



Molecular tracking of arthropod predator–prey interactions in Mediterranean lettuce crops

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ABSTRACT

The feeding habits of the generalist arthropod predators in agroecosystems are often difficult to determine, as they are small, mobile and live among the vegetation or in the soil. DNA-based gut-content analysis is a powerful tool that enables the study of arthropod predator–prey interactions. Predation on two of the main pests of Mediterranean lettuce crops, the lettuce aphid, *Nasonovia ribisnigri*, and the western flower thrips, *Frankliniella occidentalis*, as well as on Collembola, the most abundant non-pest prey, was studied. Generalist arthropods, like hoverflies, anthocorids (*Orius* spp.), coccinellids and spiders were collected in lettuce plots in two seasons (spring and summer) and analysed by conventional PCR using *N. ribisnigri*, *F. occidentalis* and Collembola-specific primers. Our results showed that in spring the main pest was *N. ribisnigri*, which was consumed by hoverfly larvae and coccinellids. In summer, the main pest was *F. occidentalis*, which was mainly predated by *Orius* spp. followed by hoverfly larvae. Spiders, which fed mainly on Collembola, did not seem to contribute to control of either target pest. This study offers a deeper knowledge of the trophic relationships present in Mediterranean lettuce crops, laying the groundwork for implementing biological control programmes based on the conservation of natural enemies.

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1. Introduction

Generalist predators can play a major role in controlling pest populations and preventing pest outbreaks in many agroecosystems (Symondson, 2002). Detailed knowledge about generalist predator diets is fundamental in the development of conservation biological control programmes. In Mediterranean lettuce crops, the lettuce aphid, *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae) and the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) are two of the main pests (Alomar et al., 2008; Gomez-Polo et al., 2015; Mou, 2008). Their biological control is based on the use of generalist predators (Díaz et al., 2010; Pascual-Villalobos et al., 2006).

Hoverflies (Diptera: Syrphidae) are usually present in Mediterranean lettuce crops, with *Episyrphus balteatus* (De Geer), *Scaeva pyrastris* (L.), *Eupeodes corollae* (F.), *Meliscaeva auricollis* (Meigen), *Sphaerophoria scripta* (L.) and *Sphaerophoria rueppellii* (Wiedemann) being the most common species (Gomez-Polo et al., 2014; Morales et al., 2007; Pascual-Villalobos et al., 2006). Whereas adults mainly consume pollen and nectar, larvae of many species are polyphagous predators of a broad

range of soft-bodied insects, such as aphids, which have been described as the preferred prey of most hoverflies (Rojo et al., 2003). *Orius* spp. (Hemiptera: Anthocoridae) are common polyphagous predators in agroecosystems of the Mediterranean area, where they have usually been associated with thrips (Castañé et al., 1999; Riudavets, 1995; Riudavets and Castañé, 1998). Seven *Orius* species have been reported to colonise Mediterranean vegetable crops naturally, often in a species complex that colonises the same crop: *O. majusculus* (Reuter), *O. laevigatus* (Fieber), *O. niger* (Wolff), *O. albidipennis* (Reuter), *O. minutus* (L.), *O. horvathi* (Reuter) and *O. laticollis* (Reuter) (Ferragut and González-Zamora, 1994; Gomez-Polo et al., 2013, 2016; Goula et al., 1993; Riudavets and Castañé, 1998; Riudavets et al., 1995; Tommasini et al., 2004). Spiders are ubiquitous in terrestrial ecosystems, both in natural and agricultural habitats (Nyffeler and Benz, 1987) and they have been suggested to decrease herbivore abundance (Chapman et al., 2013; Greenstone and Shufran, 2003; Harwood et al., 2004). Whereas linyphiids are a major component of the generalist predator community within arable crops (Agusti et al., 2003a; Romero and Harwood, 2010), other spider families have also been found to be present in Mediterranean agroecosystems (Mestre et al., 2013). Other generalist predators like lady beetles (Coleoptera: Coccinellidae) are also abundant in vegetable crops. In the Mediterranean area, one of the most common species present in lettuce crops is *Coccinella septempunctata* (Linnaeus) (Sengonca et al., 2002), which has been described as feeding on hemipterans such as aphids and scale insects (Urbaneja et al., 2005). All these generalist predators may feed not

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only on pests but also on non-pest arthropods, which may be an important food source when pests are scarce. One of the main non-pest food in arable ecosystems are springtails (Collembola), which may serve as alternative prey for the predators present in the crop (Agusti et al., 2003a; Kuusk and Agusti, 2008).

