (\text{Stones} \text{Grove})$	Stones	1	77.087	0.005*	
		Vegetation	1	0.4032	0.525	550 220
Abundance of immatures (I)		Management	1	0.2724	0.602	559.520
		Moisture	1	0.0021	0.964	
	$\mathbf{I} \sim \alpha + \beta_1 \times \mathbf{Stones} + \beta_2 \times (\mathbf{Stones}   \mathbf{Grove})$	Stones	1	73.745	0.007*	554.088
Abundance of ambush hunters (AH)	<b>rs</b> AH ~ $\alpha + \beta_1 \times \text{Stones} + \beta_2 \times \text{Vegetation} + \beta_3 \times \text{Management} + \beta_4 + \text{Moisture} + \beta_5 \times (\text{Stones} \text{Grove})$	Stones	1	0.7482	0.387	
		Vegetation	1	0.2613	0.609	307.356
		Management	1	0.7961	0.372	

		Moisture	1	17.323	0.188	
	$\mathbf{AH} \sim \alpha + \beta_1 \times \mathbf{Stones} + \beta_2 \times (\mathbf{Stones}   \mathbf{Grove})$		1	0.5829	0.445	303.812
		Stones	1	11.217	0.290	
	C.W	Vegetation	1	50.529	0.025*	147 544
Abundance of space web builders	, Spw ~ $\alpha + \beta_1 \times \text{Stones} + \beta_2 \times \text{Vegetation} + \beta_3 \times \text{Management} + \beta_4 \times \text{Moisture} + \beta_5 \times (\text{Stones} \text{Grove})$		1	0.6079	0.436	147.544
(3444)		Moisture	1	12.245	0.268	
	<b>SpW</b> ~ $\alpha + \beta_1 \times$ Vegetation + $\beta_2 \times$ (Stones Grove)	Vegetation	1	6.2141	0.012*	143.885
		Stones	1	0.6900	0.406	
A hundance of specialists	$\Delta S = r + \theta + Stance + \theta + Vacatation + \theta + Management + \theta + Maistrue + \theta + (Stance)Crosse)$	Vegetation	1	0.0104	0.919	460 514
(AS)	$F_3 \sim a + p_1 \times \text{Stories} + p_2 \times \text{vegetation} + p_3 \times \text{Waitagement} + p_4 \times \text{Wotsture} + p_5 \times (\text{Stories} \text{Stories} $	Management	1	10.993	0.294	407.514
(A5)		Moisture	1	0.6553	0.418	
	$\mathbf{AS} \sim \alpha + \beta_1 \times \mathbf{Stones} + \beta_2 \times (\mathbf{Stones}   \mathbf{Grove})$	Stones	1	0.4697	0.493	463.648
		Stones	1	106.402	0.001*	
	ShW ~ $\alpha + \beta_1 \times \text{Stones} + \beta_2 \times \text{Vegetation} + \beta_3 \times \text{Management} + \beta_4 \times \text{Moisture} + \beta_5 \times (\text{Stones} \text{Grove})$	Vegetation	1	0.0036	0.952	334.392
		Management	1	104.531	0.001*	
		Moisture	1	57.999	0.016*	
		Stones	1	0.004	0.948	
Abundance of sheet web builders	Organic: ShW ~ $\alpha + \beta_1 \times$ Stones + $\beta_2 \times$ Vegetation + $\beta_3 \times$ Moisture + $\beta_4 \times$ (Stones Grove)	Management	1	1.281	0.258	145.258
(ShW)		Moisture	1	0.000	0.984	
	$\mathbf{ShW}_{\mathbf{Organic}} \sim \alpha + \beta_1 \times \mathbf{Stones} + \beta_2 \times (\mathbf{Stones}   \mathbf{Grove})$	Stones	1	0.045	0.832	142.651
		Stones	1	23.647	<0.001*	
	Integrated: ShW ~ $\alpha + \beta_1 \times \text{Stones} + \beta_2 \times \text{Vegetation} + \beta_3 \times \text{Moisture} + \beta_4 \times (\text{Stones} \text{Grove})$	Management	1	0.122	0.727	198.699
		Moisture	1	0.903	0.342	
	$ShW_{IPM} \sim \alpha + \beta_I \times Stones + \beta_2 \times (Stones Grove)$	Stones	1	53.952	<0.001*	194.590
		Stones	1	84.232	0.004*	
Abundance of ground hunters	$GH_{a}, a + b, \times Stones + b, \times Vegetation + b, \times Management + b, \times Maisture + b, \times (Stones Group)$	Vegetation	1	0.0032	0.955	764 188
Abundance of ground numers	$p_1 \sim a + p_1 \times \text{stories} + p_2 \times \text{vegetation} + p_3 \times \text{waitagement} + p_4 \times \text{worsture} + p_5 \times (\text{stories} \text{Orove})$	Management	1	0.0827	0.774	/04.100
(GH)		Moisture	1	0.4589	0.498	
	<b>GH</b> ~ $\alpha + \beta_1 \times$ <b>Stones</b> + $\beta_2 \times$ ( <b>Stones</b>   <b>Grove</b> )	Stones	1	86.355	0.003*	758.596
Abundance of other hunters (OH)	OH ~ $\alpha + \beta_1 \times \text{Stones} + \beta_2 \times \text{Vegetation} + \beta_3 \times \text{Management} + \beta_4 \times \text{Moisture} + \beta_5 \times (\text{Stones} \text{Grove})$	Stones	1	0.7915	0.374	
		Vegetation	1	24.038	0.121	207 642
		Management	1	0.0007	0.978	287.042
		Moisture	1	0.0092	0.923	
	$OH \sim \alpha + \beta_1 \times Stones + \beta_2 \times (Stones   Grove)$	Stones	1	0.6255	0.429	284.140

**Table S7**. Statistics of the optimal generalized linear mixed models of the different components of spiders diversity considered (Response) against different crop variables in olive groves at Trás-os-Montes. Estimate: coefficients derived from the regression; SE: standard error of the estimates; Stones: number of stones /  $m^2$ ; Vegetation: percentage of vegetation cover; Stones|Grove: random effect component for olive grove. Organic: Organic management; IPM: Integrated Pest Management. An asterisk indicates statistical significance at  $\alpha < 0.05$  for the target within-grove explanatory variables.

Response	Final model	IV	Estimate	SE	Z-value	Р
Total spider abundance (TSA)	TSA ~ Stones + (Stones Grove)	Intercept	225.643	0.134	16.810	< 0.001
		Stones	0.0165	0.006	2.880	0.004*
Species richness (S)	S ~ Stones + (Stones Grove)	Intercept	157.343	0.064	24.460	< 0.001
		Stones	0.00613	0.003	2.310	0.021*
Abundance of adults	lts A ~ Stones + (Stones Grove)	Intercept	208.509	0.084	24.960	< 0.001
(A)		Stones	0.00667	0.004	1.890	0.058
Abundance of		Intercept	0.3109	0.393	0.790	0.429
immatures (I)	$1 \sim \text{Stolles} + (\text{Stolles} \text{Otove})$	Stones	0.0451	0.017	2.720	0.007*
Abundance of ambush hunters (AH)	AH ~ Stones + (Stones Grove)	Intercept	-0.4861	0.366	-1.330	0.180
		Stones	0.0117	0.015	0.760	0.450
Abundance of space	SpW ~ Vegetation + (Stones Grove)	Intercept	-0.59737	0.394	-1.520	0.129
web builders (SpW)		Vegetation	-0.01937	0.008	-2.490	0.013*
Abundance of specialists (AS)	AS ~ Stones + (Stones Grove)	Intercept	0.7411	0.384	1.930	0.054
		Stones	-0.0201	0.029	-0.690	0.493
Abundance of sheet web builders (ShW)	$ShW_{Organic} \sim Stones + (Stones Grove)$ $ShW_{IPM} \sim Stones + (Stones Grove)$	Intercept	-0.59726	0.305	-1.960	0.051
		Stones	0.00226	0.011	0.210	0.832
		Intercept	-0.5445	0.209	-2.600	0.009
		Stones	0.0647	0.009	7.350	<0.001*
Abundance of ground hunters (GH)	GH ~ Stones + (Stones Grove)	Intercept	165.194	0.174	9.510	< 0.001
		Stones	0.02169	0.007	2.940	0.003*
Abundance of other hunters (OH)	OU Stones (Stones Grove)	Intercept	-0.50547	0.211	-2.400	0.016
	$OII \sim Stolles + (Stolles   OI 0 ve)$	Stones	0.00688	0.009	0.790	0.429

# **CHAPTER 4**

Spiders actively choose and feed on nutritious non-prey food resources

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#### **Contribution of authors:**

J.B.M., S.A.P.S., J.A.P. and J.P.S. conceived and designed the experiment, J.B.M collected the spiders, performed the laboratory experiments and analyzed the data, S.A.P.S. and J.A.P. provided assistance during the laboratory experiments, and all the authors contributed to writing the paper.
## **CHAPTER 4**

## Spiders actively choose and feed on nutritious non-prey food resources

## Abstract

Spiders are generalist predators adapted to consume a wide range of prey although their ability to exploit non-prey foods such as pollen, nectar, and honeydew has been referred but less studied. In this work, the effect of different non-prey food items (Glucose at 0.5 M; aphid honeydew; black scale honeydew; a mixture of glucose 0.5 M, phenylalanine 0.1 mM, proline 0.1 mM, and tryptophan 0.1 mM; honey at 10%, and pollen at 10%) on the survival of immature spiders of two functional groups represented by Haplodrassus rufipes (ground hunters) and Synema globosum (ambushers), and their feeding choices were investigated in laboratory experiments. The overall survival of both species fed on non-prey foods significantly increased compared to individuals fed on water. The black-scale honeydew was the best food for H. rufipes increasing longevity up to 117 days. The highest survival reached by S. globosum was observed when fed on a mixture of glucose 0.5 M and three amino acids. When different non-prey food items were offered together, the exploring rate was significantly higher for H. rufipes than for S. globosum. H. rufipes chose to feed on honey whereas S. globosum chose the mixture treatment. The most chosen food items corresponded with those that provided the highest longevities in both species. These results suggest that spiders could search, recognize and actively select the most beneficial nonprey food. Habitat management practices such as maintaining weed strips in the crop may provide these valuable supplementary food resources within agroecosystems contributing for biological pest control.

#### 4.1. Introduction

Natural enemies feeding on different supplementary food resources such as nectar, sugar, and pollen experience higher levels of fitness and can enhance biological pest control (Van Rijn et al., 2002). Generally, the advantages of a prey-based diet for entomophagous predators surpass those of a diet based on non-prey foods in terms of nutrient contents (Lundgren, 2009). However, both quantity and quality of non-prey food resources available for natural enemies can influence different life-history parameters such as survival, reproduction and number of offsprings, consequently affecting their efficiency in pest suppression (Villa et al., 2016).

Among predators, the role of spiders within agricultural landscapes is well documented and several studies showed that spiders are important natural enemies of pests (Benhadi-Marín et al., 2016b; Picchi et al., 2017). Spiders have been considered strictly carnivores, however, evidences related with their capacity to feed on floral resources such as nectar and pollen have been regularly reported (Vogelei & Greissl, 1989; Sanders, 2012).

Glucophagy in spiders can improve longevity and fitness and affect molting (Taylor & Pfannenstiel, 2009). Wu et al. (2011) observed, in laboratory experiments, that the crab spider *Ebrechtella tricuspidata* (Fabricius) (Thomisidae) significantly spent more time feeding on honey solution (20%) than on water. Moreover, spiders fed on the honey solution significantly showed higher survival rates, shorter developmental time and pre-oviposition period, and laid more eggs than those spiders fed on water only. Different studies proved that feeding on pollen also increased the fitness of the spiders especially during the first instars (Vogelei & Greissl, 1989) and observations of spiders feeding on pollen have been reported for cursorial spider families such as Thomisidae, Salticidae, Clubionidae and Eurichuridae, and web-builders such as Araneidae, Linyphiidae and Theridiidae (Nyffeler et al., 2016). Also, it has been suggested that pollen availability could lead to the association between spiders and different species of plants (Rurhen & Handel, 1999).

Another important alternative food resource and one of the most abundant in infested crops as well as in spontaneous non-crop plants is the honeydew excreted by hemipteran insects (Vollhardt et al., 2010; Pfannenstiel & Patt, 2012). Pfannenstiel (2015) found, in laboratory experiments, that the whitefly (Hemiptera: Aleyrodidae) honeydew significantly extended the survival of spiders belonging to different functional groups such as *Apollophanes punctipes* (O.P. Cambridge) (Philodromidae), *Cesonia bilineata* (Hentz) (Gnaphosidae), *Dictyna* sp. (Dictynidae), *Hibana futilis* (Banks) (Anyphaenidae), and *Thiodina sylvana* (Hentz) (Salticidae). Several observations were also made in the field, for example, different species of *Myrmarachne* (Salticidae) have been seen feeding on honeydew while tending scale coccid insects (Nyffeler et al., 2016).

In terms of behavior, spiders show different patterns to exploit alternative non-prey foods. *Misumenoides formosipes* (Walckenaer) (Thomisidae) actively squeeze nectaries in flowers with the fangs (Pollard et al., 1995). Orb-weaver spiders such as *Araneus diadematus* Clerck (Araneidae) can ingest their orbicular web covered with pollen grains taking advantage of this type of food (Ludy & Lang, 2006). Jumping spiders also exploit extrafloral nectaries since they are easily accessed (Rurhen & Handel, 1999) and ambusher spiders such as *Thomisus onustus* Walckenaer (Thomisidae) use flowers to hunt pollinators, and in laboratory studies, immatures of this species survived for 40 days when fed on pollen only (Vogelei & Greissl, 1989).

Both visual cues and odor are related with spider feeding behavior. Heiling et al. (2004) demonstrated that odor seems to be the floral signal that bees use to identify high-quality flowers and that crab spiders exploit to encounter honeybees; and testing at the same time the predator and the prey, both preferred the flowers that emitted olfactory signals. Patt and Pfannenstiel (2008) pointed out that nectarivorous spiders may have mechanisms for detecting, recognizing and locating nectar sources.

Although vegetarianism and glucofagy in spiders has aroused interest, the way in which non-prey food such as nectar, pollen and honeydews affect the fitness of different guilds of spiders as well as the preference for some non-prey foods is still largely unknown. The

objective of this work was to assess the effect of different non-prey foods on the survival and food selection behavior of two species of spiders, *Haplodrassus rufipes* (Lucas) (Gnaphosidae) and *Synema globosum* (Fabricius) (Thomisidae), encompassing two functional groups, the ground hunters and ambushers respectively. The working hypotheses were that (1) non-prey foods will significantly increase the survival of immature spiders and (2) the spiders will preferentially choose some food items in multiple-choice experiments.

*Haplodrassus rufipes* and *S. globosum* are two widespread spider species in the Iberian Peninsula (Morano et al., 2014), the former has a Mediterranean distribution and the latter has a Palearctic distribution (Nentwig et al., 2018). Both species are generalist predators and in this study they were selected as representatives of two functional groups according to their hunting strategies. *Haplodrassus rufipes* is a nocturnal ground runner spider that builds its nests under stones and uses them as shelters during the day (Benhadi-Marín, J., personal observation). *Synema globosum* is an ambusher species that hunts insects on flowers during the day and nests under the flower or by joining two nearby flowers (Ajuria & Reader, 2014).

#### 4.2. Material and methods

## 4.2.1. Spider origin and rearing

Subadults of both sexes of *H. rufipes* and *S. globosum* were hand collected in Valbom-dos-Figos (41°32'58"N) and Cedães (41°29'17"N), two villages located in the vicinities of the municipality of Mirandela (northeast of Portugal) in the beginning of May 2015 and 2016. Specimens of *H. rufipes* were searched under soil stones in an olive grove in Valbom-dos-Figos and specimens of *S. globosum* were captured by inspecting flowers of *Cistus ladanifer* L. in a semi-natural shrubland area in Cedães.

The spiders were transported into the laboratory in perforated plastic tubes (volume - 15 mL), transferred individually into plastic Petri dishes (5.2 cm in diameter and 1.2 cm height) and placed in a climate chamber at 21°C ( $\pm$  1°C), 70% ( $\pm$  5%) of relative humidity

and a photoperiod of 16:8 (L:D) h. Every week, half of a 2 cm sphere of sodium acrylate hydrogel was placed on the bottom of each Petri dish to provide water.

Haplodrassus rufipes was fed on the first nymphal stages of the house cricket, Acheta domesticus (L.) (Orthoptera: Gryllidae). Acheta domesticus specimens were initially purchased in a pet shop and maintained in the laboratory. The initial cricket population ( $\approx$ 100 individuals) was placed into a plastic box (35 cm x 25 cm on the base and 30 cm in height) covered with a multiperforated cap. A layer of 2 cm in height of cat litter was provided as substrate. A plastic Petri dish (5.2 cm in diameter and 1.2 cm height) was placed on each box corner and filled with meat-based dried biscuit-like minced cat food as nourishment. Water was provided with 10 spheres of sodium acrylate hydrogel (2 cm in diameter) placed randomly on the litter surface. Eight egg cartons were aligned in the enclosure to provide shelter. Six small plastic boxes (6 cm x 6 cm on the base and 3.5 cm in height) covered with metal mesh and filled with moistened cat litter were placed randomly on the substrate as egg laying boxes. After a week, the laying boxes were removed from the culture and placed on a tray previously filled with a substrate consisting of a mixture of cat litter and minced cat food (1 cm in height). The tray carrying the laying boxes was maintained in a 30°C climate chamber until egg hatching. After hatching, the small juveniles spontaneously jump from the laying boxes and fall into the tray, becoming ready to be used.

*Synema globosum* was fed on adults of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) that were collected from the stock colony maintained at the School of Agriculture (ESA), Bragança since September 2012, as described before by Dinis et al. (2016). Both prey species were provided *ad libitum* to spiders during the rearing stage.

When the spiders reached the adult stage, 20 couples of both species were placed individually in Petri dishes (9 cm in diameter and 2 cm height) and observed until mating. After that, each female was reallocated in its Petri dish and males were released in the field. Cocoons built by females were sequentially taken from the dishes, placed into plastic Petri

dishes (5.2 cm in diameter and 1.2 cm height) and maintained with a moistened filter paper strip until spiderlings hatched.

## 4.2.2. Non-prey food

The non-prey food used in the experiments were: (1) three different saccharides, i.e., (a) glucose (D-(+)-glucose > 99%), (b) sucrose (D-(+)-sucrose > 99%), both purchased from Fisher Scientific, Loughborough, UK and (c) fructose (D-(-)-fructose > 99%) purchased from PanReac AppliChem, Darmstadt, Germany, (2) three different amino acids, i.e., (a) phenylalanine (purchased from PanReac AppliChem, Darmstadt, Germany), (b) proline and (c) tryptophan (both purchased from Acros Organics<sup>TM</sup>, New Jersey, USA), (3) commercial multifloral organic honey (as a mimic for nectar) obtained from Agrupamento de Produtores de Mel do Parque, Lda. (Bragança, Portugal), (4) bee-collected pollen obtained from an organic beekeeper (Manuel C. Martins Chêta, Pereiras, Portugal), (5) yeast extract (purchased from Biolab<sup>®</sup>Zrt., Budapest, Hungary), (6) black scale *Saissetia oleae* (Olivier) honeydew and (7) aphid Aphis craccae L. honeydew. The black scale honeydew was collected from infested leaves of small olive trees grown in a climatic chamber at 24°C (± 1°C), 70% (± 5%) of relative humidity and a photoperiod of 16:8 h (L:D) and the aphid honeydew was collected from infested plants of Vicia villosa Roth collected in the field and maintained in the same climatic chamber. In both cases, several Parafilm strips were placed under the infested parts of the plants and used in the experiments when they were mostly covered by small drops of honeydew after 24h on average.

## 4.2.3. Experimental design

## 4.2.3.1. Longevity experiments

A preliminary longevity experiment was performed in order to select which of the saccharides will be used as a positive control in the following experiments. Thus, recently hatched spiderlings of *H. rufipes* (< 24 h) were transferred individually into each well of a 24-well plate. Each specimen was provided with a filter paper strip (1 cm x 1 cm) which was embedded in a sugar solution and placed at the bottom of the well. Sugar solutions of glucose, sucrose and fructose at concentrations of 0.25 M, 0.5 M and 1 M were tested. Water was used as negative control. Each well was closed with a cotton ball wrapped in

Parafilm to prevent the spider from escaping. Since glucose at 0.5 M gave the higher mean longevity (shown in Results section), this solution was selected as the positive control.

For the following longevity experiments, the spiderlings of each spider species were kept, in the 24-well plates, with one of the non-prey foods, i.e., (1) water as negative control, (2) solution of glucose at 0.5 M as positive control, (3) solution of honey at 10% v/v, (4) solution of pollen at 10% w/v, (5) a mixture of glucose 0.5 M, phenylalanine 0.1 mM, proline 0.1 mM and tryptophan 0.1 mM (hereafter mix), (6) the black scale honeydew, and (7) aphid honeydew. Each specimen was provided with a filter paper strip (1 cm x 1 cm) embedded in the respective solution or with a Parafilm strip (1 cm x 1 cm) covered with drops of honeydew and a filter paper strip embedded in water under the Parafilm strip, that were placed at the bottom of the well.

Each non-prey food was changed twice a week to prevent fungal growth. Experiments were maintained in a climate chamber at 21°C ( $\pm$  1°C), 70% ( $\pm$  5%) of relative humidity and a photoperiod of 16:8 h (L:D) and spiderling survival was checked and registered on a daily basis. Between 47 and 55 individuals were used in each treatment.

#### 4.2.3.2. Food choices and behavior during multiple-choice experiments

Recently hatched spiderlings of each species (< 24 h) were tested in order to check if they actively searched and/or chose a specific non-prey food. In this experiment, glass Petri dishes (5.2 cm in diameter and 1.2 cm height) were used and the vertices of a hexagon (1.5 cm radius) were drawn on the bottom surface. One drop of moistened activated charcoal powder (0.02 g/drop) (AppliChem<sup>®</sup>PanReac, Darmstadt, Germany) was placed, with a syringe, on each vertex of the hexagon. After the activated charcoal dried, a drop of each non-prey food solution was deposited onto the surface using a syringe and offered to the spiderling.

Six non-prey foods were tested simultaneously in each Petri dish (multiple choice experiment): (1) water as negative control (2) glucose at 0.5 M as positive control (3) honey

at 10% v/v, (4) pollen at 10% w/v, (5) mixture of glucose 0.5 M, phenylalanine 0.1 mM, proline 0.1 mM and tryptophan 0.1 mM and (6) yeast extract at 10% w/v.

The spiderlings were maintained at 4°C for 10 minutes to reduce activity. Then, after one minute at room temperature (23°C), a spiderling was placed individually using a paintbrush in the center of the Petri dish and all the movements were recorded for 30 minutes using a Computar<sup>®</sup> lens (H2Z0414C-MP, f=4-8mm, F 1.4, ½", CCTV lens) mounted on a Basler<sup>®</sup> GigE HD Camera (acA1300-60gc with e2v EV76C560 CMOS sensor) (Noldus, 1991). The Noldus Observer XT 11.5 software (Noldus ObserverXT, 2013a) was used to encode the ethogram and to gather the data. The ethogram was encoded in two behaviors, "inactive" and "active", the latter included seven modifiers: "honey", "pollen", "glucose", "mix", "yeast", "water" and "exploring". A resource was considered selected when a spiderling climbed onto a non-prey food drop and touched it with at least the prosoma.

The Media Recorder 2.5 software (Noldus Media Recorder, 2013) was used and the Petri dishes illuminated with fluorescent direct light. Fifty-four individuals of each species were tested and were video-recorded in nine blocks of six Petri dishes per block.

#### 4.2.4. Data analysis

#### 4.2.4.1. Longevity experiments

The mean, median, interquartile range and standard error of the longevity were calculated for each non-prey food. Firstly, the longevity was modeled using a Poisson GLM in order to test for interactions between the type of sugar and the different concentrations. Since the variance was larger than the mean, the standard errors were corrected using a quasi-GLM model given by:

$$Y_i \sim P(\mu_i); E(Y_i) = \mu_i \text{ and } \operatorname{var}(Y_i) = \varphi \times \mu_i$$
$$\log(\mu_i) = \alpha + \beta_1 \times Sugar_{i1} + \beta_2 \times Concentration_{i2} + \beta_3 \times Sugar_{i3}: Concentration_{i3} \qquad [Eq. 1]$$

where  $\varphi$  is the dispersion parameter and  $\beta_3 \times Sugar_{i3}$ :*Concentration*<sub>i3</sub> the interaction term. Models were validated by checking lack of fit and patterns in the deviance residuals (Zuur et al., 2009). Since the interaction term of Eq. 1 was significant, the treatment overall effect was assessed through survival curves using log-rank tests for all sugars and concentrations. Then, the differences between non-prey foods were evaluated by a full sequential pairwise comparison analysis. Finally, differences between spider species were assessed using the Wilcoxon signed-rank test. The significance level was established at p = 0.01 in all cases.

#### 4.2.4.2. Food choices and behavior during multiple-choice experiments

The rate per minute (hereafter RPM) of the analyzed observation duration (i.e. the mean number of occurrences of a behavior over the total of the analyzed duration) was calculated for each modifier and Petri dish as:

$$\text{RPM}_i = (\text{Total number of occurrences}_i \times 60) / \text{Analyzed duration}_i (s)$$
 [Eq. 2]

The exploring rate within the Petri dish was firstly compared between species in order to assess the activity rate of each species with the Welch's t-test due to unequal variances (D). Then, the RPM of the modifiers honey, pollen, glucose, mix, yeast, and water were compared by developing general linear mixed models (GLMMs) according to Zuur et al. (2009) followed by a *post hoc* multiple comparisons analysis ( $\alpha = 0.05$ ). Block was included as a random factor ( $Z_i \times b_i$ ) and water was used as intercept. In the case of *H. rufipes*, a second term for the time spent in each resource (Duration) was included in the model to correct the exploring rate effect. Models were validated checking for nonlinear patterns in the residuals. The final models are given by:

Finally, for both species and experiment the mean of each treatment was centered around zero, scaled by dividing the centered values by their standard deviations and plotted

together to check for patterns between the most selected non-prey foods and the treatments that provided the highest longevity.

All statistical analyses and modeling were performed in R (R Core Team, 2018). The functions used outside the {base} and {stats} packages were: Anova{*car*}, effect{*effects*}, glht{*multcomp*}, lmer{*lme4*}, lsmeans{*lmerTest*}, mcp{*multcomp*}, multcompLetters{*multcompView*}, plotCI{*plotrix*}, std.error{*plotrix*}, survdiff{*survival*} and survfit{*survival*}.

## 4.3. Results

### 4.3.1. Longevity experiments

In the preliminary experiment, the interaction between the type and the concentration of the sugar significantly affected the longevity of *H. rufipes* ( $\chi^2 = 99.345$ , df = 4, P < 0.01). The overall effect was also statistically significant ( $\chi^2 = 159.70$ , df = 9, P < 0.01) and all sugars at any concentration significantly increased the survival of spiders (Table 4.1) (Fig. 4.1A). Among sugars and concentrations, glucose 0.5 M was the combination that provided the highest mean longevity (Table 4.1), hence it was included as a positive control in the following experiments.

The overall survival of spiderlings of both species fed on non-prey foods significantly increased (*H. rufipes*:  $\chi^2 = 309.50$ , df = 6, P < 0.01 and *S. globosum*:  $\chi^2 = 196.00$ , df = 6, P < 0.01). In all cases, water caused the lowest values of longevity, however, it was not significantly different from the survival of *H. rufipes* fed on aphid honeydew (Table 4.1). For this species, four groups of non-prey foods differed significantly in terms of curve fitting and represented an increasing survival gradient. The first group included the spiderlings fed on water and aphid honeydew, the second one those fed on pollen, the third group those fed on glucose, honey and the mixture of glucose 0.5 M and three amino acids, and the last group those spiderlings fed on the black-scale honeydew (Table 4.1) (Fig. 4.1B). A spiderling of *H. rufipes* fed on the black-scale honeydew survived for 117 days thus being the highest longevity among the whole study (Fig. 4.1B).

**Table 4.1.** Longevity (mean  $\pm$  standard error - SE) obtained for each treatment of the preliminary test (sugars) and non-prey foods experiments supplied to each spider species and log rank statistics. AH: aphid honeydew; BSH: black scale honeydew; Mix: glucose 0.5 M + phenylalanine 0.1 mM + proline 0.1 mM + tryptophan 0.1 mM. Different letters for longevity means significant differences between treatments within each assay (pairwise comparisons) (p < 0.01).

Accov	Treatment	Longevity	Obsomvad	Exposted	(O-E)^2/E	
Assay	Treatment	(mean ± SE)	Observeu	Expected		
	Water	$25.47 \pm 1.23$ a	53	13.89	110.11	
	Fructose 0.25 M	$42.73\pm1.81\text{ bc}$	55	49.24	0.67	
	Fructose 0.5 M	$34.77\pm2.21~b$	47	28.29	12.38	
	Fructose 1 M	$45.20\pm3.03~cd$	54	69.09	3.30	
ars fipes	Glucose 0.25 M	$44.65 \pm 1.42 \text{ bc}$	54	50.44	0.25	
Sug . ruj	Glucose 0.5 M	$51.02 \pm 2.41 \ d$	55	79.98	7.80	
(H	Glucose 1 M	$45.65\pm2.82~cd$	51	62.27	2.04	
	Sucrose 0.25 M	$42.19\pm1.62\ bc$	54	46.39	1.25	
	Sucrose 0.5 M	$45.48\pm3.01\ cd$	50	63.43	2.84	
	Sucrose 1 M	$44.98\pm3.06\ cd$	48	57.97	1.72	
	AH	$29.74 \pm 0.99$ a	55	18.27	73.81	
S	BSH	$72.20\pm2.62~d$	51	108.32	30.33	
cood es)	Glucose 0.5 M	$51.02\pm2.41~c$	55	60.93	0.58	
rey f ufip	Honey 10%	$59.22\pm2.42\ c$	51	74.30	7.31	
n-fu	Mix	$51.54\pm2.86\ c$	55	64.61	1.43	
NC NC	Pollen 10%	$37.92\pm2.25~b$	53	33.74	10.99	
	Water	$25.47 \pm 1.23$ a	53	12.82	125.93	
	AH	$32.87 \pm 2.35 \text{ e}$	55	70.40	3.37	
$\mathbf{s}$	BSH	$26.67\pm2.28\ bc$	55	46.80	1.44	
food wm)	Glucose 0.5 M	$28.11\pm0.68\ cde$	55	55.50	0.00	
rey f obos	Honey 10%	$20.13\pm1.55~\text{b}$	55	31.00	18.50	
on-p S. gli	Mix	$41.61 \pm 2.55 \; f$	55	110.60	28.00	
Ŭ Ŭ	Pollen 10%	29.38 ± 1.31 d	55	55.60	0.01	
	Water	$15.00 \pm 0.53$ a	55	15.10	105.00	



**Figure 4.1.** Survival curves for each non-prey food tested. A: Sugars tested on *Haplodrassus rufipes*; B: Non-prey food tested on *Haplodrassus rufipes*; C: Non-prey food tested on *Synema globosum*. AH: aphid honeydew; BSH: black scale honeydew; Mix: glucose 0.5 M + phenylalanine 0.1 mM + proline 0.1 mM + tryptophan 0.1 mM. Glucose at 0.5 M, honey and pollen at 10%. Different letters in legends mean significant statistical differences between treatments (p < 0.01).

For *S. globosum*, the mixture of glucose 0.5 M and three amino acids was the non-prey food that gave the highest mean longevity followed by the aphid honeydew, whereas the highest number of days survived by a spiderling (67 days) corresponded to the mixture of glucose 0.5 M and three amino acids treatment (Fig. 4.1C) (Table 4.1). In this species, four significantly different non-prey foods gave an increasing longevity, namely honey < pollen < aphid honeydew < mix, with two intermediate treatments, black-scale honeydew, and glucose (Table 4.1) (Fig. 4.1C).

