Biological Control 115 (2017) 18–22

Contents lists available at ScienceDirect

Biological Control

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

Provisioning of food supplements enhances the conservation of phytoseiid mites in citrus

Aleixandre Beltrà[a], Altea Calabuig[a], Cristina Navarro-Campos[a], María José Ramírez-Soria[b], Antonia Soto[a], Ferran Garcia-Marí[a], Felix L. Wäckers[b], Apostolos Pekas[b,*]

[a] Institut Agroforestal Mediterrani, Universitat Politècnica de València, València, Spain
[b] R & D Department, Biobest Belgium N.V, Westerlo, Belgium

ARTICLE INFO

Keywords:
Biological control
Pollen
Sugar
Non-prey food
Mediterranean Basin
Euseius stipulatus

ABSTRACT

Phytoseiid mites (Acari: Phytoseiidae) are amongst the most abundant and important predators in citrus. They range from specialist species feeding exclusively on spider mites to generalist species that can feed and reproduce on several prey as well as non-prey foods such as pollen, fungi and honeydew. Feeding on non-prey foods is crucial for the conservation of phytoseiids especially during periods when prey are scarce or absent. In a greenhouse experiment using citrus plantlets we tested the impact of provisioning cattail pollen (*Typha angustifolia* L.) and a sucrose solution, alone or in combination, on the population development of the pollen adapted *Euseius stipulatus* (Athias-Henriot) (Acari: Phytoseiidae). The highest *E. stipulatus* populations were registered in the treatment where the pollen was supplied in combination with the sucrose solution. The phytoseiid abundance in the sugar and pollen treatment was significantly higher than the sum of the abundances when either the pollen or the sugars were supplied separately. Thus, pollen and sugar acted synergistically on the populations of *E. stipulatus*. Subsequently, we tested the combination of pollen and sugar in a six month field study in two commercial citrus orchards in eastern Spain. Provisioning of pollen and sugar resulted in significantly higher abundance of the resident phytoseiid mites compared to the control trees that received no food supplements. In both orchards this increase was higher from the end of May until the end of June and from September until the end of October. We conclude that the provisioning of sugar and pollen enhances the conservation of the resident phytoseiid mites in citrus.

1. Introduction

Phytoseiid mites (Acari: Phytoseidae) are abundant and widespread in citrus ecosystems, playing a major role in biological control (McMurtry, 1977). They are considered the most important family of predators of spider mites and contribute to the control of other pests such as whiteflies, thrips and scale insects (Ferragut et al., 1992; Grout, 1994; Huffaker et al., 1969). The species complex of phytoseiid mites in citrus orchards in eastern Spain (Valencia) is composed of a limited number of species. *Euseius stipulatus* (Athias-Henriot) is the most abundant species and usually accounts for more than 80% of the records (Ferragut et al., 1988; Abad-Moyano et al., 2009). Other phytoseiid species such as *Typhlodromus phialatus* Athias-Henriot, *Neoseiulus californicus* (McGregor) and *Phytoseiulus persimilis* Athias-Henriot are also frequently found in Spanish citrus, although in much lower numbers (Abad-Moyano et al., 2009; Ferragut et al., 1983). The seasonal dynamics of these predatory mites in the Mediterranean Basin are influenced by biotic and abiotic factors. Among the abiotic factors, high temperature and low relative humidity in summer have been shown to negatively affect phytoseiid abundance in citrus (Ferragut et al., 1988, 1987). Among the biotic factors, the availability of prey and non-prey food in the ecosystem has an important impact on the overall phytoseiid abundance and on their community composition (Abad-Moyano et al., 2009; Aguilar-Fenollosa et al., 2011; Ferragut et al., 1987).

