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MOVEMENT OF PREDATORS IN ARABLE CROP SYSTEMS

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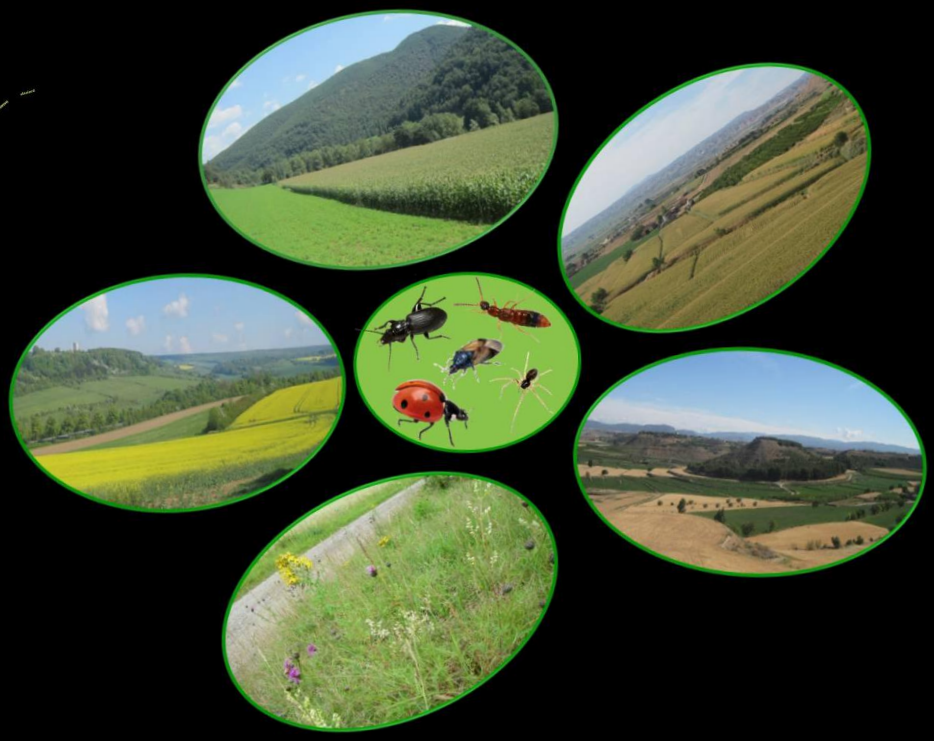

UNIVERSITAT DE LLEIDA

Departament de Producció Vegetal i Ciència Forestal

MOVEMENT OF PREDATORS IN ARABLE CROP SYSTEMS

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"...the task is not so much to see what no one has yet seen, but to think what nobody yet has thought about that which everybody sees."

Arthur Schopenhauer, 1788-1860

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Summary

The arable crop landscape in many regions is formed by a combination of annual and perennial crops. Arthropod predators are important for natural pest control in arable crops. A better understanding of the dynamics of predator movement and of the ecological role each crop plays for these predators will help to improve crop management strategies, which can enhance biological control. In this context, this study aimed to examine the movement of some of the most abundant predators that inhabit arable crop landscapes formed by maize and alfalfa (*Orius majusculus, Coccinella septempunctata* and carabids) and by winter cereals, meadows and semi-natural habitats (carabids, staphylinids and spiders).

Rubidium as a marker proved to be an effective method for tracking the movement of *O. majusculus* within maize and the movement of carabids between alfalfa and maize. Carbon and nitrogen stable isotopes also were an effective method for tracking the movement of *O. majusculus* and *C. septempunctata* between alfalfa and maize. Carbon stable isotope analysis was able to detect a diet shift of *O. majusculus* from C3 to C4 crops in less than five days, and vice versa. Traces of the old diet were still detectable more than twenty days after a diet shift.

The abundance and dispersal activity of *O. majusculus* were not different in maize plots with moderately high or low weed density, suggesting that maize weeds do not supply enough alternative resource for affecting this predator's abundance and movement. The carabids *C. fuscipes*, *P. rufipes*, *P. cupreus* and *Metallina* sp. and the anthocorid *O. majusculus* presented bidirectional movements between adjacent alfalfa and maize crops during the season. In contrast, *C. septempunctata* only moved from alfalfa to maize. The patterns of movement differed between species since the roles that the two crops played for these predators were different during the season. The plant-dwelling *O. majusculus* and *C. septempunctata* that colonized maize at early vegetative stages came from alfalfa, indicating that alfalfa acted as a source of predators towards maize. However, in the reproductive growth stage period, maize acted as a source for *C. fuscipes, P. rufipes, P. cupreus, Metallina* sp. and *O. majusculus* moving to alfalfa, mainly after cuttings. After an alfalfa cutting, margins also acted as a refuge for carabids. Spillover of carabids, staphylinids and spiders was stronger from winter

cereals to semi-natural habitats than from meadows to semi-natural habitats, indicating that neighbourhood identity shapes spillover effects to adjacent semi-natural habitats. Therefore, meadows can act as buffers around protected reserves so that spillover from arable crops does not compromise the structure and functioning of endangered communities.

The present study substantially widens the knowledge about the movement of predators between adjacent arable crops and natural habitats and clarifies the ecological role that each crop/habitat plays for predators, thereby allowing to improve habitat and landscape management at the farm scale by enhancing predators' biological control functions.

Resum

En moltes regions, el paisatge de cultius extensius està format per cultius anuals i perennes. Els depredadors són importants per el control biològic de plagues en aquests cultius. Una millor comprensió de la dinàmica i moviment dels depredadors així com de la funció ecològica que cada cultiu juga per aquests depredadors ajudarà a millorar les estratègies de maneig dels cultius que poden fomentar el control biològic. En aquest context, aquest estudi va tenir com objectiu examinar el moviment d'alguns dels depredadors més abundants presents en paisatges formats per panís i alfals (*Orius majusculus, Coccinella septempunctata* i caràbids.) i per cereals d'hivern, prats i hàbitats semi naturals (caràbids, estafilínids i aranyes).

El rubidi com marcador d'insectes va demostrar ser un mètode eficaç per al seguiment del moviment d'*O. majusculus* en el panís i també del moviment de caràbids entre camps d`alfals i panís. Les anàlisis de isòtops estables de carboni i nitrogen també van mostrar ser un mètode eficaç per al seguiment del moviment d'*O. majusculus* i de *C. septempunctata* entre camps d'alfals i panís. L'anàlisi d'isòtops estables de carboni serveix per detectar el canvi de dieta d'*O. majusculus* de cultius C3 a C4, i viceversa, en menys de cinc dies però també per saber que es detecten traces de la dieta antiga més de vint dies després del canvi de dieta.

L'abundància i dispersió d'*O. majusculus* no va ser diferent entre parcel-les de panís amb moderadament alta o baixa densitat de males herbes, suggerint que les males herbes del panís no subministren prou recursos alternatius per afectar l'abundància i el moviment d'aquest depredador. Els caràbids, *C. fuscipes*, *P. rufipes*, *P. cupreus* i *Metallina* sp. i l'antocòrid *O. majusculus* presenten moviment bidireccional entre cultius adjacents de alfals i panís durant l'estació. En contrast, *C. septempunctata* només es va moure des de l'alfals cap al panís. Els patrons de moviment difereixen entre espècies ja que el paper que exerceixen els dos cultius per aquests depredadors és diferent durant l'estació. Els individus d'*O. majusculus* i *C. septempunctata* que van colonitzar el panís a l'estadi vegetatiu provenien de l'alfals, indicant que l'alfals va ser la font d'aquests depredadors. No obstant, en el període reproductiu, el panís va actuar com una font de *C. fuscipes*, *P. rufipes*, *P. cupreus*, *Metallina* sp. i *O. majusculus* cap a l'alfals, principalment després de dall. També després del o dall d'alfals, els marges van

actuar com refugi per als caràbids. El moviment de caràbids, estafilínids i aranyes va ser més intens des dels cereals d'hivern cap als hàbitats semi naturals que des dels prats cap als hàbitats semi naturals, indicant que l'hàbitat adjacent modela el moviment dels depredadors cap als hàbitats semi naturals. Per tant, els prats poden actuar com amortidors del moviment de depredadors des dels cultius extensius al voltant de les reserves protegides perquè no posin en perill l'estructura i funcionament de les comunitats en amenaça d'extinció.

Aquest estudi amplia considerablement el coneixement sobre el moviment dels depredadors entre cultius extensius adjacents i els hàbitats naturals i aclareix el paper ecològic que cada cultiu / hàbitat juga per als depredadors, permetent millorar el maneig de l'hàbitat i del paisatge a nivell d'explotació agrícola així com millorar les funcions de control biològic dels depredadors.

Resumen

En muchas regiones, el paisaje de cultivos extensivos es formado por cultivos anuales y perennes. Los depredadores son importantes para el control biológico de plagas en los cultivos extensivos. Una mejor comprensión de la dinámica y movimiento de los depredadores así como la función ecológica que cada cultivo juega para estos depredadores ayudará a mejorar las estrategias de manejo de los cultivos que pueden fomentar el control biológico. En este contexto, este estudio tuvo como objetivo examinar el movimiento de algunos de los depredadores más abundantes presentes en paisajes formados por maíz y la alfalfa (*Orius majusculus, Coccinella septempunctata* y carábidos) y por cereales de invierno, prados y hábitats semi-naturales (carábidos, estafilinidos y arañas).

El rubidio como marcador de insectos demostró ser un método eficaz para el seguimiento del movimiento de *O. majusculus* en el maíz así como del movimiento de carábidos entre campos de alfalfa y maíz. Los análisis de isotopos estables de carbono y nitrógeno también mostraron ser un método eficaz para el seguimiento del movimiento de *O. majusculus* y *C. septempunctata* entre la alfalfa y el maíz. El análisis de isótopos estables de carbono sirvó para detectar el cambio de dieta de *O. majusculus* de cultivos C3 a C4, y viceversa en menos de cinco días pero también para saber que se detectan trazas de la dieta antigua más de veinte días después del cambio de dieta.

La abundancia y dispersión de *O. majusculus* no fué diferente entre parcelas de maíz con moderada alta o baja densidad de las malas hierbas, sugiriendo que las malas hierbas del maíz no suministran suficientes recursos alternativos para afectar la abundancia y el movimiento de este depredador. Los carábidos, *C. fuscipes*, *P. rufipes*, *P. cupreus* y *Metallina* sp. y el anthocorido *O. majusculus* presentan movimiento bidireccional entre cultivos adyacentes de alfalfa y maíz durante la estación. En contraste, *C. septempunctata* sólo se movió desde la alfalfa hacia al maíz. Los patrones de movimiento difieren entre especies ya que el papel que desempeñan los dos cultivos para estos depredadores es diferente durante la estación. Los individuos de *O. majusculus* y *C. septempunctata* que colonizaron el maíz en el estadio vegetativo provinieron de la alfalfa, indicando que la alfalfa actúo como fuente de estos depredadores. Sin embargo, en el período reproductivo, el maíz actuó como una fuente

de *C. fuscipes*, *P. rufipes*, *P. cupreus*, *Metallina* sp. y *O. majusculus* hacia la alfalfa, principalmente después de corte. También después del corte de la alfalfa, los márgenes actuaron como refugio para los carábidos. El movimiento de carábidos, estafilínidos y arañas fué más intenso desde los cereales de invierno hacia los hábitats semi-naturales que desde los prados hacia a los hábitats semi-naturales, indicando que el hábitat adyacente moldea el movimiento de los depredadores hacia los hábitats semi-naturales adyacentes. Por lo tanto, los prados pueden actuar como amortiguadores del movimiento de depredadores desde de los cultivos extensivos alrededor de las reservas protegidas para que no pongan en peligro la estructura y funcionamiento de las comunidades en peligro de extinción.

El presente estudio amplía considerablemente el conocimiento acerca del movimiento de los depredadores entre cultivos extensivos adyacentes y los hábitats naturales y aclara el papel ecológico que cada cultivo / hábitat juega para los depredadores, permitiendo mejorar el manejo del hábitat y del paisaje a nivel de explotación agricola así como mejorar las funciones de control biológico de los depredadores.

Resumo

Em muitas regiões, a paisagem de culturas arvenses é formada por culturas anuais e perenes. Os artrópodes predadores são importantes para o controle biológico de pragas nas culturas arvenses. Uma melhor compreensão da dinâmica do movimento dos predadors e do papel ecológico que cada cultura tem para estes predadores vai ajudar a melhorar as estratégias de manejo das culturas, o que pode melhorar o controle biológico. Neste contexto, este estudo teve como objetivo analisar o movimento de alguns dos predadores mais abundantes que habitam em paisagens de culturas arvenses formadas por milho e alfafa (*Orius majusculus*, *Coccinella septempunctata* e carabídeos) e por cereais de inverno, prados e habitats semi-naturais (carabídeos, estafilínideos e aranhas).

O rubídio como marcador provou ser um método eficaz para rastrear o movimento de *O. majusculus* no milho assim como o movimento de carabídeos entre alfafa e milho. Os isótopos estáveis de carbono e azoto também mostraram ser eficazes para o seguimento do movimento de *O. majusculus* e *C. septempunctata* entre a alfafa e o milho. O isótopo estável de carbono foi capaz de detectar uma mudança de dieta de *O. majusculus* entre culturas C3 e C4, e vice-versa, em menos de cinco dias, mas, vestígios da antiga dieta foram ainda detectáveis passado vinte dias depois da mudança de dieta.

A abundância e dispersão de *O. majusculus* não foram diferentes em parcelas de milho com média-alta ou baixa densidade de infestantes, o que sugere que as infestantes do milho não fornecem suficiente recurso alternativo para afetar a abundância e o movimento deste predador. Os carabídeos, *C. fuscipes*, *P. rufipes*, *P. cupreus* e *Metallina* sp. e o antocorídeo *O. majusculus* apresentaram movimento bidirecional entre culturas adjacentes de alfafa e milho durante a estação. Em contraste, *C. septempunctata* apenas se moveu desde alfalfa para o milho. O padrão de movimento difere entre espécies, visto que o papel que as duas culturas exercem para estes predadores foi diferente durante a estação. Os indivíduos de *O. majusculus* e *C. septempunctata* que colonizaram o milho no estádio vegetativo vieram da alfafa, indicando que alfafa atuou como fonte de predadores para o milho. No entanto, durante o período reprodutivo, o milho atuou como fonte de *C. fuscipes*, *P. rufipes*, *P. cupreus*, *Metallina* sp. e *O. majusculus* para alfafa, principalmente após o corte da alfalfa. Depois de um corte, as margens também atuaram como um refúgio para carabídeos. O movimento de

carabídeos, estafilínideos e aranhas foi mais intenso desde cereais de inverno para os habitats semi-naturais do que desde prados para os habitats semi-naturais, indicando que o habitat adjacente molda o movimento dos predadores para os habitats semi-naturais. Portanto, os prados podem amortecer o movimento de predadores desde culturas arvenses para reservas protegidas de modo a não comprometer a estrutura e funcionamento das comunidades ameaçadas.

O presente estudo amplia substancialmente o conhecimento sobre o movimento dos predadores entre culturas arvenses adjacentes e habitats naturais e clarifica o papel ecológico que cada cultura / habitat desempenha para os predadores, permitindo melhorar a gestão de habitat e paisagem assim como melhorar as funções do controle biológico de predadores.

GENERAL INTRODUCTION

General introduction

In the last decades, agriculture has been intensified at local and regional scales worldwide, increasing the proportion of monocultures (mainly arable crops), field sizes, and the degree of fragmentation of natural and semi-natural habitats, causing changes in the agricultural landscape (Tscharntke et al. 2005a; Baessler and Klotz 2006). Simplification of land use and destruction of natural areas are considered to be an important cause of decline in farmland biodiversity and may affect ecosystem services such as biological control since non-crop habitats can provide refuge and alternative food resources for a broad spectrum of natural enemies (Andow 1991; Bianchi et al. 2006; Tscharntke et al. 2007; Kleijn et al. 2009). These changes can also cause a decline in the exchange of natural enemies between crop and non-crop habitats in landscapes dominated by arable crops (Bianchi et al. 2006). This is especially true for annual crops, because the success of natural pest control relies on the yearly establishment of natural enemies that immigrate from more stable habitats (Landis and Marino 1999; Denys and Tscharntke 2002). Conservation biological control involves habitat manipulation to improve natural enemy fitness and this can occur at the within-crop, within-farm or landscape levels (Landis et al. 2000).

Many agricultural landscapes are formed by a mosaic of annual and perennial crops with some margins and remaining natural areas. Traditionally, annual crops are described as "sink" habitats because they are subject to frequent and severe disturbances and need to be colonised by arthropods every year. By contrast, perennial crops and natural areas are described as "source" habitats because they are systems with relatively little disturbance and potentially more amenable for arthropod population stability (Wissinger 1997). However, some perennial crops such as alfalfa and meadows suffer periodical cuts during the growing season, which cause a disruption to the resident arthropods, forcing them to move to field margins or to alternatives habitats. Moreover, these perennial crops need to be recolonized by natural enemies after regrowth. In this way, in arable landscapes formed by annual and perennial crops the ecological role that annual crops play during season may be being underestimated because they can act as alternative habitat for natural enemies after cuts but also act as a "source" for the perennial crop after its regrowth.

In the irrigated land of the northeastern (NE) Iberian Peninsula, the arable crop landscape is formed mainly by annual crops such as winter cereals and maize, and perennial crop such as alfalfa, building a mosaic with field margins, uncultivated lands, woody areas and fruit orchards. These arable crops partially overlap: alfalfa and winter cereals in spring, alfalfa and maize in summer, and all three crops in late spring and autumn. Only winter cereals and maize share some herbivores, but natural enemies can be observed within all three crops (Pons and Eizaguirre 2009). During summer, the arable crop landscape is mainly shaped by alfalfa and maize, and there is a broad spectrum of predators that can be found in both crops, such as heteropterans (Anthocoridae, Nabidae and Miridae), neuropterans (Chrysopidae), coleopterans (Coccinellidae, Carabidae, Staphylinidae and Cantharidae), dipterans (Syrphidae) and several spider families (Pons and Eizaguirre 2009). The temporal coincidence of alfalfa and maize in the landscape, the fact that these crops share predators and the comparison of predator abundances in both crops during the season led to hypothesize that predators can move between these crops (Pons et al. 2005). However, this hypothesis needs to be tested to clarify if really there is predator population movement between these crops. Even though predator movement between adjacent crops or from natural habitats to crops has been observed in other systems (Prasifka et al. 1999, 2004; Forbes and Gratton 2011), in the landscape context of the NE Iberian Peninsula little is known about the movement capacity of natural enemies, both within crop and between crops. There are many questions that need to be elucidated, e.g. aspects related with species-specificity, dispersal capacity, timing and magnitude of predator movement between crops, as well as the ecological role that each crop plays during the season for predators ("source vs. sink").

Another point that has received little attention is the movement from managed to natural habitats. A previous review about predator spillover reveals that this topic has been understudied, especially when it is compared with the number of studies focusing on movement from natural to agricultural habitats (Rand et al. 2006).

In the last decades, a wide variety of marking techniques have been developed to study arthropod movement (Reynolds et al. 1997; Lavandero et al. 2004). Such techniques should be environmentally safe, cost-effective, easy to use and persist without affecting the insects' behaviour (Hagler and Jackson 2001). However, the selection of a technique should depend on the insect species, environmental conditions,

nature of the experiment and the study objectives. Therefore, if possible, preliminary studies should be conducted before use any technique (Hagler and Jackson 2001).

Depending on the type of study, insects can be marked on purpose or they can mark themselves by contacting marking materials that are natural to their environment or materials that have been strategically added in their environment (Reynolds et al. 1997). The markers can be external, where the mark is usually applied into the insect surface and recognised by visual methods; or internal, where recognition of markers requires specialised equipment (Lavandero et al. 2004). However, external markers can be difficult to apply on small animals (Hobson and Norris 2008; IAEA 2009) but are commonly used in mark-release-recapture studies (Hagler and Jackson 2001). Nowadays, however, in mark-capture studies, the most commonly employed techniques are the analysis of rare earth elements and stable isotopes (Scherber et al. 2012).

Rubidium in its chloride form is the most common rare elemental marker used for tracking insects (Hagler and Jackson 2001). This elements is located between potassium and caesium in the periodic table and replaces the potassium in the tissues of insects feeding on a treated source (Berry et al. 1972). Rubidium is present in nature at very low concentrations and techniques using it have to raise the natural level through an application of an aqueous solution of rubidium chloride (Berry et al. 1972). The solution can be applied in a specific habitat where it is absorbed by soil/plants and moves between trophic-levels. It is absorbed by herbivores when feeding on or getting into contact with marked plants and is transferred to natural enemies when feeding on marked herbivores (Corbett et al. 1996; Prasifka and Heinz 2001). Predators and parasitoids can also be marked or self-marked through contact with marked soil, plants and through feeding on floral nectar or pollen from rubidium-marked plants (Lavandero et al. 2004). At moderate concentrations, rubidium has no adverse affect on the insects' physiology or behaviour (Jackson et al. 1988). Many studies have been using this technique to measure the movement of herbivores and natural enemies in the field (Fernandes et al. 1997; Prasifka and Heinz 2001; Albajes et al. 2004; Tillman et al. 2007; Scarratt et al. 2008; Perović et al. 2011). The rubidium content of insects is determined following a specific laboratory protocol (see Chapter 1 and 2) and is usually analysed using flame emission spectroscopy.

The use of stable isotopes for tracking the movement of predators has increased in the last decade (Prasifka et al. 2004; Girard et al. 2011; Forbes and Gratton 2011;

Ouyang et al. 2014) and has proven to be a powerful tool for obtaining qualitative and quantitative information on predation and movement of insects between crops, to be environmentally safe, and to have no effect on movement patterns (Nienstedt and Poehling 2000; Hagler and Jackson 2001; Lavandero et al. 2004; Hood-Nowotny and Knols 2007). Plants may be used as markers for tracking insects through their natural concentration in stable isotopes of chemical elements. In particular, carbon stable isotope ratios ($\delta^{13}C$) have recently been proven to be suitable for tracing the plant origin of phytophagous and predatory insects (Prasifka et al. 2004; Prasifka and Heinz 2004; Vialatte et al. 2006; Traugott et al. 2008; Forbes and Gratton 2011; Schallhart et al. 2011; Ouyang et al. 2012) and this is especially true when the system is mainly formed by two isotopically distinct crops (C3 and C4 plants) because C3 plants and C4 plants fix CO_2 using differing pathways. Carbon isotope ratios are expressed as parts per thousand (‰) and the ^{13}C signature of C4 plants is distinctly higher than the one of C3 plants. The carbon isotope ratios are transferred with low fractionation (0 ‰ ± 1 ‰) between trophic levels (DeNiro and Epstein 1981; Ostrom et al. 1997; Post 2002). The herbivores that feed on C3 or C4 plants reflect the overall isotopic composition of their food, and the predators that feed on these herbivores will also reflect the isotopic concentration of their prey's diet (DeNiro and Epstein 1978; Oelbermann and Scheu 2002). The isotopic composition of predators moving between and feeding on isotopically distinct crops such as maize (C4) and alfalfa (C3) will gradually move from the original source towards the signature of the new food source (Ostrom et al. 1997; Prasifka et al. 2004; Ouyang et al. 2012). Moreover, nitrogen stable isotopes, which are generally used as an indicator of trophic position (DeNiro and Epstein 1981), can also improve information about the incorporation of prey from different resources, with an enrichment between predators and their prey of 3.4 ‰ ± 1 ‰ (Post 2002).

Another way to infer predator movement between habitats can be through differences in their abundance at consecutive time intervals in the season and or according crop phenology/management. This is typically used in studies focusing on ground-dwelling insects' (carabids, staphylinids and spiders) activity and density using pitfall traps as sampling method (Thomas and Marshall 1999; Pluess et al. 2008; Wamser et al. 2011).

In arable crops, natural enemies can provide valuable biological control services, but for this they need to complete their life cycle in the crops or other habitats that form

the arable crop landscape. Crop manipulation in order to enhance the performance and abundance of the existing natural enemies in arable farms is one of the key strategies in conservation biological control (Landis et al. 2000) and is currently the only cost-effective pest control method in arable crop conditions of many Mediterranean regions (Pons et al. 2013). A better understanding of the natural enemies' movement dynamics will help to improve the conservation biological control strategies (Barbosa and Wratten 1998; Dent 2000; Landis et al. 2000). Moreover, detailed information on crop / habitat functionality (as source *vs*. sink of predators) and the movement patterns of predator populations can be useful for decision making in order to improve local habitat management and also to increase the potential of natural enemies. Therefore, there is the need to study the dynamic of movement of the most abundant predators present in the arable crop landscape.

In this thesis, I examine the movement of some of the most representative and abundant predators in maize and alfalfa and in winter cereals. I selected the generalist predator *Orius majusculus* Reuter (Heteroptera: Anthocoridae) and the specialised aphidophagous *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) since they are among the most common and abundant plant-dwelling predators in maize (De la Poza et al. 2005; Pons and Eizaguirre 2009) and alfalfa (Pons et al. 2005). On the other hand, carabid beetles (Coleoptera: Carabidae) are among the most common and abundant ground-dwelling predators in maize (De la Poza et al. 2005; Pons and Eizaguirre 2009; Albajes et al. 2009) and in alfalfa (Nuñez 2002). For tracking the movement of *O. majusculus* within maize and the movement of carabids between alfalfa and maize crops and vice versa, I used rubidium as a marker. For tracking the movement of *O. majusculus* and *C. septempunctata* between maize and alfalfa and vice versa I used carbon and nitrogen stable isotopes. These experiments were carried out in fields located at the NE Iberian Peninsula. The habitats used, the predators studied and the direction of movement that was found are depicted in the Figure 1A.

I also selected the most important ground-dwelling predators (carabids, staphylinids and spiders) in winter cereals (Thies et al. 2011). I inferred the spillover of these ground-dwelling predators from winter cereals and meadow to calcareous grassland (a semi-natural habitat type) through the differences in abundance of these predators during consecutives samplings. This study was conducted in the vicinity of

Göttingen, Lower Saxony, Germany. The habitats used, the predators studied and the direction of movement that was found are represented in Figure 1B.

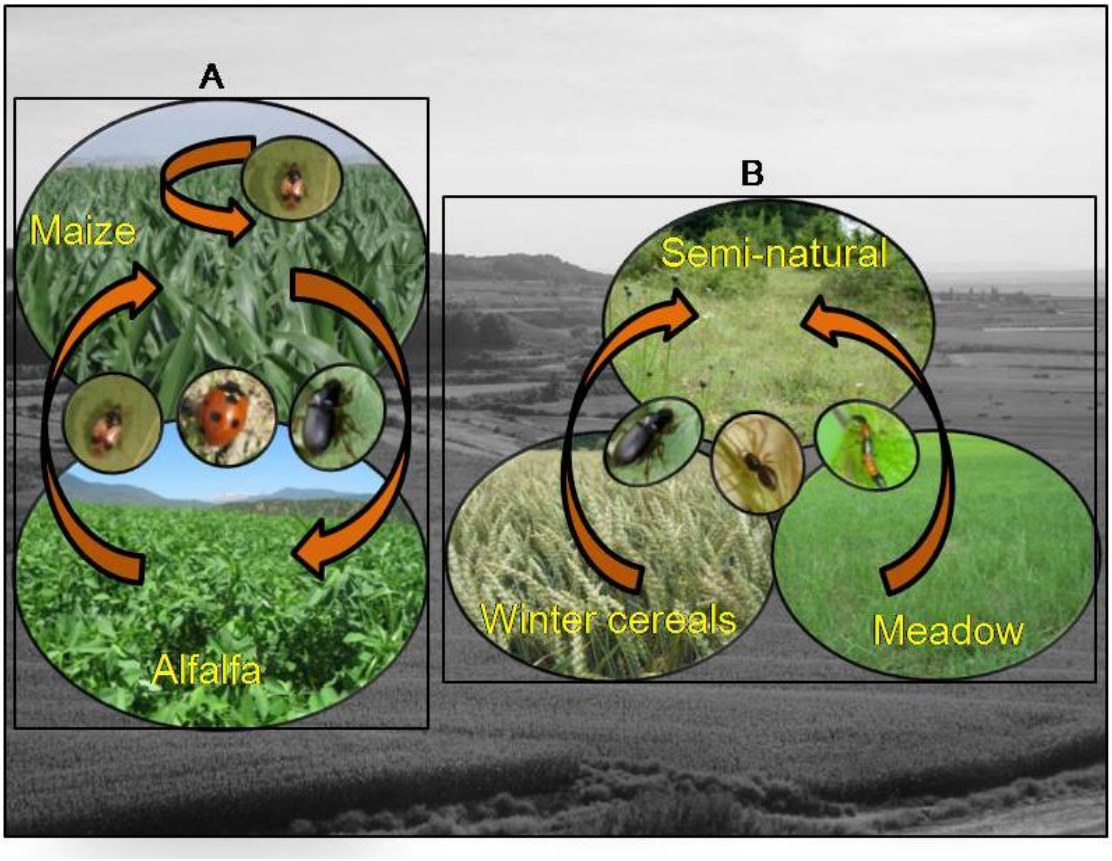

Figure 1. Diagram with crop / habitat and predators movements that were evaluated. Arrows represent directions of movement that were evaluated. **A)** Assays carried out in NE of the Iberian Peninsula with the crops and the direction of predator movement studied. **B)** Assay carried out in Lower Saxony (Germany) with crops/habitats and direction of predator movements studied.

Research objectives

The general aim of this thesis was to study multiple aspects of predatory insect movement in arable farms and to clarify the ecological role of each crop/habitat as donor or receptor for predators.

The specific objectives of this thesis were:

1. To assess the abundance and dispersal of *Orius majusculus* in plots with high and low weed density.
2. To determine the seasonal movement activity of ground-dwelling predators between alfalfa and maize.
3. To determine the spillover of plant-dwelling predators between maize and alfalfa.
4. To clarify the spillover of ground-dwelling predators from winter cereals or managed meadows to calcareous grasslands.

Outline of chapters

Chapter 1: Effects of maize weed changes on the abundance and dispersal of *Orius majusculus* Reuter (Het., Anthocoridae)

To achieve the first objective, I carried out a field study in the NE Iberian Peninsula to examine the abundance and dispersal capacity of *O. majusculus* in maize plots with moderately high and low weed density. Insects were collected using yellow sticky traps and/or a bug-vac aspirator. Dispersal activity was assessed using rubidium as a marker.

