

**Evaluation of *Amitus fuscipennis* as biological control agent of
Trialeurodes vaporariorum on bean in Colombia**

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**Evaluation of *Amitus fuscipennis* as biological control agent of
Trialeurodes vaporariorum on bean in Colombia**

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op gezag van de rector magnificus
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in het openbaar te verdedigen
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des namiddags te half twee in de Aula

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To Carlos and Gladys, my parents

BOEKEN- en
LAND-BOUWBOEKSTUUR
WAGeningen

Manzano M., Maria R.

Evaluation of *Amitus fuscipennis* as biological control agent of *Trialeurodes vaporariorum* on bean in Colombia.

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Propositions

1. We often think about insecticides as the only chemical control measures that strongly interfere with biological control agents. However, fungicides and herbicides also may have a strongly negative influence on natural enemies.

Hassan *et al.* 1991. Results of the fifth Joint Pesticide Testing Programme carried out by the IOBC Working Group "Pesticides and Beneficial Organisms". *Entomophaga* 36: 55-67.

This thesis

2. It would be a mistake to believe that biological control is not an effective management tool if it does not completely control a pest on all affected crops.

K. A. Hoelmer. 1996. Whitefly parasitoids: Can they control field populations of *Bemisia*? In: *Bemisia* 1995: taxonomy, biology, damage control and management. D. Gerling & R. T. Mayer (eds.) Intercept, Andover.

This thesis

3. Biological pest control is not a panacea and is not always effective on its own. It must, therefore, often be integrated with other pest control techniques in integrated management programmes.

D. J. Greathead. 1991. Biological control in the tropics: present opportunities and future prospects. *Insect Science and its Application* 12(1/2/3): 3-8.

This thesis

4. Farmers' knowledge should be used more often to develop integrated or biological pest control programmes in order to prevent unnecessary research.

This thesis

5. In developing countries, the bias towards biotechnological research is draining the very limited funding from critically needed basic biological work and its application.

M. J. Way & H. F. van Emden. 2000. Integrated pest management in practice – pathways towards successful application. *Crop protection* 19: 81-103.

6. *Amitus fuscipennis* is a fast walking parasitoid but, "more haste, less speed". August Caesar 63 BC.

Bartlett, J. 1968. *Familiar Quotations*. 5th edition. Lowe & Brydone, London, p. 124.

This thesis

7. As Wageningen University is developing into an international university, it should be a rule that all Ph.D. candidates defend their thesis in the international scientific language: English.

8. To write down a thesis is not an easy task. Even Gabriel García Márquez realized after publishing "One Hundred Years of Solitude", that he wrote 42 contradictions and 6 big grammatical mistakes.

García Márquez, Gabriel. 1987. *Cien Años de Soledad*, 4a. edición, J. Joset (ed), Ediciones Cátedra, Madrid. p. 52.

9. A one-half cup of Colombian cooked beans contains 131 calories, less than 1-g fat, 8.5-g protein, 10-g fiber and measureless hours of intensive, risky and badly paid farmer labor.

Propositions with the thesis "Evaluation of *Amitus fuscipennis* as biological control agent of *Trialeurodes vaporariorum* on bean in Colombia" by Maria R. Manzano.

Wageningen, October 30 2000.

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1. Introduction

The research described in this thesis concerns the study of a natural enemy of whiteflies, *Amitus fuscipennis* MacGown & Nebeker under Colombian field and laboratory conditions. The general aim of this research project is to study whether biological control of whiteflies with *A. fuscipennis* can be added to the integrated control program for bean (Prada *et al.*, 1993). First I will provide data about the pest, the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood), and its natural enemies. Then I will describe the aims of my Ph.D. project in more detail.

The greenhouse whitefly

The greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) was first described by Westwood in 1856 from individual insects found on presumably imported living plants or in the packings of Orchidaceae imported into England from Mexico (Mound and Halsey, 1978). Although Brazil or Mexico is a likely possibility, the specie's origins are not definitely established (Vet *et al.*, 1980). The whitefly is polyphagous and has been recorded on about 898 plant species from 121 families and 469 genera (Xu *et al.*, 1988). In many areas, it is a major glasshouse pest (Dowell, 1990), but it also attacks outdoor plants in temperate and sub-tropical regions (Martin, 1985), and in the tropics (Cardona and Karel, 1990).

Among the more important hosts are many vegetables (members of the Solanaceae, Cucurbitaceae), beans (Leguminosae), and ornamentals. *T. vaporariorum* has six life stages: the egg, the first nymphal instar (first as crawler, later sessile), two sessile nymphal instars (second- and third-instar nymphs), a sessile "pupa" (fourth instar), and the adult (Gill, 1990; van Roermund and van Lenteren, 1992). Crop damage is caused partially by adult and immature whiteflies sucking nutrients from the plant and reducing crop productivity. Besides, whiteflies excrete large quantities of honeydew on leaves, fruits and flowers, which favor the development of sooty moulds that, in their turn, cover leaf surfaces, reduce photosynthesis and results in cosmetic damage of fruit and flowers (van Roermund, 1995). Additionally some whitefly species transmit viral diseases (van Roermund, 1995). Extensive reviews about *T. vaporariorum* can be found in van Lenteren and Noldus (1990) and, van Roermund and van Lenteren (1992).

The natural enemies of whiteflies

The population growth rate of *Trialeurodes vaporariorum* is exponential as long as host plants remain in good physiological condition and temperatures are favorable (van Lenteren and Noldus, 1990). In natural ecosystems and agro-ecosystems where pesticides are either selectively or not used, several

natural enemies keep the whiteflies at low numbers, as illustrated by two cropping systems: one of tomato, in the 1960s in California, and the other of cotton, from 1925 onwards in Sudan (van Lenteren *et al.*, 1996). But under extensive pesticide use, natural enemies were killed and whiteflies frequently reached pest status.

The three main groups of the whitefly's natural enemies are:

1. Fungi: from the genera *Aschersonia*, *Verticillium*, *Beauveria* and *Paecilomyces*.
2. Predators from the families Coccinellidae (Coleoptera); Cecidomyiidae and Drosophilidae (Diptera); Chrysopidae and Hemerobiidae (Neuroptera); Anthocoridae and Miridae (Heteroptera).
3. Parasitoids from the families Aphelinidae, Eulophidae, and Platygasteridae (Hymenoptera).

Fungi. Entomopathogenic fungi infest their aleyrodid hosts by penetrating the cuticle after the conidiospores germinate. Subsequently, various fungal structures are produced, which circulate in the haemolymph, invading the insect's organs and probably producing lethal toxins (Fransen, 1990). One major limitation of entomopathogenic fungi as biological control agent is their need for high humidity to germinate and infect the host (Lacey *et al.*, 1995). Another limitation comprises the restrictions placed on the use of so-called exotic strains that could harm non-target organisms (Lacey *et al.*, 1995).

Two categories of fungi that attack whitefly can be distinguished: fungi specialized on Aleyrodidae like *Aschersonia* spp and *Conidiobolus* spp (Fransen, 1990; Gidin and Ben Ze'ev, 1994), and "broad-spectrum fungi", containing various genera like *Beauveria*, *Verticillium*, and *Paecilomyces*, which can infect insects belonging to different orders.

Aschersonia fungi have an essentially tropical and sub-tropical distribution. They have been successfully applied and established to control whiteflies on citrus and additionally, they have been tested against the greenhouse whitefly in glasshouses of Central and Eastern Europe and some Oriental countries (Fransen, 1990). *Aschersonia* isolates may kill up to 90% of *T. vaporariorum* and *Bemisia argentifolii* Bellows & Perring at greenhouse experimental conditions (Meekes, personal communication). Although the fungi's biological and physical properties indicate positive features, they have not yet been developed into formulated biological control products (Fransen, 1990). *Aschersonia* spp equally attacked *T. vaporariorum* and *B. argentifolii* and caused the same mortality levels for both whitefly species (Meekes *et al.*, 1996). In fact, each fungus that is introduced to control *Bemisia*, will almost certainly affect *T. vaporariorum*.

Most research has been done on *Beauveria*, *Paecilomyces* and *Verticillium* because they are broad-spectrum fungi, and therefore, more attractive for commercial production. *Verticillium lecanii*

(Zimm) Viegas seems promising for controlling greenhouse whitefly, although an effective infection requires high relative humidity but moderate temperatures (Ekbohm, 1979). *Paecilomyces fumosoroseus* var. *beijingsis* attacks *T. vaporariorum* on glasshouse cucumbers at moderate temperatures and high relative humidity (Fransen, 1990).

So far *Aschersonia* is more difficult to produce commercially than the other fungi. It takes 14 days to mass-produce *Aschersonia* spp spores, whereas it only takes 5 days to produce millions of *Beauveria* spores. Using fungi to control insects is not only a question of using the best fungus species, but also a question of using the best commercial formulation (E. Meekes, personal communication).

When reporting these fungi's interactions with other natural enemies, Fransen (1990) observed that populations of the parasitoid *Encarsia formosa* Gahan survived treatments with *Aschersonia aleyrodis* Webber and adult parasitoids did not become infected. *Encarsia formosa* females may even help with fungal transmission by probing infested hosts, carrying fungal structures on their ovipositors and injecting uninfested hosts. Vargas *et al.*, (1995) reported that *A. aleyrodis* was innocuous to *E. formosa* and was found to be neither in competition nor in association with the parasitoid. *V. lecanii* did not directly infect the parasitoid *Amitus fuscipennis* when they were tested together on *T. vaporariorum*, but the fungus seemed to decrease the parasitoid's parasitic activity (Pachón and Cotes, 1997).

Currently *B. bassiana* is commercially the most interesting species for whitefly control and is sold as Botanigard or Naturalis.

Predators. Several polyphagous predators have been recorded as enemies of the greenhouse whitefly (Vet *et al.*, 1980) but not much has been published about their use as biological control agents. The question arises as to their efficiency in reducing and controlling whitefly populations (Gerling, 1990). None of the examined predator species in the families Coccinellidae, Chrysopidae, Hemerobiidae, Anthocoridae, and most Miridae are able to maintain the greenhouse whitefly numbers below damaging levels when acting alone (Onillon, 1990). Only some of the predatory mirid species belonging to the genera *Macrolophus* and *Dicyphus* could reduce whitefly populations to low densities (Onillon, 1990). Their polyphagy allows them to feed on other prey when whitefly densities are low, which might be advantageous (Onillon, 1990).

Parasitoids. Based on the present knowledge of the control capacity of different categories of natural enemies of whiteflies, parasitoids still have the best potential for obtaining long-term control through inoculative or seasonal inoculative releases (Gerling, 1990).

Whitefly parasitoids belong to the families Eulophidae, Aphelinidae, and Platygasteridae. The only whitefly parasitoid genus in Eulophidae is *Euderomphale*. Parasitoid genera in Aphelinidae comprise *Azotus*, *Cales*, *Encarsia*, and *Eretmocerus* but only the last two parasitize *T. vaporariorum*. The parasitoid genus *Amitus* from the Platygasteridae also parasitizes whiteflies. The genera *Encarsia* and *Eretmocerus* have been studied and used much more than has been *Amitus* (van Lenteren *et al.*, 1997).

Biological control of whitefly

Aphelinid parasitoids. Several aphelinid species are important regulators of pest populations and are sometimes and successfully used in biological and integrated control. Aphelinid parasitoids of the greenhouse whitefly are described by van Lenteren *et al.*, (1997). Both the biological control of whiteflies and the integration of the whitefly parasitoids into pest management programs have been highly successful on evergreen perennials such as citrus (Dowell, 1990). The aphelinids *Encarsia opulenta* Silvestri, *E. clypealis* Silvestri, and the platygasterid *Amitus hesperidium* Silvestri were released in 1976 (Hart *et al.*, 1978) to control the citrus blackfly (*Aleurocanthus woglumi* Ashby), achieving outstanding success by 1978 (DeBach and Rosen, 1991).

An IPM program was developed with *E. formosa* to control *T. vaporariorum* on glasshouse tomato (van Lenteren and Woets, 1988). The program's success was such that the parasitoid was also used to protect glasshouse crops of cucumber and poinsettia (Dowell, 1990). The program's technology has now extended to more than 20 countries of the 35 countries with a glasshouse industry (van Lenteren *et al.*, 1996), where this parasitoid is mass-reared and used to control greenhouse whitefly on various vegetable crops and some ornamentals. The technology's success was a result of basic research on the relationships among *E. formosa*, greenhouse whitefly, and host plants, including work on how the parasitoid locates and attacks its hosts and how glasshouse climate and plant architecture can influence the host finding behavior and parasitization efficiency (van Lenteren *et al.*, 1996). An individual based simulation model, integrating elements of the local searching and parasitization behavior of individual *E. formosa* parasitoids in a greenhouse-whitefly infested tomato-crop was developed by van Roermund (1995). This model assisted in identifying causes for failure or success of biological control.

Platygasterid parasitoids. Because parasitoids of the *Amitus* genus belong to the less studied Platygasteridae, a more extensive review on this family is presented here. Most platygasterids are known as parasitoids of gall midges (Diptera: Cecidomyiidae), but some genera specialize in other

groups of insects like the Coleopterous families Curculionidae and Cerambycidae, and the Homopterous families Pseudococcidae and Aleyrodidae (Vlug, 1995).

The genus *Amitus* Haldeman has worldwide distribution except for New Zealand and an uncertain status in Australia (Masner and Huggert, 1989). Some *Amitus* species are near cosmopolitan in distribution (Masner and Huggert, 1989). Although about 20 *Amitus* species have been described, the host species for only about 18 are known (Table 1). The list shows that *A. fuscipennis* has been recorded so far as being the only *Amitus* parasitoid specializing in *T. vaporariorum*. The host range may be wider, however, as this parasitoid has not been well studied.

Morphology and biology of Platygasteridae

Female platygasterids are small, black, robust wasps, between 0.8 and 0.9 mm long, with a swollen thorax and forewings with marginal and stigmal veins absent. Males can be distinguished from females by their antennae, as occurs in other parasitoids. Male antennae are filiform, whereas female antennae have 10 segments, including a 3-segmented club. Scanning electron microscopy reveals that the outer side of the fourth antennal segment in males has a paddle-like or plate-like process, which may have a mechanical function (MacGown and Nebeker, 1978) or it may have a gland to release sexual pheromones (Isidoro and Bin, 1995).

Medina *et al.*, (1994) described that the platygasterid *A. fuscipennis* has seven life stages: the egg; the first, second, and third larval stages; the pre-pupa; the pupa; and the adult. The egg is oval (0.089×0.02 mm in size), translucent, and narrow at one extreme, forming a pedicel. The first instar is transparent, apodous, curved, and highly mobile. It has 12 segments, the last one being narrow and pointed. The second instar is sessile, thick (0.46×0.23 mm), and unsegmented, with its mouth opening in the middle of the prosoma. The third instar (0.51×0.25 mm) is quiescent and the anal opening is visible. The prosoma presents two lateral processes close to the mouth opening. At this stage, the host is whiter than a normal unparasitized nymph is and, under the stereoscope, the developing parasitoid can be observed.

The pre-pupa is translucent, 0.63×0.28 mm in size, and presents constrictions at pro-, meso-, and meta-somas. The thorax is swollen and curves dorsally. As the parasitoid changes from larva to pupa, the host's color changes from white to light brown. The pupa, 0.78×0.37 mm in size, presents a very well defined head, thorax, and abdomen, and is exarate. Initially, it has a gelatinous

Table 1. Species of the parasitoid *Amitus* genus with known host species of insect pests. Blank spaces indicate information not mentioned.

Parasitoid species	Host insect	Host plant	Reference
<i>A. aleurodinis</i> Haldeman	<i>Tetraneura corni</i> (Haldeman), <i>Tetraneura mori</i> (Quaintance), <i>Aleurodus forbesii</i> (Ashmead), <i>Aleurochiton aceris</i> (Modeer), <i>Aleuroplatus plumosus</i> (Quaintance), <i>Tetraneura abutiloneus</i> (Haldeman), <i>Tetraneura fernaldi</i> (Morrill)	<i>Acer dasycarpum</i> Ehrh	Vlug, 1995
<i>A. aleuroglanduli</i> Viggiani & Evans	<i>Aleuroglandulus malangae</i> Russell	<i>Xanthosoma</i> sp	Viggiani & Evans, 1992
<i>A. aleurolobi</i> Mani	<i>Aleurolobus barodensis</i> (Maskell)	<i>Saccharum officinarum</i> L	Vlug, 1995
<i>A. aleurotubae</i> Viggiani & Mazon	<i>Aleurotrachelus jelineki</i> (Frauenfeld)	<i>Viburnum tinus</i> L	Vlug, 1995
<i>A. arcuatus</i> Whittaker			MacGown & Nebeker, 1978
<i>A. bennetti</i> Viggiani & Evans	<i>Bemisia tabaci</i> (Gennadius)	<i>Euphorbia heterophylla</i> L	Viggiani & Evans, 1992
<i>A. croesus</i> Huldén	<i>Aleurotuberculatus similis</i> Takahashi	<i>Vaccinium vitis-idaea</i> L	Vlug, 1995
<i>A. fuscipennis</i> MacGown & Nebeker	<i>Tetraneura vaporariorum</i> (Westwood)	<i>Conyza</i> sp. <i>Parietaria</i> sp., <i>Sonchus</i> sp., <i>Urtica</i> sp. ornamentals, <i>Phaseolus vulgaris</i> L	Viggiani, 1991; Medina <i>et al.</i> , 1994
<i>A. gibbosus</i> MacGown & Nebeker			MacGown & Nebeker, 1978
<i>A. granulatus</i> MacGown & Nebeker	<i>Tetraneura perileucae</i> (Cockerell)	<i>Quercus</i> sp	MacGown & Nebeker, 1978
<i>A. hesperidum</i> Silvestri	<i>Aleurocanthus woglumi</i> Ashby, <i>Aleurocanthus citripedus</i> Quaintance & Baker	<i>Citrus</i> sp	Vlug, 1995
<i>A. longicornis</i> (Förster)	<i>Aleurocanthus spiniferus</i> (Quaintance) <i>Aleurochiton aceris</i> (Modeer), <i>Aleurochiton complanatus</i> Baerensprung, <i>Aleurolobus wuini</i> (Ryberg), <i>Aleurolobus asaris</i> Wünn, <i>Bemisia silvatica</i> Danzig, <i>Pealius quercus</i> (Signoret), <i>Asterobemisia carpinii</i> (Koch)		Vlug, 1995
<i>A. macgowni</i> Evans & Castillo	<i>Aleurotrachelus socialis</i> Bondar	<i>Manihot esculenta</i> Crantz	Evans & Castillo, 1998
<i>A. minervae</i> Silvestri	<i>Aleurolobus olivinus</i> (Silvestri), <i>Aleurolobus wuini</i> (Ryberg), <i>Aleurochiton aceris</i> (Modeer)	<i>Clematis vitalba</i> L, <i>Acer platanoides</i> L	Vlug, 1995
<i>A. pigeanus</i> MacGown & Nebeker	<i>Aleurodicus pigeanus</i> = <i>Metaleurodicus pigeanus</i> (Baker & Moles) comb. n.		MacGown & Nebeker, 1978
<i>A. rugosus</i> Viggiani & Mazon	<i>Simplaneura hemisphaerica</i> Goux	<i>Phyllirea latifolia</i> L	Vlug, 1995
<i>A. sculpuratus</i> Viggiani & Evans	<i>Tetraneura mori</i> Quaintance	<i>Ilex</i> sp	Viggiani & Evans, 1992
<i>A. spiniferus</i> (Brèthes)	<i>Aleurothrix floccosus</i> (Maskell)		Vlug, 1995
<i>A. vesuvianus</i> Viggiani & Mazon	<i>Aleuroviggianus adrianae</i> Iacc, <i>Pealius</i> sp.	<i>Quercus ilex</i> L	Vlug, 1995

consistency and is colorless but later it becomes dark, except for the antennae and legs, which remain colorless. When the formation of the pupa starts, two brown lateral dorsal spots on the host begin growing into two lateral lines. When the pupa is completely pigmented, the whitefly nymph is gray. The parasitoid emerges from the parasitized whitefly nymph by making a small round hole, 0.29 mm in diameter, with its mouth.

Most studies on the *Amitus*-whitefly relationships have been conducted with *A. hesperidium*, parasitoid of the citrus blackfly (CBF, *Aleurocanthus woglumi*), but only a few dealt with this specie's life history (Flanders, 1969) and searching and ovipositional behavior (Dowell *et al.*, 1981). The life cycle of *A. hesperidium* is markedly influenced by that of its blackfly host. The parasitoid apparently pupates only after the host attains an advanced stage, which may be delayed until the rainy season (Flanders, 1969). During host searching, *A. hesperidium* moves randomly about the leaf on first arriving until it encounters the leaf margin. It then follows the margin to the mid-vein at one or the other end of the leaf. Once hosts are found, the walking pattern changes to short walks with several turns. Females prefer first-instar hosts and can distinguish hosts already parasitized by conspecific females (Dowell *et al.*, 1981).

Viggiani and Battaglia (1983) observed that antennal contact plays an important part in courtship and mating behavior of both *Amitus rugosus* Viggiani & Mazzone and *A. vesuvianus* Viggiani & Mazzone. After copulation, the female of *A. vesuvianus* strokes the substrate with her antennae and moves slowly forwards, whereupon the male dismounts. In contrast, *A. rugosus* females remain stationary. Other biological aspects of *A. hesperidium* and the few known data about *A. fuscipennis* and *A. longicornis* (Förster) are presented later in this chapter.

Whitefly control by *Amitus* spp

Authors studying control of Aleyrodidae by *Amitus* found that the parasitoid's field performance improved in combination with *Encarsia opulenta* (Hart *et al.*, 1978; Dowell *et al.*, 1979; Cherry and Pastor, 1980; Meyerdirk *et al.*, 1980; Selhime, 1980; Nguyen *et al.*, 1983; French *et al.*, 1990; Al-Mjeni and Sankaran, 1991; Meagher *et al.*, 1991; Tsai and Steinberg, 1992).

Smith *et al.*, (1964) found that, *A. hesperidium* was the only parasitoid to attack the citrus whitefly in Pakistan where, in some areas, parasitism had reached 90%. In India, *A. hesperidium* was well distributed and parasitized between 30% and 70% of *A. woglumi* larvae, although, in general, it appeared much less effective than *Encarsia clypealis* (Smith *et al.*, 1964). *A. hesperidium*, *E. opulenta*, and *E. clypealis* have been part of outstandingly successful biological control projects against *A. woglumi* on citrus (DeBach & Rosen, 1991). All three parasitoids became established in Mexico in the

1950 spring after the release of adults reared from *A. woglumi* pupae collected in India and Pakistan. Of these parasitoids, *A. hesperidium* was the most generally pro-ovigenic, that is, with a type of reproduction that enabled it to reproduce at a remarkably rapid rate at high host densities (Flanders, 1969).

The environmental conditions at that time—very high humidity and temperatures—produced high instar mortality (Flanders, 1969). Even though, Flanders (1969) suggested that *A. hesperidium* cannot efficiently locate its hosts at low host population levels. This may explain why both *E. opulenta* and *E. clypealis* replaced it. Full-fed larvae and pupae of *A. hesperidium* even serve as hosts for the *Encarsia* males (Flanders, 1969). Later, in one Mexican area, *E. clypealis* and *A. hesperidium* were observed to occur together, with parasitism by the former being at 90%, and by the latter at 5% or less (Flanders, 1969). Despite the intensity of competition between these three species, host population regulation was enhanced (DeBach and Rosen, 1991).

Besides high rates of oviposition at high host densities, *A. hesperidium* can readily disperse, travelling up to 3.7 km within 6 to 7 months after release (Selhime *et al.*, 1982). The CBF invaded the Lower Rio Grande Valley, Texas, in 1971 and, because eradication failed, the U.S. Department of Agriculture started a biological control project (DeBach and Rosen, 1991). *E. opulenta*, *A. hesperidium*, and *E. clypealis* were introduced from Mexico and established in 1974/75. A general decline of CBF occurred before 1978 (Hart, 1978) and, later, surveys indicated complete biological control of CBF in the area (Summy *et al.*, 1983). Results of extensive parasitoid surveys, conducted between 1977-1982, showed widespread distribution of *E. opulenta* and extreme scarcity or absence of *E. clypealis* and *A. hesperidium* in most populations (Summy *et al.*, 1983). This outcome suggests competitive displacement of these species by *E. opulenta* (Summy *et al.*, 1983). Nevertheless, the overall effect was a highly successful biological control project.

In 1976, an infestation of CBF was discovered in Florida and, again, chemical eradication programs failed. *A. hesperidium* and *E. opulenta* were therefore released and they became well established. Seven months later, 100% of pupae of *A. woglumi* were parasitized at some release sites (Hart *et al.*, 1978), reducing infestation by 98% within 8 months, that is, to a level of one infested leaf per citrus grove (Dowell *et al.*, 1979). This spectacular decline of CBF is credited largely to *A. hesperidium* (Hart, 1978; Hart *et al.*, 1978), although *E. opulenta* increased gradually and has now become the predominant parasitoid in low-density host populations (Selhime, 1980; Dowell *et al.*, 1981).

E. clypealis was released in small numbers in Florida, but it never became established (DeBach and Rosen, 1991) and, although poorly competitive, it appeared not to have detracted in any way from the excellent overall biocontrol results (Summy *et al.*, 1983; DeBach and Rosen, 1991). Recently, *A.*

hesperidium and *E. opulenta* were released in the Caribbean island of Dominica to control CBF (Martin, 1999).

In a different biological control project of *A. woglumi* and *Aleurothrixus floccosus* Maskell (woolly whitefly) in Mexico, *A. hesperidium*, *Encarsia* sp., and *Eretmoceris serius* Silvestri were released in areas where levels of parasitism were low (Garza, 1979). A later survey showed average levels of *A. woglumi* parasitism of 73.9%, of which 52.0% was attributable to *Encarsia* sp., 20.5% to *A. hesperidium*, and 1.6% to *E. serius*. For *A. floccosus*, average parasitism was 67.0% of which 35.5% was attributable to *E. serius* and 31.5% to *A. hesperidium* (Garza, 1979). To biologically control *A. floccosus* on citrus, other *Amitus* species like *A. spiniferus* (Brèthes), imported from Mexico and released in California (Koch, 1977; Meyerdirk *et al.*, 1980) were used.

After repetitive releases of these and other parasitoid species like *Cales noacki* DeSantis and two new species of *Eretmoceris*, the whitefly population was greatly reduced (DeBach and Rose, 1976). In some areas, complete biological control was achieved in the initial colonization sites within two growing seasons (DeBach and Rosen, 1991). Parasitoids, particularly *A. spiniferus* and *C. noacki* (imported from Chile), were estimated to reduce, in certain areas, woolly whitefly populations by more than 95% of the original peak populations (DeBach and Rose, 1976). On demonstration trees, which were kept free of interference from ants, dust, insecticides, and isolation, reductions of 99.9% were measured (DeBach and Rose, 1976). Both parasitoid species proved particularly effective in reducing whitefly populations and maintaining them at very low levels (DeBach and Rosen, 1991). These programs in California were such outstanding examples of modern biological control (DeBach and Rosen, 1991) that *Cales* and *Amitus* adults were collected from field study sites in California and released on infested citrus trees in Spain and France where they succeeded in controlling the woolly whitefly (DeBach and Rosen, 1991).

Also for citrus, *Amitus longicornis* (Förster) has been recorded as a parasitoid of *Aleurocanthus spiniferus* (Quaintance), a common pest of oranges in China (Li, 1993). Besides orchards, *Amitus* spp. can also be found on sugar cane crops infested with whiteflies. Both *Encarsia flava* (Shafee) and *Amitus* sp. parasitize *Aleurolobus barodensis* (Maskell) on sugar cane (Inayatullah, 1984). In an Indian crop survey, *Amitus* sp. was the most abundant parasitoid, representing 1125 individuals of a total 1281 insects sampled, including *Encarsia* spp., *Azotus* spp, and *Euderomphale* sp. (Tiwari *et al.*, 1978).

While sampling for parasitoids of the sweet potato whitefly, *Bemisia tabaci* (Gennadius) and *T. vaporariorum* on some outdoor crops in Costa Rica it was found that unidentified *Amitus* species parasitized both whitefly species (Hanson *et al.*, 1993). *Amitus bennetti* Viggiani & Evans (Drost *et*

al., 1999; Joyce *et al.*, 1999; Drost *et al.*, 2000; Joyce *et al.*, 2000) has been reared and released in Florida against *B. tabaci* (Viggiani and Evans, 1992).

***Amitus fuscipennis* MacGown & Nebeker**

In 1978, MacGown & Nebeker described *A. fuscipennis* according to specimens collected from Central America, South America, and the Caribbean. Medina *et al.*, (1994) also gathered biological and morphological information on the parasitoid. Later, *A. fuscipennis* was introduced from Colombia into Italy to control the whitefly under field conditions (Viggiani, 1991). The parasitoid's performance under glasshouse conditions is now being tested in Colombia (de Vis *et al.*, 1999).

The taxonomy of this species, according to Masner & Huggert (1989) and Vluc (1995), is as follows:

Order:	Hymenoptera
Superfamily:	Platygastridae
Family:	Platygasteridae
Subfamily:	Sceliotrachelinae
Genus:	<i>Amitus</i>
Species:	<i>fuscipennis</i> MacGown & Nebeker (1978)

A. fuscipennis has been observed to parasitize *T. vaporariorum* on various host plant species like *Conyza* sp., *Parietaria* sp., *Sonchus* sp., *Urtica* sp. (Viggiani, 1991), some outdoor and glasshouse crops like tomato in Colombia (Medina *et al.*, 1994; Vis *et al.*, 1999), and ornamentals and beans (Medina *et al.*, 1994).