#### 4.3.2. Food choices and behavior during multiple-choice experiments

Significant differences were found among the choices of non-prey food items offered to *H*. *rufipes* (F = 22.65, df = 5, P < 0.01) and *S. globosum* (F = 4.34, df = 5, P < 0.01) when provided together. The exploring rate was significantly higher in *H. rufipes* than in *S. globosum* (t = 6.71, df = 53, P < 0.01) (Fig. 4.2A).



**Figure 4.2.** A: Comparison between the mean exploring rate of each spider species. B: Selection rate of the different non-prey food offered to *Haplodrassus rufipes*. C: Selection rate of the different non-prey foods offered to *Synema globosum*. HR: *H. rufipes*; SG: *S. globosum*. Mix: glucose 0.5 M + phenylalanine 0.1 mM + proline 0.1 mM + tryptophan 0.1 mM, glucose at 0.5 M. Honey, yeast and pollen at 10%. Different letters over bars mean significant statistical differences (p < 0.05).

The RPMs measured on spiderlings of *H. rufipes* feeding on drops of water, glucose, pollen and mixture of glucose 0.5 M and three amino acids were significantly lower than for those feeding on the honey drop (Fig. 4.2B). In the case of *S. globosum*, the RPMs measured on

the individuals feeding on water, glucose and honey drops were significantly lower than on those feeding on the mixture of glucose 0.5 M and three amino acids drops (Fig. 4.2C). For both species, the yeast drop was the second most accessed non-prey food but it did not significantly differ from the others (Fig. 4.2B & 4.2C).

Regarding both the longevity and multiple food-choice experiments, the highest longevity was provided by the preferred non-prey food in each spider, these being honey for *H*. *rufipes* and the mixture of glucose 0.5 M and three amino acids for *S. globosum* (Fig. 4.3).



**Figure 4.3.** Overlap of the longevity experiment and the multiple choice experiment for each spider species. Points and triangles correspond to the centered mean for each treatment and experiment at the same scale. The central area between the dashed lines corresponds to the intersection of the two experiments. The left area groups the treatments not used in the multiple choice experiments and the right area groups the treatments not used in the longevity experiment. A: *Haplodrassus rufipes*; B: *Synema globosum*; AH: aphid honeydew; BSH: black scale honeydew; Mix: glucose 0.5 M + phenylalanine 0.1 mM + proline 0.1 mM + tryptophan 0.1 mM, glucose at 0.5 M, honey, pollen and yeast at 10%. Among the treatments used in both experiments (central area), the ellipses encompass the treatment that provided the highest survival along the longevity experiment and the most selected treatment during the multiple-choice experiment.

## 4.4. Discussion

The results obtained in this work confirmed that (1) different non-prey foods affected differently the lifespan of immature spiders when provided as the only food source and (2) the spiders select the non-prey food that benefited more the longevity according to its lifestyle.

The longevity of the ambusher *S. globosum* increased significantly when fed on glucose, pollen and nectar compared with individuals fed on water-only. These results agree with those obtained by Vogelei & Greissl (1989) who fed spiderlings of the ambusher species *T. onustus* in laboratory and found that pollen and artificial nectar (a sucrose solution 30% w/v) significantly increased their mean lifespan when compared with starved spiderlings.

Suetsugu et al. (2014) reported that *Clubiona* spp. (Clubionidae), a foliage runner spider, actively removed pollen from flowers of the nectariferous orchid *Neottianthe cucullata* (L.). The pollen offered to *H. rufipes* and *S. globosum* was embedded in water but the fact that active hunting spiders actually exploit this food source supports the results found in this work suggesting that pollen is effectively digested.

The spiderlings of *H. rufipes* tested in the present work survived significantly longer when fed on aphid honeydew and honey than those fed on water. In the case of cursorial spiders these results agree with Pfannenstiel & Patt (2012) who provided different non-prey foods to spiderlings of *Cheiracanthium inclusum* (Hentz) (Eutichuridae), in laboratory experiments, and reported an average increase of 870% on survival of spiders fed on extrafloral nectar of cotton plants, and of 626% on mealybug honeydew (Hemiptera: Pseudococcidae).

Honey (a mimic to nectar) was the second best treatment for *H. rufipes* and an intermediate one in the case of *S. globosum* in terms of spider survival. In both cases, it provided significantly better results than water, which seems to be a constant pattern among studies. Ruhren and Handel (1999) showed that foraging spiders spent 86% more of their time on

plants with nectar than without active extrafloral nectaries in a controlled-environment experiment, and Taylor & Pfannenstiel (2009) found, in laboratory, that the survivorship and the number of molts of *C. inclusum* spiderlings significantly increased when a supplement of nectar of *Terminalia catappa* L. was provided. Also, Chen et al. (2010) using the cold anthrone test confirmed that the ambusher *E. tricuspidata* and eight other spider families (Oxyopidae, Pisauridae, Salticidae, Lycosidae, Tetragnathidae, Araneidae, Nephilidae and Agelenidae) consumed nectar in the field.

Considering the two types of honeydew tested in this work, an opposite pattern was observed between spider species; the black-scale honeydew was the treatment that provided the highest longevity for *H. rufipes* whereas the aphid honeydew provided the second best result for S. globosum. This could be explained in terms of honeydew chemical composition. The major component of the black scale honeydew is trehalose (193.3 mg  $g^{-1}$ ) followed by sucrose (25.0 mg g<sup>-1</sup>), glucose and melibiose (24.9 mg g<sup>-1</sup>) (S. Santos, data not published). Trehalose is a naturally occurring 1-alpha sugar consisting of two molecules of glucose and known to be a stabilizer of proteins that helps to maintain the activity of enzymes in solution as well as in the freeze-dried state (Kaushik & Bhat, 2003). Also, beyond the energy obtained from sugar, trehalose provides effective protection against desiccation in invertebrates (Watanabe, 2006) which could explain the results achieved with the black-scale honeydew for both tested species. Moreover, H. rufipes is a species that spends the winter under stones, protected from cold, since it is considered less resistant to lower temperatures than spiders living unprotected in vegetation such as S. globosum (Nentwig, 1987). Accordingly, spiderlings of *H. rufipes* could benefit more from a diet rich in trehalose regarding the nutritional requirements necessary to overcome cold periods (e.g. daily temperature fluctuations and overwintering).

The honeydew of *A. craccae* fed on *V. villosa* also resulted in opposite patterns between *H. rufipes* and *S. globosum* and it was not significantly different from water for the former species. Despite differences between species, this result corroborates the previous idea since trehalose is a minor component (< 10%) occurring in the honeydew produce by *Aphis fabae* Scopoli fed on *Vicia faba* L. (Fischer et al. 2005).

The three amino acids, phenylalanine, tryptophan and proline, provided together with glucose resulted in the best treatment in terms of survivorship for *S. globosum* and an intermediate one for *H. rufipes*. Proline varies up to 11% amino acids in silk and is thought to give elasticity (Creager et al., 2011). Silk is critical for many ecological functions of spiders and its production occurs during all developmental states representing a great energetic investment (Creager et al., 2011). Although the genetically encoded metabolic pathways remain unchanged by amino acid enrichment or deprivation, Zax et al. (2004) found that spiders of the species *Argiope argentata* (Fabricius) (Araneidae), *Nephila clavipes* (Linnaeus) (Araneidae), and *Latrodectus hesperus* (Chamberlin & Ivie) (Theridiidae) subjected to dietary deprivation incorporated less proline into the silk fiber than spiders provided with crickets. Moreover, silk profiles obtained from *N. clavipes* and *L. hesperus* fed on proline-rich diets showed clear proline peaks, while in natural conditions these peaks are difficult to discriminate above the baseline. This fact highlights the importance of this amino acid in the diet of spiders since the vast majority of spider silk is made up of alanine, glycine, proline, glutamic acid, and serine (Wilder, 2011).

Spiders use venoms to subdue prey and the combination of venom and extra-oral digestion allows spiders to exploit a wide spectrum of insect prey. Both secretions contain an array of active proteins and an overlap of some components has been reported and quantified (Walter et al., 2017). Phenylalanine is the second most important N-terminal amino acid residue of the cytolytic peptides of spider venom (Kuhn-Nentwig et al., 2011) which highlights its importance for the spiders' extra-oral digestion. Since thomisids may bite several times to subdue the larger prey (e.g., bees and bumblebees) (Pollard, 1990; Foelix, 1996) and they can regulate the quantity of injected venom according to the prey size (Pollard, 1990), the amount of venom used during an attack may be high. In addition, it has been demonstrated that thomisids do not chew their prey, digestion taking place entirely extra-orally (Foelix, 1996) which should imply strong extra-oral fluids and venom (Foelix, 1996, 2011) that could make *S. globosum* preferentially choose a solution rich in phenylalanine such as the mix treatment.

Regarding the multiple choice experiment, it has been argued that spiders could determine the chemical properties of a substance merely by touching it with the chemosensitive hairs located in their tarsi (i.e. the seventh and last segment of the leg and sixth and last segment of the palp) (Foelix, 2011). This behavior called "taste-by-touch" and the chemical sensitivity of these sensilla has been proved using electrophysiological methods (Drewes & Bernard, 1976). Vallet et al. (1998) tested the sensitivity of chemoreceptors of *Eratigena atrica* (C.L. Koch) (Agelenidae) to 14 amino acids (0.01 M in 0.001 M NaCl) and found that proline was the most stimulating one. Also, they found that chemoreceptors were functional in newly emerged individuals which can explain the selections did by spiderlings of *H. rufipes* and *S. globosum* when non-prey foods were offered together during the multiple choice experiment.

Mix was the most selected treatment by *S. globosum* whereas *H. rufipes* selected honey. *H. rufipes* showed a significantly higher activity rate and as an active hunter, honey could provide higher energy supply for this species. Although the total sugar content could be nearly the same in the honey treatment ( $\approx 8.2\%$  w/w, reference value from Pasupuleti et al., 2017) and mix treatment ( $\approx 8\%$  w/w), the diversity of sugars, proteins, vitamins and other compounds in honey together with a composition in amino acids dominated by proline (50–85%) (Hermosín et al., 2003) could make the artificial nectar more attractive for *H. rufipes*.

On the other hand, *S. globosum* is a sit-and-wait predator with a presumably lower resting metabolic rate than the ground runner *H. rufipes*. Sit-and-wait spiders spend very little time in active locomotion and may be able to remain motionless by keeping constant hydrostatic pressure using a few small muscles instead of continually activating leg musculature (Wilder, 2011) thus reducing energy loss. In this sense, the non-prey food selection of the sit-and-wait species *S. globosum* could be driven by a lower sugar requirement and higher proline content of the mix treatment ( $\approx 0.012\%$  w/w) when compared with the artificial nectar ( $\approx 0.004\%$  w/w, reference value from Hermosín et al., 2003).

The yeast treatment was the second most selected by the two species. Spiders have been reported living in association with fruiting bodies of mushrooms and other fungi referring

the latter as an excellent source of proteins (Lundgren, 2009) but fungus also contains lipids, minerals, carbohydrates, structure-fibers and the yeast may even act as a B-vitamin supplement (Kurtzman, 1997). According to these results, spiders may also exploit the ubiquitous biofilms present in nature as an alternative source of nutrients based on microorganisms.

Considering the non-prey foods provided in food choice experiments, both *H. rufipes* and *S. globosum* selected the food item that provided them the highest longevity during the non-choice experiment. These results agree with Patt & Pfannenstiel (2009) that demonstrated that the nocturnal cursorial *H. futilis* (Anyphaenidae) fed on non-prey foods not only to drink water but also to obtain nutrients.

Most biological control agents are omnivores since they usually feed on plant-derived foods and spiders are not an exception (Wäckers et al., 2008). Sap sucking hemipterans can be predated by spiders and the honeydew that they produce can also represent a supplementary source of energy. For example, the ambusher *Philodromus albidus* Kulczynski (Philodromidae) was observed feeding on a honeydew drop produced by the olive psyllid *Euphyllura olivina* Costa (Hemiptera) on the olive tree canopy (Benhadi-Marín, J., personal observation) and psyllids may encompass a great proportion of the canopy dwelling spiders diet (Barreales et al., data not published). Among other natural enemies, the lifespan of the parasitoid wasp *Aphidius colemani* (Dalman) fed on *A. craccae* honeydew was 2.5 times higher compared with longevity on water (Wäckers et al. 2008).

The results found in this work suggest that different spider guilds react differently to nonprey foods and may process the nutrient composition in different ways. For example, honeydews can be less suitable and/or toxic for some consumers (Lundgren, 2009) and spiders could have different metabolic routes to deal with toxic compounds such as alkaloids or cardenolides. In fact, Bilde & Toft (2001) found a toxic effect on *Erigone atra* Blackwall (Araneae: Linyphiidae) when fed on *Sitobion avenae* Fabricius (Hemiptera: Aphididae) adults in laboratory. Thus, food selection during multiple-choice experiments could be driven by avoidance (due to toxic or inhibitory components) rather than by

preference; however, the effect of this kind of substances in spiders remains unknown (Nyffeler et al., 2016). Further research on the chemical composition of non-prey foods involved in spider diets is mandatory as well as on the physiological mechanisms involved in the detection and selection of such alternative food sources.

### 4.5. Conclusions

The results found in this work demonstrate that different functional groups of spiders actively search and consume the most beneficial non-prey food among those offered and provide strong evidence for the relevance of non-prey foods on diet. Spontaneous plants (flowers) and honeydew may provide important nutrients to immature spiders; however, each non-prey food alone is probably not enough to fulfill the nutritional requirements of an individual. Moreover, the fact that each species belongs to a different functional group with a significantly different activity rate (i.e. different metabolic requirements) and each one selected a different non-prey food according to the best results in terms of longevity provides a cross-validation for the interpretation. However, predictions in this sense should be made carefully because it is still not clear if this pattern is consistent within and across functional groups since only one species per guild was tested. In addition, variations in the nutritional quality of non-prey foods (e.g. pollen and honey) may play a role on the lifespan of spiders, and the effect of the availability of alternative food for predators is not clear. For example, Harwood & Obrycki (2005) suggested that alternative prey might lead to a reduction in pest control provided by Erigone autumnalis (Emerton) (Araneae: Linyphildae). On the other hand, habitat management practices such as maintaining weed strips in the crop may provide this valuable source of alternative non-prey foods helping to keep the communities of natural enemies healthy within the agroecosystem, which can contribute for biological pest control.

# CHAPTER 5

Functional responses of three guilds of spiders: comparing single and multi-prey approaches.

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## **Contribution of authors:**

J.B.M., S.A.P.S., J.A.P. and J.P.S. conceived and designed the experiment, J.B.M collected the spiders, performed the laboratory experiments and analyzed the data, and all the authors contributed to writing the paper.

#### **CHAPTER 5**

Functional responses of three guilds of spiders: comparing single and multi-prey approaches.

#### Abstract

Spiders are successful natural enemies of pests occurring throughout the different strata of an agroecosystem. The study of their functional responses can provide information related to the potential effectiveness of different species and guilds on reducing a pest population. However, multiple prey availability may change the functional response of a predator. In this study, the functional responses of three species of spiders in single-prey and multipleprey experiments were modeled. The spider species were Haplodrassus rufipes, Araniella cucurbitina, and Synema globosum, and were chosen as being representatives of ground runners, orb-weavers and ambushers respectively. When the selected target prey, the Mediterranean fruit fly C. capitata was supplied in the single-prey experiments, the most and the least efficient spider species were H. rufipes and S. globosum respectively. However, opposite results were obtained when alternative preys were supplied. Araniella *cucurbitina* significantly changed its functional response with the presence of alternative preys. Acheta domesticus, used as representative of a heavy, long-sized and highly motile prey was avoided. Ceratitis capitata, the target pest used as representative of light, smallsized and moderately motile prey was preferred by the three species of spiders. Each guild could include efficient predators against pests according to its hunting strategies and the ecological exploited niches. Orb-weavers could be efficient predators against flying pests; ambushers could contribute to the reduction of the populations of flower-visiting pests, whereas active ground hunters may also play an important role preying on pests that develop a part or all of its life cycle in the ground. Further research on feeding behavior such as prey switching is needed for a better understanding of the effectiveness of spiders as natural enemies as biological control agents.

## **5.1. Introduction**

Agroecosystems support a high diversity of arthropods with different functions (e.g., herbivores, omnivores and predators) (Altieri, 1999). Pest control ecosystem service can be exerted by natural enemies occurring throughout the different strata of the agroecosystem (e.g. canopy, soil or spontaneous vegetation). Spiders are among those natural enemies and, since they are extremely diverse (Turnbull, 1973; Uetz et al., 1999), this diversity can be translated into guilds (i.e. groups of species using the same class of resources in a similar way) with different hunting strategies (Uetz et al., 1999).

Agroecosystems with multiple prey species allow predators to switch between prey species. Since spiders are generalist predators, their consumption of prey may depend on the density of the potential available preys or driven by preference. A predator switches between prey when two species are provided together and it starts attacking more the most abundant and stops attacking the prey species that is becoming rare (Murdoch, 1969). In the context of biological control, knowledge on prey preferences by a potential natural enemy is essential in evaluating its pest suppression success and for predicting predation in the field (Hassanzadeh-Avval et al., 2018).

A common approach to investigate the potential of predators for biological control is the study of their functional response. The functional response of a predator-prey system is defined by the relationship between the number of prey eaten per predator and unit time and the prey density, and it is specific of each system (Solomon, 1949; Jeschke et al., 2002). Factors such as different predator foraging strategies, prey handling and digestion time, prey escape or avoidance behavior, alternative prey, resources, habitat structure, and environmental conditions can actually change the functional response (Chan et al., 2017). Moreover, other parameters related to food intake such as digestion and energy should be considered as well because the functional response may depend on them (Jeschke et al., 2002).

Digestion-limited predators process their prey items slower than they handle them (Jeschke et al., 2002). This fact could make spiders inefficient natural enemies, however, the consequence of this unbalance between digestion and handling times can be bypassed through a behavior called overkilling, wasteful killing, or unnecessary killing (i.e. killing without feeding or discarding partially consumed prey) (Johnson et al., 1975).

Different guilds of spiders such as wandering sheet/tangle weavers, ground runners, and specialists proved to be successful natural enemies in laboratory against aphids, flies, and ants respectively, showing an amount of wasteful killing positively correlated with prey density (Mansour & Heimbach, 1993; Samu & Bíró, 1993; Pékar, 2005). Although the role of spiders as natural enemies has been already addressed following a functional response approach, the influence of multiple prey availability on the functional response of spiders has been largely neglected. Also, due to their great diversity, information on the feeding parameters and hunting behavior of spiders is still extremely scarce especially in the context of biological control.

The objective of this work were (1) to assess and compare the functional response of three guilds of spiders using both a single and a multiple-prey approach, (2) to investigate the potential of each functional group as natural enemies in terms of prey biomass extraction from the ecosystem, (3) to calculate the prey preference of each functional group for different prey types, and (4) to link the predator food preference to prey traits.

The selected species of spiders were *Haplodrassus rufipes* (Lucas, 1846) (Gnaphosidae), *Synema globosum* (Fabricius, 1775) (Thomisidae) and *Araniella cucurbitina* (Clerck, 1757) (Araneidae). All the species are generalist predators and in this study they represent three functional groups according to their hunting strategies following Uetz et al. (1999). *Haplodrassus rufipes* is a nocturnal ground runner spider with a Mediterranean distribution that builds its nests under stones and uses them as shelters during the day (Benhadi-Marín, J., personal observation) (body length female: 12.5 mm) (Nentwig et al., 2018). *Synema globosum* is a palearctic ambusher species (body length male: 4 mm; body length female: 6.8 – 8 mm) (Nentwig et al., 2018) that hunts insects on flowers during the day and nests

under the flower or by joining two nearby flowers (Ajuria & Reader, 2014). *Araniella cucurbitina* is an orb-weaver spider that ranges from Europe, Turkey, Central Asia to China and Korea and uses its orbicular web to hunt at different strata on vegetation (body length male: 3.5 - 4.5 mm; body length female: 4.5 - 9.5 mm) (Nentwig et al., 2018).

Three prey species were selected for the functional response experiments, a target prey and two alternative preys. The target prey was *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), the Mediterranean fruit fly, that is a worldwide distributed harmful pest attacking more than 250 species and affecting economically relevant fruit crops (Weldon, 2014). This species was selected as model for a pest that uses different strata during its life cycle. Adults are free-living flying insects; the larvae feed on the fruit pulp and once mature drop to the ground, tunnel into the soil and pupate. Finally, the adult develops within the pupa and emerges towards the soil surface (Thomas et al., 2010). The two alternative prey species were the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and the house cricket *Acheta domesticus* (Linnaeus) (Orthoptera: Gryllidae) as representatives of a flying prey and a soil-inhabiting prey species, respectively.

## 5.2. Material and methods

## 5.2.1. Origin and rearing of spiders

Several subadult spider females of *H. rufipes*, *S. globosum* and *A. cucurbitina* were individually hand captured in the municipalities of Mirandela and Bragança (northeast of Portugal) in early May 2016 and 2017. Specimens of *H. rufipes* were searched under soil stones in an olive grove in Valbom-dos-Figos (41°32'58"N; 7°08'44"O), specimens of *S. globosum* were captured by inspecting flowers of *Cistus ladanifer* L. in a semi-natural shrubland area adjacent to an olive grove in Cedães (41°29'17"N; 7°07'31"O) and specimens of *A. cucurbitina* were captured by inspecting low sized pine trees around Bragança (41°50'19"N; 6°44'49"O) during the night.

The spiders were transported into the laboratory in perforated plastic tubes (volume - 15 mL). Afterwards, specimens of *A. cucurbitina* and *S. globosum* were transferred

individually into plastic Petri dishes (5.2 cm in diameter and 1.2 cm height). The specimens of *H. rufipes* were individually placed into plastic cages (7.7 cm in diameter and 4.3 cm height) and provided with an inverted v-shaped filter paper strip (2 cm in length and 4 cm in width) to provide shelter.

Spiders were maintained in a climate chamber at  $21 \pm 1^{\circ}$ C,  $70 \pm 5\%$  RH and a photoperiod of 16:8 (L:D) h. Every week, half of a 2 cm sphere of sodium acrylate hydrogel was placed on the bottom of each Petri dish to provide water. Spiders were fed *ad libitum* to maturity with adults of *C. capitata* obtained from laboratory cultures maintained at the School of Agriculture of the Polytechnic Institute of Bragança since 2012 (Dinis et al., 2016). Each spider was starved for seven days prior to the functional response assays.

## 5.2.2. Origin and rearing of prey

*Ceratitis capitata* was selected to perform single prey (non-choice) functional response assays, and two other prey species were used as alternative food in the multiple prey functional response assays, the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and the house cricket *Acheta domesticus* (Linnaeus) (Orthoptera: Gryllidae). Moths were reared on a mixture of wheat bran and corn flour (3:1) half-filling 10 uncovered plastic beakers (1 L) that were maintained in two poly-methyl-methacrylate cages ( $40 \times 30 \times 30$  cm) under controlled conditions at  $24 \pm 2^{\circ}$ C;  $60 \pm 5\%$  RH and a photoperiod of 16:8 (L:D). Crickets were initially purchased in a pet shop and maintained in the laboratory at  $21 \pm 1^{\circ}$ C,  $70 \pm 5\%$  RH. The cricket population was placed into a plastic box ( $35 \times 25 \times 30$  cm) covered with a multiperforated cap. A layer of 2 cm in height of cat litter was provided as substrate. A plastic Petri dish (5.2 cm in diameter and 1.2 cm height) was placed at each box corner and filled with minced cat food as nourishment. Water was provided with 10 spheres of sodium acrylate hydrogel (2 cm in diameter) placed randomly on the litter surface.

#### 5.2.3. Experimental arenas

For functional response assays, three kinds of arenas were prepared according to each functional group of spiders. All the arenas were composed by a round plastic cage (7.7 cm

in diameter and 4.3 cm height) with a hole (1 cm in diameter) made in the lateral side of each cage and covered with a lid of a glass Petri dish (9 cm in diameter); each hole was closed with a cotton ball to facilitate the introduction of preys. For *A. cucurbitina*, each arena was left empty since the spiders build their orb-web on the glass Petri dish. For *S. globosum*, an S-shaped paperboard strip (2 cm width in the base, 3 cm in height and 2 cm width in the upper horizontal segment) was glued to the bottom of each cage in order to simulate a flower used as shelter. For *H. rufipes* an inverted v-shaped filter paper strip (2 cm in length and 4 cm in width) was placed on the bottom of the cage in order to simulate a stone used as shelter.

## 5.2.4. Functional response assays

A total of ten arenas for each initial prey density, one spider per arena, and six initial prey densities (N = 3, 5, 10, 15, 25 and 40 individuals) were used to assess the single-prey functional response of each spider species fed on adults of *C. capitata*. Assays were conducted at 21°C and 70  $\pm$  5% relative humidity and a photoperiod of 16:8 (L:D) h. The flies were anesthetized to facilitate manipulation by exposing them to diethyl ether (Panreac, Barcelona, Spain) for 20 seconds before being transferred into the arenas (once in the arenas, flies recovered their activity after 5 minutes on average).

The multiple-prey functional responses were assessed by including two alternative prey species into each arena (the flour moth and the house cricket) together with the target prey *C. capitata*. Ten arenas were used for each initial prey density and one spider per arena and per spider species. Increasing prey densities were given to the spiders maintaining the target prey / alternative prey ratio at  $\approx 1.5$  (N = 3, 6, 10, 15, 25 and 40 for *C. capitata*, and N = 1, 2, 3, 5, 8 and 13 for *E. kuehniella* and *A. domesticus*) and the assays were conducted as explained above.

## 5.2.5. Prey biomass suppression

The potential of spiders to remove prey biomass from the ecosystem (i.e. arenas) was evaluated along with the single-prey functional response assays. Each spider was weighed before and after each trial. For each arena, two groups of flies were used. Each one

contained the same number of individuals (i.e., initial prey density), one group was weighted accounting for the initial fresh weight of the prey (see section 2.7.2.) and the other one was offered to the spiders. Once each functional response assay finished, the remains of the killed prey specimens in each arena were also weighted accounting for the remains of captured prey weight (see section 2.7.2.).

#### 5.2.6. Spider food preference and prey traits

The number of flies, moths and crickets killed by each spider species was recorded at each prey density after the multiple-prey functional response assays and the count of the number of killed individuals of each prey species was used to assess the spider's food preference. Three traits were selected for the prey species that could be related to the spiders food preferences; (1) the prey biomass and (2) prey body length as a representative trait for the hunting risk of predators and (3) the prey activity rate as a trait involved in the encounter rate. The fresh body weight was measured using a precision scale (RDWAG-XA52/2X, d = 0.01 mg) and the body length (from the vertex to the most posterior part of the abdomen in dorsal view) using LAS software (Leica Microsystems Ltd. v.3.8.0.) with a Leica DFC295 camera mounted on a Leica MZ95 stereomicroscope (Leica Microsystems Ltd., Switzerland). Body weight and length were measured in 20 individuals of each prey species.

The activity rate was assessed using a video-tracking system in 45 individuals due to a higher variation within individuals. Adults of each prey species were maintained at 4°C during 10 minutes to reduce activity. Then, after one minute at room temperature (21°C), each specimen was placed individually in the center of a glass Petri dish (5.2 mm in diameter) and the activity was analyzed for 20 minutes using a Computar<sup>®</sup> lens (H2Z0414C-MP, f = 4-8mm, F 1.4, ½", CCTV lens) mounted on a Basler<sup>®</sup> GigE HD Camera (acA1300-60gc with e2v EV76C560 CMOS sensor). The recording tool used was the Media Recorder 2.5 software (Noldus Media Recorder, 2013) and the Petri dishes were illuminated from below (negative contrast) using a computer screen placed horizontally with a full white Microsoft PowerPoint slide loaded. The Noldus Ethovision XT 11.5 software (Noldus et al., 2001) was used to gather the mean velocity (cm/s) and the total

time moving (s) of each individual. Three replicates for each species were video-recorded in five blocks of nine Petri dishes per block (45 tracks per species).

### 5.2.7. Data analysis

#### 5.2.7.1. Functional response assays

Statistical analyses were performed using R software (R Core Team, 2018), based on the tools available from the "frair" package (Pritchard, 2017) and followed Benhadi-Marín et al. (2018a). For each dataset (empirical or simulated), the type of functional response was assessed in four steps:

Firstly, the number of prey killed during the time of the experiment was fitted to a generic type-I functional response model (a linear increase in attack rate with greater prey density) as:

$$N_e \sim a \times N_0 \times T$$
 [Eq. 1]

where  $N_e$  represents the number of prey killed,  $N_0$  represents the initial prey density, *a* represents the attack rate (searching efficiency per time), and T the time of the experiment. For the datasets that did not fit the type-I response, the *frair\_test* function from "frair" package was used to determine the final shape of the functional response of the remaining cases. The *frair\_test* function proceeds by fitting two models by logistic regression:

Killed / not-killed ~ $\alpha + \beta \times \text{Density}$	[Eq. 2]
Killed / not-killed ~ $\alpha + \beta_1 \times \text{Density} + \beta_2 \times \text{Density}^2$	[Eq. 3]

where Density represents the initial prey densities, Killed is the number of prey killed, and not-killed is the difference between them. The function's output provides the sign and *p*-values of the coefficients ( $\beta_1$  and  $\beta_2$ ) used to select between type-II or type-III response.

A negative linear coefficient ( $\beta_I$ ) means a better adjustment to type-II functional response (the number of prey killed hyperbolically approaches to an asymptote which represent the maximum attack rate) in which case the most commonly used models are (1) the Holling's type-II predator-prey function (for experiments with prey replacement) (Holling, 1959):

$$N_{e} \sim (a \times N_{0} \times T) / 1 + (a \times N_{0} \times T_{h})$$
[Eq. 4]

where  $N_e$  represents the number of prey killed, *a* is the attack rate (searching efficiency per time),  $N_0$  is the initial prey density, T is the time of the experiment and  $T_h$  is the handling time (time to attack, kill and eat each prey), and (2) the Rogers's type-II decreasing prey function (for experiments without prey replacement, i.e. prey depletion) (Rogers, 1972):

$$N_e \sim N_0 \{1 - \exp[a(N_e \times T_h - T)]\}$$
 [Eq. 5]

where  $N_e$  represents the number of prey killed,  $N_0$  is the initial prey density, *a* is the attack rate (searching efficiency per time),  $T_h$  is the handling time and T the time of the experiment. The fact that  $N_e$  appears in both sides of the equation is due to lacking of prey replacement during the experiment and is solved using the Lambert's transcendental equation (Bolker, 2008).

A positive linear coefficient  $(\beta_1)$  and a negative quadratic coefficient  $(\beta_2)$  imply that the data fit a type-III functional response (the attack rate (*a*) is a hyperbolic function of prey density) (see Juliano, 2001) in which case a scaling exponent on the attack rate (*a*) can be implemented as:

$$a \sim b \times N_0^{\ q}$$
 [Eq. 6]

where *a* is the attack rate, *b* is a search coefficient,  $N_0$  is the initial prey density, and as *q* increases ( $q \ge 0$ ), the functional response becomes progressively sigmoid (Vucic-Pestic et al., 2010). This applies for both the Holling's type-II predator-prey function (i.e. assuming prey replacement) and the Rogers's type-II decreasing prey function (not assuming prey depletion).

Secondly, once the type of functional response (type-II or type-III) was selected, the *frair\_fit* function was used to fit the data. The *frair\_fit* function fits non-linear predatorprey curves to integer data using the "Nelder-Mead" method for the selected functional response type estimating its parameters (e.g. attack rate (*a*) and handling time ( $T_h$ ) in the case of a type-II response).