This chapter has been submitted to Insect Science.
Madeira, Filipe and Pons, Xavier

Chapter 2: Determination of intercrop movement of ground beetles (Col., Carabidae) between adjacent alfalfa and maize using rubidium as a marker

In this chapter (objective 2) I investigated the seasonal movement of four of the most abundant ground beetle species in the NE Iberian Peninsula (*Calathus fuscipes*, *Pseudophonus rufipes*, *Poecilus cupreus* and *Metallina* sp.) between adjacent alfalfa

and maize fields. To asses the movement of carabids we used rubidium as a marker. To determine the influence of alfalfa cutting in the movement, samplings were carried out before and after alfalfa cutting in maize, alfalfa and in margins.

This chapter has been submitted to International Journal of Pest Management.
Madeira, Filipe and Pons, Xavier

Chapter 3: Change in carbon stable isotope ratios of the predatory bug *Orius majusculus* after dietary shifts

In order to achieve objective 3, there was the need to carry out a laboratory experiment, since no information was available on the use of stable isotope analysis with small predators such as *O. majusculus*.

This chapter reports changes in $\delta^{13}C$ of *O. majusculus* over time after a diet switch from aphids feeding on C3 plants to aphids feeding on C4 plants or vice versa. The laboratory experiment consisted of feeding *O. majusuculis* nymphs with aphids (*Rhopalosiphum padi*) living on C3 (barley) or C4 (maize) plants until adult emergence. Afterwards half of the adult individuals were switched to feeding on aphids reared on the other type of plant. The other half maintained their original diet (as a control). Samples of *O. majusculus* for each diet were collected for isotopic analysis after the diet switch. The results of this experiment helped to better understand isotopic field data.

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Madeira, Filipe; di Lascio, Antonella; Carlino, Pasquale; Costantini, Maria Letizia and Pons, Xavier

Chapter 4: Stable carbon and nitrogen isotope signatures to determine predator spillover between alfalfa and maize

To achieve objective 3, I examined the movement of *O. majusculus* and *C. septempunctata* between maize and alfalfa fields using carbon $\delta^{13}C$ and nitrogen $\delta^{15}N$ stable isotope analysis at two locations of the NE Iberian Peninsula. Aphids of maize and alfalfa were selected as herbivore isotopic reference of the host plant. Predators' spillover would be accepted or rejected if individuals from one crop showed differences or not in $\delta^{13}C$ from the aphids of the same crop in which they were sampled. Predators were categorized into three groups (*local, switching* and *migrant*) according to the percentage of assimilation ($\delta^{13}C$) of the two resources (aphids on alfalfa and maize).

$\delta^{15}N$ isotope was taken into account to improve the information about incorporation of prey from alfalfa and maize resources. Field samplings of both crops were performed during the vegetative and reproductive maize growth periods.

Preparation and analysis of stable isotopes were carried out during a three month training stay at the Laboratory of Trophic Ecology, University of Rome "La Sapienza".

This chapter has been submitted to Biological Control.
Madeira, Filipe; di Lascio, Antonella; Carlino, Pasquale; Costantini, Maria Letizia; Rossi, Loreto and Pons, Xavier

Chapter 5: Spillover of predatory and non-predatory arthropods from cultivated land to calcareous grasslands

To meet objective 5; carabid, staphylinid and spider spillover effects from winter cereals or meadows to adjacent semi-natural calcareous grassland were assessed. The spillover of ground-dwelling predators was examined through changes in their abundance in consecutives samples. The study was carried out near Göttingen (Germany) in semi-natural calcareous grassland fragments (adjacent to winter cereals and adjacent to meadows) before and after wheat harvest and hay cutting.

This study was performed during an eight month training stay at the Agroecology group, Georg-August University, Göttingen, Germany.

This chapter has been submitted to Journal of Applied Ecology.
Madeira, Filipe; Tscharntke, Teja; Elek, Zoltán; Kormann, Urs; Pons, Xavier; Rösch, Verena; Samu, Ferenc; Scherber, Christoph; Batáry, Péter

CHAPTER 1

EFFECTS OF MAIZE WEED DENSITY ON THE ABUNDANCE AND DISPERSAL OF *ORIUS MAJUSCULUS* REUTER (HET., ANTHOCORIDAE)

Filipe Madeira and Xavier Pons

This chapter has been submitted to Insect Science.

Effects of maize weed density on the abundance and dispersal of *Orius majusculus* Reuter (Het., Anthocoridae)

Abstract

Orius majusculus Reuter (Heteroptera: Anthocoridae) is the most common and abundant generalist predator in Spanish maize crops and is believed to be sensitive to changes in weed density. A two-year study carried out in the NE Iberian Peninsula examined the abundance and dispersal capacity of *O. majusculus* in maize plots with high and low weed density. Insects were collected using yellow sticky traps and/or a bug-vac aspirator. Dispersal activity was assessed using rubidium as a marker. We detected differences in the capture efficiency between sexes according to the sampling method used. Yellow sticky traps captured more males than females, whereas bug-vac aspirators captures were females biased. However, for each sex there were no differences in the abundance and dispersal of *O. majusculus* in plots with high or low weed density. The dispersal activity of *O. majusculus* differed between sampling periods, with high movement between the V16 and R3 maize growth stages. Our findings suggest that the abundance and dispersal of *O. majusculus* within a maize field were not linked to weed density and may be much more related to other aspects such as the abundance of prey on maize. We conclude that herbicide treatment regimes with different efficacies will not affect the abundance and dispersal of *O. majusculus* in maize.

Key words: weeds, maize, generalist predator, movement, rubidium, Heteroptera.

1. Introduction

Maize is one of the most important arable crops in the world (James 2012). In many agricultural systems weeds are the main cause of maize yield reduction and economic losses (Meissle et al. 2010). Weed management is usually based on mechanical methods, conventional herbicide spraying and in countries where it is allowed the use of transgenic herbicide-tolerant varieties that permit post emergence spraying with broad spectrum herbicides. The abundance, composition and phenology of the crop weed community depends on weed management practices (Fried et al. 2010). In arable crops, weeds may support a wide range of beneficial arthropods because they can provide a variety of food resources such as pollen and nectar or they can serve as hosts to alternative prey (Norris and Kogan 2000). Therefore, changes in weed density may influence beneficial arthropod abundance, affecting natural pest control (Hawes et al. 2003; Lundgren et al. 2009; Albajes et al. 2009). It is also known that some natural enemies can show cyclic movements between weeds and crops (Burgio et al. 2006; Atakan and Tunç 2010) and that modifications in the vegetation they use as a refuge can alter their dispersal activity (Grez et al. 2008). Although the effects of weed changes on natural enemy abundance have received a great deal of attention (Norris and Kogan 2000; Hawes et al. 2003; Lundgren et al. 2009; Albajes et al. 2009), other potential effects on mechanisms such as dispersal are still poorly understood and have rarely been studied.

In Spain, maize is one of the main summer arable crops and in 2011 it occupied 16% of the total arable crop area (MAGRAMA 2013). The composition and abundance of natural enemies of maize herbivores is well documented (Asín and Pons 2001; Albajes et al. 2003, 2009; De la Poza et al. 2005) and *Orius majusculus* Reuter (Heteroptera: Anthocoridae) is the most common and abundant generalist predator on maize and is considered to play a major role in preventing homopteran insects from reaching economic thresholds in Spanish maize (Albajes et al. 2003; De la Poza et al. 2005). Albajes et al (2009) observed that *Orius* spp. were among the most responsive predators to changes in weed abundance and they reported higher densities in plots treated with herbicide than in untreated ones. However, they did not find differences in abundance when differences in weed densities were lower (Albajes et al. 2011). On the other hand, it seems that the dispersal capacity of *Orius* spp. is high in the agricultural

landscape (Pons et al. 2005), but little is known about the dispersal of *O. majusculus* within maize fields and how weed density may affect its dispersal.

As herbicide-tolerant crops and subsequent herbicide treatment with broad spectrum herbicides may alter the abundance of weeds, potential effects of herbicide-tolerant crops on the arthropod food web needs to be studied. To this end, *O. majusculus* was selected because it is the most abundant predator in maize stands in our area (Albajes et al. 2003).

The aim of this study was to find out whether the density of weeds, at moderate levels as usually occurs under current management, affects the abundance and dispersal of *O. majusculus*. We compared abundance and dispersal of *O. majusculus* at several distances and sampling periods in plots with high and low weed density. To track the movement of *O. majusculus* we used rubidium as a marker.

2. Material and methods

2.1 Study area

The study was carried out during the maize growing seasons of 2009 and 2010 on fields located in the surroundings of Lleida (Catalonia, NE Spain), under the traditional maize cultivation practices used in the region (Piqué et al. 1998). To determine the dispersal capacity of *O. majusculus*, we used rubidium as a marker. Rubidium is a ubiquitous element chemically similar to potassium but in nature it is found at very low concentrations and has been signalled as a useful and valid method for tracking *Orius* spp. Movement (Reynolds et al. 1997; Prasifka and Heinz 2001).

In 2009, the size of the maize field used was approximately 4 ha. It was located at Pla de la Font (41º41’28”N, 0º23’43”E), belonging to the network of experimental assays to determine the impact of genetically modified herbicide-tolerant maize on non-target insects, funded by the Spanish Ministry of Environment, the National Institute for Agricultural and Food Research and Technology and the University of Lleida. In the experimental field maize had been grown for the three previous years and the same herbicide regime had been applied on the plots throughout the three years. The field was sown in mid-May using the variety TEB652-E including the transformation event NK603, which confers tolerance to applications of glyphosate herbicides on the foliage. Seeds were coated with imidacloprid.

The field was divided into three blocks and within each block two types of plots (85 x 70 m) with two randomly different herbicide regimes were assigned: one with two treatments of glyphosate and the other with conventional herbicide treatment. The glyphosate treatments (MON 78044) were made when the maize had developed four and eight leaves (maize growth stages V4 and V8, respectively, according to Ritchie et al. (2005) at a rate of 1.08 kg active ingredient/ha. The conventional treatment was at maize pre-emergence with a mixture of 1.26 kg/ha acetochlor (Harness® Plus Monsanto, Spain, 1.5 l/ha) and 0.5 kg/ha aclonifen mixed with 0.075 kg/ha isoxaflutol (Lagon®, Bayer, Spain, 1 l/ha). It was expected that two treatments with glyphosate would result in better weed control (low density) than the one conventional herbicide treatment (high density). The abundance of weeds per square meter was estimated by counting the number of individuals within a 0.25 m^2 ring. On each plot, rings were randomly distributed 16 times on each principal diagonal. Weed samplings were carried out 10 to 15 days after the last herbicide treatment. The weeds were classified into monocotyledons and dicotyledons.

To measure the dispersal capacity of *O. majusculus* we delimited a square area (25 m^2; 5 × 5 m) in the centre of each plot where plants were sprayed with a solution of rubidium chloride (RbCl). The rubidium areas at each block were separated by at least 65 meters. Rubidium solutions were prepared using solid RbCl at a minimum of 99% purity (Sigma® - Aldrich, Madrid, Spain) and water in a concentration of 4 g/l. The rubidium solutions were applied using a manual pressure sprayer (Matabi® 16 litres, Goizper, Gipuzkoa, Spain).

To collect *O. majusculus* we placed chromotropic yellow sticky traps (21 × 31 cm, Serbios®, Italy) within the rubidium-marked area and at each side of the square at 5, 15 and 25 m away from the marked area (Figure 1.1A). The number of traps placed varied according to the distance: 4 traps in the marked area, 8 traps at 5 m (2 per side of the square), 16 traps at 15 m (4 per side of the square) and 32 traps at 25 m (8 per side of the square), resulting in 60 traps per plot. We sampled five times during the season at the maize growth stages V16-18, R1, R2, R3 and R4 (nomenclature according to Ritchie et al. (2005)), when *O. majusculus* is abundant (Albajes et al. 2003, 2009). The traps remained in the field for seven days. The rubidium applications were performed three days before the 1st and 3rd samplings.

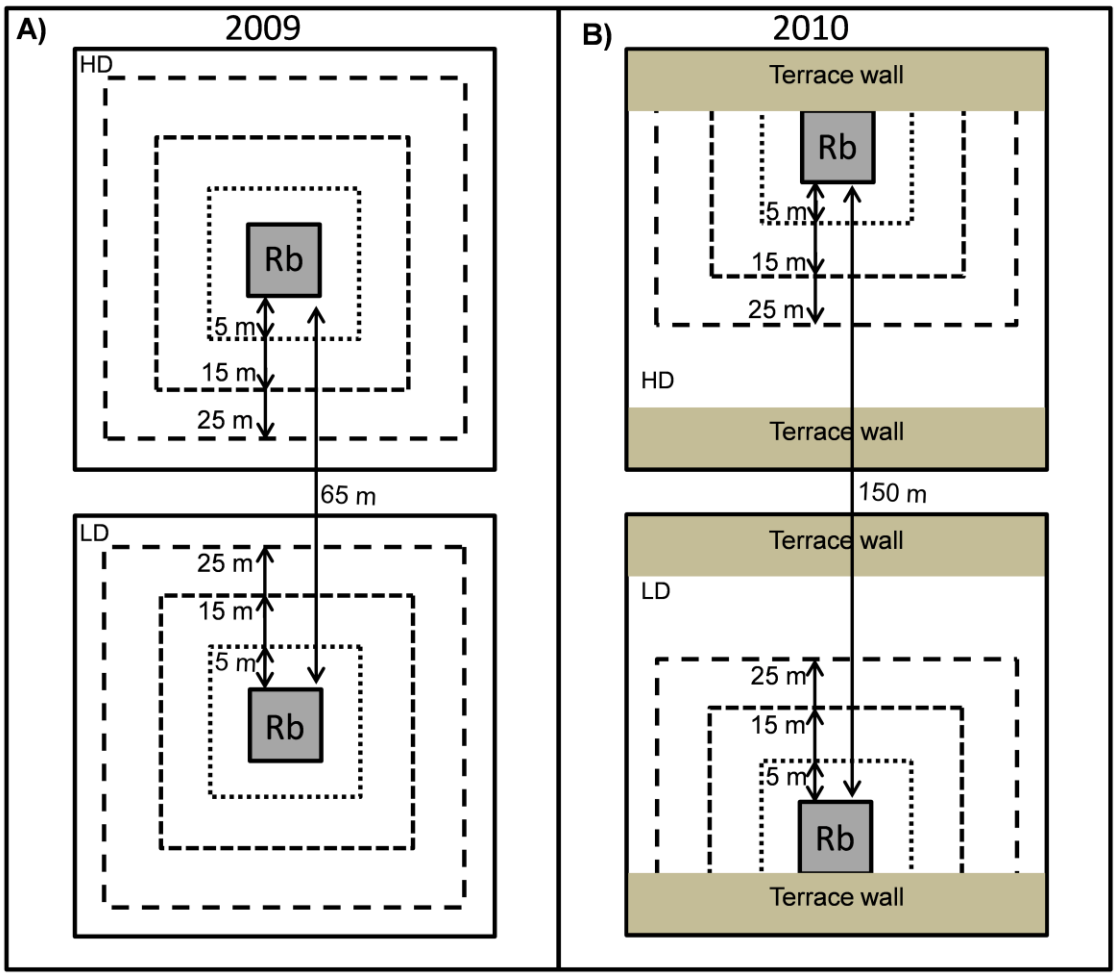

Figure 1.1. Field block design for *O. majusculus* movement studies. **A**) Assay in 2009 with two pairs of plots with high (HD) and low weed density (LD), and square transects at 5, 15 and 25 m from the rubidium-treated area (Rb). **B**) Assay in 2010 with two pairs of plots with high (HD) and low weed density (LD) and rectangular transects at 5, 15 and 25 m from the rubidium-treated area (Rb). Diagram is not to scale.

In the laboratory, the traps were kept in a refrigerated chamber at 6°C until processing. Insects were carefully extracted from the traps and placed in a beaker with xylene for a few minutes to remove residual trap glue. The insects were separated into males and females and individually stored at -18°C until rubidium analysis.

In order to increase the catches, we collected *O. majusculus* directly on the plants using a bug-vac aspirator (Standard Bug-Vac#2®, Rose Entomology, Benson, AZ, USA) during the vegetative maize growth stages R1, R2 and R3 (Ritchie et al. 2005). To this end, we walked for 20 min per distance and 10 min inside of the rubidium treated square. In the laboratory *O. majusculus* were separated by sex and stored at -18°C until rubidium analysis.

In 2010, as the experimental field used in 2009 was not available because the study promoted by the Spanish Ministry of Environment had ended, we had to select a new field. The study was carried out in an 8-ha commercial grain maize field situated in Almenar (41º46'44"N, 0º31'27"E), where the maize field had been included for many years in the farm's crop rotation with winter wheat. The field was sown in mid-May using the variety Dekalb 6451 (Monsanto, Spain) with the seeds coated with imidacloprid. The experimental design consisted of three blocks and within each block two types of plots with randomly different weed regime controls were established: one with conventional treatment and one with conventional treatment and additional manual weeding when the maize plants were at the V3-V5 maize growth stage. Because the commercial field used was formed by terraces, the plot size varied from 0.6 to 1 ha, but was uniform within each block. The conventional herbicide treatment was performed at maize pre-emergence with a mixture of 1.26 kg/ha acetochlor (Harness® Plus Monsanto, Spain, 1.5 l/ha) and 0.5 kg/ha aclonifen mixed with 0.075 kg/ha isoxaflutol (Lagon®, Bayer, Spain, 1 l/ha). It was expected that plots with additional manual weeding would have a better weed control (low density) than plots with only conventional herbicide treatment (high density). Weed samplings were carried out 10 to 15 days after manual weeding and the methodology followed was the same as in 2009.

To track the movement, on each elementary plot we delimited a square area (25 m^2; 5 × 5 m) in one of the margins adjacent to the terrace wall, where plants were sprayed with a solution of RbCl. The rubidium areas were at least 150 m apart in a straight line. Insects were collected 5, 15 and 25 m from the treated area (Figure 1.1B). To do this, we inspected plants visually and collected the insects using a bug-vac. The time dedicated per distance was different; 10, 15 and 30 min at distance 5, 15 and 25 m, respectively, and 5 min within the marked area. We exclusively used a bug-vac in 2010 because in 2009 we observed a bias between sexes in the catches with the use of yellow sticky traps. In the laboratory, *O. majusculus* were separated by sex and stored at -18°C until rubidium analysis. Similarly to 2009, we sampled six times during the season at the maize growth stages V17-19, R1, R2, R3, R4 and R5 (nomenclature according to Ritchie et al. (2005). The rubidium solutions, preparation and procedure were the same as in 2009 and were performed three days before the 1st, 3rd and 5th sampling.

In both years, in each insect sampling, three leaves of one maize plant were randomly collected at four points within the sprayed rubidium area and per distance. All

plant samples were preserved in an icebox in the field and in the laboratory they were transferred to a freezer at -20ºC until rubidium analysis.

2.2 Sample preparation and rubidium analysis

Prior to chemical digestion, the frozen plant leaves and *O. majusculus* were placed in beakers individually and then dried in an oven at 70 ± 3ºC for approximately 24 hours and their dry weight was determined. Chemical digestion consisted in adding 0.5 ml of HNO_3 (69%) and 0.4 ml of H_2O_2 (30%) to each sample. The beakers were placed in a sand bath at 70 ± 5°C, until complete sample digestion. The beakers were then withdrawn from the sand bath and a redilution was performed by adding 0.3 ml of HNO_3 (1:1) and 1 ml of distilled water to obtain a total volume of 1.3 ml. Finally, the total volume was transferred to 10 ml tubes, which were kept in the refrigerator (6ºC) until rubidium analysis.

To measure the rubidium content, the samples were automatically pipetted and injected into a nebulizer of inductively coupled plasma optical emission spectrometry (ICP-OES) (Horiba Jobin Yvon, Longjumeau, France). The rubidium content of each sample was then measured by summing the amount of energy absorbed at 780.0 nm, the most sensitive wavelength for atomic absorption detection of rubidium.

The total absorbance was integrated as the average of three measurements over a period of three seconds, with the absolute rubidium concentration established by calibration of standard solutions. The standard solutions used were 0, 5, 10, 25, 50 and 100 μg/ml. They were prepared by dilution of a standard sample of 995 μg RbCl/ml.

To determine the endogenous concentration of rubidium in plants and insects, five samples were included as a control in each analysis, Control plants came from fields that had never been sprayed with rubidium and *O. majusculus* control came from laboratory rearings that had never been in contact with any source of rubidium. A plant or insect was considered as marked if its rubidium concentration exceeded the mean of control samples plus three standard deviations, following the criterion of Stimmann (1974).

2.3 Statistical analysis

The abundance of weeds and *O. majusculus* was analyzed with a generalized linear model (GLM) assuming a Gaussian distribution. Weed density was analysed

according to weed control regime. The abundance of *O. majusculus* was analysed in relation to weed density (high vs. low), sex (male vs. female) and distance (5, 15 and 25 m) as fixed factors and two-way interactions of the above-mentioned factors. Maximum likelihood models were simplified with a stepwise model selection based on akaike information criteria (AIC) using the 'stepAIC' function of the MASS package (Venables and Ripley 2002). Data were log-transformed in order to achieve normality.

The proportion of *O. majusculus* marked in 2009 was calculated using the catches of yellow sticky traps and bug-vac, while the proportion of *O. majusculus* marked in 2010 was calculated only using the catches collected by bug-vac. Statistical analyses of the proportion of *O. majusculus* marked were carried out with GLMs, assuming a Gaussian distribution. Data on the proportion marked were normalised using the transformation $y = \arcsin(x)^{1/2}$. We analysed the proportion of marked individuals in relation to weed density (high vs. low), distance (5, 15 and 25 m), sampling (V16-18, R1, R2, R3, R4 and R5), sex (male vs. female) and method (trap vs. bug-vac, only in 2009) and all possible two-way interactions of the above-mentioned explanatory variables. Maximum likelihood models were simplified with a stepwise model selection based on AIC using the 'stepAIC' function of the MASS package (Venables and Ripley 2002). A least significant difference test was used to separate means when needed. Statistical analyses were performed using R version 2.15.0 (R Development Core Team 2011).

3. Results

3.1 Weed density

In both years, the most abundant monocotyledonous weeds were *Echinochloa crus-galli* L., *Setaria verticillata* L. and *Sorghum halepense* L. The most abundant dicotyledonous weeds were *Abutilon theophrasti* Med., *Amaranthus retroflexus* L. and *Chenopodium album* L. The density of monocotyledonous and dicotyledonous weeds in 2009 and 2010 is shown in Table 1.1. In 2009, contrary to expectations we did not always obtain better weed control with glyphosate than with the conventional herbicide. We observed significantly higher densities of monocotyledonous weeds in conventionally treated plots than in plots treated with glyphosate. The opposite occurred

with dicotyledonous weeds. In 2010, as expected, the plots with additional manual weeding showed fewer weeds than plots only treated with conventional herbicide.

Table 1.1. Mean number (± SE) of monocotyledonous and dicotyledonous weeds per m^2. In 2009, the highest densities of monocotyledonous weeds were observed in plots treated with a conventional pre-emergence herbicide and the highest densities of dicotyledonous weeds were observed in plots treated twice with glyphosate. In 2010, the highest densities of monocotyledonous and dicotyledonous weeds were observed in plots only treated with a conventional pre-emergence herbicide. Fisher *F* and probability levels are included in the right column. Values within a row followed by a different letter are significantly different (P < 0.05) according to the least significant difference test.

	Weed density		
	High	Low	*F; P*
2009			
Monocotyledonous	13.26 ± 0.84 a	7.13 ± 0.73 b	30.32; <0.001
Dicotyledonous	13.54 ± 1.87 a	5.86 ± 1.18 b	12.12; 0.01
2010			
Monocotyledonous	33.71 ± 7.72 a	9.33 ± 0.76 b	9.87; 0.01
Dicotyledonous	9.53 ± 2.42 a	1.17 ± 0.64 b	11.19; 0.01

df = 1, 11

3.2 Orius majusculus density

In 2009, the abundance of *O. majusculus* was not significantly different between plots with high and low weed density, both when caught by yellow sticky traps ($F_{1;170}$ = 0.13; P = 0.72) and when collected directly on the plant by bug-vac ($F_{1;98}$ = 0.42; P = 0.21) (Figure 2.1a and 2.1b). We observed that yellow sticky traps collected more males (82%) than females ($F_{1;170}$ = 252.94; P < 0.001). Catches performed with bug-vac showed no differences in sex ratio ($F_{1;98}$ = 0.58; P = 0.45). Also, the number of *O. majusculus* catches per trap and the number of insects captured per minute (bug-vac) were not significantly different between distances ($F_{2;170}$ = 0.24; P = 0.79 and $F_{2;98}$ = 0.28; P = 0.76, respectively).

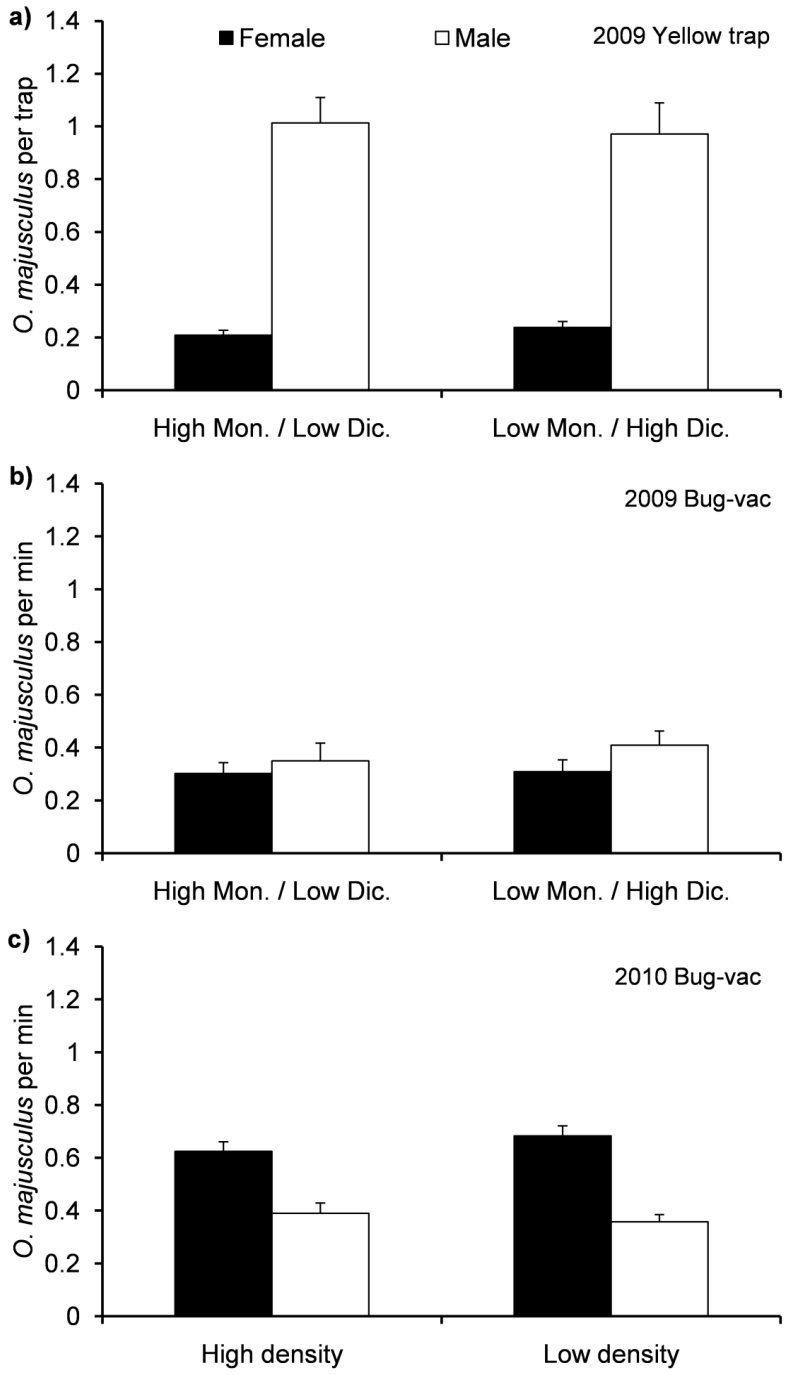

Figure 2.1. Mean number (± SE) of *O. majusculus* males and females. **a)** represents individuals collected in 2009 in plots with a high density of monocotyledonous and a low density of dicotyledonous weeds (High Mon./Low Dic.) and in plots with a low density of monocotyledonous and a high density of dicotyledonous weeds (Low Mon./High Dic.) using yellow sticky traps; **b)** individuals collected in 2009 in plots with a high density of monocotyledonous and a low density of dicotyledonous weeds (High Mon./Low Dic.) and in plots with a low density of monocotyledonous and a high density of dicotyledonous weeds (Low Mon./High Dic.) using a bug-vac; and **c)** individuals collected in 2010 in plots with a high density of monocotyledonous and dicotyledonous weeds (High density) and in plots with a low density of monocotyledonous and dicotyledonous weeds (Low density) using a bug-vac.

In 2010, the abundance of *O. majusculus* between plots with high and low weed density was not significantly different either ($F_{1;206}$ = 0.34; P = 0.56) (Figure 2.1c). However, significantly more females (64%) than males (36%) were collected with the bug-vac ($F_{1;206}$ = 69.18; P < 0.001), (Figure 2.1c). The number of *O. majusculus* catches per minute was not significantly different between distances ($F_{2;206}$ = 2.52; P = 0.08).

3.2 Orius majusculus dispersal

During all sampling periods in both years, all plants collected in the rubidium-reated area were marked with rubidium, while plants collected at distances 5, 15 and 25 m were never marked. In the case of *O. majusculus*, about 80% of catches in the rubidium area were marked and no differences between sexes were observed. Therefore, we confirmed that all *O. majusculus* marked with rubidium found at different distances could only come from the rubidium-treated area, which allows movement to be inferred.

In both years, we observed *O. majusculus* marked with rubidium at all three distances sampled. In 2009, the method used to collect insects showed no significant differences in the proportion of marked individuals ($F_{1;200}$ = 2.83; P = 0.09). Therefore, we pooled the catches of yellow traps and bug-vac corresponding to the samplings performed simultaneously. The proportion of marked individuals was significantly different between sexes ($F_{1;150}$ = 17.36; P < 0.001), with a higher proportion of marked males than marked females. For this reason we represent the results by sex (Figure 3.1). However, the proportion of males and females marked with rubidium was not significantly different between plots with different weed density, distances and two-way interactions of main factors. The sampling period was the only factor that was significantly different (Table 2.1). We observed a significantly higher proportion of marked males in the sampling periods V16-18 and R3 than in R1 and R4 (Figure 3.1), whereas we observed a significantly higher proportion of females in the sampling period R3 than in V16-18, R1 and R4 (Figure 3.1).