Phytoseiid mites can feed on different food types, including arthropod prey, as well as non-prey food such as pollen, nectar, honeydew or fungi (McMurtry and Rodríguez, 1987; Wäckers, 2005). Their diet range has also been the basis for their classification (McMurtry et al., 2013; McMurtry and Croft, 1997). The abundance of those species that are specialist predators of spider mites is typically highly correlated with the dynamics of their prey (McMurtry and Croft, 1997). On the other end of the spectrum are the generalist species that can prey on various prey and non-prey items, and finally the species able to exploit pollen. Crucially, pollen feeding phytoseiids can survive and even

* Corresponding author at: Biobest Belgium NV, Ilse Velden 18, 2260 Westerlo, Belgium.
E-mail address: tolis@biobest.be (A. Pekas).

http://dx.doi.org/10.1016/j.biocontrol.2017.09.007
Received 24 February 2017; Received in revised form 11 September 2017; Accepted 14 September 2017
Available online 18 September 2017

reproduce on pollen when prey is scarce or absent. Thus, non-prey food can play an important role in maintaining the populations of generalists and pollen adapted species and thus help prevent pest outbreaks (Ferragut et al., 2013; James, 1990, 1989). Several studies have shown that the presence of pollen improves the survival and oviposition of phytoseiid mites and reduces pest populations (Ferragut et al., 1987; Maoz et al., 2014; Nomikou et al., 2010, 2003; Pijnakker et al., 2016; van Rijn et al., 2002). In citrus, pollen can be provided by using wind pollinated cover crops and hedges (Aguilar-Fenollosa et al., 2011; Duso et al., 2004; Smith and Papacek, 1991; Villanueva and Childers, 2004). Alternatively, pollen can also be dusted directly on the crop (Maoz et al., 2014; Montserrat et al., 2013). Sugar feeding has been shown to benefit phytoseiid mites as well, by increasing survival (Ferragut et al., 1987; McMurtry and Scriven, 1966; van Rijn and Tanigoshi, 1999). Different sugar sources can be naturally available in the ecosystem, such as, nectar and honeydew. Alternatively, sugar supplements can also be provided by spraying sugar solutions on the trees (Tena et al., 2015; Wade et al., 2008). Moreover, from a parallel study (Pekas and Wäckers, 2017) we observed that the provisioning of sugar sprays in combination with pollen and/or fibers increased the abundance of phytoseiid mites on citrus seedlings.

Therefore, in this study we first tested the impact of *Typha angustifolia* pollen provided alone or in combination with a sucrose solution on the population dynamics of the phytoseiid mite *E. stipulatus* in a greenhouse experiment. Subsequently, the impact of the combined application of pollen and sucrose on the populations of resident phytoseiids was tested in two commercial citrus orchards in eastern Spain.

2. Materials and methods

2.1. Greenhouse experiment

2.1.1. Greenhouse and plants

All trials were performed at the Green Lab facilities of Biobest N.V. in Westerlo (Belgium). Temperature in the greenhouses was automatically controlled (Compri 100, type CD 750, Priva®, UK) at 24 °C and 65 % HR. *Euseius stipulatus* used in the trials were obtained from a rearing at Biobest facilities. The experiment was conducted on sour orange *Citrus aurantium* L. seedlings of 24 ± 1.15 (mean ± SE) cm in height and with approximately 50 fully grown leaves. Before the trials, all plants were inspected to assure that they were free of other predators, pests or diseases.

2.1.2. Experimental design

We assessed the impact of supplying i) sugars, ii) pollen, iii) pollen plus sugar and iv) no food (control) on the abundance of *E. stipulatus* over time. Every treatment was repeated on five plants, using a randomized complete block distribution. Each block consisted of one table (600 cm length × 80 cm height × 100 cm wide) containing one replicate of all treatments. On the upper side of the table a water barrier was created, by covering the 8 cm deep frame with a plastic white sheet and filling this with 5 cm of water. Subsequently, the citrus plants for each treatment were placed individually on a plastic container (26 cm length × 26 cm width × 10 cm height) positioned upside down in the water. Plants were placed at sufficient distance to prevent contact of leaves between plants and together with the water barrier this effectively precluded the migration of phytoseiid mites between plants.

