Biological Control 67 (2013) 227–234

Contents lists available at ScienceDirect

Biological Control

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

Biological control of the greenhouse whitefly by *Amitus fuscipennis*: Understanding the role of extrafloral nectaries from crop and non-crop vegetation

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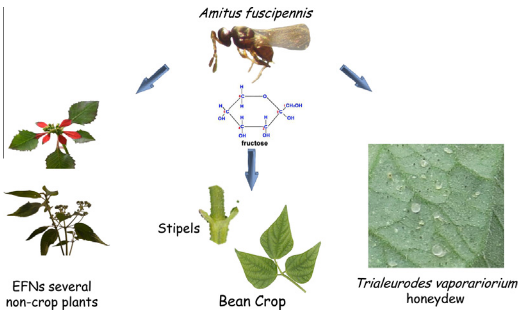
HIGHLIGHTS

- Several non-crop plant species around bean enhance reproduction of *Amitus fuscipennis*.
- *A. fuscipennis* feeds on extrafloral nectaries of non-crop wild plants.
- *Phaseolus vulgaris* stipels are both extrafloral nectaries and food for *A. fuscipennis*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 September 2012
Accepted 1 August 2013
Available online 9 August 2013

Keywords:
Phaseolus vulgaris
Conservation biological control
Extrafloral nectaries
Anthrone
Parasitoids

ABSTRACT

To determine the potential role of non-crop vegetation extrafloral nectaries (EFNs) in the presence and feeding of *Amitus fuscipennis*, a parasitoid of *Trialeurodes vaporariorum*, non-crop vegetation in and around ten fields of common beans (*Phaseolus vulgaris*) was studied in the Central Andes of Colombia. Using 0.25 m^2 quadrants located randomly both within the bean fields and at a distance of 3 m from the crop, the non-crop vegetation was identified and counted. The abundance of both *T. vaporariorum* and *A. fuscipennis* was also determined. Furthermore, plants separated by more than 3 m from the crop were examined for 30 min to determine the presence of *A. fuscipennis*. There were 92 plant species associated with bean crops: 46 within the crop, 73 at 3 m from the crop border, and an additional 18 species at more than 3 m from the crop. The occurrence of *A. fuscipennis* was influenced by the presence of EFNs and whitefly nymphs. Some plants allowed reproduction of both insect species. Experimental data confirmed that *A. fuscipennis* fed on EFNs. The role of the crop itself as a sugar source for the parasitoid was studied. It was confirmed that *P. vulgaris* stipels are EFNs and a source of sugar for *A. fuscipennis*. Several non-crop plant species in the agroecosystem and within the bean crop play a role in the whitefly/parasitoid interaction as alternate hosts for *T. vaporariorum* and as a source of concentrated sugar in EFNs for *A. fuscipennis*.

1. Introduction

Conservation biological control is the modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests (Eilenberg et al., 2001). Conservation biological control usually involves changing the farm landscape by establishing shelter habitats such as grass-sown raised earth banks, which provide a suitable over-wintering site for predatory beetles and Araneae (Thomas et al., 1991), strips of flowering plants for food resources for natural enemies (Tylianakis et al., 2004), or cover crops for

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http://dx.doi.org/10.1016/j.biocontrol.2013.08.003

conserving natural enemies (Lundgren and Fergen, 2011) although cover crops can also benefit herbivores (Lavandero et al., 2005) or only certain parasitoid species in agricultural landscapes (Pfiffner et al., 2009).

Agricultural landscapes include a mosaic of crop and non-crop habitats and are inhabited by crop pests and their natural enemies, which depend on the large-scale landscape structure (Gardiner et al., 2009). Bean (*Phaseolus vulgaris* L.) agroecosystems in the highland tropics (altitude >1000 m) of the Andes in Colombia include non-crop wild plants that are present only during the crop season or grow permanently in the area. Vegetation diversity of non-crop habitats may enhance indirect crop defense against arthropod pests by providing resources for predators and parasitoids (Cortesero et al., 2000) and improving conditions for their conservation (Gardiner et al., 2009; Griffiths et al., 2008). Access to sources of sugar such as floral and extrafloral nectar in extrafloral nectaries (EFNs) has a positive impact on the fecundity and longevity of beneficial insects (Lee and Heimpel, 2008; Nafziger and Fadamiro, 2011).

Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae), the greenhouse whitefly, is a key pest of *P. vulgaris* common beans and snap beans (Rodríguez and Cardona, 2001), causing crop losses of up to 16.5% in the interandean Cauca River Valley of Colombia (Rendón et al., 2001). Plant damage is caused by both immature and adult whiteflies, which suck plant nutrients, reduce plant productivity and excrete honeydew on the leaves, flowers and fruits, thereby inducing the growth of fungi and reducing photosynthesis (Lindquist et al., 1972). This whitefly is also a known vector of many crop viruses (Wisler and Duffus, 2001).