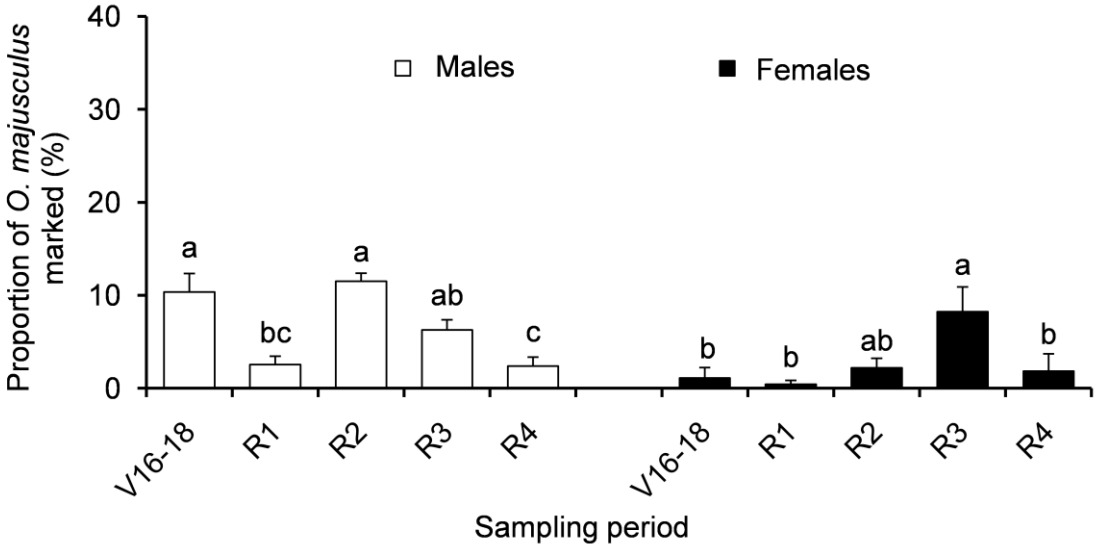

Figure 3.1. Proportion (± SE) of *O. majusculus* males and females marked with rubidium collected in 2009 in five sampling periods of maize growth vegetative stages (V16-18, R1, R2, R3 and R4 according to the nomenclature of Ritchie et al. (2005)). Differences in the proportion of marked individuals ($P < 0.05$) between sampling periods are indicated by different letters for each sex.

Table 2.1. Results of generalized linear model testing the effects of weed density (W), sampling (S), distance (D) and two-way interactions of explanatory variables in the proportion of *O. majusculus* males and females marked with rubidium collected in plots with a high density of monocotyledonous weeds and a low density of dicotyledonous weeds (High Mon./Low Dic.) and in plots with a low density of monocotyledonous weeds and a high density of dicotyledonous weeds (Low Mon./High Dic.) in 2009 at three distances (5, 15, and 25 m from the treated rubidium area) and at five sampling periods of maize growth vegetative stages (V16-18, R1, R2, R3 and R4 according to the nomenclature of Ritchie et al. (2005)).

		Males		*Females*	
	df	*F*	*P*	*F*	*P*
Weed density (W)	1; 68	0.43	0.51	1.67	0.20
Sampling (S)	4; 68	10.82	<0.001	3.98	0.006
Distance (D)	2; 68	0.08	0.92	0.04	0.96
W x S	4; 68	0.27	0.89	1.44	0.23
W x D	2; 68	1.65	0.20	2.43	0.09
S x D	8; 68	0.61	0.76	1.24	0.29

In 2010, the proportion of *O. majusculus* marked was not significantly different between plots with different weed densities, distances, sexes and all two-way interactions of explanatory variables (Table 3.1). As in 2009, the only explanatory variable that was significantly different was the sampling period (Table 3.1). We

observed the highest proportion of marked individuals in the sampling period R1 but the difference was significant only in comparison with R4 and R5 (Figure 4.1).

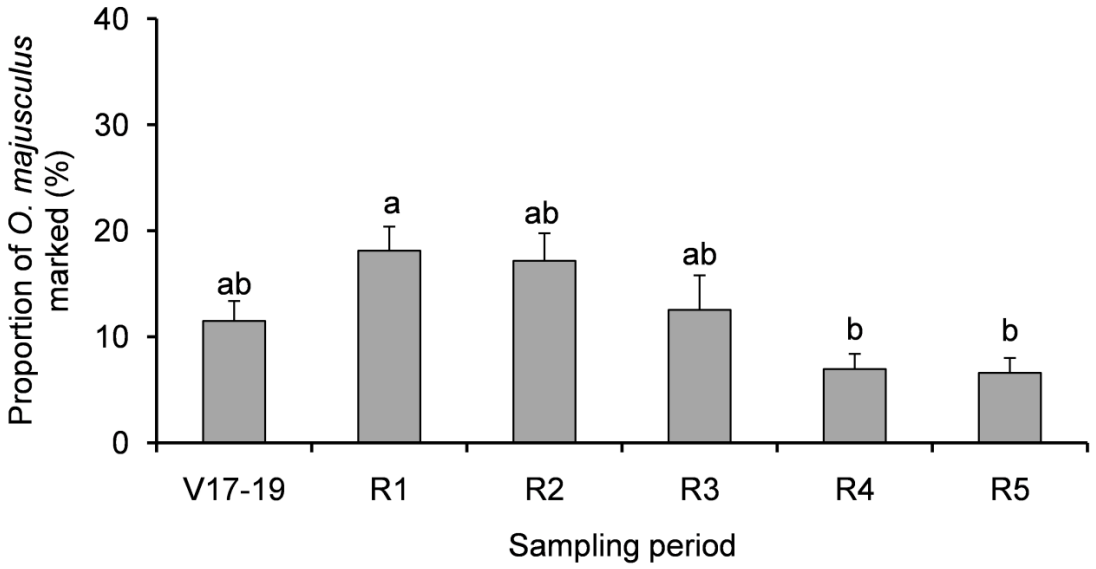

Figure 4.1. Proportion (± SE) of *O. majusculus* marked with rubidium collected in 2010 in six sampling periods of maize growth vegetative stages (V17-19, R1, R2, R3, R4 and R5 according to the nomenclature of Ritchie et al. (2005)). Differences in the proportion of marked individuals ($P < 0.05$) between sampling periods are indicated by different letters.

Table 3.1. Results of generalized linear model testing the effects of weed density (W), sampling (S), distance (D), sex and two-way interactions of explanatory variables in the proportion *O. majusculus* marked with rubidium collected in plots with a high density of monocotyledonous and dicotyledonous weeds (High weed) and in plots with a low density of monocotyledonous and dicotyledonous weeds (Low weed) in 2010, at three distances (5 , 15, and 25 m from the treated rubidium area) and during six sampling periods of maize growth vegetative stages (V17-19, R1, R2, R3, R4 and R5 according to the nomenclature of Ritchie et al. (2005)).

		Total	
	df	*F*	*P*
Weed density (W)	1; 181	2.70	0.10
Sampling (S)	5; 181	2.96	0.01
Distance (D)	2; 181	0.75	0.47
Sex	1;181	2.09	0.15
W x S	5;181	0.68	0.64
W x D	2; 181	1.69	0.19
W x Sex	1; 181	1.00	0.32
S x D	10; 181	1.29	0.24
D x Sex	2; 181	0.17	0.84
S x Sex	5; 181	0.41	0.84

4. Discussion

Many studies point out that high plant diversity can affect the abundance of herbivores, natural enemies and ecosystem services such as biological control (Andow 1991; Altieri 1999; Taylor et al. 2006; Tscharntke et al. 2012). However, most of these studies focus on the comparison between simple landscapes mainly formed by monocultures and complex landscapes with a high proportion of non-crop area. In maize, weeds compete with the crop at the early stages of growth, leading to serious yield losses (Olson and Sander 1988; Meissle et al. 2010), so they must be controlled. Most weed management practices can produce great changes in weed abundance and composition that may affect organisms at higher trophic levels, such as herbivores and their natural enemies. Herbicides are the most common weed management practice used in maize in Europe, and weed communities are shaped according to the herbicide and its application regime (Dewar 2009). However, it has been suggested that weeds provide prey for predators and other resources, such as nectar and pollen, shelter and breeding sites, thus enhancing integrated pest management on crops (Altieri 1999; Norris and Kogan 2000; Atakan and Tunç 2010). Some studies carried out in maize suggest that a high density of weeds enhances the diversity and abundance of natural enemies (Penagos et al. 2003; Hawes et al. 2003; Hough-Goldstein et al. 2004). However, Albajes et al. (2009) observed that not all predators respond in the same way to changes in maize weed flora.

In our study, though we had plots with both moderately high and low densities of weeds, the abundance of *O. majusculus* was not affected by weed density. Albajes et al. (2009, 2011) reported higher abundances of *Orius* spp. in herbicide-treated than in untreated plots, but no differences were reported when there was similar weed density between plots (Gianoli et al. 2006; Albajes et al. 2011). This, together with our results, suggests that a low or moderate density of weeds does not affect the abundance of *O. majusculus*. Contrary to our results, Lundgren et al. (2009) reported that *Orius insidiosus* (Say) was more abundant in soybean plots with high than with low weed vegetation, and attributed this result to the more favourable environment that weeds provide for nymphs. The difference from our results could be explained by differences in species within the genus *Orius*, the crops involved (plant architecture, thickness of

external plant tissues, trichome density, etc.), the crop growth stage at which the studies were made and the resources that *Orius* species could find in each crop environment.

In our study, although no difference in the abundance was observed between plots with high and low weed density for both sexes, the catches by sex were biased according to the sampling method used. Yellow sticky traps always captured more males than females, whereas the bug-vac collected the same proportion of males and females in 2009 but more females in 2010. The fact that yellow traps capture more males can be related to different attraction to colour or/and dispersal behaviour between sexes, males being more active fliers in order to mate, and females being more sedentary because they are engaged in egg-laying behaviour (Blackmer et al. 2008). In the case of the leafhopper *Carelmapu ramosi* Linnavuori and DeLong (Arismendi 2009) and the mirid *Lygus* spp. (Blackmer et al. 2008), more males than females were also captured on yellow sticky traps.

Our findings also suggest that moderate alterations in weed density do not affect the dispersal of *O. majusculus* in maize, at least at the distances measured. It is known that *Orius* spp. in cotton can move approximately 15 m/day (Prasifka et al. 2004). However, there are no data available on how far *O. majusculus* is able to move in maize. These results show that at least 25 m from a source in one direction in a week is possible, but surely it could be more. If the capacity of dispersal is higher than 25 m, then the fact that no differences between plots were found in 2009 could be due to the fact that rubidium-marked individuals from one type of plot (high or low weed density) moved to the next plot with different weed density. To minimize these potential masking effects, in 2010 the rubidium-treated areas were separated by at least 150 m. Nevertheless, once again no differences were found in the proportion of individuals marked between distances, suggesting no effects of weed density on *O. majusculus* dispersal, at least at these distances. The high and low weed density also showed no effect on the dispersal capacity between sexes.

In 2009, independently of weed density, we observed a higher proportion of marked males than marked females. This finding may be related to the high number of male (80%) catches on the yellow sticky traps. The movement was usually higher between V16 to R3 maize growth stages and lower at R4 and R5 maize growth stages. These results indicate that *O. majusculus* movement behaviour varies during maize growth stages. An explanation for this could be resource availability. Prey is quite

abundant before pollination between maize growth stages V14 and R1 (Albajes et al. 2011) and pollen is available during stages R1 to R2. For instance, *Orius* spp. aggregates to maize during anthesis, where it is known to consume maize pollen (Corey et al. 1998; Pons et al. 2005).

Orius majusculus, like its congeners, is a polyphagous predator that mainly preys on pests such as thrips, aphids and leafhoppers (Péricart 1972; Riudavets 1995; Lattin 1999). However, Albajes et al. (2011) pointed out that the presence of *Orius* spp. in maize was mainly related to the abundance of maize leafhoppers (mainly *Zyginidia scutellaris* Herrich-Schäffer) and to a lesser extent to the abundance of aphids. Although these herbivore species can use the main monocotyledonous weeds (i.e. *Setaria* sp., *Echinochloa* sp., *S. halepense*) as alternative hosts, weed density is much lower than maize density. *Orius majusculus* also feeds on plant resources such as pollen and nectar (Naranjo and Gibson 1996; Armer et al. 1998; Lattin 1999). Some studies report that some adventitious plants can enhance the fitness of *Orius* spp. by supplying prey, pollen or nectar (Atakan and Tunç 2010; Pumariño 2012; Pumariño et al. 2012). However, apart from *A. theophrasti* (Keeler 2008), the most abundant monocotyledonous and dicotyledonous weed species in maize are plants with a short pollination period and without extra floral nectarines. Furthermore, very few individuals of *O. majusculus* have been observed on that weed (Madeira 2011). It has also been reported that grasses and other wind-pollinated plants do not provide floral resources for beneficial insects (Russell 2013).

Therefore, these results suggest that the abundance and dispersal of *O. majusculus* within a maize field are not linked to weed density and may be far more related to other aspects such as the abundance of prey on maize. We can conclude that herbicide treatment regimes with different efficacies will not affect the abundance and dispersal of *O. majusculus* in maize.

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CHAPTER 2

DETERMINATION OF INTERCROP MOVEMENT OF GROUND BEETLES (COL., CARABIDAE) BETWEEN ADJACENT ALFALFA AND MAIZE USING RUBIDIUM AS A MARKER

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Determination of intercrop movement of ground beetles (Col., Carabidae) between adjacent alfalfa and maize using rubidium as a marker

Abstract

Rubidium was used as a marker to investigate seasonal movement of four of the most abundant ground beetles in the NE Iberian Peninsula, *Calathus fuscipes, Pseudophonus rufipes, Poecilus cupreus* and *Metallina* sp., between adjacent alfalfa and maize crops and the field margin before and after alfalfa cutting. Alfalfa cutting affected the movement but the pattern of movement differed between species. *Calathus fuscipes* and *P. cupreus* increased their movement from alfalfa to the margin after an alfalfa cutting; *P. rufipes* only moved to maize after an alfalfa cutting; and *Metallina* sp. only moved to the margin (from both crops) after an alfalfa cutting. Margins and maize can act as a refuge and source for carabids to recolonize alfalfa after cuttings and could act as an alternative to alfalfa strip bands, especially in areas with small fields, as in many Mediterranean regions. Arable crop rotation including alfalfa and maize combined with the maintenance of margins may conserve and enhance the populations of carabids and therefore contribute to their effectiveness in conservation biological control.

Key words: Conservation, biological control, predators, spillover, arable crops, crop management

1. Introduction

In many agricultural landscapes perennial and annual crops are adjacent and exchange of natural enemies between crops and surrounding areas may occur (Forbes and Gratton 2011). However, dispersal to another habitat could be forced by mechanical crop management practices such as crop mowing/cutting (Thorbek and Bilde 2004). Clarification of the role that margins and adjacent crops play in arable agricultural landscapes is urgently needed in order to develop conservation management practices that enhance biological control.

In the irrigated lands of the NE Iberian Peninsula, as in other regions of the Mediterranean area, the arable crop landscape in summer is characterized by a mosaic of crops in which alfalfa and maize are predominant. Alfalfa is a perennial crop and has been described as a source for predators that colonize nearby annual crops (Pons et al. 2005). Moreover, during the growing season alfalfa is periodically cut, a practice that can affect natural enemy populations, forcing them to move to field margins or other habitats. Maize is an annual crop that needs to be colonized by natural enemies every year (a sink crop). However, Madeira et al. (submitted) have shown that the role of each crop as a source or sink is species-specific and for two of the most common plant dwelling predators, *Orius majusculus* Reuter (Hemiptera: Anthocoridae) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), this role changes during the growing season. Ground beetles (Col. Carabidae) are described as one of the main predator groups in arable crops and have a high potential to reduce pest populations (Carmona and Landis 1999; Tscharntke et al. 2007). In the NE Iberian Peninsula, carabids are one of the most common and abundant ground-dwelling predators in maize (De la Poza et al. 2005; Pons and Eizaguirre 2009; Albajes et al. 2009) and in alfalfa (Nuñez 2002), but little attention has been devoted to studies about their movement between the two crops. Nevertheless, it is known that in order to improve conservation biological control strategies it is crucial to understand predator spillover and the ecological role of each habitat in the landscape (Rand et al. 2006).

Several studies have explored the issue of predator movement from adjacent habitats using methods such as the application of marking techniques (e.g. Lavandero et al. 2004). Elemental marking using rubidium, proposed by Berry et al. (1972), is one of the most frequently used ones and is considered a powerful and versatile technique

(Wolfenbarger et al. 1982; Prasifka et al. 2001; Albajes et al. 2004; Scarratt et al. 2008; Perović et al. 2011). Rubidium is an element with chemical properties similar to those of potassium, and is generally present in nature in very low concentrations. In ecological studies it is used in the form of a simple salt, rubidium chloride (RbCl). Plants can be marked by augmentation of environmental rubidium through direct spraying on soil or plants (Guillebeau et al. 1993); herbivores can be marked by direct application, feeding or contact with rubidium-marked plants (Graham et al. 1978; Fernandes et al. 1997); and predators can be marked by feeding them with rubidium-marked herbivores or through contact with rubidium-marked soil/plants (Graham et al. 1978; Johnson and Reeves 1995).

The aims of this study were (1) to measure the seasonal movement of carabids between adjacent alfalfa and maize; (2) to determine whether cutting affected the movement, (3) to determine whether different species show different movement patterns, and (4) to clarify the ecological role of each crop as a donor or receptor for carabids in the alfalfa-maize crop system complex. To this end, we used the rubidium marking technique to quantify the individuals of the most abundant carabid species from a source crop that were collected in the margin of, or inside, an adjacent crop.

2. Material and methods

2.1 Study area

The study was conducted from June to September 2010 in La Seu d'Urgell (La Seu, hereinafter) (42º 20'24" N, 1º25'48" E), 15 km south of the Pyrenees in the province of Lleida (Catalonia, NE Iberian Peninsula). It is located at 750 m altitude in a mountain area with 650 mm rainfall per year and 17ºC average temperature from April to October. In the study area from May to the end of September the arable agricultural landscape is mainly made up of maize and alfalfa. Two adjacent fields of maize and alfalfa, separated only by a narrow annual herbaceous margin (approximately 1 m wide) were selected. The maize was sown after the harvesting of the winter cereal in the same field in a double crop and non-tillage system. Maize cv. Franki (Caussade Semences, Caussade, France) was cultivated with manure fertilization, sown in mid-May and harvested in late September. Total herbicide glyphosate and chlorpyriphos (the only insecticide) were applied before maize germination and one additional herbicide

spraying was conducted after maize germination. Alfalfa cv. Aragon was three years old, was fertilized only with manure during the winter, was free of pesticide treatments, and underwent five cuttings during the growing season, from mid-March to mid-October. Both crops were sprinkler-irrigated. The margin between the two crops was formed by annual herbaceous vegetation, such as *Echinochloa crus-galli* L., *Setaria adhaerens* Forssk., *Digitaria sanguinalis* L., *Panicum dichotomiflorum* Michx., *Polygonum lapathifolium* L., *Chenopodium album* (L.), *Calystegia sepium* L., *Aster squamatus* Spreng., *Senecio vulgaris* L. *Sonchus oleraceus* L. and *Amaranthus* spp. During the experimental season this herbaceous vegetation was not subjected to any kind of management.

2.2 Carabid and plant sampling

Maize and alfalfa were sampled seven times during the period when both crops were growing at the same time. As alfalfa underwent cuttings, which involves temporary but drastic changes to the system, we mainly carried out samplings according to the management of this crop: three samplings were performed before alfalfa cutting at the 2nd, 3rd and 4th intercut, when alfalfa was 30-40 cm high, and three samplings after alfalfa cutting at the 3rd, 4th and 5th intercut, when alfalfa was 0-10 cm high. An additional sampling was made after maize harvesting in September, when alfalfa was 40-50 cm high (5th intercut). Alfalfa growing periods between cuttings are named "intercuts" in this paper, following Pons et al. (2009). At the first sampling (before alfalfa cutting at the 2nd intercut) the pitfall traps were destroyed by wild boar and it was not possible to collect insects.

In order to assess the movement of carabids, within each crop we delimited a rectangular area (100 m^2; 5 × 20 m) approximately 0.5 m from the margin, where plants and soil were sprayed with a solution of rubidium chloride (RbCl). The sprayed rectangles were separated by at least 100 m (Figure 1.2). To avoid drift and contamination of soil and plants near the sprayed rectangles, we protected the area with a 1-m-high plastic barrier. Rubidium solutions were prepared using solid RbCl at a minimum of 99% purity (Sigma®-Aldrich, Madrid, Spain) and water in a concentration of 4 g/l. The solutions were applied using a manual pressure sprayer (Matabi® 16 l, Goizper, Gipuzkoa, Spain). In order to ensure rubidium marker presence in the rubidium areas, the treatment was repeated before each sampling.

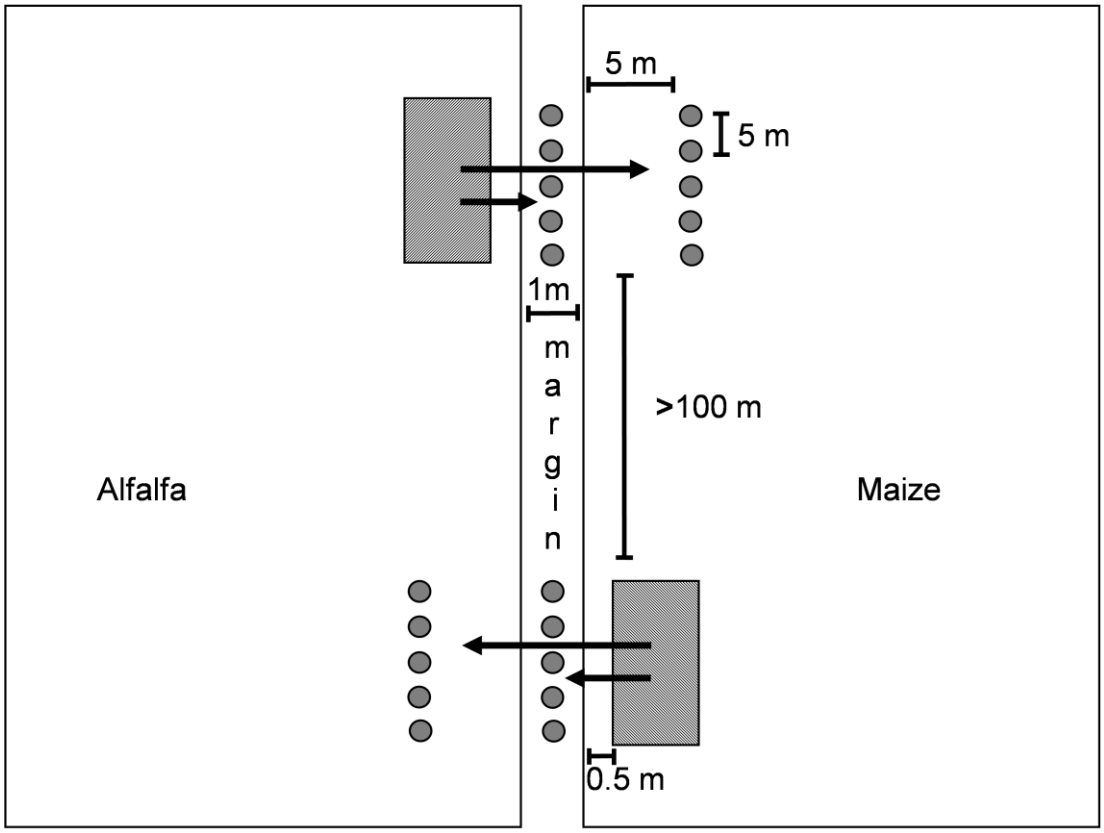

Figure 1.2. Field arrangements for carabid movement study. The white rectangles represent alfalfa and maize with an herbaceous margin between them. Rubidium-treated plots are indicated by grey rectangles and the grey circles represent pitfall traps. Arrows indicate the predator movement assessed. Diagram is not to scale.

Carabid samples were collected in two transects, in the margin and 5 m inside the opposite crop from the sprayed rubidium area (Figure 1.2). Five pitfall traps were placed in each transect (glass cups 16 cm deep and 6.5 cm in diameter) separated by 5 m. Traps contained a mixture of ethylene glycol and water (15% vol., one third of the cup height) and were covered with a polystyrene foam sheet (15 × 20 cm). They remained in the field for seven days.

The carabids collected were kept in a refrigerated chamber at 4ºC until sorting. After species identification they were stored in ethanol (70%) until rubidium analysis. Only the four most abundant carabid species collected were used in the rubidium analysis.

Plant samples were randomly collected at five points within the sprayed rubidium area, the margin and the adjacent crop. Samples of alfalfa plants consisted of one entire stem, whereas three leaves of one maize plant were collected at each

sampling point. All plant samples were preserved in an icebox in the field. In the laboratory they were transferred to a freezer at -20ºC until rubidium analysis.

2.3 Sample preparation and rubidium analysis

Plants and carabids were prepared for rubidium analysis by a method similar to the one described in Albajes et al. (2004). Carabid and plant samples were placed in beakers individually and then placed in an oven at 70 ± 3ºC for approximately 24 hours. Samples were later placed in a desiccator at room temperature for approximately 20 minutes and weighed on a precision microbalance (Mettler Toledo, Barcelona, Spain) with a weight range of 0.0001 to 5.1 g. The samples were then subjected to acid digestion by adding to each sample 0.5 ml of nitric acid (HNO_3) at 69% and 0.4 ml of hydrogen peroxide (H_2O_2) at 30%. The beakers were placed in a sand bath at 70 ± 5 °C until complete sample digestion. The beakers were then withdrawn from the sand bath and a redilution was performed by adding 0.5 ml of HNO_3 (1:1) and 2 ml of distilled water to obtain a total volume of 2.5 ml. Finally, the total volume was transferred to 10-ml tubes, which were kept in the refrigerator (6ºC) until rubidium analysis.

To measure the rubidium content, the samples were automatically pipetted and injected into a nebulizer of inductively coupled plasma optical emission spectrometry (ICP-OES) (Horiba Jobin Yvon, Longjumeau, France). The rubidium content of each sample was then measured by summing the amount of energy absorbed at 780.0 nm, the most sensitive wavelength for atomic absorption detection of rubidium.

The total absorbance was integrated as the average of three measurements over a period of three seconds, with the absolute rubidium concentration established by calibration of standard solutions. The standard solutions used were 0, 5, 10, 25, 50 and 100 μg/ml, prepared by dilution of a standard sample of 995 μg RbCl/ml.

In order to determine the endogenous level of rubidium in insects and plants, in each analysis five samples (insects/plants) that had never been in contact with a source of rubidium were included as a control. These control samples came from fields located in La Seu that had never been sprayed with rubidium. Samples were considered as marked if their rubidium level exceeded the mean of the control samples plus three standard deviations, following the criterion of Stimmann (1974).

2.4 Statistical analysis

In order to calculate the relative frequency of species in each crop and in the margin, carabids were pooled across the sampling periods and standardized by the number of traps per habitat.

In order to assess whether alfalfa management affected the movement between adjacent alfalfa and maize fields or margins, we compared the number of marked individuals and the number of unmarked individuals between sampling periods (after vs. before alfalfa cutting) within the same intercuts and also between sampling periods at consecutive intercuts. We also compared the number of marked/unmarked individuals between the margin and the crops (hereafter magnitude of movement). To achieve this, Fisher's exact tests were performed.

3. Results

During all sampling periods, plants collected in the rubidium-treated area were marked with rubidium, while plants collected at the crop edge or within the adjacent crop were never marked. Therefore, we assumed that all carabids marked with rubidium found in the margin or in the adjacent crop could only originate from the rubidium-treated area, a finding that allowed us to infer movement.

As reported in material and methods, the first sampling was destroyed by wild boar, so no results are reported. During the next six sampling periods we recorded a total of 4291 carabids. The four most abundant species selected for the rubidium analysis, representing 90% of the total catches, were *Calathus fuscipes* Goeze (51%), *Pseudophonus rufipes* De Geer (19%), *Metallina* sp. (11%), and *Poecilus cupreus* L. (9%). Within *Metallina* sp. there are two species (*M. properans* Herbst and *M. lampros* Stephans) that can easily be confused. In order to avoid error, we represent them as *Metallina* sp.

Calathus fuscipes and *P. cupreus* showed continuous movement between alfalfa, maize and the field margin before and after alfalfa cutting because marked individuals were always collected. However, the patterns of movement were different for each species. *C. fuscipes* was collected in all sampling periods (Figure 2.2a and 2.2b), in the margin (46%) and in maize (39%) and alfalfa (15%). The movement from alfalfa to the margin always increased significantly after an alfalfa cutting event. This significant

increase was observed even when we compared samplings within the "intercut" or when we compared consecutive "intercuts" before and after alfalfa cutting (Table 1.2). The significant results on the movement from alfalfa to maize were not always in the same direction. We observed higher movement before than after alfalfa cutting when we compared sampling within the 4th intercut and between consecutive samplings at the 3rd and 4th intercut. However, the movement was higher after than before alfalfa cutting if we compared consecutive samplings at the 4rd and 5th intercut (Table 1.2).

Table 1.2. Results of Fisher's exact test of movement of carabid species from adjacent crops (alfalfa, maize) or margin between samplings after alfalfa cutting (Ac) vs. before alfalfa cutting (Bc) within intercut and between consecutive cuttings.