The greenhouse whitefly problem on Colombian bean crops

Several whitefly species have been found in Colombia. The greenhouse whitefly, *T. vaporariorum*, has been registered as a pest of economic importance. Not only does it attack ornamental and horticultural crops in glasshouses (Medina *et al.*, 1994), but it also causes direct damage, in some areas, on field crops, for example by transmitting the potato golden virus. In Colombia it attacks more than 100 plant species belonging to 37 plant families, composed mainly of Asteraceae, Solanaceae, Malvaceae, Cucurbitaceae, Leguminosae, and Lamiaceae. In Colombia, *T. vaporariorum* is the predominant whitefly in the tropical highlands (altitudes above 1000 m) and inter-Andean valleys (altitudes ranging from 400 to 1000 m), where it attacks bean, tomato, and potato crops (Cardona *et al.*, 1998).

Bean cultivation is a major activity for many small farmers (farm size < 1 ha) in Colombia. Because *T. vaporariorum* is a key pest that reduces the yield of snap bean crops by about 50% (Prada *et al.*, 1993), farmers routinely apply pesticides as the only way to control it. Over the bean's 4-month cropping season, 11 or 12 applications of broad-spectrum insecticides and fungicides are carried out (Prada *et al.*, 1993), creating two major problems: pesticide resistance (Prada *et al.*, 1993; Buitrago *et al.*, 1994; Cardona *et al.*, 1998), and secondary pest outbreaks of the leafminer *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) (Prada *et al.*, 1993). In addition to economic problems, pesticides also lead to environmental pollution and health problems (Pachico, 1993). To help solve these problems and develop an alternative to chemical treatments, a guided control system was recently developed to control both whitefly and leafminer on bean. As a basic step, an action threshold for whitefly was determined: the appearance of first-instar nymphs on the lower third of the plant (Cardona *et al.*, 1993). This threshold refers to that density of the whitefly population at which control measures should be started to prevent the population from causing economic injury. The guided control system involves the:

1. Elimination of residues of previous crops before sowing,
2. Installation of yellow sticky traps to capture both whitefly and leafminer adults,
3. Application at the 'threshold level' of insecticides against whiteflies,
4. Attention, at the right moment, to control weeds and cut off lower leaves, and the proper management of soil and irrigation,
5. Removal and destruction of pruned materials,
6. Use of *Bacillus thuringiensis* (commercial toxin) to control certain herbivorous insects, and
7. Removal and destruction of the stubble after the last harvest.

These guided control components, when applied and compared with the conventional pesticide spray program in areas where the whitefly is key pest, resulted in a 54% reduction in insecticide use and an 18% reduction in costs (Prada *et al.*, 1993). So far, the program has not included any natural enemies of whiteflies, although several fungi like *Bauveria bassiana* (Balsamo) Vuillemin, *Verticillium lecanii* (Vuillemin), *Paecilomyces fumosoroseus* (Wize) Brown and Smith, and *Aschersonia* spp. are being tested (C. Cardona, personal communication).

Of the parasitoids, *Encarsia* spp. and *A. fuscipennis* have been found in whitefly infested areas and recovered from *T. vaporariorum* on bean plants. *A. fuscipennis* is often found on field crops of beans in the Colombian Andes and seems partially responsible for the present low infestation by *T. vaporariorum* in the Rionegro (Antioquia) area. The author's personal observations are that *A.*

fuscipennis is abundant on crops where no chemical treatments have been applied. According to C. Cardona (personal communication), it is even still active after heavy pesticide treatment. *A. fuscipennis* is also often found on wild plants close to crop areas, and is able to naturally colonize a glasshouse crop of chrysanthemums, replacing *E. formosa* parasitoids that had been imported from England and released (L. E. Pérez, personal communication).

A. fuscipennis also colonizes glasshouse tomato plants infested with *T. vaporariorum* in Colombia, maintaining presence and keeping *T. vaporariorum* at low densities (R. de Vis, personal communication). The observations made by Cardona, Perez, de Vis and the author suggest that *A. fuscipennis* may be a promising control agent of *T. vaporariorum* on Colombian bean crops.

Determining the role of natural enemies in pest control

The identification of an effective natural enemy or complex of natural enemies is a basic step in each biological control program. Evaluation and selection of natural enemies can be performed with an empirical or an analytical approach. In the empirical approach, one simply releases all potential natural enemies which happen to be available, with the hope that the proper species or combinations of species will be sorted out in the field (Ehler, 1990). Although historically most introduction programs were carried out on a largely empirical basis and yielded a large number of successes, the outcome not always can be predicted because it is generally unknown why one project succeeds and the other fails. Consequently, on the one hand promising agents may be rejected without knowing the causes of failure and on the other hand, inefficient agents may be released (van Lenteren and Woets, 1988).

At the other extreme is the analytical approach whereby one conducts extensive pre-introductory research to determine what species or group of species may be released (Ehler, 1990). Within the analytical approach, two different ways can be distinguished (Waage, 1990): 1) the reductionist and, 2) the holistic. The reductionist approach dissects the natural enemies into a simple set of characteristics which embody their potential as control agents and which can be viewed, and compared independently (Waage, 1990). Implicit to the reductionist approach is the notion that any combination of life history parameters is possible. However, under natural conditions these ideal agents do not occur. Because of the limitations of the reductionist approach, an alternative holistic approach appeared. The holistic approach may evaluate natural enemies i) based on ecosystem studies and, ii) based on integration of individual attributes. The holistic approach based on ecosystem studies proceeds from theoretical notions of how natural enemies fit into the broad ecology of the pest and its other mortality factors (Waage, 1990). Although it seems to be a sound approach it is not applicable yet. On the other hand, evaluation based on integration of individual attributes has been applied and has proved

to be of value. Van Lenteren and Woets (1988) and van Lenteren and Manzaroli (1999) give several of these attributes for pre-introduction evaluation of natural enemies for biological control in greenhouses (Table 2). The importance of each characteristic will more or less depend on the type of control that has to be achieved. In the inundative release method, beneficial organisms are periodically released in large numbers to obtain an immediate control effect (van Lenteren and Martin, 2000). In seasonal inoculative release method, natural enemies are mass reared and released in moderate numbers periodically, to obtain both an immediate control effect and also to build-up a natural enemy population for control later during the season, while in inoculative releases small numbers are released and the aim is long-term control (van Lenteren and Martin, 2000).

Concerning *A. fuscipennis*, the aim is not to introduce and establish the parasitoid because it occurs naturally on bean crops in Colombia, but to assess whether the parasitoid can be used in such a way that whiteflies will be kept at sufficiently low densities on bean. The attributes listed in Table 2 will first be used to evaluate *A. fuscipennis* as potential natural enemy of *T. vaporariorum* on bean crops in Colombia based on the knowledge available at the start of this Ph.D. project. This evaluation will point at limitations in our knowledge, which will lead to a list of characteristics to be determined will a reliable estimate of the role of *A. fuscipennis* become possible.

Seasonal synchronization with host

Seasonal synchronization of the parasitoid with its host is important because the natural enemy has to be around when the pest occurs. For the Colombian field situation this means that *A. fuscipennis* has to be in the field when the first generation of whitefly occurs in the bean crop. If the parasitoid does not migrate sufficiently fast into the bean fields from surrounding crops or wild plants, seasonal inoculative releases have to be made at the moment that the right whitefly stages for parasitism – 1st and 2nd nymphs – occur in the field. This can be achieved by following the greenhouse whitefly arrival and development in the bean crop.

Internal synchronization with the host

The natural enemy must be able to develop to the adult stage on the pest insect, and development should be synchronous with that of the pest species so that ongoing control can be obtained. It is an important aspect to consider in the *T. vaporariorum* - *A. fuscipennis* system, because mainly the first nymphal stages are used for parasitization and the parasitoid's developmental time is relatively long compared to that of the host. These factors might be causing problems early in the bean crop season, when whitefly generations are still separated.

Later in the season and at high greenhouse whitefly infestation, different instars of the pest may be

Table 2. Criteria for pre-introductory evaluation of natural enemies for biological control in greenhouses. (After van Lenteren and Manzaroli, 1999).

Criterion	Release Program		
	Seasonal inoculative	Inoculative	Inundative
Seasonal synchronization with host	+	–	–
Internal synchronization with host	+	+	–
Climatic adaptation	+	+	+
No negative effects	+	+	+
Good culture method	–	+	+
Host specificity	+	–	–
High kill-rate potential	+	+	–
Good searching efficiency	+	+	±

+ = Important; – = Not important; ± = Less important

present simultaneously, and internal synchronization is no longer a problem. Early in the season, synchronization could be obtained by the farmer, through releasing parasitoids in the crop at a moment when most hosts are in the developmental stage suitable for parasitization.

Climatic adaptation

Because of Andes mountain range presence in Colombia, cropping of dry and snap beans is possible at different altitudes as tropical highlands (altitudes above 1000 m) and inter-Andean valleys (altitudes around 1000 m) (Cardona *et al.*, 1998). This range implies as well, climatic variations, going from warm, dry areas to cooler, more humid ones. Therefore, it is important to know how certain climatic conditions influence the biology and performance of *A. fuscipennis*. Knowledge about functioning at different climates can be used to predict whether the parasitoid will be effective in broad or narrow geographic areas in Colombia.

Negative effects

A. fuscipennis should not attack other beneficial organisms or non-pest hosts of importance in the area where it will be used. *A. fuscipennis* has been recovered from *T. vaporariorum* hosts (MacGown and Nebeker, 1978; Viggiani, 1991). In certain bean crop areas of Colombia, *A. fuscipennis* can be found together with several *Encarsia* species that also parasitize *T. vaporariorum*. Currently, there is no information about negative interactions between these parasitoids, but studies have been very limited.

A. fuscipennis seems to reproduce by thelytokous parthenogenesis (Viggiani, 1991): *A. fuscipennis* males are rare. Further, there are no reports that this parasitoid is using other parasitoid species for hyperparasitism.

Some information is available about interactions between *A. fuscipennis* and entomopathogenic fungi of whiteflies on bean crops, and they were positive as far as whitefly control concerns. A stronger reduction of whiteflies was obtained when then the parasitoid was used together with the fungus *V. lecanii* for control of *T. vaporariorum* in field assays (Pachon and Cotes, 1997). Details about the interaction between parasitoid and fungus are, however, not available.

Good culture method

Due to the occurrence of high densities of *T. vaporariorum* at field conditions in Colombia, one of the strategies to bring or increase naturally occurring populations of *A. fuscipennis* in the field, could be by using initially inundative releases of the parasitoid. This means that large numbers of parasitoids will be required and that a good and economic culture method will have to be developed. In Colombia, a preliminary study about the mass rearing of *A. fuscipennis* showed that the parasitoid was not difficult to rear in *T. vaporariorum* as host species and bean as host plant (Garcia *et al.*, 1995). For mass rearing purposes, it is important to know the host stages that are preferred by the parasitoid, its developmental time, sex ratio and kind of reproduction. Most of these data are not yet available. Viggiani (1991) suggests that reproduction of *A. fuscipennis* is through thelytokous parthenogenesis.

Host specificity

In crops where different insect species may occur, it is important to introduce natural enemies that preferentially attack the key pest species in order to obtain adequate pest reduction (van Lenteren and Manzaroli, 1999). *A. fuscipennis* has been recovered from *T. vaporariorum* hosts (MacGown and Nebeker, 1978; Viggiani, 1991). However, at laboratory conditions we found that *A. fuscipennis* may parasitize another important whitefly pest, *Bemisia tabaci*. Although we have not found this outcome at field conditions in Colombia, we think that it would be a positive characteristic of *A. fuscipennis*, because *T. vaporariorum* and *B. tabaci* populations may occur simultaneously in the same crop at certain climate conditions. No information is available about other whiteflies or other insects that could be parasitized by *A. fuscipennis*.

Great reproductive potential

Life-history parameters on immature and mature life stages of insects can be integrated to determine the intrinsic rate of population increase or r_m value of an organism (e.g. Birch, 1948). According to van

Lenteren and Woets (1988) the r_m value or host kill rate (i.e. the r_m with the addition of hosts killed as a result of host feeding by the parasitoid) of *A. fuscipennis* should at least equal the population growth rate of *T. vaporariorum*, for the parasitoid to keep up with growth of the *T. vaporariorum* population in inoculative or seasonal inoculative programs. Due to the fact that Colombia is typified by three mountain ranges and beans are cropped from mid to high altitudes, it is important to compare *A. fuscipennis* and *T. vaporariorum* r_m values at different climatic conditions that simulate those of mid and high altitudes. If the r_m value of *A. fuscipennis* is higher than the r_m value of *T. vaporariorum* at both conditions, the parasitoid could be used and released for whitefly control over a broad climatic area.

Good searching efficiency

Searching efficiency is a required characteristic of natural enemies because they should be able to locate and reduce pest populations before they have crossed economic threshold densities (van Lenteren and Woets, 1988; van Roermund, 1995). Some parasitoids are efficient searchers because they are capable of detecting chemicals produced by the host or as a result of the interaction between the host and its host plant, at long distances (Vet and Dicke, 1992). However, other parasitoids, as the whitefly parasitoid *E. formosa*, searches at random, and is also able to sufficiently reduce its pest populations (van Lenteren *et al.*, 1996). *E. formosa* does not detect infested leaves even from short distances, and lands at random on infested or uninfested leaves (e.g. Sütterlin and van Lenteren, 2000). It is not known whether *A. fuscipennis* locates *T. vaporariorum* using long-distance cues or by random search. Reaction to long-distance host related cues may result in shorter search times, which in turn can lead to high attack rates (Sütterlin and van Lenteren, 2000).

Once the parasitoid is on the infested leaf, searching over relatively distances occurs. At bean fields in Colombia, whitefly density is usually very high and under this condition *A. fuscipennis* does not have to spend much time searching for hosts. However, at the beginning of the crop season or when applying a guided control program for *T. vaporariorum*, the whitefly density decreases and *A. fuscipennis* will have to search for hosts at low densities.

The searching efficiency criterion has been well studied in the whitefly parasitoid *E. formosa* by van Roermund and co-workers (1995). In commercial greenhouses, whitefly densities have to be very low for biological control to be successful, therefore effective host searching is a critical parameter. Many developmental, reproduction and host searching data have been collected over the past 30 years for the system greenhouse whitefly, *Encarsia formosa* and vegetable host plants (van Lenteren and van Roermund, 1999), but their effect on biological control cannot be evaluated independently because the attributes of natural enemies are often found in particular combinations (Waage, 1990). An "integration

of attributes" approach was followed for *E. formosa* and an individual based stochastic simulation model was developed for a better understanding of the performance of this parasitoid. The model includes a) the detailed search behavior of the parasitoid and, b) the demographics and distribution of whitefly and parasitoids in relation to host plant and greenhouse climate (van Lenteren *et al.*, 1996). The model was developed to be able (1) to explain the capability of *E. formosa* to reduce whiteflies in large greenhouses, (2) to improve introduction schemes of parasitoids for crops where control was difficult, and (3) to predict effects of changes in cropping practices on the reliability of biological control (van Lenteren *et al.*, 1996; van Lenteren and van Roermund, 1999). Of more than 20 parasitoid parameters related to searching and development that were evaluated experimentally and with simulation models, some (e.g. walking speed, percentage time actively searching, side of leaf on which parasitoid searches, and percentage of encounters with hosts resulting in oviposition) appeared to influence the searching and parasitization efficiency of *E. formosa* much more than others (e.g. immature mortality, maximum egg load, host handling time, probability of host feeding, residence time on leaves with remains of hosts, and flight distance between plants) (van Roermund, 1995; van Lenteren and van Roermund, 1999). Knowledge about these parameters for *A. fuscipennis* is very limited; most data were not available before this Ph.D. project was started.

In conclusion, for most of the attributes concerning the evaluation of an effective natural enemy, knowledge for *A. fuscipennis* is too limited or completely lacking.

Research aims

The general aim of this thesis is to evaluate *A. fuscipennis* as a potential biological control agent of *T. vaporariorum* on field crops of beans in Colombia. If results are positive, this parasitoid will be included into the recommended guided control practices in order to create an Integrated Pest Management program. From the criteria for pre-introductory evaluation of natural enemies given by van Lenteren and Manzaroli (1999), this thesis will address mainly climatic adaptation, culture method, reproductive potential and searching efficiency. In addition, the side effects of a fungicide used in bean crops will be tested on *A. fuscipennis*.

Climatic adaptation. Colombia is typified by three mountain ranges, and beans are cropped from mid to high altitudes. Climatic variation may influence parasitoid performance. Development time and longevity of *A. fuscipennis* will be determined under different climatic conditions – temperature and humidity - simulating the environmental conditions of crops grown at different altitudes. The effect of different combinations of temperature and relative humidity in the longevity and developmental time of

A. fuscipennis was tested. We hypothesize that *A. fuscipennis* might be able to develop and reproduce on whitefly at a limited range of temperatures and humidities, specific for a limited part of the area where beans are grown in Colombia. Results of studies presented in chapters 2 and 7 will be used to test the hypothesis.

Culture method. For mass rearing purposes, it is important to know the host stages that are preferred by the parasitoid, its developmental time, sex ratio and kind of reproduction. Literature information indicated that developmental time of this parasitoid is longer than that of *E. formosa*, which may lead to desynchronization of development of pest and natural enemy. The limited information available suggests that reproduction of *A. fuscipennis* is through thelytokous parthenogenesis. This is a positive aspects as pure female populations of parasitoids are cheaper to produce than sexual ones (Stouthamer, 1993). We hypothesize that mass rearing of *A. fuscipennis* is on the one hand complicated by its long developmental time, while on the other hand it is simplified by its thelytokous way of reproduction. Results of experiments described in chapters 2 and 5 will be used to test this hypothesis.

Reproductive potential. If a natural enemy has to be used for inoculative or seasonal inoculative releases, then its r_m value should be equal to or higher than the r_m value of its host. Information for calculating the r_m value of *A. fuscipennis* could not be obtained from the literature. We hypothesize that the r_m value of *A. fuscipennis* is higher than that of *T. vaporariorum* only at a limited range of climatic conditions at which beans are cropped in Colombia, and in a smaller area than where the parasitoid is able to develop and reproduce. Results of studies given in chapters 2, 3, 4 and 7 will be used to test this hypothesis.

Searching behavior. High searching efficiency is an important characteristic of natural enemies will they be able to keep pest populations under the economic threshold density. For *A. fuscipennis*, hardly any information on long-distance searching is available. Also, there is no information about the searching and parasitization behavior after the parasitoid has landed on the plant. We hypothesize that *A. fuscipennis* (1) is using long-distance volatile cues produced by the host or the host plant to locate infested plants, and (2) shows a type of searching behavior once landed on the plant that is adapted to occurrence of aggregated host stages. Results of studies presented in chapters 5 and 6 will be used to test these hypotheses.

Side effects of pesticides on the parasitoid. Even if *A. fuscipennis* meets the above criteria, it is necessary to find out if the parasitoid can be used in the present guided pest control program used in

Colombia. Colombian farmers spray pesticides for insect and fungus control, and we wanted to know the effect of these pesticides on *A. fuscipennis*. As a first approach to this aspect, the field performance of the parasitoid was tested in both, sprayed and unsprayed bean crops. We hypothesize that pesticides used in bean in Colombia for control of insects and fungi negatively effect the performance of the parasitoid *A. fuscipennis*. Results of studies given in chapters 6 and 7 will be used to test this hypothesis.

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2. Developmental time, sex ratio and longevity of *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae) on the greenhouse whitefly

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Abstract

Amitus fuscipennis MacGown & Nebeker (Hymenoptera: Platygasteridae) is being evaluated as a potential biological control agent of *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) on bean crops in Colombia. The life history of this natural enemy is presented in this paper. The developmental time and longevity of fed and unfed adult parasitoids were compared under different temperature and relative humidity combinations. Mean longevity of the parasitoid in absence of hosts was highest (42.2 d) at 15 °C and 75 ± 5% RH, and lowest (3.9 d) at 15 °C and 45% RH. Longevity at 25 °C and 75 ± 5% RH was also low (10.1 d). *A. fuscipennis* had the longest mean developmental time (65 d) at 15°C and 75 ± 5% RH, and the shortest (23.2 d) at 25 °C and 55 ± 5% RH. Combinations of moderate temperature and low relative humidity negatively affected parasitoid longevity. At constant high humidity, an increase of temperature leads to a decrease of longevity. Sex ratio was determined by a) collecting adults in the field and rearing them to the next generation, b) exposing different proportions of pairs of parasitoids to whitefly nymphs, and c) examining the offspring of pairs of parasitoids. Sex ratios of populations collected in the field and reared in the laboratory were strongly female-biased. Reproduction of the parasitoid is through thelytokous parthenogenesis. The species carries *Wolbachia*, the rickettsia that induces thelytoky. Results suggest that *A. fuscipennis* could be a potentially good biological control agent of *T. vaporariorum* in environments that are not overly dry or warm.

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), is a serious pest of vegetables and ornamental crops around the world (van Lenteren and Martin, 1999). Interest in biological control of this pest is growing because of concerns about insecticide resistance (Dittrich *et al.*, 1990; Prada *et al.*, 1993; Buitrago *et al.*, 1994; van Lenteren, 1995; Cardona *et al.*, 1998). Many parasitoids, predators and pathogens are known as natural enemies of the greenhouse whitefly (van Lenteren and Martin, 1999). Aphelinid parasitoids of the greenhouse whitefly are summarized in van Lenteren *et al.*, (1997). Based on our present knowledge of the control capacity of the different categories of natural enemies, we propose that parasitoids are still the best for obtaining long-term control through inoculative or seasonal inoculative releases (Gerling, 1990).

In the light of this knowledge, the parasitoid *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae) is being evaluated as a potential biological control agent of the greenhouse whitefly on bean (*Phaseolus vulgaris* L.) crops in Colombia. *A. fuscipennis* has been found in the northern part of South America and the Caribbean. It is abundant on bean crops in the Andes mountains of Colombia and Ecuador where no chemical treatments have been applied, and it is still active even after heavy pesticide use. The parasitoid seems to be partially responsible for the present low infestation by *T. vaporariorum* in the Rionegro area of Colombia. The parasitoid is commonly found on some plants close to crop areas, and was able to naturally colonize a greenhouse chrysanthemum crop

where *Encarsia formosa* Gahan, imported from England, had been previously released. *A. fuscipennis* replaced the *E. formosa* populations (L. E. Perez, personal communication). Although *A. fuscipennis* is abundant on bean crops, few studies have been conducted on the biology or efficacy of this species as a biological control agent against the greenhouse whitefly (Velez *et al.*, 1994; Marquez and Valencia, 1991). *A. fuscipennis* also parasitizes *Bemisia tabaci* Gennadius under laboratory conditions. Viggiani (1991) suggests that *A. fuscipennis* reproduction is through thelytokous parthenogenesis. Additional information about the sex ratio of this species is not available.

This paper represents part of a research effort to collect life history data needed to understand the capabilities and limitations of this parasitoid. Here we report the developmental time, sex ratio and longevity of *A. fuscipennis* at different temperature and relative humidity combinations.

Materials and methods

Parasitoids used in this study came from a rearing facility established in September 1996 at the International Center for Tropical Agriculture, CIAT, in Cali, Colombia. Parasitoids were initially collected in a bean field heavily infested with *T. vaporariorum* at Regadero, Cerrito, Valle, Colombia, located in the central range of the Andes mountain chain at 1700 m. This material was sent for identification to G. Viggiani (Institute of Agricultural Entomology, University of Naples, Italy) and to H. J. Vlug (Institute of Plant Protection, IPO-DLO, Wageningen, The Netherlands). The parasitoids were reared on first and second instars of *T. vaporariorum* on bean plants ('ICA- Pijao' cultivar) and kept at 20-22°C, 40-80% RH, 12L: 12D. The host was reared for 4 years on the same bean cultivar at 20.5 ± 2 °C, 45-90% RH, 12L: 12D.

To study the developmental time of the parasitoid, bean plants infested with first instars of *T. vaporariorum* were enclosed by using an acetate cone, and exposed to 40 or 60 unmated females that were allowed to oviposit for either 24 or 48 h. Two plants were used for every combination of temperature and humidity. Every leaflet was infested with whitefly nymphs and the whole plant was exposed to the parasitoids. The plants were then transferred to climate-controlled chambers at different combinations of temperatures and relative humidities (Table 1). Subsequently, development of immature stages was checked twice per day until all adults had emerged. Days to first nymph coloured gray (indicating clear evidence of parasitization), days to adult emergence, number of progeny, sex and mortality of immature parasitoids were recorded. Differences in developmental time were tested using one-way analysis of variance (ANOVA).

To determine the sex ratio (males to total parasitoids) of *A. fuscipennis*, three different approaches were followed. 1) *A. fuscipennis* adults were collected from different dry bean and snap bean

Table 1. Number of *A. fuscipennis* adults exposed to first instar nymphs of *T. vaporariorum* at different temperatures (°C) and relative humidities (%) for developmental time and sex ratio experiments.

		Temperature					
		15 °C		19 °C		25 °C	
		45 ± 5 %RH	75 ± 5%RH	75 ± 5%RH	90 ± 5%RH	55 ± 5%RH	75 ± 5%RH
Number of plants		2	2	2	2	2	2
Number of leaflets		7	6	6	7	8	6
Time of exposition (h)		48	24	24	48	48	24
Average host density per leaflet	100		80	150	140	100	100
Number of parasitoids exposed							
Plant 1		60 ♀ ♀	40 ♀ ♀	60 ♀ ♀	39 ♀, 1 ♂	60 ♀ ♀	60 ♀ ♀
Plant 2		60 ♀ ♀	40 ♀ ♀	60 ♀ ♀	40 ♀ ♀	60 ♀ ♀	44 ♀ ♀
Sex ratio of the offspring							
Plant 1		0.0	0.0	0.0	0.0	0.0	0.0
Plant 2		0.0	0.0	0.0	0.0	0.0	0.03

crops (both of them are cultivars of *Phaseolus vulgaris* L.) infested with *T. vaporariorum*. One sample was taken from every crop at different date and crop phenologies (Table 2). For some of the crops, a portion of adult parasitoids were collected using an aspirator and killed immediately in alcohol to easily and quickly determine their sex ratio under laboratory conditions. The other portion was collected alive as both adults and parasitized pupae.

Table 2. Sex ratio of *A. fuscipennis* adults collected in the field on dry bean (DB) and snap bean (SB) between September 1997 - March 1998, and reared on bean under laboratory conditions.

Field-collected adults				Progeny of emerged adults				
Crop	Crop phenology	Season	Sex Ratio	% ♀ ♀	N	Sex Ratio	% ♀ ♀	N
DB	Earlypod setting	Early rainy	0.02	97.9	377	0.02	97.8	325
DB	Crop residue	Dry	0.09	90.0	431	0.08	91.8	97
DB	Pre-flowering	Dry	0.09	99.0	101	0.0	100.0	147
DB	Mature	Dry	0.05	94.6	147	-	-	-
SB	Mature	Rainy	0.0	100.0	115	-	-	-

After emergence of the pupae, adults were allowed to parasitize 80-100 first and second instars of *T. vaporariorum* per bean leaflet. *A. fuscipennis* prefers first and second instars of *T. vaporariorum* for oviposition (Manzano, unpublished results). Parasitoid development took place at 20-22 °C, 40-80% RH, 12L:12D. The sex ratio of the offspring was determined. Differences in sex ratio between field collected individuals and their offspring reared at laboratory conditions were tested using Chi-square. 2) The sex ratios of emerging adults in experiments aimed at calculating developmental times were determined (Table 1). 3) Pairs of male and female *A. fuscipennis* were isolated after they showed courtship and mating behaviour similar to that described for *Amitus* by Viggiani and Battaglia (1983). Male parasitoids were reared from parasitized pupae on bean leaves enclosed in petri dishes. Sex of emerged parasitoids was checked until males emerged. Pairs of female and male *A. fuscipennis* that appeared to have mated were kept in leaf-clip cages and exposed to 30-40 second instar nymphs of *T. vaporariorum*. Four different pairs were tested. Every 24 h each parasitoid pair was transferred to a new location on the bean leaf until the female died. Parasitized hosts were kept at 20-22 °C, 40-80% RH, 12L: 12D. Sex ratio of the F₁ offspring was determined. Emerged females were isolated individually in leaf cages and exposed daily to 30-40 second instars until the female died. Parasitized hosts were kept under the same conditions as above. The sex ratio of the F₂ generation was also determined. This procedure was performed to determine if parasitoid reproduction is only by thelytokous parthenogenesis as suggested by Viggiani (1991), or if

sexual form of reproduction can also be found.

Female longevity without hosts was determined by keeping female parasitoids of less than 24 h old in individual glass vials (11 ml). The insects were provided with honey and moisture three times per week. Moisture was offered by setting a small piece of wet clay inside the vial. The vials were stored in climate chambers at different conditions: 15 °C, 45 ± 5% RH; 19 °C, 90 ± 5% RH; 25 °C, 55 ± 5% RH. Another batch of insects was kept at 15, 19 and 25 °C, and uniform 75 ± 5% RH. Female longevity without hosts and food was also determined at 15, 19, and 25 °C, and uniform 75 ± 5% RH. Male survival in the absence of females with food was determined at 15 and 19 °C, 75 ± 5% RH. Survival was recorded daily. Differences in female longevity were analyzed using ANOVA and Duncan's Multiple Range test. Differences in longevity due to differences in temperature or food were tested using two-way analysis of variance (ANOVA).

Results

Developmental time of the parasitoid differed significantly between the temperatures and relative humidities tested (Table 3; one-way ANOVA, $P < 0.0001$). Developmental time decreased when temperature increased. At 15 °C, developmental time increased as relative humidity increased (from 51.0 to 65.6 d) but at 19 °C it decreased from 38.1 to 31.3 d at very high humidity. Immature mortality was less than 5% at most of the environmental conditions tested (Table 3), but it was high at 15 °C and 75 ± 5% RH due to the presence of the fungus *Oidium* on the bean plants. The fungus covered the parasitized pupa and parasitoids could not emerge.

