

Bemisia tabaci* biotype B in bean: Life history parameters and absence of host-parasitoid interaction with *Amitus fuscipennis

Estadísticos vitales de *Bemisia tabaci* biotipo B en frijol e interacción con el parasitoide *Amitus fuscipennis*

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Abstract

Life-history parameters of the whitefly *Bemisia tabaci* biotype B were determined under laboratory conditions at; 26 °C ± 2 °C; 80 ± 5 % Relative Humidity on the bean cultivar ICA-Pijao. Mean longevity of the whitefly was 34 ± 1.64 days, mean total fecundity was 211.4 ± 15.1 eggs per female with a daily oviposition rate of 6.1 ± 0.28 eggs per day. Net reproduction rate was 137.8. Generation Time was 43.8 days and the intrinsic rate of population increase r_m was 0.125. These values are concordant with previous ones reported and support the usefulness of ICA-Pijao bean for *B. tabaci* rearing. It was not possible to rear *Amitus fuscipennis* from *B. tabaci*. Behavior studies showed that this parasitoid stayed only an average 617.3 s on whitefly infested leaflets. It invested most of the time walking (46.4%), preening (35.6%) and standing still (16.7%). *A. fuscipennis* just touched accidentally one nymph once but it neither drummed it with the antenna nor laid an egg. Results indicated that *A. fuscipennis* is not a potential natural enemy of *B. tabaci* biotipo B.

Key words: *Bemisia tabaci*, sweet potato whitefly, population increase, biological control.

Resumen

Se determinaron los estadísticos vitales de la mosca blanca *Bemisia tabaci* biotipo B en condiciones de laboratorio (26 °C ± 2 °C y 80 ± 5 % de HR) en frijol cultivar ICA-Pijao. Los valores promedios fueron: longevidad 34 ± 1.64 días, fecundidad de 211.4 ± 15.1 huevos/hembra y tasa de oviposición de 6.1 ± 0.28 huevos/día. La tasa neta reproductiva fue de 137.8. El tiempo generacional de 43.8 días y la tasa

intrínseca de crecimiento poblacional (r_m) de 0.125. Estos valores son coincidentes con los reportados en la literatura e indicaron la utilidad del frijol (*Phaseolus vulgaris* L) ICA-Pijao para la multiplicación de *B. tabaci*. No se logró la reproducción de *Amitus fuscipennis* en *B. tabaci*. Estudios de comportamiento mostraron que el parasitoide permaneció solo 617.3 seg en promedio en folíolos infestados con la mosca blanca, la mayor parte del tiempo lo invirtió en caminar (46.4%), limpiarse el cuerpo (35.6%) y permanecer quieta (16.7%). *A. fuscipennis* solo tocó una vez accidentalmente una ninfa de *B. tabaci*, pero no la examinó con las antenas ni la ovipositó. Los resultados indican que *A. fuscipennis* no es un enemigo natural potencial de *B. tabaci* biotipo B.

Palabras clave: *Bemisia tabaci*, hemíptera, insecta, *Amitus*, himenóptera, mosca blanca de la batata; crecimiento poblacional, control biológico.