		Movement from alfalfa to		Movement from maize to	
Species	Intercut	margin	maize	margin	alfalfa
C. fuscipes	3rd (Ac vs Bc)	P < 0.001	P = 0.117	P = 0.078	P = 0.032
	4th (Ac vs Bc)	P = 0.033	P = 0.006	P < 0.001	
	3rd Bc vs 4th Ac	P < 0.001	P = 0.013	P < 0.001	
	4th Bc vs 5th Ac	P < 0.001	P < 0.001	P = 0.007	P = 0.019
P. rufipes	3rd (Ac vs Bc)				
	4th (Ac vs Bc)	P < 0.001	P = 0.455	P < 0.001	P < 0.001
	3rd Bc vs 4th Ac				
	4th Bc vs 5th Ac	P = 1	P < 0.001	P = 0.998	P = 0.117
Metallina sp.	3rd (Ac vs Bc)		P = 0.259		P = 0.779
	4th (Ac vs Bc)				P = 0.020
	3rd Bc vs 4th Ac	P = 0.348	P = 0.245		P = 0.010
	4th Bc vs 5th Ac				P = 0.159
P. cupreus	3rd (Ac vs Bc)	P = 0.009	P = 0.004	P = 0.010	
	4th (Ac vs Bc)	P < 0.001	P = 0.586	P = 0.226	P = 0.593
	3rd Bc vs 4th Ac	P = 0.013	P = 0.612	P < 0.001	P = 0.353
	4th Bc vs 5th Ac	P < 0.001		P = 0.182	P = 0.210

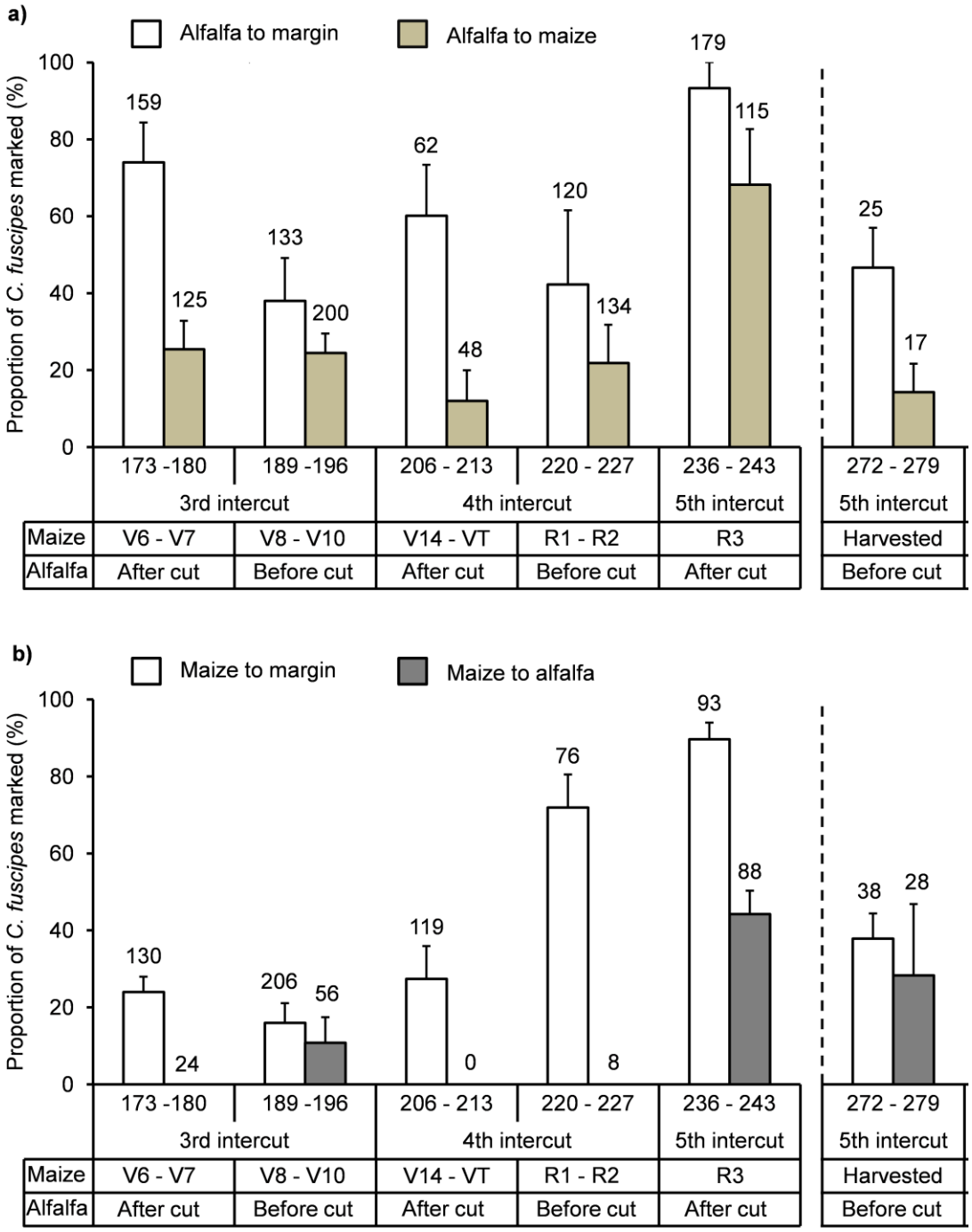

Figure 2.2. Mean proportion (± SE) of *Calathus fuscipes* marked with rubidium in the sampling periods expressed in Julian calendar days, collected from the 3rd to 5th alfalfa intercuts. Maize development growth stage is presented according to Ritchie et al. (2005) and alfalfa is expressed according to management, before (30-45 cm high) and after (0-10 cm high) cutting. (**a**) Movement from alfalfa to margin (white bars) and to maize (filled bars) and (**b**) movement from maize to margin (white bars) and to alfalfa (filled bars). The numbers above the standard error bars indicate the number of insects collected. Alfalfa cutting dates were days 170, 203 and 233 and maize harvesting date was at day 270 of the Julian calendar.

In the case of maize, after alfalfa cutting we observed a significant increase in the number of insects marked in the margin but only when we compared consecutive "intercuts". Otherwise, if we compared samplings within intercuts, we only observed a significant increase in the number of marked individuals in the 4th intercut (Table 1.2). The movement from maize to alfalfa was significantly higher before than after alfalfa cutting when we compared within the 3rd intercut. However, opposite results were observed when we compared consecutive samplings at the 4rd and 5th intercuts (Table 1.2). Comparison within the 4th intercut and between consecutive samplings at the 3rd and 4th intercuts was not possible because *C. fuscipes* was not caught in maize after alfalfa cutting at the 4th intercut (Figure 2.2b). The magnitude of movement from alfalfa to the margin was always higher than movement from alfalfa to maize in all sampling periods. Similar results were observed on the magnitude of movement from maize, the only exception being at the 3rd intercut before alfalfa cutting (Table 2.2). No differences in the magnitude of movement after maize harvest were found (Table 2.2).

Pseudophonus rufipes was only collected during the 4th and 5th intercut in the margin (53%) and in alfalfa (39%) and maize (8%). We observed movement between crops and from crops to the margin (Figure 3.2a and 3.2b). At the 4th intercut the movement from crops to the margin and from maize to alfalfa was significantly higher before than after cutting (Figure 3.2a and 3.2b, Table 1.2). Movement from alfalfa to maize was only observed after alfalfa cutting but significant differences were only observed between intercuts (Figure 3.2a, Table 1.2). The magnitude of movement from alfalfa to the margin or to maize was only significantly different in the sampling at the 4th intercut before alfalfa cutting, with more movement to the margin than to maize (Table 2.2). The movement from maize to the margin was always significantly greater than the movement from maize to alfalfa (Table 2.2). After maize harvesting, *P. rufipes* was only collected in alfalfa but no movement was observed.

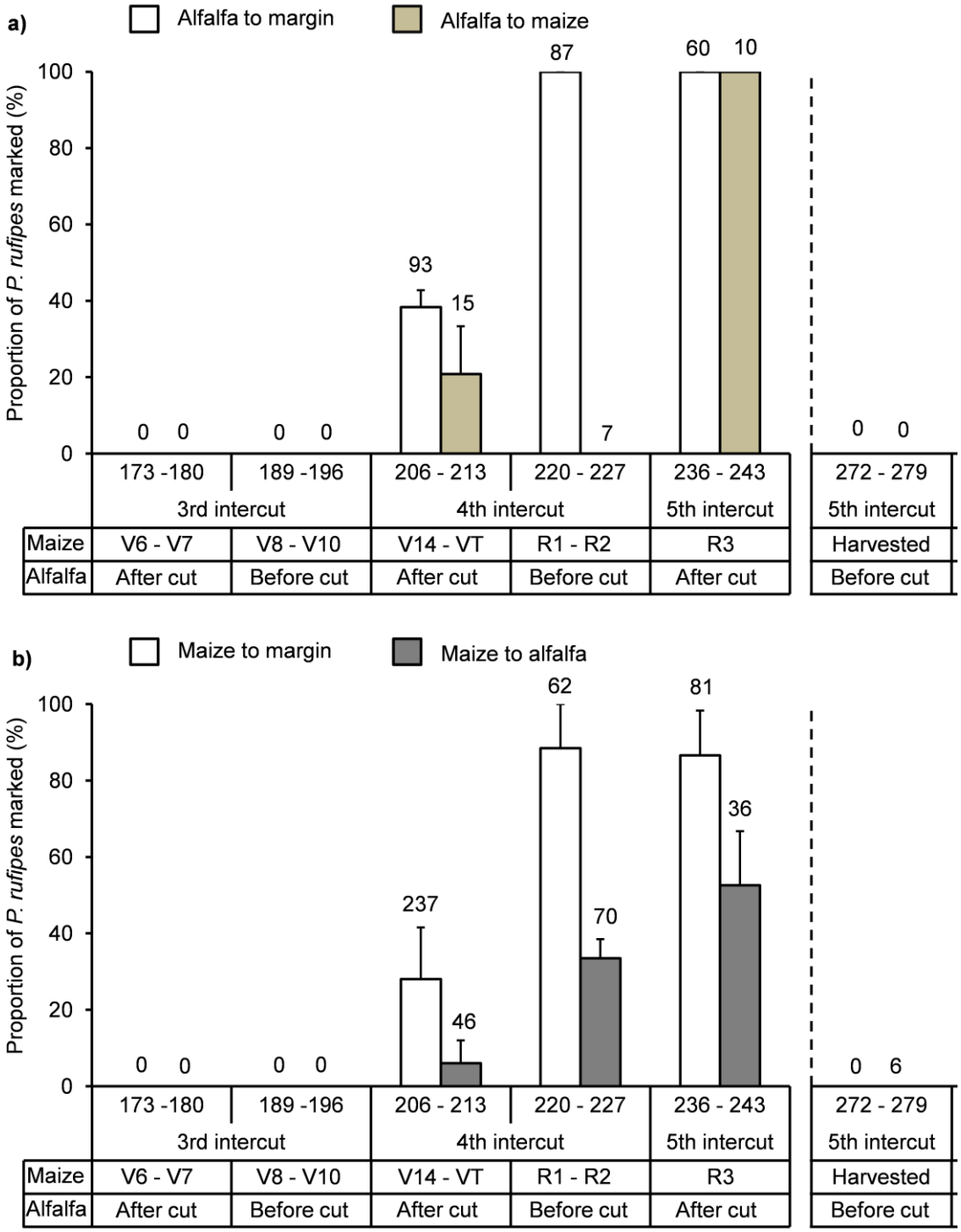

Figure 3.2. Mean proportion (± SE) of *Pseudophonus rufipes* marked with rubidium in the sampling periods expressed in Julian calendar days, collected from the 3rd to 5th alfalfa intercuts. Maize development growth stage is presented according to Ritchie et al. (2005) and alfalfa is expressed according to management, before (30-45 cm high) and after (0-10 cm high) cutting. (**a**) Movement from alfalfa to margin (white bars) and to maize (filled bars) and (**b**) movement from maize to margin (white bars) and to alfalfa (filled bars). The numbers above the standard error bars indicate the number of insects collected. Alfalfa cutting dates were days 170, 203 and 233 and maize harvesting date was day 270 in the Julian calendar.

Metallina sp. was collected in alfalfa (65%) in all sampling periods, in maize (22%) in all samplings except in the 4th intercut before alfalfa cutting, and in the margin (13%) mainly after alfalfa cutting. Movement from alfalfa to the margin was only observed after alfalfa cutting. Movement from alfalfa to maize was only recorded at the 3rd intercut before alfalfa cutting and the consecutive sampling after alfalfa cutting at the 4th intercut, and no differences in the proportion of marked individuals were found (Table 1.2, Figure 4.2a). The movement from maize to the margin was only observed after alfalfa cutting (Figure 4.2b). Movement to alfalfa was always observed (Figure 4.2b). Movement was not enhanced by alfalfa cutting since a significantly higher proportion of marked individuals were found before than after alfalfa cutting (Figure 4.2b, Table 1.2). No significant differences in the magnitude of movement to the margin or to adjacent crops were found (Table 2.2). After maize harvesting we only observed movement from maize to alfalfa (Figure 4.2b).

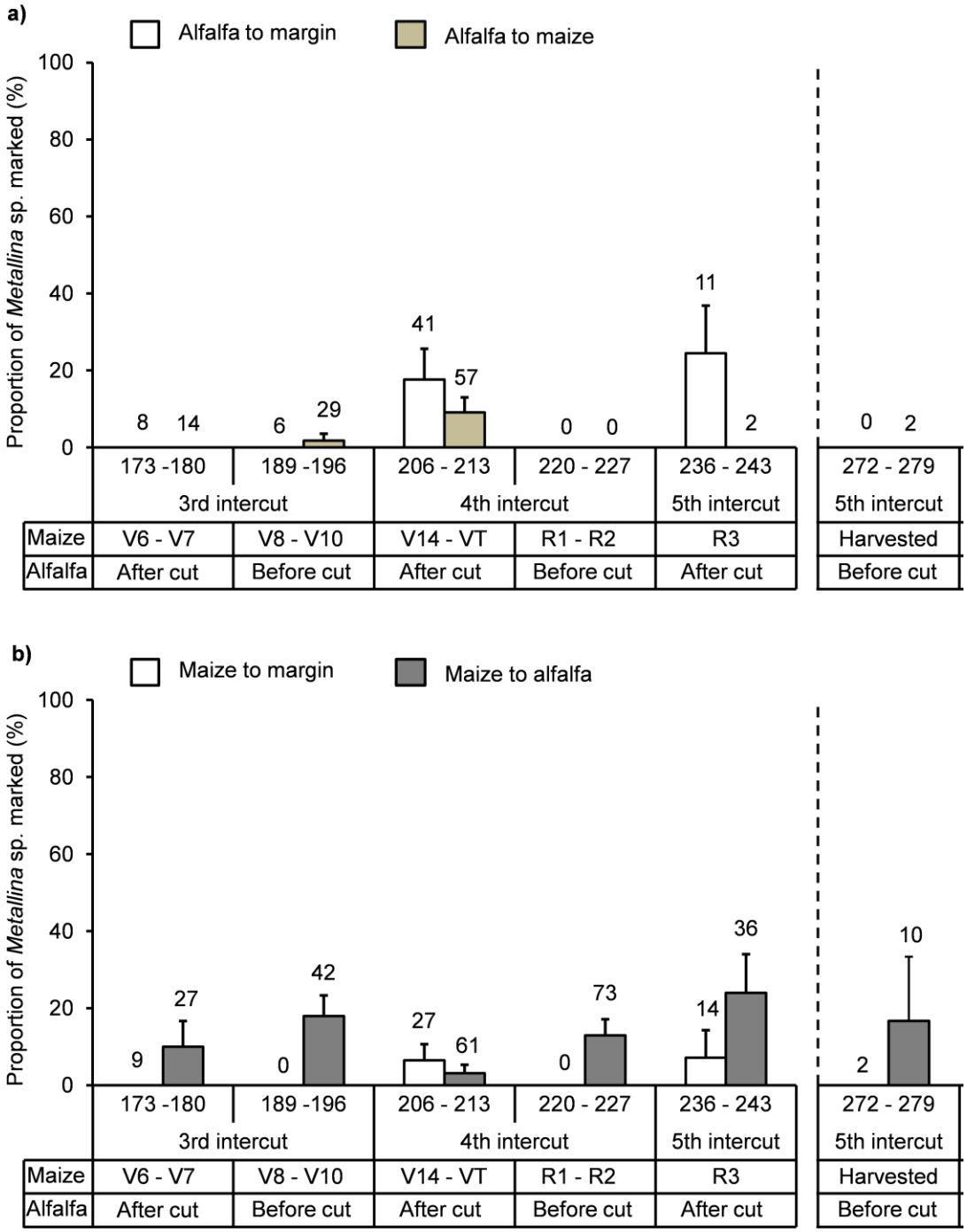

Figure 4.2. Mean proportion (± SE) of *Metallina* sp. marked with rubidium in the sampling periods expressed in Julian calendar days, collected from the 3rd to 5th alfalfa intercuts. Maize development growth stage is presented according to Ritchie et al. (2005) and alfalfa is expressed according to management, before (30-45 cm high) and after (0-10 cm high) cutting. (**a**) Movement from alfalfa to margin (white bars) and to maize (filled bars) and (**b**) movement from maize to margin (white bars) and to alfalfa (filled bars). The numbers above the standard error bars indicate the number of insects collected. Alfalfa cutting dates were days 170, 203 and 233 and maize harvesting date was day 270 of the Julian calendar.

Poecilus cupreus was collected in the margin (45%) in all sampling periods, in maize (29%) during the 3rd and 4th intercut, and in alfalfa (26%) in all samplings except after alfalfa cutting at the 3rd intercut (Figure 5.2a and 5.2b). We observed movement from alfalfa to the margin in all sampling periods, and a significant increase of movement after alfalfa cutting (Figure 5.2a, Table 1.2). The movement to maize decreased during the samplings. Significant differences on the proportion of individuals marked between samplings were only observed within the 3rd intercut, with higher movement after alfalfa cutting (Figure 5.2a, Table 1.2). Movement from maize to the margin was observed in all sampling periods except after maize harvesting. We observed more movement after than before alfalfa cutting. However, this difference was only significant in the 3rd and 4th intercuts (Table 1.2, Figure 5.2b). No differences in the proportion of individuals marked in alfalfa were observed between sampling before and after alfalfa cutting (Table 1.2, Figure 5.2b). Alfalfa cutting had little influence on the magnitude of movement to maize or to the margin but, when it occurred, a higher proportion of individuals moved to the margin. Similar results were found for individuals leaving maize (Table 2.2). After the maize harvest, we observed movement from maize to alfalfa and from alfalfa to the margin and no individuals of *P. cupreus* were collected in maize (Figure 5.2a and 5.2b).

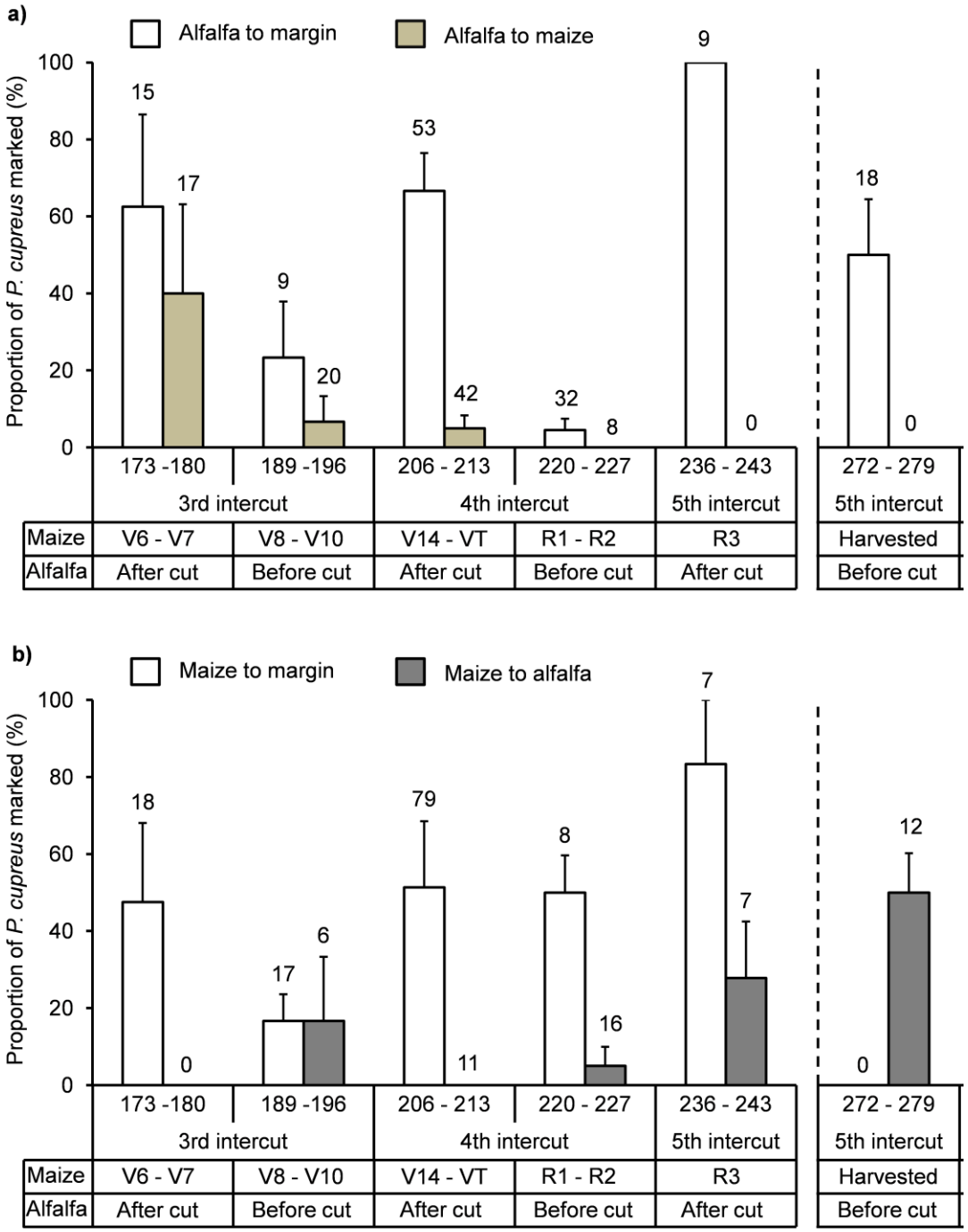

Figure 5.2. Mean proportion (± SE) of *Poecilus cupreus* marked with rubidium in the sampling periods expressed in Julian calendar days, collected from the 3rd to 5th alfalfa intercuts. Maize development growth stage is presented according to Ritchie et al. (2005) and alfalfa is expressed according to management, before (30-45 cm high) and after (0-10 cm high) cutting. (**a**) Movement from alfalfa to margin (white bars) and to maize (filled bars) and (**b**) movement from maize to margin (white bars) and to alfalfa (filled bars). The numbers above the standard error bars indicate the number of insects collected. Alfalfa cutting dates were days 170, 203 and 233 and maize harvesting date was day 270 of the Julian calendar.

Table 2.2. Results of Fisher's exact test of the magnitude of movement of carabid species to adjacent crop vs. to the margin at each sampling period. Ac, after alfalfa cutting; Bc, before alfalfa cutting; Mh, maize harvesting

Species	Intercut	Sampling	Move from Alfalfa to margin vs maize	Move from maize to margin vs alfalfa
C. fuscipes	3rd	Ac	P < 0.001	P = 0.004
		Bc	P = 0.002	P = 0.629
	4th	Ac	P < 0.001	
		Bc	P < 0.001	P < 0.001
	5th	Ac	P < 0.001	P < 0.001
		Mh	P = 0.463	P = 0.053
P. rufipes	3rd	Ac		
		Bc		
	4th	Ac	P = 0.055	P < 0.001
		Bc	P < 0.001	P < 0.001
	5th	Ac	P = 1	P < 0.001
		Mh		
Metallina sp.	3rd	Ac		P = 0.298
		Bc	P = 0.829	
	4th	Ac	P = 0.259	P = 0.360
		Bc		
	5th	Ac		P = 0.153
		Mh		
P. cupreus	3rd	Ac	P = 0.058	
		Bc	P = 0.220	P = 0.730
	4th	Ac	P < 0.001	P < 0.001
		Bc	P = 0.644	P = 0. 028
	5th	Ac		P = 0.084
		Mh		

4. Discussion

In Spain, carabids are one of the most prevalent ground-dwelling predator groups recorded in pitfall traps in alfalfa (Nuñez 2002; Pons et al. 2005) and in maize (De la Poza et al. 2005; Albajes et al. 2009). In our study, four species represent 90% of all carabids captured. *Pseudophonus rufipes*, *Metallina* sp. and *P. cupreus* have been reported as common in maize (De la Poza et al. 2005; Farinós et al. 2008; Pons and Eizaguirre 2009; Albajes et al. 2013) and in alfalfa (Nuñez 2002; Pons et al. 2005). However, in our study *C. fuscipes* was the most frequently captured species. It has been

reported to be widely distributed in the Iberian Peninsula (Nieto 2008; Ruiz et al. 2012) but this is one of the first reports in alfalfa and maize in Spain. This finding may be related to study location conditions (latitude, weather, landscape features, etc.), since our study was located in a mountain area and biodiversity of carabids in crops can be affected by these factors (Weibull et al. 2003).

Variations in the vegetation cover cause microclimatic differences and can influence carabid activity and species composition in the agricultural landscape (Cole et al. 2005; Eyre et al. 2013). Although maize and alfalfa have different canopy structures and vegetation covers, we observed that species composition was similar in both crops, which shared the four most abundant carabid species. While alfalfa is typically assumed to be a source of predators colonizing annual crops (Summers 1998; Nuñez 2002), little attention has been given to the opposite direction, especially after periodical cuttings of this perennial crop.

The exchange of generalist predators among adjacent habitats is well documented (Landis and Marino 1997; Bommarco and Fagan 2002; Tscharntke et al. 2005a; Blitzer et al. 2012). However, to our knowledge this work is the first study in the Iberian Peninsula that examines the movement of carabids between alfalfa and maize, taking into account alfalfa cutting. Movement patterns can help to identify habitats that act as a sink/source for carabid species at certain periods during the crop growing season.

We observed that the carabids moved continuously between alfalfa and maize during the growing season. Continuous movement of carabids between adjacent habitats is widely reported (e.g. Goulet 2003). Moreover, our results provide evidence of species-specific patterns. In studies using mark-recapture methods, species-specific movement between two adjacent cereal fields have also been observed. *Nebria brevicollis* Fabricius (Fernandez et al. 2000) and *Pterostichus melanarius* Illiger (Thomas et al. 1998) move between crops but *P. rufipes* remain within the hedgerow and field margins (Thomas et al. 1997). In our study we observed that *P. rufipes* also showed some preference to move from crop to margin.

Crop management practices such as crop cutting/harvesting can affect abundance of carabids (Kotze and Samways 1999; Rainio and Niemelä 2003) and also force them to move to alternative habitats (Ribera et al. 2001; Thorbek and Bilde 2004; Eyre et al. 2013). In our case, alfalfa cutting did not affect the movement of all carabids

in the same way. After an alfalfa cutting the margin proved to be the main refuge habitat for all carabid species since a consistent increase in movement to the margin was observed. Field margins have been reported as an important habitat for carabids, providing refuge from adverse agricultural activities as well as for overwintering (Holland and Luff 2000; Holland et al. 2005; Yu and Liu 2006; Benjamin et al. 2008).

The movement of *C. fuscipes*, *Metallina* sp. and *P. cupreus* between alfalfa and maize and vice versa does not seem to be strictly linked to alfalfa cutting because movement was variable throughout samplings and inconsistent effects after cutting were observed. On the other hand, *P. rufipes* only moved to maize after alfalfa cutting, which may indicate that maize can act as a refuge habitat. Landis et al. (2000) and Hossain et al. (2002) identified alfalfa strip cutting as a useful crop management system for conserving natural enemies after alfalfa cutting. However, in conditions in which the landscape is made up of small fields, this technique is not easy to apply (Pons and Eizaguirre 2009) and with our findings margins and adjacent maize can be seen as an alternative for these alfalfa strips.

In the case of movement from maize, after an alfalfa cutting we expected an increased movement to the margin and a decreased movement to alfalfa since carabids that usually move to alfalfa remain in the margin. However, only *Metallina* sp. increased the movement to the margin and no consistent differences in the movement towards alfalfa were observed before and after alfalfa cutting. The fact that we observed movement from maize to alfalfa after cutting could be related to food source availability due to aphids/prey insects dropping onto the ground after cutting and/or the time that pitfall traps remained in the field (seven days), enough time for alfalfa regrowth and carabid recolonization.

Carabid movement between crop fields and other habitats can also be related to the search for appropriate overwintering sites (Pfiffner and Luka 2000). In our study we can assume that alfalfa can be an overwintering habitat for maize carabids, particularly *P. rufipes* and *P. cupreus*, since movement from maize to alfalfa increased during the growing season and after maize harvesting. *P. rufipes* was only collected in alfalfa and *P. cupreus* was collected both in alfalfa and in the margin. This finding agrees with those of (Brewer and Goodell 2012), who describe alfalfa as an overwintering habitat for carabids.

Other causes of local differences in carabid activity could be linked to the dietary preferences of the species and food availability (Frampton et al. 1995). Carabids are described as one of the most important generalist predator groups in pest management strategies (Lövei and Sunderland 1996). In our study, all species analyzed have been described as carnivorous, although *P. rufipes* can also feed on seeds (Toft and Bilde 2002; Purtauf et al. 2005b). Aphids are one of the main prey of several carabid species (Lövei and Sunderland 1996). Consequently, movement of carabids may be related to the availability and distribution of aphids (Winder et al. 1994; Schmidt et al. 2003; Al Hassan et al. 2013). In Spain, aphids are one of the main pests of alfalfa and maize (Pons et al. 2005; Meissle et al. 2010) and are present in alfalfa throughout the growing season (Pons et al. 2013). However, we could not exclusively relate the movement of carabids between crops with typical peaks of aphids in these crops. Seed availability can also affect the movement of carabids (Jonason et al. 2013) and non-crop habitats such as margins and grasslands can be an important source of seed to carabid seed predators (Purtauf et al. 2005a), possibly explaining the high abundance of *P. rufipes* in the margin, which can provide more weed seeds than crops.

In conclusion, carabid spillover between alfalfa and maize is highly species-specific and continuous but may be affected by crop management. The association of alfalfa and maize with the presence of margins may conserve and enhance the populations of carabids and therefore contribute to the effectiveness of conservation biological control practices. Margins and maize can act as a refuge and source to recolonize alfalfa after cuttings and could act as an alternative to alfalfa strip cutting, especially in areas with small fields, as in many Mediterranean regions. Margins and alfalfa can act as overwintering habitats for carabids after maize harvesting and may foster the early colonization of maize. Our results also suggest that rubidium can be used as a marker to elucidate carabid movement between alfalfa and maize.