Sex ratios of *A. fuscipennis* collected on dry bean and snap bean field crops are shown in Table 2. *A. fuscipennis* had a female-biased ratio, males were scarce. In some of the field surveys, only females were collected. There was no significant difference between the sex ratio of the offspring reared under laboratory conditions and the parental generation at field conditions (Table 2). In the developmental time experiments, unmated females occasionally produced males and none of the probably mated females produced males (Table 1). From the presumably mated females, the offspring consisted only of female parasitoids. These virgin females were allowed to parasitize on first and second instars of *T. vaporariorum*. The offspring consisted of 37 females and 2 males.

At different combinations of temperatures and relative humidities, the longevity without hosts for honey fed females differed significantly (Table 4, $F = 34.89$; $P < 0.0000$; all means are different, Duncan's test $P < 0.05$). Longevity was longest at 19 °C, 90 ± 5% RH (18.1 d) and shortest at 15 °C, 45 ± 5% (3.9 d). At constant relative humidity of 75 ± 5%, longevity without hosts and with food differed among the three tested temperature conditions (Table 4, one-way ANOVA $F = 16.0$; $P < 0.0001$; all means were

Table 3. Developmental time (days) and immature mortality (%) of *A. fuscipennis* in relation to temperature, T (°C) and relative humidity, RH (%).

RH	Temperature							
	15 °C			19 °C		25 °C		
	45±5	75±5	90±5	75±5	55±5	75±5		
mean	51.0	--*-- 65.6a	31.3	--*-- 38.1b	23.4	--ns-- 24.9c		
N	450	120	1030	390	260	180		
SE	0.1	0.2	0.06	0.1	0.06	0.1		
Range (days)	45-62	63-71	27-40	35-45	22-32	22-28		
First nymph colored gray**	37	37	21	27	16	17		
Immature mortality	2.3	15.8***	1.0	0.8	3.8	0		

*All means differed significantly (one-way ANOVA, $p < 0.0001$).

Values at 75±5% RH followed by different letters are significantly different (ANOVA, followed by Tukeys's β -test, $\alpha = 0.05$).

The effect of humidity was significant within 15°C and 19°C but not within 25°C (ns, test for independent variable, $\alpha = 0.05$).

** days after parasitization.

*** leaves infected by the fungus *Oidium*.

different, Duncan's test, $p < 0.05$). The longevity without hosts for fed and unfed females at 15, 19 and 25 °C and uniform relative humidity of $75 \pm 5\%$ differed significantly. Both food and temperature affected longevity independently or through their interaction (Table 4, two-way ANOVA, food: $F = 127.7$; $df = 1$ $p < 0.000$ independently or through their interaction (Table 4, two-way ANOVA, food: $F = 127.7$; $df = 1$ $p < 0.000$ 1; temperature: $F = 35.1$, $df = 2$; $p < 0.0001$; interaction: $F = 26.3$, $df = 2$; $p < 0.0001$). The only values not significantly differently were longevity of fed and unfed females at 25 °C. Female longevity was short without food at any temperature, and short at 25 °C when food was available (Table 4). Longevity of *A. fuscipennis* with food was strongly affected by the combination of temperature and relative humidity conditions (Fig.1). Longevity was low at both low relative humidity and low temperature, but high at higher relative humidity ($75 \pm 5\%$) and temperatures around 15-19 °C. Due to scarcity of males, only some individuals could be used in male longevity experiments. At 15 °C and $75 \pm 5\%$ RH, one fed male lived for 31 d. At 19 °C and $75 \pm 5\%$ RH, 5 fed males lived on average for 24.8 d.

Discussion

Developmental time of *A. fuscipennis* at 19 °C, $90 \pm 5\%$ RH (31.3 d) was similar to the value measured on bean at 19 °C, 82 % RH (35.9 d) by Medina *et al.*, (1994). Developmental times of *A. fuscipennis* at $75 \pm 5\%$ RH are shorter than the values found for *Amitus bennetti* Viggiani & Evans at 15 °C (65.6 d and 72.2 d, respectively) and at 25°C (24.9 d and 27.6 d, respectively; Drost *et al.*, 1996). At 17 ± 1 °C, the developmental time of *Encarsia formosa* is on average 31.6 d (Vet and van Lenteren, 1981), which is considerably shorter than the developmental time of *A. fuscipennis* at 15 °C (65.6 d) and 19 °C (38.1 d).

A. fuscipennis shows a strongly female biased ratio. This characteristic was also noticed by Viggiani (1991) who reported only females from a sample of 400 individuals collected in Colombia and introduced to Italy. Based on this, he concluded that the species reproduces by thelytokous parthenogenesis. The occasional production of males within a strong female-biased populations as it occurs in *A. fuscipennis*, is a normal observation for thelytokous lines (R. Stouthamer, personal communication).

We tested *A. fuscipennis* for the presence of *Wolbachia*, the rickettsia that induces thelytoky (Stouthamer, 1993), and the result was positive (M. van Meer, personal communication). Therefore, the presence of *Wolbachia* probably explains the female-biased sex ratio of the species. To be absolutely sure that *Wolbachia* induces thelytoky, *A. fuscipennis* females should be 'cured' by feeding antibiotics so that both males and females will be produced (Stouthamer *et al.*, 1990; Pijls, 1996), but these tests have not yet been performed. *Wolbachia* has been found also in *E. formosa* and *Eretmocerus staufferi* Rose and Zolnerowich, both parasitoids of whitefly (M. van Meer, in preparation).

Table 4. Longevity (days) of *A. fuscipennis* females with and without food in relation to temperature T, (°C) and relative humidity, RH(%).

With food ¹							Without food ²		
T	15	15	19	19	25	25	15	19	25
RH	45±5	75±5	90±5	75±5	55±5	75±5	75±5	75±5	75±5
mean	3.9	42.2*	17.3	18.1*	6.2	10.1*	5.2*	4.2*	2.6*
n	91	97	78	72	71	71	80	78	81
SE	0.1	1.3	0.6	0.8	0.4	0.4	0.1	0.1	0.07
Range (days)	1-7	3-67	3-29	1-37	1-13	1-17	1-11	2-8	1-4

¹All means differed significantly (one-way ANOVA, Duncan's test $p < 0.05$).

²All means differed significantly (one-way ANOVA, Duncan's test $p < 0.05$).

*All means differed significantly (two-way ANOVA, $p < 0.001$); except for fed and unfed females at 25 °C.

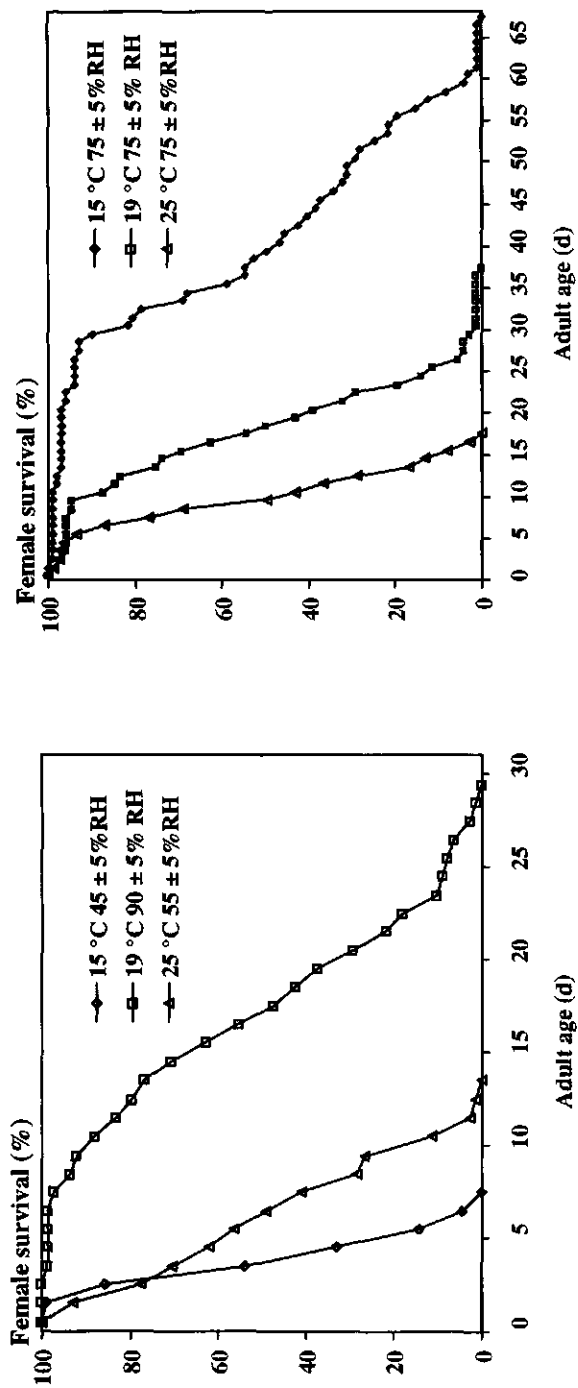


Figure 1. Survivorship (%) of female *A. fuscipennis* in relation to temperature (°C) and relative humidity (% RH)

During *A. fuscipennis* courtship behavior observed under laboratory conditions, antennal contact between both sexes was evident. Viggiani and Battaglia (1983) described the courtship and mating behavior of *A. vesuvianus* Viggiani and Mazzone and *A. rugosus* Viggiani and Mazzone where antennal contact between male and females also plays an important role. However, it is not known whether sperm is actually transferred in *A. vesuvianus* and *A. rugosus* and the sex ratio of the offspring was not determined. The release and spread of a sexual pheromone through a gland located in the fourth antennomere of the male antenna of *A. spiniferus* has been suggested (Isidoro and Bin, 1995).

In the genus *Amitus*, the male antennae are thread-like with cylindrical antennomeres, except for the fourth antennomere where a gland is located, which has a paddle-shaped area (MacGown and Nebeker, 1978; Masner and Huggert, 1989). Recently it has been found that the male antennae of *A. fuscipennis* also show morphological evidence of these special glands located in the same antennomere (A4) as in *A. spiniferus* (N. Isidoro, personal communication). This may indicate that *A. fuscipennis* uses sexual pheromones for mating. However, we also have not yet determined whether sperm is transferred in *A. fuscipennis*. An indication that sperm was not transferred in the isolated pairs is the high proportion of females obtained in the F₂ generation. According to Stouthamer (1993), virgin females of sexual lines produce male offspring exclusively and those of asexual lines produce almost all female offspring. We obtained almost pure female offspring from 'pairing pairs'. This might indicate that *A. fuscipennis* consists of asexual forms only. Additionally, due to the small proportion of males found, sexual reproduction may be rare in *A. fuscipennis*. However, coexistence of both asexual and sexual populations in the same habitat is possible and has been reported earlier for *Trichogramma* spp. (Stouthamer, 1993).

Sex ratios in other *Amitus* vary considerably. *A. hesperidium* Silvestri is biparental with a sex ratio of 1:1 (Flanders, 1963), but its sex ratio varies from 5:1 to 1:1.4 depending on host quantity and nutritional conditions (Zhang *et al.*, 1982). *A. bennetti* has thelytokous parthenogenetic reproduction (Viggiani and Evans, 1992; Drost *et al.*, 1996). If *A. fuscipennis* has to be mass reared for biological control programs, its thelytokous reproduction is an advantage because, among others, asexual wasps do not "waste" expensive hosts for the production of males (Stouthamer, 1993). This is particularly advantageous for parasitoids used in inundative releases (Stouthamer, 1993).

Average longevity of fed *A. fuscipennis* females reported here at 19 °C are higher (17.3, 18.1 d) than the values reported for this species (11 d) by Medina *et al.*, (1994) at 18 °C, 80 % RH.

Average longevity of *A. fuscipennis* at 75 ± 5 % RH and at any of the three tested temperatures is longer than that reported for *A. hesperidium* (Flanders, 1966; Zhang *et al.*, 1982). Mean longevity of *A. fuscipennis* without hosts at 15 °C and 90 ± 5% RH (42.2 d), is longer than that reported for *A. bennetti* (29.2 d) and *Eretmocerus californicus* Howard (38.7 d; Drost *et al.*, 1996). However, it is shorter than that of *E. formosa* (46.9 d) and *Eretmocerus* sp (Texas) (47.5 d; Drost *et al.*, 1996); *E.*

formosa (199.3 d) and *E. pergandiella* Howard (80 d; Vet & van Lenteren, 1981). Mean longevity of *A. fuscipennis* at 25 °C is also longer than that reported for *Eretmocerus* sp at 26 °C (3.9 d) by Lopez and Botto (1997). In the latter study however, relative humidity was variable (30-70 %), and this probably negatively affected the parasitoid longevity. Longevity of *A. fuscipennis* in the absence of whitefly hosts and in the presence of honey follows the same trend as found for *E. formosa* (van Roermund, 1995): longevity decreases exponentially when temperature increases. Although only a few males were tested, the results suggest that males probably live as long as females do.

Longevity is longer for females that have the opportunity to feed than for females without food. The importance of sugar intake for the longevity, fecundity and high attack rates of adult parasitoids is documented, among others, by Powell (1986). Under field conditions, *A. fuscipennis* may consume honeydew excreted by whitefly nymphs and probably sugar from extra-floral nectaries present on some of the plants that surround the bean crop. Under laboratory conditions, honey can be offered to *A. fuscipennis* adults. This is especially necessary because the first and second instars of *T. vaporariorum* preferred by the parasitoid for oviposition (Manzano, unpublished results) excrete only a small amount of honeydew.

A. fuscipennis lives long at high relative humidity conditions ($75 \pm 5\%$) and temperatures around 15-19 °C, conditions which are similar to the environmental conditions of hillside areas (> 1400m) where beans are grown in Colombia. Low relative humidity areas (< 60% RH) will hamper the establishment and reproduction of the parasitoid, and these conditions should be avoided during mass releases of the parasitoid. Different surveys carried out by the Research Group on Whiteflies at CIAT on tomato, snap bean and sweet pepper, showed that the parasitoid is also present in the piedmont at 1060 m where the average temperature is 22 °C and average relative humidity is 75%. At these conditions, we have found that parasitized pupae of *A. fuscipennis* can be present on the same snap bean leaflet where *Eretmocerus* sp and *Encarsia* spp also have parasitized *T. vaporariorum* hosts. However, at higher dry bean and snap bean cropping areas (> 1400 m), *A. fuscipennis* is the dominant species and other parasitoid species of *T. vaporariorum* have hardly ever been found.

In conclusion, different combinations of temperature and relative humidity affect developmental time and longevity of *A. fuscipennis*. Combinations of low relative humidity and low temperature, and low relative humidity and high temperature, negatively affected the parasitoid longevity. These conditions limiting parasitoid development and reproduction should be taken into account when developing habitat manipulation tactics. In the bean canopy, however, temperature is lower and relative humidity higher and the parasitoid would probably not be continuously exposed to severe ambient conditions. Reproduction of the parasitoid is probably only by parthenogenesis thelytokous. This kind of reproduction is an advantage if the parasitoid has to be mass reared, because it is cheaper to produce than sexual forms. Additional

research concerning the performance of *A. fuscipennis* as biological control agent of *T. vaporariorum* based on demographic parameters such as intrinsic rate of increase at hillside and lowland areas conditions is desired and now performed. This information will allow us a better understanding of the potential of this parasitoid.

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3. Intrinsic rate of population increase of *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) at different temperatures and relative humidities on two bean cultivars

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Abstract

Life-history parameters of the whitefly *Trialeurodes vaporariorum* (Westwood), an important pest of bean crops in Colombia, were studied in environmental control chambers on two dry bean (*Phaseolus vulgaris* L.) cultivars. Mean longevity, fecundity and oviposition rates were found to be statistically different between the bean cultivars. Mean longevity on cv. Chocho ranged from 22.6 d (at 19°C), through 17.5 d (at 22°C), to 5.9 d (at 26°C). On cv. ICA-Pijao, it was 35.5 d at 19°C. On cv. Chocho, mean total fecundity ranged from 8.6 to 33.3 eggs for the three temperatures. On cv. ICA-Pijao, fecundity at 19°C was much higher (127.2 eggs). Intrinsic rate of increase (r_m) on cv. Chocho was highest at 22°C (0.061), intermediate at 19°C (0.044) and lowest at 26°C (0.035). On cv. ICA-Pijao, it was 0.072 at 19°C. Because the r_m of *T. vaporariorum* is much lower on cv. Chocho than on cv. ICA-Pijao at 19°C, we suggest that cv. Chocho be used in IPM programs for hillside areas. The whitefly's population growth is slower and the rate of death from natural enemies is usually higher.

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), is an important problem, especially in vegetable and ornamental crops (van Lenteren and Martin, 2000). Adults and nymphs feed on phloem sap, excreting large amounts of honeydew that favor the development of black mould that, in its turn, hampers the plant's photosynthesis. The honeydew also reduces yields and causes economic losses of fruits and ornamentals (van Roermund and van Lenteren 1992).

Greenhouse whitefly is also a key pest on dry and snap bean (*Phaseolus vulgaris* L.) crops in Colombia (Prada *et al.*, 1993). Colombian bean crops are therefore heavily sprayed with chemical insecticides to control this, and other, pests. Snap beans may be sprayed as many as 14 times per cropping season (Prada *et al.*, 1993). Because the greenhouse whitefly has developed resistance to several insecticides (Buitrago *et al.*, 1994; van Lenteren, 1995; Cardona *et al.*, 1998), biological control is desired. Many natural enemies of whitefly are known (Gerling, 1990; Gerling and Mayer, 1996), including several parasitoids have been successfully used in classical and seasonal inoculative releases for controlling the whitefly (Gerling, 1990; van Lenteren *et al.*, 1997).

Within the Whitefly Project of the CGIAR IPM Program, field surveys have been carried out on tomato at the International Center for Tropical Agriculture (CIAT), Cali, Colombia, and on dry and snap bean crops in some areas of the Department of Valle del Cauca, Colombia (CIAT, 1999). These surveys have shown that *T. vaporariorum* is distributed in the hillsides of the Andean region, between 1400 and 2255 m above sea level, where the average temperature is 19°C and the average relative humidity is 80%. This pest is also present in lowland areas, on crops located between 920 and 1200 m,

where the average temperature ranges between 22°C and 24°C and the relative humidity drops to about 74%.

At least 37 commercial bean cultivars are grown in Colombia and more than 60 bean lines have been improved agronomically (Voyssest, 1983). To test the potential effect of host plant on the r_m value of *T. vaporariorum*, two different cultivars were used. One was the cv. Chocho, an important commercial cultivar that is cropped, among other places, in the hillside areas of Tenerife, Valle del Cauca. The other cultivar was ICA-Pijao, which is well adapted to greenhouse conditions and is less susceptible to microbial infections.

In Colombia, the greenhouse whitefly is parasitized by an endemic natural enemy, the parasitoid *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae), which is currently being evaluated as a potential biological control agent of the greenhouse whitefly on bean crops in Colombia (Manzano et al., 2000). One criterion used to evaluate the potential of natural enemies for controlling a pest is that the intrinsic rate of population increase (r_m) of the control agent is at least equal to the pest's population growth rate, thereby making biological control feasible (van Lenteren and Woets, 1988). In this paper, the r_m values of *T. vaporariorum* were measured in environmental control chambers set to simulate the climates found in hillside and lowland bean-cropping regions. Once the r_m values of the parasitoid and its host are known and compared, we can determine those altitudinal zones where *A. fuscipennis* is likely to be most efficient as a biological control agent of the greenhouse whitefly.

Materials and methods

Plants and whitefly cultures. Plants used in this experiment were dry bean (*Phaseolus vulgaris* L.) cultivars Chocho and ICA-Pijao. They were grown in a greenhouse at CIAT at 20°C-30°C, 40%-80% RH and with at least 12 h of light per day. Plants used in experiments were 15 days old and were not fertilized or sprayed with chemical products.

The adults of *T. vaporariorum* used in this study came from a colony that was established at CIAT 5 years ago on bean plants (cv. ICA-Pijao) from insects collected from snap bean fields in Fusagasugá, Colombia. New generations were reared at 22°C-24°C, 40%-80% RH and 12L:12D.

Longevity and reproduction of *Trialeurodes vaporariorum*. Newly emerged adults of *T. vaporariorum* were sexed and caged by pairs in clip-cages (inside diameter = 2.5 cm). Every 48-h, the clip-cage with test insects was moved to a new leaf area until the female died. Fecundity was estimated by counting the number of eggs laid in each period of 48 h. The caged adults were kept in environmental control chambers under different temperature and humidity conditions, as follows:

Bean cultivar	Temperature (°C)	Relative humidity (%)
Chocho	19	75 ± 5
Chocho	22	70 ± 5
Chocho	26	75 ± 5
ICA-Pijao	19	75 ± 5

*Development time and survival rate of immature individuals, and proportion of females of *Trialeurodes vaporariorum*.* About 40 adults of *T. vaporariorum* were placed in clip-cages (inside diameter = 2.5 cm), which were put on leaves of each cultivar. After 10 h, the adults were removed and spots of 100 eggs of *T. vaporariorum* were allowed to develop under the same conditions as described for the longevity experiment until the adult whiteflies emerged. Development time and survival rate of immature individuals and proportion of females of *T. vaporariorum* were recorded.

Demographic parameters. Development time and survival rate of immature individuals and proportion of females were combined with reproduction experimental data to create 'lx-mx' life tables to calculate demographic parameters for *T. vaporariorum*. For each experiment, the following demographic parameters, as defined by Price, (1975) were calculated: the net reproductive rate (R_0 , which represents the number of female descendants that an average female leaves in one generation), and the generation time (T , which is equivalent to the mean period between the birth of parents and birth of offspring). The intrinsic rate of population increase (r_m) for *T. vaporariorum* under the different environmental conditions was also calculated, using the equation given by Carey, (1993):

$$\sum \exp(-r_m x) l_x m_x = 1$$

where x is the age,

l_x is the age-specific survival,

m_x is the proportion of female offspring of a female at age x

Following Carey, (1993), pivotal age, which is $x + 0.5$, was used to calculate r_m values.

The r_m values of *T. vaporariorum* at 19°C and 75 ± 5% RH, and on bean cultivars ICA-Pijao and Chocho were measured simultaneously. For treatment 22°C and 70 ± 5% RH, however, the r_m value

was calculated on the assumption that the proportion of females was 25%. The assumption was based on the proportions already found for 19°C and 26°C.

Data analysis. Differences among values for longevity and for fecundity were compared, using the Kruskal-Wallis One Way Analysis of Variance on Ranks test at a significance level of $P < 0.0001$. Oviposition rate values were compared by one-way ANOVA at a significance level of $P < 0.001$. For these three parameters if significance differences were detected, multiple comparisons were made using the Student-Newman-Keuls method ($P < 0.05$). Differences in development time were tested by Kruskal-Wallis One Way Analysis of Variance on Ranks test at a significance level of $P < 0.0001$, followed by multiple comparisons (Dunn's method, $P < 0.05$). For comparing survival rates, χ^2 tests were used.

Results

Longevity and fecundity of *Trialeurodes vaporariorum*. Longevity (d) of *T. vaporariorum* females decreased as temperature increased. It was shortest on cv. Chocho at 26°C (5.7 d). It was longest on cv. ICA-Pijao at 19°C (35.5 d). The average longevity differed significantly among temperatures, relative humidities and bean cultivars, except for values on Chocho at 19°C and 22°C (Student-Newman-Keuls method $P < 0.05$, after Kruskal-Wallis $P < 0.0001$; Table 1). Adult longevity curves on cv. Chocho are shown in Figure 1a.

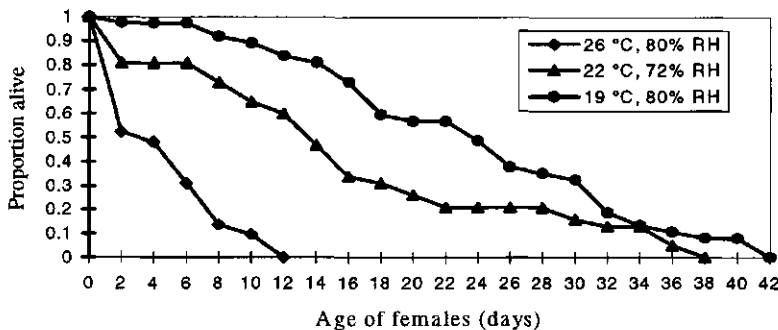


Figure 1a. Survival curves of *T. vaporariorum* on cv. Chocho at different climatic conditions.

Mean fecundity ranged from 8.6 eggs at 26 °C on cv. Chocho to 127.2 eggs at 19 °C on cv. ICA-Pijao. The mean fecundity was significantly different among the different temperatures, relative humidities and bean cultivars (Kruskal-Wallis, $P < 0.0001$), except for values on cv. Chocho at 19 °C and 22 °C (Student-Newman-Keuls method $P < 0.05$, Table 1). Reproductive curves on cv. Chocho are shown in Figure 1b. The difference between fecundity on cv. Chocho and cv. ICA-Pijao at 19 °C can be clearly observed in the reproduction curves shown in Figure 2. Daily oviposition in cv. ICA-Pijao is much higher and egg laying continues for many more days (Figure 2).

Mean oviposition rate (eggs per female per 2 days) on cv. Chocho increased from 1.4 at 19 °C to 1.9 at 22 °C and decreased to 1.4 at 26 °C. It was highest on cv. ICA-Pijao (3.6) (Table 1). Average oviposition rate was significantly different among treatments (one-way ANOVA, $P < 0.0001$), except for the comparison between females on cv. Chocho at 19 °C and 26 °C (1.4 for both conditions) (Student-Newman-Keul method, $P < 0.05$).

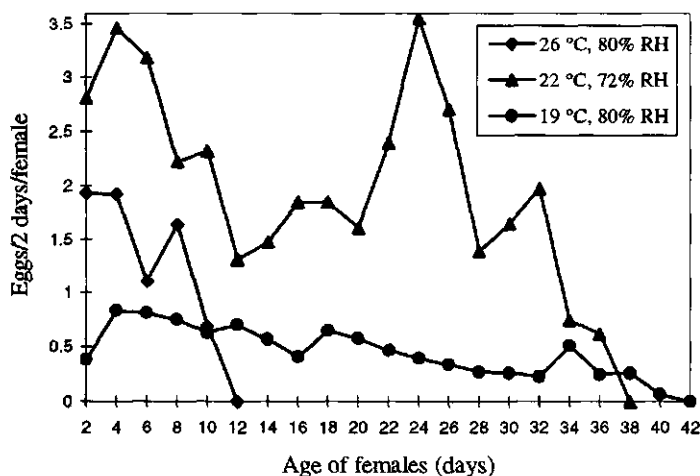


Figure 1b. Reproduction curves of *T. vaporariorum* on cv. Chocho at different climatic conditions

Table 1. Mean longevity (d), mean fecundity (eggs) and oviposition rate (eggs/female/2 days) of *Trialeurodes vaporariorum* at different temperatures ($T = ^\circ\text{C}$), relative humidities (RH = %) and bean cultivars (cv.)^a

Parameter	T = 19 RH = 75 \pm 5 ICA-Pijao	19 75 \pm 5 Chocho	22 70 \pm 5 Chocho	26 75 \pm 5 Chocho
Mean longevity*	35.5 a	22.6 b	17.5 b	5.9 c
SE	2.3	1.5	1.8	0.5
Range	14-52	6-40	6-36	2-10
No. of insects	31	36	31	27
Mean fecundity*	127.2 a	32.6 b	33.3 b	8.6 c
SE	9.7	3.9	4.3	1.5
Range	33-261	5-89	8-94	1-31
Mean Oviposition rate [†]	3.6 a	1.4 bd	1.9 c	1.4 d
SE	0.2	0.1	0.1	0.1
Range	2.3-5.06	0.29-4.5	0.61-3.67	0.3-3.3

^aFigures followed by different letters across columns indicate significant differences

*Kruskal-Wallis test $P < 0.0001$, followed Student-Newman-Keul's method $P < 0.05$

[†]One-Way Anova $P < 0.0001$, followed Student-Newman-Keul's method $P < 0.05$

Development time and survival rate of immature individuals, and proportion of females of Trialeurodes vaporariorum. Development time of *T. vaporariorum* was around 30 d at 19°C on both cultivars, Chocho and ICA-Pijao. It was 24.7 and 20.3 d on cv. Chocho at 22°C and 26°C, respectively (Table 2). The development times were significantly different between environmental conditions and bean cultivars (Kruskal-Wallis $P < 0.0001$, followed by Dunn's method, $P < 0.05$). Survival rate of immature individuals was highest on cv. Chocho (about 97%) and decreased to about 52% on the same cultivar at 26°C (Table 2). Significant differences in survival rates were present between environmental conditions and bean cultivars ($\chi^2 = 77.7$, 3 df, $P < 0.0001$), except for cv. ICA-Pijao at 19°C and cv. Chocho at 22°C (Table 2). The proportion of emerged females was low, being around 20% females at 19°C on both bean cultivars, and 28% at 26°C on cv. Chocho (Table 2).

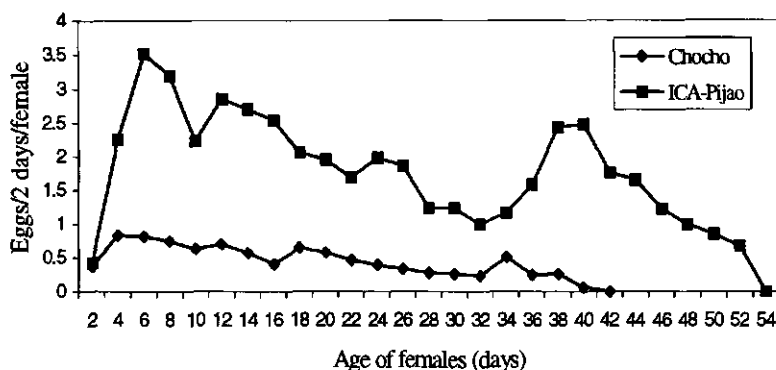


Figure 2. Reproduction curves of *T. vaporariorum* at 19 °C, 80% RH on ICA-Pijao and Chocho bean cultivars.