Thirdly, once estimated, the attack rate (*a*) and handling time ( $T_h$ ) were bootstrapped using the *frair\_boot* function which resamples a previously fitted predator-prey functional response model and provides the 95% confidence intervals for each parameter. The whole empirical confidence interval for each curve was generated using the *drawpoly* function that draws a closed polygon delineated by the upper and lower edges given by *frair\_boot* over the plotted curve.

Finally, the maximum attack rate  $T/T_h$  (i.e. the maximum number of prey that can be attacked by a predator during the time interval considered) was simulated for each spider species as 24/T<sub>h</sub> and its confidence intervals generated using the simaR package (Benhadi-Marín et al., 2018a). In order to test the effect of additional prey species within the arenas, the multiple-prey functional response analysis was performed for each spider species as described for the single-prey functional responses considering *C. capitata* as target prey ignoring the other prey species, and then compared with the single-prey functional response assays through the confidence intervals.

## 5.2.7.2. Prey biomass suppression

The prey biomass removed from the arenas of spiders was assessed by calculating the following parameters:

Spider weight gain = Final spider weight – Initial spider weight	[Eq. 7]
Captured prey biomass = (Initial prey fresh weight / Initial prey density) × Number of prey killed	[Eq. 8]
Consumed prey biomass = Prey biomass removed from the arena – Remains of captured prey weight	[Eq. 9]
Metabolism = Consumed prey biomass – Spider weight gain	[Eq. 10]
Unexploited prey biomass = Captured prey biomass – Consumed prey biomass	[Eq.11]
% Unexploited prey biomass = (Unexploited prey biomass $\times$ 100) / Captured prey biomass	[Eq. 12]

The captured prey biomass, consumed prey biomass, metabolism (the difference between the consumed prey biomass and the spider weight gain was assumed as the amount of resources spent in terms of metabolism) and spider weight gain were modeled for each spider species as a function of the initial prey density. For it, a series of linear and polynomial models were developed following (Zuur et al., 2009) being the final models:

 $\mathcal{E}_i \sim N(0, \sigma^2)$ 

Spider weight gain ~ $\alpha + \beta \times$ Prey density + $\mathcal{E}_i$	[Eq. 13]
Captured prey biomass ~ $\alpha + \beta_1 \times \text{Prey density} + \beta_2 \times (\text{Prey density})^2 + \mathcal{E}_i$	[Eq. 14]
Consumed prey biomass ~ $\alpha + \beta_1 \times \text{Prey density} + \beta_2 \times (\text{Prey density})^2 + \mathcal{E}_i$	[Eq. 15]
Metabolism ~ $\alpha + \beta_1 \times \text{Prey density} + \beta_2 \times (\text{Prey density})^2 + \mathcal{E}_i$	[Eq. 16]
Consumed prey biomass ~ $\alpha + \beta_1 \times \text{Prey density} + \beta_2 \times (\text{Prey density})^2 + \mathcal{E}_i$	[Eq. 17]

The percentage of unexploited prey biomass (%UPB) was log-transformed to reduce the variance effect and modeled for each spider species using third-grade polynomials as follows:

 $\mathcal{E}_i \sim N(0, \sigma^2)$ log<sub>10</sub>(%UPB) ~  $\alpha + \beta_1 \times Prey \text{ density} + \beta_2 \times (Prey \text{ density})^2 + \beta_3 \times (Prey \text{ density})^3 + \mathcal{E}_i$  [Eq. 18]

Finally, percentage of unexploited prey biomass (%UPB) was compared between spider species using a one-way analysis of variance (ANOVA) followed by a *post hoc* multiple comparisons analysis as:

% UPB ~ 
$$\alpha + \beta_1 \times$$
 Spider species +  $\mathcal{E}_i$  [Eq. 19]

## 5.2.7.3. Spider food preference

The count of the number of individuals killed of each prey species was used to assess the spider's food preference by calculating D, a modification of the Ivlev's Electivity Index

(Ivlev, 1961) proposed by Jacobs (1974) that is independent of the relative abundance of each food type at the initial food density. Using Pearre (1982) notation:

$$D_a = (a_d \times e) - (a_c \times d) / (a_d \times e) + (a_c \times d) - 2 \times (a_d \times a_c)$$
[Eq. 20]

were  $a_d$  is the number of the killed individuals of the species "a", d is the sum of the killed individuals of each species,  $a_c$  is the initial number of the species "a", and e is the sum of the initial number of each species. D ranges from -1 (prey type avoided) to +1 (prey type preferred) and it was calculated for each initial prey density and prey species and provided together with its standard error. The food preference results (D) were pooled together for each spider and prey species along the different initial prey densities (n = 60), the mean and its 95% confidence interval was calculated and for each spider-prey combination, a one sample t-student tests (p < 0.01) was used to check if D was significantly different from zero (i.e. no-preference or avoidance for a prey species).

## 5.2.7.4. Prey functional traits

The activity rate (AR) was calculated for each individual as the percentage of time moving corrected by the mean velocity:

$$AR = (Time moving (s) \times 100 / Total track duration (s)) \times Mean velocity (cm/s) [Eq. 21]$$

Finally, the prey biomass (PB) and length (BL) were compared between prey species using a one-way analysis of variance (ANOVA) and the activity rate was compared analogously using the block and replicate as random factor ( $Z_i \times b_i$ ) as follows:

$$b_i \sim N(0,D)$$
 and  $\mathcal{E}_i \sim N(0,\Sigma_i)$ 

$PB \sim \alpha + \beta_1 \times Prey \text{ species } + \mathcal{E}_i$	[Eq. 22]
BL ~ $\alpha + \beta_1 \times \text{Prey species} + \mathcal{E}_i$	[Eq. 23]
$AR \sim \alpha + \beta_1 \times Prey \text{ species } + Z_i \times b_i + \mathcal{E}_i$	[Eq. 24]

After modeling each response, a *post hoc* multiple comparisons analysis was carried out to test for significant differences between prey species.

## 5.2.7.5. Relationship between food preference and prey traits

The link between food preference and the prey traits considered in this work was established through a distribution of the proportion of prey individuals representing each trait within each arena. A qualitative dichotomous partition of each trait was considered (e.g. lower vs. higher) according to the results obtained in the previous section; then, the proportion of individuals was assigned to each new trait category accounting for the initial amount of each prey species within the arenas. The proportion of individuals 3:1:1 (fly:moth:cricket) of the first initial prey density within each area during the multiple-prey experiment was used as reference. Finally, each branch of the distribution of traits was related to the Electivity index and a category was assigned (from avoided to preferred) for each upstream combination of traits.

#### **5.3. Results**

## 5.3.1. Functional response assays

The estimated parameters for the analyses of the empirical proportion of prey killed by *A*. *cucurbitina* and *S. globosum* fitted type-II functional response models when fed only on *C*. *capitata* adults while *H. rufipes* fitted a type-I functional response model (Table 5.1).

During the single-prey experiment, *S. globosum* was the species that killed the lowest number of *C. capitata* adults at the end of the experiment followed by *A. cucurbitina*; *H. rufipes* killed the highest number of flies and the number of prey killed was statistically significant different among the three spider species according to the 95% confidence intervals obtained for the curves (Fig. 5.1A).

Considering the overlap of the 95% confidence intervals, the coefficients of attack rate (*a*) were significantly different among the species. *A. cucurbitina* showed the highest *a* value followed by *S. globosum* and *H. rufipes* (Table 5.1). The estimated handling times ( $T_h$ ) of

**Table 5.1**. Estimated parameters for the linear and logistic regression analysis of the number of prey killed against prey density for the three spider species studied in the single and multiple-prey functional response experiments. SPS: single-prey experiment; MPS: multiple-prey experiment; Estimate: coefficients derived from the models used to select between type-II and type-III type of functional response; Type: Empirical response type; *a*: coefficient of attack rate and 95% confidence interval (*a*CI); T<sub>h</sub>: estimated handling time and 95% confidence interval (T<sub>h</sub>CI); sMar: mean of the simulated maximum attack rate (T/T<sub>h</sub>) and 95% confidence interval (sMarCI). Different letters for *a* (attack rate) and T<sub>h</sub> mean significant differences between spider species within each experiment. Asterisks indicate statistical significant differences between parameters comparing the two types of experiments. <sup>F</sup> indicates the F-value (1 degree of freedom) corresponding to the type-I functional response fitting model.

Modality	Predator	Estimate	Std. Error	z-value	Pr(> z )	Туре	а	aCI	T <sub>h</sub>	T <sub>h</sub> CI	sMar	sMarCI
	Haplodrassus rufipes	0.845	0.0423	389.1 <sup>F</sup>	< 0.001	Ι	0.031 a	0.028, 0.034	-	-	-	-
SPS	Araniella cucurbitina	-0.080	0.007	-11.037	< 0.001	II	0.179 b	0.143, 0.225	0.897 a	0.734, 1.039	26.674 a	26.311, 27.038
	Synema globosum	-0.047	0.005	-8.731	< 0.001	II	0.075 c	0.050, 0.126	1.443 a	0.914, 2.207	16.445 b	16.088, 16.807
SdW	Haplodrassus rufipes	-0.046	0.005	-8.562	< 0.001	II	0.090 a*	0.061, 0.130	1.201 a	0.890, 1.509	22.272 a	21.824, 22.743
	Araniella cucurbitina	0.064	0.031	424.1 <sup>F</sup>	< 0.001	Ι	0.028 b*	0.026, 0.030	-	-	-	-
	Synema globosum	-0.047	0.005	-8.731	< 0.001	Π	0.055 a	0.041, 0.073	0.514 b*	0.167, 0.848	41.279 b*	39.699, 42.852


**Figure 5.1**. Functional response models obtained for *Haplodrassus rufipes* (ground runner), *Araniella cucurbitina* (orb weaver) and *Synema globosum* (ambusher) fed on *Ceratitis capitata* adults during 24 h single-prey (non-choice) experiments (A), and functional response models obtained for single prey species (SPS) experiments and for multiple-prey species (MPS) experiments with *Haplodrassus rufipes* (B), *Araniella cucurbitina* (C) and *Synema globosum* (D). In SPS experiments, only *C. capitata* adults were provided and in MPS, *C. capitata, Ephestia kuehniella* and *Acheta domesticus* adults were provided together at ratios of 3:1:1 respectively. Thick lines represent the fitted values of data and the polygons that surround them represent the limits of the 95% confidence interval of the empirical curves.

*A. cucurbitina* and *S. globosum* were not significantly different while the maximum attack rate  $(T/T_h)$  of *A. cucurbitina* was significantly higher than that of *S. globosum* (Table 5.1). When the three prey species were offered together, the results obtained for *S. globosum* also fitted a type-II functional response, whereas *H. rufipes* and *A. cucurbitina* switched their functional responses to type-II and type-I, respectively (Table 5.1).

In the presence of multiple prey species, *H. rufipes* killed significantly less *C. capitata* adult individuals at the end of the experiment when compared with the single prey experiment (Fig. 5.1B) and no statistically significant differences were observed for *A. cucurbitina* (Fig. 5.1C). On the contrary, the number of flies killed by *S. globosum* was significantly higher (Fig. 5.1D).

The coefficients of attack rate obtained for *H. rufipes* significantly increased when multiple prey species were offered whereas for *A. cucurbitina*, it significantly decreased and no significantly differences were observed for *S. globosum* (Table 5.1). The estimated handling time for *H. rufipes* was significantly higher than that of *S. globosum* during the multiple-prey experiment whereas the maximum attack rate was significantly lower (Table 5.1).

Comparing the coefficients of attack rate obtained in single and in multiple-prey experiments, the former was significantly higher than the latter in the case of *H. rufipes*, significantly lower for *A. cucurbitina* and was not significantly different for *S. globosum* (Table 4.1). However, the estimated handling time obtained for *S. globosum* significantly decreased and the maximum attack rate significantly increased (Table 5.1).

# 5.3.2. Prey biomass suppression

The captured prey biomass, consumed prey biomass and metabolism of *H. rufipes* increased significantly as the initial prey density increased (Table 5.2) (Fig. 5.2A). The prey biomass captured by *S. globosum* also increased significantly, and in both species the maximum values for these parameters were reached at the maximum initial prey density (N = 40) (Table 5.2) (Figs. 6.2A & 6.2C). The captured prey biomass, consumed prey biomass

**Table 5.2**. Statistics of the models developed for the spider weight gain, captured prey biomass, consumed prey biomass, metabolism, and percentage of unexploited prey biomass (%UPB) as a function of the initial prey density along the single prey species (*Ceratitis capitata*) functional response experiments. IV: independent variable; df: degrees of freedom.

Predator	Model	IV	Estimate	Std. Error	t-value	<b>Pr(&gt; t )</b>	df	F-statistic	<b>Pr(&gt;F)</b>
	Spider weight gain	Initial fly density	0.149	0.130	1.145	0.257	1	1.311	0.257
	Contured prey biomass	Initial fly density	563.590	22.777	24.740	< 0.001	$\mathbf{r}$	325.220	< 0.001
es	Captured prey biomass	(Initial fly density) <sup>2</sup>	140.774	22.777	6.180	< 0.001	2		
difn	Consumed pray biomass	Initial fly density	376.862	22.821	16.514	< 0.001	r	151.600	< 0.001
ı snı	Consumed prey biomass	(Initial fly density) <sup>2</sup>	126.002	22.821	5.521	< 0.001	2		
lrass	Matchaliam	Initial fly density	343.748	22.059	15.583	< 0.001	r	153.200	< 0.001
Haploa	Metabolisiii	(Initial fly density) <sup>2</sup>	144.014	22.059	6.445	< 0.001	Z		
		Initial fly density	0.343	0.284	1.206	0.233			
	% UPB	(Initial fly density) <sup>3</sup>	-0.653	0.284	-2.296	0.026	3	2.490	0.070
		(Initial fly density) <sup>2</sup>	nitial fly density) <sup>2</sup> -0.245 $0.284$		-0.862	0.392			
	Spider weight gain	Initial fly density	-0.051	0.120	-0.422	0.674	1	0.179	0.674
itina	Captured prev biomass	Initial fly density	356.165	22.303	15.970	< 0.001	2	141.270	< 0.001
Araniella cucurbi	Captured prey biolilass	(Initial fly density) <sup>2</sup>	-116.959	22.303	-5.244	< 0.001	2		
	Consumed prey biomass	Initial fly density	140.019	18.689	7.492	< 0.001	2	51 724	<0.001
	Consumed prey biomass	(Initial fly density) <sup>2</sup>	-128.579	18.689	-6.880	< 0.001	2	51.754	<b>\U.UU</b>
	Matabolism	Initial fly density	121.623	19.048	6.385	< 0.001	2	42.057	<0.001
	WELAUUIISIII	(Initial fly density) <sup>2</sup>	-135.040	18.699	-7.222	< 0.001	L	43.737	<0.001

			Initial fly density	2.278	0.250	9.111	0.000			
		% UPB	(Initial fly density) <sup>2</sup>	-0.818	0.250	-3.273	0.002	3	33.545	< 0.001
			(Initial fly density) <sup>3</sup>	0.658	0.250	2.631	0.011			
_		Spider weight gein	Initial fly density	67.397	11.954	5.638	< 0.001	2	22 157	<0.001
Synema globosum	Spider weight gam	(Initial fly density) <sup>2</sup>	-35.258	11.508	-3.064	0.003	Ζ	22.137	<0.001	
	Captured prey biomass	Initial fly density	1.942	0.274	7.092	< 0.001	1	50.302	< 0.001	
	Consumed prey biomass	Initial fly density	0.575	0.201	2.860	0.006	1	8.181	0.006	
	Metabolism	Initial fly density	0.135	0.128	1.049	0.299	1	1.101	0.299	
		Initial fly density	1.479	0.235	6.293	< 0.001				
	% UPB	(Initial fly density) <sup>2</sup>	-1.620	0.235	-6.891	< 0.001	3	35.590	< 0.001	
			(Initial fly density) <sup>3</sup>	1.043	0.235	4.437	< 0.001			



**Figure 5.2.** Variation of the captured and consumed prey biomass, metabolism, spider weight gain (upper panels) and percentage of unexploited prey biomass of three functional groups of spiders fed for 24 h on differing densities of adults of *Ceratitis capitata*. *Haplodrassus rufipes* (ground hunter) (A & D), *Araniella cucurbitina* (orb-weaver) (B & E) and *Synema globosum* (ambusher) (C & F). Asterisks in A, B and C indicate a slope significantly different from zero.

and metabolism of *A. cucurbitina* were also significantly affected by the initial prey density reaching a maximum (159.730 mg, 96.113 mg, and 82.777 mg,) at an initial prey density of  $\approx 37, \approx 27$  and  $\approx 26$  flies respectively (Fig. 5.2B). The weight gain of *S. globosum* was also significantly affected by the initial prey density and the maximum value was obtained (30.318 mg) at  $\approx 31$  flies (Fig. 5.2C). On the contrary, there was no significant variation in the weight gain of *H. rufipes* and *A. cucurbitina* with the initial prey densities (Table 5.2) (Fig. 5.2A & 6.2B). Also, the metabolism and consumed prey biomass of *S. globosum* were not significantly affected by the initial number of flies (Table 5.2) (Fig. 5.2C). The percentage of unexploited prey biomass (%UPB) was not significantly different between spider species (F = 0.361; df = 2; P = 0.698) and did not changed significantly with the initial prey density in the case of *H. rufipes* (37.33% in average) (Table 5.2) (Fig. 5.2D). On the contrary, this parameter varied significantly with the initial prey density for *A. cucurbitina* and *S. globosum* reaching its maximum at different prey densities, 40 flies in the case of *A. cucurbitina* (%UPB = 52.59%) (Fig. 5.2E) and  $\approx 16$  flies for *S. globosum* (%UPB = 61.74%) (Fig. 5.2F).

# 5.3.3. Spider food preference and prey traits

The three spider species showed the same pattern in terms of food preferences (Fig. 5.3A, 5.3B & 5.3C). The Electivity Index (D) was positive and significantly different from zero for *C. capitata*, negative and significantly different from zero for *A. domesticus* and did not differ significantly from zero for *E. kuehniella* (Table 5.3).

The activity rate was significantly different between the three prey species ( $\chi^2$ = 66.603; df: 2; P < 0.001). The highest percentage of activity rate corresponded to *A. domesticus* whereas the lowest mobile prey was *E. kuehniella* (Fig. 5.3D). The body length (BL) was also significantly different between the three prey species (F = 303.270; df: 2; P < 0.001) being *A. domesticus* the longest one followed by *C. capitata* and finally *E. kuehniella* (Fig. 5.3E). The prey biomass (PB) was significantly different between prey species, however, no significant differences were found between *C. capitata* and *E. kuehniella*, being *A. domesticus* significantly heavier than the two previous species (F = 140.120; df: 2; P < 0.001) (Fig. 5.3F).



**Figure 5.3.** Feeding preference (electivity index, D) among three prey species (the fly *Ceratitis capitata*, the moth *Ephestia kuehniella* and the cricket *Acheta domesticus*) showed by three functional groups of spiders (upper panels), and functional traits of each prey species (lower panels). A: *Haplodrassus rufipes* (ground runner); B: *Araniella cucurbitina* (orb-weaver); C: *Synema globosum* (ambusher); D: activity rate; E: body length, and F: prey biomass.

**Table 5.3.** Statistics of each *t*-test performed to compare the Electivity Indexes (D) calculated for assessing food preferences of the spider species *Haplodrassus rufipes* (HR) (ground runner), *Araniella cucurbitina* (AC) (orb weaver) and *Synema globosum* (SG) (ambusher) fed on each prey species used in the multiple-prey functional response experiments.; df: degrees of freedom; Estimate: mean of D for the complete pool of samples (n = 60); DCI: 95% confidence interval for the mean of D.

Predator	Prey	t-value	df	Estimate	DCI	Р
HR	Ceratitis capitata	5.882	59	0.274	0.181, 0.368	< 0.001
	Ephestia kuehniella	-2.094	59	-0.046	-0.089, -0.002	0.041
	Acheta domesticus	-7.552	59	-0.153	-0.194, -0.112	< 0.001
	Ceratitis capitata	10.494	59	0.311	0.252, 0.370	< 0.001
AC	Ephestia kuehniella	0.564	59	0.010	-0.025, 0.044	0.575
	Acheta domesticus	-15.594	59	-0.248	-0.280, -0.216	< 0.001
	Ceratitis capitata	8.684	59	0.305	0.235, 0.375	< 0.001
SG	Ephestia kuehniella	1.937	59	0.037	-0.001, 0.074	0.058
	Acheta domesticus	-23.422	59	-0.271	-0.295, -0.248	< 0.001

# 5.3.4. Relationship between food preference and prey traits

The first trait considered was the prey biomass and it was categorized as heavy prey (crickets) and light prey (flies and moths) (Fig. 5.3F & Fig. 5.4); since there were 60% of flies and 20% of moths within the arenas, the light prey category represented p = 0.6 + 0.2= 0.8 of the total prey density and thus the heavy prey (crickets) represented p = 0.2. Then, the prey length was divided downstream into medium-sized prey (moths represented 25% of 0.8 = 0.2) and small-sized prey (flies represented 75% of 0.8 = 0.6) (Fig. 5.3E & Fig. 5.4). Within prey length level, the heavy prey (crickets) inherited its upstream proportion p = 0.2 and was categorized as long-sized prey (Fig. 5.3E & Fig. 5.4). The last one trait considered was the activity rate. The former long-sized prey (crickets) inherited its proportion p = 0.2 and was categorized as highly mobile prey (Fig. 5.3D & Fig. 5.4). The medium-sized prey (moths) inherited its proportion p = 0.2 and was categorized as highly mobile prey (Fig. 5.3D & Fig. 5.4). The medium-sized prey (Fig. 5.3D & Fig. 5.4) whereas the small-sized prey (flies) inherited its



**Figure 5.4.** Relationship between prey traits, relative abundance, Electivity index, and food preference of three functional groups of spiders fed on three alternative prey species (*Acheta domesticus, Ephestia kuehniella* and *Ceratitis capitata*) during a series of multiprey functional response laboratory experiments. *p* represents the proportion of individuals of each category within an arena. P represents the p-values of a *t*-test performed to compare the Electivity Index with the null hypothesis (no preference). HR: *Haplodrassus rufipes* (ground runner), AC: *Araniella cucurbitina* (orb-weaver), and SG: *Synema globosum* (ambusher).

proportion p = 0.2 and was categorized as moderately mobile prey (Fig. 5.3D & Fig. 5.4). Considering the values of the Electivity index and each trait together, three prey classes were defined: (1) heavy, long-sized and highly mobile prey, (2) light, medium-sized and

low mobile prey, and (3) light, small-sized and moderately mobile prey corresponding to the avoided, occasionally consumed, and preferred prey respectively (Fig. 5.4).

# 5.4. Discussion

In this work, each of the three guilds of spiders studied showed a different functional response on *C. capitata* and the functional response significantly changed when alternative preys were available. These results correspond with those obtained by Benhadi-Marín et al. (2018a) using *C. capitata* in non-choice laboratory experiments performed at 21°C. At the highest prey density (N = 40 flies), Benhadi-Marín et al. (2018a) found that *A. cucurbitina* killed 20.75  $\pm$  2.75 individuals (mean  $\pm$  standard deviation) and *S. globosum* 7.5  $\pm$  1.73 individuals, whereas in this study, 21.10  $\pm$  4.97 and 11.50  $\pm$  4.46 individuals were killed by each species, respectively. Although a different functional response type for *A. cucurbitina* was found, the confidence intervals for both the attack rate and handling time of *S. globosum* overlapped with those obtained by Benhadi-Marín et al. (2018a).

Regarding the multiple-prey experiments, the results clearly demonstrated that the presence of alternative prey can shift the predatory efficiency of a generalist predator on a target prey species. For the single-prey experiments, the most and the least efficient spider species against *C. capitata* were *H. rufipes* and *S. globosum* respectively. However, opposite patterns were found when alternative preys were available. In the presence of alternative prey species *H. rufipes* shifted from a type-I to a type-II functional response killing approximately half of the flies at the maximum prey density, *A. cucurbitina* shifted from a type-II to a type-I functional response killing significantly more flies at the highest prey density, and *S. globosum* showed a type-II functional response in both experiments significantly increasing the number of flies killed at the highest prey density.

Few studies have addressed the switching behavior in spiders and suggested that spiders can actually switch between alternative preys according to prey density (e.g. Provencher & Coderre, 1987; Hardwood et al., 2004). Since the experimental design of this work focused on keeping the proportion "target prey / alternative prey" constant, the switching effect was not tested directly. Due to the massive preference of all spiders for the most abundant prey

(flies) during the multiple-prey experiments (especially at the highest prey density) it would be reasonable to think that the three species may switch; however, it is still unclear if it corresponds to active (due to changes on behavior) or passive (due to a constant prey preference) switching (see Begon et al., 1996; Gentleman et al., 2003; Vallina et al., 2014).

A clear gradient on the evolution of the prey biomass captured by spiders was found along the increased prey density during the single-prey experiments. While the prey biomass captured by *H. rufipes* increased exponentially, *A. cucurbitina* reached a plateau at the highest prey densities and *S. globosum* showed a linear increase. In general, spiders kill more prey than that they consume (Mansour & Heimbach, 1993), however different guilds may handle differently their resources according to their nutritional requirements and hunting strategies. Although the amount of overkilling usually increases with prey density (Mansour & Heimbach, 1993; Samu & Bíró, 1993), the results obtained in this work did not support this correlation for *H. rufipes*. As ground runner, *H. rufipes* actively pursue its prey and due to its high body size presumably needs a high amount of energy to hunt; however it was not found a significant increase in the percentage of unexploited prey biomass towards the highest prey densities.

Multiple-prey capture could explain this lack of correlation, *Haplodrassus rufipes* was observed to pursue and kill several prey items in the arena before consumption. According to Bailey (1985) multi-prey captures should occur at high prey densities, when hungry predators (in this work starved spiders before the experiments) are exposed to high numbers of prey items during short periods (e.g. a high prey density within an experimental arena). It has been suggested that this behavior could be derived from an attempt to increase the size of each meal, thus allowing the spider to feed less frequently (Williams, 1979). Since *H. rufipes* represents the longest body size among the three species of spiders tested in this work, the results and observations agree with those made by Williams (1979) on the active hunter *Dolomedes* Latreille, 1804 (Pisauridae) both in the field and in laboratory supporting that multi-capture is performed by bigger spiders that are capable to handle several small preys.

Since the density of alternative preys (moths and crickets) proportionally increased across the increasing target prey densities (flies), changes on the functional response and predatory behavior between the single and multiple-prey experiments could be explained in terms of predator-prey encounter rates (Scharf et al., 2008; Smout et al., 2010). Because of the higher number of total prey items within the arenas in the multiple-prey experiments (i.e. the same number of flies plus the alternative preys) the predator-prey encounter rate can increase when compared with the single-prey experiments.

Considering the preference of *H. rufipes* for flies (especially at high prey densities), selecting each individual among an increased number of prey items can be more costly. The handling time includes the time necessary to pursue, subdue, feed and restart hunting (Holling, 1959), and in fact, the handling times of *H. rufipes* increased from a theoretical zero (type-I response) during the single-prey experiments to 1.2 h during the multiple-prey experiments. Moreover, the spiders' functional response can be reduced, as it was found for *H. rufipes*, if the nutrient composition of the prey is far from the spider's intake target (Toft, 2013) which suggests that *C. capitata* could be an easy-to-catch but low-quality prey for this species.

In the case of *A. cucurbitina* (orb-weaver), the web most likely enhanced the chances to trap flies (especially within a closed environment such as an experimental arena) thus decreasing the handling time from 0.89 h to theoretically zero when multiple preys were available and significantly increasing its functional response. For this species, the percentage of unexploited prey biomass reached its maximum at the maximum prey density. In this case, these results agree with the general assumption that partial consumption increases with prey density.

*Araniella cucurbitina* could be considered as an actual queueing predator (i.e. predators for which handling and capture prey are not mutually exclusive, see Juliano (1989) for details) due to the use of their sticky webs for hunting purposes. In fact, several spiders were observed handling and eating flies while other flies were stacked still alive or wrapped in the web. In this sense of queueing prey, although spiders could develop aversion against

certain types of abundant prey (Toft, 1997; 2013), orb-weavers may prioritize the highest quality prey to the head of the queue among the available prey (Cox & Smith, 1961). Accordingly, a kind of preference could be achieved if flies were a high-quality prey for *A. cucurbitina*. However, Provencher & Coderre (1987) found that the behavior of the orb-weaver *Tetragnatha laboriosa* Hentz, 1850 (Tetragnathidae) in experimental arenas created a refuge for prey, as spiders occupied the upper part of the arenas while prey remained in the lower part. Since the same behavior was found in *A. cucurbitina*, flies were significantly more active than moths, and crickets mostly used the bottom of the arenas, the preference for flies could be artificial and still remains unclear for *A. cucurbitina*.

*Synema globosum* is a sit-and-wait predator. Ambusher predators can actually search while eating and while waiting empty handed (Lucas, 1985), however *S. globosum* was not observed searching for other prey items during the consumption period. Probably due to an increase of the encounter rate, *S. globosum* increased the number of flies killed during the multiple-prey experiments significantly reducing its handling time from 1.43 to 0.514 h. This behavior could be due to partial consumption. *Synema globosum* presented its maximum of unexploited prey biomass at the lowest number of prey density among the three species of spiders studied in this work during the single-prey experiments; however, the amount of consumed prey biomass was not significantly affected by prey density.

Since *S. globosum* captures its prey one by one, two reasons not mutually exclusive may explain the decrease of its handling time and the lack of a significant increase of the consumed prey biomass with prey density; on one hand, it could be due to a temporary filling of the foregut instead from satiation (Johnson et al., 1975), and on the other hand, the increased response could be done by the disturbance caused by a prey while the spider was handling another one, thus making the spider release the former and capture the new prey as Haynes & Sisojević (1966) suggested for the ambusher *Philodromus rufus* Walckenaer, 1826 (Philodormidae) when fed on *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) in laboratory.

#### **5.5.** Conclusions

Considering the prey preference and prey traits, the three functional groups of spiders studied in this work surprisingly showed the same pattern: the heavy, long-sized and highly mobile preys (crickets) were avoided; the light, medium-sized and low mobile preys (moths) were occasionally killed; and the light, small-sized and moderately mobile preys (flies) were preferred. However, the hunting behavior and feeding parameters clearly differed according to each spider guild. Summarizing, when different prey species were available, orb-weavers could be efficient predators against flying pests such as C. capitata due to their web device. This is of capital importance for biological control since orbweaver spiders encompass cosmopolitan species (such as A. cucurbitina) frequently observed inhabiting crops (e.g. Mestre et al., 2012) at different vertical strata including the canopy of trees, bushes and the herbaceous layer (Nentwig et al., 2018). On the other hand, flower-living ambushers such as S. globosum could help to reduce the population of pest flies that feed on pollen and/or nectar, whereas active ground hunters with a lower functional response on flies such as *H. rufipes* may also play an important role preying on soil-inhabiting life cycle stages of pests (e.g. crawling or digging larvae and teneral stages of dipteran pests).

Laboratory experiments based on a single predator-prey system may lead to a limited understanding of its functional response in the field and multiple-prey laboratory experiments are been strongly recommended (e.g. Smout et al., 2010). The consideration of different sources of noise in laboratory experiments including multiple prey availability, arenas of different size, and variation in temperature is mandatory toward a more realistic evaluation of a predator functional response. Although efforts have been made to assess the role of predator traits on the predation rates in agroecosystems (e.g. Rusch et al., 2015), research focused on the body parameters of prey is required to disentangle the role of their traits in a context of biological control. Also, further research in terms of feeding parameters such as the amount of overkilling and prey switching is needed for a better understanding of the effectiveness of spiders as natural enemies as biological control agents.

# **CHAPTER 6**

A simulation-based method to compare the pest suppression potential of predators: a case study with spiders.

