# Biological control of whitefly on greenhouse tomato in Colombia:
## *Encarsia formosa* or *Amitus fuscipennis*?

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# Propositions

1. The statement that biological control in greenhouses in temperate zones is easier than in tropical areas (e.g. Gullino et al., 1999; van Lenteren, 2000) is not valid (Bueno & van Lenteren, submitted; this thesis).

   Gullino, M.L., et al. 1999. In: Albajes, R., et al. (Eds.). Integrated pest and disease management in greenhouse crops. Kluwer Academic Publishers, The Netherlands. p 1-13.
   van Lenteren, J.C. 2000. Crop Protection 19: 375-384.
   Bueno, V.H.P. & J.C. van Lenteren, submitted.
   This thesis.

2. The application of relatively safe pesticides (Hassan et al., 1991) by water sprays can still be very harmful for natural enemies. This may also apply for biopesticides.

   Hassan, S.A., et al. 1991. Entomophaga 36: 55-67.
   This thesis.

3. The intrinsic rate of increase of many whitefly parasitoids has probably been seriously underestimated.

   van Roermund, H.J.W. & J.C. van Lenteren. 1992. Wageningen Agricultural University Papers 92(3): 103-147.
   van Lenteren, J.C., et al. 1997. J. Appl. Ent. 121: 473-485.
   Drost, Y.C., et al. 1996. Proc. Exper. & Appl. Entomol, N.E.V. 7: 165-170.
   This thesis.

4. The requirement that growers' organisations participate in research projects in Colombia is an excellent way to force applied scientists to resolve real problems instead of producing science for science.

5. Most computer databases on agriculture or biology include only recent references exemplifying that our society has more respect for recent developments than for those of our predecessors.

6. The fact that acquiring scientific papers in "developed" countries is a question of few mouse clicks, while in "developing" countries it is a difficult to impossible task, is a serious limitation and disadvantage for scientists in the latter countries.

7. In contrast to the impression one gets from the media, the vast majority of Colombian people is very peaceful.


Propositions with the thesis "Biological control of whitefly on greenhouse tomato in Colombia: *Encarsia formosa* or *Amitus fuscipennis*?" by Raf M. J. De Vis.

Wageningen, December 18, 2001.

Raf M. J. De Vis

**Biological control of whitefly on greenhouse tomato in Colombia:**
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*Ter nagedachtenis van mijn ouders*

**Table of contents**

# 1. General introduction

## From chemical control to integrated pest management

Chemical control became the panacea of pest management in the 40's and 50's because of its easy use and the highly increased benefits. However, the appearance of a series of negative effects has made it now the black sheep of crop protection. These negative effects are mainly the impact on the environment and non-target organisms. Additionally, the use of chemicals put the growers at risk when applying chemicals in the crop and the consumers when eating products with chemical residues. But certainly, the appearance of resistance was the most important drive to develop new ways of pest control. This lead to the development of integrated pest management (IPM) programs where several ways of pest control, including physical, mechanical, ethological, chemical and biological control are combined. However, the way pest management is focused in most of these programs is still therapeutic and, although the newly used control measures may be less harmful, pest outbreaks still occur. Crop protection should therefore focus on a total system approach, based on agroecosystems that limit in a natural way the development of pests and where therapeutic measures are only used as a corrective rather than a standard procedure. Habitat manipulation and host plant resistance are likely to play an important role in this approach. Therefore, knowledge of the agroecosystems and interactions between the different trophic levels is very important (Lewis et al., 1997; van Lenteren, 1997).

The success of integrated approaches such as that of The Netherlands (Lewis et al., 1997), stimulated governments to develop rules to reduce the use of pesticides in favour of biologically based pest control. In this way, pesticide use has been reduced by 25-50 % or more in relatively short periods of less than a decade in Sweden, Denmark, Germany and The Netherlands (van Lenteren, 1997).