Agricultural habitats are artificially created and often characterised by high levels of disturbance as a consequence of frequent harvesting and planting regimes. In particular, Mediterranean lettuce crops are short-term crops, which makes them a simple model for studying predator–prey links in those disturbed habitats, an important step in developing predator conservation programmes for focal pests. Traditional methods of visual observation have been used for determining trophic linkages through gut dissection and microscopic characterisation of the gut contents, but they can only be applied when solid remains are present (Moreno-Ripoll et al., 2012; Symondson, 2002). Conversely, when the studied generalist predators are fluid feeders (e.g. hoverfly larvae, *Orius*, and spiders), PCR-based techniques are more suitable approaches, as they detect prey DNA within predator guts (Agusti et al., 2003b; King et al., 2008; Sint et al., 2011).

The overall purpose of this work is to describe the trophic interactions present in Mediterranean lettuce crops in two different seasons (spring and summer). A PCR-based gut content analysis was conducted to study predation under non-manipulated field conditions by the most common generalist predators (hoverflies, *Orius* spp., spiders and coccinellids) on the two main pests of lettuces (*N. ribisnigri* and *F. occidentalis*) and the most abundant alternative prey (Collembola). This study provides dietary information about the main predatory natural enemies for improving CBC programmes in Mediterranean lettuce crops.

2. Materials and methods

2.1. DNA amplification and primer specificity

DNA was extracted from individual predators with the DNeasy Tissue Kit (QIAGEN; Hilden, Germany; protocol for animal tissues). Total DNA was eluted into 100 µl of AE buffer and stored at -20°C . Negative controls were added to each DNA extraction set. Predation on *N. ribisnigri*, *F. occidentalis* and Collembola was analysed by conventional PCR using specific primers previously developed for the detection of *N. ribisnigri* (Nr1F/Nr2R) and *F. occidentalis* (Fo1F/Fo1R) (Gomez-Polo et al., 2015). They were designed from the cytochrome oxidase I (COI) mitochondrial region and produced amplicons of 331 bp and 292 bp for *N. ribisnigri* and *F. occidentalis*, respectively. Collembola-specific primers (Co14F/Co15R) were designed from the 18S region and produced an amplicon of 177 bp (Kuusk and Agusti, 2008). PCR reactions (25 µl) contained 4 µl of template DNA, 0.6 U of *Taq* DNA polymerase (Life Technologies, CA, USA), 0.2 mM of dNTPs (Promega Corporation, WI, USA), 0.6 µM of each primer and 5 mM of MgCl_2 (50 mM) in $10\times$ manufacturer's buffer. Samples were amplified in a 2720 thermal cycler (Applied Biosystems, CA, USA) for 35 cycles at 94°C for 30 s, 58°C (Fo1F/Fo1R) or 62°C (Nr1F/Nr2R and Co14F/Co15R) for 30 s and 72°C for 45 s. A denaturation cycle of 94°C for 2 min initiated the PCR, and a final cycle extension was conducted at 72°C for 5 min. Target DNA and water were always included as positive and negative controls, respectively. PCR products were separated by electrophoresis in 2.4% agarose gels stained with ethidium bromide and visualised under UV light.

N. ribisnigri, *F. occidentalis* and Collembola primers were screened by PCR for specificity against two to five individuals of several non-target species (see Table 1), like other prey, predators and parasitoids potentially present in agroecosystems of the studied area.

