Biological Control 77 (2014) 66–75

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Stable carbon and nitrogen isotope signatures to determine predator dispersal between alfalfa and maize

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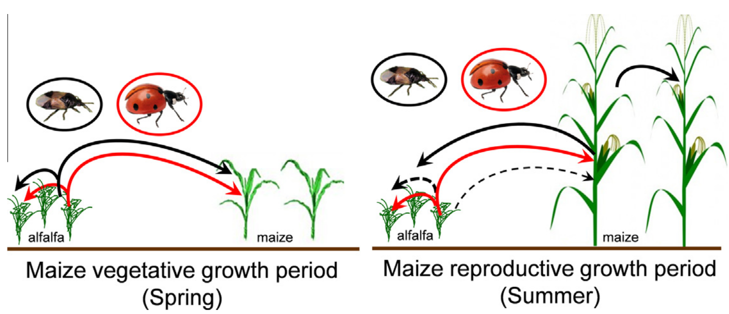
HIGHLIGHTS

- $\delta^{13}C$ and $\delta^{15}N$ analysis allow tracking predator movement between alfalfa and maize.
- Alfalfa is a source of *C. septempunctata* and *O. majusculus* towards maize in spring.
- Dispersal patterns differ between predators in summer.
- Maize acts as a source crop of *O. majusculus* towards alfalfa in summer.
- *C. septempunctata* does not disperse from maize to alfalfa.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 March 2014
Accepted 24 June 2014
Available online 2 July 2014

Keywords:
Orius majusculus
Coccinella septempunctata
Conservation biological control
Aphids
Natural enemies

ABSTRACT

The dispersal 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, which mainly becomes a sink crop. All of the *C. septempunctata* collected on alfalfa were local and those collected on maize came from alfalfa, but there was no dispersal 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.

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 (Pons and Eizaguirre, 2009; Pons et al., 2011). 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.

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http://dx.doi.org/10.1016/j.biocontrol.2014.06.009
1049-9644/

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. Dispersal 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 dispersal between adjacent crops.

Information on natural enemy dispersal can be acquired through the application of marking and tracking techniques (Lavandero et al., 2004; Reynolds et al., 1997), 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 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. Hagler and Jackson, 2001; Lavandero et al., 2004; Nienstedt and Poehling, 2000, 2004). Plants themselves may be used as markers for tracking insects through their natural C and N stable isotopes. In particular, carbon stable isotope ratios (δ^{13}C) have recently been proven to be suitable for tracing the plant origin of phytophagous and predator insects (Forbes and Gratton, 2011; Prasifka and Heinz, 2004; Prasifka et al., 2004; Schallhart et al., 2011, 2009; Traugott et al., 2008; Vialatte et al., 2006) and this is especially true when C3 and C4 plants are compared because of the clearly different δ^{13}C values. 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 dispersal 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 analyses 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 dispersal would be rejected if individuals from one crop showed no difference in δ^{13}C from the prey (aphids) of the same crop in which they were sampled.

2. Materials and methods

2.1. Study area

The study was conducted in 2009 at two 100-km apart 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 first location was Almenar (41°46′22″N, 0°30′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 17 °C from April to October. The second location was in La Seu d'Urgell (42°20′24″N, 1°25′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 17 °C 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 3 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.