Introduction

The epidemics of the begomoviruses causing the golden mosaic and the golden yellow mosaic in the common bean (*Phaseolus vulgaris* L.), causing yield losses up to 100% (Cuéllar y Morales, 2006), constitute the main damage in Latin America caused by the biotype B of the whitefly *Bemisia tabaci* (Gennadius) (Hemíptera:Aleyrodidae). *Bemisi tabaci* is a pest on hundreds of wild and cultivated plants in tropical and subtropical regions (Schuster et al., 1996). Based on bio-ecological differences of the insect and the damage symptoms in the plants, *B. tabaci* has been separated into two biotypes: A and B (Brown et al., 1995). Due to its resistance to pyrethroids and organophosphates (Tsagkarakou et al., 2009) the biotype Q is also recognized (Martínez-Carrillo & Brown, 2007). The biotype B was proposed as a different species, called *Bemisia argentifolii* Bellows & Perring (Perring et al., 1993). In the bean (*Phaseolus vulgaris*), the *B. tabaci* biotype B produces chlorosis in the petioles and the pods (Rodríguez et al., 2005) as both the nymphs and the adult whiteflies suck the phloem, and weaken the plant. Furthermore, in order to eliminate the excess of ingested fluids, the insects excrete a type of molasses, promoting the growth of the fungi *Cladosporium* and *Capnodium* known as soot molds due to the black color of their mycelium (Fox, 1997). Soot molds interfere with photosynthesis, reducing crop yield, and causing economic losses in fruits and ornamental plants (Schuster et al., 1996). However, the most important damage caused by the *B. tabaci* biotype B is the transmission of begomoviruses. This transmission is of a persistent circulative type, with persistence varying between only a few days or weeks, to a permanent presence (Cuéllar & Morales, 2006).

In addition to its polyphagy, one of the most important adaptive traits of *B. tabaci* compared with other whitefly such as *Trialeurodes vaporariorum* (Westwood) (Hemiptera:Aleyrodidae) is its resistance to high temperatures (Wan et al., 2009) that is seen in beans and other biannual crops in the Cauca Valley, Colombia (Rodríguez et al., 2005). These authors recognized the variation in both the altitudinal distribution as well as in the quantity of biannual crops attacked by *T. vaporariorum* and by the biotypes A and B of *B. tabaci* between 1997 and 2003. In 1997 the following were found: *T. vaporariorum* (73% of samples), *B. tabaci* A (15.5 %), *B. tabaci* B (11.5%), and in not one sample was the combination *T. vaporariorum* – *B. tabaci* found. In 2003, the presence of *T. vaporariorum* fell to 22.7% of the samples, that of *B. tabaci* B increased drastically to 62.6%, the combination *T. vaporariorum* – *B. tabaci* appeared, at 14.6% and *B. tabaci* biotype A disappeared.

This variation suggests the displacement of *T. vaporariorum* and the A biotype by the B biotype, with the latter extending its range of climatic adaptation by 152 m.a.s.l., from 995 to 1147 m.a.s.l. (Rodríguez et al., 2005). Additionally, the number of economically important host plants attacked by the *B. tabaci* biotype B increased from three to fourteen, including the bean, tomato, and cotton (Rodríguez et al., 2005). In the Cauca Valley, this biotype is controlled through the application of insecticides; this insect has shown high levels of resistance to these commercial products, but susceptibility to others, more novel products, such as neonicotinoids and growth hormones (Rodríguez et al., 2005). There is not sufficient information available for population parameters of *B. tabaci* biotype B in bean for the climatic conditions studied (Tsai y Wang, 1996; Romberg, 1998).

The biocontrol agents of *Bemisia* B in Colombia include *Encarsia nigricephala* Dozier (Hymenoptera:Aphelinidae) in egg plant, melon, cabbage, bean, cassava and pumpkin in both Colombia and Ecuador (López-Ávila et al., 2001). Gerling et al. (2001) present a revision of parasitoids and predators of *B. tabaci*, where they state that non-identified species of *Amitus* have also been found parasitizing this fly in Central America, specifically in Honduras (Bogran et al., 1998), Nicaragua (Nunes et al., 2006) and Costa Rica (Mora-Arias, 1998). Outside of Central America, *A. bennetti* Viggiani & Evans has been studied in the USA (Joyce & Bellows, 2000) and Holland (Drost et al., 1999) as a regulating agent for *B. argentifolii*.