Demographic parameters. The values for the different demographic parameters for *T. vaporariorum* are shown in Table 2. On cv. Chocho, no large differences were found between reproduction data at 19°C ($R_0 = 6.2$) and those at 22°C ($R_0 = 8.3$). The r_m value was lower (0.044) for the first than for the second (0.061). The reproduction data decreased for the same cultivar at 26°C ($R_0 = 2.3$ and $r_m = 0.035$). The main difference was found for values of cv. Chocho at 19°C ($R_0 = 6.2$, $r_m = 0.044$) and those of cv. ICA-Pijao under the same environmental conditions ($R_0 = 26.7$ and $r_m = 0.072$) (Table 2). For individuals on cv. ICA-Pijao, generation time was highest (49.6) and shortest (24.7) on cv. Chocho at 26°C (Table 2).

Discussion

To compare the results for longevity and fecundity was presented in this paper with those described in the literature, only those parameters that were tested at similar temperatures during the females' whole life span were used. These data were taken from the life-history parameters revised by van Roermund and van Lenteren (1992), (Tables 3 to 5). The authors did not give data on the relative humidities used in the experiments.

Mean longevity of *T. vaporariorum* on cv. ICA-Pijao reported here at 19°C (35.5 d) was close to the value (37.3 d) reported by Madueke, (1979) at 18°C (Tables 1 and 3). Our results showed a

Table 2. Demographic parameters for *Trialeurodes vaporariorum* on bean cultivars ICA-Pijao and Chocho under different temperature ($T = ^\circ\text{C}$) and relative humidity ($\text{RH} = \%$) conditions. Different letters between columns indicate significant differences (Kruskal-Wallis $P < 0.0001$, followed by Dunn's method, $P < 0.05$) for development time; $\chi^2 = 77.7$, 3 df, $P < 0.0001$ for survival rate of immature individuals).

Parameter	T = 19 RH = 75 ± 5 cv. ICA-Pijao	19 75 ± 5 Chocho	22 70 ± 5 Chocho	26 75 ± 5 Chocho
Development time (d)	30.4 a	29.3 b	24.7 c	20.3 d
Survival rate (%)	74 a	97.4 b	81 ac	52.3 d
Proportion of females (%)	21	19	25 ^d	28
r_m^a	0.072	0.044	0.061	0.035
$\Sigma l_x m_x (R_0)^b$	26.7	6.2	8.3	2.4
T^c	49.6	42.3	36.1	24.7

^aIntrinsic rate of natural increase

^bNet reproductive rate

^cGeneration time (d)

^dValue assumed

statistically significant reduction in longevity of *T. vaporariorum* with increasing temperature at 80% RH. We reported a longevity value of 5.9 d at 26°C, equal to the one obtained by Collman and All, (1980) at the same temperature, and similar to the value (4.8 d) reported by Zabudskaya, (1989) at 27°C (1989) (Table 3).

Few data exist in the literature on the fecundity of *T. vaporariorum* on dry bean under conditions similar to the ones we tested. Maducke, (1979) reported a mean fecundity of 118.4 eggs at 18°C on cv. Canadian Wonder. This result was higher than the mean fecundity found on cv. Chocho under any of the conditions tested (33.3, 32.6 and 8.6), but lower than on cv. ICA-Pijao (127.2) at 19°C (Table 4). Fecundity on two sweet pepper cultivars reported by van Lenteren *et al.*, (1989) and Zabudskaya, (1989) were close to the fecundity on CV. Chocho at 26°C reported in this study. Fecundity on CV. Chocho at 19°C and 22°C (32.6 and 33.3, respectively) were close to the fecundity of a resistant tomato cultivator (37.0, Romanoff *et al.*, 1991). Fecundity on CV. ICA-Pig at 19°C (127.2) was close to fecundity on a susceptible tomato cultivator (130.2, Romanoff *et al.*, 1991, Table 4). Fecundity values

on cucumber and aubergine (van Lenteren *et al.*, 1977; Boxtel *et al.*, 1978; Boxtel 1980) were much higher than those found in this study for cv. Chocho at 22°C (Tables 1 and 4).

Table 3. Mean longevity (d) of *Trialeurodes vaporariorum* at different temperatures (T = °C) and host plants as compiled by van Roermund and van Lenteren, (1992).

T	Females tested (no.)	Mean longevity	Host plant	Cultivar	Reference
27	4	4.8	Sweet pepper	Novi	Zabudskaya, 1989
18	15	37.3	Bean	Canadian Wonder	Madueke, 1979
22.5	15	25.3	Bean	Canadian Wonder	Madueke, 1979
26	82	5.7	Bean	Contender	Collman & All, 1980
19	17	18.2	Tomato	Allround	Romanow <i>et al.</i> , 1991
				'susceptible'	
19	20	11.5	Tomato	'Resistant' accession	Romanow <i>et al.</i> , 1991
21	44	58.7	Tomato	Tiny Tim	Curry & Pimentel, 1971
27	4	20.3	Tomato	Immunity	Zabudskaya, 1989
22	40	20.2	Cucumber	IVT 71-240	Boxtel, 1980
22	44	30.4	Aubergine	Mammouth	Boxtel, 1980; Boxtel <i>et al.</i> , 1978; van Lenteren <i>et al.</i> , 1977; Woets & van Lenteren, 1976

The mean oviposition rate values of the greenhouse whitefly reported on dry bean of 3.5, 4.7 and 3.0 at 18°C, 22.5°C and 27°C, respectively, by Madueke, (1979; Table 5) were higher than the ones reported here on cv. Chocho, but were similar to the value found for cv. ICA-Pijao (3.6) (Table 1). On sweet pepper cv. Tisana at 22°C, Woets and van Lenteren (1976), van Lenteren *et al.*, (1977), Boxtel *et al.*, (1978) and Boxtel, (1980) reported a value of 0.72, all less than the values reported in this paper (Tables 1 and 5). Boxtel, (1980) and Boxtel *et al.*, (1978) reported on the same cultivar Tisana, at the same temperature, a value of 3.8. This is higher than the values reported on cv. Chocho, but it is close to that for cv. ICA-Pijao (3.6). Oviposition rates on tomato, cucumber and aubergine reported by van Lenteren *et al.*, (1977), Boxtel *et al.*, (1978) and Boxtel, (1980) were higher than those reported here for either bean cultivar (Tables 1 and 5). Net reproduction (Ro) values at 27°C on different sweet pepper cultivars ranged between 1.34 and 25.92 (Zabudskaya, 1989).

Table 4. Mean fecundity (eggs) of *Trialeurodes vaporariorum* at different temperatures ($T = ^\circ\text{C}$) and host plants as compiled by van Roermund and van Lenteren (1992).

T	Females tested (no.)	Mean fecundity	Host plant	Cultivar	Reference
23.2	53	9.1	Sweet pepper	Tisana	van Lenteren <i>et al.</i> , 1989
23.4	53	82.7	Sweet pepper	Angeli Emleke	van Lenteren <i>et al.</i> , 1989
27	4	8.5	Sweet pepper	Podarok Moldovy	Zabudskaya, 1989
27	4	163.0	Sweet pepper	Lastochka	Zabudskaya, 1989
18	15	118.4	Bean	Canadian Wonder	Madueke, 1979
19	--	130.2	Tomato	Allround 'susceptible'	Romanow <i>et al.</i> , 1991
19	--	37.0	Tomato	'Resistant' accession	Romanow <i>et al.</i> , 1991
20	25	210.7	Tomato	Money Maker	Dorsman & van de Vrie, 1987
22	40	176.4	Cucumber	IVT 71-240	van Lenteren <i>et al.</i> , 1977; Boxtel <i>et al.</i> , 1978; Boxtel, 1980
22	44	416.5	Aubergine	Mammoth	van Lenteren <i>et al.</i> , 1977; Boxtel <i>et al.</i> , 1978; Boxtel, 1980

Ro values on cv. Chocho are within this range (2.4, 6.2 and 8.3). The Ro value on cv. ICA-Pijao (26.7) is close to the highest value of the range. Yano (1989), using different temperature regimes during day and night on tomato, found Ro values for *T. vaporariorum* between 17.4 and 39.9. The Ro value on cv. ICA-Pijao was within this range (26.7). The r_m values reported by Zabudskaya, (1989) ranged between 0.008 and 0.104. The r_m values on cv. Chocho (0.035, 0.044 and 0.061) and cv. ICA-Pijao (0.072) reported here was in this range. The r_m values reported by Yano, (1989) ranged between 0.0455 and 0.0932. The r_m values of 'Chocho' at 19°C (0.044) and 22°C (0.061), and ICA-Pijao (0.072) were in this range. The r_m values of the greenhouse whitefly on resistant and susceptible cultivars were 0.066 and 0.097, respectively (Romanow *et al.*, 1991). The r_m value of the susceptible tomato was similar to the value reported here for cv. ICA-Pijao. Van Lenteren and Hulspar-Jordaan, (1983) cited and reported different r_m values for *T. vaporariorum*. At 18°C , r_m values were 0.096 and 0.127; at 21°C , 0.077 and 0.139; at 22.5°C , 0.112; at 27°C , 0.201.

In our experiments, a strongly male-biased sex ratio was found. We did not find the 'normal' sex ratio of 1:1 mentioned by van Lenteren and Noldus, (1990). Our result cannot be easily explained

Table 5. Mean oviposition rate (eggs/female/2 days) of *Trialeurodes vaporariorum* at different temperatures ($T = ^\circ\text{C}$) and host plants as compiled by van Roermund and van Lenteren, (1992).

T	Females tested (no.)	Oviposition rate	Host plant	Cultivar	Reference
22	47	0.72	Sweet pepper	Tisana	Woets & van Lenteren, 1976; van Lenteren <i>et al.</i> , 1977; Boxtel <i>et al.</i> , 1978; Boxtel, 1980
22	14	3.8	Sweet pepper	Tisana	Boxtel <i>et al.</i> , 1978; Boxtel, 1980
18	15	3.5	Bean	Canadian Wonder	Madueke, 1979
22.5	15	4.7	Bean	Canadian Wonder	Madueke, 1979
27	17	3.0	Bean	Canadian Wonder	Madueke, 1979
22	41	4.4	Tomato	Money-maker	Woets & van Lenteren, 1976; van Lenteren <i>et al.</i> , 1977; Boxtel <i>et al.</i> , 1978; Boxtel, 1980
22	40	8.7	Cucumber	IVT 71-240	Woets & van Lenteren, 1976; van Lenteren <i>et al.</i> , 1977; Boxtel <i>et al.</i> , 1978; Boxtel, 1980
22	21	9.5	Aubergine	Mammoth	van Lenteren <i>et al.</i> , 1977; Boxtel <i>et al.</i> , 1978; Boxtel, 1980

Table 6. Intrinsic rate of increase (r_m) of *Trialeurodes vaporariorum* at different temperatures ($T = ^\circ\text{C}$) and host plants as recorded by different authors.

T	r_m	Host plant	Cultivar	Reference
18	0.096	--	--	van Lenteren & Huispas-Jordaan, 1983
18	0.127	--	--	van Lenteren & Huispas-Jordaan, 1983
19	0.097	Tomato	Allround 'susceptible'	Romanow <i>et al.</i> , 1991
19	0.066	Tomato	'Resistant' accession	Romanow <i>et al.</i> , 1991
21	0.068	Tomato	Delicious	
21	0.077	--	--	van Lenteren & Huispas-Jordaan, 1983
21	0.139	--	--	van Lenteren & Huispas-Jordaan, 1983
22.5	0.112	--	--	van Lenteren & Huispas-Jordaan, 1983
27	0.008	Sweet pepper	Podarok Moldovy	Zabudskaya, 1989
27	0.035	Sweet pepper	Topolyok	Zabudskaya, 1989
27	0.104	Sweet pepper	Lastochka	Zabudskaya, 1989
27	0.201	--	--	van Lenteren & Huispas-Jordaan, 1983
D30/N20	0.084	Tomato	--	Yano, 1989
D30/N20	0.093	Tomato	--	Yano, 1989
D25/N10	0.059	Tomato	--	Yano, 1989
D25/N10	0.045	Tomato	--	Yano, 1989

because sex ratio in *T. vaporariorum* is thought to be independent of temperature and host-plant species (van Lenteren and Noldus, 1990).

Differences in suitability for whiteflies of bean cultivars may account for the greater longevity and fecundity of *T. vaporariorum* on cv. ICA-Pijao (35.5 d and 127.2 d, respectively), compared with cv. Chocho (22.6 d and 32.6 d, respectively) under the same conditions of 19°C, 75 ± 5% RH. Van Lenteren and Noldus, (1990) have found that, for *T. vaporariorum* on a range of host plants, the more a plant is preferred, the larger the total number of eggs laid per female, the higher the oviposition rate, the higher the longevity of females, the shorter the development time from egg to adult, and the lower the mortality rates in all stages. According to longevity and fecundity values, they ranked host plant preference of whitefly as follows: aubergine > cucumber = gherkin = melon = gerbera = tobacco > bean = tomato > sweet pepper.

Our results suggest that for the Colombian *T. vaporariorum* population, bean is in a range of suitability between sweet pepper and tomato, but more interesting is our finding that large differences exist among bean cultivars at 19°C: cv. Chocho appears to be much less suitable for *T. vaporariorum* than cv. ICA-Pijao. On cv. Chocho, longevity, fecundity and oviposition rate of *T. vaporariorum* were lower than on cv. ICA-Pijao, which reflected a much lower r_m value (0.044) on cv. Chocho than on cv. ICA-Pijao (0.072).

Variation in host-plant suitability may affect the biological control of the greenhouse whitefly. For example, van Lenteren and Noldus, (1990) found that the more suitable a plant is for the whitefly, the more difficult it is to control it with the parasitoid *Encarsia formosa* Gahan. Van Lenteren and Woets, (1988) also found that whitefly control is easy on sweet pepper and tomato and more difficult on good host plants such as aubergine or cucumber. Reasoning in the same way, we may expect that the biological control of *T. vaporariorum* by the parasitoid *A. fuscipennis* will be easier on cv. Chocho because of the whitefly's lower population growth rate.

During our fieldwork, we observed that, without having our scientific basis, farmers knew that, of the bean cultivars, *T. vaporariorum* least prefers cv. Chocho. On one field trip to the El Dovio area of Valle del Cauca, we asked to a farmer why he preferred cropping cv. Chocho. He answered, 'because the greenhouse whitefly does not like this cultivar too much'. (Additionally, this cultivar produces a big red seed, much appreciated by Colombian consumers.)

The reasons behind the differences in suitability between the two bean cultivars tested in this study are unknown and deserve attention because any resulting knowledge may provide insight into resistance mechanisms that could then be exploited.

Climatic conditions influence population development of *T. vaporariorum*. According to our results on cv. Chocho, *T. vaporariorum* has similar longevity and fecundity in hillside areas (19°C, 80% RH) as in lowland areas (22°C, 74% RH). These results agree with our field survey results. However, at a constant temperature of 26°C, combined with a high relative humidity of 75%, longevity, fecundity and survival rates decrease dramatically. The latter environmental parameters represent the climate in the Colombian North Coast, where RH may be even higher than 80%: *T. vaporariorum* is absent here, but other whitefly species do occur: *Bemisia tabaci* (Gennadius) and *B. argentifolii* Bellows and Perring on vegetable crops, and *B. tuberculata* Bondar, *T. variabilis* (Quaintance) and *Aleurotrachelus socialis* Bondar on cassava (Cardona *et al.*, 1998).

A useful study would be to determine the r_m value of *T. vaporariorum* on cv. Chocho directly under field conditions to compare with laboratory results. Until now, difficult conditions have prevented us from performing field experiments. Often, r_m values determined in the field are lower than in the laboratory, because many factors, not appearing in the laboratory, play a role in mortality rates.

Currently, we are measuring the population growth of the parasitoid *A. fuscipennis* on *T. vaporariorum* feeding on both bean cultivars. Results will be compared with data found for whitefly. Based on preliminary experiments, the population growth rate of this parasitoid is expected to be higher than that of *T. vaporariorum*, especially on cv. Chocho. This indicates that *A. fuscipennis* could be a promising candidate for biological control of greenhouse whitefly on beans.

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4. Intrinsic rate of population increase of *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae) according to climatic conditions and bean cultivar

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Abstract

Data on adult longevity, reproduction and intrinsic rate of population increase of insect parasitoid *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae) were obtained under different climatic conditions on two bean cultivars. The insect host was the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). On cv. Chocho, at 19°C, 75 ± 5% RH and at 22°C, 70 ± 5% RH, mean longevity of adult *Amitus* females on the host insect was 9.3 and 5.7 days; mean fertility was 64.1 eggs and 52.7 eggs; and r_m values were 0.102 and 0.144 for the respective conditions. On cv. ICA-Pijao, at 19°C, 75 ± 5% RH, mean longevity was 15.2 days; mean fertility was highest (162.8 eggs), and r_m value was 0.117. The r_m values of *A. fuscipennis* were higher than those of its host under the same tested conditions. This parasitoid may therefore be a suitable candidate for use in inundative biological control programs against *T. vaporariorum*.

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) is an important pest, especially in vegetable and ornamental crops (van Lenteren and Martin, 1998). It is a key pest of dry and snap beans (both *Phaseolus vulgaris* L.) in Colombia (Prada *et al.*, 1993).

Because the greenhouse whitefly has developed resistance to several insecticides (Buitrago *et al.*, 1994; van Lenteren, 1995; Cardona *et al.*, 1998), an alternative control mechanism, a guided pest control system, has been implemented. It consists of combining cultural control practices with insecticide applications at a pre-established action level (Prada *et al.*, 1993). When the system of guided pest control was tested in a series of trials conducted on farmers' fields, it resulted in a 54% reduction in insecticide use and an 18% reduction in costs, compared with the conventional pesticide spray program (Prada *et al.*, 1993).

The system of guided pest control lacks a biological component. To improve the system, the native parasitoid *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae) is being evaluated as a potential biological control agent of the greenhouse whitefly on bean crops in Colombia (Manzano *et al.*, 2000). *Amitus fuscipennis* has been found in northern South America (including in the Andes) and the Caribbean, and seems to be partly responsible for the present low infestation of *T. vaporariorum* in field crops in Rionegro, Department of Antioquia, Colombia (C. Cardona, personal observation). The performance of *A. fuscipennis* is also being tested on tomatoes in greenhouses in Colombia (Vis *et al.*, 1999).

The Andes separate into three ranges in Colombia, making the cropping of dry and snap beans possible at different altitudes. Within the Whitefly Project of the CGIAR IPM Program, field surveys have been carried out on tomato, and dry and snap beans at the International Center for Tropical

Agriculture (CIAT) and in some areas of the Department of Valle del Cauca (CIAT, unpublished, 1999). Results of these surveys showed that *A. fuscipennis* and *T. vaporariorum* can be found together in tropical high-altitude cropping areas located above 1000 m, or at mid-altitude areas, between 400-1000 m (CIAT, 1999 unpublished results). Around 1500 m, average temperature is 19°C and relative humidity 80%. In midland areas, average temperature increases to between 22°C and 24°C and average relative humidity decreases to about 74%.

The aim of the research reported here was to determine the reproductive capacity of *A. fuscipennis*, measured as the intrinsic rate of population increase (r_m), under two sets of environmental conditions that simulated those of the highland and midland areas mentioned above. At least 37 commercial bean cultivars are cropped in Colombia and more than 60 bean lines have been improved agronomically (Voysest, 1983). Because testing all these materials would be impossible, two were chosen: the cv. Chocho, which is commercially grown on hillside areas of the Andes (e.g., Tenerife and El Dovio, Valle del Cauca), and cv. ICA-Pijao that is well adapted to greenhouse conditions, being less susceptible to microbial infections.

The r_m values of the parasitoid were compared with those of its host, the greenhouse whitefly, estimated under similar conditions. If the reproductive capacity of *A. fuscipennis* is similar or exceeds that of its host under comparable conditions, *A. fuscipennis* could be a promising candidate for the biological control of *T. vaporariorum* (van Lenteren and Woets, 1988), and thus for becoming a component to implement an IPM system for the greenhouse whitefly over a broader area.

Materials and methods

Plants, and whitefly and parasitoid cultures. The plants used in the experimental trials were 15-day-old plants of the bean (*Phaseolus vulgaris* L.) cultivars Chocho and ICA-Pijao. The plants received no fertilization or other chemical applications.

Whiteflies (*Trialeurodes vaporariorum*) were initially collected 4 years ago from snap bean crops in Fusagasugá, Department of Cundinamarca, Colombia. They have since been reared at CIAT on the bean cultivar ICA-Pijao at 22°C-24°C, 40%-80% RH, and 12L:12D.

A colony of *A. fuscipennis* was established in September 1996, also at CIAT. These parasitoids originated from bean crops in Tenerife, Valle del Cauca. They were reared on first and second instars of *T. vaporariorum*, using the same host-plant species as the whitefly colony and under the same conditions: 22°C-24°C, 40%-80% RH, and 12L:12D.

Longevity and reproduction of Amitus fuscipennis. Small clip-cages (inside diameter measures 1.8 cm) were used to create two or three-*T. vaporariorum* infested spots per leaflet. Twenty whitefly adults were confined within the cage for 8 h. After the adults were removed, the eggs were manipulated to later obtain spots with at least 50 nymphs.

When the nymphs reached first instar, unmated female parasitoids of less than 24 h old were introduced individually into a larger clip-cage (inside diameter = 2.5 cm) that entirely covered the infested spot. Every 24 h, the introduced parasitoids were transferred via an oral aspirator to a new whitefly infested spot until the female parasitoids died. Parasitized whiteflies were maintained until the parasitoids emerged. Only single females were caged because *A. fuscipennis* reproduces by thelytoky (Manzano *et al.*, 2000).

The trials were conducted in environmental control chambers, as follows:

Females (no.)	Temperature (°C)	Relative humidity (%)	Daylength (L:D)	Bean cultivar
23	19	75 ± 5	12:12	Chocho
21	22	70 ± 5	12:12	Chocho
19	19	75 ± 5	12:12	ICA-Pijao

We recorded the number and sex of parasitoid offspring emerging from the parasitized whitefly nymphs.

Development time, survival rate and proportion of females of Amitus fuscipennis. Spots of whitefly first-instar nymphs on cv. Chocho were each exposed to *A. fuscipennis* females during 24 h. After the parasitoids were removed, some spots of whiteflies were kept at 22°C and 70 ± 5% RH and others at 19°C and 75 ± 5% RH until the parasitoids emerged.

Development time and proportion of females of *A. fuscipennis* were also recorded for cv. Chocho. These parameters had previously been calculated for *A. fuscipennis* at 19°C on cv. ICA-Pijao (Manzano *et al.*, 2000).

The survival of individuals from the pupa-adult period was estimated by the ratio of dead pupae to the imago parasitoids that emerged from the parasitized spots. Survival rate, development time and proportion of females were also used to create 'lx-mx' life tables for *A. fuscipennis*.

The intrinsic rate of population increase. Experimental data on the development time of immature individuals and reproduction were combined to create 'lx-mx' life tables that were used to calculate

demographic parameters for *A. fuscipennis*. For each experiment, the following demographic parameters, as defined by Price (1975), were calculated: the net reproductive rate, R_0 (which represents the number of female descendants that an average female leaves in one generation), and the generation time (T , which is equivalent to the mean period between the birth of parents and the birth of offspring).

The intrinsic rate of population increase (r_m) for *A. fuscipennis* under the different environmental conditions was calculated, using the equation given by Carey (1993):

$$\sum \exp(-r_m x) l_x m_x = 1$$

where x is the age,

l_x is the age-specific survival, and

m_x is the proportion of females in the offspring of a female at age x .

Following Carey (1993), pivotal age, which is $x + 0.5$, was used to calculate r_m values.

Data analysis. Differences between the mean values of treatments were tested for significance by one-way ANOVA for normally distributed populations and, when normality failed, by the Kruskal-Wallis One Way Analysis of Variance on ranks test. If significance differences were detected, multiple comparisons were made using the Student-Newman-Keuls method or the Dunn's method ($P < 0.05$, for both of them). The development time of immature individual was compared using the Mann-Whitney test.

Results

Longevity and reproduction of *Amitus fuscipennis*. Mean female longevity was highest (15.2 days) on cv. ICA-Pijao at 19°C (Table 1); differences can clearly be seen in the survival curves (Figure 1). A Student-Newman-Keuls method $P < 0.05$, after Kruskal-Wallis One Way ANOVA test $P < 0.0001$ indicated a difference in female longevity between the three sets of environmental conditions.

A. fuscipennis did not demonstrate any pre-oviposition period, oviposition starting immediately after emergence (Figure 2). The number of eggs/day/female decreased continuously on cv. Chocho, whereas it increased on cv. ICA-Pijao when females were between 5 and 15 days old, then decreased again (Figure 2).

Mean life time fertility was significantly higher (162.8 eggs) on cv. ICA-Pijao at 19°C (Kruskal-Wallis test, $P < 0.001$; Table 1) but did not differ significantly on cv. Chocho between 22°C and 19°C (Dunn's method after Kruskal-Wallis test, $P < 0.05$; Table 1).

Table 1. Longevity and fertility of *Amitus fuscipennis* at different temperatures ($T = ^\circ\text{C}$), relative humidities (RH = %), and bean cultivars

Parameter	T = 19	T = 19	T = 22
	RH = 75 \pm 5	RH = 75 \pm 5	RH = 70 \pm 5
	ICA-Pijao	Chocho	Chocho
Mean longevity [†] \pm SE (days)	15.2 a \pm 1.2	9.3 b \pm 0.8	5.7 c \pm 0.4
Range	4-23	4-18	3-9
Number of insects	19	23	21
Mean lifetime fertility [‡] \pm SE (eggs per female)	162.8 a \pm 18.7	64.1 b \pm 12.6	52.7 b \pm 6.9
Range	43-299	7-262	14-124
Mean daily fertility* \pm SE (eggs per female)	10.7 a \pm 0.8	6.8 b \pm 1.0	9.2 ab \pm 0.8
Range	4.6-17.3	1.2-19.2	1.8-17.3

[†]Student-Newman-Keuls method, $P < 0.05$, after Kruskal-Wallis One Way ANOVA on ranks, $P < 0.0001$

[‡]Dunn's method, $P < 0.05$, after Kruskal-Wallis One Way ANOVA on ranks, $P < 0.001$

*Student-Newman-Keuls method, $P < 0.05$, after One Way ANOVA, $P < 0.001$

Mean daily fertility was highest on cv. ICA-Pijao (10.7 eggs) and lowest (6.8 eggs) on cv. Chocho at 19°C (Table 1). The mean daily fertility was not significantly different under the two sets of environmental conditions for cv. Chocho, but became significantly different between cv. Chocho and cv. ICA-Pijao at 19°C (Student-Newman-Keuls $P < 0.05$, after One Way ANOVA, $P < 0.001$; Table 1). Offspring were females only.

Development time of immature individuals, survival rates and proportion of females of Amitus fuscipennis. On cv. Chocho, the shortest development time for *A. fuscipennis* was 25.5 days at 22°C,

being significantly different to that of 37.5 days at 19°C (Man-Whitney test, $P < 0.0001$; Table 2). No significant difference in the survival rate was present on cv. Chocho between 19°C and 22°C. All offspring were females (Table 2).

Demographic parameters. The values for the different demographic parameters of *A. fuscipennis* are shown in Table 3. On cv. Chocho, the net reproductive rate decreased as temperature increased, being 64.1 at 19°C but 52.7 at 22°C. The intrinsic rate of natural increase (r_m) was also lower at 19°C (0.102) than at 22°C (0.144). For cv. ICA-Pijao, the r_m was 0.117 at 19°C. Generation time was similar at 19°C for both Chocho (41.1) and ICA-Pijao (44.6), but decreasing to 27.5 at 22°C on cv. Chocho.

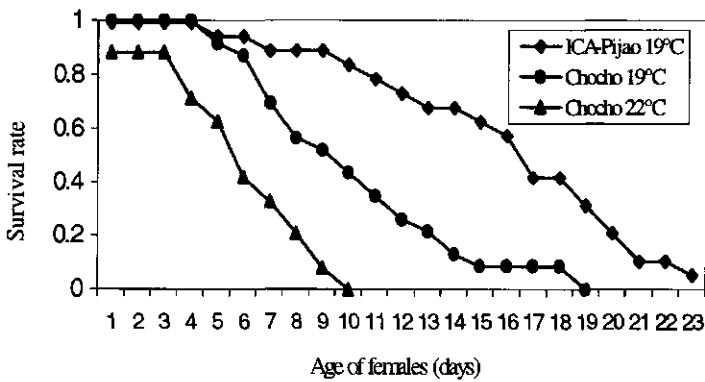


Figure 1. Survival rate curves of *A. fuscipennis* females under different environmental conditions on bean cultivars ICA-Pijao and Chocho

Table 2. Mean pre-imaginal development time (DT) in days, survival rate (%), and percentage of females of *A. fuscipennis* on bean cultivars Chocho and ICA-Pijao. Statistical comparisons made within cv. Chocho. Values across columns followed by the same letter are not statistically different

Cultivar	Climatic conditions (°C, RH%)	Number of insects	Mean of DT* \pm SE	Range	Survival rate [†]	Females (%)
Chocho	22, 70 \pm 5	34	25.5 a \pm 0.31	23-28	88.2 a	100
Chocho	19, 75 \pm 5	32	37.5 b \pm 0.24	35-41	100.0 a	100
ICA-Pijao ^a	19, 75 \pm 5	390	38.1 \pm 0.1	35-45	99.2	100

^aThese results come from Manzano *et al.*, 2000.