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# **Contribution of authors:**

J.B.M., S.A.P.S. and J.A.P. conceived and designed the functional response experiments, J.B.M. developed the R functions and analyzed the data, D.B. collected the spiders in the field and provided assistance during the laboratory experiments, J.P.S. reviewed and tested the code and all the authors contributed to writing the paper.

# **CHAPTER 6**

A simulation-based method to compare the pest suppression potential of predators: a case study with spiders.

# Abstract

Assessing and comparing the pest killing capacity of predators is a crucial but laborious task during the implementation of sustainable farming systems. Critical attributes of assessment include quantifying predator's attack rate (a) and handling time (T<sub>h</sub>). The maximum attack rate (T/T<sub>h</sub>) (i.e. the maximum number of prey that can be attacked by a predator during the time interval (T) considered) could be a more precise and interpretable indicator of the potential suppression of pests exerted by a predator; however, its calculation only provides a point estimator usually derived from incomplete datasets (e.g. unbalanced or low replicated experimental designs) that could lead to draw wrong conclusions. The "simaR" (simulation of maximum attack rates using R) package was developed. The simaR package generates 95% confidence intervals around estimates of the maximum attack rate that can be easily and intuitively used to compare across species. The simulation method was validated and the empirical results of a controlled laboratory experiment were used to compare the maximum attack rates of spiders across a range of Medfly prey densities and illustrate how to use simaR with non-replicated partial data. Applying this method, a significant effect of temperature was found on the maximum attack rate of two different guilds of spiders, the orb-weaver A. cucurbitina and the ambusher S. globosum that was not relevant regarding their attack rate and handling time. This method compares different predator species and/or experimental conditions in a simple and reproducible procedure through an accurate, easy-to-use, fast and statistically robust analysis, based on simulation and bootstrapping that can be used to assess the pest suppression potential of predators by simulating their functional responses from low-effort laboratory trials.

#### **6.1. Introduction**

A classic way of characterizing the predation behavior of a species in terms of prey consumption is through modeling its functional response, i.e. the number of prey consumed or killed by a predator as a function of prey density (Bolker, 2008; Holling, 1959; Solomon, 1949). Predation can be a critical factor affecting prey population dynamics of pest species, thus its accurate description is crucial for practical and applied aspects of biological control (VanLeeuwen et al., 2007).

The attack rate (*a*) and handling time ( $T_h$ ) are the most common parameters obtained from the functional response models and are widely used to compare the efficiency of a predator species as a natural enemy, or how such an efficiency is affected by differing variables (e.g. Dinis et al., 2016; Gilioli et al., 2005; Pasandideh et al., 2015; Tello et al., 2017). However, variation within these two parameters can be difficult to interpret in terms of bioecology since they are estimated non-independently of each other. In addition, the amount of overlap of their corresponding dispersion measures or interval estimates (e.g. standard deviation and confidence interval) may conceal important ecological patterns only due to low quality datasets (i.e. low replicated and/or incomplete datasets). This fact could lead to misinterpretation of results and affect decision making, for example, selecting a predator as the most efficient against a pest or assessing the effects of a pesticide on the mortality of a selected predator species.

The maximum attack rate  $(T/T_h)$ , which is the maximum number of prey that can be attacked by a predator or a parasitoid during an interval of time, is a parameter easily calculated using the time interval considered (T) of a functional response experiment and the estimated handling time  $(T_h)$  obtained from modeling the functional response of a predator. This measure also allows evaluating the suppression potential of a species and it could be used as a stand-alone parameter to make comparisons within a pest control framework since it is directly related to the number of prey killed. However, most published works describe non-replicated experiments and the modeling of a single functional response dataset generates a single point estimator for the maximum attack rate lacking any dispersion measure (e.g. a laboratory experiment conducted during 24h and an estimated handling time of 1.5 h, gives a maximum attack rate of 24/1.5 = 16 prey/day). Accordingly, providing a point estimator without a mean and its dispersion measure reduces its usefulness, especially when comparing experiments with a different x and y scales. In fact, in many published works the maximum attack rate is calculated without any dispersion measure or is compared using statistical techniques available from expensive software packages which may limit the number of researchers that could have access to them (e.g. Ahn et al., 2010; Dinis et al., 2016; Mercado et al., 2017).

Simulation based methods have been widely used by ecologists and the number of packages, code and data have been constantly increasing (e.g. Delmas et al., 2017; Réjou-Méchain et al., 2017). Also, within the context of functional response analysis useful tools have been already developed to help estimate the parameters derived from a functional response experiment such as the "frair" package (Pritchard, 2017) for R software (R Core Team, 2018). However, a simulation method to assess and compare directly (e.g. through the maximum attack rate) the potential of different species as natural enemies is still lacking. In addition, the laboratory trials necessary to model a functional response are usually arduous and time consuming because of the difficulty of rearing not only a particular species (e.g. species strongly linked to ecological processes hard to reproduce in laboratory) but also the necessary number of individuals to perform the experiment. This can result in a low number of samples, replicates, or incomplete datasets (e.g. a low number of initial prey densities) that usually causes poor data fitting to the functional response models.

The objectives of this work were (1) to develop a simulation-based method to assess the predatory potential of a species based on the comparison of the maximum attack rate, (2) validate the method, (3) provide an R package to apply the method, and (4) apply the method to two generalist predators fed with a cosmopolitan pest at three temperatures using empirical data as starting point.

Globally, spiders are among the most abundant predators, and their ubiquitous feeding habits upon invertebrate prey qualify them as potential pest control agents in agroecosystems (Benhadi-Marín et al., 2016b). The generalist predators selected as model species to apply the simulation method were *Araniella cucurbitina* (Clerck) (Araneae: Araneidae) and *Synema globosum* (Fabricius) (Araneae: Thomisidae). The distribution of *A. cucurbitina* ranges from Europe, Turkey, Central Asia to China and Korea (Nentwig et al., 2018). *A cucurbitina* is an orb-weaver spider that uses its orbicular web to hunt on trees, bushes and the herbaceous strata (body length male: 3.5 - 4.5 mm; body length female: 4.5 - 9.5 mm) (Nentwig et al., 2018). *S. globosum* is a palearctic ambusher spider (body length male: 4 mm; body length female: 6.8 - 8 mm) (Nentwig et al., 2018). Females can be easily observed on flowers, during the day, hunting insect pollinators (Ajuria & Reader, 2014). The selected prey species model was *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *C. capitata*, the Mediterranean fruit fly, is a worldwide distributed harmful pest that attacks more than 250 species and affects economically relevant fruit crops such as apple, citrus, figs, peach and pear (Carroll et al., 2002; Weldon et al., 2016).

#### 6.2. Material and methods

#### 6.2.1. Origin and rearing of organisms

Subadult spider females of *S. globosum* were individually hand captured from the flowers of *Cistus ladanifer* L. located in Cedães (41° 29'17"N; 7° 07'31"W), northeast of Portugal, in early May 2016. Subadults of *A. cucurbitina* were captured by inspecting low size pine trees around Bragança (41°50'19"N; 6°44'49"O), northeast of Portugal, during the night in early April 2016.

The spiders were transported into the laboratory in perforated plastic tubes (volume 15 mL). Afterwards, they were transferred individually into plastic Petri dishes (5.2 cm in diameter and 1.2 cm height) and maintained in a climate chamber at  $21 \pm 1^{\circ}$ C,  $70 \pm 5\%$  relative humidity and a photoperiod of 16:8 (L:D) h. Every week, half of a 2 cm sphere of sodium acrylate hydrogel was placed on the bottom of each Petri dish to provide water. Spiders were fed *ad libitum* to maturity with adults of *C. capitata* obtained from laboratory

cultures maintained at the School of Agriculture of the Polytechnic Institute of Bragança since 2012 (Dinis et al., 2016).

# 6.2.2. Experimental arenas

Adult spiders were transferred into plastic cages (7.7 cm in diameter and 4.3 cm height) and starved for seven days. For *A. cucurbitina*, no other elements were introduced in the arena since the spiders build their orb-web under the glass Petri dish top. In the case of *S. globosum* a paperboard strip folded in three parts (S-shaped) was placed in the center of each cage to simulate a flower for shelter. The first part ( $2 \text{ cm} \times 2 \text{ cm}$ ) was adhered horizontally to the cage base, the second part (2 cm in width and 3 cm in height)left vertically to the former and acted as stem, and the third one ( $2 \text{ cm} \times 2 \text{ cm}$ ) left horizontally the second one acting as flower. Each cage was covered with a glass Petri dish top (9 cm in diameter) and was used as arena. A hole (1cm in diameter) was opened in the side of each cage and sealed with a cotton ball to facilitate the introduction of prey.

# 6.2.3. Functional response assay

Four arenas, one spider per arena, and four initial prey densities ( $N_0 = 3, 5, 10$  and 40) were used to assess the functional response of *A. cucurbitina* and *S. globosum* on adults of *C. capitata.* Each of the four spiders was used once (i.e. one replicate) per prey density and temperature giving a total of four individuals × four prey densities × three temperatures = 48 individuals per species. Assays were conducted at three different, but constant temperatures (21 °C, 24 °C and 27 °C) at 70 ± 5% relative humidity and a photoperiod of 16:8 (L:D) h. For the temperatures 24 °C and 27 °C, the spiders were subjected to an acclimatization period of 7 days, and in all cases, a 7 days period of starvation preceded the functional response experiment. The flies were anesthetized to facilitate manipulation by exposing them to diethyl ether (Panreac, Barcelona, Spain) for 20 seconds before being transferred into the arenas (once in the arenas, flies recovered their activity behaving normally after 5 minutes in average). After 24 h, the number of flies killed was recorded.

#### 6.2.4. Data analysis

### 6.2.4.1. Functional response analyses

Statistical analyses were performed using R software (R Core Team, 2018) and were based on the tools available from the "frair" package (Pritchard, 2017). For each dataset (empirical or simulated), the type of functional response ), was selected in four steps:

Firstly, the number of prey killed during the time of the experiment was fitted to a generic type-I functional response model (a linear increase in attack rate with greater prey density) as:

$$N_e \sim a \times N_0 \times T$$
 [Eq. 1]

where  $N_e$  represents the number of prey killed,  $N_0$  represents the initial prey density, *a* represents the attack rate (searching efficiency per time), and T the time of the experiment. For the datasets that did not fit the type-I response, the *frair\_test* function from "frair" package was used to determine the final shape of the functional response of the remaining cases. The *frair\_test* function proceeds by fitting two models by logistic regression:

Killed / not-killed ~ 
$$\alpha + \beta \times$$
 Density [Eq. 2]

Killed / not-killed ~ 
$$\alpha + \beta_1 \times \text{Density} + \beta_2 \times \text{Density}^2$$
 [Eq. 3]

where Density represents the initial prey densities, Killed is the number of prey killed, and not-killed is the difference between them. The function's output provides the sign and *p*-values of the coefficients ( $\beta_1$  and  $\beta_2$ ) used to select between type-II or type-III response.

A negative linear coefficient ( $\beta_1$ ) means a better adjustment to type-II functional response (the number of prey killed hyperbolically approaches to an asymptote representing the maximum attack rate) in which case the most commonly used models are (1) the Holling's type-II predator-prey function (for experiments with prey replacement) (Holling, 1959):

$$N_{e} \sim (a \times N_{0} \times T) / 1 + (a \times N_{0} \times T_{h})$$
[Eq. 4]

where  $N_e$  represents the number of prey killed, *a* is the attack rate (searching efficiency per time),  $N_0$  is the initial prey density, T is the time of the experiment and  $T_h$  is the handling time (time to attack, kill and eat each prey), and (2) the Rogers's type-II decreasing prey function (for experiments without prey replacement, i.e. prey depletion) (Rogers, 1972):

$$N_e \sim N_0 \{1 - \exp[a(N_e \times T_h - T)]\}$$
 [Eq. 5]

where  $N_e$  represents the number of prey killed,  $N_0$  is the initial prey density, *a* is the attack rate (searching efficiency per time),  $T_h$  is the handling time and T the time of the experiment. The fact that  $N_e$  appears in both sides of the equation is due to lacking of prey replacement during the experiment and is solved using the Lambert's transcendental equation (Bolker, 2008).

A positive linear coefficient  $(\beta_1)$  and a negative quadratic coefficient  $(\beta_2)$  imply that the data fit a type-III functional response (the attack rate (*a*) is a hyperbolic function of prey density) (see Juliano, 2001) in which case a scaling exponent on the attack rate (*a*) can be implemented as:

$$a \sim b \times N_0^q$$
 [Eq. 6]

where *a* is the attack rate, *b* is a search coefficient,  $N_0$  is the initial prey density, and as *q* increases ( $q \ge 0$ ),the functional response becomes progressively sigmoid (Vucic-Pestic et al., 2010). This applies for both the Holling's type-II predator-prey function (i.e. assuming prey replacement) and the Rogers's type-II decreasing prey function (not assuming prey depletion).

Secondly, once the type of functional response (type-II or type-III) was selected, the *frair\_fit* function was used to fit the data. The *frair\_fit* function fits non-linear predatorprey curves to integer data using the 'Nelder-Mead' method for the selected functional response type estimating its parameters (e.g. attack rate (*a*) and handling time ( $T_h$ ) in the case of a type-II response).

Thirdly, once estimated, the attack rate (*a*) and handling time ( $T_h$ ) were bootstrapped using the *frair\_boot* function which resamples a previously fitted predator-prey functional response model and provides the 95% confidence intervals for each parameter. The whole empirical confidence interval for each curve was generated using the *drawpoly* function that draws a closed polygon delineated by the upper and lower edges given by *frair\_boot* over the plotted curve.

Finally, the experimental maximum attack rate was calculated as  $T/T_h$  and a series of replicates were simulated to generate its confidence interval (see section 6.2.4.2).

# 6.2.4.2. Simulation for assessing and comparing the prey killing potential of a predator: the simaR package.

An R package consisting of six core functions was developed to run the whole simulation process (Fig. 6.1). simaR requires a matrix with two columns as input, the first one containing a vector of the initial prey densities and a second one containing a vector of the proportion of prey killed at each initial prey density. The process described in Fig. 6.1 is performed by simaR once by dataset (in this case study with spiders it is applied six times, one for each of the six species-temperature relationships).

Since each of the prey individual has a certain probability of dying, the pool of discrete data (i.e. the number of prey killed at each initial prey density) is simulated by extracting random values from a binomial distribution with N equal to the total number of available flies and p equal to the proportion of prey that were killed at each initial prey density. By default, a set of 20 simulated samples and 50 datasets (i.e. 50 simulated replicates) is generated for each study case.

Each set of simulated datasets is then passed to the *newTests* function to perform and inspect the test of fitting to a type-II or type-III functional response.



**Figure 6.1.** Workflow with the simaR package. The six core functions are shown in bold. The expected input and the output generated by each function are shown in boxes. The processes carried out by each function are shown in italic. In this case study with spiders, the whole process was done six times, one for each of the six species-temperature relationships.

Then, the user can select between a series of functional response models already implemented in the "frair" package (see below). The handling time ( $T_h$ ) can be approximated by the time of the experiment (T) divided by the number of prey killed as T/Ne<sub>max</sub>, where Ne<sub>max</sub> is the number of prey killed at the highest prey density (Juliano, 2001) and the mean of this value is used by the next function (*getFitData*) as starting value for the handling time ( $T_h$ ) to be optimized. By default, the starting value for the attack rate (*a*) is 0.001.

According to the results of *newTests*, the *getFitData* function allows to select between the following functional response models provided by the "frair" package: (1) Holling's type-II

predator-prey function (assuming prey replacement), (2) Rogers's type-II decreasing prey function(not assuming prey replacement), (3) Hassell's original type-III response (assuming prey replacement), (4) Hassell's type-III response (not assuming prey replacement), (5) Scaling exponent response (assuming prey replacement), (6) Scaling exponent response (not assuming prey replacement), and (7) The 'Ecological Models and Data in R' type-II decreasing prey function for more than one predator (assuming prey replacement) (Table S8).

During the former process, some outliers could be generated due to the simulation of datasets that poorly fitted the selected functional response model. For example, if the experimental data fit a type-II response and *simData* generates by chance a simulated dataset that fits better a type-I than a type-II functional response, *getFitData* will fit the simulated dataset by brute force (since the response type is selected by the user) as a type-II response. Consequently, the handling time ( $T_h$ ) for that simulated dataset will be significantly underestimated and accordingly, the value for its estimated maximum attack rate ( $T/T_h$ ) will be overinflated. The consequence is that the mean of the simulated maximum attack rates may be significantly altered and this bias trespassed to the subsequent bootstrapping process. It is not necessary to inspect the entries in the output of *newTests* one by one, instead, it is suggested to check the presence of outliers within the list of simulated maximum attack rates using the *boxplot.stats* function and remove them (if any) manually from the *getFitData* output (that will be passed to *Max\_attackRates*) to ensure accuracy of further calculations.

The simulated curves (50 curves by default according to the number of replicates) can be plotted using the *plotCurves* function to visually inspect the simulation results. Then, the function *Max\_attackRates* calculates the maximum attack rate for each simulated replicate as  $T/T_h$ .

Finally, the mean of the simulated maximum attack ratesisbootstrapped using the *MARbootstrapping* function that calls the *boot* function from "boot" package running a selected number of bootstrap replicates to generate its 95% confidence intervals.

#### 6.2.5. Validation of simulation method

The validation of the method was carried according to three objectives: (1) to assess the correspondence between the known (true) maximum attack rate obtained from an artificial dataset and the estimated value provided by simaR, as well as the proportion of cases in which the simulated confidence interval provided by simaR overlapped the known (true) value, (2) assess the performance of the method using partial data, and (3) assess the correspondence between the maximum attack rate calculated by simaR and the point estimator for it obtained from literature across different study cases.

# 6.2.5.1. Correspondence between the known and simulated maximum attack rate calculated by simaR.

Since the maximum attack rate is a point estimate calculated from the handling time, an artificial dataset generated from a functional response model with known parameters was used, and then the parameters were estimated back using simaR. For this, 11 initial prey densities were considered ( $N_0 = 3, 5, 10, 15, 20, 25, 30, 35, 40, 45$  and 50) assuming prey replacement and their corresponding number of prey killed ( $N_e$ ) were calculated using the Holling's type-II predator-prey function.

It was assigned a time of experiment T = 24h, an attack rate a = 0.05, and a handling time  $T_h = 1.30$ , i.e. a known maximum attack rate  $T/T_h$  of 24/1.30 = 18.46 prey/day, so that the number of prey killed for each initial prey density was  $N_e = 3.00$ , 4.53, 7.27, 9.11, 10.43, 11.43, 12.20, 12.82, 13.33, 13.76, and 14.12 respectively (Fig. S1A). Then 1000 simulations were run (50 replicates of 20 samples per initial prey density per simulation, i.e. 50.000 simulated datasets) using the simaR package to estimate the maximum attack rate, and the number of times the known maximum attack rate fell within the simulated confidence interval was calculated. Finally, a one-sample Student's t-test was carried out to check if the real maximum attack rate differed significantly from the simulated ones.

#### 6.2.5.2. Performance of simaR on partial data.

Once again, 11 initial prey densities were considered ( $N_0 = 3, 5, 10, 15, 20, 25, 30, 35, 40, 45$  and 50) now without assuming prey replacement, a proportion of prey killed was

assigned for each density ( $p_c = 1$ , 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.3, 0.3 and 0.3), and a time of experiment of T = 24h was assumed. Then, the number of prey killed for each initial prey density was simulated by extracting 10 random values (i.e. 10 samples) from a binomial distribution with N = N<sub>0</sub> and p = p<sub>c</sub>. Then, two series of five and six new datasets respectively were created in two ways: the first series by arbitrarily removing a number of initial prey densities (N<sub>0</sub>) from the complete original simulated dataset keeping constant the number of samples (i.e. n = 10), and the second one by removing the same initial prey densities together with the 50% of the samples (i.e. n = 5). The *frair\_test* function was used to assess the type of functional response of the original dataset (N<sub>0</sub> = 11; n = 10) (Fig. S2A) and its corresponding sample-reduced dataset (N<sub>0</sub> = 11; n = 5) (Fig. S2B). For each of the two datasets, the attack rate (*a*) and handling time (T<sub>h</sub>) were estimated using the *frair\_fit* function from the "frair" package and the estimated handling time was then used to calculate the empirical maximum attack rates (T/T<sub>h</sub>). Finally, the 11 new partial datasets were used together with the original one to apply the simulation method with simaR (Figs. S3 & S4).

Ten simulation runs were carried out for each of the 12 datasets (20 samples and 50 replicates per simulation run) and the effect of the simulation run and number of samples (n = 10 or n = 5) on the simulated maximum attack rate was assessed. The Shapiro-Wilk test was used to check if the simulated data were normally distributed (W = 0.853; P < 0.001) and then the simulated maximum attack rate was linearly modeled following Zuur et al. (2009) as:

$$\mathcal{E}_i \sim N(0, \sigma^2)$$
  
SMAR ~  $\alpha + \beta_1 \times \text{Run} + \beta_2 \times \text{Samples} + \beta_3 \times \text{Run*Samples} + \mathcal{E}_i$  [Eq. 7]

were SMAR represents the simulated maximum attack rates, Run represents the number of simulation run, Samples represents the number of samples used at each initial prey density in the original dataset and Run\*Samples represents the interaction term between the two independent variables.

6.2.5.3. Correspondence between the simaR estimation of the maximum attack rate and point estimators obtained from literature.

A search for papers focused on the functional response of different natural enemies in different conditions (e.g. response of a natural enemy to different temperatures or response of different natural enemies against the same pest) providing the mean number of prey attacked at different prey densities was carried out. The proportion of attacked prey was used to replicate the analyses conducted in the literature using simaR, and the simulated maximum attack rates  $(T/T_h)$  together with their 95% confidence intervals were generated. Then, in order to check the correspondence between the simulated maximum attack rates obtained using simaR and the corresponding ones obtained from literature, the latter were linearly modeled as:

$$\mathcal{E}_i \sim N(0, \sigma^2)$$
  
LiteratureMAR ~  $\alpha + \beta \times \text{simaRMAR} + \mathcal{E}_i$  [Eq. 8]

where MAR represents the maximum attack rates.

#### 6.2.6. Application of the simulation method to experimental data with spiders.

The "frair" package was used to select, fit the functional response model, and estimate the corresponding parameters (as described in section 2.4.1) of each spider-temperature relationship. Then, the simaR package was used to simulate and generate the 95% confidence interval (as described in section 2.4.2), and compare the maximum attack rate of each spider species between the three selected temperatures. A type-I functional response is supposed to be independent from the handling time, which could be considered unrealistic because the consumption rates cannot continue increasing indefinitely regardless of food density. On the other hand, Batzli et al. (1981) argued that after providing the test organism with the highest food densities found in their natural habitat it would be reasonable to conclude that a type-I response can be exhibited. However, this range of higher prey densities in nature could be unknown for the test organism, which is the case of the two selected spider species selected for this work. Because of this, the type-I responses found

along this study were fitted as the increasing part of a Rogers's type-II decreasing prey function (i.e. a type-II response that did not reached the asymptote).

# 6.3. Results

# 6.3.1. Correspondence between the known and estimated maximum attack rate calculated by simaR.

The known value for the maximum attack rate of the artificial dataset generated to test the correspondence between the known and the simulated parameter was 18.46 prey/day and the maximum attack rate calculated by 1000 simulation runs using simaR was 18.459  $\pm$  0.003 (mean  $\pm$  SE). The lower and upper limit of its simulated 95% confidence interval were 18.274  $\pm$  0.003 (mean  $\pm$  SE) and 18.645 $\pm$  0.003 (mean  $\pm$  SE) respectively. The known maximum attack rate fell 955 times of 1000 simulation runs within the simulated confidence intervals giving a method accuracy of 95.50% (Table S9) (Fig. S1B) and did not differed significantly from the simulated value (t = -0.805, df = 999, P = 0.421)

#### 6.3.2. Performance of simaR on partial data.

The original complete dataset (N<sub>0</sub> = 11; n = 10) (Fig. S2A) and the corresponding samplereduced dataset (N<sub>0</sub> = 11; n = 5) (Fig. S2B) generated to test the method performance on partial data fitted a type-II functional response ( $\beta_1$  = -0.0515; Z = 15.473; P < 0.001, and  $\beta_1$ = -0.052; Z = -10.982; P < 0.001 respectively). Accordingly, the Rogers's type-II decreasing prey function was fitted for each dataset giving a coefficient of attack rate of *a* = 0.148 ± 0.020 (estimate ±SE) (Z = 7.552; P < 0.001), an estimated handling time of T<sub>h</sub> = 1.68 ± 0.071 (estimate ±SE) (Z = 23.719; P < 0.001), and an empirical maximum attack rate of (T/T<sub>h</sub>) of 14.29 prey/day for the original complete dataset. The sample-reduced dataset (N<sub>0</sub> = 11; n = 5) generated a coefficient of attack rate (*a*) of 0.149 ± 0.030 (estimate ±SE) (Z = 5.130; P < 0.001), an estimated handling time (T<sub>h</sub>) of 1.742 ± 0.105 (estimate ±SE) (Z = 16.553; P < 0.001), and an empirical maximum attack rate of (Z = 16.553; P < 0.001), and an empirical maximum attack rate ±SE) (Z = 16.553; P < 0.001), and an empirical maximum attack rate ±SE) (Z = 16.553; P < 0.001), and an empirical maximum attack rate ±SE) (Z = 16.553; P < 0.001), and an empirical maximum attack rate ±SE) (Z = 16.553; P < 0.001), and an empirical maximum attack rate (T/T<sub>h</sub>) of 13.777 prey/day. The simulated maximum attack rates were  $14.23 \pm 0.01$  (mean  $\pm$  SE) and  $14.11 \pm 0.08$  (mean  $\pm$  SE) for the pool of datasets with 10 samples and 5 samples respectively (Table S10). The simulation run number had not a significant effect on the simulated maximum attack rate estimation (F = 0.779; df = 1; P = 0.379) (Fig. S5A). The number of samples (n) of each dataset did not affected significantly the estimation of the parameter (F = 1.535; df = 1; P = 0.218) (Fig. S5B), and the interaction between the two variables was not significant as well (F = 0.494; df = 1; P = 0.484).

# 6.3.3. Correspondence between the simaR estimation of the maximum attack rate and point estimators obtained from literature.

Regarding the values for the maximum attack rate extracted from literature, six papers focused on the functional response of different groups of natural enemies (seven species of predators and one parasitoid) in different conditions using three types of functional response to fit the data provided the mean of the number of attacked prey at each initial prey density (Table S11). The results reported in these papers were used together with the re-analysis of the functional response data of a paper on the pest suppression potential of two species of carabids (Dinis et al 2016; Table S11). The linear regression carried out between the simulated maximum attack rates obtained using the simaR package on their corresponding bibliographic maximum attack rates gave a coefficient of determination ( $\mathbb{R}^2$ ) of 0.932 (Fig. S6).

# 6.3.4. Application of the simulation method to experimental data with spiders.

The estimated parameters from the logistic regression analysis of the empirical proportion of *C. capitata* adults killed by adult females of *A. cucurbitina* and *S. globosum* indicated a type-II functional response at each tested temperature except for the former species at 21°C which showed a type-I functional response (Table 6.1). The highest number of killed preys was obtained at 21°C with *A. cucurbitina* (Fig. 6.2A) whereas the lowest one was observed in *S. globosum* at 21°C (Fig. 6.2B). Almost the total of the simulated curves ranged within the empirical confidence intervals of the empirical curves for both *A. cucurbitina* and *S. globosum* (Fig. 6.3) although the simulated range of curves differed slightly from the empirical one in the case of *A. cucurbitina* at 21°C (Fig. 6.3A).

**Table 6.1.** Estimated parameters from the linear and logistic regression analysis of the number of prey killed against prey density for the two spider species studied. Estimate: coefficients derived from the logistic regression used to select between type-II and type-III type of functional response; *a*: coefficients of attack rate and their confidence interval (*a* CI); T<sub>h</sub>: estimated handling times and their confidence interval (T<sub>h</sub> CI); MAR: maximum attack rates (T/T<sub>h</sub>) of the empirical datasets; mMARs: simulated maximum attack rate means and their confidence intervals (mMARs CI). AC: *Araniella cucurbitina*; SG: *Synema globosum*. E.type: Empirical response type; S.type: simulated response type. Asterisks indicate statistical significance at p < 0.05. <sup>F</sup> indicates the F-value (1 degree of freedom) corresponding to the type-I functional response fitting.

Predator	Temperature	Estimate	Std. Error	Z-value	Р	а	a CI	$\mathbf{T}_{\mathbf{h}}$	T <sub>h</sub> CI	MAR	mMARs	mMARs CI	E.type	S.type
	21°C	0.5031	0.0267	354.36 <sup>F</sup>	<0.001*	0.0230	0.0210, 0.0250	-	-	-	86.7388	78.5528, 95.0345	Ι	II
AC	24°C	-0.0439	0.0093	-4.7303	<0.001*	0.0620	0.0370, 0.0910	1.2820	0.8800, 1.6250	18.7207	18.9839	18.4930, 19.4728	II	II
	27°C	-0.0333	0.0090	-3.7097	< 0.001*	0.0470	0.0280, 0.0820	1.2820	0.7200, 2.0500	18.7207	19.1139	18.4856, 19.7602	II	II
SG	21°C	-0.0507	0.0095	-5.3174	< 0.001*	0.0570	0.0440, 0.1000	2.7010	2.1860, 4.1220	8.8856	8.9710	8.6191, 9.3314	II	II
	24°C	-0.0286	0.0091	-3.1590	0.0016*	0.0370	0.0180, 0.1230	1.5820	0.5760, 6.8580	15.1707	15.7094	15.0262, 16.3917	II	II
	27°C	-0.0188	0.0088	-2.1363	0.0327*	0.0320	0.0230, 0.0480	0.8040	0.1110, 1.6320	29.8507	31.8333	30.0105, 33.6730	II	II



**Figure 6.2.** A: Functional responses of adult females of *Araniella cucurbitina*, and B: *Synema globosum* fed for 24 h on differing densities of adults of *Ceratitis capitata* at three temperatures. Points represent the number of flies killed at each prey density. Thick lines represent the fitted values of empirical data and the polygons that surround them represent the limits of the confidence interval of the empirical curves for each temperature.

The coefficient of attack rate (*a*) corresponding to the type-I response of *A. cucurbitina* at 21°C was the lowest one across the six spider-temperature relationships (Table 6.1), however it was not significantly different along the gradient of temperature for this species (Fig. 6.4A). The estimated handling time ( $T_h$ ) of *A. cucurbitina* was not significantly different between 24 °C and 27 °C (Table 6.1) (Fig. 6.4B). The simulated maximum attack rate of *A. cucurbitina* was significantly higher at 21 °C when compared to 24 °C and 27 °C; however, it was not significantly different between the two latter temperatures (Fig. 6.4C).

In the case of *S. globosum*, both the attack rate and handling time decreased across the increasing gradient of temperatures (Table 6.1). However, based on the overlapping of the 95% confidence intervals, no significant differences in attack rate or handling time could be observed (Fig. 6.4D & 5.4E), except for the handling time at 21 °C and 27°C where the former was significantly higher than the latter (Fig. 6.4E). Finally, significant differences between



**Figure 6.3.** Comparison between the empirical and simulated functional responses of adult females of *Araniella cucurbitina* (A: 21°C; B: 24°C and C: 27°C) and *Synema globosum* (D: 21°C; E: 24°C and F: 27°C) fed for 24 h on differing densities of adults of *Ceratitis capitata*. The mass of thin lines represent the fitted values of each simulation run using the Rogers's type-II decreasing prey function. Thick lines (red lines) represent the fitted values of empirical data for each temperature. The shaded areas represent the 95% confidence interval obtained using "frair" for each empirical curve (based on 999 bootstrap replicates). Dots were omitted to facilitate visualization.