In Colombia, *A. fuscipennis* MacGown & Nebeker (Hymenoptera:Platygasteridae) is a natural enemy with potential for controlling *T. vaporariorum* in beans, due to its thelytoky type of parthenogenetic reproduction (Manzano et al., 2002a), adaptation to the climatic conditions of the tropical Andes, with an intrinsic population growth rate (r_m) greater than that of *T. vaporariorum* (Manzano et al., 2002a), and being

easily found (Manzano et al., 2002b). Although there are no published studies that support *A. fuscipennis* as a parasitoid of *B. tabaci*, from the presence of the parasitoids in this genus in Central America, it may be suggested that *B. tabaci* is an alternative host. The present study in beans was undertaken in laboratory conditions with the objective to determine the population parameters of the *B. tabaci* biotype B and determine whether *A. fuscipennis* is a potential natural enemy of this fly.

Materials and methods

Culture of *B. tabaci* biotype B and the majority of the experiments were performed in laboratory of the Experimental Center of the National University of Colombia, Palmira campus (CEUNP): 3° 24' N; 76° 26' O; 26 °C ± 2 °C; 80 ± 5% RH. Only one reproduction test for *A. fuscipennis* was made under different conditions.

Plants, whitefly and parasitoid. 15 day old plants of *Phaseolus vulgaris*, cultivar ICA-Pijao were used. The plants did not receive neither fertilizer nor insecticide applications, only an application of the fungicide Elosal® (1 cm³/lt) against the fungi *Oidium* sp.

The whitefly *B. tabaci* Biotype B was obtained from a colony held at CIAT (Cali, Colombia). Adults and pupas of *A. fuscipennis* were obtained from nymphs of *T. vaporariorum* cultured in CIAT, and from bean crops from the localities of Regaderos (municipality El Cerrito, 03° 38' 6.8" N; 76° 6' 5.1" O, 1743 m.a.s.l.) and Santa Helena (municipality Darién, 03° 56' 03" N; 76° 29' 18" O, 1485 m.a.s.l.), Cauca Valley, Colombia.

Longevity and reproduction of *B. tabaci* biotype B. In order to estimate the longevity and reproduction of *B. tabaci* biotype B clamp-cages (2 x 2 cm) were used as experimental units, where pairs of recently emerged whitefly (male-female) were placed; 25 pairs were used. Each day the cages were moved to new foliage areas until the females died. Measured variables were number of eggs/female/24 hours, and longevity of female. Each clamp-cage with a mating pair was considered as a repetition. Variables were only measured for the females.

Development and survival of nymphs and the proportion of females of *B. tabaci* biotype B. In order to record the development time and survival of nymphs, and the proportion of females of *B. tabaci* biotype B 30 adults were placed for 12 hours in clamp-cages (2 cm x 2 cm) for them to oviposit. A group of 300 eggs on a leaflet was selected randomly and their development was followed to adult emergence. Development time, was determined by averaging the duration (days)

emergence to adult. Survival (%) was calculated as the number of adults emerged from 300 eggs

Demographic parameters. Information on the development and survival time of immature individuals and the proportion of females was combined with that of reproduction to construct life tables ' $lxmx$ ', which were used to calculate demographic parameters for *B. tabaci*. Following Carey (1993) the following parameters were determined: net reproductive rate (R_0); generation time (T); and the intrinsic rate of population (r_m). This last was calculated according to the relationship: $\sum \exp(-r_mx)lxmx = 1$ where x is age, lx is survival at a specific age, and mx is the proportion of descendants of a female of age $s = x$. The pivotal age of the female of *B. tabaci* was considered as $x + 0.5$ according to Carey (1993).

Study of the interaction of *A. fuscipennis* with *B. tabaci* biotype B. Two different procedures were used. First, whitefly nymphs were exposed to parasitism by *A. fuscipennis*. Secondly, the searching behavior of individual females of *A. fuscipennis* was studied in order to determine the reaction of this parasitoid in the presence of nymphs of the *B. tabaci* biotype B.