Acknowledgements

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CHAPTER 3

CHANGE IN CARBON STABLE ISOTOPE RATIOS OF THE PREDATORY BUG ORIUS MAJUSCULUS AFTER DIETARY SHIFTS

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Change in carbon stable isotope ratios of the predatory bug *Orius majusculus* after dietary shifts

Abstract

Orius majusculus Reuter (Hemiptera: Anthocoridae) is an important component of the pest predatory complex in arable crops in Mediterranean areas. It moves between crops searching for prey, and improving knowledge on its dispersal abilities will help to develop conservation biological control strategies. Stable isotope ratios may be used as a tool for tracking insect movements, as the isotopic composition of insect tissues changes to reflect that of their diet when they undergo dietary shifts on moving between isotopically distinct crops. We carried out laboratory diet switch experiments with a stable isotope approach to infer information on dispersal of *O. majusculus* individuals among C3 and C4 crops to better understand isotopic field data collections. Switching the aphid food source caused a quick change in $\delta^{13}C$ signatures, regardless of the original and final food source. Changes in the $\delta^{13}C$ ratio of *O. majusculus* after diet switching fitted with an exponential model that showed similar turnover rates, and thus half-lives, between shifting diets up to 20 days. Subsequently, whereas individuals feeding on C4 aphids did not survive, turnover rate decreased in individuals that switched from C4 to C3 aphids. However, $\delta^{13}C$ traces from the original source remained in the predator until 25 days after switching, and this is enough time to help determine the movement of *O. majusculus* between crops in the field and to plan the timing of predator sampling and crop practices that may enhance predator ecological services. *Orius majusculus* that switched to a maize aphid diet showed different turnover rates between sexes, although this did not influence the pattern of switchover.

Key words: Hemiptera, Anthocoridae, Aphididae, sucking predators, aphids, barley, maize, predator movement, conservation biological control.

1. Introduction

Conservation biological control consists in fostering the action of the established natural enemies in a given agroecosystem in order to maintain pests below the economic thresholds (Ehler 1998; Landis et al. 2000). Many of the natural enemies that inhabit arable crops share preys in the different crops that form the agricultural landscape, and spill-over of natural enemies between crops occurs. Determining the precise time when natural enemies shift between crops will improve the management of resources for implementing conservation biological control.

In many Mediterranean areas, the agricultural landscape of arable crops is characterized by a dynamic mosaic of maize, alfalfa, and winter cereals. Growth cycles of the three crops are partially overlapping: alfalfa and winter cereals in spring, alfalfa and maize in summer, and the three crops in late spring and autumn. The coincidence of crop cycles and field neighbourhood allows arthropods to move from one crop to the other, but whereas only winter cereals and maize may share some herbivores, their natural enemies (predators and parasitoids) feed on herbivores from the three crops (Pons and Eizaguirre 2009).

Orius majusculus Reuter (Hemiptera: Anthocoridae) and other species of the genus are a major component of the complex of predators in arable crops in Mediterranean areas (Bourguet et al. 2002; Pons et al. 2004; De la Poza et al. 2005; Pons and Eizaguirre 2009). *Orius majusculus* is a generalist predator that feeds on soft-bodied arthropods such as thrips, aphids, leafhoppers, and mites, in addition to the eggs and young larvae of lepidopterans (Péricart 1972; Reid 1991; Riudavets 1995; Lattin 1999).

In the north-east of the Iberian Peninsula, field population surveys have been used to hypothesize that *O. majusculus* individuals spend the autumn and winter in alfalfa crops, moving to maize in spring and returning to alfalfa in late summer (Pons et al. 2005). To test this hypothesis and to determine when movement between crops occurs, we must ascertain the movement of the insect; several techniques for marking and tracking movement are available (Reynolds et al. 1997; Hagler and Jackson 2001; Lavandero et al. 2004).

Plants themselves may be used as a marker for tracking insects through their natural ratio of stable isotopes. Insects moving between isotopically distinct food webs

can carry information on the location of previous feeding sites that helps to trace their movement and dispersal (Gould et al. 2002). In particular, carbon stable isotope ratios have been tested for tracking the plant origin of herbivores and predator insects (Prasifka et al. 2004; Prasifka and Heinz 2004; Vialatte et al. 2006; Traugott et al. 2008; Schallhart et al. 2009, 2011; Forbes and Gratton 2011; Ouyang et al. 2012). Maize plants differ fromwinter cereals and alfalfa because they follow Hatch-Slack's photosynthetic pathway (C4 plants) instead of Calvin's pathway (C3 plants) and this implies differences in the relative abundance of the stable isotope ^{13}C. The bodies of the herbivores feeding on such plants reflect the overall isotopic composition of their food, and this composition is maintained to the upper trophic levels in the food web with a fractionation of about 0 ± 1‰ at each trophic level (DeNiro and Epstein 1978; Ostrom et al. 1997; Post 2002). However, when an animal changes its food source from a C3 to a C4 plant (or vice versa), the isotopic composition of its body gradually moves towards the value associated with the new food source (Ostrom et al. 1997; Prasifka et al. 2004).

The time required to complete exchange of carbon isotope ratio from one source to another or the rate at which the change occurs is very important for determining when the insects shift between crops. Knowing how food source switching affects the isotope signature of a species will give information about how stable isotope data collected in the field should be interpreted (Traugott et al. 2007) and will improve the implementation of conservation biological control. Laboratory experiments are therefore a very useful tool for obtaining this type of information (Ostrom et al. 1997; Prasifka et al. 2004; Gratton and Forbes 2006).

The aim of this laboratory study was to determine the isotopic ^{13}C turnover rate of *O. majusculus* shifting its diet from prey fed on C3 to prey fed on C4 source plants, and vice versa, and to determine how long traces of the old diet remain. Estimating the timing of diet shift is crucial to interpret stable isotope data in field studies of the movement and dispersion of predators between isotopically different crops and the time when the shift between crops occurs. In field studies, we recorded significantly more catches of males than females in yellow sticky traps placed on maize (F Madeira and X Pons, unpubl.), suggesting different dispersion capacity between sexes. Therefore, we also aimed to determine whether the turnover was influenced by sex. For this purpose, we developed a laboratory based diet shifting experiment using aphids fed on maize as

C4 and barley as C3 food sources, which were supplied ad libitum to *O. majusculus* individuals.

2. Materials and methods

Experiments were conducted in the Laboratory of Entomology of the Department of Crop and Forest Sciences of the University of Lleida (Spain). Isotope analyses were performed at the Laboratory of Applied Ecology of the Environmental Biology Department of the University of Rome – Sapienza (Italy).

The pirate bug, *O. majusculus*, as the predator, and the aphid *Rhopalosiphum padi* L. (Hemiptera: Aphididae), as the herbivore prey, were used in the experiment. The aphid *R. padi* is one of the most common species living on Poaceae including winter cereals and maize worldwide (Blackman and Eastop 2000). All insects were obtained from long-dated cultures maintained in the Laboratory of Entomology of the University of Lleida. The predators were reared in 2-l glass jars in a climatic chamber at 24 ± 1 °C, 75 ± 10% r.h., and L16:D8 h photoperiod, fed ad libitum with frozen eggs of *Ephestia kuehniella* Zeller (Biotop, Livron sur Drome, France) and provided with water for drinking and green bean pods as an egg-laying substrate. The culture originally consisted of individuals collected in the Lleida area and was restocked yearly with field individuals. Aphids came from a culture maintained on barley in the greenhouse for more than 5 years. In order to have two food sources for feeding *O. majusculus*, two colonies of *R. padi* were reared in a greenhouse at 25 ± 5 °C and natural photoperiod: one on plants of maize cv. DKG 6450 (Monsanto, Madrid, Spain) and the other on plants of barley cv. Graphic (RAGT Ib_erica, Palencia, Spain). Maize plants for the experiment were grown in plastic pots (25 cm diameter, 20 cm high) using Tray substrate (Klasmann-Deilmann, Geeste, Germany) inside a cage (1.8 × 1.5 × 1.5 m). The cage walls were built with mesh to avoid the escape or entry of insects. Maize plants were infested with aphids from the rearing when they were at the 12-leaf stage and aphids were maintained in the cage for 3 weeks before the experiment started. This period was long enough to ensure that all aphids used in the experiment were born and fed all their life on maize and no traces of barley remained (Asín and Pons 2001). Pots with 12 to 15 leaf stage maize plants free of aphids and other insects were added to the cage every 2 weeks in order to supply fresh food and to maintain the aphid colony.

Barley was grown inside cylindrical cages (40 cm diameter, 60 cm high), in clay pots (16 cm diameter, 15 cm high) with the same type of substrate as that used for maize. Each cage was protected from outside contamination by aphids or other insects with a mesh bag. Pots with seedling barley plants were added weekly to the cage to maintain the aphid barley culture in good condition before the feeding experiment started.

The feeding experimental design is shown in Figure 1.3. Green bean pods with eggs laid the same day from the *O. majusculus* culture were collected and separated in glass jars. The jars were covered with a mesh for aeration and supplied with frozen eggs of *E. kuehniella* and vials containing water. The nymphs that hatched were maintained in the jars for 3 days to reduce mortality. After this time, 300 nymphs were collected and randomly assigned to clean jars covered with a mesh for aeration and to prevent escaping until adult emergence. Each jar contained wrinkled paper to provide refuges and to prevent cannibalism, and one 5-ml vial containing water covered with cotton wool for drinking. Every day, each jar was supplied with a sufficient amount of aphids reared on maize (C4) or barley (C3) plants and water. When insects reached the adult stage, the individuals of each jar were separated into two groups of equal numbers; one continued being fed with aphids from the same type of plant (C3 or C4 plants) and the other was placed in a new jar and fed with aphids from the other type of plant. Therefore, we established four *O. majusculus* food source treatments: (1) always fed with aphids from C3 barley plants (C3 control); (2) fed with aphids from C3 barley plants until adult emergence and switching to feeding with aphids from C4 maize plants for 25 days; (3) always fed with aphids from C4 maize plants (C4 control); and (4) fed with aphids from C4 maize plants until adult emergence and switching to feeding with aphids from C3 barley plants for 25 days, which is about 80% of the average adult life span in laboratory cultures (Lattin 1999; Pumariño and Alomar 2012). Although we used ‘always’ to designate the C3 or C4 controls, note that the *O. majusculus* were feeding on *E. kuehniella* during the first 3 days of their life. Samples of leaves, aphids, eggs of *E. kuehniella*, and *O. majusculus* from the stable culture were periodically analysed for $\delta^{13}C$ ratio as a reference to compare $\delta^{13}C$ fractionation between trophic levels (Table 1.3).

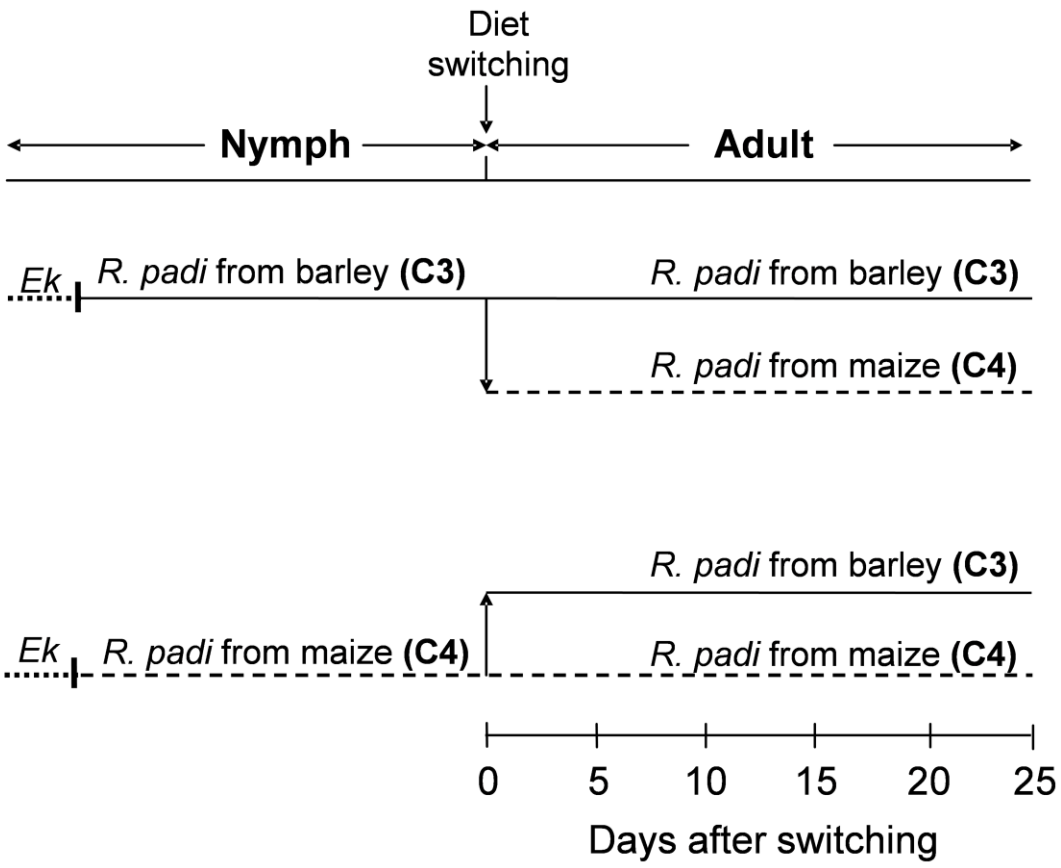

Figure 1.3. *Orius majusculus* diet switching experimental design. Nymphs were fed with eggs of *Ephestia kuehniella* (Ek) during their first 3 days and then fed with aphids (*Rhopalosiphum padi*) living on C3 (barley) or C4 (maize) plants until adult emergence, when 50% of the individuals were switched to feeding on aphids rearing on the other type of plant and the other 50% maintained their diet (as experimental control). Samples of adults of *O. majusculus* for each diet were collected for isotopic analysis on days 0, 5, 10, 15, 20, and 25 after diet switching.

In order to determine the temporal changes in the $\delta^{13}C$ isotope signature, sets of 5–10 individuals of *O. majusculus* were collected for each of the four treatments at 0, 5, 10, 15, 20, and 25 days after adult emergence and sex was determined. Bugs that died during the experiment were discarded.

Samples were placed in an oven at 60 °C for at least 72 h. Entire individuals of *O. majusculus* were analysed singly, whereas aphids and *E. kuehniella* eggs were transformed into a fine, homogeneous powder using an agate mortar. Leaves were transformed to a fine, homogeneous powder using a ball-mill (Mini-Mill Fritsch Pulverisette 23 with a zirconium ball; Fritsch Instruments, Idar-Oberstein, Germany).

An aliquot of each powdered sample was analysed (0.160–0.190 and 0.700–0.800 mg dry weight for aphids/*E. kuehniella* eggs and leaves, respectively).

Carbon isotope analyses were carried out using a continuous flow isotope ratio mass spectrometer coupled with an elemental analyser (IRMS Finnigan Delta Plus and FlashEA 1112 series; Thermo Fisher Scientific, Waltham, MA, USA). The carbon isotopic contents were expressed in "δ" units as the relative difference of the isotopic ratio (in parts per thousand) between the sample and international standard of Vienna PeeDee Belemnite (PDB) according to the formula: $\delta R(‰) = [(Rsample - Rstandard)/Rstandard] \times 10^3$ (Ponsard and Arditi 2000; Vander Zanden and Rasmussen 2001), where R is the ratio $^{13}C/^{12}C$.

2.1 Data analysis

Differences in $\delta^{13}C$ signatures between C3 and C4 plants and between just-moulted (day 0) adults of *O. majusculus* continuously reared as nymphs with eggs of *E. kuehniella* and adults reared with aphids fed on maize or barley from the 3rd day of nymphal development were analysed with a Student's t-test. Before the statistical analysis, the Shapiro–Wilk test was used to test normality of data.

In order to know the proportion of $\delta^{13}C$ change at a given time after diet switching (Ct), we considered the isotopic difference between the new and the old resource as 100% and Ct was calculated as $Ct = [(\delta Ct - \delta 0) / (\delta n - \delta 0) \times 100]$, where $\delta Ct = \delta^{13}C$ of *O. majusculus* at time t, $\delta 0 = \delta^{13}C$ of the old diet, and $\delta n = \delta^{13}C$ of the new diet.

Several studies have demonstrated that tissue isotopic composition changes after a diet shift follow an exponential model (Phillips and Eldridge 2006; Cerling et al. 2007; Klaassen et al. 2010). We fitted $\delta^{13}C$ changes over time of *O. majusculus* according to diet switch from C4 to C3 diet or from C3 to C4 diet to exponential decay or growth curves, respectively, using the following equation:

$$\delta(t) = \delta(n) + (\delta(0) - \delta(n))e^{-\lambda t}, \qquad \text{(Equation 1)}$$

where δ(t) is the *O. majusculus* isotopic signature at some time t (days) after the diet switch, δ(n) and δ(0) are the *O. majusculus* isotopic signature in equilibrium with the new and old diet, respectively, and λ is the turnover rate of the isotope in *O. majusculus* as a result of metabolic and growth processes. As *O. majusculus* adults were used in the experiments, the turnover rate (λ) was all attributed to the metabolic process, assuming

that growth is zero. This constant can also be used to calculate the turnover half-life [HL = ln(2) ⁄ λ], where HL is the half-life in days (Phillips and Eldridge 2006). Exponential curves (Equation 1) were calculated separately for each shift diet treatment (C3 to C4 vs. C4 to C3) using the nonlinear least squares function (nls) (Rossiter 2009). The estimated isotopic turnover rate between shift diet treatments was compared using a t test (Motulsky and Christopoulos 2003).

To calculate the estimated time since the diet shift, we used equation 2, which is a rearrangement of Equation 1 (Klaassen et al. 2010):

$$t = \frac{\ln\left(\frac{\delta(0) - \delta(n)}{\delta(t) - \delta(n)}\right)}{\lambda}$$

(Equation 2)

This estimated time was compared with the actual time obtained in the experiments in order to determine whether the model over- or underestimated the time at which a $\delta^{13}C$ value occurs.

To determine whether the turnover was influenced by sex, we fitted males and females data separately to the exponential model. Differences in the isotopic turnover ratios between sexes were analysed using an F-test (Motulsky and Ransnas 1987). Only the significant results between sexes were represented graphically. Statistical analysis was performed using R (R Development Core Team 2011).

3. Results

As expected, barley and maize plants had different isotope ratios ($t = -37.20$, d.f. = 6, $P < 0.0001$), which were reflected in aphids feeding on plants and in *O. majusculus* feeding on aphids (Table 1.3). Aphids reared on maize were 1.56‰ enriched, and aphids reared on barley were 0.79‰ depleted in $\delta^{13}C$ in comparison with their respective food source (Table 1.3). *Orius majusculus* that fed on aphids reared on maize showed 0.24‰ enrichment in $\delta^{13}C$, whereas those fed on aphids reared on barley showed an enrichment of 2.59‰. The emerged adults (at day 0) always fed on eggs of *E. kuehniella* had significantly different $\delta^{13}C$ signatures than emerged individuals reared with barley or maize aphids from the 3rd day of their nymphal development (t =

-4.61, d.f. = 17, P < 0.001, and t = 54.66, d.f. = 6, P < 0.001, respectively). This feeding time was enough to ensure the loss of the signature of the artificial diet.

Table 1.3. Carbon isotope ratios (mean $\delta^{13}C \pm SE$) of eggs of *Ephestia kuehniella*, plants, aphids, and predators that were always fed with the same diet (controls) and isotope shifts ($\Delta\delta^{13}C$) between sources and consumers.

Plants and insects	n	$\delta^{13}C$	$\Delta\delta^{13}C$
a. Maize	5	-14.27 ± 0.19	
b. *Rhopalosiphum padi* on maize	6	-12.71 ± 0.11	1.56 (b-a)
c. *Orius majusculus* always fed with maize *R. padi*	48	-12.47 ± 0.14	0.24 (c-b)
d. Barley	5	-29.96 ± 0.38	
e. *Rhopalosiphum padi* on barley	6	-30.75 ± 0.20	-0.79 (e-d)
f. *Orius majusculus* always fed with barley *R. padi*	64	-28.16 ± 0.07	2.59 (f-e)
g. Eggs of *E. kuehniella*	6	-26.55 ± 0.13	
h. *Orius majusculus* always fed with eggs of *E. kuehniella*	6	-26.84 ± 0.03	-0.29 (h-g)

n = number of samples (from day 0 to the last day of the experiment).

Temporal variation of *O. majusculus* $\delta^{13}C$ during the adult life span, when individuals were fed with aphids from maize or barley and when their diet was switched, is shown in Figure 2.3. The predators reared on, or switched to, aphids from maize were able to survive only until 20 days after adult emergence, whereas the predators reared on, or switched to, aphids from barley survived 25 days after adult emergence.

The $\delta^{13}C$ values changed very fast when diet switched, regardless of the initial food source (Figure 2.3). Five days after switching from a C3- to a C4-based diet, an enrichment in $\delta^{13}C$ of 8.85‰ was observed, in comparison with individuals maintained always on barley aphids, whereas the change from C4 to C3 resulted in a depletion of -8.71‰, in comparison with the individuals maintained always on maize aphids. In both cases, the $\delta^{13}C$ shift was ca. 60% of the difference between the new and old food source (Figure 2.3), more specifically 60.3% from C3 to C4, and 61.4% from C4 to C3.

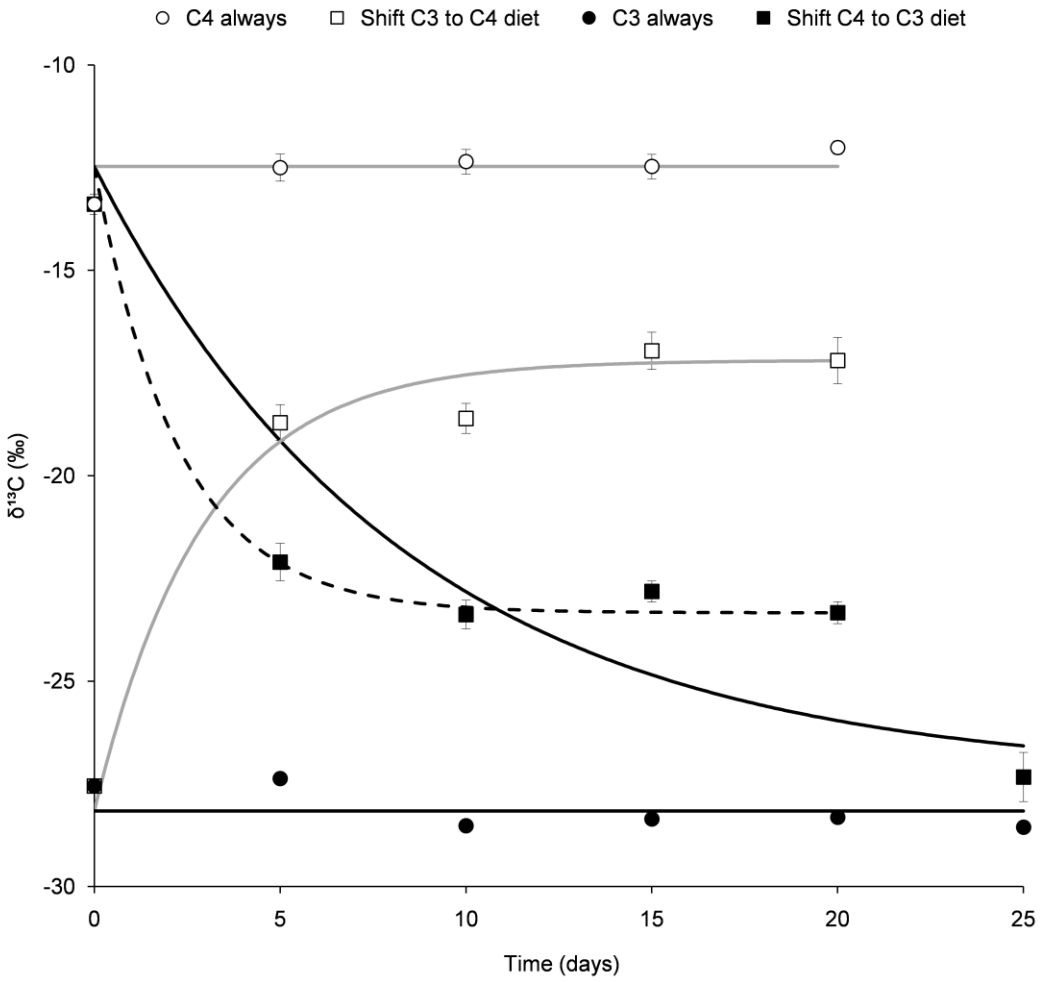

Figure 2.3. Carbon isotope ratios (mean $\delta^{13}C \pm SE$) (‰) of *Orius majusculus* adults fed over time (days) with aphids (*Rhopalosiphum padi*) reared on maize (C4 always; straight grey line) or barley (C3 always; straight black line) and after switching the diet (from C3 to C4 or from C4 to C3) during the feeding experiment. The grey curve represents an exponential growth model fitted to the experimental data when *O. majusculus* were shifted from C3 to C4 diet. The black drawn and dashed curves represent an exponential decay model fitted to the experimental data when *O. majusculus* were shifted from C4 to C3 diet up to 25 days and up to 20 days after switching, respectively.

Afterwards, the change in $\delta^{13}C$ slowed down, and 20 days after the diet switching, the shift was about 70% of the $\delta^{13}C$ difference between the new and old food source (69.9% from C3 to C4, 69.2% from C4 to C3). Until the end of the experiment, the complete turnover was not observed, but after 25 days, the $\delta^{13}C$ of predators switching from maize to barley aphids was -27.3 ± 0.60‰, which represented 92.4% of the difference between the new and old food source.

3.1 Model data fitting and model validity

Model fitting equations for each shifting diet treatment and the corresponding parameters are shown in Table 2.3. The $\delta^{13}C$ values of *O. majusculus* sampled up to 20 days after switching from a C3 to a C4 diet and from a C4 to a C3 diet fitted quite well with the exponential curve ($F_{1,48} = 44.58$ and $F_{1,41} = 38.08$, respectively; both $P < 0.0001$ and $R^2 = 0.93$), showing similar turnover rates ($t = 1.33$, d.f. = 87, $P = 0.19$) and half lives (Figure 2.3, Table 2.3). When C4 to C3 diet data were fitted up to 25 days, the fit with the simple exponential model was still good ($R^2 = 0.71$, $F_{1,57} = 40.28$, $P < 0.0001$), but the turnover rate was lower, with a higher half-life, than in the C3 to C4 diet shift ($t = 4.44$, d.f. = 101, $P < 0.001$; Table 2.3). However, the exponential models used to fit switched diet from C3 to C4 and from C4 to C3 (up to 20 days) generated, from day 10 onwards, an increasing underestimation of the time at which the $\delta^{13}C$ values were actually observed (Figure 3.3A and 3.3B). In the model used to fit switched diet from C4 to C3 up to 25 days, the underestimation occurred only from day 15, before which the values were overestimated (Figure 3.3C).

Table 2.3. Mean parameters (± SE) and equations of the exponential models [δ(t) =] describing $\delta^{13}C$ changes in time of *Orius majusculus* after diet shift treatments. Shift from C3 to C4: total (both sexes), males, and females. Shift from C4 to C3 up to 25 days and up to 20 days: total (both sexes).

	Shift from C3 to C4 diet			Shift from C4 to C3 diet	
Parameter	Total	Males	Females	Total (25 days)	Total (20 days)
λ (day^{-1})	0.31 ± 0.03	0.24 ± 0.02	0.27 ± 0.02	0.12 ± 0.01	0.44 ± 0.06
δ(0) (‰)	-28.16 ± 0.07	-28.13 ± 0.08	-28.21 ± 0.11	-12.47 ± 0.14	-12.47 ± 0.14
δ(n) (‰)	-17.19 ± 0.57	-18.17 ± 0.49	-15.57 ± 0.75	-27.33 ± 0.60	-23.33 ± 0.27
HL (days)	2.22	2.93	2.55	5.81	1.58
δ(t) =	$-17.19 - 10.96e^{-0.31t}$	$-18.17 - 9.96e^{-0.24t}$	$-15.57 - 12.64e^{-0.27t}$	$-27.33 + 14.87e^{-0.12t}$	$-23.33 + 10.86e^{-0.44t}$

λ, turnover rate; δ(0), isotopic signature of *O. majusculus* in equilibrium with the old diet; δ(n), isotopic signature of *O. majusculus* in equilibriumwith the new diet; HL, half-life.

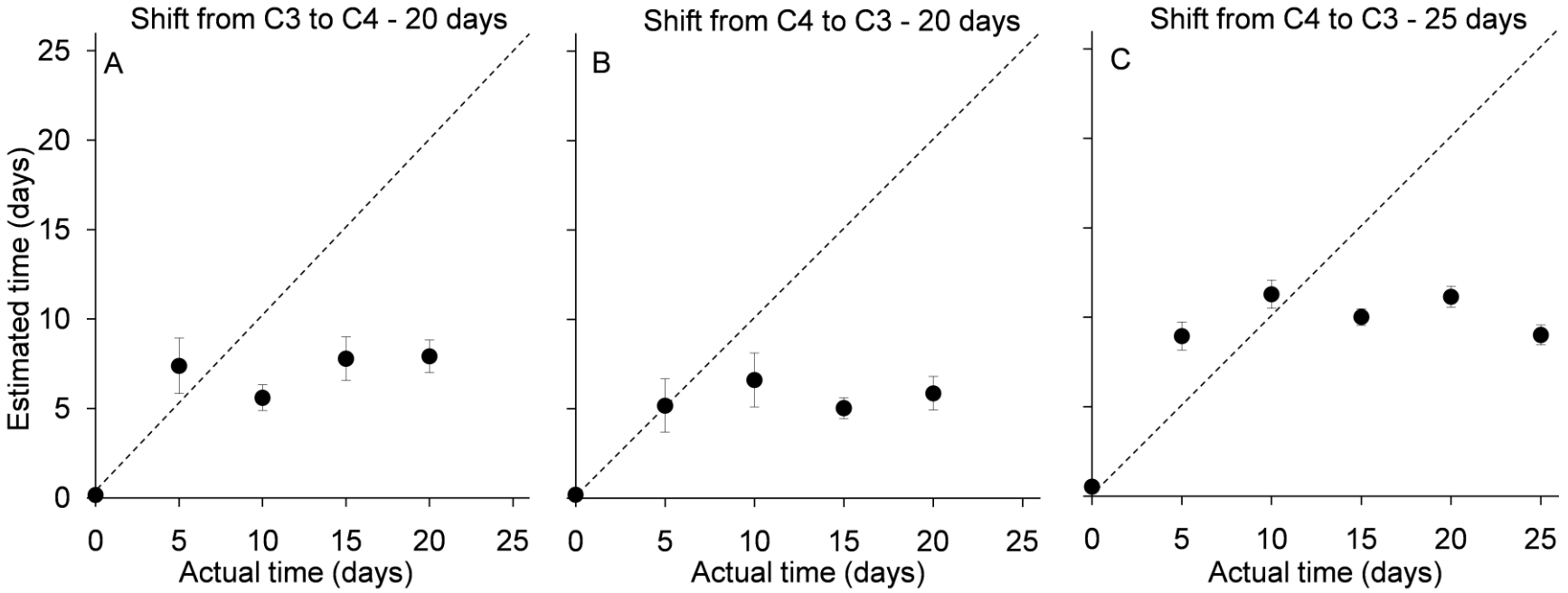

Figure 3.3. Model estimates of the mean (± SE) time required for $\delta^{13}C$ signatures of *Orius majusculus* since the diet switch (**A**) from C3 to C4 diet, (**B**) from C4 to C3 up to 20 days, and (**C**) from C4 to C3 up to 25 days, vs. actual times. Values on the left or on the right of the equality line show model over- and underestimation, respectively. Estimated time was calculated using equation 2 for each *O. majusculus* individual.

