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An introduction device for the aphidophagous hoverfly *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae)

Pascal D. Leroy *, François J. Verheggen, Quentin Capella, Frédéric Francis, Eric Haubruge

Department of Functional and Evolutionary Entomology, University of Liege, Gembloux Agro-Bio Tech, Passage des Déportés 2, B-5030 Gembloux, Belgium

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ABSTRACT

Augmentative biocontrol constitutes a safe option to reduce pest populations through the enhancement of natural enemies' activity. In this context, the aphidophagous syrphid *Episyrphus baltetaus* (De Geer) (Diptera: Syrphidae) is a promising candidate for aphid biological control: larvae of this syrphid attack and consume a wide range of aphid species and are found on many vegetable crops.

Because natural populations of beneficial insects are not always sufficient to regulate the pest infestations, this work has focused on the conception of a biological control device containing syrphid eggs which ones can easily be introduced in fields or greenhouses. Using semiochemicals [E-(β)-farnesene, R-(+)-limonene and (Z)-3-hexenol], honeydews and "artificial honeydews" (10% or 30% aqueous solutions of sucrose, fructose and glucose), the syrphid oviposition was artificially induced on an inert surface. Specifically, E-(β)-farnesene and concentrated mono-sugars (30%) were identified as the most efficient ovipositional stimulants. To test and validate the biological control device described above, laboratory and field experiments were performed: a plastic lamella covered with syrphid eggs was suspended on aphid infested plants in order to measure the efficiency of the device. The results obtained were promising since populations of 500 aphids were eliminated in 10 days when 15 syrphid eggs were introduced. The use of such a biological control device could certainly contribute to the biological control to reduce the aphid infestations.

1. Introduction

Augmentative biocontrol is one of the possible biological control strategies that is focused on enhancing the number and/or activity of natural enemies in agroecosystems. This strategy involves mass multiplication and periodic release or introduction of natural enemies in fields (Koul and Dhaliwal, 2003). Indeed, the equilibrium population size and dynamic behavior of many phytophagous insects are largely determined by their natural enemies (Waller, 1987). In this sense, many authors have demonstrated the importance of natural enemies in the regulation of pest populations (Price, 1987; Van Driesche and Bellows, 1996). Augmentation of natural enemies provides a biological solution to pests' problems in crops where naturally occurring beneficial organisms fail to respond quickly enough to control populations of pests (King et al., 1993). In the context of integrated pest management, an ideal natural enemy is one that consumes sufficient preys at the right time to maintain a pest population below the economic injury threshold for the crop considered (Michaud and Belliure, 2000).

Several predators have been studied as efficient beneficial insects to reduce the aphid damages. The importance of generalist predators in reducing the population density of aphids is widely recognized (Hughes, 1989; Dixon, 1998; Jervis and Kidd, 1996). Among them, Coccinellidae and Chrysopidae are certainly the most documented predators since numerous studies have described predator–prey interactions involving these predators (Powell and Pell, 2007; Volkl et al., 2007; Latham and Mills, 2009). Furthermore, predation by Coccinellidae and Chrysopidaes contributes to the suppression of aphids in several agricultural systems (e.g., potatoes, sugar beets, alfalfa, cotton and wheat) (Coderre, 1999; Barbosa et al., 2008).

* Corresponding author. Fax: +32 81 62 23 12.
E-mail addresses: entomologie.gembloux@ulg.ac.be, p.leroy@ulg.ac.be (P.D. Leroy).

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In this study, the hoverfly *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae) was chosen because this syrphid is considered as the most abundant in agroecosystems and natural habitats in central Europe (Tenhumberg and Poehling, 1995; Colignon et al., 2001), as the most efficient aphid predators (Tenhumberg and Poehling, 1991) but also because this hoverfly is associated with different aphid–plant complexes (Bargen et al., 1998). Data from Rojo et al. (2003) indicate that *E. balteatus* larvae feed on a large variety of aphid species (234 taxa) with strong evidence for best adaptation to aphids on Gramineae. Also, *E. balteatus* is the most abundant aphidophagous predator in vegetable crops such as broad beans and carrots (respectively, 70% and 80% of the aphidophagous species) (Colignon et al., 2001, 2002).

Episyrphus balteatus larvae are particularly voracious feeders, often eating hundreds aphids during their development. Adults have strong abilities to forage for aphid colonies using vision and semiochemicals released from their preys or prey host–plants (Verheggen et al., 2008, 2009b; Almohamad et al., 2007, 2008, 2009). Furthermore, even first instar larvae can move to new aphid colonies: they are capable of covering about 1 m what allows them to move between plants (Banks, 1968) and even if ladybirds appear to be more active among aphid colonies (Brodsky and Barlow, 1986), it has been shown that syrphid larvae, acting in more restricted area without excessively disturbing aphids, significantly reduce the dispersal of aphids (Niku, 1976) and so the spread of viruses in fields.

The aim of this work was to obtain a non-expensive biological control device containing eggs after having artificially induced the syrphid oviposition without using any aphids or host–plants parts. So obtained eggs could be introduced in fields to reduce and control the aphid populations. Because natural insect predators are difficult to maintain in situ and because it is not easy to maintain the natural enemy populations at a sufficiently high number in any given area, the introduction of eggs is advantageous since the emerging larvae directly act in situ.