In the first experiment, approximately 50 nymphs of *B. tabaci* biotype B per leaflet in stages n_1 y n_2 were confined for 24 hours (Manzano et al., 2002b) with recently emerged females of con *A. fuscipennis*, and subsequently moved to plants contained in two tulle cages (50 cm x 50 cm x 50 cm -9 plants/cage) to allow the development of the parasitoid. The experimental unit was the clamp-cage with a female of *A. fuscipennis* and the nymphs of *B. tabaci* biotype B. In this case 50 different females were used.

In the second experiment 10 female *A. fuscipennis* were confined in a transparent acetate cone on individual plants ($n = 6$) of the bean ICA-Pijao with n_1 (first) and n_2 (second) nymphal stages of *B. tabaci* biotype B (10 wasps per plant, 9 leaflets/plant, approximately 20 nymphs/ plant). After 24 hours the parasitoids were removed and the plants with the *B. tabaci* B nymphs moved to another cage to allow the development of the parasitoid.

In the third experiment a group of nine plants of the bean ICA-Pijao with a mean of 35 first and the second instars *B. tabaci* nymphs per leaflet (12 leaflet/plant) were exposed to an saturating release of recently emerged females of *A. fuscipennis* (> 30 parasitoids/leaflet) over 48 h. Both plants and insects were maintained at 19 °C, 75 ± 5% RH, conditions at which *A. fuscipennis* is normally maintained in CIAT.

Search behavior of *Amitus fuscipennis*. Nine plants of the bean ICA Pijao with three leaflets were exposed for 12 hours to adults of *B. tabaci*. When the nymphs reached developmental stages n_1 y n_2 the excess were removed with masking tape to leave a mean of 20 nymphs/leaflet. The leaflet still joined to the plant was placed upside down under a stereomicroscope. The number of nymphs per leaflet was counted and a map constructed to then determine the parasitized nymphs. A recently emerged female of Se *A. fuscipennis* was released in the center of the leaflet, and her behavior was followed for 45 minutes, and registered in the program The Observer 3.0 (Noldus, 1995) under conditions of $26\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and $80 \pm 5\%$ RH. The following behavior of 21 females was recorded H: walking (C), jumping (S), remaining still (P), body cleaning (L), finding a nymph (E), palpating the nymph with antennas (A), adopting oviposition position (O). If the nymph was acceptable for oviposition, an egg was laid, and then parasitism signs were recorded, such as: creamy white or dark gray color seen in nymphs of *T. vaporariorum* parasitized by *A. fuscipennis* (Manzano et al., 2002b). After removing the parasitoid, the plants were moved to a tulle cage until the signs of parasitism were seen, of an adult emerged.

Results and discussion

Longevity and reproduction

Mean longevity of *B. tabaci* biotype B was 34 days, during which time a mean fecundity of 2111 eggs was observed, with an oviposition rate of 6.09 eggs/day ([Box 1](#)). The fecundity observed was less than that reported by Romberg (1998) of 385.3 ± 114.73 eggs for the bean ICA-Pijao, but greater than that reported by Tsai y Wang (1996) of 83.5 eggs/female in a bean crop. Female survivorship is presented in [Figure 1](#). After day 16 the females began to die, and at day 76% of the population was still alive. At 43 days all 25 females were dead.

Cuadro 1. Parámetros demográficos de 25 hembras de *Bemisa tabaci* biotipo B en frijol ICA-Pijao (en laboratorio a $26\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ y $80 \pm 5\%$ de HR).

Longevidad (días) (Promedio \pm ES)	34 \pm 1.64
Rango	16-42
Fecundidad (huevos/hembra) (Promedio \pm ES)	211.4 \pm 15.10
Rango	53-347
Tasa promedia de oviposición (huevos/hembra por día) (Promedio \pm ES)	6.1 \pm 0.28
Rango	3.12 – 9.13
Tasa intrínseca de crecimiento poblacional, r_m	0.125
Tasa neta reproductiva, R_0	137.8
Tiempo generacional, T	43.8

The reproduction curve for the cohort of 25 females is presented in [Figure 2](#). The females of 39 days of age presented an increase in the amount of eggs deposited,

possibly explained by individual variation in the population (7 females). The intrinsic rate of population growth (r_m) was 0.125, the net reproductive rate (R_0) was 137.8, and the total lifespan (T) for those that survived at the beginning of each life cycle was 43.8 days.