3.2 Sex effect

The food source based on aphids fed on barley caused no differences in the turnover rate between males and females of *O. majusculus*. No significant differences were observed between sexes when the diet was always based on barley aphids ($F_{2,66}$ = 0.67, P = 0.51). When the adult predators that were fed with maize aphids during the nymphal development were switched to the barley aphid food source, no significant differences in turnover rate between sexes were observed either (F2,54 = 1.41, P = 0.25).

On the other hand, turnover rates were significantly different between sexes when *O. majusculus* was continuously fed with ($F_{2,44}$ = 10.20, P < 0.0001), or switched to ($F_{2,45}$ = 6.57, P = 0.003), aphids reared on maize (Figure 4.3). *Orius majusculus* females switched to maize aphids showed a significantly higher turnover rate and consequently a lower half-life than males (Table 2.3). Although significant differences were observed in the turnover rate, the pattern of $\delta^{13}C$ growth curves was similar (Figure 4.3).

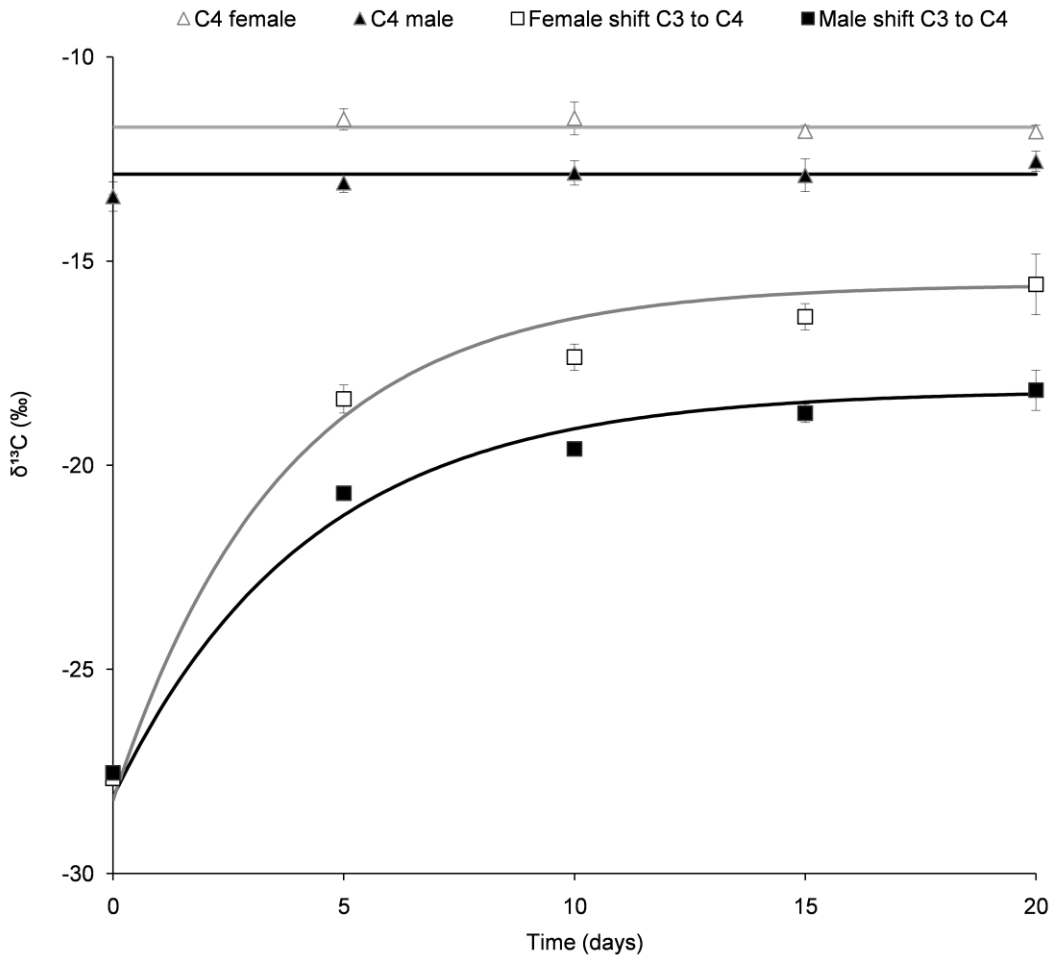

Figure 4.3. Carbon isotope ratios (mean $\delta^{13}C \pm SE$) (‰) of *Orius majusculus* males and females fed over time (days) on aphids (*Rhopalosiphum padi*) reared on maize (C4 females, straight grey line; C4males, straight black line) and after the switch from barley to maize aphids (C3 to C4). The grey and black curves represent an exponential growth model fitted to the experimental data when females and males, respectively, were shifted from C3 to C4 diet.

4. Discussion

The study of switching food sources based on $\delta^{13}C$ signature can help to delineate the temporal history of dietary intake of insects over a fairly long period and to trace the energy flow within agroecosystems (Ostrom et al. 1997; Gratton and Forbes 2006; Traugott et al. 2007). It also improves insect field sampling performance and interpreting field results, and helps to manage natural enemies within biological control strategies in arable crops (Ostrom et al. 1997; Prasifka et al. 2004; Forbes and Gratton 2011).

Several studies deal with food source switching in herbivores and predators, focusing on grasshoppers (Webb et al. 1998), wireworms (Traugott et al. 2007), and ladybirds (Ostrom et al. 1997; Prasifka et al. 2004; Gratton and Forbes 2006; Ouyang et al. 2012). Our study is the first one dealing with *O. majusculus*, a generalist and omnivorous predator that feeds on soft-bodied arthropods by sucking their contents (Lattin 1999) and that is very common in arable crops in the Mediterranean region (Pons and Eizaguirre 2009; Pons et al. 2009). Our study shows that the analysis of ^{13}C stable isotope is a useful method for determining the food source in small sucking predators such as *O. majusculus*. It also allows us to speculate about some aspects of their feeding behaviour and to determine the timing of the diet shift.

Exponential and other more complex models have been used to estimate the time since a diet shift for migratory vertebrates and errors in time calculation have also been observed (Phillips and Eldridge 2006; Oppel and Powell 2009; Klaassen et al. 2010). Our experimental data set fitted quite well to a simple exponential model although this kind of model is only a rough approximation of the real turnover processes (Martínez Del Rio and Anderson-Sprecher 2008). The model can be used to estimate some basic parameters of the diet shifting process such as the rate of turnover and the half-life, which provide important information for better understanding the field movement of *O. majusculus* between isotopically different crops. However, the model showed some limitations because it underestimated the time since the diet switch, especially in the asymptotic portion of the turnover curve. Specifically, the time estimated by the model was reliable for a relatively short period after diet shifting and the prediction errors increased after 10 days. In spite of these limitations, our results showed that traces of the old diet in *O. majusculus* were still detectable more than 20 days after diet shifting. This time is long enough to know whether *O. majusculus* has moved into a crop from another isotopically different one, and this information can be used in management strategies to adjust the timing of insect sampling and crop practices in order to improve conservation biological control.

The fitting of the experimental data set to the exponential model showed a higher turnover rate and a lower half-life in the C3 to C4 switching treatment (lasting 20 days) than in the C4 to C3 switching treatment (lasting 25 days). However, when data from the latter treatment were fitted to the exponential model up to 20 days, the turnover rates did not differ significantly. Indeed, a similar conclusion could be obtained by

calculating the switching proportion at the same time intervals, in which for both treatments 60% of the difference between new and old diet food sources occurred within 5 days after adult emergence. All these fast changes suggest that the shift of this insect between C3 and C4 crops, and vice versa, is rapidly detectable in the field if prey is available. After this quick change, there was a slower progression towards the isotope signature of the new diet (10% increase in 15 days). Although individuals fed maize aphids died 5 days earlier than those fed barley aphids, given the similarity between the exponential curves up to 20 days and the switching proportions, this result suggests that the switchover pattern of *O. majusculus* between crops could be similar. Differences in the rate of change of $\delta^{13}C$ before and after day 5 may be due to the different rates of carbon assimilation by specific insect tissues (especially fatty and reproductive tissues) in young adults as they feed and mature to reproduction (Gratton and Forbes 2006). The incomplete turnover of the $\delta^{13}C$ signature to the new food source until nearly 4 weeks reveals the origin of an insect that has moved from one crop to another many days before. This interpretation is only possible if the adjacent crops do not share pests, as in the case of maize and alfalfa (Pons et al. 1994, 2011) and other C3 crops, and may help to understand insect spillover between these kinds of crop.

Our results on food source switching from maize aphids to barley aphids are consistent with those reported by Ostrom et al. (1997), in which the coccinellid *Hippodamia variegata* (Goeze) was switched from a diet based on aphids raised on sorghum to an artificial diet consisting of pork liver, and with those of Prasifka et al. (2004), in which the coccinellid *Hippodamia convergens* Guérin-Méneville was changed from a diet based on sorghum aphids to one based on cotton aphids. In all cases, when the insects switch from a C4 to a C3 food source, a high rate of change in $\delta^{13}C$ signature occurs in the first days after switching, followed by a lower rate of change. Similar results were observed with the coccinellid *Propylea japonica* Thunberg when adults were shifted from aphids reared on cotton (C3) to aphids reared on maize (C4) (Ouyang et al. 2012). All these authors only performed one-way shifting experiments (that is, from C4 to C3 or from C3 to C4 diets), whereas our experiment compares both diet shifts in the same experimental conditions. In spite of the different form of preying by *O. majusculus* (sucking) and coccinellids (chewing), these results suggest that the pattern of change of $\delta^{13}C$ signature of predators is similar when they shift froma C4 to a C3 crop and vice versa.

Gratton and Forbes (2006) found no differences in $\delta^{13}C$ signatures of different body tissues between males and females of the coccinellid *Harmonia axyridis* Pallas maintained with the soybean aphid *Aphis glycines* Matsumura. We also found no sex-induced differences in *O. majusculus* fed with aphids reared on barley, a C3 plant like soybean. However, the carbon isotope ratio of *O. majusculus* varied with sex when adults were fed on aphids from maize. Because this unexpected result was not observed in *O. majusculus* fed on aphids from barley, we speculate that the food source may influence these isotopic differences between sexes. Maize has been reported to have low nutritional quality for insects (Webb et al. 1998), and Honek (1994) found a better performance of the aphid *Metopolophium dirhodum* Walker on wheat than on maize, suggesting that aphids reared on maize are a poorer food source for their predators than aphids reared on winter cereals. An observation that supports this hypothesis is that, in our experiment, the adults of *O. majusculus* fed with maize aphids survived for fewer days than those fed with barley aphids. The resource allocation of predators may be different under suboptimal than under optimal food conditions, and this may have a greater effect on females, which need to allocate energy resources to egg production. Under suboptimal food conditions, females develop physiological strategies for reproduction and maintenance of somatic functions (Retnakaran and Percy 1985; Rosenheim et al. 2000; Ferrer et al. 2011; Lundgren 2011). These strategies could affect the carbon isotopes being assimilated in the body tissues and result in differences in $\delta^{13}C$ signatures between sexes of *O. majusculus* when they are raised on maize aphids but not when they are raised on barley aphids.

Our results show that the change in the carbon isotope ratio of *O. majusculus* switched from a C3 to a C4-based diet follows the same pattern in males and females but that rates of enrichment were different between sexes, particularly during the first days after diet switching. Gratton and Forbes (2006) found substantial differences between sexes in the isotopic shift to a new food source when *H. axyridis* was changed from a C3 to a C4 aphid-based diet and their results are consistent with ours in that females showed a higher rate of enrichment than males a few days after switching. However, these authors found a downturn of the $\delta^{13}C$ signature in females 10 days after switching that did not occur in males, and they attributed this depletion to an increase in resource allocation to egg production. We did not find such a downturn in females, but only a decrease in the rate of enrichment that was also observed in males. These

differences cannot be explained by the fact that we analysed entire insects, because a downturn was observed by Gratton and Forbes (2006) in different tissues of *H. axyridis* females, though it was evident only in the reproductive and fat tissues of the abdomen. One possible explanation is that females of *O. majusculus* in our experiment were not allowed to lay eggs normally because no suitable substrate (plant tissue) was included in the rearing jar. The difficulty of females in laying eggs could have contributed to the oosorption (Retnakaran and Percy 1985) and thus to the alteration of reproductive metabolism resulting in the absence of $\delta^{13}C$ depletion (DeNiro and Epstein 1977). This hypothesis, however, deserves further investigation.

In conclusion, switching the food source led to a rapid change in $\delta^{13}C$ signatures of adults of *O. majusculus*, regardless of the original and final food source. However, $\delta^{13}C$ traces from the aphid's original feeding crop remain for sufficient time, and this helps to determine the feeding sources and movement of the predator and to track shifts between C3 and C4 crop for at least 20 days after crop change. Moreover, it is valuable for determining the timing to perform predator sampling and crop practices that may enhance predator ecological services. Our results show that sex has no influence on the pattern of switchover, but it can affect the rate of isotopic turnover in conditions of poor or limiting resources. The results of this laboratory experiment can help to define when and how the shift of predators between C3 and C4 crops occurs in the field and to determine the role that these crops play as a source or sink for pest predators if they do not share pests. This information makes a valuable contribution to crop and landscape management by improving the action of pest natural enemies and fostering biological control in arable crops.

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CHAPTER 4

STABLE CARBON AND NITROGEN ISOTOPE SIGNATURES TO DETERMINE PREDATOR SPILLOVER BETWEEN ALFALFA AND MAIZE

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Abstract

Spillover of *Orius majusculus* and *Coccinella septempunctata* between maize and alfalfa during the vegetative and reproductive maize growth periods was investigated, using carbon and nitrogen stable isotope analysis and aphids as herbivore prey at two locations of the NE Iberian Peninsula. The dispersal pattern of *O. majusculus* and *C. septempunctata* differs between these crops and also between different moments of the maize growth period. In the vegetative period the pattern was similar for the two predators and alfalfa acted as a source of predators towards maize. During the reproductive period, the dispersal of *O. majusculus* between the two crops was continuous but biased from maize to alfalfa. Maize acts as a source of *O. majusculus*, supplying individuals of this predator to alfalfa, which mainly becomes a sink crop. Most of the *C. septempunctata* collected on alfalfa were local and those collected on maize came from alfalfa, but there was no spillover from maize to alfalfa. The present study increases knowledge of the dispersion of predators between arable crops and can improve habitat and landscape management at the farm scale by enhancing their biological control functions.

Keywords: *Orius majusculus*, *Coccinella septempunctata*, conservation biological control, aphids, natural enemies

1. Introduction

Conservation biological control involves the manipulation of the environment to enhance the effectiveness of natural enemies (Landis et al. 2000) and is currently the only cost-effective biological control method in arable crop conditions of many Mediterranean regions. The management of the environment may focus on the habitat, farm or landscape levels (Landis et al. 2000), with scale-dependent effects on natural enemies (Tscharntke et al. 2007).

Arable crops largely determine the agricultural landscape in irrigated areas of the north-eastern Iberian Peninsula, where winter cereals, maize and alfalfa predominate, forming a mosaic together with field margins, uncultivated lands, woody areas and fruit orchards.

Growth-cycles of the three main crops partially overlap: alfalfa and winter cereals in spring, alfalfa and maize in summer, and all three crops in late spring and autumn. The coincidence of crop cycles and field proximity allows arthropods to move from one crop to the other, but whereas only winter cereals and maize share some herbivores, their natural enemies (predators and parasitoids) feed on herbivores from all three crops (Pons and Eizaguirre 2009).

Pons et al. (2005), after monitoring the occurrence and abundance of predators in alfalfa and maize, hypothesized about their movement between these two crops. They suggested that *Orius* spp. (Heteroptera, Anthocoridae), one of the most abundant predators, spend the autumn and winter in alfalfa, which becomes a source of individuals dispersing in spring to maize, and after maize pollination they come back to alfalfa where they overwinter. Spillover from alfalfa to maize of other common predators such as ladybirds (Coleoptera, Coccinellidae) according to field abundance has also been suggested (Pons and Eizaguirre 2009) but no conclusive data exist to confirm this. If the full potential of natural enemies is to be realized in a conservation biological control programme, it is necessary to understand their population dynamics and the factors that influence them, including the role of refuges (Hossain et al. 2002) and the potential spillover between adjacent crops.

Information on natural enemy dispersal can be acquired through the application of marking and tracking techniques (Reynolds et al. 1997; Lavandero et al. 2004), which should be easy to use, cost-effective and environmentally safe and should persist

under different environmental conditions (Hagler and Jackson 2001). However, conventional exogenous markers can be difficult to apply for small animals (Hobson and Norris 2008; IAEA 2009) and may lead to confusing results as they can affect insect performance (Hagler and Jackson 2001), and therefore their movement. Moreover, such approaches determine whether the movement occurred but do not measure the effective predation of natural enemies on prey from the different crops. Stable isotopes, which are naturally abundant in individuals, were recently shown to be a powerful tool for obtaining qualitative and quantitative information on predation and movement of insects between crops, to be environmentally safe, and to have no effect on movement patterns (e.g. Nienstedt and Poehling 2000, 2004; Hagler and Jackson 2001; Lavandero et al. 2004). Plants themselves may be used as markers for tracking insects through their natural concentration in stable isotopes of chemical elements. In particular, carbon stable isotope ratios ($\delta^{13}C$) have recently been proven to be suitable for tracing the plant origin of phytophagous and predator insects (Prasifka et al. 2004; Prasifka and Heinz 2004; Vialatte et al. 2006; Traugott et al. 2008; Schallhart et al. 2009, 2011; Forbes and Gratton 2011) and this is especially true when C3 and C4 plants are compared because of the clearly different signatures in their $\delta^{13}C$. Given the low fractionation of carbon isotopes between trophic levels (DeNiro and Epstein 1978; Ostrom et al. 1997; Post 2002), these differences remain up in the food web and can be traced in herbivores and then in their predators. Isotopic composition of predators moving and feeding between isotopically distinct crops such as maize (C4) and alfalfa (C3) will gradually move from the local source towards the new food source signature (Ostrom et al. 1997; Prasifka et al. 2004). Moreover, nitrogen stable isotopes, which are generally used as an indicator of trophic position (DeNiro and Epstein 1981), can also improve information about incorporation of prey from different resources, with an enrichment between predators and their prey of 3.4‰ ± 1‰ (Post 2002).

The aim of this study was to investigate the spillover between maize and alfalfa of the two common predators *Orius majusculus* Reuter and *Coccinella septempunctata* L., in order to test the hypotheses suggested by Pons et al. (2005) and Pons and Eizaguirre (2009) and reported above. Specifically, *O. majusculus* is a polyphagous predator and is the most abundant predator in maize fields in Spain (Albajes et al. 2003; Pons et al. 2004), whereas the aphid-specific *C. septempunctata* is very common in alfalfa stands, especially in spring (Pons et al. 2005). Knowledge of the shifting of these

two species can give information about movement and crop interchange of predators in Mediterranean areas with similar crop conditions in order to enhance landscape management and biological control functions.

To this aim, we carried out analysis of carbon and nitrogen stable isotopes on samples of alfalfa, maize, aphids and the two predators (*O. majusculus* and *C. septempunctata*) collected at two locations and two sampling times in north-eastern Spain. Predator spillover would be rejected if individuals from one crop showed no difference in $\delta^{13}C$ from the prey (aphids) of the same crop in which they were sampled.

2. Material and methods

2.1 Study area

The study was conducted in 2009 at two locations of Lleida province (Catalonia, NE Iberian Peninsula) in areas where the arable crop rotation of winter cereals, maize and alfalfa under irrigation is predominant. The two locations are about 100 km apart. The first location was Almenar (41º46'22"N, 0º30'51"E), 30 km west of Lleida in the Segrià county, in an area where maize is grown for grain and alfalfa is dehydrated for production of pellets, both for feeding livestock. This is a fairly flat area with low hills, about 200 mm rainfall and an average temperature of around 17ºC from April to October. The second location was in La Seu d'Urgell (42º 20'24"N, 1º25'48"E), 15 km south of the Pyrenees in the Alt Urgell county. It was in a mountain area at 750 m altitude with 650 mm rainfall and an average temperature of 17ºC from April to October. In this area both maize and alfalfa are used for silage.

At Almenar the alfalfa and maize study fields covered an area of 7 and 8 ha, respectively, and were separated by a 5-m-wide road. Maize cv. Dekalb 6451 (Monsanto, Spain) was cultivated under traditional crop conditions consisting in conventional tillage. It was sown in late April with chemical fertilization and one preemergence and one postemergence herbicide treatment. Maize seeds were coated with imidacloprid but no more insecticides were applied. Maize was harvested in November. Alfalfa (cv. Aragon) was three years old and managed according to the traditional system in the area. Potassium fertilization before the start of the growth period was applied and the crop underwent five cuttings during the productive period (March-October). One insecticide treatment with chlorpyriphos was made in April

against the alfalfa weevil (*Hypera postica* Gyllenhall) but no more pesticides were applied. Both alfalfa and maize fields were sprinkler irrigated. The landscape surrounding the study fields was mainly composed of other fields with the same crops, with a low proportion of the area covered by orchards, pine forest and uncultivated lands.

At La Seu d'Urgell (hereinafter La Seu), alfalfa and maize study fields covered an area of 4 and 5 ha, respectively, and were separated by a 1-m-wide herbaceous margin. Maize cv. Franki (Caussade Semences, France) was cultivated in a no-tillage system with manure fertilization, sown in mid-May and harvested in September. Total herbicide glyphosate and chlorpyriphos (the only insecticide) were applied at maize preemergence and one additional herbicide spraying was made at maize postemergence. Alfalfa (cv. Aragon) was three years old, only fertilized with manure during the winter and free of pesticide treatments. Alfalfa was cut five times during the productive period (March-October), as is usual in the region. Both alfalfa and maize crops were sprinkler irrigated. The study fields were located in a valley between mountains where maize, alfalfa, and natural and cultivated grasslands dominated the agricultural landscape during the study period.

Although winter cereals are extensively cultivated at both locations, the fields were all harvested at Almenar and most of them at La Seu before the study started. At both locations maize is the only C4 crop cultivated on a large hectarage. Fields of sorghum, another C4 crop, are very scattered and small at Almenar and this crop is not cultivated at La Seu.

2.2 Plant and insect sampling

Fields at both locations were sampled on the basis of maize crop growth (Ritchie et al. 2005), once at both locations during the vegetative growth period (6-12 leaves, hereinafter T1), and three times at both locations in summer during the reproductive stages of maize (tasseling to physiological maturity, hereinafter T2). At each location alfalfa and maize were sampled on the same day. Samples of *O. majusculus* and *C. septempunctata* in each crop were collected at 3 distances (1, 15 and 35 m) from the field margin with 5 replicate points 15 m apart per distance. As the comparison of predator abundance between crops was not taken into account, and given the different architecture of maize and alfalfa plants, two different sampling methods were chosen

for their efficiency in collecting the target predators alive and suitable for stable isotope analysis.

Predators in alfalfa were collected using a 38-cm-diameter sweep net and five sweeps were made for each sampling point. Catches were put in labelled plastic bags and stored in an icebox. Maize plants were inspected visually and predators were collected using a bug-vac aspirator (Standard Bug-Vac#2®, Rose Entomology, Benson, AZ, USA) individually placed in Eppendorf vials, and stored in an icebox. At each maize sampling point, for 10 min a variable number of plants were inspected. Colonies of aphids were collected in the sampled crops in order to determine the herbivore prey isotope signature. Aphids were chosen as the reference of the crop $\delta^{13}C$ signature, as they are one of the most important pests of alfalfa and maize (Summers 1998; Pons et al. 2005; Meissle et al. 2010) and crop-specific (Blackman and Eastop 2000), so no switching between the two crops occurs. Aphids are a prey shared by the aphid-specific *C. septempunctata* and the polyphagous predator *O. majusculus*, and are easy to collect because they live in colonies.

Plant samples were also taken at each sampling point. Among the different parts of the maize plant, we considered leaves as the isotopic plant reference because they are present throughout the sampling period. For alfalfa, entire stems with leaves were taken as plant samples. One leaf or one stem from three plants of maize and alfalfa, respectively, were collected at each sampling point. The collection of these parts of the plants does not seriously affect the plant development.

In the laboratory, samples of insects and plants collected were dehydrated in an oven at 60ºC for at least 72 h. The plant samples were transformed into a homogeneous fine powder using a ball mill (Fritsch® with a zirconium ball), while for aphids and *C. septempunctata* an agate mortar was used. All powdered samples were weighed in tin capsules on a microbalance (0.700–0.800 mg for plant samples and 0.160–0.190 mg for samples of *C. septempunctata* and aphids, dry weight). In the case of aphids, to obtain sufficient dry matter each analysis consisted of a group of individuals from the same colony. Entire *O. majusculus* individuals were analysed in tin capsules as one specimen did not exceed the dry weight needed for the analysis (0.160–0.190 mg).

2.3 Stable isotope analysis

Analyses were carried out using a continuous flow isotope ratio mass spectrometer coupled with an elemental analyser (IRMS Finnigan Delta Plus and FlashEA 112 series, Thermo Fisher Scientific, Waltham, MA, USA). Isotopic contents were expressed in "δ" units as the relative difference (in parts per thousand) between the sample and international standards (atmospheric N_2 (Air) for nitrogen and Vienna PeeDee Belemnite (PDB) for carbon, according to the formula:

$$\delta R(‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$$

(Ponsard and Arditi 2000; Vander Zanden and Rasmussen 2001), where R is the ratio of heavy/light isotope content for the element ($R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$).

2.4 Predator movement between crops

In order to contrast the null hypothesis of absence of movement, for each of the two predator species we tested whether:

$$\delta^{13}C_{predators(x)} = \delta^{13}C_{aphids(x)},$$

where *x* represents the crop in which the animals were sampled.

For each of the sampled individuals, mixing models were applied in order to individually discriminate percentage contribution of the two prey resources (aphids from C3 and C4 plants) in the diet of predators, and therefore to qualify and quantify the movement between the two crops. As a system with *n* isotopes requires a minimum of *n+1* resources, we used a standard linear system based on one isotope (carbon), following Phillips (2001), to mathematically solve for the unique combination of proportions (f_A, f_B) of source isotopic signatures (δ_A, δ_B) which coincide with the observed signature for the mixture (δ_M):

$$\delta_M = f_A\delta_A + f_B\delta_B$$

$$f_A + f_B = 1$$

where δ_M is the $\delta^{13}C$ of the predator individual, A is the C3 source (aphids in alfalfa) and B is the C4 source (aphids in maize). δ_A and δ_B sources for predators were calculated as:

$$\delta_A = (\delta^{13}C + SE)_{C3aphid} + 1\ ‰ \quad (1)$$

$$\delta_B = (\delta^{13}C - SE)_{C4aphid} - 1\ ‰ \quad (2)$$

where ± 1‰ is the standard error for a $\delta^{13}C$ fractionation of 0‰ between a prey and its consumer during digestion and assimilation (DeNiro and Epstein 1978; Ostrom et al. 1997; Post 2002; Phillips and Gregg 2003). All δ_M values below (1) were considered as 100% assimilation of C3 (alfalfa) and all values above (2) were considered as 100% assimilation of C4 (maize). To avoid overestimation of assimilation, when more than one species of aphid was present, δ_A and δ_B were calculated on the basis of the species with the highest $\delta^{13}C$ value for alfalfa and the lowest one for maize aphids.

The percentage of assimilation of the two resources (prey on alfalfa and maize) was then determined for each of the sampled individuals. The values obtained were categorized into three groups for each of the two resources, following Forbes and Gratton (2011):

a) 90%-100% assimilation (*local,* L) = an individual that fed completely on resources of the crop in which it was sampled, with no movement assumed;

b) 10%-90% assimilation (*switching,* S) = an individual with intermediate assimilation of the two resources, assumed to have moved between the crops;

c) 0%-10% assimilation (*migrant,* M) = an individual that has not fed on resources of the crop in which it was sampled, assumed to have moved recently from the opposite crop.

The threshold for a) and c) was calculated as an additional 10% of the difference between δ_A and δ_B (Equations (1) and (2)):

$$\text{Alfalfa: } \delta_A + [(\delta_B - \delta_A) \times 0.10]$$

$$\text{Maize: } \delta_B - [(\delta_B - \delta_A) \times 0.10]$$

These thresholds, corresponding to a $\delta^{13}C$ of about ± 1‰, were applied also on the basis of results from a previous laboratory experiment for *O. majusculus* (Madeira et al. 2013) and from the literature for coccinellids (Ostrom et al. 1997; Prasifka et al. 2004; Ouyang et al. 2012). In these studies, standard errors of $\delta^{13}C$ of individuals maintained on the same diet were lower than 0.2 ‰, but we kept a larger value in order to take into account the isotopic variability of the diet, which may be higher in field experiments and lead to an overestimation of movements.