*Man-Whitney test, $P < 0.0001$ for pre-imaginal development time

[†]Fisher exact test

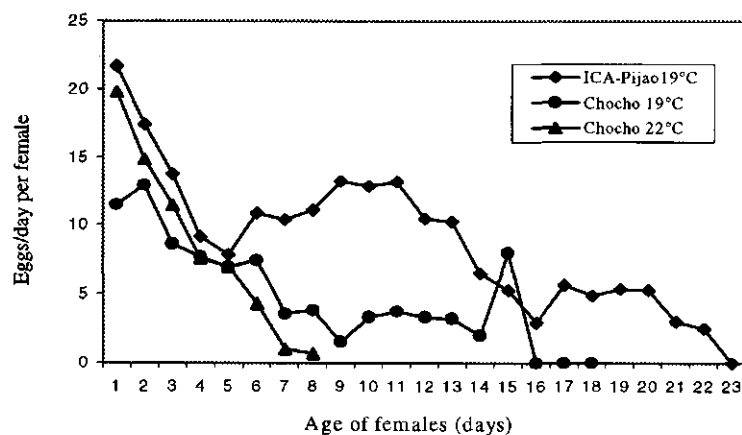


Figure 2. Reproduction curves of *A. fuscipennis* on *Trialeurodes vaporariorum* under different environmental conditions and bean cultivars.

Table 3. Demographic parameters for *Amitus fuscipennis* on bean cultivars Chocho and ICA-Pijao at different temperatures ($T = ^\circ\text{C}$) and relative humidities ($\text{RH} = \%$)

Parameter	$T = 19$	$T = 19$	$T = 22$
	$\text{RH} = 75 \pm 5$	$\text{RH} = 75 \pm 5$	$\text{RH} = 70 \pm 5$
	cv. ICA-Pijao	cv. Chocho	cv. Chocho
r_m^a	0.117	0.102	0.144
Ro^b	162.7	64.1	52.7
T^c	44.8	41.2	27.5

^a Intrinsic rate of natural increase^b Net reproductive rate^c Generation time in days

Discussion

Adult longevity of *A. fuscipennis* in the presence of hosts on cv. ICA-Pijao was higher than on cv. Chocho. Longevity of 15.2 d reported here for cv. ICA-Pijao is closer to the value of 13.6 d reported for *A. fuscipennis* on tomato at 20°C by Vis *et al.*, 1999. The short longevity of *A. fuscipennis* can be related to its reproductive physiology. *Amitus* females are pro-ovigenic, and live only a few days (Gerling, 1990) when hosts are present, for example, *A. hesperidium* Silvestri lives 4–5 d (Nguyen and Hamon, 1993); *A. bennetti* Viggiani & Evans 5–8 d, depending on temperature (Drost *et al.*, 1999); and *A. fuscipennis* 6, 9 or 15 d (this study). Synovigenic species like the whitefly parasitoid *Encarsia formosa* Gahan usually live longer (Gerling, 1990). Kajita, (1979) reported that, at 20°C , *E. formosa* lived for 31.9 d, laying eggs on *T. vaporariorum*.

Mean fertility of *A. fuscipennis* was also higher on cv. ICA-Pijao than on cv. Chocho. On cv. Chocho, most eggs were laid on days 0–6, whereas on cv. ICA-Pijao oviposition increased again after day 6. This result is not easily explained, but probably the abundance of hosts, under all three environmental conditions, may have led to the entire egg load being laid before the females died.

Mean lifetime fertility reported here of 162.8 eggs per female for *A. fuscipennis* on cv. ICA-Pijao was lower than the value of 270 eggs per female reported on tomato at 20°C by Vis *et al.*, (1999). The fertility of *A. fuscipennis* on cv. ICA-Pijao was in the same range as that of *A. hesperidium*, according to Nguyen and Hamon, (1993). They reported that each female of this species could produce as many as 70 offspring in 4–5 d. Joyce *et al.*, (1999) reported an average daily fertility of 78.8 eggs per day for *A. bennetti*. Pre-imaginal development at 19°C on cv. Chocho (37.5 d) was similar to that

on cv. ICA-Pijao at the same temperature (38.1 d; Manzano *et al.*, 2000). A longer preimaginal development of 41.7 d at 20°C was reported for *A. bennetti* by Drost *et al.*, (1999). *E. formosa* took 32.2 d at 20° C (Dorsman and van de Vrie, 1987) for pre-imaginal development. This difference between both genera is influenced by the host instar preferred for oviposition; *Amitus* prefers first instars (Joyce *et al.*, 1999; Drost *et al.*, 1999) and *Encarsia* prefers third and fourth instars, and the prepupae (van Lenteren *et al.*, 1980). Offspring consisted only of females, a result consistent with the assumption of thelytoky reproduction of *A. fuscipennis*. Pupal survival rate was high and unaffected by temperatures ranging between 19°C and 22°C. Nevertheless, the total survival rate of immature individuals was underestimated because mortality at egg or larval stages could not be determined.

The net reproductive (R_o) values were lower than the value of 264 reported, also for *A. fuscipennis*, on tomato by Vis *et al.*, (1999). R_o values reported here were higher than those recorded for an arrhenotokous population of *Eretmocerus* sp. (12.09, 23.02) at 20°C (Powell and Bellows, 1992). The R_o rates of our study (on cv. Chocho) fall within the range of 43.8-73.1 reported for *A. bennetti* by Joyce *et al.*, (1999).

The lowest mean daily fertility of *A. fuscipennis* (6.8 eggs) was higher than that of *A. bennetti* (6.3 eggs) reported by Joyce *et al.*, (1999). It was also higher than the range recorded for arrhenotokous (2.67-3.34) and thelytokous (1.79-4.04) populations of *Eretmocerus* sp. at 20°C (Powell and Bellows, 1992).

Reproductive differences for *A. fuscipennis* females kept under the same environmental conditions and on different cultivars could be related to host-plant characteristics such as leaf morphology, chemical composition or other interactions not examined in this study.

Despite these differences, the r_m values of *A. fuscipennis* were higher than those of *T. vaporariorum* under the same environmental conditions and on the same bean cultivars. At 19°C and 22°C on cv. Chocho, r_m values of *T. vaporariorum* were 0.043 and 0.078, respectively (M. R. Manzano, unpublished results). The r_m value of *A. fuscipennis* on cv. Chocho was highest at 22°C, partly because the parasitoid has a shorter generation time at this temperature. At 19°C, on cv. ICA-Pijao, the r_m value was 0.066 (M. R. Manzano, unpublished results).

The r_m values of *A. fuscipennis* were higher than those of *T. vaporariorum* because, first, half of the whitefly eggs were female, whereas all parasitoid eggs were females. This, combined with a low mortality rate for immature parasitoids, permitted higher R_o values for the parasitoid than for the whitefly.

Second, on cv. Chocho, which is of lower quality for *T. vaporariorum* than ICA-Pijao (M. R. Manzano, 2000 unpublished results), the total amount of eggs laid by *A. fuscipennis* was even larger

than the total amount of eggs laid by the greenhouse whitefly. Vis *et al.*, (1999) also reported a higher r_m value for *A. fuscipennis* (0.160) than for *T. vaporariorum* (0.064) on tomato at 20°C.

The r_m values reported for *A. fuscipennis* compare favorably with other whitefly parasitoids used for biological control. For *E. formosa* laying eggs on *T. vaporariorum*, Arakawa (1982) reported a r_m value of 0.2. Vet and van Lenteren, (1981) reported a r_m value of 0.137 for the same parasitoid-host interaction.

Van Lenteren and Woets, (1988) proposed that an efficient parasitoid should have a potential maximum rate of population increase (r_m) equal to or larger than that of its host. When this criterion is applied to *A. fuscipennis* and its host, *T. vaporariorum*, we conclude that *A. fuscipennis* would be an efficient biological control agent of *T. vaporariorum* in Colombia in tropical highland areas above 1000 m (average conditions of 19°C, 80% RH), and in midland areas around 400-1000 m (average conditions of 22°C, 74% RH).

Life-history data suggest that biological control of *T. vaporariorum* could be successful on the commercial cv. Chocho. This cultivar is a less suitable host for the whitefly than is cv. ICA-Pijao (M. R. Manzano, 2000 unpublished results). The intrinsic rate of increase is higher for *A. fuscipennis* than it is for *T. vaporariorum* under the two sets of environmental conditions. Under field conditions, should *T. vaporariorum* be densely populated, an initial mass release of *A. fuscipennis* may effectively and quickly reduce the whitefly.

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5. Searching behavior of *Amitus fuscipennis*, a parasitoid of the greenhouse whitefly

Abstract

Amitus fuscipennis MacGown & Nebeker is a parasitoid of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). It is found on several crops at field and greenhouse conditions in Colombia. Knowledge about the foraging behavior of parasitoids is essential to estimate the efficiency of biological control agents under various conditions and to develop parasitoid release strategies. Therefore, the searching and oviposition behavior of *A. fuscipennis* was studied in the laboratory, using *T. vaporariorum* as a host on bean leaves. The parasitoid's basic search pathway consisted of walking, encountering the host, and drumming and probing it. While walking, the parasitoid stopped for short periods of times, partly to preen herself. Walking while searching comprised 61% of the adults' time budget, and probing hosts represented 16 %. The remainder of the time consisted of standing still, encountering and drumming hosts, and preening. After a host nymph was parasitized by *A. fuscipennis*, the parasitoid remained close by and continued searching for new hosts in the immediate vicinity. Such behavior suggests area-restricted searching. First and second nymphal instars of *T. vaporariorum* were preferred by the parasitoid for oviposition. *Amitus fuscipennis* on average walked faster before an oviposition (1.4 mm s^{-1}) than do other whitefly parasitoids. *A. fuscipennis* had a high percentage of host acceptance resulting in a high percentage parasitism (60 %) of all encountered hosts. The parasitoid preferred first instars of *T. vaporariorum* for oviposition, resulting in long developmental times. The implications of these findings for the control of *T. vaporariorum* are discussed.

Introduction

Parasitoids can help make sustainable agriculture successful by regulating pest populations without the need for chemical pesticides. Basic knowledge of parasitoid biology and ecology is essential to be able to evaluate their usefulness as a biological control agent. The performance of parasitoids in the field or greenhouse can be estimated by studying those behavioral parameters that indicate their efficacy and potential application as biological control agents (e.g. van Roermund, 1995; Mattiacci *et al.*, 1999).

In this study, the tritrophic system we investigated consisted of dry bean (*Phaseolus vulgaris* L.), one of its key pests—the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae)—and its nymphal parasitoid, *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae).

Beans are important crops in the developing world, not only because they are the cheapest source of protein in many countries, but also because they are a very economical source of calories (Pachico, 1993). Out of nearly 10 million tons of dry beans produced annually, 46.2% is produced by Latin America (Pachico, 1993). Because of the beans' economic importance in Latin American countries such as Colombia, a guided pest control system has been developed for this crop (Prada *et al.*, 1993).

The greenhouse whitefly is worldwide a major pest of vegetable and ornamental crops. Many natural enemies of whitefly are known (Gerling, 1990; Gerling and Mayer, 1996), and several species of parasitoids have been used successfully in both classical and seasonal inoculative releases for whitefly control (Gerling, 1990; van Lenteren *et al.*, 1997). At present, biological control of *T. vaporariorum* by the parasitoid *Encarsia formosa* Gahan is commercially successful for several greenhouse vegetables and ornamentals (van Lenteren *et al.*, 1996; van Lenteren, 2000).

T. vaporariorum frequently reaches key pest status on bean crops in Colombia, resulting in intensive application of chemical pesticides. Pest resistance to pesticides began appearing in 1978/79. Recently, whitefly resistance to several insecticides has been detected (Cardona *et al.*, 1998; Rodríguez and Cardona, in press), accompanied by farmer intoxication during chemical application (Rodríguez and Cardona, in press). Even so, chemical control of whiteflies is still the method most favored by Colombian farmers. As an alternative, a system of guided control was implemented. It consists of combining cultural control practices with the application of insecticides at pre-established action levels (Prada *et al.*, 1993). The guided practices were tested in a series of trials conducted in farmers' fields, resulting in a 54% reduction in insecticide use and an 18% reduction in costs, compared with the conventional pesticide spray program (Prada *et al.*, 1993). *Bacillus thuringiensis* (commercial toxin) was applied for the control of insects that consume the snap bean foliage.

As an important addition to the guided control program, the parasitoid *A. fuscipennis* is being evaluated as a potential control agent of *T. vaporariorum* on beans in Colombia. The parasitoid is abundant, and widely distributed in Colombia in some areas of the Andes, and in inter-Andean valleys. The species has also been found in Central America, northern South America, and the Caribbean (MacGown & Nebeker, 1978). *A. fuscipennis* has a strongly female-biased sex ratio (Manzano *et al.*, 2000; chapter 2) and shows intrinsic rates of population increase (r_m) higher than those of *T. vaporariorum* at climatical conditions in Colombian fields (Chapter 4). These biological characteristics support the hypothesis that *A. fuscipennis* has potential as a useful biological control agent of *T. vaporariorum*. Some few data on biology and longevity of *A. fuscipennis* are provided by Medina *et al.*, (1994). The usefulness of *A. fuscipennis* for control of whiteflies is also being tested on tomatoes in greenhouses in Colombia (de Vis *et al.*, 1999).

Effectiveness of parasitoids as biological control agents depends to a great extent on their host finding behavior. Therefore, examination of the searching behavior of a parasitoid species is a basic step toward its evaluation as a biological control agent. In this paper, we report on the searching behavior over short distances of *A. fuscipennis* on bean leaves infested with *T. vaporariorum* nymphs. In particular, we (1) analyze the oviposition behavior and instar preference for oviposition, (2) quantify the searching behavior, and (3) measure the walking speed of the parasitoid before an

oviposition event. Results will be compared to those of other successful biological control agents such as *E. formosa* and other *Amitus* species.

Materials and methods

Plants. Bean plants (*Phaseolus vulgaris* L. cv. ICA-Pijao) were grown under greenhouse conditions at 22 ± 0.4 °C, 80.7 ± 19.2 % RH, and 12L:12D. Plants did not receive fertilizers or chemical treatments. The plants were cut down when they were 15 to 20 days old to bear only one trifoliate leaf.

Parasitoids. *A. fuscipennis* was reared for 3 years at the International Center for Tropical Agriculture (CIAT), Colombia. *A. fuscipennis* was reared by infesting bean plants (cultivar ICA-Pijao) with *T. vaporariorum* adults for 24 h. Subsequently, whitefly adults were removed and plants were set in cages until whitefly eggs hatched and nymphs reached first or second instar. At these stages, the hosts were exposed to parasitoid adults for 48 h. Later, plants were left in a cage until parasitized pupae became dark gray, indicating that parasitoids were close to emergence. After parasitoid emergence, the cycle was repeated. To obtain adult parasitoids for our experiments, leaves bearing parasitized pupae were cut one day before observation and placed in glass petri dishes (15 cm in diameter) on dry tissue paper at 19 °C, 80 ± 10 % RH, L12:D12. Most parasitoids emerged in the early morning hours. No food was added. For each observation, a recently emerged female of *A. fuscipennis*, without oviposition experience, was used.

Searching and oviposition behavior. A bean plant was cut down to bear three leaflets. Each leaflet was infested with *T. vaporariorum* by caging whitefly adults in clip-cages (2.5 cm diameter) for 4 h. Leaflets were re-infested every 4 days for a total of four infestations to obtain similar proportions of 1st, 2nd, 3rd, and 4th (including red-eyed nymphs) instars of *T. vaporariorum*.

The leaflet still attached to the plant and infested with different instars of *T. vaporariorum* was placed with the abaxial side up under a stereomicroscope. The number of each instar type per leaflet was counted and mapped to permit later identification of instar parasitism. One female parasitoid was then released in the middle of the leaflet, and its searching and oviposition behavior recorded for 1 h, using a hand-held computer (Psion Workabout) with the software *The Observer 3.0* (Noldus, 1991).

The behavioral components recorded were (a) walking (i.e., searching for a host), (b) standing still, (c) preening, (d) encountering a host (i.e., making contact with the host with the antennae), (e) drumming a host (i.e., inspecting the host with the antennae and (f) adopting the oviposition posture (i.e., feeling about and entering the host with the ovipositor; also addressed as "probe"). If the host is

accepted for oviposition, an egg is laid inside it by the parasitoid. Although parasitization was recorded only when the parasitized nymph turned gray - as it is almost impossible to find the very small *A. fuscipennis* eggs- we cannot definitely state that each oviposition posture (= probe) resulted in an oviposition.

Time for oviposition was calculated for those hosts that were probed only once. Hosts with consecutive probes and drumming events were not included because we could not know exactly after which probe an egg was laid.

Parasitoid females feed from the host plant's stipels (tiny leaves located at the base where each leaflet joins the main leaf stem), but this feeding was not recorded in this study, and will be the subject of future research (Chapter 6)

Observations were carried out on 15 females at 19 °C and 80 ± 10% RH and the plants bearing parasitized hosts were kept under the same environmental conditions after observations to allow the parasitoids to develop.

Data from all females were combined, and frequency, duration, and percentage of time spent on each behavior calculated. Tests for significant differences among the means for duration of behavior were carried out using t-tests. Frequencies of oviposition in each type of whitefly instar were compared using χ^2 analysis.

Walking speed. The experiment was set up and walking was recorded according to Drost *et al.*, (2000). Just before the experiments, a 4.5-cm diameter disc was cut from a bean leaf and put, upside down, on a 1% agar solution that filled a petri dish (5-cm diameter) to a height of 0.5 cm. The agar solution prevented the leaf tissue from wilting. The petri dish with the experimental arena was placed under a video camera. It was lit from the underside by a circular fluorescent tube, separated by a 1-cm thick Plexiglass plate to prevent heating. The walking process was recorded, using a videocamera (Panasonic CCTV), a videorecorder (Panasonic AG-6200), and a color video monitor (Panasonic TC 1470-y) connected to a computer. Video tracking and motion analyzes were carried out with the Ethovision^R program (version 1.7, Noldus Information Technology, Wageningen, Netherlands).

A female was released onto the leaf disc and recording of walking started immediately. Analyzes were carried out using a sample frequency of 2 frames per second, chosen from the Down-Sampling-Step (DSS) option of Ethovision^R. Ten recently emerged parasitoid females were individually tested, using them only once. Thirteen long tracks from these 10 females were analyzed. Experiments were carried out at 24 ± 1 °C and 70 ± 10% RH.

Results

Searching patterns. After *A. fuscipennis* females were placed on infested bean leaves, they immediately began walking and searching for whitefly nymphs. They were fast moving while drumming the leaf surface. The distal parts of the antennae were folded with the ventral side kept flat, touching the leaf surface. Females normally exhibited the following sequence of behavior: walking on the leaf surface with short stops of standing still, encountering a host, drumming the host with her antennae, probing or not probing the host, and walking away from the encountered host. In addition, they also preened and took longer stops of standing still. An ethogram was developed from the sequences observed (Figure 1).

Walking was the most frequently observed activity (720 times), followed by standing still (509 times, 70.6%) and encountering host (209 times, 29.0 %). Preening just twice followed walking. After preening, the females restarted walking 85.5% of the time. Standing still was followed by walking 67.4% of the time or by preening 32.6% of the time. Drumming always followed a host encounter. After drumming the host, the female probed the host 62.9% of the times or walked away from it 37.1% of the time. After probing the host, the parasitoid started walking again 61.1% of the time or drummed the host again 38.9% of the time.

On comparing the mapped distribution of the whitefly nymphs on every leaflet with that of gray parasitized pupae, we saw that 80% of the parasitoid females laid at least their first three consecutive eggs on close by hosts, and that 47% did the same with their first five eggs.

Feeding upon leaf surface water was occasionally observed during the stops but, as was mentioned before, feeding was not recorded in this study. The parasitoid was never observed to feed on the host insect.

Frequency and duration of behavior. Females of *A. fuscipennis* spent most of their time walking the leaf (60.8%). In round numbers, the other activities took: for probing 16% of the time; for standing still 10%; for drumming 7%; for preening 6%; and for encounters less than 1% (Table 1). During each bout of activities, a female spent, on the average, 49.1 s walking; and 67.2 s for handling (drumming and probing) the host, of which 52.9 s were spent probing. Average oviposition time (i.e. all cases where the oviposition posture = probe, resulted in a real oviposition based on gray nymph color) across all whitefly instars was 63.7 ± 4.7 s (no. of hosts = 48), being significantly different between first and second instars (t-test = 3.636; df = 19.5; $P = 0.002$), being longer on the second instar (82.1 ± 9.1 s, $n = 19$) than on the first instar (48.4 ± 1.9 s, $n = 25$, Table 2). Average oviposition time on third instars took 68.3 ± 22.9 s. However, we did not include third instars in the statistical analysis because of the low numbers of probes (4). Drumming a host took about 13 s.

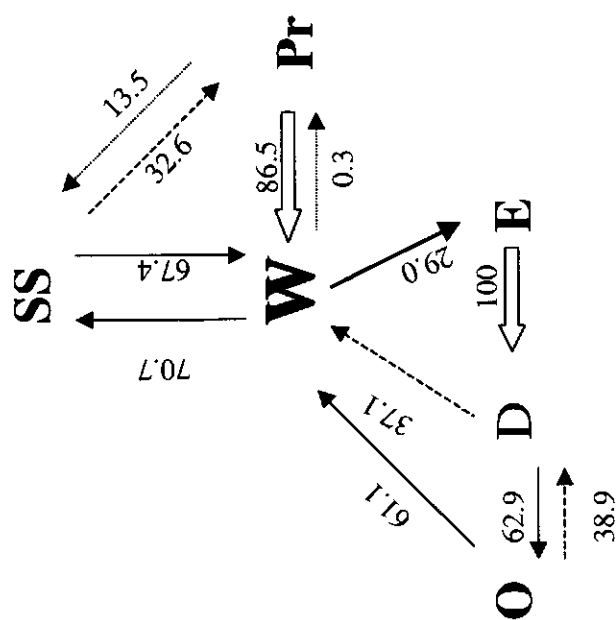


Figure 1. Behavioral sequences (% times a sequence was followed) of *A. fuscipennis* (n = 15). W, walking; Pr, preening; SS, standing still; E, host encounter; D, drumming; O, oviposition posture. Differences in percentages are indicated by different arrows and numbers

When we considered only encounters with unparasitized hosts, time of drumming leading to probing did not differ significantly to that of drumming leading to host rejection. Drumming time did not differ significantly between whitefly instars.

Oviposition behavior and host-instar preferences. *A. fuscipennis* tried to oviposit in all four instars of *T. vaporariorum*, but eggs were laid in only the first three instars. Occasionally, a female would try to oviposit in an empty pupal cage or to probe an egg. Encounters with hatched eggs resulted in faster walking activity in that specific area. When a host nymph was encountered, the female touched its edges with her antennae. She walked over the host's body and inserted her ovipositor without facing the host.

Table 1. Frequencies and duration of behavioral events of *A. fuscipennis* (n = 15) across all nymphal stages of *T. vaporariorum*. Means are presented with their standard errors.

Behavior	Abundance (no. of times)	Mean duration (seconds)	Percentage of total observed time
Walking	732	49.1 ± 3.2	60.8
Probing	178	52.9 ± 4.9	16.1
Standing still	549	10.5 ± 0.8	10.2
Drumming	279	14.3 ± 0.9	6.9
Preening	179	17.9 ± 1.7	5.7
Encountering	210	0.87 ± 0.1	0.3

During oviposition the front legs were on the leaf, the hind legs secured the host or were on the leaf, the antennae were on the leaf or free, and the wings were raised. The host nymphs were probed on all sides. The percentage acceptance (the ratio between the number of ovipositions and the number of encounters) was similar for first and second instars (60%, n = 47 encounters; and 58%, n = 43 encounters, respectively), and lower for the third instar (32%, n = 28 encounters). No oviposition was observed for the fourth instar.

Based on the number of available first, second and third instars (the instars that were parasitized) and the number of each instar parasitized after 1 h of observation (Table 2), we may conclude that instars were not parasitized according to their quantity ($\chi^2 = 6.63$, df = 2, $P < 0.05$). Partitioning the degrees of freedom, third instars were not parasitized as often as other instars ($\chi^2 = 5.6$, df = 1, $P < 0.025$); so they were less preferred for parasitization than first and second instars. First and second instars seem to be equally accepted for parasitization ($\chi^2 = 0.69$, df = 1, $P < 0.001$).

Table 2. Observed and expected number of ovipositions, and mean time (s) of oviposition on *T. vaporariorum* nymphs by *A. fuscipennis* females after 1 h of observation. Means are presented with their standar error values.

Whitefly instar Number/ Time	First		Second		Third	
	(no.)	(time)	(no.)	(time)	(no.)	(time)
Nymphs available ^a	152		106		98	
Observed no. parasitized	28	48.4 ± 1.9	25	82 ± 9.1	9	68.3 ± 22.9
Expected no. parasitized ^b	26		18		17	

^aTotal number of *T. vaporariorum* nymphs exposed to *A. fuscipennis* during 1 h of observation.

^bExpectation based on the number available of each instar.

Walking speed. The walking speed of *A. fuscipennis* at 24 °C was 1.4 mm s⁻¹ ± 0.11 SE, n = 13 tracks.

Discussion

Search patterns. While females of *A. fuscipennis* searched, the distal parts of their antennae were held folded, with the ventral part pressed flat against the leaf surface. This behavior is similar to that of *A. bennetti* (Drost *et al.*, 1999) and contrasts with that of *E. formosa* females, which touch the leaf surface with the tips of their antennae (van Lenteren *et al.*, 1980). As suggested by Drost *et al.*, (1999), this difference may be because either the sensilla differ among the insects or their positions in the antennae are different.

The search pathway of *A. fuscipennis* can be divided into two main cycles (Figure 1). The first, which occurs when a host is encountered, consists of walking, encountering the host, drumming, probing, and return to walking. The second occurs when hosts are not encountered, and consists of walking, standing still, and preening. This second sequence differs from that of *A. bennetti* where the parasitoid frequently stops searching to preen herself so that preening was followed by searching 55.3% of the time (Joyce *et al.*, 1999). *A. fuscipennis* stops for short times to restart searching (67.4% of the time) or to preen itself (32.6%). Preening after probing a host, normal in *A. hesperidium* (Dowell *et al.*, 1981), was never observed in *A. fuscipennis*, and only infrequently in *A. bennetti* (Joyce *et al.*, 1999).

Once an *A. fuscipennis* female parasitizes a nymph, the parasitoid remains close to this area and continues searching for new hosts in the immediate vicinity. Such behavior suggests area-restricted searching which is an adaptation to searching for hosts that are distributed in a clumped manner (Godfray, 1994). Under Colombian bean field conditions, *T. vaporariorum* density per leaflet

can be very high and the distribution of the different whitefly instars is random. The distribution of *T. vaporariorum* on a leaf or leaflet is aggregated in all instar stages (Noldus and van Lenteren, 1990) and area-restricted searching therefore seems advantageous for *A. fuscipennis*. The end result of area restricted search by a small population of *A. fuscipennis* at high whitefly densities is that some whitefly patches are completely parasitized whereas others remain unparasitized.

As mentioned before, in *A. fuscipennis* every host encounter leads to drumming as is the case in *Encarsia formosa*, where 99.6% of encounters lead to drumming (van Lenteren *et al.*, 1980). In contrast, *A. bennetti* and *Eretmocerus* sp. nr. *californicus* show drumming in only 70.6% and 34% of cases, respectively (Headrick *et al.*, 1995; Joyce *et al.*, 1999).

After drumming a host, the *A. fuscipennis* female probed it in 62.9% of the cases. This result may be overestimated because the parasitoids, although they encountered late fourth instars and tried to probe them, did not always introduce the ovipositor, probably because the hard wax filaments covering the host in this stage hampered entry. Probing and oviposition in most of the hosts that *A. fuscipennis* encountered may be an adaptation of pro-ovigenic parasitoids like *A. fuscipennis*, whose short-lived adult females emerge with their life-time egg load ready to oviposit (Donaldson and Walter, 1988). The egg-load of a parasitoid often influences, among others, its search intensity, oviposition rate, and host acceptance (Minkenberg *et al.*, 1992).

For *A. bennetti*, host drumming resulted in probing 75% of the time (Joyce *et al.*, 1999). Also the absence of host feeding for *A. bennetti* and *A. fuscipennis* is a characteristic of pro-ovigenic species parasitoids (Drost *et al.*, 1999; Joyce *et al.*, 1999; this study). For *Amitus* species, oogenesis does not depend on nutrition as it does in synovigenic species which feed on their hosts like *E. formosa* (van Lenteren *et al.*, 1980) and *Eretmocerus* sp. nr. *californicus* (Headrick *et al.*, 1995).

Frequency and duration of behavior. Probing a host took 52.9 s in *A. fuscipennis* and was the activity in which most time was invested (16%) after walking. *Amitus fuscipennis* spent more time on probing than *A. bennetti* (22.6 s, according to Joyce *et al.*, 1999 or 39.1 s, according to Drost *et al.*, 1999). Oviposition in *A. fuscipennis*, taking into account all whitefly instars, took longer (63.7 s) than did probing (52.9 s). Flanders (1969) reported a short oviposition time of 15 s for *A. hesperidium*. Dowell *et al.*, (1981) reported that *A. hesperidium* spent more time ovipositing on second whitefly instars (39.3 s) than on first instars (27.6 s), as we found for *A. fuscipennis* (82.1 s and 48.4 s, respectively). For all three *Amitus* species, oviposition time was shorter than it was for *E. formosa* (>100 s, van Lenteren *et al.*, 1980).