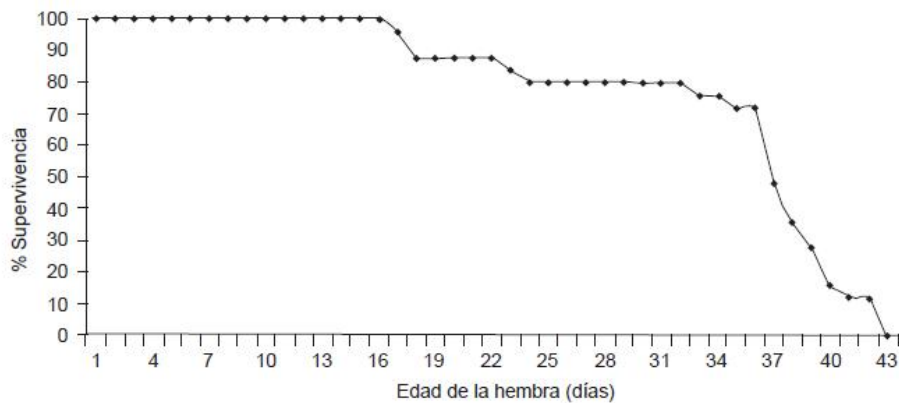


Figura 1. Curva de supervivencia de *Bemisa tabaci* biotipo B en *Phaseolus vulgaris* a $26 \pm 2 \text{ }^\circ\text{C}$ y $80 \pm 5 \%$ de HR (n = 25 hembras).

The observed r_m (0.125) was similar (0.12) to that reported by Tsai & Wang (1996) for *B. argentifolii* in bean at $25 \pm 1 \text{ }^\circ\text{C}$, but greater than that reported by Romberg (1998) of 0.098. These differing values may reflect the adaptability of the biotype B to the bean ICA-Pijao, a crop which has increased: nine years have passed between the two studies. In fact, ICA-Pijao is a variety of bean that may be recommended for the culture of the *B. tabaci* biotype B. On the other hand, these results may reflect the adaptation of *B. tabaci* to the climatic conditions of the Cauca Valley, facilitating its distribution and displacing the other species of whitefly *T. vaporariorum* (Rodríguez et al., 2005).

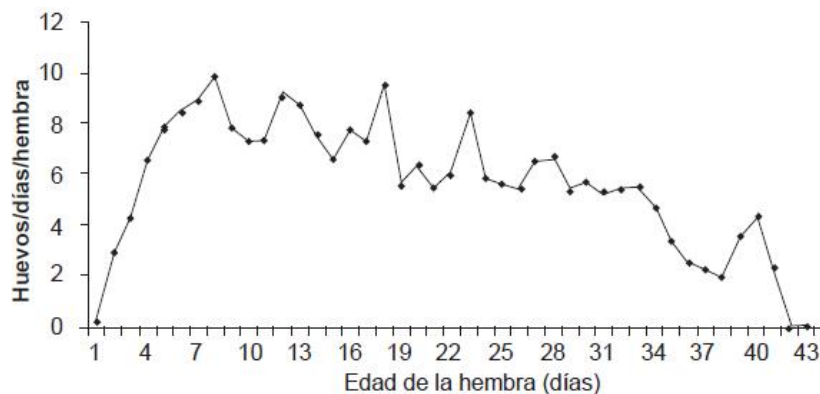


Figura 2. Curva de reproducción de *Bemisa tabaci* biotipo B en *Phaseolus vulgaris* a $26 \pm 2 \text{ }^\circ\text{C}$ y $80 \pm 5 \%$ de HR

Development, sex ratios and adult survival (%)

Based on 247 emerged adults, the mean development time for *B. tabaci* biotype B was 26.9 days (n = 247, SE = 0.18; range 23 - 35 days), the proportion of females was 66%, and the survival egg-adult was 82.3%. Development time for the Biotype B was shorter than that reported by Romberg (1998) of 29.6 days at a lower temperature (24.5 °C) and greater than 20.9 days, reported in bean (*Phaseolus vulgaris* L.) at 25 ± 1 °C by Tsai & Wang (1996).