As a difference in nitrogen isotopic signature between alfalfa and maize can be expected (Ostrom et al. 1997), $\delta^{15}N$ was taken into account to improve the information

about incorporation of prey from alfalfa and maize resources, considering an enrichment between predators and their prey of 3.4‰ ± 1‰ (Post 2002).

2.5 Statistical analysis

Data for plants and insects are reported as mean ± standard error within each crop, location and sampling period. Before the statistical analysis, the Shapiro-Wilk test was used to test for normality of distributions. Three-way ANOVA followed by Tukey's unequal N HSD test were used to assess the effect of sampling period, location and crops on $\delta^{13}C$ and $\delta^{15}N$ plant values. The Student t-test was used to assess differences in (1) $\delta^{13}C$ and $\delta^{15}N$ of aphids within and between locations; (2) $\delta^{13}C$ values between predators and aphids; and (3) $\delta^{15}N$ and $\delta^{13}C$ values of predators between locations and sampling periods and between predators captured at 1 m distance and the inner field sampling distances. When the assumptions for performing parametric tests were not met, the Mann-Whitney U test was performed. Statistical significance was accepted at $\alpha = 0.05$.

3. Results

3.1 $\delta^{13}C$ and $\delta^{15}N$ in plants

$\delta^{13}C$ values of alfalfa and maize plants, which were typical of C3 and C4 plants, respectively, were highly distinguishable from each other and did not differ between locations within crops and sampling times (Figure 1.4, Table 1.4). Differences between sampling periods (T1 and T2) emerged only for maize, which exhibited higher $\delta^{13}C$ values in T1 independently from locations (Table 1.4a and 1.4b).

Alfalfa and maize were different also in their $\delta^{15}N$ (Figure 1.4, Table 1.4a). Within crop, $\delta^{15}N$ of alfalfa differed significantly between locations in both sampling periods, being lower at Almenar (Table 1.4a and 1.4c). No significant differences between locations were observed in $\delta^{15}N$ of maize (Figure 1.4, Table 1.4a and 1.4c) whereas values were higher in T1 than T2, particularly at La Seu (Figure 1.4, Table 1.4c).

Table 1.4. Three-way ANOVA (**a**) and HSD Tukey tests (**b**, **c**) evaluating effects of Sampling period (T1,T2), Location (Almenar, La Seu) and Crop (Alfalfa, Maize) on $\delta^{13}C$ and $\delta^{15}N$ values of plants.

a)

	$\delta^{13}C$		**$\delta^{15}N$**	
	F	*P*-level	F	*P*-level
1. Sampling period	24.72	<0.0001	8.80	0.01
2. Location	1.16	0.29	25.06	<0.0001
3. Crop	16693.48	<0.0001	454.60	<0.0001
1*2	4.30	0.04	0.59	0.45
1*3	55.91	<0.0001	23.67	<0.0001
2*3	4.23	0.05	37.65	<0.0001
1*2*3	1.73	0.20	2.68	0.11

df = 1,40

b)

$\delta^{13}C$

Unequal N HSD

Probabilities for Post Hoc Tests, *=<0.05 **=<0.01 ***=<0.001

	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1. T1 La Seu alfalfa		***	0.16	***	0.27	***	0.32	***
2. T1 La Seu maize			***	1.00	***	***	***	***
3. T1 Almenar alfalfa				***	1.00	***	1.00	***
4. T1 Almenar maize					***	***	***	***
5. T2 La Seu alfalfa						***	1.00	***
6. T2 La Seu maize							***	0.92
7. T2 Almenar alfalfa								***
8. T2 Almenar maize								

c)

$\delta^{15}N$

Unequal N HSD

Probabilities for Post Hoc Tests, *=<0.05 **=<0.01 ***=<0.001

	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1. T1 La Seu alfalfa		***	*	***	0.65	***	**	***
2. T1 La Seu maize			***	1.00	***	***	***	0.14
3. T1 Almenar alfalfa				***	***	***	1.00	***
4. T1 Almenar maize					***	*	***	0.11
5. T2 La Seu alfalfa						***	***	***
6. T2 La Seu maize							***	0.85
7. T2 Almenar alfalfa								***
8. T2 Almenar maize								

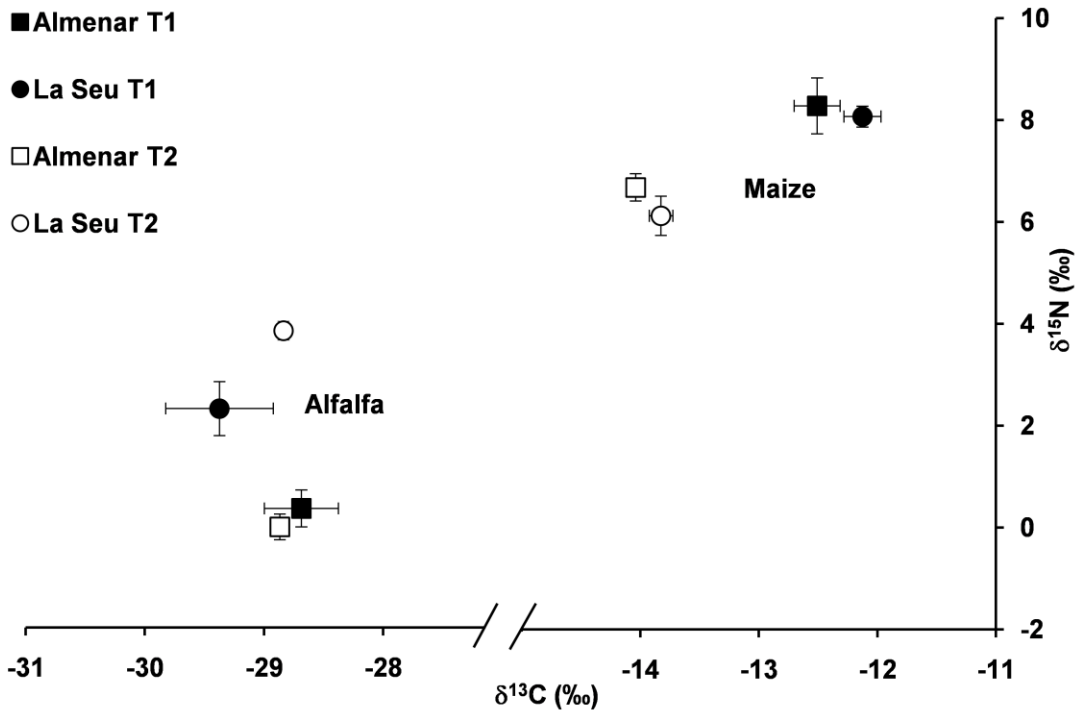

Figure 1.4. $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) bi-plot showing mean values (± SE) of alfalfa and maize plants from Almenar (squares) and La Seu (circles) during maize vegetative (T1; filled symbols) and reproductive (T2; open symbols) growth periods. The x-axis has been split for visual clarity.

3.2 $\delta^{13}C$ and $\delta^{15}N$ in aphids

Aphid species collected in maize and alfalfa were different due to the aphid-plant specificity (Table 2.4). *Aphis craccivora* Koch, *Acyrthosiphon pisum* Harris and *Therioaphis trifollii* Monell were the species collected on alfalfa. No aphids were found on maize in T1, whereas *Aphis fabae* Scopoli and *Sipha* sp. at Almenar, and *Rhopalosiphum padi* L. at La Seu were collected on maize in T2.

$\delta^{13}C$ values of aphids reflected those of the plants on which they were collected, with typical C3 signatures for aphids on alfalfa and C4 signatures for aphids on maize (Table 2.4, Figure 2.4 and Figure 3.4). On alfalfa $\delta^{13}C$ of *A. craccivora* and *A. pisum* differed at La Seu, where they co-occurred in T2 (t-test for unequal variances, $t_2 = 5.72$, $P = 0.03$), whereas no significant differences were found in *A. craccivora* between La Seu and Almenar (t-test $t_6 = 0.20$, $P = 0.85$).

Aphid species exhibited a variable $\delta^{15}N$ fractionation with respect to the host plant, some of them being enriched and others depleted (Table 2.4).

Table 2.4. $\delta^{13}C$ and $\delta^{15}N$ mean values (± SE) of aphids and predators in the two crops and locations in (**a**) the maize vegetative growth period (T1) and (**b**) the maize reproductive period (T2). Values in square brackets show isotopic signatures of plants. -- = species not found; brackets below $\delta^{13}C$ predators show Mann-Whitney or t- test statistics for differences between predator and prey; * = obtained values (standard errors were calculated from the standard deviation, on the basis of a minimum n=2; see results section for calculation details).

		$\delta^{13}C$ (‰)				**$\delta^{15}N$ (‰)**			
		Alfalfa		**Maize**		**Alfalfa**		**Maize**	
	Plant	**Almenar** [-28.69 ± 0.31]	**La Seu** [-29.37 ± 0.45]	**Almenar** [-12.51 ± 0.19]	**La Seu** [-12.12 ± 0.16]	**Almenar** [0.29 ± 0.35]	**La Seu** [2.21 ± 0.52]	**Almenar** [8.28 ± 0.55]	**La Seu** [8.07 ± 0.21]
a) T1	**Aphids**								
	A. craccivora	-28.53 ± 0.53	--	--	--	-0.45 ± 0.57	--	--	--
	A. pisum	--	-27.81 ± 0.01	--	--	--	0.01 ± 0.36	--	--
	Other herbivores*			-12.51 ± 0.71	-12.12 ± 0.71			11.68 ± 0.71	11.47 ± 0.71
	Predators								
	O. majusculus	-27.45 ± 0.54 (U =4, *P* = 0.10)	-27.00 ± 0.32 (t_4 =2.51, *P* =0.07)	-23.31 ± 0.71 (t_5 =8.66, *P* <0.001)	-21.84 ± 0.74 (t_{15} =4.63, *P*<0.001)	7.83 ± 0.31	4.36 ± 0.30	12.44 ± 0.41	6.51 ± 0.75
	C. septempunctata	-27.40 ± 0.17 (U =2, *P* =0.12)	-27.67 ± 0.35 (t_7 =0.41, *P* =0.70)	-26.24 ± 0.19 (t_3=23.64, *P*<0.001)	-26.21 ± 0.54 (U =50, *P* =0.006)	5.13 ± 0.71	5.59 ± 0.80	10.66 ± 0.60	6.18 ± 0.68
	Plant	[-28.87 ± 0.07]	[-28.84 ± 0.09]	[-14.04 ± 0.07]	[-13.82 ± 0.10]	[-0.07 ± 0.25]	[3.70 ± 0.18]	[6.68 ± 0.27]	[6.12 ± 0.38]
b) T2	**Aphids**								
	A. craccivora	-26.89 ± 0.34	-26.79 ± 0.35	--	--	0.19 ± 0.24	3.52 ± 0.54	--	--
	A. pisum	--	-28.83 ± 0.05	--	--	--	1.49 ± 0.30	--	--
	T. trifolli	--	-29.23 ± 0.96	--	--	--	4.27 ± 0.76	--	--
	A. fabae		--	-12.70 ± 0.46	--	--	--	9.78 ± 0.79	--
	Sipha sp.	--	--	-12.96 ± 0.12	--	--	--	10.27 ± 1.10	--
	R. padi	--	--	-	-12.72 ± 0.49	--	--	--	5.19 ± 0.11
	Predators								
	O. majusculus	-17.53 ± 1.10 (U =5, *P* =0.008)	-18.91 ± 0.78 (U =6, *P* <0.0001)	-14.49 ± 0.31 (U =25, *P* =0.005)	-13.95 ± 0.18 (U =30, *P* =0.05)	9.45 ± 0.93	6.66 ± 0.29	12.80 ± 0.48	8.26 ± 0.20
	C. septempunctata	-26.78 ± 0.23 (t_7 =0.24, *P* =0.81)	-27.07 ± 0.28 (t_{14} =2.09, *P* =0.06)	-29.27 ± 0.16 (t_6 =45.13,*P*<0.001)	-24.41 ± 1.30 (t_8 =5.64,*P* <0.001)	3.54 ± 1.00	4.71 ± 0.74	2.95 ± 0.76	5.11 ± 1.24

3.3 $\delta^{13}C$ and $\delta^{15}N$ in predators

No significant differences in isotopic signatures of *O. majusculus* and *C. septempunctata* were found between the outer field distance (1 m, where the distance from the adjacent crop was the lowest) and the inner sampling distances at both locations and for both crops and sampling dates (t-test, P > 0.05 in all cases). Therefore, there was no edge effect and distance was not considered as a factor of variation for the subsequent analyses.

3.3.1 O. majusculus

Differences in carbon isotope ratios ($\delta^{13}C$) of *O. majusculus* were observed between sampling periods (Mann-Whitney U test; alfalfa in Almenar, *P* = 0.03; maize in Almenar, P = 0.0005; alfalfa in La Seu, P = 0.003; maize in La Seu, P < 0.0001) (Table 2.4, Figure 2.4).

In T1, *Orius* individuals sampled in alfalfa had $\delta^{13}C$ signatures close to those of alfalfa-specific aphids with no difference between locations (Figure 2.4a; t-test t_6 = 0.77, P = 0.47). Mixing model output confirmed the very low preference for C4-based prey at both locations (Table 3.4a), attributing all individuals to the *local* assimilation range group (Figure 2.4a). Aphids were not found on maize in T1 and $\delta^{13}C$ values of *O. majusculus* individuals in this crop were significantly different from the expected $\delta^{13}C$ of herbivore prey estimated from the consumer-resource fractionation of about 0‰ ± 1‰ s.d. (DeNiro and Epstein 1978; Ostrom et al. 1997; Post 2002) with respect to maize. Specifically, 20% of individuals displayed a C3-based diet (*migrant*) and the remaining 80% had intermediate values (*switching* between crops), at both locations (Figure 2.4a). Mixing models output confirmed the high mean contribution (>60%) of C3 food in their diet (Table 3.4a).

In T2, $\delta^{13}C$ of individuals sampled in alfalfa was highly variable (Figure 2.4b) and a significant difference was found between predators and alfalfa-specific aphids (Table 2.4). In particular at Almenar, all but one of the sampled individuals showed $\delta^{13}C$ values that reflect a diet highly based on maize (72% in, Table 3.4a, Figure 2.4b). Though at La Seu $\delta^{13}C$ of individuals covered a wider range of values between alfalfa and maize signatures (Figure 2.4b), and the mean percentage of assimilation of maize aphids was lower than at Almenar (Table 3.4a), the percentage of individuals that fell in

the three groups of assimilation range was similar: a small portion of the total population were *local* (8% at Almenar and 11% at La Seu), a great portion were *switching* (67% at Almenar and 60% at La Seu) and the remaining portion (25% and 29%, respectively) were *migrant* (Figure 2.4b).

In maize, although individuals of *O. majusculus* sampled in T2 had $\delta^{13}C$ values statistically different from those of the aphids (Table 2.4), most of them were *local* (86.1% at Almenar and 90.2% at La Seu) and the remainder were *switching* (13.9% at Almenar and 9.8% at La Seu). The percentage of alfalfa prey in their diet was under 7% (Table 3.4a).

Table 3.4. Relative contribution of aphids sampled in maize and alfalfa in the maize vegetative (T1) and reproductive (T2) periods for **a**) *O. majusculus* and **b**) *C. septempunctata* at the two locations and in the two crops. For each of the sampled individuals a value of relative contribution was calculated with a linear mixing model and averaged for each crop and location. * as no aphid value on maize was found in T1, "aphids" should be read as generic "herbivores" (see results for details).

a)

O. majusculus		T1	T2
	Locations	Relative contribution (%) of maize aphids in diet (mean ± SE)	
Alfalfa	Almenar	1.18 ± 1.18*	72.05 ± 7.70
	La Seu	1.36 ± 0.98*	59.54 ± 6.12
		Relative contribution (%) of alfalfa aphids in diet (mean ± SE)	
Maize	Almenar	71.09 ± 5.56	6.45 ± 2.37
	La Seu	61.72 ± 5.72	3.10 ± 1.21

b)

C. septempunctata		T1	T2
	Locations	Relative contribution (%) of maize aphids in diet (mean ± SE)	
Alfalfa	Almenar	0.00 ± 0.00*	0.00 ± 0.00
	La Seu	0.58 ± 0.58*	0.00 ± 0.00
		Relative contribution (%) of alfalfa aphids in diet (mean ± SE)	
Maize	Almenar	94.07 ± 1.45	100.00 ± 0.00
	La Seu	90.95 ± 3.43	83.82 ± 8.43

As regards $\delta^{15}N$, *O. majusculus* individuals sampled in T1 had higher values at Almenar than at La Seu in both alfalfa and maize crops (alfalfa $t_6 = 7.46$, $P = 0.0003$; maize $t_{18} = 4.39$, $P = 0.0003$) (Table 2.4). In alfalfa, fractionation between predators and

aphids was +8.28‰ and +4.35‰ at Almenar and La Seu respectively. In maize, an enrichment of +0.76‰ at Almenar and a depletion of -4.96‰ at La Seu were observed with respect to the expected prey herbivore $\delta^{13}C$, which was estimated by adding 3.4 ± 1‰ to the plant signature according to the literature, as aphids were absent in T1.

In T2 $\delta^{15}N$ of *O. majusculus* in alfalfa was different between locations, with the highest values being found in individuals sampled at Almenar (t-test for unequal variances; $t_{13} = 2.86$, $P = 0.01$) (Table 2.4). Higher than expected fractionation values between predators and local aphids (i.e. +3.4 ± 1‰) were observed (+9.26‰ at Almenar and a maximum of +5.17‰ at La Seu, Table 2.4). In maize $\delta^{15}N$ of *O. majusculus* also differed between locations, with higher values found in individuals sampled at Almenar (t-test for unequal variances; $t_{57} = 8.7$, $P < 0.0001$), consistently with differences between locations found for aphids.

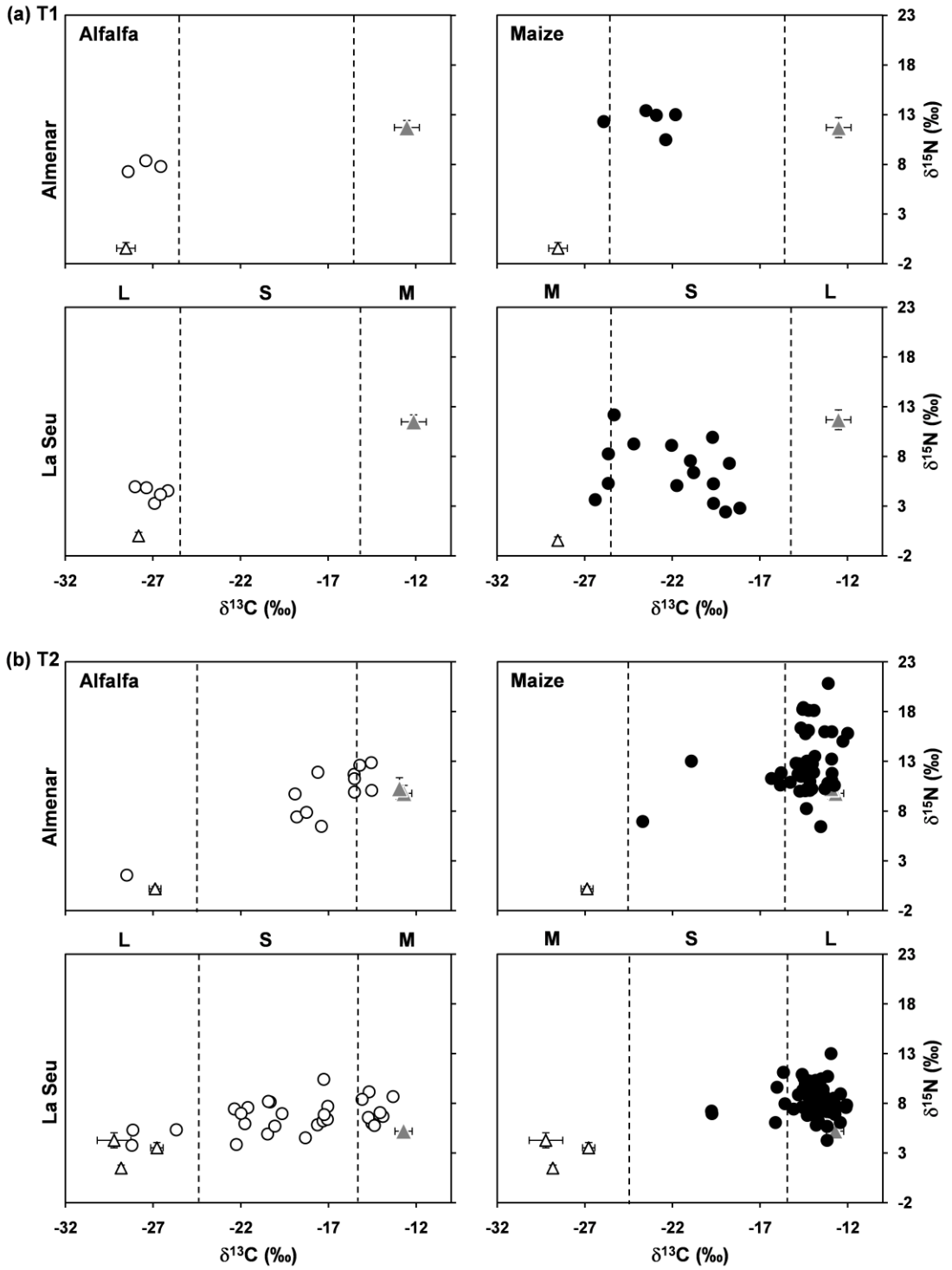

Figure 2.4. $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) bi-plots showing values of *O. majusculus* individuals sampled in alfalfa (open circles) and maize (filled circles) at Almenar and La Seu in (**a**) T1 and (**b**) T2. Isotopic values of aphids (one point for each species with standard errors) from both alfalfa (open triangles) and maize (grey triangles) are reported for each crop as a reference of possible prey. Dashed lines separate Local (L), Switching (S) and Migrant (M) predators (see methods for classification).

3.3.2 C. septempunctata

Individuals sampled in alfalfa showed very low variability in their $\delta^{13}C$ signatures (Figure 3.4), with values consistent with a C3-based diet at both locations and sampling times (Table 2.4). No difference was observed between locations (T1: t-test, $t_8 = 0.36$, $P = 0.73$; T2: Mann-Whitney U test, $P = 0.67$). At both locations and sampling periods, the percentage of *local* individuals in alfalfa was 100% (Figure 3.4a and 3.4b) and therefore there was no contribution of maize aphids in the *C. septempunctata* diet as also confirmed by mixing model outputs (Table 3.4b).

In maize, $\delta^{13}C$ of *C. septempunctata* was significantly different from expected values for maize-specific herbivores in T1, when aphids were not found (Table 2.4). Differences between locations in $\delta^{13}C$ of *C. septempunctata* emerged in T2 (t-test for unequal variances, $t_6 = 3.72$, $P = 0.009$), with lower values observed at Almenar with respect to La Seu. No *local* individuals were observed at either location or sampling period. In particular, at Almenar all of the individuals collected in maize were *migrant* in T1 and T2, whereas at La Seu 76% of the individuals collected were *migrant* and 24% *switching* in T1, and 43% *migrant* and 56% *switching* in T2 (Figure 3.4a and 3.4b). The relative contribution of alfalfa prey was globally more than 80% (Table 3.4b).

While a temporal decrease in $\delta^{15}N$ enrichment between *C. septempunctata* and aphids was observed in alfalfa (T1: +5.58‰ both at Almenar and La Seu; T2: 3.35‰ at Almenar and from 0.44‰ to 3.22‰ at La Seu), a more variable depletion was recorded in maize (T1: -1.02‰ at Almenar and -5.29‰ at La Seu; T2: from -6.83‰ to -7.32‰ at Almenar and -0.08‰ at La Seu).

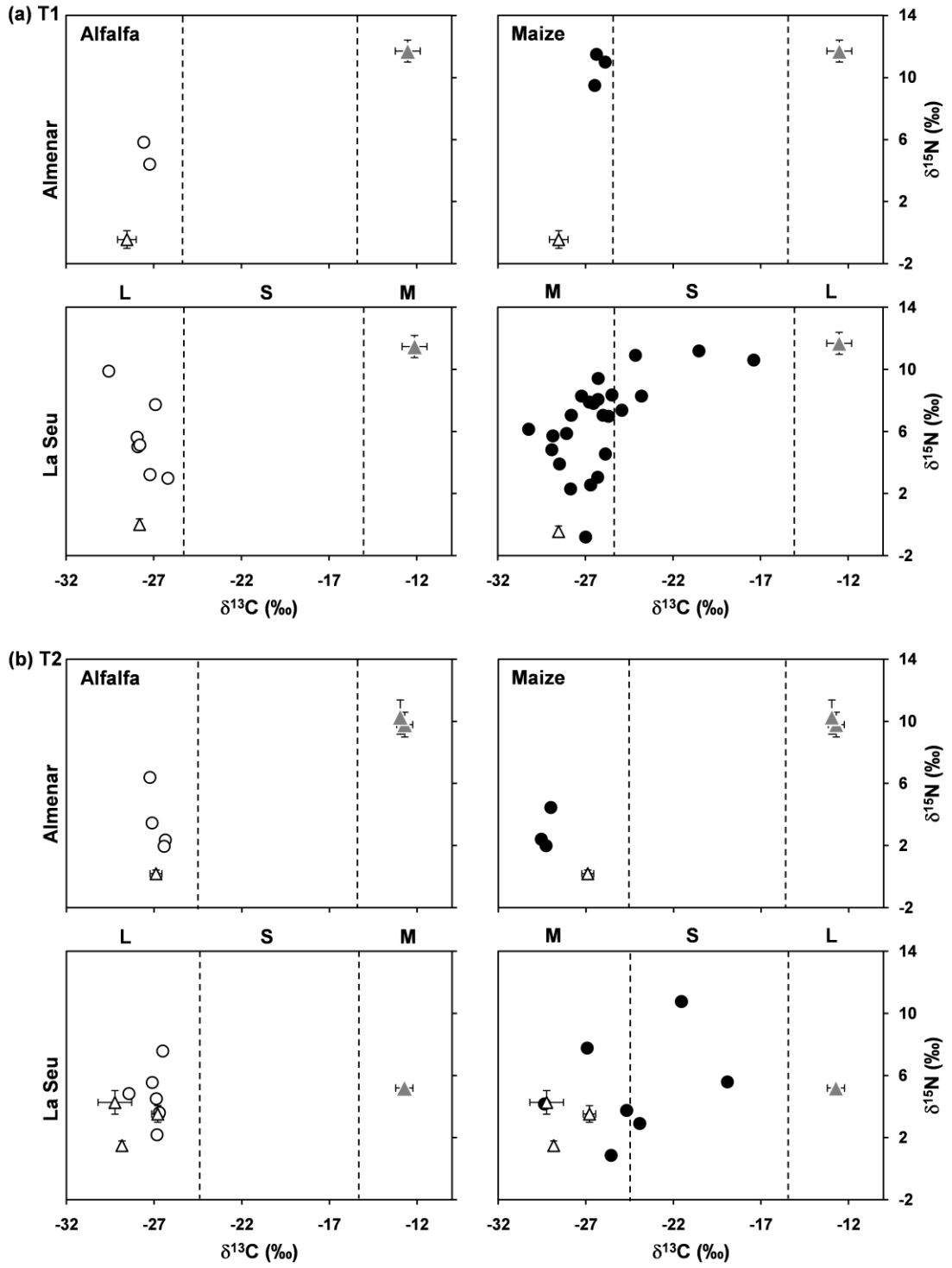

Figure 3.4. $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) bi-plots showing values of *C. septempunctata* individuals sampled in alfalfa (open circles) and maize (filled circles) at Almenar and La Seu in (**a**) T1 and (**b**) T2. Isotopic values of aphids (one point for each species with standard errors) from both alfalfa (open triangles) and maize (grey triangles) are reported for each crop as a reference of possible prey. Dashed lines separate Local (L), Switching (S) and Migrant (M) predators (see methods for classification).

4. Discussion

Ephemerality and disturbance of most agricultural landscapes are challenges to pest control by natural enemies and this is especially true for annual crops which, in addition, are characterized by their limited suitability for over-wintering after harvest and subsequent tillage (Tscharntke et al. 2007). Cyclic colonization of annual crops by natural enemies from more stable habitats that act as reservoirs is well established (Wissinger 1997). Perennial crop systems are potentially more amenable to conservation biological control than are ephemeral annual systems because they are subject to lower levels of disturbance and resident populations of natural enemies may persist from year to year (Landis et al. 2000).

Alfalfa is a perennial crop considered as a great reservoir of natural enemies (Pimentel and Wheeler 1973; Summers 1998; Núñez 2002) and a source of these for other neighbouring crops. Therefore, spillover of natural enemies from alfalfa to annual crops at farm level can be predicted, as can the potential return to alfalfa when the annual crop is harvested. Nevertheless, we lack sufficient knowledge of the role of the annual crop during the growing season, that is, whether it acts simply as a "sink" or also as a "source" habitat, supplying natural enemies to alfalfa.

In this study, through a stable isotope analysis approach applied at plant, prey and predator levels, we have found that:

1) the dispersal pattern of predators between alfalfa and maize differs between the vegetative and reproductive maize growth periods;

2) the pattern differs between *O. majusculus* and *C. septempunctata*; and

3) maize is a sink crop, but should also be seen, in some periods of its growth development, as a source of insect predators to alfalfa.

4.1 Plants

As expected, the two crops showed different $\delta^{13}C$ values as their different photosynthetic pathways leads alfalfa (C3) to have much lower values than maize (C4) (O'Leary 1988). Indeed the successful application of stable isotope analysis to tracking animal movement requires a discrimination factor of baseline resources, which have to be isotopically highly distinguishable (Gannes et al. 1998; IAEA 2009; Hobson et al. 2010). In addition to $\delta^{13}C$, alfalfa and maize were also highly distinguishable from their

$\delta^{15}N$. Differences in $\delta^{15}N$ between locations for alfalfa were also observed, probably as an effect of differences in fertilization regimes. The higher values found for alfalfa at La Seu, where crop undergoes manure fertilization, were probably due to the known enrichment of organic fertilizers in ^{15}N (Shearer et al. 1974; Choi et al. 2003; Yun et al. 2006). Maize $\delta^{15}N$ decreased during the growing period. It has been shown that, as a result of growth, maize leaves lose nitrogen content (N%) translocating it to the developing ear (Hay et al. 1953; Crawford et al. 1982) and become depleted in ^{15}N (Weiland 1989; Ta and Weiland 1992). As happened with carbon isotopes, a lower depletion was observed for $\delta^{13}C$ from the vegetative to the reproductive stage of maize leaves, in accordance with Cliquet et al. (1990), who observed a lower remobilization of ^{13}C than ^{15}N to kernels.