Oviposition behavior and host-instar preferences. The oviposition behavior described here for *A. fuscipennis* is similar to that described for *A. bennetti* by Drost *et al.*, (1999). Slight differences were

observed when ovipositing: the hind legs of *A. fuscipennis* secure the host or are on the leaf. *A. bennetti*'s hind legs always secure the host. When ovipositing, the antennae of *A. fuscipennis* are on the leaf surface or free. In contrast, *A. bennetti* keeps them pressing on the leaf surface (Drost *et al.*, 1999). Similarly to *A. bennetti*, *A. fuscipennis* probes the host on all sides (Drost *et al.*, 1999) and does not turn before inserting her ovipositor, as has been reported for *A. hesperidium* (Dowell *et al.*, 1981).

A. fuscipennis prefers equally first and second instars for oviposition, above third instar. This preference might be based on relative easiness to oviposit in these young nymphs, resulting in short duration of oviposition which is critical for time limited, pro-ovigenic parasitoids. But this preference results in a long development time. Fourth instars were probed but never received eggs. This result differs from *A. bennetti*, which prefers first instars of *B. argentifolii* for oviposition, but oviposited in all four instars (Drost *et al.*, 1999). Joyce *et al.*, (1999) also reported that *A. bennetti* prefers probing first instars. A previous study on *A. fuscipennis* reported no statistical difference in the preference for the first three instars of *T. vaporariorum* (Medina *et al.*, 1994). Flanders (1969) and Dowell *et al.*, (1981) reported that *A. hesperidium* prefers to oviposit in first instars, but also oviposits in second and third instars. No oviposition in fourth instars was reported for *A. hesperidium* from either study.

When *A. fuscipennis* encounters hatched eggs, it reacts by walking faster, probably because the eggs indicate the probable presence of first instars. The same outcome was observed for *A. bennetti* by Drost *et al.*, (1999), who suggested that it was a good strategy for increasing encounter rates with first instars.

Walking speed. The walking speed of *A. fuscipennis* before an oviposition event was the same as for *A. bennetti* (1.4 mm s^{-1}) at 23–24 °C on poinsettia (Drost *et al.*, 2000). Both *A. bennetti* and *A. fuscipennis* are fast walking, more so than other whitefly parasitoids. Fast walking in recently emerged *A. fuscipennis* females may be related to its pro-ovigenic nature, leading to high motivation for finding hosts when carrying a full egg load. However, fast walking may also be an adaptative strategy to make up for the lower probability of encountering the small first and second instars of *T. vaporariorum*. Van Lenteren *et al.*, (1976) found that the parasitoid *E. formosa* had a much lower probability of encountering first and second instars of *T. vaporariorum* than larger instars.

Walking speed of whitefly parasitoids can be influenced by temperature and leaf hairiness of the host plant. A negative linear relationship between walking speed of *E. formosa* and trichome density on cucumber was observed (van Lenteren *et al.*, 1995). In contrast to this outcome, the walking speed of *E. formosa* was not affected by trichome density on gerbera leaves. The mean walking speed of *E. formosa* at 20 °C on gerbera ranged between 0.20 and 0.29 mm s^{-1} and, at 25 °C, between 0.35 and 0.43 mm s^{-1} (Sütterlin and van Lenteren, 1997). In tomato, the walking speed of *E. formosa* ranged between 0.18 and 0.62 mm s^{-1} as temperatures increased from 15 to 30 °C (van

Roermund, 1995). The walking speed on poinsettia before oviposition for *Eretmocerus mundus* Mercet was 0.74; of *Er. eremicus* Rose and Zolnerowich, 0.77; and of *Er. staufferi* Rose and Zolnerowich, 0.88 mm s⁻¹ (Drost *et al.*, 2000). The influence of leaf hairiness or temperature on the walking speed of *A. fuscipennis* was not determined in this research.

The preference for the first and second instars of *T. vaporariorum* for oviposition implies a long developmental time and a potential slow population development of the parasitoid. This trait may negatively influence the parasitoid's effectiveness as a biological control agent. For example in *E. formosa* the developmental time in the preferred nymphal host stage (third and fourth) is 25 d at 20 °C (Laska *et al.*, 1980), while it is 38 d at 19 °C for *A. fuscipennis* in first nymphal stages. Nevertheless, the parasitoid's intrinsic rate of increase (r_m value), a parameter that is influenced by developmental time and reproduction, was always higher than that for its host, when measured under various climatical conditions (Chapter 4). For a natural enemy to be an effective biological control agent in inoculative or seasonal inoculative releases, the difference in r_m values with the host should always be in the agent's favor (van Lenteren and Woets, 1988). With *T. vaporariorum*, under Colombian conditions, this parameter is in the parasitoid's favor, thus indicating its potential as an effective control agent.

Our findings suggest that *A. fuscipennis* has several behavioral traits that positively influence its performance as a biological control agent, such as fast walking and high host acceptance resulting in high percentages parasitism. Whether the combined effects of a high r_m , fast host finding and high host acceptance will result in efficient reduction of the whitefly pest population will have to be tested in the field.

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**6. First flights responses and residence times of *Amitus fuscipennis*
MacGown & Nebeker on bean plants**

Abstract

First flight of individual *Amitus fuscipennis* MacGown & Nebeker parasitoids to bean leaves or plants was studied. Plants or leaves were either uninfested or infested with the parasitoid's host, the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood). The parasitoid almost always flew upwards, seemingly not attracted by infested bean leaves or plants, even at distances as short as 30 cm. The behavior of *A. fuscipennis* when searching for *T. vaporariorum* nymphs on bean plants was also studied on plants that were (1) unsprayed and uninfested, (2) sprayed with fungicide and uninfested, or (3) unsprayed and whitefly-infested. Observations stopped when the parasitoids flew away. Residence time on infested plants was higher than on uninfested plants. Residence time was the shortest on fungicide sprayed plants, indicating that the fungicide disturbed *A. fuscipennis*. The residence time of *A. fuscipennis* was prolonged by both, encounters with unparasitized hosts and feeding from the plant. Parasitoids searched more often on a leaflet's underside than on the upside for all treatments.

Introduction

The tritrophic system dry bean (*Phaseolus vulgaris* L., Leguminosae), its key whitefly pest *Trialeurodes vaporariorum* (Westwood), and the nymphal parasitoid *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae) was studied to evaluate possibilities for biological control of whitefly. Dry bean is an important crop in the developing world because it is the cheapest source of protein in many countries (Pachico 1993). Nearly 10 million tons of dry beans are produced worldwide annually. Of this production 46.2% occurs in Latin America (Pachico, 1993). Because of the economic importance of beans to Latin American countries such as Colombia, a system of guided pest control is being tested for this crop (Prada *et al.*, 1993), to obtain a reasonable production with careful pesticide use.

The greenhouse whitefly, *Trialeurodes vaporariorum* is a cosmopolitan pest, which is responsible for heavy bean crop losses in Colombia. Average losses vary during time and locations, but values up to 13 t/ha (50% yield potential loss) on snap bean crops have been reported by Rodríguez and Cardona, (1990). Many natural enemies of whitefly are known (Gerling, 1990; Gerling and Mayer, 1996), including several parasitoids that have been successfully used in classical and seasonal inoculative releases for controlling this whitefly pest (Gerling, 1990; van Lenteren *et al.*, 1997).

A recently discovered parasitoid, *A. fuscipennis*, native to Colombia could be a potential biological control component within a system of guided control for *T. vaporariorum* on bean crops (Prada *et al.*, 1993). To date, the guided control system consists of a combination of cultural control practices with the application of insecticides at pre-established action levels (Prada *et al.*, 1993). When the system was tested in a series of on-farm trials, it resulted in a 54% reduction in insecticide

use and an 18% drop in costs, compared with the conventional spraying program (Prada *et al.*, 1993). The use of a biological component could improve these results even more.

Biological control of *T. vaporariorum* on bean crops in Colombia is also desirable because whitefly is showing resistance to certain insecticides (Cardona *et al.*, 1998). Farmers use sulfur-based fungicides against certain species of fungi that attack the bean plant. Although it is generally believed that sulfur fungicides do not affect the whitefly, in this paper, we study the effect of one of these fungicides on the searching behavior of its parasitoid, *A. fuscipennis*.

A. fuscipennis is a pro-ovigenic parasitoid, with a strongly female-biased ratio (Manzano *et al.*, 2000; Chapter 2), and has higher intrinsic rates of increase (r_m) than does *T. vaporariorum* at conditions which prevail in the field (Chapter 4). These biological characteristics support the potential use of *A. fuscipennis* as a biological control agent of *T. vaporariorum*. The parasitoid's performance is also being tested on greenhouse tomatoes in Colombia (Vis *et al.*, 1999). Successful parasitism and consequent regulation of the whitefly population under the economic injury level depend in addition to its reproduction capacity, for an important part on the parasitoid's searching behavior. Studying the searching behavior of a parasitoid species is therefore a basic step towards complementing its evaluation as a biological control agent (e. g. van Roermund, 1995).

Little is known about how *A. fuscipennis* disperses in a bean crop. For example, we do not know if *A. fuscipennis* can detect whitefly hosts before landing on the plant. For certain parasitoids, long-range searching is mediated through volatile infochemicals (Vet and Dicke, 1992), but others like the whitefly parasitoid *Encarsia formosa* Gahan search at random (van Lenteren *et al.*, 1996). Our first objective was to study whether *A. fuscipennis* is attracted to infested or clean leaves or plants over short distances. We observed the first flight of *A. fuscipennis* to clean and infested leaves or infested plants.

Once *A. fuscipennis* arrives on a leaf, it starts searching for hosts by walking about and drumming the leaf with its antennae, like *E. formosa* does (van Lenteren *et al.*, 1980). When it finds a host, it either rejects the host after an antennal examination, or accepts or rejects it for oviposition after inserting the ovipositor (Chapter 5). Our second objective was to test the hypothesis that the searching behavior of *A. fuscipennis* is influenced by the quality of the plant on which the search is carried out. Plant quality differed with respect to presence or absence of whitefly hosts, and presence or absence of fungicides. In particular, we analyzed (1) the time *A. fuscipennis* spent on bean plants, and (2) its walking activities.

Materials and methods

Plants. Bean plants (*Phaseolus vulgaris* L. cv. ICA-Pijao) were grown under greenhouse conditions at 22 ± 0.4 °C, 80.7 ± 19.2 % RH, and 12L:12D. Plants did not receive fertilizers or chemical treatments. The plants were cut down when they were 15 to 20 days old to bear only one trifoliate leaf. Plants for experiments were treated as follows: (1) one group of the plants was kept intact (2) another group was sprayed with a sulfur-based fungicide (3×10^{-3} ml), using a hand sprayer, and (3) a third group was infested with *T. vaporariorum*. Each leaflet on which the observations were carried out averaged $34.3 \text{ cm}^2 \pm 0.7 \text{ SE}$ in size ($n = 62$).

Greenhouse whiteflies. The adults of *T. vaporariorum* used in this study came from a colony that had been established at CIAT 5 years ago on bean plants (cv. ICA-Pijao) with insects collected from snap bean fields in Fusagasugá, Colombia. New generations were reared under the following conditions: 22-24 °C, 40-80% RH, and 12L:12D. A low density of four unparasitized nymphs per leaflet to be infested was obtained by leaving clip cages (2.5 cm), containing about 20 adults of *T. vaporariorum* on the leaflet for 4 h. The adults were then removed. The number of eggs laid was manipulated so those four nymphs per leaflet would be obtained.

Parasitoids. *A. fuscipennis* females used in these experiments came from a rearing established in September 1996, at CIAT. These parasitoids originated from bean crops in Tenerife, Valle del Cauca, Colombia. They were reared on first and second instars of *T. vaporariorum*, using the same host-plant species as the whitefly colony and under the same conditions: 22°C-24°C, 40%-80% RH, and 12L:12D.

Flight experiment

Wind tunnel. Flight response experiments were carried out in a wind tunnel constructed for diurnal flying insects as shown in Figure 1 and described by Geervliet *et al.*, (1994). The wind tunnel offered the possibility of producing an odor stream coming from plants and hosts that could be detected by the parasitoid. Because of the parasitoid's small size, only part of the total area (200 l x 60 w x 60 h cm) of the flight compartment was used. Experiments were carried out at the end of the wind tunnel opposite the wind release zone; the gate was removed for handling purposes. The area of the flight compartment used was 40 x 60 x 60 cm. Light intensity in the flight compartment during testing was 2000 lux, and temperature varied between 22.0 °C and 26.8 °C in the tunnel. The wind, when used, was held constant at a speed of 15 cm/s.

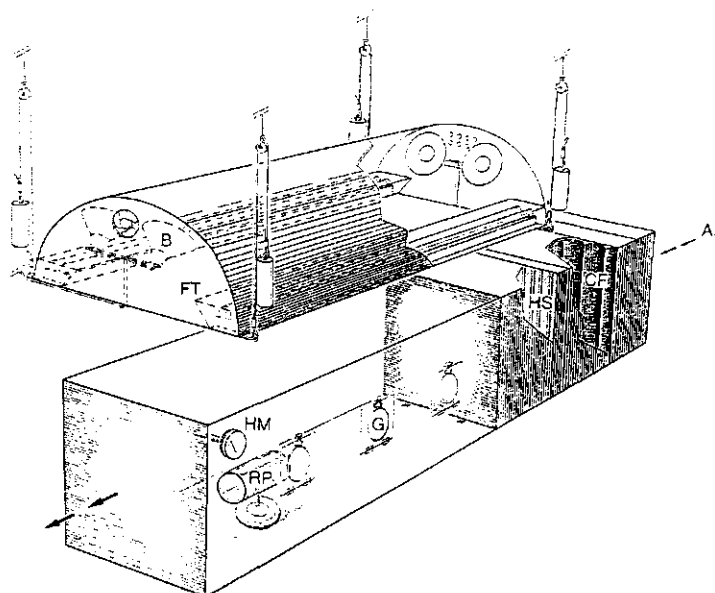


Figure 1. Wind tunnel according to the set-up of Geervliet (1997): AI, air-inlet; CF, charcoal filter; HS, heating system; RP, parasitoid release point; FT high-frequency fluorescent tubes; B, bulbs; X, position of the odor source; HM, hygrometer; G, gate. For the set-up of *A. fuscipennis* experiments, see the text.

The parasitoid's release site consisted of a wooden cube (9 cm^3) placed on supports 7 or 14 cm high. Individual females were placed in polysaccharide capsules on the wooden cube for easy release.

Cut trifoliolate leaves. Trifoliolate bean leaves of similar area and shapes were put into small vases filled with water. They were then placed in the wind tunnel described above. Two leaves (i.e., 6 leaflets) were uninfested and six leaves (i.e., 6 leaflets) were infested with 65 first and second instars of *T. vaporariorum*. The two groups of leaves were placed 30 cm apart. The parasitoid was released 30 cm equidistantly from both groups of leaves and at 7 cm above the tunnel floor, at the same level as the leaves. *A. fuscipennis* females used in this experiment had not flew before.

Parasitoids received two different kind of treatments: a) one group of 100 parasitoids used in this experiment was allowed to oviposit on first and second instars of *T. vaporariorum* before starting the experiment, b) another group of 50 tested parasitoids was not permitted to first oviposit but, they were in contact with whitefly exuviae after emerging from parasitized mummies on bean leaves. The first flight of the parasitoid was observed.

Infested plants. In order to increase the odor source, we used 1 bean plant, bearing 4 trifoliolate leaves infested at high density with first and second instars of *T. vaporariorum*. Parasitoids were released 30 cm away from the plant at the leaf-base level of the plant. Parasitoids used in this experiment had not previously oviposited, but had been in contact with whitefly exuviae after emerging from parasitized mummies on bean leaves. Forty parasitoids were tested with wind flow and 40 without wind flow. The first flight of the parasitoid was observed.

Searching behavior experiment

Basic design. Observations were carried out on a bean plant in a room at 19°C and $80 \pm 10\%$ RH. These values simulate the conditions of hillside areas at around 1500 m above sea level, where the parasitoid is commonly found. Light shone directly on the experimental plant at an intensity of 2700 lux. Four clean plants surrounded the experimental plant to mimic a cropping environment. At the start of each observation, a single *A. fuscipennis* female without any oviposition experience was introduced onto a leaflet, using a polysaccharide capsule (2.5×0.8 cm). Parasitoids were always introduced in the center of the underside of an infested or uninfested leaflet.

The parasitoid was observed immediately after introduction and continuously followed by eye. An observation stopped when the parasitoid flew from the plant. When no foraging activity occurred for more than 15 min or the parasitoid flew away within 2 min (probably because of disturbance), the observation was stopped and was not included in the analysis. Behavioral components were recorded, using a hand-held computer (Psion Workabout) with The Observer 3.0, window version computer program (Noldus, 1991).

The following behavioral elements were distinguished: walking, standing still, preening, host encountering, host drumming, oviposition posturing, and jumping. The direction of the parasitoid's departure from the leaflet was recorded as up, down, or horizontal. Locations of the parasitoid on the leaflet were recorded as on leaf upper center, leaf lower center, or on the leaf's edge. Edge width was defined as being 0.5 cm. The parasitoid's locations on the plant were recorded as on plant stem (including leaf petiole), or stipel.

Treatments. Three types of treatments were carried out. In treatment I, uninfested unsprayed plants were used. In treatment II, uninfested plants were sprayed with a sulfur-based fungicide to test the effect of fungicide on the parasitoid's searching behavior. In treatment III, unsprayed plants, infested with unparasitized first-instar nymphs of *T. vaporariorum*, were used. The plants had four unparasitized nymphs in one out of the three leaflets, except for two plants that had six unparasitized nymphs. For each experiment, 20 plants and 20 *A. fuscipennis* females were tested.

Measurements. We measured (1) residence time, i.e., the total time spent by *A. fuscipennis* females on plants; (2) walking or searching activities of *A. fuscipennis*, which were expressed as a percentage of the total time spent on the plant. When hosts were offered to the parasitoid, handling time (host encountering, host drumming, plus host oviposition) was not included in the total time spent on the plant. For infested plants, we also measured (3) the acceptability ratio of unparasitized hosts, i.e. the number of encounters of unparasitized hosts that resulted in successful parasitization. We also measured (4) giving-up time (GUT), which is the time spent by *A. fuscipennis* after encountering its last host and before leaving the plant.

Data analysis. Percentages of times were transformed to squared root arcsine values, because they were not distributed normally (Murdie, 1972). Transformed data were exposed to an analysis of variance at a significance level of 0.0001 or 0.001. If significant differences were found, multiple comparisons were made, using the Student-Newman-Keuls procedure ($P < 0.05$). Correlations were calculated using Pearson's or Spearman's procedures ($\alpha = 0.05$).

Results

First flight

The activities of *A. fuscipennis* immediately after release consisted of flying or walking. In all experiments, most flying parasitoids flew upwards to the ceiling of the wind tunnel without landing on infested leaflets or plants (Table 1). *A. fuscipennis* was not attracted to clean or infested leaflets or plants, even at the short distance of 30 cm.

Searching behavior

Residence time. The residence time of *A. fuscipennis* visiting plants was affected by treatment (Table 2). Residence times were highest when hosts were discovered by the parasitoid (2.7 h) on infested, unsprayed plants. The *A. fuscipennis* females abandoned uninfested fungicide sprayed plants more quickly (0.9 h) than they did uninfested unsprayed plants (1.7 h). The mean residence time between the three treatments was significantly different (Kruskal-Wallis One Way Analysis of Variance on Ranks test, $P < 0.001$, Table 2).

Table 1. Activities (percentages of all tested females) of *Anisus fuscipennis* after release in a wind tunnel containing bean leaflets or plants.

Plant part	Females (no.)	Walking to point (%)	Flying up to tunnel ceiling (%)	Flying to clean leaflet or plant (%)	Flying to infested leaflet or plant (%)	Flying to tunnel walls (%)
Cut trifoliolate leaves						
Parasitoid with oviposition experience	100	25	75	0	-	-
Parasitoid without oviposition experience	50	28	60	6	4	2
Infested plants						
With wind	40	15	80	-	2.5	2.5
Without wind	40	10	87.5	-	0	2.5

Table 2. Mean residence time (s) and mean time (%) allocated to walking in *Amitus fuscipennis* on uninfested fungicide-sprayed, uninfested unsprayed, and infested unsprayed bean plants. Means are followed by standard error values.

Activity	Uninfested sprayed	Uninfested unsprayed	Infested unsprayed
Residence time [†]	3184.6 a ± 670	5996.6 b ± 888	9881.9 c ± 1022
Walking activity [‡]	54.7 a ± 4.0	62.1 b ± 4.2	78.3 c ± 3.2

[†] Different letters in a row indicate significant differences (Student-Newman-

Keuls method, $P < 0.05$, after Kruskal-Wallis one Way ANOVA on ranks, $P < 0.0001$)

[‡] Different letters in a row indicate significant differences (Student-Newman-

Keuls method, $P < 0.05$ after Kruskal-Wallis one Way ANOVA on $\sqrt{\text{arcsine}}$ -transformed data, $P < 0.0001$)

The amount of time (%) *A. fuscipennis* searched (i.e., walking) while on the plant was also affected by treatment (Table 2). The percentage of time spent walking on uninfested-sprayed leaves (54.7%) was similar to that on uninfested unsprayed leaves (62.1%) but it was significantly higher on infested leaves (78.3%, Table 2). On infested leaves, the high walking activity of *A. fuscipennis* remained the same during a leaf visit and did not increase or decrease significantly after ovipositions.

The time allocated by females to different activities on infested leaves, different to walking, is given in Table 3. Host handling took up only about 3% of residence time on the plant (Table 3). On average, the time spent on a plant by a parasitoid, apart from handling hosts, was 9611.3 s (almost 2.7 h). The parasitoid spent its time standing still, preening, or feeding.

On leaflets carrying four hosts, individual *A. fuscipennis* females had up to seven encounters with up to four different hosts, and laid a maximum of 3 eggs during their visit (Table 3). When six hosts were present, they had up to 9 encounters and laid a maximum of 5 eggs. Mean giving-up time was 7385.4 s (2.05 h, Table 3), that is, about 75% of mean residence time, including host handling. On infested plants, all the parasitoids encountered the hosts because the wasps were released in the center of the infested leaflet close to the hosts. The percentage of hosts accepted for oviposition at first contact was 80%. In this experiment, the number of encounters or ovipositions did not influence residence time.

Locations on infested plants. *A. fuscipennis* moved over through the whole plant during searching. Females began searching when placed on the underside of a bean leaflet and walked quickly, constantly moving among the leaflets. This was done by walking to the leaflet base and via the leaf stem, reaching a different leaflet, or by walking from one leaflet to another when leaflet borders were very close or touched each other. The parasitoid also walked in up-and-down directions through the

Table 3. Mean residence time (excluding host-handling); host-handling time; mean number of host encounters and oviposition postures; acceptability ratio of unparasitized hosts; and mean giving-up time (GUT; n = 20) of *Amitus fuscipennis* on leaves infested with four whitefly hosts. Means are presented with their standard error values.

Residence time (s)	Host handling (% of entire residence time)	Host encounters (no.)	Oviposition postures (no.)	Host acceptance (%)	Giving Up Time (s)
9611 ± 993	2.8 ± 0.5	4.5 ± 0.6	2.4 ± 0.2	80 ± 0.5	7385 ± 1009

petioles and stems, which served as access corridors to other trifoliate leaves. The four main parts of the plant visited by the parasitoids were trifoliate leaves, petioles, stems, and stipels. The parasitoids stopped frequently on the stipels, mostly to feed but also to rest.

For all treatments, the parasitoids consistently spent more time searching on leaves than on other plant parts: 95.6% (as a percentage of total time, excluding host-handling) for sprayed plants, 95.3% for unsprayed plants, and 93.9% for infested plants. Time spent on petioles and stems was 1.7% on sprayed plants, 4.3% on unsprayed plants, and 4.5% on infested plants. Time spent on stipels was 2.7% on sprayed plants, 0.4% on unsprayed plants, and 1.6% on infested plants.

The average number of leaflets visited (2.3); (Table 4) was significantly lower for sprayed plants because the parasitoid abandoned the plant more quickly. When leaves were visited, *A. fuscipennis* walked all over an entire leaflet, changing from one side to another while searching. The time-spent (sec) on leaf lower side was significantly higher for infested unsprayed plants (7800.7 sec) than for infested unsprayed (5081.2 s) or uninfested sprayed (2644.6 sec) plants (Table 4). Time spent (sec) on the leaflet's lower center was significantly higher for infested unsprayed leaflets (6443.2 s) compared two other treatments (Table 4, Student-Newman-Keuls method $P < 0.05$, after Kruskal-Wallis, One-Way ANOVA on ranks $P < 0.0001$).

Table 4. Number of bean leaflets visited and mean time spent (sec) by *Amitus fuscipennis* at different sites on a given leaflet when searching. Means are presented with their standard error values.

Leaflets	Uninfested sprayed	Uninfested unsprayed	Infested unsprayed*
Number visited†	2.3 ± 0.5 a	5.9 ± 1.1 b	8.9 ± 1.0 b
Time spent on			
Lower center‡	1356.8 ± 308.7a	2356.5 ± 322.6b	6443.2 ± 681.0c
Lower side§	2644.6 ± 558.9a	5081.2 ± 788.5b	7800.7 ± 766.0c
Upper side¶	389.9 ± 134.7a	673.9 ± 142.4b	724.2 ± 159.5b

* Excluded host handling

† Different letters in the row indicate significant differences (Student-Newman-Keuls method, $P < 0.05$, after Kruskal-Wallis one Way ANOVA on ranks, $P < 0.001$)

‡ Different letters in the row indicate significant differences (Student-Newman-Keuls method, $P < 0.05$, after Kruskal-Wallis one Way ANOVA on ranks, $P < 0.0001$)

§ Different letters in the row indicate significant differences (Student-Newman-Keuls method, $P < 0.05$, after Kruskal-Wallis one Way ANOVA on ranks, $P < 0.0001$)

¶ Different letters in the row indicate significant differences (Student-Newman-Keuls method, $P < 0.05$, after Kruskal-Wallis one Way ANOVA on ranks, $P = 0.0445$)

When hosts were present, the parasitoid spent 83% of lower side (excluding host-handling time) on the leaflet's lower center (Table 4), where it was introduced and where most hosts were located. On leaflets of uninfested sprayed plants this percentage declined to 51.3% and to 46.4% on uninfested unsprayed plants (Table 4). The time spent on the leaflet's upper edge was not significantly different between infested unsprayed (724.2 s) and uninfested unsprayed (673.9 s) leaflets (Table 4). Time spent on upper leaflet side was significantly shorter for sprayed uninfested (389.9 s) leaflets compared to the other two treatments (Student-Newman-Keuls method, $P < 0.05$, after Kruskal-Wallis one Way ANOVA on ranks $P = 0.045$, Table 4).

On infested plants, petioles and stems were visited by all parasitoids, whereas on clean plants the number of visiting parasitoids was lower (Table 5). The average time (s) spent on petioles and stems was 54.5 ± 17.1 SE on sprayed plants, 257.3 ± 104.1 SE on unsprayed plants, and 426.1 ± 138.7 SE on infested plants. These three values were significantly different (Student-Newman-Keuls method, $P < 0.05$, after Kruskal-Wallis one way ANOVA on ranks, $P = 0.0006$).

Stipels were more visited by parasitoids on infested plants than on clean ones (Table 5). Feeding occurred on the stipels, especially on infested plants (50% of parasitoids), taking an average of 85.0 s (Table 6). The percentage of feeding time was significantly correlated with residence time (excluding host-handling time) on infested leaflets. This trend was not found for clean unsprayed leaflets. Although the parasitoids visited the stipels on sprayed plants, they did not feed on them.

The site on the plant from which *A. fuscipennis* flew away was similar for all treatments. Most parasitoids (85.0%) left from the leaflet upper edge, 8.3% from the stem, 3.3% from the lower leaf edge, and 1.7% either from lower or upper leaf center. The direction of the flight was upwards for most parasitoids (86%), downwards for 12% and horizontal for 2%.

Table 5. *Amitus fuscipennis* females (%) that visited the petioles and stems, and stipels of bean leaves ($n = 20$).

Position	Uninfested sprayed	Uninfested unsprayed	Infested unsprayed
Petiole and stem	50	80	100
Stipel	40	50	80

Discussion

Flight experiment

In our experiments, *A. fuscipennis* was not attracted to uninfested or infested leaves or plants, but flew upwards, strongly attracted by the light. For *E. formosa*, Noldus and van Lenteren (1990) and,

Table 6. Mean feeding duration (s) of females of *Amitus fuscipennis* on stipels of unsprayed bean leaves and number of parasitoids that fed out of 20 for each treatment. Means are followed by standard error values.

Parameter	Uninfested	Infested
Mean duration [†]	9.63 ± 6.9 a	85.04 ± 25.5 b
Number of feeding parasitoids	4	10

† Different letters in the row indicate significant differences (Mann-Whitney test, $P < 0.0001$).

Sütterlin and van Lenteren (2000), found, in a choice experiment that the parasitoid did not discriminate between uninfested and host-infested leaves and plants from a distance.

In our experiments, *A. fuscipennis* was not attracted to uninfested or infested leaves or plants, but flew upwards, strongly attracted by the light. For *E. formosa*, Noldus and van Lenteren (1990) and, Sütterlin and van Lenteren (2000), found, in a choice experiment that the parasitoid did not discriminate between uninfested and host-infested leaves and plants from a distance. Guerrieri (1997) reported that *E. formosa*, in a no-choice experiment, could locate potential hosts from a distance. Although, in our experiments, *A. fuscipennis* was not attracted to infested leaves or plants, whether in choice or no-choice experiments, probably, after the first landing, the parasitoid would redistribute and be arrested on infested leaves, as was observed for *E. formosa* (Sütterlin and van Lenteren, 2000). Our results and observations suggest that, after emerging, *A. fuscipennis* flies upward within the crop canopy and lands at random on the underside of bean leaves.