Development of *A. fuscipennis* in *B. tabaci* biotype B

In the three experiments, it was not possible to reproduce *A. fuscipennis* in nymphs of the *B. tabaci* biotype B, despite the fact that the parasitoid passed through three types of confinement: clamp-cage, acetate cone, and tulle cage. These results confirm that *A. fuscipennis* does not parasitize *B. tabaci* under laboratory conditions.

Search behavior of *Amitus fuscipennis*

Search patterns. As soon as the female *A. fuscipennis* was placed on the leaflet infested with nymphs of *Bemisia* B it started to walk. The female moved quickly, and exhibited the following behavioral sequence: walking on the surface of the leaf with frequent pauses in order to clean its body – mainly from the wax coming from the whitefly nymph– or to examine the surface of the foliage with the antennae. When walking the parasitoid sometimes jumped and occasionally after jumping stayed still. Only on one occasion was contact made with a nymph of *B. tabaci* but it did not examine it with the antennae nor with the ovipositor. The ethogram that describes the sequence of behavioral events of *A. fuscipennis* is shown in [Figure 3](#). This ethogram is more complete than that reported for *A. fuscipennis* searching for nymphs of *T. vaporariorum* (Manzano et al., 2002b). The principal differences are the presence of jumps and the absence of antennae movement and also oviposition in *B. tabaci*. Nymphal color changes that indicate parasitism were not observed, Nor were individuals of *A. fuscipennis* retrieved. This confirms that *A. fuscipennis* does not parasitize *B. tabaci* biotype B, as is shown in the ethogram.

Frequency and duration of behavior. During the search, the parasitoid spent the majority of the time in walking (145 times, 46.4%), body cleaning (88 times, 35.6%) and staying still (122 times, 16.7%) ([Box 2](#)). *Amitus fuscipennis* was more active when looking for nymphs of *T. vaporariorum* (60.8%) and spent less time cleaning its body (5.7% in this activity) (Manzano et al., 2002b).