Amitus fuscipennis MacGown and Nebeker (Hymenoptera: Platygasteridae) is a solitary koinobiont and small (<1 mm) parasitoid of the first and second instars of the greenhouse whitefly (Manzano et al., 2002). The parasitoid exhibits area-restricted searching behavior that facilitates finding nymphs of *T. vaporariorum*, which are distributed in a clumped pattern (Manzano et al., 2002). *A. fuscipennis* is adapted to highland tropical climate conditions similar to those of *T. vaporariorum* (Manzano and van Lenteren, 2009) and is abundant on common bean and snap bean crops, especially at the end of the growing season when farmers stop insecticide spraying. Parasitism on bean crops can reach levels of up to 68.5% (Manzano, 2000). Field experiments in Pradera (Cauca Valley State, Colombia), showed that nymphs of *T. vaporariorum* parasitized by natural populations of *A. fuscipennis* appeared 20 days after planting, suggesting that the parasitoid remained on the surrounding plants and crops, dispersing early to new crop fields (Manzano et al., 2003b). Due to a heavy insecticide spraying regimen and the removal of a semiannual crop such as beans, the role of surrounding wild plants as a refuge may be a requirement for maintaining *A. fuscipennis* populations locally and thereby decreasing focal crop susceptibility to whitefly attack (Barbosa et al., 2009). A significant decrease in *A. fuscipennis* longevity from 18.1 to 4.2 days in the absence of sugar under lab conditions (Manzano et al., 2000) suggests that under field conditions the parasitoid must feed on sugar sources. When foraging on *P. vulgaris* leaflets, the parasitoid typically walks, exhibiting area-restricted searching behavior for *T. vaporariorum* nymphs, adopting a feeding position, but apparently feeding on secretions from *P. vulgaris* stipels and increasing its residence time on the plant although it does not feed upon the host (Manzano et al., 2003a). The presence of EFNs has been recorded for several *Phaseolus* spp. but not for *P. vulgaris* (Keeler, 2008). These observations and the absence of this parasitic wasp on 420 flowers of 13 plant species around bean crops in the study area (unpublished results) suggest the importance of extrafloral nectar as a source of sugar from both the primary host crop and surrounding vegetation. Although parasitoids with short mouth parts might not always have access to floral nectar (Jervis, 1998), several studies have demonstrated their ability to feed on floral nectar (Nafziger and Fadamiro, 2011; Winkler et al., 2006), extrafloral nectar (Röse et al., 2006) and honeydew in the field (Wäckers et al., 2008). Nothing is known about the use of extrafloral nectar by *A. fuscipennis*. After extrafloral nectar feeding, the fructose consumed by the parasitoid wasp can be detected by biochemical assays of carbohydrate metabolism; e.g., anthrone tests (Lavandero et al., 2005; Nafziger and Fadamiro, 2011; Wyckhuys et al., 2008). Knowing what plant species harbor *A. fuscipennis* would allow habitat manipulation to maintain plants that provide food and shelter for the parasitoid. Habitat manipulation could enhance *A. fuscipennis* performance if key plants that offer food or refuge to the parasitoid are identified.

The goal of this study was to evaluate the availability of extrafloral nectars in the bean landscape. Specifically, we tested (i) the effect of EFNs on *A. fuscipennis* abundance, (ii) the presence of extrafloral nectaries (EFNs) on *P. vulgaris*, and (iii) whether the parasitoid feeds on EFNs sugars.

2. Materials and methods

The study was conducted in bean crops (*P. vulgaris*), located from 1400 to 2000 masl on the eastern slope of the Central Andes (3°38′N, 76°06′W) in the town of Cerrito, Cauca Valley State, Colombia. The area is a subtropical dry forest with temperatures ranging from 17 to 24 °C and an average annual rainfall ranging from 500 to 1000 mm (Espinal, 1968). The agricultural landscape consists primarily of monocrops surrounded by non-crop vegetation that can be broadly divided into three groups: (i) primarily weeds that grow within the crop area with the bean plants; (ii) mostly herbaceous plants that grow within 3 m of the crop border, some of which are reduced by herbicides applied by farmers at the end of the cropping season; and (iii) permanent shrub and tree vegetation that grows more than 3 m from crop borders and is apparently free of herbicide or pesticide residue from crop management practices. All three groups were studied.

2.1. Plants sampled within the crop and at 3 m from the crop border

Ten small crop farms that were sown with *P. vulgaris* dry bean ‘Cargamanto’ and ‘Calima’ and snap bean ‘Lago Azul’, with crop ages ranging from 1 to 3.5 months, were chosen, and their area was estimated. All farmers controlled whiteflies with a weekly application of thiametoxam (Actara® 25WG) and methomyl (Methavin® 90 SP). To study the influence of non-crop vegetation on the presence of *A. fuscipennis* under current pest management practices, pesticide spraying was not stopped.

To sample plants within and at 3 m from the crop on each farm, a wood frame (0.25 m^2) was launched randomly (backwards) four times, both within the crop area and at 3 m from crop borders (one on each side). The experimental unit was the wood frame that was launched 80 times (40 times within the crops and 40 times in surrounding vegetation) in ten different *P. vulgaris* agroecosystems. Each crop and its surrounding plants were visited once from 19 February to 26 September 2009.The crop cycle lasts from 3 to 4 months. The total richness and abundance of plant species inside the squares were recorded. The number of *A. fuscipennis* adults, parasitized hosts and non-parasitized whitefly nymphs found on all leaves were also recorded. The sample unit was the plant. The position of *A. fuscipennis* adults on certain plant structures (leaf, stem, petiole, peduncle, EFNs) was determined. The number of *A. fuscipennis* adults on plants with EFNs and/or *T. vaporariorum* nymphs was compared in a log-linear analysis for a $2 \times 2 \times 2$ table of cross-categorized frequency data ($p < 0.05$). In addition, the number of *A. fuscipennis* adults present within and outside the crop