2. Material and methods

2.1. Rearing plants and insects

In a climate-controlled room (16 h light photoperiod; 60 ± 5% RH; 20 ± 2 °C), the host–plants – *Vicia faba* L. – were grown in 9 × 8 cm plastic pots containing a mixture of vermiculite and perlite (1/1) and were infested with the aphid *Acyrthosiphon pisum* Harris, *Aphis fabae* Scopoli, *Megoura viciae* Buckton or *Myzus persicae* Sulzer. In the same climatic conditions, but in a different room, *E. balteatus* larvae were obtained from a mass-production: the hoverflies were reared with sugar, pollen and water and the oviposition was induced by the introduction of infested host–plants in the rearing-cage (75 × 60 × 90 cm) during 3 h. The complete life cycle took place on the host–plants daily re-infested with aphids. Syrphid pupae were provided by Katz Biotech AG (Baruth, Germany).

2.2. Oviposition induction with semiochemicals

Several experiments were conducted to obtain syrphid eggs without using host–plants and aphids. First, the following semiochemicals were tested to induce the oviposition: *E*-(β)-farnesene [the major component of many aphids alarm pheromones, (Francis et al., 2005)], R-(+)-limonene [a common plant monoterpene (Paré and Tumlinson, 1999)] and (Z)-3-hexenol [a green leaf alcohol released by plants in response to mechanical damages or infestations (Paré and Tumlinson, 1999)]. All chemicals were purchased from Sigma–Aldrich (Steinheim, Germany) and had a chemical purity >97% (GC analyses).

The previously cited semiochemicals were tested individually or mixed with each other as follow: E-(β)-farnesene + R-(+)-limonene (10/90; 50/50; 90/10 v/v); E-(β)-farnesene + (Z)-3-hexenol (10/90; 50/50; 90/10 v/v). As proposed by Verheggen and colleagues (2008), a rubber septum was used as a dispenser to release continuously the volatile chemicals. The dispenser was placed into a plastic container (50 cm^3) (VWR International) closed with a piece of net for aeration and filled with a 100-µl paraffin oil solution (400 ng/µl final concentration) of the tested chemical. As a positive control, 50 *A. pisum* aphids (adults on a piece of plant) were placed into containers 24 h before the experiments.

2.3. Oviposition induction with sugars

A second set of experiments consisted in the evaluation of the oviposition activity of the main aphid honeydew sugars. Only sucrose, fructose and glucose were tested in this study because the other typical honeydew sugars such as melezitose, melibiose and trehalose are too expensive in the context of a mass-production of syrphid eggs. To do so, solutions of the main honeydew sugars (sucrose (S), fructose (F) and glucose (G)) at 10 g/100 ml and 30 g/100 ml in distilled water (for each sugar), were prepared. For both concentrations, single solutions were tested (S; G; F). Different sugar combinations were also evaluated where sugars were added in the same proportions: S + F; S + G; F + G; S + G + F. A 50 µl volume of these solutions was sprayed onto small plastic lamellas (1 × 5 cm) that were placed into a plastic container (50 cm^3).

2.4. Oviposition induction with natural honeydews

The third set of experiments consisted in the evaluation of the oviposition activity of natural honeydew. Natural honeydew was obtained from four aphid species (*A. pisum*, *M. viciae*, *M. persicae* and *A. fabae*). It was collected on plastic lamellas (1 × 5 cm) placed under infested *V. faba* during 24 h. Lamellas covered with 10 mg (mass difference between tarred and honeydew covered lamellas) of honeydew were used to study the syrphid oviposition into plastic containers (50 cm^3).

In all sets of experiments, one gravid *E. balteatus* female was introduced into a plastic container and was allowed to lay eggs during 3 h. Gravid females were separated from no-gravid ones when they contained mature eggs easily seen through transparent abdominal pleurites (Sadeghi and Gilbert, 2000).

Twenty replications, for each experiment, were performed. These tests were conducted in a climate-controlled room at 22 ± 2 °C and 60 ± 5% RH. The gravid *E. balteatus* females were 15–20 days old and were deprived of aphids for 24 h before the experiments.

2.5. Biological control device: laboratory and field experiments

Syrphid eggs were artificially obtained using semiochemicals, natural honeydew or honeydew sugar solutions as described above.

During the laboratory experiments, the biological control device consisted in a plastic lamella (1 × 5 cm) covered with 5, 10 or 15 eggs. At the beginning of the assay (Day 0), *V. faba* plants (15–20 cm; 2 leaves) were separately potted and then infested with exactly 50 aphids *A. pisum* (adults). One biological control device covered with syrphid eggs (lamella) was suspended on the plant. The control consisted in an infested *V. faba* plant without the biological control device. To evaluate its efficiency, the number of hoverfly larvae and the number of surviving aphids found on the plant were counted after 2, 5 and 7 days. Fifteen replications were performed for each density of eggs. These observations were conducted in a climate-controlled room at 22 ± 2 °C and 60 ± 5% RH.