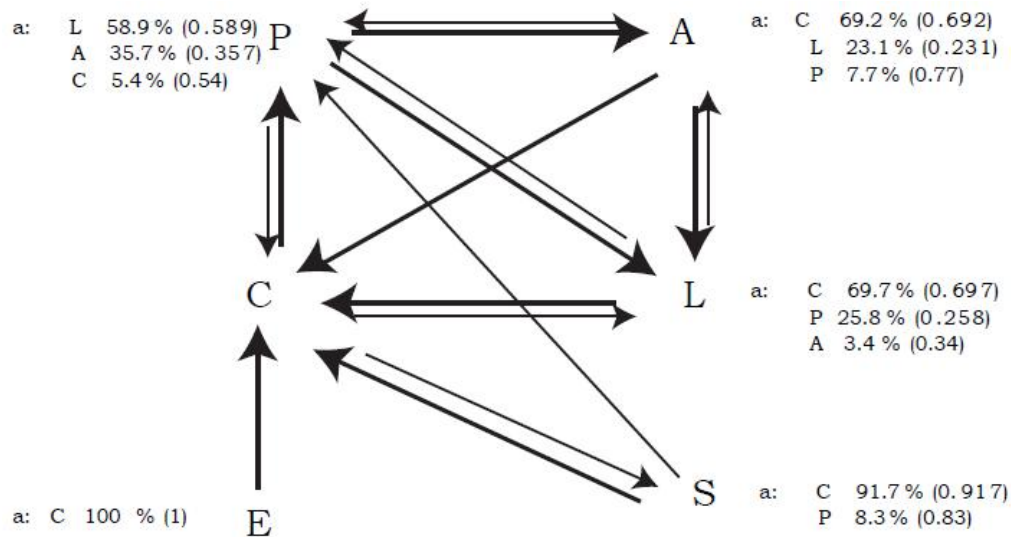


Figura 3. Secuencias, frecuencia y porcentaje de eventos de comportamiento de *Amitus fuscipennis* (n = 19 hembras) en foliolo de *Phaseolus vulgaris* infestado con ninfas de *Bemisa tabaci* biotipo B. C, caminar; P, permanecer quieta; A, antenas; L, limpiarse; S, saltar y E, encontrar el hospedero.

Only on one occasion did *A. fuscipennis* meet a nymph of *B. tabaci* (Box 2), although this was apparently accidental as it did not proceed to examine it with its antennae, as it does on meeting a nymph of *T. vaporariorum* (Manzano et al., 2002b; De Vis et al., 2003). When *A. fuscipennis* searches for nymphs of *T. vaporariorum*, the general pattern is to walk while moving its antennae, to find a host, examine it with antennae, oviposit and continue to walk, although the parasitoid may also remain still, clean itself with the antennae, and feed from liquid and molasses from the whitefly (Manzano et al., 2002b; De Vis et al., 2003). In contrast, when *A. fuscipennis* walked on the leaflets infested with *B. tabaci*, the parasitoid did not detect the presence of the nymphs of this species, as it walked between them without meeting them. This may be because the nymphs of *B. tabaci* are flatter than those of *T. vaporariorum* or because they emit a chemical substance that perturbs the parasitoid. In fact, the duration of presence on leaflets infested with four *T. vaporariorum* nymphs (Manzano et al., 2002b) was greater (9882 sec) than the mean duration reported in the present study (617.3 sec SE = 88.75) with 20 nymphs of *B. tabaci* per leaflet.

Cuadro 2. Frecuencia y duración de los eventos de comportamiento de hembras de *Amitus fuscipennis* (n = 19) en foliolos de frijol cv. ICA-Pijao infestado con ninfas de *Bemisia tabaci* biotipo B.

Comportamiento	Abundancia	Promedio de duración \pm	Porcentaje del tiempo
	(número de veces)	ES (segundos)	total observado
Caminar	145	299.7 \pm 36.79	46.4
Limpiarse el cuerpo	88	230.1 \pm 45.15	35.6
Permanecer quieta	122	108.2 \pm 23.12	16.7
Antenar	8	5.5 \pm 3.61	0.86
Saltar	17	2.2 \pm 1.05	0.35
Encontrar hospedero	1	0.095	0.01

The results indicate that *A. fuscipennis* does not parasitize *B. tabaci* under laboratory conditions, and there are no reports that it does so in the field. Previous observations (unpublished) indicate the opposite, but in this study no evidence was found for the parasitism of the Biotype B by *A. fuscipennis*. Due to its climatic adaptation *B. tabaci* biotype B increasing its altitudinal distribution to the high tropics, *A. fuscipennis* would not be a biological control for this bean crop pest.

Conclusions

- *Phaseolus vulgaris* cultivar ICA-Pijao is an adequate host to culture *Bemisia tabaci* biotype B at 26 ± 2 °C and 80 ± 5 % RH. Under these conditions, the intrinsic population growth rate of *B. tabaci* was 0.125, a similar value to those reported in other studies in bean.
- *Amitus. fuscipennis* does not parasitize *Bemisia tabaci* biotype B and this result supports the specificity of *A. fuscipennis* to greenhouse whitefly *Trialeurodes vaporariorum*. Thus, the species reported in Central America as *Amitus* sp. parasitizing *B. tabaci* cannot be *Amitus fuscipennis*.

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