For the sugar treatment a 10% sucrose solution (Sigma-Aldrich) was uniformly sprayed on the plants. For the pollen treatment, 0.2 g of *T. angustifolia* pollen (Nutrimite™) was dusted uniformly with a salt shaker on each plant. For the combination of sugar and pollen, we first sprayed the sugar solution and once the sugar had dried we dusted the pollen (0.2 g /plant). The control treatment did not receive any food supplement. Pollen and sugar were re-applied every ten days. Ten adult females of *E. stipulatus* were gently released on each plant using a small paintbrush on May 22, 2015. The number of *E. stipulatus* motile forms was assessed on twenty leaves per seedling using an eye-piece magnifier. Countings were performed every two weeks during two months.

2.2. Field experiment

2.2.1. Orchards

The study was conducted in two commercial citrus orchards in Carcaixent (Valencia, Spain) (39.074751 N, −0.425678 W; 39.068921 N, −0.44946 W) in 2014. Both orchards consisted of clementine *Citrus clementina* Tanaka var. 'Clemenules'. The orchards were drip irrigated and were maintained free of weeds throughout summer by mowing (orchards 1 and 2) and spot treatment against problem weeds by applying herbicide (Glyphosate®; Bayer CropScience, Valencia, Spain). Orchard 1 had a surface of 0.73 ha, with a distance of five meters between trees as well as between rows. Trees were 2.5 m in height. Orchard 2 had a surface of 0.91 ha, with a distance of five meters between trees and nine meters between rows. Here trees were 3 m in height. No insecticides were applied before or during the study.

2.2.2. Experimental design

We assessed the influence of supplying pollen plus sugar, versus no food (control) on the abundance of a resident phytoseiid population. In each orchard, two groups of eight trees were selected for the pollen plus sugar and control treatments respectively. To avoid interference between treatments (e.g. pollen drift or migration of predatory mites) the experimental trees were separated by two buffer trees in a row (15 meters) and two trees between rows (15–20 m).

Sugar and pollen were applied biweekly starting from the 21st of May until the end of the experiment (November 14th). We used a 10% solution of the commercial sugar Biogluc®, (71.5% w/w) containing fructose (37.5%), glucose (34.5%), sucrose (25%), maltose (2%) and oligosaccharides (1%). The Biogluc solution was sprayed at a dose of 400 ml per tree. When the sugar dried, 3 g of *T. angustifolia* pollen (Nutrimite ™) was uniformly applied on each tree using a dusting applicator (Nutrigun, Biobest N.V.)®.

To record the number of motile stages of predatory mites per leaf, forty interior leaves were randomly taken from each tree and examined immediately in the field under a stereomicroscope. Samplings were performed biweekly in both orchards starting one week after the first application on May 28th and running until the 14th of November 2014.

Two additional samplings were done in order to identify the phytoseids present in the citrus trees to species level. The first sampling was carried out one week after the beginning of the trial (28th May), and the second one at two weeks before the end of the samplings (27th October). Twenty leaves were randomly collected from the inside of the canopy of each tree (five for each orientation), i.e. 160 leaves per treatment and orchard. In the laboratory, adult females were slide-mounted (Heinze PVA) and identified under a microscope following Ferragut et al. (2010).

2.3. Statistics

In both greenhouse and field experiments we used repeated measures ANOVA to compare the number of motile predatory mites per leaf among treatments. Treatment and time were the fixed factors and tree nested within treatment was the random factor. When significant differences were found among treatments, means were separated according to Fisher's LSD. All analyses were carried out with Statgraphics Centurion XVII (v. 16.1.11) (Statpoint Technologies Inc., 2010).

3. Results

3.1. Greenhouse experiment

Treatment had a significant effect on the abundance of *E. stipulatus* ($F = 31.42$; df = 3, 16; $P < 0.0001$) (Fig. 1). The number of motiles