4.2 Aphids

We expected that no intermediate $\delta^{13}C$ signature between alfalfa and maize would be found for aphids, as these crops do not share aphid species (Pons et al. 1994, 2011; Blackman and Eastop 2000) and that potential maize colonization by aphids from winter cereals (C3) would occur sufficient time beforehand to ensure that colonies reached the maize signature (Asín and Pons 2001; Vialatte et al. 2006). In our study aphids from maize and alfalfa crops were extremely different in their carbon isotope ratios, with values similar to their host plant taking into account fractionation for assimilation. This result confirms that they can successfully be used as an isotopic reference of the host plant rather than the plant itself when the movement of a predator has to be studied, allowing more precise data based on the real isotopic fractionation value between the predators and their prey.

A fractionation of $\delta^{15}N$ different from the expected 3.4‰ (Post 2002) was often observed, as aphids showed similar or even depleted values in comparison with the plant. As sap feeders, these insects are frequently reported to be depleted in $\delta^{15}N$ in comparison with their diets (Ostrom et al. 1997; McCutchan et al. 2003; Wilson et al. 2011). Ostrom et al. (1997) hypothesize that this could be a function of the degree of plant N assimilation by aphids.

We did not find aphids in maize at vegetative stage. Their low abundance at Almenar could be related to the imidacloprid seed dressing treatment, which reduces maize aphid densities from maize emergence up to a month later (Pons and Albajes

2002) and we can assume that chlorpyriphos applied at sowing at La Seu had a negative effect on aphid colonization as well.

4.3 Predators

The C4- or C3-based signature of predators can be confidently related to the two sampling crops. Indeed, the size and proximity (1-5 m) of alfalfa and maize fields and the characteristics of the surrounding landscape, together with the abundance and species of weeds within the fields, reduced very much the potential effect of other plant sources inside or around the fields. It should be taken into account that after herbicide treatments in maize and alfalfa cuttings weed population and abundance were very small and represented a low percentage of the field biomass. In the study areas, C4 plants other than maize were mainly restricted to *Sorghum halepense* (L.) Pers. in field margins. Though Johnsongrass may be colonized by aphids (especially during period T2 when aphids are mainly present in this weed), which can be preyed on by *O. majusculus* and *C. septempunctata*, the probability that individuals collected on alfalfa came from this weed rather than maize is low if the populations of predators are compared with those of maize. For the same reason, also the probability that individuals collected on maize came from C3 edge weeds is low. Among external sources, orchards and forests are not a preferred habitat for *O. majusculus* and *C. septempunctata* (Hodek and Michaud 2008; Avilla et al. 2009). On the contrary, predators could move from winter cereals to maize (or alfalfa) looking for aphids or other prey, but this could occur only 2-3 weeks before the first sampling and, in this case, individuals would have had enough time to change their isotopic signatures to the new diet (Madeira et al. 2013).

As we expected, a different movement pattern of predators occurred in the two maize growth periods, and similar results emerged at the two locations. We found that in maize vegetative phase (T1) the pattern was similar for the two predator species, and alfalfa acted as a source of predators towards maize. All the individuals of *O. majusculus* and *C. septempunctata* sampled in maize came from alfalfa, where instead only *local* individuals were found. This result was also confirmed by a lower than expected fractionation of $\delta^{15}N$ of predators in comparison with the local prey, giving the lower $\delta^{15}N$ of aphids in alfalfa than those of maize. In a similar experimental design, Prasifka and Heinz (2004) and Prasifka et al. (2004) observed that adults of the ladybird *Hippodamia convergens* Guérin-Méneville began to move from the adjacent

grain sorghum to colonize cotton at the earliest stage growth, and continued there for several weeks. A spillover of ladybirds from alfalfa to maize according to field abundance was also suggested by Pons and Eizaguirre (2009). The observed movement pattern of *O. majusculus* is in agreement with the hypothesis of Pons et al. (2005), who proposed that *Orius* spp. would spend the autumn and winter in alfalfa, which becomes a source of individuals dispersing in spring to maize. Moreover, our results suggest that in Mediterranean agricultural systems, where the presence of alfalfa is common, colonization of *Orius* spp. to other crops occurred earlier than in other Central European agro-ecosystems (Veres et al. 2012).

The presence of *C. septempunctata* in a crop when few or no prey were available, as we found for maize, has been reported in alfalfa (Van der Werf et al. 2000) and barley (Ninkovic and Pettersson 2003) and the presence of the coccinellid *H. convergens* has been reported in cotton Prasifka et al. (2004). It is known that natural enemies may still be attracted to crop habitats when prey are absent (Price 1986); the reasons for this behaviour include chemicals released by plants even when they are not attacked by herbivores or pathogens (Glinwood et al. 2011), volatile compounds released by conspecifics that foster aggregation (Van der Werf et al. 2000), and the chance of finding shelters, pollen or nectar (Hodek and Honek 1996). However, seeking pollen or nectar cannot explain our results, as they were not available when maize was in the vegetative growth period.

During the reproductive period of maize (T2), the two target predator species collected in this crop showed different movement behaviour: while most *O. majusculus* individuals were *local*, indicating a high preference for maize prey, the nearly C3-δ^{13}C values of coccinellid individuals indicated a diet highly based on alfalfa prey, with some individuals showing only partial assimilation of maize prey and therefore having recently moved, and others that did not yet feed on any C4 food, particularly at Almenar.

In this period, an opposite movement pattern for the two species was observed also in alfalfa. For *O. majusculus*, maize acted as a source towards alfalfa, as the majority of the sampled individuals were *migrant* from maize or *switching* between the two crops. As in the vegetative period of maize, δ^{15}N lower fractionation of predators in comparison with the local prey confirmed these results. Prasifka et al. (1999) observed for *Orius* spp. a continuous movement between annual adjacent crops. Our results

partially agree with theirs because we found a continuous movement but it was biased from the annual (maize) to the periannual crop (alfalfa). Regarding *C. septempunctata*, as occurred in T1, the totality of *local* individuals indicated a full preference for the alfalfa food source and no movement from maize emerged. Also our results show that there is no spillover of *C. septempunctata* from maize to alfalfa in summer, and this predator may disperse to other hosts to spend the autumn and overwinter. *Coccinella septempunctata* can overwinter on shelters provided by hedgerows, forest edges, shrubs, tree trunks or other landscape refuges (Majerus 1994; Hodek and Honek 1996). Prasifka et al. (1999), differently from our results, observed a continuous movement of *H. convergens* between adjacent crops. Forbes and Gratton (2011) observed that *C. septempunctata* is faithful to the C3 habitat (alfalfa and soybean) during summer, unlike *Harmonia axyridis* Pallas, which showed frequent C3-C4 (maize) inter-crop movements. These findings suggest that the differences in patterns of dispersal may be the result of species-specific traits, a factor that deserves further investigations.

Knowing the isotopic turnover rate, erroneous interpretations of data due to species-specific fractionations can be avoided (Hobson et al. 2010), and information about time of integration of the exploited resources can be obtained, allowing temporal movement dynamics to be inferred (Ostrom et al. 1997; Prasifka et al. 2004; Gratton and Forbes 2006; Ouyang et al. 2012). In a laboratory experiment, (Madeira et al. 2013) showed that *O. majusculus* switching from a C3 to a C4 food diet (*R. padi* aphids), and vice versa, displayed more than 60% of carbon isotopic signature of the new diet as early as 5 days after the switching, but the rate of approximation to the new diet then decreased without completely reaching the final signature after nearly four weeks. Similar results have been obtained for coccinellids (Ostrom et al. 1997; Prasifka et al. 2004; Gratton and Forbes 2006; Ouyang et al. 2012). This turnover pattern applied to the present work reveals that migrant individuals of both predators may have recently moved to the sink crop, but also makes it possible to trace the origin of an individual that has moved many days before from one crop to the other.

In addition to the differences or similarities to other similar studies, our results supply additional information. As stable isotope analysis indicates effective assimilation (Cabana and Rasmussen 1994; Post 2002), it can be used to determine whether the movement occurred, when it occurred (for a relatively long period, up to 4 weeks) and whether natural enemies actually had preyed, which is obviously essential in pest

control. All these things together could be difficult to assess with exogenous or other endogenous markers, so the isotopic approach is confirmed as a powerful tool in this type of studies.

4.4 Conclusions

Carbon and nitrogen stable isotope analysis has been confirmed as a powerful tool for inferring qualitative and quantitative information on predator movement between alfalfa and maize, taking aphids as a herbivore-prey reference. Using this approach we have been able to determine the pattern of dispersal of predators and to infer the role of crops as a source or sink of predators.

In particular, we observed that the dispersal pattern of two of the most common predators in alfalfa and maize, *O. majusculus* and *C. septempunctata*, differs between these crops and the pattern also varies during the maize growth period. Alfalfa may be considered a source and maize a sink crop for *C. septempunctata* during the growing season of both crops. During the maize vegetative growth period (spring), alfalfa is a source crop also for *O. majusculus*. However, in the maize reproductive growth period (summer), maize acts as a source of *O. majusculus*, supplying individuals of this predator to alfalfa, which mainly becomes a sink crop.

These results suggest that an association of alfalfa and maize may have potential for improving pest management, facilitating an early colonization of predators in maize from populations coming from alfalfa. However, this crop association is even more valuable because maize also acts as a source of some predators for alfalfa during the maize reproductive growth period. Alfalfa undergoes cuttings during the summer, causing disturbances to predator populations that may, as occurred with *O. majusculus*, find temporary refuge in maize and later move back to alfalfa. Alfalfa strip-cutting has been proposed as a good crop management system for conserving natural enemies after cutting (Landis et al. 2000; Hossain et al. 2002), but the risk of this practice may be that strips may also be a refuge for pests. A mosaic landscape including adjacent fields of alfalfa and maize could be complementary or an alternative to alfalfa strips, especially in areas with small fields as in many Mediterranean regions, where this conservation technique is not easy to apply (Pons and Eizaguirre 2009).

In conclusion, this study improves knowledge of the switching processes of predators of alfalfa and maize and can help to better manage these crops at habitat and

farm scales in order to conserve *O. majusculus* and *C. septempunctata* and foster their biological control functions.

Acknowledgments

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CHAPTER 5

SPILLOVER OF PREDATORY AND NON-PREDATORY ARTHROPODS FROM CULTIVATED LAND TO CALCAREOUS GRASSLANDS

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Aquest capítol s'ha extret de la tesi ja que correspon a un article encara no publicat.

GENERAL DISCUSSION

General discussion

Advances in conservation biological control strategies require an understanding of the population dynamics of natural enemies (predators and parasitoids), their movement within and between habitats (Thomas et al. 1992) as well as an insight into the role each habitat plays in the landscape. This is especially true when the landscape is mainly formed by annual and perennial crops that suffer several disturbances during the season. The experimental work presented in this thesis allows a better understanding of movement dynamics of some of the most abundant predators that inhabit arable crop landscapes formed by alfalfa and maize and by winter cereals, meadows and semi-natural habitats. Such an understanding will help to improve habitat manipulation, which is a methodology for enhancing conservation biological control (e.g. Landis et al. 2000). To assess the movement of predators, different methods were used. All of them allowed to track the predators' movements successfully.

To reach the objectives 1 and 2 (Chapter 1 and 2), I used rubidium as a marker to track the movement of some predators within maize and between alfalfa and maize. This technique confirmed to be effective in tracking both the movement of small insects, such as *O. majusculus* and *Metallina* sp., and the movement of much larger insects, such as *C. fuscipes*, *P. rufipes* and *P. cupreus*. These findings agree with many studies that have been using this technique to measure the movement of insects in the field (e.g. Fernandes et al. 1997; Prasifka et al. 2001; Pons et al. 2004; Perović et al. 2011). This tracking technique also proved to be highly versatile because it could track the distance of movement within a crop and also to measure the movement between two crops.

Alfalfa and maize are isotopically distinct plants, therefore I could use stable isotope analysis for tracking the movement of plant-dwelling predators between these crops (objective 3, Chapter 4). When an insect changes its food source from a C3 to a C4 plant (or vice versa), the isotopic composition of its body gradually moves towards the value associated with the new food source. However, data using this technique is mainly available in larger predators such as coccinellids (e.g. Ostrom et al. 1997; Prasifka et al. 2004; Ouyang et al. 2014) and no data were available for small predators such as *O. majusculus*. In order to improve and better understand isotopic field data of *O. majusculus*, I carried out laboratory diet switch experiments (Chapter 3) with a

carbon stable isotope approach to deduce information on the dispersal of *O. majusculus* individuals among C3 and C4 crops. Findings show that five days after the diet shift, approximately 60% of the difference between new and old diet food sources occurred. This allows to assume that *O. majusculus* collected in a crop with a completely distinct isotopic signature has moved to this crop in less than five days. Moreover, I found out that traces of the old diet in *O. majusculus* were still detectable more than twenty days after a diet shift, revealing whether *O. majusculus* has moved into a crop from an isotopically different one. In spite of the different ways of prey consumption of *O. majusculus* (sucking) and coccinellids (chewing), these results show that the pattern of change in the $\delta^{13}C$ signature of predators is similar when they shift from a C4 to a C3 crop and vice versa. Findings showed that stable isotope analysis is a reliable method for tracking the movement of small predators such as *O. majusculus* between alfalfa and maize. Moreover, results also agree with many studies that have been using this technique to measure the movement of coccinellids between isotopically different crops (e.g. Prasifka et al. 2004; Forbes and Gratton 2011; Ouyang et al. 2012).

Previous work showed that *Orius* spp. were sensitive to changes in maize weed density, being more abundant in plots with low than in plots with very high weed density (Albajes et al. 2009). In this study (objective 1, Chapter 1) using rubidium as an insect marker, I found that the abundance of *O. majusculus* was not different in maize with moderately high and in maize with low densities of weeds. Moreover, I also observed that *O. majusculus* has great dispersal capacity within maize and that dispersal is not affected by different weed densities. Since effects in dispersal would be expected if insects used weeds as refuge or to find alternative resource, these findings suggest that under the usual weed management systems, the remaining weeds do not supply enough alternative resources such as prey, pollen and nectar. The results also showed that herbicide treatment regimes with different levels of efficacy did not affect the abundance and dispersal of *O. majusculus* in maize.

In this work the hypothesis that predators can move between alfalfa and maize in the usual crop conditions of the north-eastern Iberian Peninsula (Pons et al. 2005) was tested (objectives 2 and 3). I demonstrated that there really are movements between adjacent crops of both plant and ground dwelling predators, that the movement is mainly bidirectional (no matter if the crop is annual or perennial), but the pattern of movement is species-specific.

In the complex of alfalfa-maize, I determined that carabids (*Calathus fuscipes*, *Pseudophonus rufipes*, *Poecilus cupreus* and *Metallina* sp.) and the heteropteran *O. majusculus* presented bidirectional movement between these two crops, while the coccinellid *C. septempunctata* only moved from alfalfa to maize (see Chapters 2 and 4). Additionally, these studies show that predator movement can be affected by crop management and crop phenology.

Using rubidium as an insect marker (objective 2, Chapter 2), I found that the movement of the ground dwelling *C. fuscipes*, *Metallina* sp. and *P. cupreus* between alfalfa and maize was continuous, while *P. rufipes* only moved to maize after alfalfa cutting. However, after an alfalfa cutting event, the margin and maize proved to be a refuge habitat for all carabid species and acted as a source for the recolonisation of alfalfa after regrowth. This study also suggests that alfalfa can be an overwintering habitat for carabids, particularly *P. rufipes* and *P. cupreus*, since movement from maize to alfalfa increased during the growing season and after maize harvesting. The result that margins act as refuge and source after adverse agricultural activities is in agreement with many studies (Holland and Luff 2000; Holland et al. 2005; Yu and Liu 2006; Benjamin et al. 2008). However, the role of the annual crop (maize) as a refuge and source to adjacent crops is a new finding. This study indicates that when alfalfa and maize are in adjacent fields, these crops share the most abundant carabids but that they can play a distinct ecological role during the season.

In the case of the plant-dwelling predators *O. majusculus* and *C. septempunctata* and using carbon and nitrogen stable isotopes analysis for tracking the movement (objective 3, Chapter 4), I observed that their dispersal pattern between alfalfa and maize differed between the vegetative and the reproductive maize growth stage. During the vegetative stage, all individuals of *O. majusculus* and *C. septempunctata* that colonized maize came from alfalfa. In the reproductive period of maize, the two predator species collected in maize showed different movement patterns. *Orius majusculus* continuously moved between crops but with higher flow from maize to alfalfa than from alfalfa to maize, while continuous movement of *C. septempunctata* only occurred from alfalfa to maize. The results obtained in Chapter 3 allow to confirm that individuals of *O. majusculus* that colonised maize had moved from alfalfa recently (less than five days). In the maize reproductive growth period most of the individuals collected in maize had been living in that crop for more than four weeks. Most of the

individuals collected in alfalfa had come from maize recently or in less than twenty days. These results partially confirm the hypothesis of Pons et al. (2005), who assumed that *Orius* spp. would spend the autumn and winter in alfalfa, which in spring becomes a source of individuals dispersing to maize. These findings also suggest that in Mediterranean agricultural systems, where the presence of alfalfa is common, colonization of other crops by *Orius* spp. occurred earlier than in other Central European agro-ecosystems (Veres et al. 2012). Moreover, this study confirms that maize can act as a source of *O. majusculus* towards alfalfa during the reproductive stage.

Since I used aphids as an herbivore field reference, these results supply additional information compared to other studies. This gives more confidence to the results because it considers three trophic levels (plant, herbivore and predators), allowing better tracking of isotope fractionation. Moreover, to infer predator movement I not only focused on $\delta^{13}C$ but also on $\delta^{15}N$, allowing to infer trophic specific relationships, to confirm $\delta^{13}C$ results and better explain them, given the nitrogen isotopic differences between the two resources. These stable isotope analyses indicate effective assimilation (Cabana and Rasmussen 1994; Post 2002), determine whether the movement occurred, when it occurred and whether natural enemies actually had preyed on pest insects, which is obviously essential in pest control.

All findings reported in Chapter 2 and 4 show that the rotation of alfalfa and maize in the arable crop landscape has the potential for improving pest management. Alfalfa acts as a source of predatory insects and allows an early colonization of maize by predators. During the maize reproductive growth period, maize acts as refuge and source of some predators for alfalfa, especially after alfalfa cutting. The fact that all predators can move from alfalfa to maize and that maize acts as an alternative and source habitat for predators implies that adjacent fields of maize could be a complement or even an alternative to alfalfa strip-cutting that was reported as a good technique for preserving natural enemy populations after alfalfa cutting (Landis et al. 2000; Hossain et al. 2002). This is especially true in areas with small fields, like in many Mediterranean regions, where this conservation technique would not be easy to apply (Pons and Eizaguirre 2009). An advantage of the rotation of alfalfa and maize is the fact that these crops do not share pests compared to some ecological measures such as strip-cutting and bio-corridors around agricultural fields that may also be a refuge for pests

and actually increase the densities of pest and cause reductions in yield (Hunter 2002). The management of margins can also play an important role for carabid conservation, since they can act as refuge and source for the colonisation of alfalfa, especially after alfalfa cutting.

Spillover effects from natural areas to agricultural areas are widely assumed, but little is known about the opposite (Rand et al. 2006). In Chapter 5 (objective 4), carrying out periodical sampling, I investigated the spillover of carabids, staphylinids and spiders through changes in their abundance in two different neighbourhood combinations: from winter cereals to adjacent semi-natural calcareous grasslands and from managed meadows to adjacent semi-natural calcareous grasslands. In this study, I found evidence for spillover of carabids, staphylinids and spiders from agricultural (wheat and meadows) to semi-natural habitats (calcareous grasslands). Calcareous grasslands adjacent to wheat fields presented higher abundances of all arthropod taxa (except hunting spiders) than calcareous grasslands adjacent to meadows. This implies that there is a stronger spillover effect to semi-natural habitats from annual than from perennial crops. Additionally, we observed a strong correlation in the carabid and spider abundances between wheat field edges and calcareous grassland edges providing further support for spillover. This is in accordance with another study that showed that species abundance and diversity in terrestrial habitat patches can be strongly influenced by neighbouring habitats (Cook et al. 2002). These results demonstrate that semi-natural areas can be a sink habitat for predators that move from adjacent annual or perennial crops.

The results obtained during this thesis strongly indicate that predator spillover is bidirectional, species-specific and moderated by the adjacent crop and habitat type. Furthermore, the ecological role that annual and perennial crops and semi-natural habitats play for predators within agricultural habitats varies during the season and in accordance with crop phenology and crop management.

CONCLUSIONS

Conclusions

Methods for tracking predator's movement

- Rubidium is an effective marker for tracking the movement of *O. majusculus*, *C. fuscipes*, *P. rufipes*, *P. cupreus* and *Metallina* sp.
- Stable isotope analysis is an appropriate and useful method for tracking the movement of *C. septempunctata* and *O. majusculus* between alfalfa and maize.
- Aphids are a good herbivore reference for tracking spillover of predators between maize and alfalfa using stable isotope analysis.
- Changes in the $\delta^{13}C$ of *O. majusculus* after diet switching (C3 to C4 and vice versa) are detectable within five days, but $\delta^{13}C$ traces of the previous diet are detectable up to twenty days after the diet change.

Orius majusculus and maize weed density

- The abundance and dispersal of *O. majusculus* within maize fields are not affected by weed density.
- *Orius majusculus* is able to move 25 m/week within maize fields.

Predator's movement between alfalfa and maize

- In an arable crop landscape, ground and plant-dwelling predators move between adjacent habitats whichever the crop, but the pattern of movement is species-specific.
- The ground beetles *C. fuscipes*, *P. rufipes*, *P. cupreus* and *Metallina* sp. move between adjacent alfalfa and maize fields, but alfalfa cutting can affect the movement of some carabids:
    - ✓ *P. rufipes* only move from alfalfa to maize after alfalfa cutting.
    - ✓ *Metallina* sp. only move to margin after alfalfa cutting.
- Margins and maize act as a refuge and source for all carabids after alfalfa cutting**.**
- The plant-dwelling *O. majusculus* and *C. septempunctata* move between adjacent alfalfa and maize crops but the pattern of movement differs during the season and between species:
    - ✓ During the vegetative period of maize, *O. majusculus* and *C. septempunctata* only move from alfalfa to maize. Alfalfa is the source for these predators that colonize maize.

- ✓ During the reproductive growth period of maize, *O. majusculus* moves continuously between alfalfa and maize but the flow is higher from maize to alfalfa. Maize mainly acts as a source of *O. majusculus* towards alfalfa.
- ✓ During the reproductive growth period of maize, *C. septempunctata* only moves from alfalfa to maize. Alfalfa continues to act as the source of *C. septempunctata* for maize.

- A landscape including a mosaic of adjacent fields of alfalfa and maize could be complementary and an alternative to alfalfa strip-cutting, especially in areas with small fields.

Ground-dwelling predator spillover from winter cereals and meadows to semi-natural habitats

- Carabids, staphylinids and spiders move from winter cereals and meadows to protected calcareous grassland but spillover effects are habitat and taxon specific:
  - ✓ Spillover effects of carabids, staphylinids and web-building spiders are stronger from winter cereals than from meadows to calcareous grasslands.
- Meadows can act as buffer strips around protected areas so that spillover from arable crops does not compromise the structure and functioning of endangered communities.

Conclusions

Mètodes per al seguiment del moviment dels depredadors

- El rubidi és un marcador eficaç per al seguiment del moviment de *O. majusculus, C. fuscipes, P. rufipes, P. cupreus* i *Metallina* sp.
- L'anàlisi d'isòtops estables és un mètode apropiat i útil per al seguiment del moviment de *C. septempunctata* i *O. majusculus* entre l'alfals i el panís.
- Els pugons són una bona referència per al seguiment del moviment de depredadors entre el panís i l'alfals si s'utilitza l'anàlisi d'isòtops estables.
- Els canvis en el $\delta^{13}C$ d'*O. majusculus* després del canvi de dieta (C3 a C4 i viceversa) són detectables en cinc dies, però traces de $\delta^{13}C$ de la dieta anterior es detecta fins a vint dies després del canvi de dieta.

Orius majusculus i densitat de les males herbes del panís

- L'abundància i dispersió d'*O. majusculus* en camps de panís no estan vinculades amb la densitat de males herbes.
- *Orius majusculus* és capaç de moure's 25 m/setmana dins dels camps de panís.

Moviment de depredadors entre alfals i panís

- En el paisatge de cultius extensius, els depredadors es mouen entre hàbitats adjacents sense importar quin és el cultiu, però el patró de moviment és propi de l'espècie.
- Els caràbids *C. fuscipes, P. rufipes, P. cupreus* i *Metallina* sp. es mouen entre camps d'alfals i de panís, però el dall de l'alfals pot afectar el moviment d'alguns caràbids:
  - ✓ *P. rufipes* només es mou de l'alfals al panís després del dall de l'alfals.
  - ✓ *Metallina* sp. només es mou al marge després del dall de l'alfals.
- Els marges i el panís actuen com a refugi i font de caràbids després dels dalls de l'alfals.
- *Orius majusculus* i *C. septempunctata* es mouen entre camps adjacents d'alfals i panís, però el patró de moviment varia durant la temporada i entre espècies:
  - ✓ En el període vegetatiu del panís, *O. majusculus* i *C. septempunctata* només es mouen cap al panís, esdevenint l'alfals la font d'aquests depredadors.
  - ✓ Durant el període reproductiu del panís, *O. majusculus* es mou contínuament entre l'alfals i el panís, malgrat que el moviment des del panís cap a l'alfals

és més intens. El panís actua principalment com una font d'*O. majusculus* cap a l'alfals.

- ✓ Durant el període reproductiu del panís, *C. septempunctata* només es mou de l'alfals al panís. L'alfals continua actuant com a font d'aquest depredador cap al panís.

- Un paisatge format per camps adjacents d'alfals i panís podria ser complementari i una alternativa a la sega a bandes de l'alfals, especialment en zones amb camps petits.

Moviment de depredadores epiedàfics des dels cereals d'hivern i prats cap als hàbitats semi naturals

- Els caràbids, estafilínids i aranyes es mouen des dels cereals d'hivern i prats cap als hàbitats semi naturals, però el moviment depèn de l'hàbitat i de l'espècie d'artròpode:
  - ✓ El moviment de caràbids, estafilínids i aranyes constructores de teranyines és més intens des dels cereals d'hivern cap als hàbitats semi naturals que des dels prats.
- Els prats poden actuar com franges amortidores al voltant de les àrees protegides de manera que els depredadors provinents dels cultius extensius no posin en perill l'estructura i funcionament de les comunitats vulnerables.

Conclusiones

Métodos para el seguimiento del movimiento de los depredadores

- El rubidio es un marcador eficaz para el seguimiento del movimiento de *O. majusculus*, *C. fuscipes*, *P. rufipes*, *P. cupreus* y *Metallina* sp.
- El análisis de isótopos estables es un método apropiado y útil para el seguimiento del movimiento de *C. septempunctata* y *O. majusculus* entre la alfalfa y el maíz.
- Los pulgones son una buena referencia para el seguimiento del movimiento de depredadores entre el maíz y la alfalfa si se utiliza el análisis de isótopos estables.
- Los cambios en el $\delta^{13}C$ de *O. majusculus* después del cambio de dieta (C3 a C4 y viceversa) són detectables en cinco días, pero trazas de $\delta^{13}C$ de la dieta anterior se pueden detectar hasta veinte días después del cambio de dieta.

Orius majusculus y densidad de malas hierbas del maíz

- La abundancia y dispersión de *O. majusculus* en campos de maíz no están vinculadas con la densidad de malas hierbas.
- *Orius majusculus* es capaz de moverse 25 m/semana dentro de los campos de maíz.

Movimiento de depredadores entre alfalfa y maíz

- En el paisaje de cultivos extensivos, los depredadores se mueven entre hábitats adyacentes sin importar cuál es el cultivo, pero el patrón de movimiento es propio de la especie.
- Los carábidos *C. fuscipes*, *P. rufipes*, *P. cupreus* y *Metallina* sp. se mueven entre campos de alfalfa y de maíz, pero el corte de la alfalfa puede afectar el movimiento de algunos carábidos:
  - ✓ *P. rufipes* sólo se mueve de la alfalfa al maíz después del corte de la alfalfa.
  - ✓ *Metallina* sp. sólo se mueve al margen después del corte de la alfalfa.
- Los márgenes y el maíz actúan como refugio y fuente de carábidos después del corte de la alfalfa.
- *Orius majusculus* y *C. septempunctata* se mueven entre campos adyacentes de alfalfa y maíz, pero el patrón de movimiento difiere durante la temporada y entre especies:
  - ✓ En el periodo vegetativo del maíz, *O. majusculus* y *C. septempunctata* sólo se mueven hacia el maíz, siendo la alfalfa la fuente de estos depredadores.

- ✓ Durante el período reproductivo del maíz, *O. majusculus* se mueve continuamente entre la alfalfa y el maíz, pero el movimiento desde el maíz a la alfalfa es más intenso. El maíz actúa principalmente como una fuente de *O. majusculus* hacia a la alfalfa.
- ✓ Durante el período reproductivo del maíz, *C. septempunctata* sólo se mueve de la alfalfa al maíz. La alfalfa continúa actuando como fuente de este depredador hacia el maíz.

- Un paisaje formado por campos adyacentes de alfalfa y maíz podría ser complementario y una alternativa a la siega en franjas de la alfalfa, especialmente en zonas con campos pequeños.

Movimiento de depredadores epiedáficos desde los cereales de invierno y prados hacia hábitats semi-naturales

- Los carábidos, estafilínidos y arañas se mueven desde los cereales de invierno y prados hacia hábitats semi-naturales, pero el movimiento depende del hábitat y de la especie de artrópodo:
  - ✓ El movimiento de carábidos, estafilínidos y arañas constructoras de telarañas es más intenso desde los cereales de invierno hacia los hábitats semi-naturales que desde los prados.
- Los prados pueden actuar como franjas amortiguadoras alrededor de las áreas protegidas de manera que los depredadores provenientes de los cultivos extensivos no pongan en peligro la estructura y funcionamiento de las comunidades vulnerables.

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