**Figure 6.4.** Parameters of the functional response of adult females of *Araniella cucurbitina* (A: attack rate (*a*); B: empirical handling time ( $T_h$ ), and C: maximum attack rate ( $T/T_h$ )), and adult females of *Synema globosum* (D: attack rate(*a*); E: handling time ( $T_h$ ), and F: maximum attack rate ( $T/T_h$ )) fed for 24 h on different densities of adults of *Ceratitis capitata* at three temperatures. Since the scale of the simulated maximum attack rate is very different at 21°C than at 24°C and 27°C , in C the simulated maximum attack rate at 24°C and 27°C are represented on the secondary right vertical axis to facilitate visualization. Attack rates and handling times were estimated using the "frair" package and the maximum attack rate was simulated using simaR. Dots represent the original data and bars the 95% confidence intervals given by bootstrapping (999 bootstrap replicates). Different letters over bars indicate significant differences based on the overlap of confidence intervals. \*In B, the handling time at 21 °C is empty because the fitted model was of type-I.

the simulated maximum attack rates among the three temperatures were found for *S*. *globosum* increasing from 21 °C to 27 °C (Table 6.1) (Fig. 6.4F).

# 6.4. Discussion

#### 6.4.1. Method validation and performance.

A method to assess the prey killing capacity of a predator was developed using the maximum attack rate  $(T/T_h)$ , a parameter derived from the functional response of a species under selected experimental conditions, to evaluate the capacity of a predator or a parasitoid to attack a number of prey individuals during a given time. The development of this method was born from the need to make statistical hypothesis testing using experimental data with a low number of samples, low replicated assays, or even partial data in the context of predator functional response research. The confidence interval method was selected to test hypotheses because of its ability to help quantify the magnitude of an effect in units of scientific interest (Jiroutek et al., 2003).

The way to generate the confidence intervals was through simulation, a method that allows to recreate a selected number of both samples and replicates, followed by bootstrapping that allows to resample data matrices and generate dispersal measures around an estimate (e.g. the mean) at a desired level of confidence (usually 95%). The simaR package runs the whole process using an experimental approximation of the handling time (T/Ne<sub>max</sub>) as starting value to parameterize the simulated handling time, which in turn is used to calculate the simulated maximum attack rate and its 95% confidence interval.

It is important to say that the resulting intervals should be proper confidence intervals or at least good approximations (i.e. a 95% confidence interval should overlap the known value with a probability of 95%). Commonly, the true values are not known since the parameter to be simulated and for which we want to provide confidence intervals is an estimate itself (e.g. the handling time). Accordingly, the method was validated by fabricating an artificial dataset with known parameters and the parameters were estimated back reaching an accuracy of 95.50%. Moreover, when testing the performance of the method using partial
data, it was found consistency on the parameters estimation by simulation, i.e. the maximum attack rate and the lower and upper bound of its confidence interval. This suggests that the effect of gaps in the data can be solved by using a selected number of simulated samples and replicates (in this case 20 and 50 respectively). Also, when only the means of the number of prey killed were available (i.e. bibliographical sources) the method loosed only 6.80% of information.

When comparing the experimental and simulated data for spiders a good overlap between the simulated range of curves (i.e. replicates) and the experimental data was found despite a slight difference in the case of *A. cucurbitina* at 21°C because the fitted models were different, i.e. type-I (empirical) and type-II (simulated).

### 6.4.2. Method limitations

In general, this method allows a fast and accurate comparison of the maximum attack rates of different study cases; however, some points have to be stressed. The known value of the simulated parameter was used to validate the method and an *ad-hoc* sample size and number of replicates for simulation was selected (20 and 50 respectively); since a confidence interval that includes the true parameter value may slightly differ from a confidence interval that does not include it (Beal, 1989), further combination of simulation runs should be carried out to test in which extent the number of simulated samples and simulated replicates may affect the size of the confidence interval. For example, is a simulation of 20 samples and 50 replicates significantly different from another one of 10 samples and 100 simulations? In which cases such a combination is more accurate and/or appropriate? In addition, extracting random values from the binomial distribution is an approximation and the assumption of independence between the fates of individual prey should be investigated in the future.

Simulated data was used to validate the method toward the estimation of a biological parameter such as the maximum attack rate using empirical data. Since no ecological information is represented by the initial artificial dataset beyond the fit to a selected model, it could be argued that the method can be applied to any species under any laboratory

conditions that the code allows to select (i.e. time of the experiment, number of predators, prey replacement, and type of functional response). However, conclusions derived from different functional response analyses must be carefully drawn since variation in physical and biological conditions of the studied system such as starvation before the experiment, arena size, and acclimatization period are not taken into account by the model equations and may lead to ecological misinterpretations. For instance, in which extent the conclusions of a laboratorial experiment on functional response can be extrapolated to the field?

Since natural assemblages have multiple prey species that are subjected to seasonal processes, key community-related concepts such as food and prey preference (Wise et al., 2016), behavioral differences among conspecifics (Toscano & Griffen, 2014), succession processes (Settle et al., 1996), intraguild competition, prey availability (Perkins et al., 2018), and predator-prey body size proportion (Shimazaki & Miyashita, 2005) can have implications for ecosystem functioning and stability and should also be considered within the context of functional response modeling.

Finally, the simaR package contemplates a type-II decreasing prey function for multiple predators, but multispecies functional response models are not currently included. Nevertheless, development of methods accounting for multiple prey functional responses and predator and prey behavior are of growing interest (e.g. Scharf et al., 2008; Smout et al., 2010). Accordingly, within the context of biological control, further research is needed to test under which situations the parameters of the functional response models and their derivates such as the maximum attack rate could be suitable stand-alone surrogates of the suppression potential of a biological control agent.

# 6.4.3. Bioecological interpretation of the effect of temperature on the spiders' functional response.

Since temperature is a key driver in pest control, e.g. high temperature usually hinders the natural enemies action in greenhouses (Gilioli et al., 2005; Vacante, 2000), the simaR method was applied to experimental data obtained in the laboratory at different temperatures (21, 24 and 27°C) on two widespread species of generalist predators, the orb-

weaver spider A. cucurbitina and the ambusher spider S. globosum against the pest C. capitata.

The maximum attack rate of *A. cucurbitina* decreased dramatically from 21°C to 24°C and was not significantly different from the latter temperature to 27°C. This reduction in the efficiency of capturing prey according to the temperature by orb-weavers has been well documented from different points of view. Hesselberg & Vollrath (2006) performed an experiment with *Araneus diadematus* Clerck (Araneae: Araneidae) against *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) and hypothesized that flying insects had a higher body temperature because of the flight whereas the spiders had a lower body temperature waiting in the centre on their web. In this work, this difference in body temperature could make the escape time of *C. capitata* significantly faster at 24 and 27°C. In addition, the web building architecture of *A. diadematus* was also found to be significantly different at lower temperatures by Vollrath et al. (1997) suggesting that the increase in spiral spacing could be an adaptation to larger sized prey supposedly able to fly at lower temperatures.

Hunting success of orb-weavers relies on their web efficiency. The capture threads of this web are coated with glues droplets composed by an adhesive viscoelastic glycoprotein covered by an aqueous solution. This aqueous glue plasticizes the silk and provides elasticity from the surface tension of the liquid (Vollrath & Edmonds, 1989). However, environmental changes in temperature have the potential to alter thread and web function, and Stellwagen et al. (2014) found that an increase in temperature decreased the glycoprotein viscosity of the thread build by *Argiope aurantia* Lucas (Araneae: Araneidae). This effect may also alter the mechanical properties of the webs of *A. cucurbitina* significantly decreasing the efficiency in capturing prey at high temperatures.

Moreover, biomechanical properties of silks and structural diversity of spider webs are highly variable and depends on rate of spinning, pH levels in the silk glands, humidity and temperature (Harmer et al., 2011). In fact, the dependency of spider webs on environmental and physiological conditions is not exclusive of orb-weavers. Barghusen et al. (1997)

suggested that *Achaearanea tepidariorum* C.L. Koch (Araneae: Theridiidae), an aerial space web builder (Uetz et al., 1999) optimizes web construction by temperature selection and found an optimal experimental temperature at 20 °C at which the spiders produced the heaviest webs and prey capture should be maximized.

In this work, *S. globosum*, a sit-and-wait active hunter (i.e. ambusher without web device) showed a clear significant increasing pattern of the maximum attack rate from the lowest to the highest temperature tested. This gradient in hunting success could be explained in terms of the effect of temperature in both the predator and the prey. As for orb-weavers, the fact that an ambusher spider waits for its prey can result in a difference between the body temperature of the predator and the prey thus being advantageous for the flying insect. Booster et al. (2015) hypothesized that at lower temperatures, the movement of the spider leg joints could be less coupled because of increased hemolymph viscosity thus slowing the flow of hemolymph, and demonstrated that at high temperature the spiders ran the fastest with the highest stride frequencies using *Aphonopelma hentzi* (Jean-Étienne Girard) (Araneae: Theraphosidae) as model species.

The hunting behavior of a crab spider such as *S. globosum* consists of standing still with the first and second pair of legs (raptorial forelimbs) extended while waiting for a prey. When a prey approximates enough, the spider rapidly grabs it and brings it close to the mouthparts to be bitten. The effect of temperature on the hemolymph viscosity may improve the ability of *S globosum* to catch a fly by increasing the attack movement velocity at higher temperatures. However, Schmalhofer & Casey (1999) found that temperature did not affect the hunting performance of the crab spiders *Misumenops asperatus* (Hentz) (Araneae: Thomisidae) and *Misumenoides formosipes* (Walckenaer) (Araneae: Thomisidae) on *Musca domestica* (L.) (Diptera: Muscidae). On the contrary, they reported a slight effect of temperature on the amount of time spent moving by the flies. Also, the prey were exposed to predators at set densities and within confined arenas, so that effect of encounter rate was irrelevant. As explained by Kruse et al. (2008), predator hunting success depends on the encounter rate with prey, prey escape ability, and predator agility; they also found that the predation rate of *Pardosa prativaga* (L. Koch) (Araneae: Lycosidae) and *Clubiona* 

*phragmitis* (L. Koch) (Araneae: Clubionidae) increased with temperature, their prey *D. melanogaster* being more active at the highest temperatures. This positive relationship between predation rate and prey activity on an increasing gradient of temperature can also explain the significant increase of the maximum attack rate of *S. globosum* on *C. capitata* from 21°C to 27°C.

The two spider species used to study the effect of temperature on the functional response use a sit-and-wait strategy to hunt. Overall, their attack rate and handling time were not significantly different across the studied temperatures. However, the handling time of *S. globosum* was significantly reduced from 21°C to 27°C suggesting a pattern that was then clearly uncovered by the simaR method focusing on the maximum attack rates. This highlights the relevance of the former parameter on studies related to biological pest control. Hence, differences in the functional response of these species resulted in an opposite pattern in the efficiency as predators against *C. capitata* along a gradient of temperature. Moreover, populations and food-web dynamics may depend on slight differences in functional response parameters (Vucic-Pestic et al., 2010) which may have important implications, for instance, in a climate change scenario.

### **6.5.** Conclusions

In this work, it was shown how to obtain reliable and accurate simulated data and draw conclusions through simulation starting from low-effort laboratory trials (in this case only four samples and one replicate per spider species) within the context of functional response analysis. This enables a fast and direct comparison between the efficiency of different species of natural enemies in terms of the maximum attack rate under different conditions. Accordingly, research on functional responses under different environmental conditions and for different species of predators, parasitoids and their prey is crucial to increase the efficiency of biological pest control programs. In the case of spiders, known variables affecting pest suppression, such as mechanical silk properties, web architecture, humidity, body and environmental temperature, encounter rate, and prey behavior, need to be better investigated to determine their effects upon capture rate. The simaR package is provided in

the highly extensible and Open Source R language that automates each step of a simulated functional response experiment based on real data. Using a bootstrapping process, the pest suppression potential of predators can be easily compared between potential natural enemies starting from low effort laboratory trials. This method could represent a powerful tool for using in the sustainable farming system context. However, it is not limited to pest control studies. In fact, it can be used to develop basic research on the biology of species and apply it to study the effects of climatic change on population dynamics and also to provide valuable data for powerful individual based models (IMBs) that are of increasing interest nowadays such as ALMASS (Topping et al., 2003) and Weaver (Bilbao-Castro et al., 2015).

### 6.6. Data accessibility

The empirical functional response datasets used to test the code (Synema\_dataset.txt and Araniella\_dataset.txt), the simaR package (simaR\_v06.R) and an example R file for type-II and type-III functional responses (Example\_simaR.R) are available from https://github.com/jbenma/simaR/tree/Simulation-functions.

# 6.7. Supplementary material.

**Table S8.** Functional response models provided by the "frair" package that are supported by the simaR package and can be selected to be used with the *getFitData* function. *a*: coefficient of attack rate; *b*: search coefficient; *c* and *d*: original type-III Hassell's constants; *q*: scaling exponent on the attack rate;  $N_0$ : initial prev density;  $N_e$ : number of prev killed; P: number of predators; T: time of the experiment, and  $T_h$ : handling time.

Functional response model	Nº predators	Prey replacement	Model equation	Attack rate (a)	Coefficients	Fixed parameters	References
Holling's type-II predator-prey function	1	Yes	$N_{e} \sim (a \times N_{0} \times T) / 1 + (a \times N_{0} \times T_{h})$	Constant	a, T <sub>h</sub>	Т	Holling (1959); Pritchard (2017).
Rogers's type-II decreasing prey function	1	No	$N_e \sim N_0 \{1 - exp [a (N_e \times T_h - T)]\}$	Constant	<i>a</i> , T <sub>h</sub>	Т	Bolker (2008); Pritchard (2017); Rogers (1972); Royama (1971).
Hassell's original type-III response	1	Yes	$N_{e} \sim (a \times N_{0} \times T) / 1 + (a \times N_{0} \times T_{h})$	$a \sim (d \times N_0) / (1 + c \times N_0)$	$a, d, c, T_h$	Т	Hassell et al. (1977); Pritchard (2017).
Hassell's type-III response	1	No	$N_{e} \sim N_{0} \left\{ 1 - exp \left[ a \left( N_{e} \times T_{h} - T \right) \right] \right\}$	$a \sim (d \times N_0) / (1 + c \times N_0)$	$a, d, c, T_h$	Т	Hassell et al. (1977); Pritchard (2017); Rogers (1972); Royama (1971).
Scaling exponent response (type-II)	1	Yes	$N_e \sim (a \times N_0 \times T) / 1 + (a \times N_0 \times T_h)$	$a \sim b \times {\mathrm{N_0}}^q$	$a, b, q, T_h$	Т	Holling (1959); Pritchard (2017); Real (1977); Vucic-Pestic et al. (2010).
Scaling exponent response (type-III)	1	No	$N_{e} \sim N_{0} \left\{ 1 - \exp \left[ a \left( N_{e} \times T_{h} - T \right) \right] \right\}$	$a \sim b \times N_0^q$	$a, b, q, \mathrm{T_h}$	Т	Bolker (2008); Pritchard (2017); Rogers (1972); Real (1977); Royama (1971); Vucic-Pestic et al. (2010)
"EMDR" type-II decreasing prey function	Р	No	$N_{e} \sim N_{0} \left\{ 1 - exp \left[ a \left( N_{e} \times T_{h} - T \times P \right) \right] \right\}$	Constant	a, T <sub>h</sub>	Τ, Ρ	Bolker (2008); Pritchard (2017); Rogers (1972); Royama (1971)

# <u>Chapter 6</u>

Table	S9. Simulated	ł maximum a	ttack rate	27	18.268	18.468	18.679	65	18.397	18.545	18.696	103	18.379	18.536	18.698
$(T/T_h)$	and the low	er (li) and u	pper (ui)	28	18.179	18.373	18.567	66	18.283	18.476	18.661	104	18.391	18.546	18.707
bounde	for the 95%	confidence i	nterval of	29	18.067	18.307	18.554	67	18.331	18.503	18.682	105	18.167	18.364	18.566
bounds		confidence in		30	18.298	18.505	18.724	68	18.160	18.395	18.623	106	18.252	18.423	18.591
each of	the 1000 sim	ulation runs c	arried out	31	18.283	18.459	18.644	69	18.390	18.536	18.688	107	18.377	18.523	18.672
with si	imaR to asse	ss the corres	pondence	32	18.226	18.423	18.626	70	18.278	18.471	18.673	108	18.212	18.406	18.610
between	n the simul	ated values	and the	33	18.313	18.491	18.668	71	18.213	18.410	18.603	109	18.158	18.364	18.567
known	value assig	ned to gene	erate the	34	18.113	18.338	18.560	72	18.146	18.338	18.528	110	18.157	18.349	18.545
artificio	al dataset Tri	time of the ex	pariment.	35	18.295	18.479	18.670	73	18.364	18.539	18.713	111	18.414	18.606	18.808
arument		time of the ex	perment,	36	18.133	18.318	18.506	74	18.179	18.359	18.538	112	18.064	18.274	18.490
$T_h$ : han	dling time.			37	18.225	18.414	18.609	75	18.195	18.391	18.592	113	18.313	18.475	18.643
Run	li	T/T <sub>h</sub>	ui	38	18.323	18.518	18.710	76	18.462	18.614	18.767	114	18.193	18.376	18.562
1	18.323	18.477	18.641	39	18.393	18.565	18.739	77	18.211	18.384	18.559	115	18.387	18.532	18.674
2	18.171	18.360	18.548	40	18.103	18.313	18.527	78	18.346	18.507	18.669	116	18.090	18.293	18.498
3	18.215	18.419	18.621	41	18.249	18.407	18.567	79	18.327	18.492	18.665	117	18.459	18.656	18.840
4	18.338	18.527	18.708	42	18.514	18.706	18.905	80	18.364	18.530	18.693	118	18.239	18.435	18.634
5	18.368	18.552	18.724	43	18.194	18.399	18.602	81	18.399	18.555	18.712	119	18.332	18.517	18.708
6	18.408	18.548	18.684	44	18.317	18.508	18.701	82	18.230	18.408	18.569	120	18.400	18.621	18.837
7	18.250	18.438	18.630	45	18.219	18.420	18.619	83	18.287	18.450	18.627	121	18.431	18.596	18.755
8	18.110	18.327	18.535	46	18.201	18.393	18.586	84	18.369	18.571	18.776	122	18.389	18.559	18.734
9	18.225	18.412	18.599	47	18.355	18.513	18.675	85	18.383	18.546	18.704	123	18.324	18.523	18.723
10	18.349	18.527	18.715	48	18.173	18.327	18.484	86	18.067	18.261	18.451	124	18.112	18.293	18.465
11	18.285	18.467	18.650	49	18.314	18.524	18.725	87	18.397	18.593	18.782	125	18.296	18.517	18.727
12	18.328	18.497	18.661	50	18.172	18.360	18.549	88	18.532	18.671	18.808	126	18.196	18.383	18.577
13	18.239	18.431	18.625	51	18.174	18.391	18.602	89	18.168	18.375	18.584	127	18.339	18.520	18.708
14	18.285	18.491	18.696	52	18.336	18.529	18.727	90	18.210	18.394	18.576	128	18.459	18.638	18.820
15	18.328	18.513	18.703	53	18.182	18.377	18.571	91	18.229	18.425	18.614	129	18.194	18.411	18.636
16	18.036	18.225	18.410	54	18.359	18.535	18.717	92	18.447	18.588	18.735	130	18.059	18.269	18.473
17	18.176	18.358	18.546	55	18.225	18.397	18.559	93	18.211	18.379	18.553	131	18.128	18.294	18.456
18	18.206	18.427	18.645	56	18.280	18.466	18.648	94	18.342	18.534	18.719	132	18.317	18.541	18.771
19	18.406	18.583	18.761	57	18.491	18.687	18.877	95	18.203	18.399	18.591	133	18.401	18.593	18.794
20	18.283	18.449	18.613	58	18.394	18.581	18.769	96	18.208	18.414	18.618	134	18.173	18.359	18.546
21	18.158	18.356	18.554	59	18.160	18.345	18.535	97	18.338	18.515	18.687	135	18.149	18.335	18.519
22	18.322	18.475	18.628	60	18.237	18.430	18.627	98	18.370	18.529	18.701	136	18.146	18.318	18.484
23	18.287	18.465	18.647	61	18.270	18.470	18.667	99	18.184	18.380	18.587	137	18.326	18.534	18.745
24	18.331	18.516	18.694	62	18.221	18.428	18.632	100	18.306	18.527	18.742	138	18.222	18.388	18.559
25	18.300	18.490	18.669	63	18.251	18.433	18.608	101	18.316	18.491	18.667	139	18.304	18.491	18.676
26	18.200	18.395	18.583	64	18.220	18.392	18.558	102	18.310	18.499	18.690	140	18.234	18.409	18.587

18.048	18.228	18.412	179	18.299	18.494	18.687	217	18.378	18.563	18.750	255	18.237	18.426	18.603
18.301	18.507	18.704	180	18.513	18.677	18.833	218	18.132	18.313	18.482	256	18.222	18.418	18.614
18.243	18.425	18.598	181	18.380	18.536	18.686	219	18.068	18.280	18.491	257	18.103	18.294	18.475
18.284	18.460	18.641	182	18.171	18.352	18.539	220	18.285	18.466	18.649	258	18.245	18.431	18.614
18.178	18.365	18.538	183	18.281	18.471	18.669	221	18.165	18.353	18.548	259	18.271	18.492	18.712
18.248	18.445	18.637	184	18.337	18.514	18.692	222	18.321	18.519	18.715	260	18.393	18.611	18.835
18.272	18.439	18.605	185	18.364	18.542	18.732	223	18.280	18.434	18.594	261	18.206	18.363	18.517
18.292	18.494	18.703	186	18.113	18.316	18.521	224	18.421	18.575	18.727	262	18.392	18.554	18.714
18.374	18.526	18.682	187	18.398	18.605	18.810	225	18.182	18.357	18.537	263	18.045	18.246	18.448
18.218	18.411	18.593	188	18.395	18.536	18.678	226	18.111	18.328	18.546	264	18.269	18.448	18.626
18.291	18.502	18.700	189	18.357	18.576	18.787	227	18.263	18.432	18.601	265	18.420	18.575	18.731
18.178	18.392	18.596	190	18.411	18.595	18.781	228	18.211	18.412	18.610	266	18.140	18.383	18.636
18.352	18.513	18.670	191	18.175	18.364	18.554	229	18.232	18.414	18.596	267	18.399	18.581	18.768
18.203	18.408	18.609	192	18.270	18.473	18.686	230	18.331	18.520	18.713	268	18.334	18.505	18.669
18.232	18.406	18.580	193	18.241	18.403	18.563	231	18.447	18.601	18.756	269	18.221	18.427	18.633
18.374	18.543	18.719	194	18.437	18.626	18.812	232	18.154	18.338	18.518	270	18.243	18.449	18.654
18.160	18.380	18.609	195	18.385	18.568	18.750	233	18.248	18.454	18.653	271	18.075	18.253	18.430
18.358	18.539	18.723	196	18.209	18.380	18.550	234	18.188	18.341	18.498	272	18.381	18.535	18.685
18.256	18.443	18.626	197	18.323	18.534	18.733	235	18.330	18.512	18.691	273	18.309	18.481	18.642
18.281	18.453	18.626	198	18.275	18.496	18.724	236	18.315	18.509	18.702	274	18.191	18.354	18.518
18.286	18.477	18.656	199	18.315	18.505	18.694	237	18.273	18.425	18.577	275	18.361	18.562	18.765
18.245	18.415	18.586	200	18.407	18.597	18.786	238	18.280	18.468	18.641	276	18.309	18.483	18.658
18.317	18.481	18.646	201	18.114	18.295	18.482	239	18.348	18.548	18.757	277	18.173	18.352	18.531
18.305	18.504	18.689	202	18.269	18.454	18.639	240	18.156	18.384	18.607	278	18.364	18.554	18.747
18.219	18.416	18.599	203	18.362	18.551	18.748	241	18.383	18.565	18.751	279	18.128	18.340	18.545
18.259	18.461	18.661	204	18.370	18.556	18.751	242	18.227	18.397	18.569	280	18.250	18.434	18.626
18.170	18.374	18.568	205	18.096	18.286	18.480	243	18.412	18.606	18.797	281	18.250	18.438	18.642
18.119	18.335	18.553	206	18.315	18.491	18.671	244	18.283	18.482	18.675	282	18.206	18.411	18.616
18.286	18.493	18.691	207	18.125	18.317	18.502	245	18.377	18.552	18.722	283	18.337	18.513	18.696
18.043	18.267	18.482	208	18.252	18.448	18.642	246	18.222	18.424	18.617	284	18.403	18.554	18.696
18.327	18.520	18.710	209	18.353	18.523	18.700	247	18.332	18.499	18.663	285	18.100	18.298	18.497
18.396	18.567	18.740	210	18.225	18.404	18.596	248	18.422	18.560	18.697	286	18.314	18.493	18.674
18.269	18.436	18.598	211	18.104	18.299	18.492	249	18.269	18.450	18.632	287	18.337	18.525	18.714
18.335	18.532	18.742	212	18.187	18.361	18.530	250	18.309	18.462	18.617	288	18.236	18.428	18.622

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293	18.266	18.474	18.681	331	18.448	18.628	18.806	369	18.292	18.488	18.682	407	18.255	18.492	18.740
294	18.307	18.501	18.704	332	18.152	18.384	18.622	370	18.248	18.461	18.678	408	18.389	18.581	18.778
295	18.112	18.315	18.517	333	18.266	18.428	18.589	371	18.246	18.437	18.622	409	18.265	18.412	18.560
296	18.386	18.574	18.773	334	18.330	18.525	18.722	372	18.421	18.579	18.737	410	18.292	18.467	18.646
297	17.984	18.204	18.429	335	18.439	18.586	18.727	373	18.273	18.443	18.622	411	18.197	18.387	18.575
298	18.233	18.433	18.624	336	18.376	18.576	18.786	374	18.302	18.507	18.704	412	18.288	18.474	18.659
299	18.157	18.341	18.534	337	18.352	18.562	18.765	375	18.427	18.585	18.742	413	18.115	18.295	18.471
300	18.411	18.560	18.713	338	18.406	18.580	18.749	376	18.201	18.408	18.614	414	18.144	18.342	18.535
301	18.314	18.513	18.709	339	18.354	18.503	18.656	377	18.333	18.513	18.696	415	18.442	18.594	18.748
302	18.252	18.459	18.659	340	18.228	18.414	18.598	378	18.330	18.507	18.682	416	18.195	18.384	18.581
303	18.374	18.538	18.709	341	18.190	18.389	18.604	379	18.195	18.406	18.617	417	18.405	18.571	18.732
304	18.412	18.590	18.761	342	18.466	18.640	18.805	380	18.277	18.463	18.650	418	18.333	18.525	18.707
305	18.367	18.576	18.777	343	18.149	18.337	18.522	381	18.265	18.475	18.691	419	18.261	18.433	18.613
306	18.240	18.432	18.635	344	18.372	18.523	18.681	382	18.237	18.420	18.594	420	18.209	18.417	18.621
307	18.235	18.437	18.639	345	18.435	18.602	18.773	383	18.216	18.406	18.597	421	18.328	18.518	18.713
308	18.332	18.538	18.746	346	18.338	18.518	18.708	384	18.115	18.314	18.522	422	18.190	18.389	18.590
309	18.325	18.517	18.718	347	18.230	18.417	18.601	385	18.238	18.430	18.616	423	18.254	18.431	18.614
310	18.141	18.349	18.560	348	18.351	18.548	18.743	386	18.305	18.480	18.662	424	18.195	18.379	18.555
311	18.183	18.381	18.580	349	18.254	18.464	18.666	387	18.369	18.514	18.659	425	18.224	18.391	18.566
312	18.463	18.663	18.859	350	18.324	18.486	18.647	388	18.300	18.452	18.609	426	18.093	18.262	18.429
313	18.378	18.546	18.706	351	18.239	18.440	18.642	389	18.117	18.310	18.502	427	18.366	18.545	18.721
314	18.310	18.496	18.685	352	18.326	18.537	18.753	390	18.321	18.507	18.691	428	18.475	18.605	18.739
315	18.220	18.429	18.628	353	18.131	18.301	18.471	391	18.274	18.432	18.595	429	18.053	18.252	18.448
316	18.183	18.383	18.592	354	18.273	18.445	18.614	392	18.079	18.265	18.448	430	18.286	18.461	18.637
317	18.427	18.597	18.759	355	18.156	18.347	18.535	393	18.421	18.595	18.775	431	18.186	18.388	18.581
318	18.273	18.464	18.655	356	18.213	18.391	18.568	394	18.253	18.430	18.610	432	18.206	18.365	18.523
319	18.299	18.475	18.646	357	18.205	18.392	18.576	395	18.177	18.352	18.525	433	18.208	18.393	18.586
320	18.101	18.295	18.499	358	18.180	18.369	18.557	396	18.383	18.541	18.698	434	18.321	18.468	18.620
321	18.310	18.497	18.679	359	18.248	18.426	18.606	397	18.282	18.473	18.661	435	18.375	18.565	18.750
322	18.186	18.394	18.596	360	18.211	18.390	18.564	398	18.359	18.506	18.659	436	18.318	18.485	18.662
323	18.257	18.422	18.591	361	18.304	18.473	18.637	399	18.354	18.545	18.739	437	18.414	18.571	18.731
324	18.153	18.353	18.553	362	18.479	18.673	18.862	400	18.211	18.397	18.585	438	18.363	18.538	18.716
325	18.338	18.515	18.686	363	18.224	18.416	18.605	401	18.181	18.377	18.574	439	18.302	18.529	18.744
326	18.245	18.405	18.575	364	18.158	18.343	18.525	402	18.401	18.554	18.703	440	18.361	18.530	18.703
327	18.271	18.470	18.669	365	18.387	18.569	18.768	403	18.375	18.533	18.702	441	18.124	18.315	18.512
328	18.388	18.583	18.777	366	18.301	18.484	18.669	404	18.364	18.567	18.785	442	18.227	18.410	18.593
329	18.388	18.571	18.752	367	18.256	18.473	18.686	405	18.184	18.389	18.604	443	18.193	18.370	18.547
330	18.219	18.429	18.631	368	18.594	18.730	18.872	406	18.248	18.438	18.628	444	18.196	18.392	18.591