Searching behavior

Residence time. The searching behavior of *A. fuscipennis* is influenced by the "quality" of the plant on which searching takes place. Residence time of *A. fuscipennis* was less than 1 h when leaves were not infested with whitefly hosts and had been sprayed with a sulfur-based fungicide. When the fungicide was absent, residence time increased to 1.7 h.

Colombian farmers to control certain bean plant diseases (CIAT 1999, unpublished) use sulfur-based fungicides. Although this kind of fungicide does not kill *T. vaporariorum* (E. Valencia,

personal communication), our results show that it interferes with biological control by reducing the residence time of the whitefly's natural enemies, such as *A. fuscipennis*. Apparently, the parasitoids perceive the presence of the fungicide and they abandon the leaf earlier than unsprayed leaves (residence time on uninfested unsprayed leaves is 0.7 h longer than on uninfested sprayed leaves). On fungicide-sprayed leaves, the parasitoid showed a lower walking activity. When parasitoids are used within an IPM system, special care must be paid to how fungicide applications are timed with the arrival or introduction of *A. fuscipennis* to the crop to prevent such interference.

On infested unsprayed leaves, walking activities increased, probably because host encounters and ovipositions increased the parasitoids' motivation to search.

Giving-up time of *A. fuscipennis* involved 75% of the residence time, that is, the parasitoids found the hosts quickly and spent the rest of the time walking, standing still, and feeding on stipels. All the parasitoids could discover the whitefly nymphs quickly because they were released close to them.

Locations on infested plants. The presence of unparasitized hosts on unsprayed plants stimulated the parasitoid to increase its residence time to 2.7 h. However, in this study, we could not prove statistically that residence time was correlated with the total number of host encounters or the total number of ovipositions. The number of replicates (20) was probably too low or perhaps the bean plant was infested with too low a density of whiteflies. Van Roermund and van Lenteren (1995) found that the parasitoid *E. formosa* stayed longer on the leaflet after encounters with unparasitized hosts and ovipositions.

A. fuscipennis when searching on the leaflet spent most time on leaflets underside than on upperside. On infested unsprayed plants the parasitoid spent more time searching on leaflet lower side compared to uninfested plants indicating arrestment of the parasitoid after contacts with unparasitized hosts. Considering that whitefly hosts are found almost exclusively on a leaflet's underside, preference for this side seems advantageous. When hosts were present, the leaflet's lower center was preferred, probably because of area-restricted searching. This behavior is an adaptation for finding hosts like whiteflies that occur in an aggregated fashion (Godfray, 1994).

A. fuscipennis fed mostly from stipels on infested plants, which increased residence time. Feeding was carried out by taking certain secretions from the stipels. There is no clear morphological evidence that secretions are produced from stipels of *Phaseolus vulgaris* as it occurs in other *Phaseolus* species (D. Debouck, personal communication). However, we observed that *A. fuscipennis* feeds on certain substances present on the stipels. This aspect remains to be investigated. An important effect of plant food sources is natural enemy retention (Cortesero *et al.*, 2000). Our results suggest that *A. fuscipennis* stayed on the plant to feed, probably because oviposition increased its

requirement of food. *A. fuscipennis* is a pro-ovigenic parasitoid that does not feed on whitefly hosts (Chapter 5) but takes food from the host plant. So *A. fuscipennis* may be arrested both as a result of encounters with hosts and plant food substances.

In conclusion, we discovered the following characteristics of searching behavior in *A. fuscipennis* this study:

1. The parasitoid is not attracted by infested leaves or plants, even from a short distance;
2. It prefers a leaflet's underside while searching, and the lower center when hosts are present;
3. Residence time is higher on infested plants (2.7 h) than on uninfested unsprayed (1.7 h) or on uninfested fungicide-sprayed plants (< 1 h);
4. The parasitoid is arrested on the leaflet by both encounters with, or ovipositions in unparasitized hosts and by plant food
5. Walking activity is higher on infested plants than on uninfested unsprayed or uninfested sprayed plants;
6. The parasitoid accepts 80% of the unparasitized hosts for oviposition at first encounter.

In general, all these findings with the exception of aspect 1, point at a high searching efficiency of *A. fuscipennis*, because it searches more intensively at places where hosts can be expected and it increases searching activity after host encounter. These results will to be put in a wider frame in Chapter 7.

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7. Distribution by altitude, and dynamics of whitefly and associated parasitoids in snap bean

Abstract

The distribution of the parasitoid *Amitus fuscipennis* MacGown & Nebeker, was determined by field surveys of different crops infested with the whitefly *Trialeurodes vaporariorum* (Westwood) in Valle del Cauca, Colombia. The parasitoid occurred from middle (400-1000 m) to high altitudes (above 1000) and it was the only whitefly parasitoid species observed at altitudes over 1460 m. At lower altitudes, *Encarsia* spp occurred together with *A. fuscipennis*. To determine seasonal abundance of the whiteflies *T. vaporariorum* and *Bemisia tabaci* Gennadius, and their parasitoids, we performed field experiments in sprayed and unsprayed fields during a cropping season of snap bean in Pradera, Valle del Cauca, Colombia. Substantially larger populations of whitefly nymphs occurred in the unsprayed field than in the sprayed field in this area. The parasitoid *Encarsia nigricephala* Dozier was more prevalent than *A. fuscipennis*. Parasitoids were more frequent in unsprayed than in sprayed fields and parasitoid populations in the sprayed field were larger than those of whiteflies at the end of the cropping season.

Introduction

Trialeurodes vaporariorum (Westwood) is the predominant whitefly species in the tropical highlands (altitudes above 1000 m) and inter-Andean valleys (altitudes ranging from 400 to 1000 m), whereas *Bemisia tabaci* (Gennadius), is the predominant species in the tropical lowlands (altitudes below 400 m) (Cardona *et al.*, 1998). In Colombia they co-exist in the valleys formed as the Andes split into three major ranges (Cardona *et al.*, 1998).

The pests damage plants by directly feeding on them or by contaminating leaves and fruits with honeydew on which sooty mould develop, and by virus transmission. Although *T. vaporariorum* can transmit plant viral diseases (Cohen, 1990), whether it does so for snap beans has yet to be proved. *T. vaporariorum* is a key pest of beans in Colombia, resulting in intensively applied chemical treatments. Recently, whiteflies have been observed to be resistant to certain insecticides (Cardona *et al.*, 1998), unlike the farmers, who often poison themselves when applying the chemicals (Rodríguez and Cardona, in press). Even so, Colombian farmers still prefer to control whiteflies with chemicals.

A system of guided control was offered as an alternative for this chemical regime. It consisted of combining cultural control practices with insecticide applications at pre-established action levels (Prada *et al.*, 1993). The guided control practices were tested in a series of on-farm trials, resulting in a 54% reduction in insecticide use and an 18% reduction in costs, compared with the conventional pesticide spray program (Prada *et al.*, 1993). This system did not yet include a biological component, but during field observations, natural enemies of whiteflies were regularly observed. One of the common whitefly parasitoids in Colombia, *Amitus fuscipennis* MacGown & Nebeker was, therefore, studied as a potential control agent of whiteflies.

A. fuscipennis is widely distributed throughout Colombia, in other Andean areas, and in inter-Andean valleys. The species has also been found in Central America, northern South America, and the Caribbean (MacGown and Nebeker, 1978). *A. fuscipennis* has a strongly female-biased sex ratio (Manzano *et al.*, 2000; Chapter 2) and between 19 and 22 °C it has higher intrinsic rates of population increase (r_m) than does *T. vaporariorum* (Chapter 3). Its host searching and parasitization behavior resulted in effective parasitism of greenhouse whitefly (Chapter 5). These biological characteristics support the hypothesis that *A. fuscipennis* might be a useful biological control agent of *T. vaporariorum*, which could be included as a component in the guided control program. The performance of *A. fuscipennis* is currently also tested on tomatoes in glasshouses in Colombia (Vis *et al.*, 1999).

Other whitefly parasitoids from the genus *Encarsia* and *Eretmocerus* in Colombia have been reported by Lopez-Avila *et al.*, (1999). Many natural enemies of whitefly are known (Gerling, 1990; Gerling and Mayer, 1996), and other parasitoids have been successfully used in classical and seasonal inoculative releases for whitefly control (Gerling, 1990; van Lenteren *et al.*, 1997). At present, biological control of *T. vaporariorum* by the parasitoid *Encarsia formosa* Gahan is commercially successful for several glasshouse-grown vegetables and ornamentals (van Lenteren *et al.*, 1996; van Lenteren, 2000).

In this paper we present the distribution by altitude of *A. fuscipennis* in Valle del Cauca, Colombia. Based on results of our previous research about biology of *A. fuscipennis* under laboratory conditions (Manzano *et al.*, 2000; Chapter 2), we hypothesize that *A. fuscipennis* is more commonly found at altitudes with a cool and humid climate than at altitudes with a warm and dry climate.

We also describe the regional population trends of whiteflies and their parasitoids in snap bean crops grown under two different sets of conditions. One crop was grown in a typical farmer agroecosystem with intensive insecticide sprays. The other was an experimental crop where insecticides were not used. Our hypothesis is that in unsprayed fields parasitoid populations would be larger than in sprayed fields and that parasitoids will reduce whitefly populations here.

Materials and Methods

Distribution of A. fuscipennis by altitude. To determine the distribution of *A. fuscipennis* over altitude, we surveyed different areas in the Department of Valle del Cauca, Colombia between September and October 1999, during the wet season. Table 1 shows the different locations surveyed, together with their altitudes and crops. Middle altitudes were considered those of the inter-Andean valleys (ranging from 400 to 1000 m). The tropical highlands (above 1000 m) were considered high altitudes according to Cardona *et al.*, 1998. Low altitudes (below 1000 m) were not surveyed during this study. Ranges of

these altitudes are not so strict, and a place like Lomitas, Pradera located at 1180 m, corresponds to an inter-Andean valley of middle altitude. Surveyed crops belonged to farmers who sprayed insecticides and fungicides to control whitefly and other pests and diseases. Table 1 also gives the insecticide name (trade name) and the number of days after the farmer had last applied it when we took the samples. In each chosen crop, a plot of 20 × 11 m was measured and, walking diagonally through the plot, two leaflets of the third lower part of 10 different plants were picked. These leaflets were chosen because they are the first to show symptoms of parasitism. The leaflets were taken to the laboratory in plastic bags, then cut into squares, measuring 2.54 × 2.54 cm, and preserved in petri dishes filled with an agar gel (1.2 g agar/100 ml water) to prevent leaf dehydration and to permit whiteflies and parasitoids to continue their development.

As the whiteflies matured, we counted the number of parasitized and unparasitized fourth instars. From these samples, parasitism was estimated as the proportion of fourth instars that were parasitized.

Table 1. Sites altitude (m) and crops where whitefly parasitoids were found in the Department of Valle del Cauca, Colombia. Days after last insecticide application are indicated.

Location	Altitude	Crop	Insecticide (trade name)	Days after last application
Pradera, Lomitas	1180	Snap bean	Furadan	15
Roldanillo, Cajamarca	1460	Dry bean	Unknown	12
	1440	Tomato	Evisect,	
			Copper oxychloride	20
	1440	Tomato	Curacron, Vapona,	
			Perfekthion	28
	1400	Dry bean	Unknown	Unknown
Cerrito, Tenerife	1920	Dry bean	Manzante, Furadan	15
	1980	Dry bean	Furadan	20

Adult parasitoids were allowed to emerge, sorted according to species and given to the Museum of Entomology of the International Center for Tropical Agriculture, CIAT for taxonomic verification and deposition of voucher specimens.

Field populations of whiteflies and parasitoids on bean crops. Studies were conducted at Pradera, Valle del Cauca, Colombia, located at 1080m, with an average temperature of 22 °C and average RH of 72%. Studies were carried out during the wet season (3 November 1999 to 2 January 2000) in two different fields. One of the fields, belonged to a farmer who sprayed it with commercial insecticides.

This sprayed field received a weekly application of a combination of the insecticide carbosulfan (0.12-kg ai), the fungicide mancozeb (0.10-kg ai) and 0.46 kg of nitrogen, all dissolved in 220 l of water. It was planted with snap bean cv. Ferri. The other field, was an unsprayed, experimental field. It received only an application of a systemic fungicide (eritridiazole + thiophanate-methyl, 0.096 kg a.i./ha) 2 weeks after sowing. It was planted with snap bean cv. Blue Lake ASGRO

The two fields were of the same size (37×24.4 m), with 31 rows, the rows separated by a distance of 40 cm. Each field was divided into four similar plots so that treatments (sprayed or unsprayed) were replicated four times. The distance between the sprayed and unsprayed fields was 400 m. Sugar cane and maize crops, and two houses stood between them.

For each plot, whitefly stages were counted and sampled once per week from 15 plants per plot, so 60 plants per field. To estimate the number of whitefly adults, a cotyledonary leaf was gently turned upside down and adults counted quickly before they flew away. To count the number of live whitefly eggs, one cotyledonary leaf or one leaflet was picked and carried to the laboratory. To count the number of live nymphs, one leaflet from the lower third of the snap bean plant was picked and carried to the laboratory. This last procedure was carried out to be able to detect parasitized pupa as early as possible because *A. fuscipennis* prefers first and second instars for oviposition (Chapter 5).

At the laboratory, the same procedure for preventing leaf dehydration and determining whitefly parasitization as described above was followed. Emerged adult parasitoids were also sent to the CIAT Entomology Museum for taxonomic verification and depositing of voucher specimens. To estimate the effect of insecticides and fungicides used in the field for both, distribution by altitude and field populations of whiteflies and parasitoids studies, we evaluated information on side effects on several categories of natural enemies (Koppert, <http://www.koppert.nl>, 2000).

Data analysis. For comparing the numbers of whitefly stages and number of parasitoids, two-way analysis of variance (ANOVA) was performed on $\log(x + 1)$ transformed data. To isolate the group or groups that differ from the others, an All Pairwise Multiple Comparison Procedure (Student-Newman-Keels method, $P < 0.05$) was followed.

Results

Distribution of Amitus fuscipennis by altitude. Table 2 shows the parasitoid species found on *T. vaporariorum* and the level of parasitism (%) according to the different surveyed crops. *A. fuscipennis* occurred at middle and high altitudes, and was the only species found at altitudes higher than 1460 m.

Table 2. Species of parasitoids of whiteflies found at different altitude (m), crops and their percentages of parasitism

Altitude	Crop	Parasitoids	Parasitism (%)	Number of	
				Whiteflies	Parasitoids
1180	Snap bean	<i>A. fuscipennis</i>	37.4	1133	732
		<i>E. nigricephala</i>	4.5		87
		<i>Encarsia</i> sp	0.1		2
		unidentified	0.05		1
1400	Dry bean	<i>A. fuscipennis</i>	6.3	100	8
		<i>E. nigricephala</i>	3.1		4
		<i>Encarsia</i> sp	5.5		7
		unidentified	6.3		8
1440	Tomato	<i>A. fuscipennis</i>	3.7	264	10
		<i>A. fuscipennis</i>	35.2	107	58
1460	Dry bean	<i>A. fuscipennis</i>	52.0	40	52
		<i>Encarsia</i> sp	8.0		8
1920	Dry bean	<i>A. fuscipennis</i>	68.5	28	61
1980	Dry bean	<i>A. fuscipennis</i>	30.8	766	314

Until 1460 m other parasitoid species were found such as *Encarsia nigricephala* Dozier and *Encarsia* sp.

For all crops, insecticide sprays finished at latest 12 days before the survey and we expect that this spray free period has allowed parasitism during the final weeks of our sampling. Out of the three parasitoid species found, *A. fuscipennis* showed the highest percentages of parasitism. Total percentage parasitism by *A. fuscipennis* was found to be particularly high on dry beans at 1920 m (69%) and at 1460 m (52%).

Most of the pesticides used by Colombian farmers are toxic for natural enemies of whiteflies. We could not always obtain exact information on the type of pesticides and the frequency at which they were applied during our surveys. Sometimes the farmer was not present and somebody else gave us the information, or sometimes the farmer did not give complete information about the pesticides applied. The active ingredient and degree of toxicity for natural enemies of some of these pesticides that were mentioned by farmers are presented in Table 3.

Table 3. Insecticides and fungicides applied by Colombian farmers, and fungicides used for the unsprayed experimental plot. Effects of pesticides on predators, parasitoids and the entomopathogenic fungus *Verticillium lecanii* (Zimm.) Viegas, are classified as 1: harmless; 2: slightly harmful; 3: moderately harmful; 4: very harmful. If 2, 3 or 4 values are given, it means that there were different on 2, 3 or 4 different natural enemy species. If the effect is unknown, this is indicated by an empty space. Effect of pesticides on natural enemy over time is indicated between brackets^a as follows: no, no persistence; short, < 1.5 weeks; medium, 1-4 weeks; long, > 4 weeks.

Trade name	Active ingredient	Predators	Parasitoids	<i>V. lecanii</i>
Furadan	carbofuran	4 (medium, long)		
Evisect	thiocyclam hydrogen oxalate	1,2,3,4 (no, medium)	1,2,3,4 (no, medium)	1 (no)
Curacron	profenofos	4	1,3	
Perfekthion	dimethoate	1,2,3,4 (no, long)	3,4 (long)	1 (no)
Eltra	carbosulfan	1,2,4 (no, short, long)	4 (medium)	1 (no)
Vapona	dichlorvos	1,3,4 (short)	4 (no, short)	1 (no)
Cupravit	copper oxychloride*	1,2 (no)	1 (no)	4
Banrot	thiophanate-methyl eritridiazole	1,2,3 (no, medium)	1,2 (no, short) 1 (no)	1 (no) 1 (no)
Manzate	mancozeb	1,2,4 (no)	1,4 (no)	4

* sprayed

^a Presented by Koppert B. V. Side effects Data-Base, <http://www.koppert.nl>

Populations of whiteflies and parasitoids on bean crops. Adult whiteflies of *T. vaporariorum* and *B. tabaci* were present throughout the whole cropping season. For further analysis both species were counted and considered together as a complex. Eggs appeared 20 days after sowing and were found during the next 13 days. Average numbers of whitefly adults and eggs were similar for sprayed and unsprayed fields (Table 4). The number of nymphs peaked on day 33 for both treatments, being about four times higher at unsprayed than at sprayed fields (Figures 1 and 2). Also the average number of nymphs was significantly higher in unsprayed (40.5) than in sprayed (9.7) fields ($F = 15.29$, $df = 1$, 4.14 ; $P = 0.003$; Table 4).

Table 4. Mean number of adults, eggs and nymphs of whiteflies per 1 squared inch of leaflet unsprayed and sprayed fields, Department of Valle del Cauca, Colombia. Means are presented with their standard error values.

Whitefly stage	Unsprayed [†]		Sprayed [†]	
	Mean \pm SE	n	Mean \pm SE	n
Adults	20.3 \pm 3.1a	32	18.5 \pm 3.2a	32
Eggs	44.7 \pm 7.2a	12	35.8 \pm 11.6a	12
Nymphs [†]	40.5 \pm 5.8a	28	9.7 \pm 1.8b	28

[†]Numbers within a row followed by different letters are significantly different

[†]F = 15.29, df = 1, 4.14; P = 0.003

Examination of infested leaves from sprayed and unsprayed fields revealed only two whitefly parasitoid species in substantial numbers: *E. nigricephala* and *A. fuscipennis*. Parasitism by both species of parasitoids was detected only 40 days after sowing, when parasitized pupae became black and yellow (*E. nigricephala*) or gray (*A. fuscipennis*). *E. nigricephala* was the more abundant species with 6141 parasitized pupae (88.9% of all parasitized hosts). We found 769 pupae parasitized by *A. fuscipennis* (11.1%) during the whole crop season for both fields. Populations of both species of parasitoids were higher in unsprayed than in sprayed fields (*E. nigricephala* 18.4 vs. 8.0% parasitism and *A. fuscipennis* 3.1 vs. 0.2% parasitism; Table 5).

Populations of parasitoids fluctuated in a similar pattern for both treatments and were increasing over time (Figs. 1 and 2). Population dynamics of whitefly nymphs and parasitoids differed for both treatments. In the unsprayed plots, parasitoid populations were always lower than whitefly populations, but an increase of parasitoid populations occurred simultaneously with a decrease of the nymphal population during the second phase of our observations (Fig. 1). In the sprayed plots the *E. nigricephala* population exceeds the nymphal population from day 54 onwards (Fig. 2), after the farmer stopped spraying.

The percentage parasitism was calculated for both treatments on days 54 and 59. Percentage parasitism was 45% on day 54 and in unsprayed plots and increased to 57 % on day 59. In sprayed plots, these percentages were 52% and 70%, respectively. One needs to bear in mind that there is a much lower whitefly population in the sprayed plot.

Discussion

Distribution of A. fuscipennis by altitude. *A. fuscipennis* is distributed from middle to high altitudes as had also been reported by MacGown and Nebeker (1978) when they described the species. CIAT (1999, unpublished results) found *A. fuscipennis* as parasitoid of *T. vaporariorum* in other areas of Colombia different from Valle del Cauca, at altitudes varying from 750 to 2700 m, but most commonly on hillside areas above 1400 m.

Table 5. Mean number of pupae per 1 square inch of leaflet of the whitefly parasitoids, *Encarsia nigricephala* and *Amitus fuscipennis*. Means are presented with their standard error values.

Parasitoid	Unsprayed field ^a		Sprayed field ^a	
	Mean \pm SE	n	Mean \pm SE	n
<i>E. nigricephala</i> [†]	18.4 \pm 2.1a	16	8.0 \pm 1.0b	16
<i>A. fuscipennis</i> [‡]	3.1 \pm 1.0a	16	0.2 \pm 0.08b	16

^a Numbers within a row followed by different letters differed significantly.

[†] F = 11.846; df = 1, 0.81; P = 0.002.

[‡] F = 11.449; df = 1, 1.06; P = 0.02

A. fuscipennis is also abundant and easy to find on bean crops on hillside areas around 2000 m in Rionegro, Antioquia. In Tenerife, Valle del Cauca, where we sampled at 1900 m, the parasitoid was abundant on bean crops.

Although *A. fuscipennis* has a broad distribution, it was found that a combination of high temperatures and low relative humidity decreased longevity in *A. fuscipennis* adults (Manzano *et al.*, 2000). We suggest that *A. fuscipennis* is more common in hillside areas, where temperatures are not so high (averaging 19 °C) and relative humidity is about 80%. *A. fuscipennis* was the only *T. vaporariorum* parasitoid species we found at altitudes higher than 1400 m.

E. nigricephala was found below 1400 m suggesting that this species can live in warmer areas than *A. fuscipennis*. We did not find any biological data about this species to support the distribution we observed. *E. nigricephala* also occurred naturally in experimental field trials on beans in Honduras in an area located at approximately 800 m of altitude. Beans were infested with *B. tabaci* and, *E. nigricephala* along with *E. pergandiella* Howard were the most common parasitoids collected (Bográn *et al.*, 1998).

Populations of whiteflies and parasitoids on bean crops. The whitefly population was significantly lower in the farmer's field where a weekly spray of insecticides was applied compared with the

unsprayed field. The effect of insecticides reduced the number of nymphs; the mean number of adults and eggs were similar for both treatments. It is clear from Table 3 that eggs and adults are hardly killed by the insecticides that were used, as numbers of these stages are the same in sprayed and unsprayed fields. The insecticides seem to have a rather poor effect on whiteflies because their numbers will increase quickly after a treatment as a result of oviposition and hatching of eggs.

E. nigricephala populations reached higher densities than *A. fuscipennis* populations in both sprayed and unsprayed fields. This can be explained by (1) a better adaptation of *E. nigricephala* than *A. fuscipennis* to warm environments, and maybe (2) the presence of higher resistance to pesticides in *E. nigricephala* compared with *A. fuscipennis*, because *E. nigricephala* is much more often exposed to treatments with insecticides. In Pradera, the area where this experiment was set-up, farmers spray frequently pesticides (Rodríguez and Cardona, in press).

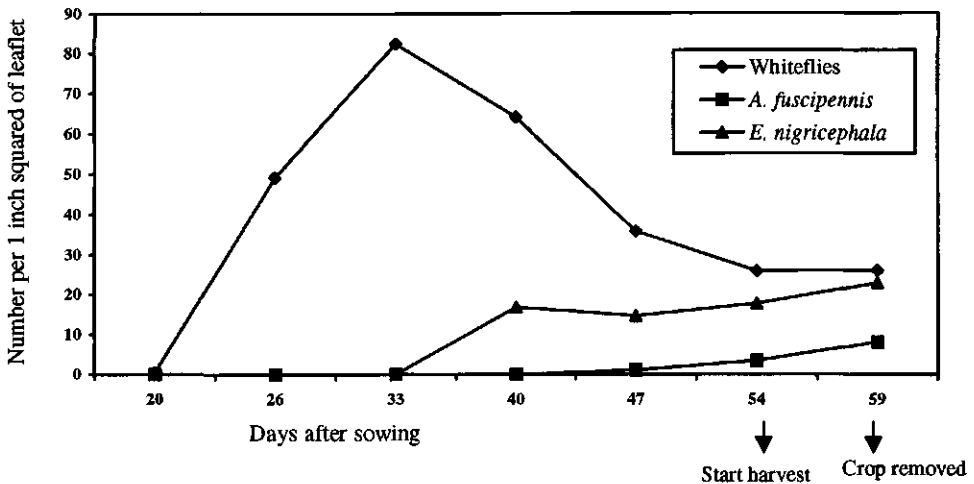
A. fuscipennis and *E. nigricephala* co-occur in the study area. They can probably do this because they oviposit in different instar stages of *T. vaporariorum*. *A. fuscipennis* prefers to oviposit the first two instars (Chapter 5) whereas *E. nigricephala* seems to prefer third and fourth instars, as does *E. formosa* (Nell *et al.*, 1976) and other *Encarsia* species (Gerling, 1990).

Based on whitefly presence and parasitoid development, we can extrapolate from our data that *A. fuscipennis* was already present when first whitefly stages appeared (*A. fuscipennis* developmental time is 25.5 d at the climatic conditions of this study area, 22 °C; 75% RH, Chapter 4). *E. nigricephala* was also present when their preferred whitefly stages occurred (third and fourth instars). So both parasitoids are early present in the crop, but their numbers were not high enough to sufficiently suppress whitefly numbers in the first generation of the pest. In unsprayed plots, the whitefly nymphal population was so high that even *E. nigricephala*, which was more abundant, never reached the levels of the whitefly population.

In contrast, in the sprayed plots, where the whitefly nymphal population was reduced by the insecticide, the population of *E. nigricephala* increased in such a way that it exceeded the whitefly population at the end of the season. This trend was reflected by an increase of parasitism from 52 to 70 %. The fact that the farmer stopped spraying insecticides 14 days before the crop was removed, also probably helped to increase the *E. nigricephala* population. However, the development of parasitoid populations occurred too late to sufficiently control the whitefly.

As snap bean is a short-term crop lasting only 60 days, this might be a practical limitation to natural biological control, unless surroundings crops and wild plants can boost parasitoid populations in snap bean. Successful examples of biological control programs of whiteflies at field conditions have been achieved in long-term crops such as citrus (DeBach and Rosen, 1991) that permitted parasitoid populations to be established on a permanent basis. Our hypothesis that, in unsprayed fields, parasitoid populations are higher than in sprayed fields is supported by the data. But the hypothesis that they

Figure 1. Dynamics of population of whitefly nymphs and parasitoids, unsprayed field

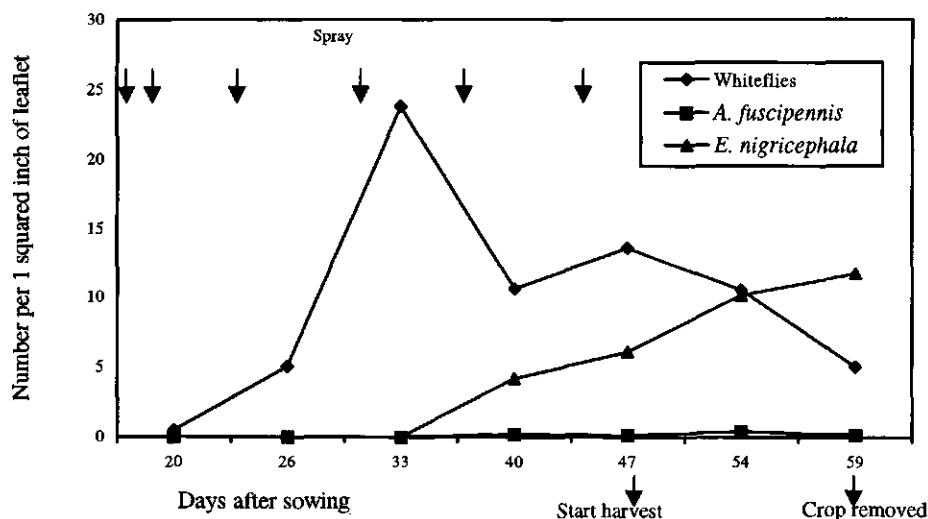


exceed whitefly populations is rejected for the unsprayed fields. Our results suggest that, while whiteflies on snap beans crops cannot be exclusively controlled by natural occurring parasitoids, they do significantly contribute to whitefly mortality.