2.2. PCR analyses of field-collected predators

One experimental lettuce plot (var. Maravilla) (plot 1) located in the El Maresme area, at the IRTA Centre in Cabrils (41.518 N, 2.377 E) was sampled. Between 17 and 30 lettuce plants were collected in this plot

in spring (18 and 19 May 2009, 11, 18 and 25 May and 1 June 2010) and in summer (7 and 14 July 2009, 13, 20 and 27 July and 3 August 2010). On the other hand, three commercial plots (plots 2, 3, and 4) were sampled at particular dates in order to complement samplings from plot 1. Twenty lettuce plants were collected once in plot 2 in summer 2010 (12 July); 25 and 14 were collected in plot 3 in spring 2009 (22 April and 5 May) and 20 more were collected once in plot 4 in spring 2009 (5 May). These plots 2, 3 and 4 were located in two different areas: plot 2 in El Maresme (in Vilassar de Mar. (41.497 N, 2.374 E)); plots 3 and 4 in Baix Llobregat (plot 3 in Castellbisbal (41.474 N, 1.959 E); and plot 4 in Molins de Rei (41.398 N, 2.024 E)). Both areas (El Maresme and Baix Llobregat) are close to Barcelona (Spain) and about 35 Km apart. All collected lettuce plants were brought individually in plastic bags to the laboratory, where each leaf was separated and visually examined. Abundances of each prey (*N. ribisnigri*, *F. occidentalis* and Collembola) were recorded for each plot and season and predators (hoverflies, *Orius*, spiders and coccinellids) were kept at -20°C for subsequent molecular analyses. Comparison of prey densities in plot 1 between spring and summer samplings conducted in both years was performed via a Student's *t*-test with aphid data transformed by $\log(x + 1)$. On 12 May 2009, some hoverfly larvae and coccinellid larvae were collected on the ground of plot 1 because of their high abundance on that date. Spiders were also collected on the ground of plot 3 on 22 April and on the ground of plot 4 on 5 May 2009. Ground predators were collected with a mouth aspirator and also stored at -20°C awaiting molecular analysis.

Prior to DNA extraction, all predators were checked for attached prey remains under a microscope and thoroughly rinsed with distilled water. After PCR analyses, predation percentages of the lettuce aphid, the thrips and Collembola and all their combinations were calculated. The identification of all *Orius* and hoverfly specimens collected in the sampled plots was conducted molecularly as described in Gomez-Polo et al. (2013) and Gomez-Polo et al. (2014), respectively. Spiders and coccinellids were morphologically identified with the identification keys of Barrientos and Ferrández (1985) and Plaza (1986), respectively.

3. Results

3.1. Specificity of the primers

When primers Nr1F/Nr2R (*N. ribisnigri*), Fo1F/Fo1R (*F. occidentalis*) and Co14F/Co15R (Collembola) were tested for cross-amplification against 36 predator species, 2 parasitoids and 6 other potential prey (some of them already tested in other previous studies), only the target species were detected, showing a high specificity (Table 1).

3.2. PCR analyses of field-collected predators

Table 2 shows the mean abundances of the three prey items recorded in the experimental plot (plot 1) sampled in 2009 to 2010 per season and in the three commercial plots at those particular moments when they were sampled. In plot 1, *N. ribisnigri* abundances were quite high in spring, dramatically decreasing in summer. Similar results were observed in plot 3 in spring, when medium abundances were found, and in plot 2 in summer with very low abundances. Abundances of the lettuce aphid in plot 4 in spring were low. Although *F. occidentalis* were present in spring, they were very scarce in plot 1, when in summer, even if they increased, they were still quite low. Similarly, low thrips densities were also present in spring in plots 3 and 4, being slightly higher in summer in plot 2. Collembola abundances varied between years in plot 1. They were scarce in plots 3 and 4 in spring and higher in plot 2 in summer. The comparison of aphid, thrip and Collembola abundances between spring and summer was only possible in plot 1, where most of the samplings were conducted. *N. ribisnigri* populations were significantly higher in spring (Student's *t*-test: $t = -5.26$; $df = 5.14$; $P = 0.003$) and *F. occidentalis* significantly higher

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