445	18.268	18.463	18.659	483	18.132	18.354	18.574	521	18.149	18.347	18.533	559	18.273	18.469	18.661
446	18.169	18.357	18.539	484	18.179	18.390	18.607	522	18.345	18.484	18.634	560	18.370	18.528	18.685
447	18.321	18.487	18.649	485	18.318	18.510	18.701	523	18.186	18.414	18.638	561	18.279	18.451	18.625
448	18.250	18.423	18.607	486	18.314	18.492	18.669	524	18.303	18.466	18.626	562	18.230	18.413	18.598
449	18.279	18.486	18.694	487	18.301	18.480	18.658	525	18.246	18.456	18.670	563	18.226	18.397	18.564
450	18.427	18.607	18.784	488	18.365	18.546	18.723	526	18.266	18.456	18.639	564	18.337	18.546	18.754
451	18.466	18.642	18.826	489	18.305	18.483	18.653	527	18.215	18.382	18.555	565	18.389	18.549	18.707
452	18.370	18.522	18.680	490	18.267	18.481	18.691	528	18.196	18.403	18.611	566	18.376	18.563	18.739
453	18.266	18.451	18.632	491	18.295	18.466	18.637	529	18.259	18.455	18.646	567	18.334	18.533	18.729
454	18.432	18.599	18.773	492	18.352	18.521	18.695	530	18.118	18.368	18.623	568	18.216	18.399	18.574
455	18.250	18.434	18.616	493	18.152	18.326	18.504	531	18.250	18.422	18.593	569	18.227	18.412	18.586
456	18.276	18.451	18.628	494	18.286	18.474	18.657	532	18.286	18.468	18.645	570	18.221	18.390	18.562
457	18.389	18.564	18.735	495	18.365	18.552	18.741	533	18.320	18.481	18.650	571	18.432	18.626	18.815
458	18.263	18.455	18.657	496	18.383	18.512	18.648	534	18.147	18.337	18.533	572	18.237	18.424	18.608
459	18.316	18.525	18.742	497	18.263	18.511	18.768	535	18.292	18.467	18.643	573	18.214	18.382	18.554
460	18.251	18.419	18.589	498	18.407	18.586	18.760	536	18.400	18.595	18.785	574	18.070	18.265	18.460
461	18.369	18.568	18.757	499	18.372	18.541	18.711	537	18.344	18.513	18.679	575	18.175	18.362	18.547
462	18.249	18.445	18.640	500	18.158	18.372	18.595	538	18.052	18.276	18.487	576	18.468	18.641	18.808
463	18.195	18.389	18.571	501	18.295	18.477	18.649	539	18.422	18.583	18.739	577	18.366	18.573	18.771
464	18.349	18.517	18.680	502	18.335	18.512	18.696	540	18.165	18.358	18.544	578	18.274	18.447	18.620
465	18.400	18.554	18.712	503	18.427	18.630	18.831	541	18.286	18.481	18.679	579	18.255	18.453	18.652
466	18.316	18.497	18.676	504	18.234	18.431	18.631	542	18.376	18.579	18.783	580	18.242	18.399	18.557
467	18.159	18.335	18.511	505	18.197	18.392	18.580	543	18.324	18.506	18.685	581	18.191	18.394	18.572
468	18.257	18.445	18.631	506	18.364	18.532	18.710	544	18.341	18.544	18.736	582	18.242	18.412	18.574
469	18.222	18.383	18.541	507	18.339	18.555	18.772	545	18.344	18.562	18.777	583	18.283	18.507	18.730
470	18.130	18.344	18.559	508	18.259	18.462	18.659	546	18.388	18.585	18.787	584	18.091	18.341	18.594
471	18.099	18.305	18.508	509	18.211	18.431	18.650	547	18.332	18.520	18.701	585	18.145	18.337	18.528
472	18.368	18.509	18.644	510	18.198	18.389	18.594	548	18.137	18.342	18.550	586	18.388	18.515	18.641
473	18.308	18.509	18.714	511	18.204	18.403	18.603	549	18.246	18.398	18.553	587	18.547	18.675	18.802
474	18.070	18.299	18.511	512	18.096	18.295	18.487	550	18.409	18.593	18.777	588	18.506	18.668	18.823
475	18.283	18.484	18.688	513	18.296	18.447	18.605	551	18.217	18.415	18.612	589	18.415	18.562	18.713
476	18.280	18.432	18.577	514	18.229	18.439	18.651	552	18.130	18.333	18.529	590	18.130	18.323	18.515
477	17.975	18.206	18.431	515	18.238	18.429	18.615	553	18.239	18.429	18.590	591	18.349	18.512	18.666
478	18.217	18.420	18.623	516	18.422	18.601	18.782	554	18.127	18.290	18.461	592	18.306	18.477	18.645
479	18.339	18.536	18.738	517	18.399	18.578	18.756	555	18.400	18.571	18.736	593	18.264	18.418	18.572
480	18.401	18.556	18.712	518	18.286	18.466	18.649	556	18.162	18.339	18.526	594	18.436	18.652	18.867
481	17.897	18.082	18.265	519	18.403	18.565	18.734	557	18.190	18.375	18.571	595	18.355	18.565	18.774
482	18.239	18.421	18.599	520	18.261	18.471	18.679	558	18.226	18.416	18.598	596	18.306	18.486	18.673

597	18.287	18.443	18.600	635	18.161	18.363	18.558	673	18.439	18.621	18.801	711	18.301	18.501	18.695
598	18.228	18.425	18.632	636	18.197	18.406	18.620	674	18.400	18.573	18.750	712	18.275	18.476	18.678
599	18.265	18.452	18.636	637	18.248	18.422	18.604	675	18.221	18.424	18.625	713	18.284	18.495	18.691
600	18.165	18.350	18.542	638	18.201	18.364	18.522	676	18.256	18.460	18.658	714	18.470	18.643	18.819
601	18.273	18.468	18.666	639	18.204	18.393	18.572	677	18.179	18.337	18.493	715	18.209	18.396	18.581
602	18.224	18.450	18.667	640	18.374	18.567	18.759	678	18.181	18.351	18.526	716	18.189	18.346	18.506
603	18.205	18.393	18.577	641	18.295	18.462	18.627	679	18.268	18.459	18.652	717	18.222	18.407	18.588
604	18.260	18.431	18.608	642	18.229	18.394	18.566	680	18.302	18.495	18.682	718	18.293	18.498	18.708
605	18.372	18.541	18.704	643	18.438	18.630	18.831	681	18.427	18.599	18.762	719	18.196	18.420	18.650
606	18.427	18.593	18.763	644	18.321	18.486	18.666	682	18.232	18.454	18.677	720	18.401	18.587	18.768
607	18.332	18.498	18.657	645	18.302	18.504	18.702	683	18.460	18.643	18.825	721	18.222	18.418	18.619
608	18.211	18.361	18.511	646	18.320	18.522	18.727	684	18.171	18.355	18.533	722	18.281	18.470	18.653
609	18.326	18.488	18.654	647	18.266	18.460	18.651	685	18.420	18.604	18.790	723	18.407	18.588	18.768
610	18.296	18.453	18.605	648	18.379	18.555	18.725	686	18.067	18.278	18.473	724	18.376	18.532	18.688
611	18.327	18.506	18.682	649	18.293	18.473	18.644	687	18.254	18.445	18.628	725	18.232	18.422	18.604
612	18.342	18.504	18.665	650	18.297	18.497	18.693	688	18.431	18.623	18.816	726	18.204	18.394	18.573
613	18.136	18.349	18.565	651	18.186	18.398	18.618	689	18.211	18.402	18.596	727	18.254	18.412	18.578
614	18.327	18.505	18.680	652	18.191	18.393	18.587	690	18.273	18.461	18.653	728	18.300	18.501	18.696
615	18.158	18.380	18.604	653	18.244	18.409	18.581	691	18.250	18.411	18.570	729	18.256	18.472	18.675
616	18.319	18.518	18.714	654	18.486	18.653	18.823	692	18.382	18.543	18.699	730	18.129	18.316	18.510
617	18.254	18.438	18.622	655	18.271	18.460	18.645	693	18.203	18.365	18.534	731	18.344	18.536	18.725
618	18.317	18.509	18.699	656	18.093	18.288	18.494	694	18.190	18.372	18.562	732	18.549	18.701	18.861
619	18.392	18.554	18.719	657	18.238	18.416	18.591	695	18.216	18.388	18.558	733	18.223	18.416	18.609
620	18.284	18.471	18.659	658	18.287	18.477	18.665	696	18.236	18.396	18.557	734	18.328	18.515	18.711
621	18.291	18.494	18.703	659	18.376	18.551	18.730	697	18.130	18.332	18.547	735	18.239	18.422	18.607
622	18.382	18.565	18.755	660	18.253	18.431	18.610	698	18.378	18.529	18.678	736	18.331	18.505	18.690
623	18.366	18.544	18.722	661	18.238	18.421	18.601	699	18.305	18.469	18.628	737	18.094	18.288	18.491
624	18.273	18.437	18.600	662	18.033	18.232	18.430	700	18.235	18.403	18.581	738	18.339	18.482	18.627
625	18.108	18.324	18.540	663	18.146	18.371	18.599	701	18.205	18.401	18.598	739	18.392	18.610	18.823
626	18.359	18.504	18.651	664	18.449	18.620	18.786	702	18.152	18.362	18.570	740	18.307	18.484	18.662
627	18.367	18.545	18.732	665	18.258	18.464	18.669	703	18.403	18.576	18.753	741	18.058	18.287	18.517
628	18.364	18.510	18.660	666	18.191	18.388	18.586	704	18.351	18.557	18.773	742	18.217	18.423	18.628
629	18.390	18.563	18.732	667	18.190	18.382	18.568	705	18.139	18.354	18.558	743	18.285	18.491	18.689
630	18.405	18.589	18.772	668	17.990	18.205	18.419	706	18.347	18.509	18.664	744	18.125	18.341	18.556
631	18.362	18.546	18.727	669	18.247	18.468	18.678	707	18.219	18.407	18.599	745	18.239	18.411	18.580
632	18.425	18.596	18.759	670	18.178	18.366	18.562	708	18.090	18.278	18.457	746	18.234	18.463	18.698
633	18.355	18.531	18.704	671	18.040	18.229	18.422	709	18.241	18.404	18.567	747	18.353	18.508	18.671
634	18.291	18.457	18.625	672	18.447	18.613	18.780	710	18.247	18.449	18.653	748	18.180	18.407	18.629

18.433	18.605	787	18.305	18.489	18.667	825	18.367	18.542	18.713	863	18.173	18.420
18.458	18.664	788	18.303	18.489	18.671	826	18.236	18.404	18.570	864	18.351	18.511
18.271	18.483	789	18.330	18.510	18.689	827	18.379	18.510	18.646	865	18.120	18.344
18.539	18.718	790	18.105	18.264	18.419	828	18.149	18.338	18.524	866	18.347	18.512
18.390	18.567	791	18.359	18.550	18.733	829	18.293	18.471	18.644	867	18.209	18.376
18.234	18.412	792	18.366	18.588	18.805	830	18.357	18.545	18.736	868	18.226	18.410
18.535	18.716	793	18.282	18.498	18.705	831	18.232	18.433	18.630	869	18.143	18.328
18.570	18.766	794	18.163	18.387	18.607	832	18.275	18.483	18.683	870	18.252	18.435
18.427	18.589	795	18.394	18.580	18.770	833	18.345	18.478	18.613	871	18.274	18.461
18.511	18.670	796	18.213	18.391	18.564	834	18.202	18.389	18.564	872	18.213	18.420
18.425	18.613	797	18.401	18.559	18.717	835	18.367	18.564	18.761	873	18.415	18.544
18.540	18.719	798	18.284	18.496	18.699	836	18.328	18.512	18.706	874	18.085	18.267
18.614	18.813	799	18.314	18.497	18.681	837	18.267	18.464	18.660	875	18.224	18.414
18.534	18.743	800	18.187	18.401	18.620	838	18.244	18.407	18.562	876	18.236	18.419
18.681	18.880	801	18.106	18.304	18.507	839	18.304	18.490	18.670	877	18.361	18.527
18.295	18.514	802	18.255	18.452	18.650	840	18.292	18.469	18.661	878	18.188	18.424
18.395	18.580	803	18.227	18.437	18.653	841	18.234	18.433	18.636	879	18.099	18.314
18.528	18.740	804	18.297	18.470	18.649	842	18.278	18.456	18.637	880	18.258	18.482
18.419	18.604	805	18.218	18.389	18.563	843	18.311	18.482	18.651	881	18.323	18.532
18.402	18.605	806	18.476	18.643	18.807	844	18.269	18.461	18.662	882	18.340	18.538
18.466	18.658	807	18.150	18.344	18.527	845	18.221	18.400	18.572	883	18.227	18.421
18.262	18.466	808	18.268	18.459	18.644	846	18.282	18.472	18.664	884	18.128	18.333
18.372	18.550	809	18.257	18.418	18.584	847	18.023	18.241	18.460	885	18.210	18.376
18.340	18.533	810	18.299	18.485	18.667	848	18.191	18.386	18.572	886	18.227	18.394
18.357	18.566	811	18.277	18.476	18.680	849	18.363	18.541	18.708	887	18.286	18.451
18.371	18.559	812	18.106	18.370	18.637	850	18.346	18.532	18.720	888	18.373	18.613
18.511	18.674	813	18.302	18.493	18.678	851	18.290	18.478	18.672	889	18.432	18.601
18.379	18.589	814	18.365	18.524	18.692	852	18.145	18.354	18.560	890	18.401	18.580
18.607	18.786	815	18.304	18.456	18.610	853	18.251	18.484	18.717	891	18.479	18.632

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901	18.168	18.347	18.528	939	18.306	18.487	18.680	977	18.141	18.351	18.567
902	18.402	18.587	18.770	940	18.318	18.513	18.710	978	18.380	18.557	18.728
903	18.324	18.522	18.714	941	18.311	18.476	18.641	979	18.353	18.521	18.682
904	18.363	18.519	18.673	942	18.388	18.528	18.674	980	18.302	18.507	18.706
905	18.338	18.510	18.673	943	18.250	18.426	18.610	981	18.257	18.462	18.675
906	18.307	18.457	18.619	944	18.159	18.355	18.558	982	18.351	18.514	18.684
907	18.327	18.531	18.729	945	18.398	18.538	18.680	983	18.301	18.493	18.681
908	18.199	18.414	18.620	946	18.557	18.702	18.848	984	18.397	18.538	18.683
909	18.337	18.560	18.788	947	18.378	18.551	18.721	985	18.289	18.448	18.613
910	18.230	18.405	18.572	948	18.060	18.261	18.468	986	18.348	18.502	18.666
911	18.261	18.441	18.607	949	18.240	18.449	18.653	987	18.208	18.391	18.580
912	18.160	18.320	18.471	950	18.342	18.522	18.699	988	18.172	18.360	18.554
913	18.337	18.562	18.789	951	18.260	18.465	18.671	989	18.259	18.433	18.614
914	18.286	18.502	18.710	952	18.241	18.442	18.636	990	18.210	18.402	18.593
915	18.197	18.362	18.534	953	18.076	18.291	18.513	991	18.115	18.300	18.477
916	18.303	18.492	18.684	954	18.339	18.492	18.649	992	18.098	18.282	18.464
917	18.371	18.520	18.674	955	18.442	18.589	18.747	993	18.340	18.524	18.717
918	18.361	18.545	18.725	956	18.304	18.454	18.606	994	18.181	18.387	18.595
919	18.142	18.343	18.537	957	18.166	18.351	18.543	995	18.199	18.414	18.647
920	18.224	18.388	18.544	958	18.123	18.343	18.557	996	18.357	18.527	18.705
921	18.525	18.669	18.810	959	18.237	18.412	18.595	997	18.391	18.571	18.749
922	18.366	18.577	18.780	960	18.308	18.480	18.657	998	18.467	18.634	18.804
923	18.168	18.343	18.526	961	18.315	18.516	18.719	999	18.204	18.413	18.624
924	18.282	18.467	18.654	962	18.315	18.480	18.644	1000	18.316	18.461	18.603
925	18.428	18.574	18.718	963	18.428	18.592	18.757				
926	18.045	18.254	18.465	964	18.439	18.637	18.838				
927	18.232	18.400	18.571	965	18.413	18.599	18.788				
928	18.336	18.559	18.779	966	18.249	18.457	18.672				
929	18.263	18.505	18.749	967	18.333	18.505	18.669				
930	18.117	18.330	18.547	968	18.233	18.470	18.716				
931	18.191	18.382	18.567	969	18.213	18.407	18.602				
932	18.325	18.503	18.670	970	18.192	18.389	18.591				
933	18.367	18.532	18.692	971	18.282	18.432	18.580				
934	18.291	18.484	18.676	972	18.333	18.517	18.693				
935	18.314	18.458	18.604	973	18.245	18.434	18.621				
936	18.226	18.401	18.585	974	18.259	18.455	18.651				
937	18.354	18.515	18.674	975	18.164	18.364	18.559				
938	18.110	18.298	18.487	976	18.412	18.554	18.703				

**Table S10.** Maximum attack rate  $(T/T_h)$  and its confidence interval  $(T/T_h \text{ CI})$  of 120 simulations run to assess the method performance over a series of incomplete datasets. Each run was carried out with 20 simulated samples and 50 replicates. n: number of samples in the original dataset;  $nN_0$ : number of kept initial prey densities (n = 11 in the original simulated dataset); T: time of the experiment;  $T_h$ : handling time; CI: confidence interval. Letters between parentheses after dataset names correspond to those used in Figs. S3 & S4.

Dataset	D	NI		n = 10		n = 5
Dataset	Kun	$\mathbf{n}\mathbf{N}_0$	T/T <sub>h</sub>	T/T <sub>h</sub> CI	T/T <sub>h</sub>	T/T <sub>h</sub> CI
Complete dataset (A)	1	11	14.18065	14.08112, 14.27672	13.68588	13.56286, 13.81539
Complete dataset (A)	2	11	14.34579	14.24325, 14.45175	13.73908	13.63097, 13.84891
Complete dataset (A)	3	11	14.30454	14.17534, 14.43613	13.82704	13.71174, 13.93983
Complete dataset (A)	4	11	14.06766	13.96067, 14.17414	13.74144	13.64259, 13.83643
Complete dataset (A)	5	11	14.33438	14.22702, 14.44239	13.84756	13.70672, 13.99120
Complete dataset (A)	6	11	14.18482	14.07638, 14.29656	13.80399	13.69420, 13.91806
Complete dataset (A)	7	11	14.38471	14.28100, 14.48500	13.84127	13.72202, 13.96234
Complete dataset (A)	8	11	14.29176	14.17497, 14.40908	13.80406	13.66987, 13.94086
Complete dataset (A)	9	11	14.29616	14.17515, 14.41205	13.72628	13.63399, 13.81865
Complete dataset (A)	10	11	14.40962	14.28876, 14.53103	13.86542	13.75463, 13.98149
Incomplete dataset (B)	11	7	14.16916	14.00709, 14.32610	14.01083	13.88739, 14.13687
Incomplete dataset (B)	12	7	14.12410	13.97336, 14.27370	14.02048	13.85822, 14.18193
Incomplete dataset (B)	13	7	14.23945	14.09833, 14.37911	13.97810	13.84593, 14.10124
Incomplete dataset (B)	14	7	14.23113	14.08341, 14.38126	13.96877	13.81770, 14.12212
Incomplete dataset (B)	15	7	14.11048	13.97775, 14.24284	14.00435	13.85709, 14.15342
Incomplete dataset (B)	16	7	14.18609	14.06572, 14.31924	13.93690	13.79975, 14.07230
Incomplete dataset (B)	17	7	14.25583	14.14517, 14.36363	14.18600	14.03283, 14.33439
Incomplete dataset (B)	18	7	14.16576	14.01564, 14.32180	14.06298	13.92559, 14.20742
Incomplete dataset (B)	19	7	14.14602	14.00623, 14.28617	14.16710	14.02150, 14.30786
Incomplete dataset (B)	20	7	14.20768	14.06744, 14.35128	14.10163	13.968543, 14.2374
Incomplete dataset (C)	21	7	14.19308	14.07351, 14.31057	14.97946	14.84907, 15.10875
Incomplete dataset (C)	22	7	14.29002	14.16222, 14.42350	14.87202	14.72241, 15.02364

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Incomplete dataset (C)	23	7	14.22479	14.09568, 14.35446	14.87021	14.72859, 15.01744
Incomplete dataset (C)	24	7	14.25726	14.13902, 14.37722	14.84145	14.69404, 14.99439
Incomplete dataset (C)	25	7	14.19374	14.07262, 14.31126	15.00729	14.86655, 15.14519
Incomplete dataset (C)	26	7	14.11486	13.98747, 14.24337	15.01257	14.87240, 15.15808
Incomplete dataset (C)	27	7	14.14540	14.02239, 14.26441	14.98461	14.84786, 15.12766
Incomplete dataset (C)	28	7	14.24457	14.11411, 14.38200	14.89254	14.74901, 15.03052
Incomplete dataset (C)	29	7	14.22781	14.10214, 14.35436	15.01523	14.79757, 15.22890
Incomplete dataset (C)	30	7	14.23918	14.14282, 14.33726	14.94620	14.80259, 15.08766
Incomplete dataset (D)	31	7	14.15534	14.06576, 14.24531	14.68680	14.58542, 14.78611
Incomplete dataset (D)	32	7	14.16696	14.05859, 14.27334	14.60252	14.49013, 14.70303
Incomplete dataset (D)	33	7	14.15724	14.05418, 14.25918	14.64390	14.53837, 14.74830
Incomplete dataset (D)	34	7	14.10664	13.99019, 14.22411	14.75931	14.63165, 14.89207
Incomplete dataset (D)	35	7	14.14085	14.04958, 14.23517	14.67757	14.59666, 14.75805
Incomplete dataset (D)	36	7	14.22591	14.12588, 14.31904	14.68957	14.58388, 14.79549
Incomplete dataset (D)	37	7	14.23610	14.13449, 14.33963	14.64080	14.54469, 14.73549
Incomplete dataset (D)	38	7	14.21277	14.09413, 14.32144	14.77905	14.65649, 14.90057
Incomplete dataset (D)	39	7	14.15562	14.06497, 14.24423	14.59704	14.47249, 14.72400
Incomplete dataset (D)	40	7	14.13142	14.02217, 14.24040	14.77438	14.66553, 14.87817
Incomplete dataset (E)	41	6	14.20265	14.10456, 14.30780	12.94551	12.85927, 13.03052
Incomplete dataset (E)	42	6	14.40083	14.28771, 14.51559	12.99838	12.90893, 13.08697
Incomplete dataset (E)	43	6	14.29725	14.20117, 14.38936	13.03428	12.93866, 13.12475
Incomplete dataset (E)	44	6	14.36309	14.26953, 14.45847	12.94129	12.84005, 13.04392
Incomplete dataset (E)	45	6	14.26244	14.15488, 14.37622	12.84711	12.74366, 12.94755
Incomplete dataset (E)	46	6	14.24481	14.09099, 14.40108	12.99173	12.90189, 13.08028
Incomplete dataset (E)	47	6	14.28690	14.17661, 14.39315	12.97156	12.85843, 13.08354
Incomplete dataset (E)	48	6	14.30517	14.18487, 14.42529	12.96321	12.87418, 13.05265
Incomplete dataset (E)	49	6	14.26201	14.15558, 14.36739	12.95982	12.86864, 13.05145
Incomplete dataset (E)	50	6	14.20763	14.09689, 14.31396	12.92144	12.82743, 13.01777
Incomplete dataset (F)	51	6	14.22314	14.12974, 14.31980	14.22078	14.11065, 14.32767

Incomplete dataset (F)	52	6	14.27321	14.15865, 14.38623	14.37324	14.28038, 14.46494
Incomplete dataset (F)	53	6	14.22901	14.13536, 14.33357	14.20415	14.09399, 14.31840
Incomplete dataset (F)	54	6	14.19861	14.08227, 14.31698	14.25568	14.15951, 14.35563
Incomplete dataset (F)	55	6	14.21047	14.11493, 14.30553	14.14786	14.03939, 14.26020
Incomplete dataset (F)	56	6	14.34042	14.23543, 14.44214	14.27058	14.15975, 14.38110
Incomplete dataset (F)	57	6	14.18531	14.07733, 14.28697	14.32703	14.22766, 14.42985
Incomplete dataset (F)	58	6	14.22917	14.11350, 14.34466	14.16537	14.07548, 14.25594
Incomplete dataset (F)	59	6	14.29325	14.17127, 14.41539	14.21763	14.11407, 14.32011
Incomplete dataset (F)	60	6	14.19896	14.07133, 14.32197	14.26001	14.15677, 14.36470
Mean ± SE			$14.23\pm0.01$		$14.11 \pm 0.08$	

**Table S11.** Maximum attack rate  $(T/T_h)$  of different predators and a parasitoid species, functional response models, experimental conditions and the corresponding references found in literature used to validate the simaR protocol, the functional response model used in simulations, the obtained maximum attack rates (simaR simulation of  $T/T_h$ ) and their confidence intervals. T: time of the experiment;  $T_h$ : handling time;  $T^a$ : Temperature; CI: confidence interval; Sernr: scaling exponent response (without prey replacement). Superscript letters within the column "Reference" indicate how the maximum attack rate was reported in the paper. <sup>a</sup>demand rate ( $\omega$ ); <sup>b</sup>maximum depredation rate (*K*); <sup>c</sup>maximum attack rate ( $T/T_h$ ); <sup>d</sup>upper processing limit; <sup>f</sup>estimated maximum attack rate ( $T/T_h$ ) and <sup>e,g</sup>maximum attack rate ( $T/T_h$ ).

Predator (Pr) / Parasitoid (Pa)	Prey	Reference	T (h)	T <sup>a</sup> (°C)	Contrast	Bibliographic model	simaR model	Prey depletion	Bibliographic T/T <sub>h</sub>	simaR simulation of T/T <sub>h</sub>	Simulated T/T <sub>h</sub> CI
				18	Temperature (18°C)	Bidimensional model	Holling II	No	1.28	1.47	1.416, 1.529
Coenosia attenuata	Drosophila	<sup>a</sup> Ciliali at al. 2005	12	24	Temperature (24°C)	Bidimensional model	Holling II	No	3.86	4.19	4.099, 4.272
(Muscidae) (Pr)	(Drosophlidae)	Ginon et al., 2005	12	30	Temperature (30°C)	Bidimensional model	Holling II	No	4.70	5.07	4.996, 5.154
				36	Temperature (36°C)	Bidimensional model	Holling II	No	6.02	6.47	6.391, 6.559
Cydnodromus				25	Egg maturity (16 h)	Holling II	Rogers II	Yes	35.12	37.84	37.623, 38.044
picanus	Tetranychus urticae (Tetranychidae)	<sup>b</sup> Tello et al., 2017	24	25	Egg maturity (42 h)	Holling II	Rogers II	Yes	26.85	31.98	31.788, 32.175
(Phytoseiidae) (Pr)	(Tettanyenidae)			25	Egg maturity (65 h)	Holling II	Rogers II	Yes	26.70	23.13	23.016, 23.242
				25	Parasitoid age (1 day)	Rogers III	Sernr	Yes	47.06	41.61	41.136, 42.085
				25	Parasitoid age (2 days)	Rogers III	Sernr	Yes	68.57	66.51	65.531, 67.503
				25	Parasitoid age (3 days)	Rogers II	Rogers II	Yes	109.09	81.04	79.738, 82.298
Praon volucre (Braconidae) (Pa)	Acyrthosiphon pisum	Desendidablet al. 2015	24	25	Parasitoid age (4 days)	Rogers II	Rogers II	Yes	126.31	106.28	103.491, 109.036
(Braconidae) (Pa)	(Aphididae)	rasandiden et al., 2015	24	25	Parasitoid age (5 days)	Rogers II	Rogers II	Yes	88.88	77.35	75.819, 78.797
				25	Parasitoid age (6 days)	Rogers II	Rogers II	Yes	70.58	63.01	61.459, 64.647
				25	Parasitoid age (7 days)	Rogers II	Rogers II	Yes	75.00	43.07	42.090, 44.084
				25	Parasitoid age (8 days)	Rogers II	Rogers II	Yes	25.53	22.21	21.834, 22.572
Amphiareus constrictus (Anthocoridae) (Pr)			24	25	Predator species	Type-III	Sernr	Yes	18.86	23.62	19.115, 28.096
Blaptostethus pallescens (Anthocoridae) (Pr)	<i>Tuta absoluta</i> (Gelechiidae)	<sup>d</sup> Queiroz et al., 2015	24	25	Predator species	Type-III	Sernr	Yes	25.42	32.55	28.256, 36.795
Orius tristicolor (Anthocoridae) (Pr)			24	25	Predator species	Type-II	Rogers II	Yes	15.20	17.23	16.847, 17.590
			24	25	Predator age (3 day)	Type-II	Rogers II	Yes	17.30	18.66	17.376, 19.941
	- · ·		24	25	Predator age (4 day)	Type-II	Rogers II	Yes	19.78	19.10	18.359, 19.843
Amblyseius swirskii (Phytoseiidae) (Pr)	(Tetranychus urticae (Tetranychidae)	<sup>e</sup> Fathipour et al., 2017	24	25	Predator age (5 day)	Type-II	Rogers II	Yes	18.16	13.99	13.604, 14.376
(1 1)	(Tetranychidae)	24 24	24	25	Predator age (6 day)	Type-II	Rogers II	Yes	60.42	67.04	63.146, 70.857
			24	25	Predator age (7 day)	Type-II	Rogers II	Yes	60.67	59.18	57.967, 60.375

			24	25	Predator age (12 day)	Type-III	Sernr	Yes	41.11	48.79	35.900, 62.104
			24	25	Predator age (17 day)	Type-II	Rogers II	Yes	57.65	63.15	62.393, 63.878
			24	25	Predator age (22 day)	Type-II	Rogers II	Yes	28.60	27.91	27.681, 28.141
			24	25	Predator age (27 day)	Type-II	Rogers II	Yes	24.69	23.53	23.255, 23.814
Phytoseiulus persimilis (Phytoseiidae)	Tetranychus urticae (Tetranychidae)	<sup>f</sup> Fathipour et al., 2018	24	25	Predator age (4 day)	Type-II	Rogers II	Yes	10.10	8.07	7.914, 8.220
			24	25	Predator age (5 day)	Type-II	Rogers II	Yes	11.41	11.07	10.925, 11.223
			24	25	Predator age (6 day)	Type-II	Rogers II	Yes	19.44	19.73	19.532, 19.936
			24	25	Predator age (10 day)	Type-II	Rogers II	Yes	39.95	39.88	39.57, 40.183
			24	25	Predator age (15 day)	Type-III	Sernr	Yes	35.19	35.59	35.28, 35.894
			24	25	Predator age (20 day)	Type-II	Rogers II	Yes	48.57	47.97	47.557, 48.366
			24	25	Predator age (25 day)	Type-II	Rogers II	Yes	42.36	40.31	40.019, 40.595
			24	25	Predator age (30 day)	Type-II	Rogers II	Yes	44.95	43.52	43.049, 43.990
			24	25	Predator age (35 day)	Type-III	Sernr	Yes	37.00	40.13	38.917, 41.308
			24	25	Predator age (40 day)	Type-III	Sernr	Yes	30.32	30.40	30.082, 30.727
Calathus granatensis (Carabidae) (Pr)	Bactrocera oleae (Tephritidae)	<sup>g</sup> Dinis et al., 2016	24	21	Predator species	Rogers II	Rogers II	Yes	7.40	8.52	8.455, 8.589
Pterostichus globosus (Carabidae) (Pr)	Bactrocera oleae (Tephritidae)	<sup>g</sup> Dinis et al., 2016	24	21	Predator species	Rogers II	Rogers II	Yes	19.60	19.01	18.893, 19.123



**Figure S1.** A: Artificial dataset with known parameters generated to assess the accuracy of the simulation method. Dots represent the artificial number of prey killed at each initial prey density. The line represents the fitted values of the Holling's type-II predator-prey function obtained using the "frair" package. B: Simulated maximum attack rates  $(T/T_h)$  and their 95% confidence interval resulting of 1000 simulation runs based on the artificial dataset. The black horizontal line represents the known value of the maximum attack rate (18.46 prey/day).T: time of the experiment; *a*: coefficient of attack rate;  $T_h$ : handling time.



**Figure S2.** Simulated datasets used to test the method performance over a series of subsequent incomplete datasets. Dots represent the artificial number of prey killed at each initial prey density. The lines represent the fitted values using the Rogers's type-II decreasing prey function obtained using the "frair" package. A: complete dataset with 10 samples (n) at 11 initial prey densities ( $nN_0$ ), and B: incomplete dataset keeping 50% samples (n = 5) of the former dataset at the same initial prey densities.



**Figure S3.** Example of complete and incomplete artificial datasets generated to assess the method performance on partial data. In all cases the original number of samples were n = 10. A: complete dataset (11 initial prey densities); B, C, D: incomplete datasets (7 initial prey densities) and E, F: incomplete datasets (6 initial prey densities). Dots represent the simulated number of prey killed at each initial prey density. The mass of lines represent the fitted values of each one of the 50 simulated replicates using the Rogers's type-II decreasing prey function in simaR. Each plot correspond to one of the 10 simulations run for each number of initial prey densities (i.e. a total of 60 simulations for n = 10).  $nN_0$ : Number of initial prey densities.



**Figure S4.** Example of incomplete artificial datasets generated to assess the method performance on partial data. In all cases the original number of samples were n = 5. A: incomplete dataset (11 initial prey densities); B, C, D: incomplete datasets (7 initial prey densities) and E, F: incomplete datasets (6 initial prey densities). Dots represent the simulated number of prey killed at each initial prey density. The mass of lines represent the fitted values of each one of the 50 simulated replicates using the Rogers's type-II decreasing prey function in simaR. Each plot correspond to one of the 10 simulations run for each number of initial prey densities (i.e. a total of 60 simulations for n = 5).  $nN_0$ : Number of initial prey densities.