We suggest testing the following control strategy for whiteflies:

- (1) Reduce the whitefly population at the beginning of the cropping season, by using a different biological control agent (e.g. an entomopathogenic fungus) or a selective pesticide that does not harm natural enemies. Certain insecticides are more toxic to whiteflies than to parasitoids and could be used, in an integrated manner, to reduce *T. vaporariorum* numbers (Zchori-Fein, *et al.*, 1994; www.koppert.nl, 2000). Also, carry out inundative releases of parasitoids early in the cropping season. The aim of biological control of whiteflies in snap bean is not so much to build up a parasitoid population (the crop will be harvested within 60 days), but to obtain immediate control (van Lenteren and Martin 2000). If an entomopathogenic fungus were tested, proper timing of the fungal spray with parasitoid introduction is essential (van Lenteren and Martin, 2000). Positive results have been obtained in field assays on bean crops by using *A. fuscipennis* in combination with the fungus *Verticillium lecanii* (Zimm) Viegas for control of *T. vaporariorum* (Pachon and Cotes, 1997).

Figure 2. Population development of whitefly nymphs and parasitoids, sprayed field.



Some of these fungi species are under study and seem to reduce whitefly populations at certain field conditions (E. Valencia, personal communication).

- (2) Build up a natural enemy population in the surrounding crops or wild vegetation. Habitat manipulation is one of the strategies used to enhance parasitoid activity in crops (e.g. Powell, 1986). Wild vegetation around the crop can supply parasitoids with essential resources (e.g. shelter, alternative hosts, food sources) that are crucial in determining their efficacy as biological control agents (Cortesero *et al.*, 2000).

We found several wild plants around bean crops that are alternative host plants for *T. vaporariorum* and *A. fuscipennis* (Manzano, unpublished results). They were *Ipomoea purpurea* (L.) Roth, *Urocarpium peruvianum* (L.) Krapovickas, *Byttneria aculeata* Jacquin, *Nicandra physaloides* (L.) Gaertn, *Tricanthera gigantea* (Hub et Bompl.) Nees van Essembeck and *Poinsettia caribaea* (Jacq.) Lavin.

- 3) Use bean cultivars that are the least suitable for whiteflies. In an earlier study, we found that under laboratory conditions *T. vaporariorum* had a low fecundity on the dry bean cv. "Chocho", and that on this cultivar, *A. fuscipennis* had a higher intrinsic rate of population increase (r_m) than did *T. vaporariorum* (Chapter 3).
- 4) If one of these three tactics still gives insufficient control, combine the tactics.

The next step will be to integrate the parasitoid into the guided control system to develop and IPM program for snap beans in Colombia.

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8. Summarizing discussion

Parasitoids of the genus *Amitus* are natural enemies of whiteflies. From the around 19 *Amitus* species described, two of them, *A. hesperidium* and *A. spiniferus* are used in large-scale application programs for biological control of *Aleurocanthus woglumi* and *Aleurothrixus floccosus*. *A. bennetti* is currently evaluated for biological control of *Bemisia argentifolii* in greenhouses (Drost *et al.*, 1999) and field (Joyce and Bellows, 2000). *A. fuscipennis* is under study for possible future use in the field and in greenhouses to control *T. vaporariorum*.

The selection of new natural enemies should preferably be based on the study of certain characteristics which indicate its potential value as biological control agent under the specific conditions where it is to be applied (van Lenteren and Woets, 1988; **Chapter 1**).

The general aim of this thesis was to investigate biological and behavioral characteristics of *A. fuscipennis* in order to be able to evaluate its potential use as biological control agent of the greenhouse whitefly, *T. vaporariorum* on field crops of beans in Colombia. In chapter 1, I gave a review on biology and use of *Amitus* species and I summarized the whitefly problem in Colombia. I also formulated the research hypotheses.

Application of evaluation criteria

Climatic adaptation. Laboratory experiments (**Chapter 2**) and field data (**Chapter 7**) showed that *A. fuscipennis* could find and parasitize *T. vaporariorum* in certain bean cropping areas of Colombia. Developmental time and longevity of *A. fuscipennis* were tested at different combinations of relative humidity and temperature where bean are produced in Colombia (**Chapter 2**). Combinations of moderate temperature and low relative humidity negatively affected longevity of *A. fuscipennis*. These results suggest that *A. fuscipennis* could be a potentially good biological control agent of *T. vaporariorum* in environments that are not very dry or warm, i.e. temperatures above 22 °C and relative humidity values bellow 75%. *A. fuscipennis* should perform well at temperatures between 16-19 °C and relative humidity values between 75-90%. This aspect is quite important for the whitefly situation in the Colombian landscape. The Andes separate into three ranges making cropping of dry and snap beans possible at different altitudes and climates. Our surveys on bean and snap bean crops at different altitudes in Valle del Cauca, revealed that *A. fuscipennis* was the only parasitoid of *T. vaporariorum* found above 1460 m (**Chapter 7**). Besides these areas, we have collected it abundantly on bean and snap bean crops in Sumapaz (Cundinamarca) at altitudes between 1550-1990 m and, in Rionegro (Antioquia) between 1800-2300 m. These areas are also located in the tropical highlands. Thus, the hypothesis that *A. fuscipennis* might be able to develop and reproduce on whitefly at a limited range of temperatures and humidities, specific for a limited part of the area where beans are

grown in Colombia is supported by my data. I expect that *A. fuscipennis* can play a role in reducing whiteflies in the tropical highlands of Colombia.

Culture method. A good culture method is an important characteristic of successful natural enemies in inundative and seasonal inoculative releases. An initial method for mass rearing of *A. fuscipennis* was presented by Garcia *et al.*, (1995). In addition, I studied the host stages that are preferred by the parasitoid, its developmental time, sex ratio and kind of reproduction (**Chapters 2 and 5**). *A. fuscipennis* prefers the 1st and 2nd nymphal stage of whitefly for parasitization (**Chapter 5**). As a result, its average developmental time is relatively long when compared with *E. formosa*, which attacks the host mainly at later nymphal stages (**Chapter 2**). The developmental time of *A. fuscipennis* is also about 8 days longer than that of *T. vaporariorum* at a rearing temperature of 19° C. This means that for efficient mass rearing special attention is needed to have sufficient whitefly nymphs of the 1st and 2nd instars have to be present. *A. fuscipennis* showed a strongly female-biased ratio as a result of thelytoky, probably caused by the endosymbiotic bacteria *Wolbachia* (**Chapter 2**). To be absolutely sure that *Wolbachia* induces thelytoky, *A. fuscipennis* females should be 'cured' by feeding it with antibiotics or be reared at high temperatures, and then both males and females should be produced (Stouthamer *et al.*, 1990; Pijls, 1996). For mass rearing, thelytokous reproduction is an advantage because expensive hosts are not "wasted" for male production, and time consuming processes like mate finding and mating are not needed (Stouthamer, 1993). This would also be particularly advantageous for initial field releases of *A. fuscipennis* at low whitefly densities in the field: field establishment of thelytokous lines is easier than that of arrhenotokous lines, because mate finding and mating is not necessary. The hypothesis that mass rearing of *A. fuscipennis* might on the one hand be complicated by its long developmental time, while on the other hand it is simplified by its thelytokous way of reproduction, is supported by my results. Good timing of the whitefly rearing will prevent the problem of desynchronization between whitefly and parasitoid, but this may make mass production more expensive than that of *E. formosa*, which is also a thelytokously reproducing parasitoid (van Lenteren and Martin, 1999).

Reproductive potential. The intrinsic rate of population increase (r_m), or host-kill rate of a natural enemy should be larger than the intrinsic rate of population growth of the pest will seasonal inoculative or inoculative lead to successful biological control. As *A. fuscipennis* does not show the habit of host feeding (**Chapter 5**), it is sufficient to calculate the r_m value, and not the host kill rate as no hosts are killed by host feeding. The reproductive capacity of *T. vaporariorum* and *A. fuscipennis* were measured as the intrinsic rate of population increase (r_m) under climatic conditions that simulated those of highland and midland areas (**Chapters 3 and 4**, respectively). This was done for different

combinations of temperature and humidity because presence in Colombia of the Andes mountain range makes cropping of dry and snap beans possible at different altitudes as tropical highlands (altitudes above 1000 m) and inter-Andean valleys (altitudes around 1000 m). The r_m values of *A. fuscipennis* were compared to those of *T. vaporariorum* at same climate conditions. The r_m values of *A. fuscipennis* were found to be higher than those of its hosts at the two climate zones for which values were determined. Based on the proposition of van Lenteren and Woets (1988), that for successful biological control the r_m of the natural enemy of the pest should be higher than that of its host, we may conclude that *A. fuscipennis* could be a good control agent of *T. vaporariorum* at tropical highland and midland areas, if the r_m value was the only criterion to consider.

It is interesting to note that the r_m value of *T. vaporariorum* was particularly low on bean cv. "Chocho" compared to cv. "ICA-Pijao", so cv. "Chocho" is partially resistant to whitefly. The cause behind this partial host-plant resistance is unknown yet, but deserves attention because any knowledge about the resistance mechanisms could be exploited for whitefly control. Based on the presence of partial resistance to whitefly, "Chocho" is the bean cultivar suggested for using in IPM for *T. vaporariorum*. It might be useful to test other bean successions for (partial) resistance to whitefly.

The hypothesis that the r_m value of *A. fuscipennis* is higher than that of *T. vaporariorum* only at a limited range of climatic conditions at which beans are cropped in Colombia, and in a smaller area than where the parasitoid is able to develop and reproduce, is rejected by my results. If the r_m value were the only criterion to consider, we might conclude that biological control of *T. vaporariorum* with *A. fuscipennis* would be possible in the climatic zones that were considered in this research project.

Searching behaviour. The r_m value of a parasitoid is an important characteristic for the biological control capacity of a parasitoid on the premise that they can lay their daily egg load, and this is only possible at very high host densities (e.g. van Roermund, 1995). Early in the bean crop season we find high host densities in the field in Colombia, so the high r_m of *A. fuscipennis* compared to that of *T. vaporariorum* is a positive attribute of this parasitoid species. But a high r_m value is often not the only criterion that an effective parasitoid should meet. After parasitoids have reduced pest numbers, their searching efficiency becomes critical in host finding, and in keeping pest numbers low. So we also need information about foraging behavior of *A. fuscipennis* on bean (**Chapters 5 and 6**).

Little was known about long-range searching in *A. fuscipennis*. For certain parasitoids, long-range searching is mediated through volatile infochemicals (Vet and Dicke, 1992), but other parasitoids appeared efficient biological control agents even when searching at random (e.g. van Roermund, 1995; van Lenteren *et al.*, 1996). The flight response of *A. fuscipennis* to infested and uninfested whitefly leaves was tested (**Chapter 6**), and it appeared that *A. fuscipennis* was not attracted to clean or infested leaves or plants, even not over a short distance. *A. fuscipennis* flew

upwards, apparently attracted by the light. Probably after emerging, *A. fuscipennis* flies upwards within the crop canopy and lands at random on the underside of the leaves.

The efficiency of the host finding process, both at long and short range, will ultimately determine the parasitoid's capability to keep whitefly populations at very low densities during the season (van Lenteren et al., 1996). When searching on the leaf, *A. fuscipennis* preferred equally first and second instars above third instars. *A. fuscipennis* showed area-restricted searching, which is an adaptation to searching for hosts that are distributed in a clumped manner as *T. vaporariorum* does. So *A. fuscipennis* seems to be adapted to search for aggregated hosts. By this kind of searching, *A. fuscipennis* remains longer on leaves where hosts are present (**Chapter 5**).

A. fuscipennis is a fast walking parasitoid that probed and oviposited in most of the hosts encountered, which may be an adaptation resulting from its pro-ovigenic way of reproduction. The percentage of unparasitized hosts accepted for oviposition was high (60%), which combined with large numbers of eggs laid over a short period results in high percentages parasitism.

Also, after having landed on a host plant, the searching behavior of *A. fuscipennis* is influenced by the quality of the plant on which searching takes place (**Chapter 6**). Residence time was higher on infested plants (2.7 h) than on uninfested unsprayed (1.7 h) or on uninfested fungicide-sprayed plants (less than 1 h). Walking activity of *A. fuscipennis* showed the same trend: it was high on infested leaves, medium on uninfested unsprayed leaves and low on uninfested fungicide-sprayed leaves.

A. fuscipennis preferred the leaflet's underside while searching and the lower center when hosts were present. *A. fuscipennis* was arrested on the plant as a result of both, encounters with hosts and feeding from plant secretions. Feeding from the plant was carried out by sipping certain secretions from stipels.

Part of the hypothesis, i.e. that *A. fuscipennis* is using long-distance volatile cues produced by the host or the host plant to locate infested plants is rejected; the parasitoid cannot discriminate between infested and uninfested host plants from a distance. The second part of the hypothesis, i.e. that *A. fuscipennis* shows a type of searching behaviour - once the parasitoid has landed on the plant - that is adapted to occurrence of aggregated host stages, is supported by my data.

Side effects of pesticides on the parasitoid. In the laboratory it was tested whether a fungicide that is regularly used by Colombian farmers to control certain plant diseases (a sulfur-based fungicide) affects the behavior of the parasitoid (**Chapter 6**), and results indicated that there was a negative effect, indeed. Apparently, the parasitoids perceive the presence of the fungicide and they abandon the leaf earlier than unsprayed leaves. Thus, when parasitoids are used within an IPM system, special care

must be paid to synchronize fungicide applications with the arrival or introduction of *A. fuscipennis* to the crop to prevent such negative interference.

In addition to laboratory experiments, fieldwork was carried out to test the effect of pesticides (Chapter 7). From this work it appeared that *A. fuscipennis* numbers might have been reduced as a result of pesticide applications.

The hypothesis that pesticides used in bean in Colombia for control of insects and fungi negatively effect the performance of the parasitoid *A. fuscipennis*, is supported by my results.

Performance of parasitoids in the field and IPM of whitefly. The distribution of *A. fuscipennis* by altitude was determined by sampling parasitized whitefly pupa from dry bean, snap bean and tomato crops (Chapter 7). *A. fuscipennis* was distributed from middle to high altitudes but it was most commonly found on hillside areas above 1400 m. This result support data from Chapter 1, i. e. that environments overly dry or warm result in short *A. fuscipennis* adult longevity.

To determine regional population trends of the whiteflies *T. vaporariorum* and *Bemisia tabaci*, and their parasitoids, we performed field experiments in sprayed and unsprayed fields during a cropping season of snap beans (Chapter 7). The parasitoid *E. nigricephala* was more abundant than *A. fuscipennis*, probably because 1) the experimental field was located in an area that did not optimally match climate conditions for *A. fuscipennis*, and/or 2) *E. nigricephala* might have a higher resistance to pesticides compared with *A. fuscipennis* in that area of Valle del Cauca.

In unsprayed plots, parasitoid populations were larger than those of sprayed plots were, but they did not reach the level of whitefly populations. In sprayed plots where whitefly populations were reduced by insecticides, the population of *E. nigricephala* exceeded the whitefly populations at the end of the cropping season. My results suggest that, while whiteflies on snap beans cannot be exclusively controlled by natural occurring parasitoids, they do significantly contribute to whitefly mortality.

Based on the results obtained in this Ph.D. project, I suggest for whitefly control, (1) to reduce the whitefly population at the beginning of the cropping season (e.g. by using an entomopathogenic fungi) and, 2) to carry out releases of parasitoids after fungus treatment.

An additional aspect to reduce whitefly populations and to make biological control more effective, could be the use of partially whitefly resistant bean plants. There is an increasing interest in combining host-plant resistance and biological control as a pest management practice (Cortesero *et al.*, 2000). I established partial resistance to *T. vaporariorum* in one line of bean, and I suggest using this bean cv. "Chocho" as one aspect of IPM for this pest.

Finally, habitat manipulation could be exploited resulting in a building up of a natural enemy population in the surrounding crops or wild vegetation. Several wild plant species were found bearing parasitized *T. vaporariorum* nymphs by *A. fuscipennis*. If these plants remain close to crops, they can

support *A. fuscipennis* populations between different crop seasons, or they can support and increase parasitoid populations during one cropping season. Habitat manipulation is one of the tactics suggested to enhance parasitoids in crops (Powell, 1986; Cortesero, 2000).

In conclusion, information collected during this Ph.D. project strongly indicates that *A. fuscipennis* might be an effective parasitoid of *T. vaporariorum* in beans under Colombian conditions. Field-testing where releases of *A. fuscipennis* are added to the guided control program for bean will give the final proof of its efficiency.

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Summary

The research described in this thesis concerns the study of a natural enemy of whiteflies, *Amitus fuscipennis* MacGown & Nebeker under Colombian field and laboratory conditions. The general aim of the project was to study whether biological control of *Trialeurodes vaporariorum* (Westwood) with *A. fuscipennis* can be included into the guided control program for pests and diseases in bean. In **Chapter 1**, natural enemies of whiteflies are reviewed (especially the biology and use of some species of *Amitus*), the whitefly problem in Colombian bean crops is described and the aims of the study are given.

When the present research project started, few biological data about *A. fuscipennis* were known. In **Chapter 2** data about longevity, sex ratio and developmental time of *A. fuscipennis* are presented. The immature developmental time and longevity of fed and unfed adult parasitoids were compared under different temperature and relative humidity combinations. Combinations of moderate temperature and low relative humidity negatively affected parasitoid longevity. Results suggest that *A. fuscipennis* could be a potentially good biological control agent of *T. vaporariorum* in environments that are not overly dry or warm. Sex ratios of populations collected in the field and reared in the laboratory were strongly female-biased. Reproduction of the parasitoid is through thelytokous parthenogenesis. The species carries *Wolbachia*; the rickettsia that induces thelytoky but it was not tested if it induces thelytoky in *A. fuscipennis*.

Because of the presence of the Andes mountain range in Colombia, cropping of dry and snap beans is possible at different altitudes as tropical highlands (altitudes above 1000 m) and inter-Andean valleys (altitudes around 1000 m). This range implies as well that there are large climatic variations, going from warm, dry areas to cooler, more humid ones. Therefore, it is important to determine how certain climatic conditions influence the biology and performance of *A. fuscipennis*. In **Chapters 3** and **4**, the reproductive capacity of the pest insect *T. vaporariorum* and the parasitoid *A. fuscipennis* were measured as the intrinsic rate of population increase r_m . These parameters were measured on bean cultivars 'Chocho' and 'ICA-Pijao', under climatic conditions that simulated those of highland and midland areas. The r_m values of *A. fuscipennis* were higher than those of *T. vaporariorum* for both kinds of simulated conditions. This result permits us to conclude that *A. fuscipennis* may therefore be a suitable candidate for use in biological control programs against *T. vaporariorum* in Colombia. An interesting additional result was the finding that the r_m of *T. vaporariorum* was much lower on bean cv. Chocho than on cv. 'ICA-Pijao'. Cv. 'Chocho' should preferably be used in IPM programs in Colombia to slow down whitefly development and ease biological control.

A high r_m value is often not the only criterion an effective parasitoid should meet. After parasitoids have reduced pest numbers, their searching efficiency becomes critical in host finding, and in keeping pest numbers low. Therefore, the foraging behavior of *A. fuscipennis* on bean was studied

Summary

(Chapter 5). *A. fuscipennis* is a fast walking parasitoid that probed and oviposited most of the hosts encountered, which may be related to its pro-ovigenic way of reproduction. The percentage of unparasitized hosts accepted for oviposition was high (60%), which combined with large numbers of eggs laid over a short period resulted in high percentages parasitism. *A. fuscipennis* showed area-restricted searching, which is an adaptation to searching for hosts that are distributed in a clumped manner as *T. vaporariorum* does.

Long-range searching in *A. fuscipennis* had not been studied yet. The flight response of *A. fuscipennis* to uninfested and whitefly infested leaves was tested in the laboratory (Chapter 6). *A. fuscipennis* was not attracted to clean or infested leaves or plants, even not over a short distance. *A. fuscipennis* flew upwards, apparently attracted by the light. Once *A. fuscipennis* had arrived on a plant, its searching behavior was influenced by the 'quality' of the plant (Chapter 6). Residence time and walking activity of *A. fuscipennis* were higher on unsprayed infested leaves than on uninfested fungicide sprayed or uninfested unsprayed leaves. *A. fuscipennis* preferred the leaflet's underside while searching and the lower center when hosts were present. *A. fuscipennis* was arrested on the plant as a result of both encounters with hosts and feeding from plant secretions.

In addition to laboratory experiments, fieldwork was carried out in Colombia (Chapter 7). *A. fuscipennis* was distributed from middle to high altitudes but it was most commonly found on hillside areas above 1400 m. To determine regional population trends of the whiteflies *T. vaporariorum* and *Bemisia tabaci*, and their parasitoids, experiments were performed in sprayed and unsprayed fields during a cropping season of snap beans. Results suggested that, while whiteflies on snap beans cannot be exclusively controlled by natural occurring parasitoids, they do significantly contribute to whitefly mortality. Based on these results, I suggest for whitefly control, (1) to reduce the whitefly population at the beginning of the cropping season e.g. by applying entomopathogenic fungi, and 2) to carry out releases of parasitoids after fungus treatment. As an additional aspect to reduce whitefly populations, the use of partially whitefly resistant bean plants and habitat manipulation tactics to build up a natural enemy population in the surrounding crops or wild vegetation, are suggested.

In Chapter 8, the research findings are discussed in a broader context. Information collected during this Ph.D. project strongly indicates that *A. fuscipennis* might be an effective natural enemy of *T. vaporariorum* in beans under Colombian conditions. Field testing of releases of *A. fuscipennis* within the guided control program for bean will give the final proof of the parasitoids usefulness.

Samenvatting

Dit proefschrift beschrijft de studie aan een natuurlijke vijand van witte vlieg, *Amitus fuscipennis* MacGown & Nebeker, zowel in het veld in Columbia als in het laboratorium. Centraal stond de vraag of biologische bestrijding van *Trialeurodes vaporariorum* (Westwood) met *A. fuscipennis* een onderdeel kan zijn van het geleide-bestrijdingsprogramma voor ziekten en plagen in bonen. In **hoofdstuk 1** wordt een overzicht gegeven van natuurlijke vijanden van witte vlieg (met nadruk op de biologie en het gebruik van enkele *Amitus*-soorten). Tevens wordt het probleem van witte vlieg in boongewassen in Columbia beschreven en worden de doelen van het onderzoek gespecificeerd.

Toen met dit onderzoek begonnen werd waren er weinig biologische gegevens over *A. fuscipennis* bekend. In **hoofdstuk 2** worden gegevens over levensduur, sexratio en ontwikkelingsduur van *A. fuscipennis* gepresenteerd. De larvale ontwikkelingsduur en de levensduur van gevoede en ongevoede volwassen sluipwespen werden vergeleken bij verschillende combinaties van temperatuur en relatieve luchtvochtigheid. Combinaties van een matige temperatuur en een lage relatieve luchtvochtigheid hadden een negatief effect op de levensduur van de sluipwesp. De resultaten suggereren dat *A. fuscipennis* een goede bestrijding van *T. vaporariorum* zou kunnen bieden in een niet al te droge of warme omgeving. De sexratio's van zowel veldpopulaties als laboratoriumkweken neigden sterk naar een overmaat van vrouwtjes. De sluipwesp plant zich voort door middel van thelytoke parthenogenese. *A. fuscipennis* is drager van *Wolbachia*, de rickettsia die thelytokie veroorzaakt; maar of *Wolbachia* de veroorzaker is van thelytokie in deze soort is niet onderzocht.

In verband met de aanwezigheid van het Andes-gebergte in Columbia is het verbouwen van droge boon en sperzieboon mogelijk op verschillende hoogten, zoals tropische hooglanden (boven 1000 m) en valleien in de Andes (rond 1000 m). Dit betekent ook dat er grote verschillen in klimaat zijn, variërend van droge, warme gebieden tot meer koele en vochtige gebieden. Daarom is het belangrijk om te bepalen hoe klimatologische omstandigheden de biologie en de effectiviteit van *A. fuscipennis* beïnvloeden. In de **hoofdstukken 3 en 4** wordt de reproductiecapaciteit van het plaaginsect *T. vaporariorum* en de sluipwesp *A. fuscipennis* gemeten, uitgedrukt als de intrinsieke populatiegroeisnelheid r_m . Deze parameters werden gemeten op de boon-cultivars 'Chocho' en 'ICA-Pijao', onder nagebootste klimatologische omstandigheden van het middengebergte. De r_m -waarden van *A. fuscipennis* waren hoger dan die van *T. vaporariorum* voor beide nagebootste omstandigheden. Deze resultaten staan de conclusie toe dat *A. fuscipennis* waren hoger dan die van *T. vaporariorum* voor beide nagebootste omstandigheden. Deze resultaten staan de conclusie toe dat *A. fuscipennis* een geschikte kandidaat is voor de biologische bestrijding van *T. vaporariorum* in Columbia. Een interessant aanvullend resultaat was het feit dat de r_m van *T. vaporariorum* lager was op cv 'Chocho' dan op cv 'ICA-Pijao'. In IPM-programma's in Columbia dient bij voorkeur cultivar 'Chocho' te

worden gebruikt om de ontwikkeling van witte vlieg af te remmen en om de biologische bestrijding te bevorderen.

Een hoge r_m -waarde is vaak niet het enige criterium waaraan een effectieve sluipwesp moet voldoen. Nadat de plaagpopulatie door de sluipwesp is gereduceerd, wordt het zoekgedrag cruciaal voor het vinden van de gastheren en voor het beperkt houden van de plaagpopulatie. Daarom is het fourageergedrag van *A. fuscipennis* op boon bestudeerd (hoofdstuk 5). *A. fuscipennis* is een snellopende sluipwesp die de meeste van de gastheren die ze ontmoet aanprikt en parasiteert, hetgeen verband kan houden met haar pro-ovigene manier van voortplanten. Het percentage ongeparasiteerde gastheren dat geaccepteerd werd voor ovipositie is hoog (60%). Dit, gecombineerd met het grote aantal eieren dat gelegd wordt gedurende een korte periode, resulteerde in een hoge parasiteringsgraad. *A. fuscipennis* vertoonde gebieds-gelimiteerd zoekgedrag, hetgeen een aanpassing is aan het zoeken naar gastheren met een geclusterde verspreiding, zoals *T. vaporariorum*.

Het zoekgedrag van *A. fuscipennis* op lange afstand was tot op heden nog niet bestudeerd. De vliegrespons van *A. fuscipennis* op onbesmette en wittevlieg-besmette bladeren is bestudeerd in het laboratorium (hoofdstuk 6). *A. fuscipennis* werd niet aangetrokken door onbesmette of wittevlieg-besmette bladeren, zelfs niet op korte afstand. *A. fuscipennis* vertoonde de neiging omhoog te vliegen, klaarblijkelijk aangetrokken door het licht. Na landing van *A. fuscipennis* op een plant, werd haar zoekgedrag beïnvloed door de 'kwaliteit' van de plant (hoofdstuk 6). De verblijfstijd van *A. fuscipennis* was langer en haar loopactiviteit hoger op besmette planten die niet met fungiciden waren bespoten dan op onbesmette planten, al dan niet met fungiciden bespoten. *A. fuscipennis* zocht bij voorkeur op de onderkant van het blad met name het midden als gastheren aanwezig waren. *A. fuscipennis* bleef langer op de plant na zowel ontmoetingen met gastheren als het voeren op uitscheidingen van de plant.

Naast laboratoriumonderzoek werd veldwerk verricht in Columbia (hoofdstuk 7). *A. fuscipennis* kwam voor op gemiddelde tot grote hoogte, maar werd voornamelijk gevonden in heuvelachtige gebieden boven 1400 m. Om regionale trends in de populaties van de witte vliegen *T. vaporariorum* en *Bemisia tabaci* en hun sluipwespen te bepalen, werden gedurende een groeiseizoen van sperziebonen experimenten uitgevoerd in bespoten en onbespoten velden. De resultaten suggereren dat, hoewel witte vlieg op sperziebonen niet bestreden kan worden door uitsluitend de natuurlijk voorkomende sluipwespen, deze sluipwespen wel substantieel bijdragen aan de mortaliteit van witte vlieg. Op basis van deze resultaten stel ik voor, met betrekking tot de bestrijding van witte vlieg, om (1) de wittevliegpopulatie te verkleinen aan het begin van het groeiseizoen, bijvoorbeeld door behandeling met entomopathogene schimmels, en (2) na schimmelbehandeling sluipwespen los te laten. Daarnaast stel ik voor om de habitat aan te passen ten bate van natuurlijke vijanden in de omringende gewassen of wilde planten en om gebruik te maken van partieel resistente boon.

In hoofdstuk 8 worden de resultaten in een breder verband besproken. De informatie die verzameld is gedurende dit promotie-onderzoek geven duidelijk aan dat, onder Columbiaanse omstandigheden, *A. fuscipennis* een effectieve natuurlijke vijand van *T. vaporariorum* in boon kan zijn. Loslaat-experimenten van *A. fuscipennis* in het veld in het kader van het geleidebestrijdingsprogramma voor boon zal uitsluitsel kunnen geven over het nut van deze sluipwesp.

Publications

One chapter of this thesis has been published as:

Manzano, M.R., J.C. van Lenteren, C. Cardona & Y.C. Drost. 2000. Development time, sex ratio and longevity of *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae) on the greenhouse whitefly. *Biological Control* 18: 94-100.

Other publications:

Manzano, M. R., J. C. van Lenteren & C. Cardona. 1999. Some biological characteristics of *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygasteridae), parasitoid of the greenhouse whitefly. *Bulletin IOBC/WPRS* 22 (1): 169-172.

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Curriculum vitae

Maria del Rosario Manzano Martinez was born on March 26 1963, in Cali, Colombia. She started studies in Biology at Universidad del Valle in Cali in 1980. After graduation she worked at the Department of Biology of the same university during two years. She did her MSc studies in Crop Protection at Wageningen University during 1990-1992. After finishing she worked at Instituto Colombiano Agropecuario, ICA in Ibagué, Colombia in control of fruit flies. She spent one year in Belgium at Université Libre in Brussels involved in research about forest entomology. In 1996 she started her studies at Wageningen University in the subject of biological control of whiteflies. Most of this research was carried out at the Centro Internacional de Agricultura Tropical, CIAT, in Cali.