**Figure S5.** Results of the linear model developed to test the simulation method performance using simaR over a series of incomplete datasets. A: Variation of the simulated maximum attack rate along the number of simulation runs. The shaded area represents the 95% confidence interval of the fitted values; B: effect of the number of samples of the initial simulated datasets. Different letters over the boxplots indicate significant differences at p < 0.05. The line that divides the box into two parts represents the median; the end of the box shows the upper and lower quartiles and the extreme lines show the highest and lowest value.



simaR simulation of T/Th

**Figure S6.** Linear regression of the simulated maximum attack rate  $(T/T_h)$  of different predators and a parasitoid using the simaR package on their corresponding bibliographic maximum attack rate. Equation and R-square are presented within the plot.

# **CHAPTER 7**

EcoPred: an educational Individual Based Model to explain biological control, a case study within an arable land.

# Chapter submitted as journal article:

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# **Contribution of authors:**

J.B.M. conceived and designed the model and wrote the code. All the authors contributed to writing the paper.

### **CHAPTER 7**

EcoPred: an educational Individual Based Model to explain biological control, a case study within an arable land.

### Abstract

Individual based models (IBMs) are up-to-date tools both in research and educational areas. In this work, it was delveloped an IBM built on NetLogo platform that simulates a topdown trophic cascade controlled by the pressure exerted by two model predators (webbuilding spiders and ground runner spiders) on a model pest (the olive fruit fly) within a hypothetical agricultural landscape (the olive crop). EcoPred is an IBM that aims to be an educational tool that can help teachers to explain concepts related to ecology in a modern, enjoyable and comprehensive way. EcoPred reflects the changes on a fly population within a simulated olive crop according to (1) the mortality rate caused by the predation of two spider species and energy loss, (2) the energy gain by feeding on flowers and (3) the reproduction rate in olive trees. The model was tested with 26 students achieving very good results in terms of acceptance and interest on the learning method. EcoPred can be used for educational purposes with 16 years old students and older to explain ecological concepts such as trophic level, species interactions, limiting factor and biological control in an interactive way simultaneously introducing students to biology oriented programming languages.

#### 7.1. Introduction

Students find practical work relatively useful and enjoyable as compared with other science teaching and learning activities (Abrahams & Millar, 2008). Cerini et al. (2003) showed that 71% of surveyed students selected "doing an experiment in class" as one of the three methods of teaching and learning science they found most enjoyable. Also, Roberts (2002) reported science, technology, engineering, and mathematics as key skills, and the students' learning experience could inspire and motivate themselves to study these subjects further at higher levels.

Individual based models (IBMs) are simulation models based on the interactions between individuals rather than on populations where population dynamics are usually a consequence of individual behavior. IBMs were developed from the need to understand and predict ecosystem complexity. Railsback (2001) explained the IBM concept as "build a model of an individual organism, build a model of the environment, and let a computer create multiple individual organisms and simulate the interactions of the individuals with each other and the environment" and stated that an IBM should address ecological concepts such as emergence, adaptation, fitness, state-based decisions, prediction, and computer implementation. Huhns & Singh (1998) defined the agents (individuals) as "active and persistent (software) components that perceive, reason, act, and communicate".

Although IBMs were born in the field of ecology, their development and use has grown steadily in recent years and the scope has been extended to several fields such as chemical engineering (Taherian & Mousavi, 2017), psychology (Schröder & Wolf, 2017) and health (Ufholz & Harlow, 2017). Within biology, IBMs have been applied to a large number of study areas including bird population dynamics (Parry et al., 2013), genetics (Pertoldi & Topping, 2004), land use effects on wildlife (Jepsen et al., 2005), microbiology (Oyebamiji et al., 2017), organic farming (Topping, 2011), plant evolution (Warren et al., 2011) and statistics (Grazzini et al., 2017).

Strong and comprehensive tools and platforms have also been developed in the context of ecological scientific research. For example, IBMs such as ALMaSS (Topping et al., 2003) and Weaver (Bilbao-Castro et al., 2015) are able to answer policy-related questions and facilitate management decision through the study of the effect of landscape structure, agricultural management and ecology on key animal species, and the evolution of their ecological interactions.

Within the educational framework, computers and computer graphics must be integrated into traditional teaching (Bio, 2010), and although efforts have been made to introduce IBMs within the educational context (Betancourt & Más, 2012) the number of IBMs specifically oriented for teaching is scarce (Ginovart, 2014). In addition, integration of concepts across levels of complexity and the ability to synthesize and analyze information that connects conceptual domains are skills that students need to develop (Vision and Change, 2011). Accordingly, the objectives of this work were (1) to develop an IBM able to represent a three-level trophic cascade in the framework of biological pest control, (2) and to test its viability and acceptance in the real world using the model as a teaching tool with a group of high school students.

### 7.2. Material and methods

### 7.2.1. Programming platform

EcoPred was developed in NetLogo (Wilensky, 1999). NetLogo is an open source multiagent modeling environment that has been widely used in research and teaching contexts and is freely available from the NetLogo website. It is a complete environment for creating and running models that enables exploration of emergent phenomena and comes with an extensive library including models in several domains.

### 7.2.2. Model description

EcoPred simulates a top-down trophic cascade controlled by the pressure exerted by two spider species (models for a web-builder spider and a ground runner spider) on a fruit fly

(model for the olive fruit fly *Bactrocera oleae* (Rossi, 1790)) in a simulated world that represents an olive orchard.

The model description follows the ODD (Overview, Design concepts, Details) protocol for describing individual- and agent-based models (Grimm et al. 2006, 2010):

**7.2.2.1. Purpose.** EcoPred is a simple and easy to use IBM built on the NetLogo platform that aims to be an educational tool that can help teachers to explain four concepts related to ecology: (1) the concept of trophic cascade including three trophic levels (primary producers, herbivores and carnivores), (2) the concept of generalist and specialist species; (3) the concept of functional group (guild), and their practical application within a simulated arable land ecosystem, (4) the concept of biological pest control.

**7.2.2.2. Agents/entities, state variables, scales, and model parameters.** The model uses three NetLogo types of entity: (1) turtles, (2) patches, and (3) an observer. Turtles correspond to the individuals, patches are spatial units composing the environment, and the observer is an entity that oversees everything that is going on in a two-dimensional world.

7.2.2.2.1. Agents (entities):

7.2.2.2.1.1. Observer: The observer is an entity that oversees everything that is going on in the world.

7.2.2.2.1.2. Patches: The world is two-dimensional and composed by a grid of patches. Each patch is a square piece of "ground" over which turtles can move.

7.2.2.2.1.3. Turtles: Turtles are agents that move around and interact in the world.

7.2.2.2.1.3.1. Ground spiders (acting as natural enemies against flies).

7.2.2.2.1.3.2. Web-females (acting as natural enemies against flies and reproduction sites for web-spiders).

7.2.2.2.1.3.3. Web-males (acting as natural enemies against flies).

7.2.2.1.3.4. Flies (acting as pest).

7.2.2.2.1.3.5. Olives (representing olive trees that act as reproduction sites for flies).

7.2.2.2.1.3.6. Flowers (acting as food source for flies).

7.2.2.1.3.7. Stones (acting as reproduction sites for ground spiders)

### 7.2.2.2.2. State variables:

7.2.2.2.1. Turtles

7.2.2.2.1.1. Sex (constant variable): Web-spiders are divided in males (allowed to move) and females (static).

7.2.2.2.1.2. Size and color (constant variables): Each turtle group presents a constant size (given in size units) and color:

7.2.2.2.1.2.1. Stones: 4 size units and color brown.

7.2.2.2.1.2.2. Olives: 1.5 size units and color violet.

7.2.2.2.1.2.3. Flowers: 3.5 size units and color yellow.

7.2.2.2.1.2.4. Flies: 2 size units and color white.

7.2.2.2.1.2.5. Web-females: 3 size units and color black.

7.2.2.2.1.2.6. Web-males: 2 size units and color black.

7.2.2.2.1.2.7. Ground spiders: 3 size units and color brown.

7.2.2.2.1.3 .Energy (this variable changes along time): flies and spiders (web-females, web-males and ground spiders) start with 50 energy units.

7.2.2.2.1.4. Heading (variable): it controls the direction the turtle is facing. Flies, web-females, web-males and ground spiders turn left and right while moving ahead according to a certain interval of degrees (see also section 7.1.2.).

7.2.2.2.1.4.1. Flies and web-males: the number (integer) of degrees to turn is randomly selected within the interval [0, 20) to the right and [0, 20) to the left.

7.2.2.2.1.4.2. Ground-spiders: the number (integer) of degrees to turn is randomly selected within the interval [0, 50) to the right and [0, 50) to the left.

7.2.2.2.1.5. Identity number (constant variable): a single identification number is assigned to each turtle.

7.2.2.2.1.6. Spatial location (this variable may change along time): the position of each type of turtle is described in section 7 (see below).

7.2.2.2.2. Patches.

7.2.2.2.2.1. Color (constant variable): Constant and green.

7.2.2.2.2.2. Spatial location (constant variable): Patches are stationary and arranged in a grid.

7.2.2.2.3. Spatial scale:

7.2.2.3.1. Patch. The model landscape is comprised by a grid of  $31 \ge 67$  square patches (i.e. 2257 area units). Space is a dimensionless variable.

7.2.2.3.2. World topology. The world has neither horizontal nor vertical limits. The world is a torus which means it is not bounded, but wraps, i.e. flies and spiders that reach the left border disappear and reappear on the right edge.

7.2.2.2.4. Temporal scale:

7.2.2.2.4.1. Tick. Time passes in discrete steps called "ticks". Time is a dimensionless variable.

7.2.2.2.5. Model parameters:

7.2.2.2.5.1. Initial number of flowers: an integer within the interval [0, 100].

7.2.2.2.5.2. Initial number of stones: an integer within the interval [0, 50].

7.2.2.5.3. Initial number of olives: an integer within the interval [0, 100].

7.2.2.2.5.4. Initial number of flies: an integer within the interval [0, 50].

7.2.2.5.5. Initial number of web-females: an integer within the interval [0, 20].

7.2.2.2.5.6. Initial number of web-males: an integer within the interval [0, 20].

7.2.2.2.5.7. Initial number of ground-spiders: an integer within the interval [0, 20].

### 7.2.2.3. Process overview and scheduling.

7.2.2.3.1. Which agents do what processes? EcoPred incorporates two levels of processes, low level processes (i.e. carried out by turtles) and high-level processes (i.e. carried out by the Observer). Each process within each category is indicated and followed by the agent that performs it:

7.2.2.3.1.1. Low level processes: move (flies and web-males), move-b (ground-spiders), fed (flies), reproduce (flies), fed-a (web-females), reproduce-a (web-females), fed-b (web-males), reproduce-b (web-males), move-b (web-males), fed-c (ground-spiders), reproduce-c (ground-spiders), death (flies, web-females, web-males and ground-spiders), and energy loss (flies, web-females, web-males and ground-spiders).

7.2.2.3.1.2. High-level processes: stop (if more than 2000 flies are counted within the crop, the system stops and show the message "Your crop have been invaded!"; if more than 1000 web-females, web-males or ground-spiders are counted within the crop the system stops; if the number of flies reaches zero, the system stops), plot (the number of flies and spiders are plotted along time at each tick), count the number of turtles (the number of flies and spiders is counted at each time tick), create the initial population of turtles, and introduce some individuals of web-males and ground-spiders (simulating immigration into the crop).

7.2.2.3.2. Order of processes. Firstly, the model checks the stop conditions described in section and one web-male and one ground-spider are created. Then, flies move, feed, reproduce, die, and alive individuals lose energy, then web-females feed, reproduce, die, and lose energy, then web-males move, feed, reproduce, die, and alive individuals lose energy. Finally, ground-spiders move, feed, reproduce, some individuals enters the world, die and lose energy. The whole order and processes are represented at Fig. 7.1.

7.2.2.3.3. When are state variables updated? During the simulation of processes or actions with the different entities, the turtles are called in a different random order in each time step (asynchronous updating). Each turtle completes the full set of actions requested before passing on control to the next agent at each time tick.

### 7.2.2.4. Design concepts.

7.2.2.4.1. Basic principles. EcoPred is based on four general concepts related to ecology, (1) the trophic cascade, (2) generalist and specialist species, (3) functional traits, and (4) biological pest control.

7.2.2.4.1.1. The concept of three-level trophic cascade was introduced by Hairstone et al. (1960) in which three trophic levels are food-limited and present interspecific competition among levels that regulates the population of each level. The model considers the primary producers (flowers), herbivores (flies) and carnivores (web and ground-spiders).

7.2.2.4.1.2. The concept of generalist or euryphagous species (i.e. a species that consumes a wide variety of prey species) and specialist or stenophagous species (i.e. a species that consume only particular parts of their prey, feed on only a narrow range of closely related species or even just a single species) (Begon et al., 2006). The model considers both groups, the generalist predators are represented by the ground-spiders that feed on flies and web-males, and specialist predators represented by web-spiders that only feed on flies.



**Figure 7.1.** Schematic view of the trophic levels and processes included in EcoPred along a time step (tick).

7.2.2.4.1.3. The concept of functional traits. Functional traits can be seen as key characteristics by which single species and groups of species influence ecosystem properties (de Bello et al., 2010) and are defined as a feature of an organism, which has demonstrable links to the organism's function (Lavorel et al., 1997). Consequently, 'functional groups' or guilds of species can be defined as an assemblage of organisms with similar functional trait attributes (Harrington et al., 2010), or in other words, species using the same class of resources in a behaviorally similar way (Simberloff & Dayan, 1991). The model represents two guilds among predators, spiders inhabiting the ground, and web-builder spiders. Each functional group behaves and feeds differently, ground spiders move freely and are generalist predators that need stones as shelter to reproduce (the model assumes that there are no sexual dimorphism), and web-building spiders are specialists. In addition, web-females are immobile and their reproduction depends on whether a male (that move freely) finds a female.
7.2.2.4.1.4. The concept of biological pest control. Natural ecosystems and their component species experience a rapid loss as habitat is destroyed for human use and invaded by species from other biogeographical areas (Van Driesche et al., 2010). In the 1990s, the insect biological control against environmental pests was raised as an independent goal (Van Driesche, 1994). The model simulates the effect of two predators (web and ground spiders) that represent natural enemies on a fly species that represent the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), the key pest of the olive tree (Boccaccio & Petacchi, 2009) in the Mediterranean basin within the simulated olive crop.

7.2.2.4.2. *Emergence*. The model represents two extreme situations. If the initial number of predators (i.e. spiders) is zero, the pest (i.e. flies) blooms and invades the crop. On the contrary, if the number of predators is set at maximum, the population plot at the end of the simulation represent the biological control of flies (i.e. their population reaches zero) exerted by the specialist predator (i.e. web-spiders) and the generalist predator (i.e. ground spiders) being the former dominated by the latter.

7.2.2.4.3. Sensing. The mechanisms by which flies and spiders obtain information about food sources and reproduction processes are modeled explicitly.

7.2.2.4.3.1. Flies only feed if the agent they found is a flower.

7.2.2.4.3.2. Flies only reproduce if the agent they found is an olive.

7.2.2.4.3.3. Web-spiders only feed if the agent they found is a fly.

7.2.2.4.3.4. Web-spiders only reproduce if the agent they found is a web-spider.

7.2.2.4.3.5. Web-females only reproduce if the agent they found is a web-male.

7.2.2.4.3.6. Ground-spiders only feed if the agent they found is a fly or a web-male spider.

7.2.2.4.3.7. Ground-spiders only reproduce if the agent they found is a stone.

7.2.2.4.4. *Interaction*. EcoPred incorporates both direct and indirect interactions; however they not involve communication between turtles.

7.2.2.4.4.1. Direct interactions:

7.2.2.4.4.1.1. Feeding of flies on flowers.

7.2.2.4.4.1.2. Predation of web-spiders over flies.

7.2.2.4.4.1.3. Predation of ground-spiders over flies.

7.2.2.4.4.1.4. Predation of ground-spiders over web-males.

7.2.2.4.4.1.5. Reproduction of flies (the fly only reproduce if an olive is found).

7.2.2.4.4.1.6. Reproduction of web-spiders (the spider only reproduces if a female or male is found respectively).

7.2.2.4.4.1.7. Reproduction of ground-spiders (the spider only reproduces if a stone is found).

7.2.2.4.4.2. Indirect interactions:

7.2.2.4.4.2.1. Competition for food (i.e. flies) among spiders. Competition for food among flies is not considered since during the time window they need to reach a pest outbreak the resources provided by flowers are assumed to be unlimited.

7.2.2.4.5. Stochasticity. Stochasticity is used in three processes related to the position of the turtles: (1) to place the turtles over the world at the initial time tick, (2) to place the hatched flies and spiders, and (3) to define the heading pattern of moving individuals. Stochasticity is not used to cause model events or behaviors to occur.

7.2.2.4.6. Collectives. The individuals do not form aggregations in EcoPred.

7.2.2.4.7. Observation. The "View" window within the user interface is a visual representation of the world of turtles and patches where the user can see the evolution of the system at real time. The number of flies, web-spiders (i.e. web-females and web-males) and ground-spiders is collected and plotted at each time tick by the observer to be interpreted. Also, the whole dataset (i.e. the results at each time tick) can be manually exported as an excel file (".csv" extension) for further analyses if desired.

**7.2.2.5. Initialization.** The aim of the model is to analyze the consequences of its initial state, i.e., how the population of flies and spiders change along time as a function of the initial number of each turtle. For this purpose, the number of individuals can be arbitrarily selected in the GUI using sliders within the following ranges:

Flowers: 0-100 individuals. Stones: 0-50 individuals. Olives: 0-100 individuals. Flies: 0-50 individuals. Web-females: 0-20 individuals. Web-males: 0-20 individuals. Ground-spiders: 0-20 individuals.

**7.2.2.6. Input data.** The model does not use input from external sources such as data files or other models to represent processes that change over time.

### 7.2.2.7. Submodels.

7.2.2.7.1. Low level processes:

7.2.2.7.1.1. Loose energy (flies, web-females, web-males, ground-spiders)

$$E_{(t+1)} = E_{(t)} - 1$$
 [Eq. 1]

where E denotes the amount of energy units the turtle owns, and t denotes the time tick.

7.2.2.7.1.2. Move (flies, web-males and ground-spiders):

Flies, web-males and ground-spiders move endlessly around the world by advancing forward one unit of distance at each time step (note that since patches are squares, a step forward could occur within the same patch). The degree of turn to each side (heading) is defined by a random integer at each time step (see section 2.5.). Movement is independent of the density of turtles and patches can house more than one turtle.

7.2.2.7.1.3. Fed (flies):  

$$E_{(t+1)} = E_{(t)} + N_{(t)} \times 30$$
 [Eq. 2]

where E denotes the number of energy units the fly has, N is the number of flowers on the patch, and t the time tick. The rule applies if the fly finds a flower at a distance of one unit along its current heading at t+1.

7.2.2.7.1.4. Reproduce (flies):

$$N_{(t+1)} = 4 \times N_{0(t)}$$
 [Eq. 3]

where  $N_0$  denotes the number of ascendants in a patch, N is the number of ascendants plus descendants (3 flies will hatch), and t the time tick. The rule applies if a fly enters a patch occupied by an olive, then the flies hatched are randomly positioned within the world. The energy is equally divided between the hatched flies and the parent.

7.2.2.7.1.5. Fed-a (web-females):

$$EWF_{(t+1)} = EWF_{(t)} + N_{(t)} \times 5$$
 [Eq. 4]

where EWF denotes the number of energy units the web-female has, N is the number of flies on the patch, and t the time tick. The rule applies if the web-female spider finds a fly at a distance of one unit along its current heading at t+1. Once the spider feeds, the flies found die.

7.2.2.7.1.6. Reproduce-a (web-females):

$$NWF_{(t+1)} = 2 \times NWF_{0(t)}$$
 [Eq. 5]

where  $NWF_0$  denotes the number of ascendants at the patch, NWF is the number of ascendants plus descendants (1 web-female will hatch), and t is the time tick. The rule applies if a web-male spider enters a patch occupied by a web-female, then the web-females hatched are randomly positioned within the world. The energy is equally divided between the hatched spiders and the parent.

$$EWM_{(t+1)} = EWM_{(t)} + N_{(t)} \times 5$$
 [Eq. 6]

where EWM denotes the number of energy units the web-male has, N is the number of flies on the patch, and t the time tick. The rule applies the web-male spider finds one fly at a distance of one unit along the turtle current heading at t+1. Once the spider feeds, the flies found die.

7.2.2.7.1.8. Reproduce-b (web-males):

$$NWM_{(t+1)} = 2 \times NWM_{0(t)}$$
[Eq. 7]

where  $NWM_0$  denotes the number of ascendants at the patch, NWM is the number of ascendants plus descendants (1 web-male will hatch), and t is the time tick. The rule applies if the patch the web-male enters is occupied by a web-female spider, and then the web-males hatched are randomly positioned within the world. The energy is equally divided between the hatched spiders and the parent.

7.2.2.7.1.9. Fed-c (ground-spiders):

$$EGS_{(t+1)} = EGS_{(t)} + NF_{(t)} \times 10 + NWM_{(t)} \times 10$$
 [Eq. 8]

where EGS denotes the number of energy units the ground-spider has, NF is the number of flies on the patch, NWM is the number of web-male spiders on the patch, and t the time tick. The rule applies if the ground-spider finds a fly at a distance of one unit along the turtle current heading, or a web-male spider in the same patch the ground-spider entered at t+1. Once the spider feeds, the flies/web-males found die.

7.2.2.7.1.10. Reproduce-c (ground-spiders):

 $NGS_{(t+1)} = 2 \times NGS_{0(t)}$ [Eq. 9]

where  $NGS_0$  denotes the number of ascendants in a patch where a stone is present, NGS is the number of ascendants plus descendants (1 ground-spider will hatch), and t the time tick. The ground-spiders hatched are randomly positioned within the world. The energy is equally divided between the hatched flies and the parent.

7.2.2.7.1.11. Death (flies, web-females, web-males, and ground-spiders).

If the condition  $E_{(t+1)} = 0$ , where E denotes the number of energy units is met, the turtle dies.

7.2.2.7.2. High-level processes:

7.2.2.7.2.1. Stop (flies, web-females, web-males, and ground-spiders):

If the condition  $NF_{(t)} = 0$  is met, where NF denotes the total number of flies and t is the time tick, the system stops. If the condition  $NF_{(t)} > 2000$  is met, where NF denotes the total number of web-females and t is the time tick, the system stops. If the condition  $NWF_{(t)} > 1000$  is met, where NWF denotes the total number of web-females and t is the time tick, the system stops. If the condition  $NWM_{(t)} > 1000$  is met, where NWM denotes the total number of web-males and t is the time tick, the system stops. If the condition  $NWM_{(t)} > 1000$  is met, where NWM denotes the total number of web-males and t is the time tick, the system stops. If the condition  $NGS_{(t)} > 1000$  is met, where NGS denotes the total number of ground-spiders and t is the time tick, the system stops.

7.2.2.7.2.2. Plot. The number of flies and spiders are plotted along time at each tick as:

$Flies = NF_{(t)}$	[Eq. 10]
Web-spiders = $NWF_{(t)} + NWM_{(t)}$	[Eq. 11]
Ground-spiders = $NGS_{(t)}$	[Eq. 12]

where t denotes the time tick,  $NF_{(t)}$  is the number of flies at time t,  $NWF_{(t)}$  is the number of web-female spiders at time t,  $NWM_{(t)}$  is the number of web-male spiders at time t, and  $NGS_{(t)}$  is the number of ground-spiders at time t.

### 7.2.3. Model performance

Twenty simulation runs were run and plotted together to test the model performance at each extreme situation, i.e., the evolution of the fly population along time in the absence of predators starting with the maximum number of flies, and the evolution of the fly population in the presence of the maximum number of predators. In order to assess the pattern stability, for each situation and key turtle (i.e. flies, web-spiders and ground-spiders) the mean and the standard error of the number of individuals were calculated at each time step (tick) and plotted together to allow visual comparison with the raw simulations. Finally, the mean of the number of individuals among simulations at each time step was modeled according to the observed pattern. Calculation of estimates and data fitting were performed in R (R CoreTeam, 2018).

#### 7.2.4. Method testing at classroom

In order to assess the acceptance, performance and interest of students on the use of IBMs and more specifically of EcoPred, it was used with 26 students of 16 years old coming from three different secondary schools of Bragança (Portugal). The students were allowed to explore and use, for 1 h, a simplified version of the model during an activity called "EcoPred: from the field to the computer, a virtual ecosystem" within the "Science and Technology Week 2017".

The activity was divided into three blocks of 20 minutes; during the first block, three aspects were explained: (1) the concept of IBMs, (2) the concept of biological pest control, and (3) how EcoPred can be used to reproduce the effect of a predator on a pest. During the second block, the students were allowed to run as many simulations as they wanted changing the initial parameters in two ways, (1) setting the initial number of predators at zero and the number of flies at maximum, and (2) setting the initial number of flies and predators at maximum. Then, the simulation results with and without predators were briefly discussed. Finally, during the third block, the students were encouraged to modify the code (accompanied by the monitor) in order to change the identity of the turtles (shape, size and color). The activity took place at the School of Agriculture (Polytechnic Institute of Bragança) and a survey was carried out at the end of the activity by asking to the students eight yes/no key questions:

- 1. Have you ever used any kind of simulation model?
- 2. Did this activity seem fun to you?
- 3. Did this activity seem complicated?
- 4. Would you like to continue creating your own ecosystem?
- 5. Did you have previous programming knowledge?
- 6. Would you like to learn how to program?
- 7. In your opinion, is a simulation model useful for learning?
- 8. Do you think that programming is useful in real life?

### 7.3. Results

### 7.3.1. Interface, simulations, and model performance

The designed graphical user interface encompasses a time speed control device, a set of devices used to select the initial number of each agent (*turtle*), a live data plot used to register the fly and spider's population changes along time, a counter for the number of flies, web-spiders and ground-spiders, and the 2D-world that represent the agents and interactions (Fig. 7.2). In general, the pattern on the evolution of each species' population was consistent among simulation runs (Fig. 7.3A & 7.3B) and supported by the amount of standard deviation (Fig. 7.3C & 7.3D). In terms of modeling, the fly population in the absence of predators followed an exponential growth until the stop condition was reached (i.e. 2000 flies) given by:

$$y \sim exp(a + b \times x)$$
 [Eq. 13]

where *y* denotes the number of flies and *x* is the time step (tick),  $a = 4.31 \pm 0.069$  (estimate  $\pm$  SE) (t = 62.88; P < 0.001), b = 0.071  $\pm$  0.002 (estimate  $\pm$  SE) (t = 43.44; P < 0.001) (Fig. 7.4A).

The evolution of the fly population in the presence of predators was fitted using local polynomial regression fitting (loess) with a degree of smoothing of  $\alpha = 0.5$ , and polynomials of degree = 1 that gave an equivalent number of parameters = 4.1, and a residual standard error = 12.60 (Fig. 7.4B). The evolution of the web-spiders' population was fitted using local polynomial regression fitting (loess) with a degree of smoothing of  $\alpha$  = 0.5, and polynomials of degree = 1 that gave an equivalent number of parameters = 4.1, and a residual standard error = 6.82 (Fig. 7.4B). The evolution of the ground-spiders' population was fitted using local polynomial regression fitting (loess) with a degree of smoothing of  $\alpha$  = 0.5, and polynomials of degree = 1 that gave an equivalent number of the ground-spiders' population was fitted using local polynomial regression fitting (loess) with a degree of smoothing of  $\alpha$  = 0.5, and polynomials of degree = 1 that gave an equivalent number of the ground-spiders' population was fitted using local polynomial regression fitting (loess) with a degree of smoothing of  $\alpha$  = 0.5, and polynomials of degree = 1 that gave an equivalent number of parameters = 4.1, and a residual standard error = 7.05 (Fig. 7.4B).

### 7.3.2. Method testing at classroom

About two thirds of the students had never used a simulation model before, however the same amount of them had some previous programming knowledge (Table 7.1). Most of the students found the activity fun, not difficult, and would like to continue developing the ecosystem (Table 7.1). Also, the vast majority of students said that they would like to learn programming, found the simulation model useful for learning, and thought that programming could be useful in real life (Table 7.1).



**Figure 7.2.** Aspect of the graphical user interface (GUI) of EcoPred. The red arrow indicates the time speed control device. The black arrows indicate the devices used to select the initial number of each agent (*turtle*). The blue arrow indicates the live data plot used to register the fly and spider's population changes along time. The green arrow indicates the individual counters for flies and spiders. The orange arrows indicate the buttons used to reset the initial conditions ("Clear") and initiate ("Go") a simulation.



**Figure 7.3.** Results of 20 simulation runs for each of two extreme situations (flies with and without predators) in EcoPred. A: evolution of the fly population at each time step (tick) (raw data) without predators; B: evolution of the fly and predators' population together at each time step (tick) (raw data); C: pattern followed by the mean number of flies along time (ticks) without predators, and D: pattern followed by the mean number of flies, web-spiders and ground-spiders along time (ticks). In all cases the initial number of individuals was established at maximum. In A and B, circles represent the abundance of turtles at each time step. In C and D, continuous lines represent the evolution of the average abundance of turtles, and dashed lines represent the evolution of its standard deviation along time.



**Figure 7.4.** Fitted models for two extreme situations (flies with and without predators) simulated in EcoPred. A: pattern of the pest (flies) population evolution without the pressure exerted by predators, and B: pattern of the pest (flies), specialist predator (webspiders) and generalist predator (ground-spiders) populations running the three-level trophic cascade with the initial number of individuals established at maximum. Dots were omitted within the charts to increase clarity.

**Table 7.1.** Results of the obtained with 26 students from high schools who tested the EcoPred model during their participation in the "Science and Technology Week 2017" in the School of Agriculture, Bragança, Portugal. N: number of students surveyed; DK/NA: Don't know / No answer.

Question	Ν	Yes (%)	No (%)	<b>DK/NA (%)</b>
1	26	30.77	61.54	7.69
2	26	96.15	3.85	0.00
3	26	19.23	80.77	0.00
4	26	88.46	11.54	0.00
5	26	69.23	30.77	0.00
6	26	96.15	3.85	0.00
7	26	92.31	7.69	0.00
8	26	96.15	3.85	0.00

### 7.4. Discussion

EcoPred represents consistently the relationship between the populations of the trophic levels of a three-level trophic cascade along time. The concepts related to population dynamics are mandatory knowledge for environmental science students, especially those related to areas such as agronomy, ecology, microbiology and zoology. EcoPred proved to be useful and interesting to students during an activity oriented to discuss the role of different types of predators on the ability to control a pest within a simulated agroecosystem.

Two extreme possible situations in the context of biological pest control were assessed during the activity using EcoPred, i.e. how the fly population evolves with and without predators during the simulation. The approach followed in this work allows a discussion of the implications of an up-to-date strategy of pest control in agricultural management such as the biological pest control at each situation in terms of relevant ecology-related concepts including trophic cascade, generalist and specialist species and species' guilds.

Beyond the basic followed approach, some more questions can be posed by teachers such as:

How would predator populations evolve if the system stop conditions were eliminated?
How would the fly population evolve if there were just the specialist predator present?
How would the fly population evolve if there were just the generalist predator present?
How would the population of flies and spiders evolve if the initial amount of resources changes?

In addition, the model parameterization in terms of initial conditions, such as the initial amount of energy, the maximum number of *turtles*, the amount of patches moved in each *tick* or the behavior of spiders was established *ad hoc*; however, the flexibility of NetLogo enables these to be easily changed and to include more agents into the trophic network such as parasitoids, more predator species and different food and reproductive resources for each

trophic level. For example, different population dynamic models such as the Lotka-Volterra equations for predator-prey systems (Yorke & Anderson, 1973) could be implemented within the trophic cascade if desired towards a more realistic model development.

Following the open source paradigm, students of higher levels (e.g. university level) or students specifically oriented to programming courses can be encouraged to statistically analyze the outputs of EcoPred in R software; for example, modeling the different responses as a function of time, or finding the optimal model among competing models for a species' population response using specific criteria such as goodness of fit ( $R^2$ ) or the Akaike information criterion (AIC) (Aho et al., 2014).

## 7.5. Conclusions

Simulation is a method increasingly used for both research and teaching. Therefore, manipulating a virtual ecosystem through a simple programming language can be a beneficial approach for students. In fact, programming and data analysis are increasingly demanded skills in a world more and more dominated by open source software. The survey suggested that students are interested in gaining programming skills, they recognized simulation-based approaches as a useful and enjoyable tool and they would like to continue learning in this context. EcoPred integrates both components at the same time, (1) ecological learning within a biological control framework and (2) programming learning, with the advantage that each user can expand the complexity of his model in an unlimited way using user-friendly code syntax on a free platform.

### 7.6. Data accessibility

EcoPred (application and code) is available from https://github.com/jbenma/EcoPred. The NetLogo software and user manual are available from https://ccl.northwestern.edu/netlogo/

# CHAPTER 8

General conclusions, conceptual overview and future perspectives.

### **CHAPTER 8**

### General conclusions, conceptual overview and future perspectives

The results obtained in this work were gathered in a conceptual scheme (Fig. 8.1) integrating the main considerations of the different aspects of the bioecology of spiders and their role as potential agents of biological control in the olive grove agroecosystem that can be summarized as:

1 - The olive grove and its surrounding semi-natural areas provided multiple habitats that supported a diverse community of spiders in terms of functional groups and species. In total, 10 functional groups of spiders (ambushers, foliage runner hunters, ground hunters, orb-web builders, sensing web builders, sheet web builders, space web builders, stalkers, wandering sheet/tangle weavers) encompassing, at least, 123 species were found to inhabit the studied olive groves located in Trás-os-Montes. Among these species, *Thanatus vulgaris* Simon, 1870 could be a potential indicator for the ground central area of the olive grove. An approach using functional counterparts of these species in other regions and agroecosystems may allow assessing their actual role as agrobionts and potential bioindicators.

2 - The number of stones on the soil of olive groves promoted the community of ground inhabiting spiders. Low-cost activities for the farmer such as building dry stone walls, and maintaining hedgerows can represent abundant ground refuges for spiders.

3 - Different functional groups of spiders actively searched and consumed the most beneficial non-prey food among those available. The black-scale honeydew was the best food for ground hunters whereas the highest survival reached by ambushers was observed when fed on a mixture of glucose 0.5 M and three amino acids. When different non-prey food items were offered together, the ground hunters explored them significantly more actively than ambushers. Ground runners chose to feed on honey whereas ambushers chose



**Figure 8.1.** Conceptual overview of the pool of significant relationships found along this work. Green arrows mean positive relationships and red arrows mean negative relationships. Size of circles is proportional to the number of relationships found. Abbreviations can be found at Table 8.1.

<b>Table 8.1.</b>	Abbreviations	corresponding	to each	"driver-dependent	variable"	pair	used	to
plot Figure	8.1.							

Alemative preyAPFunctional Response-Ground runnersFRGAlternative preyAPFunctional Response-Orb-weaversFROWAlternative preyAPFunctional Response-Arb-weaversFROAphild honeydewAHLongevity-AnthushersLABlack scale honeydewBSHLongevity-ArbushersLABlack scale honeydewBSHLongevity-ArbushersLGGlucoseGLongevity-ArbushersLGGlucoseGLongevity-ArbushersLGGlucoseGLongevity-ArbushersPGHeavy long-sized and highly mobile preyHLSHMPPrey preference-Orb-weaversPFOWHeavy long-sized and highly mobile preyHLSHMPPrey preference-ArbushersLGHoneyHLongevity-Ground namersLGLGHoneyHLongevity-Ground namersLGHoneyHSelection-Ground namersPFOLight small-iszed and moderately mobile preyLSSMMPPrey preference-ArbushersPPOWLight small-iszed and moderately mobile preyLSSMMPPrey preference-Ground namersLGMixMLongevity-Ground namersLGSAGround central area in the olive groveOGCAIndicator-Grynita paxallaZPMixMLongevity-ArbushersLASAGround central area in the olive groveOGCAIndicator-Cynita paxallaZPPollenPLongevity-ArbushersLAPrey densityPDCostuned prey biomass-Choun	Driver	Abbreviation	Dependent variable	Abbreviation
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the mixture of glucose 0.5 M and three amino acids. Different guilds of spiders most likely use alternative foods according to their lifestyle. However the way in which spiders take advantage of different alternative non-prey foods in the field is still unclear.

4 - The method developed to calculate the maximum attack rate and its 95% confidence interval of a predator through simulation can be easily used to assess and compare the success of potential natural enemies on target pests. Due to the low number of samples required to achieve a good estimation of the maximum attack rate, this is of special relevance in the case of arthropods difficult to rear in laboratory such as spiders.

5 - The three functional groups of spiders (ground hunters, orb-weavers and ambushers) studied during the functional response assays avoided the heavy, long-sized and highly mobile preys whereas the light, small-sized and moderately mobile preys (flies) were preferred. Although their prey preference was the same, the hunting behavior and feeding parameters clearly differed according to each spider guild. Since the olive fruit fly *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae) uses different strata during its life cycle, this fact promises a fruitful way to develop research towards the finding of species of spiders that could act as natural enemies at different vertical strata within the crop such as the canopy of trees, the herbaceous layer and the soil.

6 - The developed tool EcoPred proved to be successful in using individual-based models to teach the basics of biological control to students. Relevant ecology-related concepts such as generalist and specialist species and species' guilds were explained in a modern and enjoyable way within the context of biological control using EcoPred that represents a three-level trophic cascade within a simulated olive grove.

7 - Finally, the results obtained in this work strongly suggest different aspects related to the role of spiders as natural enemies in general, as well as in the olive grove agroecosystem in particular that are worth to be **further investigated** such as:

7.1 - A deeper characterization of the olive grove surrounding landscape and calculation of landscape metrics (e.g. perimeter and area of surrounding agricultural, semi-natural and built areas) measured at different buffers (e.g. 500 m and 1 km in radius). The use of these metrics can help to assess the effect of landscape on the composition of the spiders' assemblages within the olive groves.

7.2 - The inclusion of more soil-related variables (e.g. water holding capacity, soil density, row and between-row sampling), spatial effect (i.e. coordinates) and years of study in the models in order to refine the effect of drivers on the spiders' populations.

7.3 - The study of the phenology and migration patterns of spiders' populations between the olive groves and their surrounding patches towards the determination of species that could overlap in terms of life-cycle with the different olive pests.

7.4 - Measurement of the behavior and movement patterns of olive pests towards a realistic simulation of the functional responses of spiders on them using individual-based models (IBMs).

7.5 - Quantification *in situ* of the amount of honeydew produced by olive pests such as the black scale *Saissetia oleae* (Olivier, 1791) (Hemiptera: Coccidae) and assessment of the degree of utilization by spiders as alternative non-prey foods in the field.

7.6 - Laboratory experiments on the functional response of different species of spiders within different functional groups towards the investigation of characteristic patterns (traits) of each guild.

7.7 - Field experiments (e.g. mesocosms) of spider predation on non-olive-pest species towards the assessment of the effect of environmental constraints (e.g. intraguild competition) on their functional response.

7.8 - Study of the functional responses of spiders on *B. oleae* and the olive moth *Prays oleae* Bernard, 1788 (Lepidoptera: Yponomeutidae) in the laboratory.

The achievement of these goals may narrow the distance between the results obtained in laboratory and field experiments, a common issue faced by researchers in ecology, and help to disentangle further paths of work towards a broader knowledge on the role of spiders as natural enemies in the olive grove agroecosystem.

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APPENDIX 1 - Code of simaR\_v06

Code of simaR\_v06:

# Copyright (C) 2017 Jacinto Benhadi Marín

# This program is free software: you can redistribute it and/or modify

# it under the terms of the GNU General Public License as published by

# the Free Software Foundation, either version 3 of the License, or

# (at your option) any later version.

#

# This program is distributed in the hope that it will be useful,

# but WITHOUT ANY WARRANTY; without even the implied warranty of # MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. See the # GNU General Public License for more details at http://www.gnu.org/licenses/.

simData<-function(x,y,z) {</pre>

# x: an object (table) of two columns, the first one containing the number of prey offered

# in each treatment and the second one containing the mean proportion of prey consumed items for each corresponding initial prey density.

# y: number of samples per N to be simulated.

# z: number of curves to be simulated.

# Creating an intermediate data.frame to allocate columns of simulated data; 1 column = 1 initial prey density #

New\_Data<-as.data.frame(matrix(nrow=y,ncol=nrow(x))) colnames(New\_Data)<-x[,1] New\_Curves<-list()

```
for(j in 1:z){ #j counts each new curve#
```

```
#Simulating new data; i counts each new sample#
for (i in 1 : nrow(x)) {
    New_Data[,i]<-rbinom(y, size=x[i,1], prob=x[i,2])
}</pre>
```

```
# Reorganizing the new data in a list of data.frames #
```

```
New_Curves[[j]]<-
```

```
data.frame("Offered"=sort(as.integer(rep(colnames(New_Data),y))),"Consumed"=stack(Ne
```

```
w_Data)[,1])
```

```
}
return(New_Curves)
```

```
}
```

#### 

```
newTests<-function(x){
    # x: an object containing the output of simData()
    library(frair)
    New_Test<-list()
    for(i in 1:length(x)){
        New_Test[[i]]<-frair_test(Consumed~Offered, data=x[[i]])
    }
    return(New_Test)
}</pre>
```

# Types of response in "frair" used by SimaR\_v05

# Response Replacement? Parameters Description
# ------

# hollingsI	I Yes	X,a,h,T	Holling's original type II function
# rogersII	No	X,a,h,T	Roger's type II decreasing prey function
# hassIII	Yes	X,b,c,h,T	Hassell's original type III function
# hassIIInr	No	X,b,c,h,T	Hassell's type III function, not assuming replacement
# emdII	No	X,a,h,P,T	Ecological Models and Data in R type II function
# flexp	Yes	X,b,q,h,T	Flexible exponent model, assuming replacement
# flexpnr	No	X,b,q,h,T	Flexible exponent model, not assuming replacement

# Types of response in "frair" used by SimaR\_v06 -> new version!!! flexp now is real77 but both are implemented in SimaR\_v06

#Response Replacement? Parameters Description

#			
# hollingsII	Yes	X,a,h,T	Holling's original type II function
# rogersII	No	X,a,h,T	Roger's type II decreasing prey function
# hassIII	Yes	X,b,c,h,T	Hassell's original type III function
# hassIIInr	No	X,b,c,h,T	Hassell's type III function, not assuming replacement
# emdII	No	X,a,h,P,T	Ecological Models and Data in R type II function
# real77	Yes	X,b,q,h,T	Flexible exponent model, assuming replacement
# real77r	No	X,b,q,h,T	Flexible exponent model, not assuming replacement

#### 

getFitData<-function(x,y,z,Ne,NPred){

- # x: an object containing the output of simData()
- # y: the time of the experiment (T)
- # z: type of functional response ( see frair\_responses() )
- # Ne: mean of the number of prey consumed at the highest initial prey density
- # NPred: Number of predators for response type "emdII"

```
library(frair)
 options(warn=-1)
 New_Params<-list()
 if(z=="hollingsII") {
  for(i in 1:length(x)){
   New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                                data=x[[i]],
response="hollingsII",start=list(a=0.001,h=T/Ne), fixed=list(T=y))
  }
 }
 if(z=="rogersII"){
  for(i in 1:length(x)){
   New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                                data=x[[i]],
response="rogersII",start=list(a=0.001,h=T/Ne), fixed=list(T=y))
  }
 }
 if(z=="hassIII") {
  for(i in 1:length(x)){
   New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                                data=x[[i]],
response="hassIII",start=list(b=0.001,c=0.001,h = T/Ne), fixed=list(T=y))
  }
 }
 if(z=="hassIIInr") {
  for(i in 1:length(x)){
   New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                                data=x[[i]],
response="hassIIInr",start=list(b=0.001,c=0.001,h = T/Ne), fixed=list(T=y))
  }
 }
```

```
if(z=="emdII") {
  for(i in 1:length(x)){
   New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                               data=x[[i]],
response="emdII",start=list(a=0.001,h = T/Ne), fixed=list(T=y,P=NPred))
  }
 }
 if(z=="flexp") {
  for(i in 1:length(x)){
   New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                               data=x[[i]],
response="flexp",start=list(b=0.001,q=0,h = T/Ne), fixed=list(T=y))
  }
 }
 if(z=="flexpnr") {
  for(i in 1:length(x)){
   New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                               data=x[[i]],
response="flexpnr",start=list(b=0.001,q=0,h = T/Ne), fixed=list(T=y))
  }
 }
 if(z=="real77") {
  for(i in 1:length(x)){
   New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                               data=x[[i]],
response="real77",start=list(b=0.001,q=0,h = T/Ne), fixed=list(T=y))
  }
 }
 if(z=="real77r") {
  for(i in 1:length(x)){
```

```
New_Params[[i]]<-frair_fit(Consumed~Offered,
                                                                       data=x[[i]],
response="real77r", start=list(b=0.001,q=0,h = T/Ne), fixed=list(T=y))
  }
 }
return(New_Params)
}
plotCurves<-function(x,y,z){</pre>
# x: an object containing the output of getFitData()
# y: upper limit for the X-axis
 # z: upper limit for the Y-axis
for(i in 1:length(x)){
  plot(x[[i]],lty=i,pch="",xlim=c(0,y),ylim=c(0,z),ylab=c(""),xlab=c(""),axes=F)
  lines(x[[i]],lty=i)
  par(new=T)
 }
plot(x[[1]],xlim=c(0,y),ylim=c(0,z),ylab=c("Number of prey killed (N)"),xlab=c("Initial
prey density (N)"),col=0,pch="")
```

```
}
```

#new data.frame for each simulated attack rate#
simulated\_attack\_rates<-as.data.frame(matrix(nrow=length(x),ncol=1))
simulated\_attack\_rates<-data.frame(Attack\_rate=simulated\_attack\_rates[,1])</pre>

#new data.frame for allocate the calculated maximum attack rate#
Max\_attackRates<-as.data.frame(matrix(nrow=length(x),ncol=1))
Max\_attackRates<-data.frame(Max\_attack\_rate=Max\_attackRates[,1])</pre>

```
if(z=="hollingsII"){
```

```
for(i in 1:length(x)){
```

```
simulated_attack_rates[i,]<-round(x[[i]][["fit"]]@details$par[2],digits=4) #Extracting the attack rate from the frair_fit output#
```

}

```
}
```

#Calculating the maximum attack rate for each curve (T/Th)#

```
Max_attackRates<- round(y/simulated_attack_rates,digits=4) #Calculating the maximum attack rate for each curve (T/Th)#
```

```
if(z=="rogersII"){
```

```
for(i in 1:length(x)){
```

```
simulated_attack_rates[i,]<-round(x[[i]][["fit"]]@details$par[2],digits=4) #Extracting the attack rate from the frair_fit output#
```

}

}

#Calculating the maximum attack rate for each curve (T/Th)#

```
Max_attackRates<- round(y/simulated_attack_rates,digits=4) #Calculating the maximum attack rate for each curve (T/Th)#
```

```
if(z=="emdII"){
```

```
for(i in 1:length(x)){
```

```
simulated_attack_rates[i,]<-round(x[[i]][["fit"]]@details$par[2],digits=4) #Extracting
the attack rate from the frair_fit output#</pre>
```

```
}
```

}

#Calculating the maximum attack rate for each curve (T/Th)# Max\_attackRates<- round(y/simulated\_attack\_rates,digits=4) #Calculating the maximum attack rate for each curve (T/Th)#

```
if(z=="hassIII"){
```

```
for(i in 1:length(x)){
```

```
simulated_attack_rates[i,]<-round(x[[i]][["fit"]]@details$par[3],digits=4) #Extracting the attack rate from the frair_fit output#
```

```
}
```

```
}
```

#Calculating the maximum attack rate for each curve (T/Th)#

Max\_attackRates<- round(y/simulated\_attack\_rates,digits=4) #Calculating the maximum attack rate for each curve (T/Th)#

```
if(z=="hassIIInr"){
```

```
for(i in 1:length(x)){
```

simulated\_attack\_rates[i,]<-round(x[[i]][["fit"]]@details\$par[3],digits=4) #Extracting
the attack rate from the frair\_fit output#</pre>

```
}
```

#Calculating the maximum attack rate for each curve (T/Th)#

Max\_attackRates<- round(y/simulated\_attack\_rates,digits=4) #Calculating the maximum attack rate for each curve (T/Th)#

}

if(z=="real77"){

```
for(i in 1:length(x)){
```

simulated\_attack\_rates[i,]<-round(x[[i]][["fit"]]@details\$par[3],digits=4) #Extracting the attack rate from the frair\_fit output#

}

#Calculating the maximum attack rate for each curve (T/Th)#

Max\_attackRates<- round(y/simulated\_attack\_rates,digits=4) #Calculating the maximum attack rate for each curve (T/Th)#

}

```
if(z=="real77r"){
```

for(i in 1:length(x)){

simulated\_attack\_rates[i,]<-round(x[[i]][["fit"]]@details\$par[3],digits=4) #Extracting the attack rate from the frair\_fit output#

}

#Calculating the maximum attack rate for each curve (T/Th)#

Max\_attackRates<- round(y/simulated\_attack\_rates,digits=4) #Calculating the maximum attack rate for each curve (T/Th)#

}

#Replacing infinites by NAs, supressing them and allocating the results on the its own data.frame#

Max\_attackRates<-

```
as.vector(na.omit(replace(Max_attackRates[,1],is.infinite(Max_attackRates[,1]),NA))) return(Max_attackRates)
```

}

# x: a vector with the means that will be used by MARbootstrapping()

# y: a second argument required by the boot function from boot package.

### Remember, the second argument of the function "boot" needs two parameters being the second one a vector of indices.

```
mean1 = mean(x[y])
```

}

MARbootstrapping<-function(x,y,z,w){

# x: an object containing the output of Max\_attackRates()

# y: the number of bootstrap replicates.

# z: the confidence level of the required interval.

# w: A vector of character strings representing the type of intervals asked by the function boot.ci from boot package: "norm", "basic", "stud", "perc", "bca".

library(plotrix) library(boot)

boot\_res<-as.vector(matrix(nrow=length(x),ncol=1))
#bootstrapping the mean of the maximum attack rate#
boot\_res<-boot(x, own\_mean, R = y)</pre>

#extracting the lower and upper limit of the confidence interval#
return(data.frame(li=as.vector(boot.ci(boot\_res,conf=z,
type=w)[[4]])[2],ui=as.vector(boot.ci(boot\_res, conf=0.95, type="norm")[[4]])[3]))
}

APPENDIX 2 - Code of EcoPred\_v06
#### Code of EcoPred\_v06:

; Firstly all the agents are created...

breed [olives olive] ; for each agents type, both the group (plural) and individuals (singular) must be created breed [flies fly] breed [web-females spider] breed [ground-spiders spider2] breed [flowers flower] breed [stones stone] breed [web-males webmale]

; ...and attributes for agents are assigned flies-own [energy] web-females-own [energy] web-males-own [energy] ground-spiders-own [energy]

; The "Clear" block carries the code controlled by the button "Clear" in the Interface to Clear clear-all ; cleans the previous results and restart the situation

ask patches [ set pcolor 62 ] ; setting color to the ground

set-default-shape flowers "flower" ; setting shape to agents "flower" create-flowers initial-number-flowers

[ set color yellow ; setting color... set size 3.5 ; ...and size setxy random-xcor random-ycor ; flowers are randomly positioned along the scenario

```
]
```

```
set-default-shape olives "olive" ; setting shape to agents "olive"
create-olives initial-number-olives
[
set color violet ; setting color...
set size 1.5 ; ...and size
setxy random-xcor random-ycor ; olives are randomly positioned along the scenario
]
set-default-shape flies "fly" ; setting shape to agents "fly"
create-flies initial-number-flies
[
set color white ; setting color...
set size 2 ; ...and size
set energy 50 ; setting attribute energy for flies
```

setxy random-xcor random-ycor ; flies are randomly positioned along the scenario ]

set-default-shape web-females "web-female"; setting shape to agents "web-female" create-web-females initial-number-web-females

[

```
set color black ; setting color...
set size 3 ; ...and size
set energy 50 ; ...and energy
setxy random-xcor random-ycor ; spiders are randomly positioned along the scenario
]
```

set-default-shape web-males "web-male"; setting shape to agents "web-male" create-web-males initial-number-web-males

[

```
set color black ; setting color...
set size 2 ; ...and size
set energy 50 ; ...and energy
setxy random-xcor random-ycor ; spiders are randomly positioned along the scenario
]
```

set-default-shape ground-spiders "ground-spider"; setting shape to agents "ground-spider"

create-ground-spiders initial-number-ground-spiders

```
[
```

set color brown ; setting color ...

set size 3; ...and size

```
set energy 50 ; ...and energy
```

setxy random-xcor random-ycor ; spiders are randomly positioned along the scenario

]

set-default-shape stones "stone"; setting shape to agents "stone"

```
create-stones initial-number-stones
```

```
set color brown ; setting color...
```

```
set size 4 ; ...and size
```

setxy random-xcor random-ycor ; stones are randomly positioned along the scenario

]

[

```
reset-ticks ; resetting ticks to zero end
```

; The "Go" block carries the code controlled by the button "Go" in the Interface to Go

if not any? flies [stop] ; if there are no flies within the crop, the system stops

if count flies > 2000 [user-message "Your crop have been invaded!" stop] ; if more than "x" flies are counted within the crop, the system will stop and show the message if count web-females > 1000 [stop] if count web-males > 1000 [stop] if count ground-spiders > 1000 [stop]

; Simulating some immigration of spiders with the same attributes as within the initial conditions

if (initial-number-web-females > 0) or (initial-number-web-males > 0) or (initial-numberground-spiders > 0); Condition to avoid the entrance of spiders when the fly population is simulated alone

```
[create-web-males 1 [
  set color black
  set size 2
  set energy 50
  setxy random-xcor random-ycor
]
create-ground-spiders 1 [set color brown
  set size 3
```

```
set energy 50
setxy random-xcor random-ycor
]]
```

; What the different turtles will do?...

```
ask flies [move fed reproduce death set energy energy - 1]; setting behaviours for flies
ask web-females [fed-a reproduce-a death set energy energy - 1]; setting behaviours for
spiders
```

ask web-males [move fed-b reproduce-b death set energy energy - 1 ]; setting behaviours for spiders

ask ground-spiders [move-b fed-c reproduce-c death set energy energy - 1 ] ; setting behaviours for spiders

tick ; advancing one step in time end

; ... and How it will be done?

to move

right random 20 left random 20 forward 1 ; flies and web-males will move around the crop end

to move-b

right random 50 left random 50 forward 1 ; ground-spiders will move around the crop end

## to fed

if any? flowers-on patch-ahead 1 [set energy energy + (count flowers-on patch-ahead 1 \* 30)]; flies will feed if a flower is found and its energy will increase in "x" units end

to fed-a

if any? flies-on patch-ahead 1 [set energy energy + (count flies-on patch-ahead 1 \* 5)] ask flies-on patch-ahead 1 [die] end

## to fed-b

if any? flies-on patch-ahead 1 [set energy energy + (count flies-on patch-ahead 1 \* 5)] ask flies-on patch-ahead 1 [die] end

to fed-c

if any? flies-on patch-ahead 1 [set energy energy + (count flies-on patch-ahead 1 \* 10)] ask flies-on patch-ahead 1 [die]

if any? web-males-on patch-here [set energy energy + (count web-males-on patch-here \* 10)]

ask web-males-on patch-here [die] end

#### to reproduce

if any? olives-on patch-here [set energy (energy / 4) hatch-flies 3 [setxy random-xcor random-ycor]]; flies will reproduce if an olive is found hatching 3 more fly (that will move the same way than its parents) end

to reproduce-a

if any? web-males-on patch-here [set energy (energy / 2) hatch-web-females 1 [setxy random-xcor random-ycor]] end

```
to reproduce-b
```

if any? web-females-on patch-here [set energy (energy / 2) hatch-web-males 1 [setxy random-xcor random-ycor]]

end

### to reproduce-c

if any? stones-on patch-here [set energy (energy / 2) hatch-ground-spiders 1 [setxy random-xcor random-ycor]] end

to death if energy < 1 [die] ; turtles will die if energy decreases to zero end **APPENDIX 3 - Publications, communications and events derived from or related to this thesis** 

#### Publications, communications and events derived from or related to this thesis

## Articles in indexed journals:

- Benhadi-Marín, J., Pereira, J.A., Barreales, D., Sousa, J.P. & Santos, S.A.P., 2018. A simulation-based method to compare the pest suppression potential of predators: A case study with spiders. <u>Biological Control</u>, 123: 87-96.
- Benhadi-Marín, J., 2018. A conceptual framework to deal with outliers in ecology. <u>Biodiversity and Conservation</u>, 27(12): 3295-3300.
- Benhadi-Marín, J., Pereira, J.A., Barrientos, J.A., Sousa, J.P. & Santos, S.A.P., 2018. Stones on the ground in olive groves promote the presence of spiders (Araneae). <u>European Journal of Entomology</u>, 115: 372-379.
- Benhadi-Marín, J., Pereira, J.A., Sousa, J.P. & Santos, S.A.P., 2019. Spiders actively choose and feed on nutritious non-prey food resources. <u>Biological Control</u>, 129: 187-194.
- Benhadi-Marín, J., Pereira, J.A., Sousa, J.P. & Santos, S.A.P., 2019. EcoPred: an educational Individual Based Model to explain biological control, a case study within an arable land. <u>Journal of Biological Education</u>. https://doi.org/10.1080/00219266.2019.1569086.

#### **Book chapters:**

 Benhadi-Marín, J., Pereira, J.A., Bento, A., Sousa, J.P. & Santos, S.A.P., 2016. Chapter 3. Biodiversity of Spiders in Agroecosystems: Community Structure, Conservation and Roles as Biological Control Agents. pp 43-110. In: <u>Natural</u>

Enemies: Identification, Protection Strategies and Ecological Impacts. S.A.P. Santos (Ed.). Nova Science Publishers.ISBN: 978-1-63485-921-9.

### Oral communications in scientific meetings:

- Benhadi-Marín, J., Barrientos, J.A., Sousa, J.P., Pereira, J.A. & Santos, S.A.P., 2015. Patrones de diversidad de arañas a lo largo de un gradiente de prácticas agrícolas: Enlazando composición de especies, factores ambientales y rasgos funcionales. <u>XV Jornadas do Grupo Ibérico de Aracnología</u>, Rodalquilar – Níjar, Spain. 21-23 March 2015.
- Benhadi-Marín, J., Sousa, J.P., Pereira, J.A. & Santos, S.A.P., 2016. Potencial de tres gremios de arañas como agentes de control biológico en el olivar: eficacia en la captura de presas y respuestas funcionales. <u>XVI Jornadas del Grupo Ibérico de</u> <u>Aracnología</u>. Bragança, Portugal. 2-4 September 2016.
- Benhadi-Marín, J., Pereira, J.A. Barreales, D., Sousa, J.P. & Santos, S.A.P., 2017. La interacción entre presas afecta a la respuesta funcional de las arañas y a su preferencia por los distintos tipos de presa. <u>XVII Jornadas del Grupo Ibérico de</u> <u>Aracnología</u>. Plasencia, Spain. 12-15 October 2017.
- Benhadi-Marín, J., Pereira, J.A., Villa, M., Sousa, J.P. & Santos, S.A.P., 2017. La glucofagia favorece la supervivencia y afecta al comportamiento durante la selección de alimentos "no presa" en dos grupos funcionales de arañas. <u>XVII</u> Jornadas del Grupo Ibérico de Aracnología. Plasencia, Spain. 12-15 October 2017.
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- Benhadi-Marín, J., Pereira, J.A., Sousa, J.P. & Santos, S.A.P., 2018. Una aproximación al análisis de la respuesta funcional a través de modelos basados en individuos (IBMs). <u>XVIII Jornadas del Grupo Ibérico de Aracnología</u>. Barcelona. Spain. 5-7 October 2018.

#### Poster presentations in scientific meetings:

- Benhadi-Marín, J., Pereira, J.A., Gonçalves, C., Barrientos, J.A., Patanita, M.I. & Santos, S.A.P., 2013. Relaciones araña-hormiga en el olivar de Trás-os-Montes (Portugal): Mirmecofilia, mirmecomorfia y mirmecofagia. <u>XIV Jornadas del Grupo</u> <u>Ibérico de Aracnología</u>. San Xoan de Seoane do Courel, Lugo (Spain). 12-15 September 2013.
- Benhadi-Marín, J., Pereira, J.A., Sousa, J.P. & Santos, S.A.P., 2015. Effect of different non-prey foods on the longevity of the cursorial spider *Haplodrassus severus* (C. L. Koch, 1839) (Araneae: Gnaphosidae). <u>IV Encontro Iberico de</u> <u>Ecología</u>. Coimbra, Portugal. 16-19 June 2015.
- Benhadi-Marín, J., Pereira, J.A., Barrientos, J.A., Sousa, J.P. & Santos S.A.P., 2016. La presencia de piedras en el suelo afecta a la estructura de la comunidad de arañas del olivar. <u>XVI Jornadas do Grupo Ibérico de Aracnología</u>. Bragança, Portugal. 2-4 September 2016.
- 4. Benhadi-Marín J., Villa, M., Baptista, P. & Pereira, J.A., 2017. Predatory potential of two functional groups of spiders on *Philaenus spumarius* (Linnaeus, 1758), a vector of *Xylella fastidiosa* Wells et al., 1987. <u>European conference on *Xylella*</u>

*fastidiosa*: finding answers to a global problem. Palma de Mallorca, Spain. 13-15 November 2017.

- Mahzoum, M.A., Villa, M., Benhadi-Marín, J. & Pereira, J.A., 2018. A cochonilha negra, *Saissetia oleae*, como alimento para larvas e adultos de *Chrysoperla carnea* s.l. 8º Simpósio Nacional de Olivicultura. Santarém, (Portugal). 7-9 June 2018.
- Benhadi-Marín, J., Pereira, J.A., Sousa, J.P. & Santos, S.A.P., 2018. Spatial variability of the spider community in the olive grove agroecosystem in Trás-os-Montes (Portugal). <u>I Iberian Meeting on Agroecological Research</u>. Évora, Portugal. 22-23 November 2018.

#### Software development:

- Benhadi-Marín, J., 2016. EcoPred: an educational Individual Based Model to demonstrate biological control within arable lands. DOI: 10.5281/zenodo.1030140 https://github.com/jbenma/EcoPred/
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### **Organization of scientific meetings:**

- 1. Organizer of the XVI Conference of the Iberian Group of Arachnology at the Polytechnic. http://sea-entomologia.org/gia/jornadas\_gia\_xvi.html
- Organizer of the IX Iberian Congress of Agroengineering at the Polytechnic Institute of Bragança. Bragança. Portugal Bragança, Portugal. 4-6 September 2016.

 Organizer of the XVII Conference of the Iberian Group of Arachnology. Center for Ecological and Mountain Agriculture (CAEM). University of Extremadura. Plasencia (Cáceres), Spain. 12-15 October 2017.

## Scientific divulgation events:

 Benhadi-Marín, J., 2017. Ensalada de arañas. <u>Pint of Science</u>. 15-17 Mayo de 2017, León, España. *Oral communication by invitation*. https://pintofscience.es/event/artropodos