The most important general advantages of IPM, and more specifically biological control, are the reduced impact of pest control on the environment and a healthier final product. But, growers look first at the benefit of their crops and are not likely to be interested in increasing costs or risks of yield losses solely for the altruistic reason of using biological control. However, it has been shown that the currently used systems are cheaper or comparable in costs and in fact more reliable than chemical control, at least in greenhouse vegetable crops (Bolckmans, 1999; van Lenteren & Martin, 1999). On the other hand, because of the lower thresholds and the wider variety of products in ornamentals, more research and experience will be needed to come to biological systems with comparable costs and reliability as chemical control. Additionally, growers implement biological control as the main pest control strategy for many other reasons (van Lenteren, 2000a; Parella et al., 1999). These include (1) a healthy working environment; (2) beneficials do not cause phytotoxicity. Yield increases of 20-25 % have been found in greenhouses under biological control compared to chemical control in cucumber because of the reduced fytotoxicity; (3) the introduction of natural enemies is less time consuming and more pleasant; (4) natural enemies often find their way into areas of plant foliage, which may not be reached by sprays; (5) introduction of natural enemies is usually done at the start of the crop when time is available and once the natural enemies are established no further introductions are normally

necessary, in contrast with chemical control; (6) application of natural enemies does not interfere with harvest (safety period before harvest) or with activities in the crop (re-entry period after application); (7) natural enemies do not become resistant, as opposed to pesticides; and (8) producing healthy products, without damaging the environment is appreciated by the general public and improves the status of the farm and farmer.

Several strategies have been developed for the biological control of pests in agroecosystems (van Lenteren et al., 1996). Examples are given of whitefly pests that are controlled with the different strategies:

- Classical biological control or inoculative biological control: long term biological control is obtained by the introduction of a limited number of beneficials originating from an area other than where the pest occurs. This method has been used successfully to control pests that are introduced in a new habitat where no natural enemies of that pest are present or control by endemic natural enemies is insufficient. For example, the introduction of *Amitus spiniferus* and *Cales noacki* to control the woolly whitefly (*Aleurothrixus floccosus*), and *Amitus hesperidum* and *Encarsia opulenta* to control the citrus blackfly (*Aleurocanthus woglumi*), in citrus orchards in North America (DeBach & Rose, 1976; Flanders, 1969).
- Inundative biological control: high quantities of beneficials are released to obtain immediate control of the pest for a short period, without aiming to obtain an effect of these introductions for later pest generations. An example of this system is the use of *Encarsia luteola* and *Delphastus pusillus* to control *Bemisa argentifolii* in poinsettias in the USA (Heinz & Parella, 1994).
- Seasonal inoculative biological control: high numbers of beneficials are introduced once or several times at the beginning of a crop to obtain immediate and prolonged control by the establishment of the beneficials for the duration of the normally short growing season. Most of the biological control systems in greenhouse crops are based on this method. A very successful example is the use of *Encarsia formosa* to control *Trialeurodes vaporariorum* in greenhouse grown vegetables and ornamentals (van Lenteren et al., 1996).
- Conservation biological control: beneficials are conserved by several methods like habitat modification to avoid or mitigate seasonal pest outbreaks or to avoid costly reintroductions of the beneficials after crop renewal. This control method has gained much attention recently. An example is the creation of special habitats between greenhouses in Spain to conserve the natural enemies of whiteflies. At the end of the crop, whiteflies and enemies migrate to the between-greenhouse-habitat and after crop renewal immigrate into the greenhouse again (Alomar et al., 1991).

An important way of pest control, less frequently mentioned because of the lack of detailed studies is natural control. In greenhouse tomatoes on the Bogota plateau, aphids are usually under control during the complete cropping season by naturally occurring parasitoids of the genus *Lysiphlebus, Praon* and *Aphelinus*. Similarly, leafminers are controlled naturally by *Diglyphus begini* (De Vis & van Lenteren, 1999, De Vis & Fuentes, 2001). In greenhouse chrysanthemums in Brazil, natural enemies spontaneously invade greenhouses and seem to provide efficient control of most pests (Bueno & van Lenteren, 2001).

**Greenhouses and biological control**

Before the appearance of chemical pesticides biological control was already applied on large areas in greenhouses. After its discovery in England in 1927 (Spreyer, 1927), *E. formosa* was mass-reared at the Chestnut Experiment Research Station and used to control *T. vaporariorum* biologically in about 800 nurseries in the United Kingdom. During the 30's it was also shipped to European countries and even overseas to Canada and New Zealand (van Lenteren & Woets, 1988). Because of the appearance of synthetic organic pesticides, its use was discontinued in the 40's and 50's until resistance problems in the 70's caused severe outbreaks of *T. vaporariorum* in greenhouses (Wardlow et al., 1972) and the parasitoid regained the interest of farmers and scientists. In the 60's, the two-spotted spider mite *Tetranychus urticae* became resistant to pesticides and experiments showed that *Phytoseiulus persimilis* was an efficient predator (Bravenboer, 1963). By the end of the 60's, commercial application of biological control started and in less than two decades, *P. persimilis* was applied on more than 5000 ha. Also the use of *E. formosa* increased quickly and reached about 1000 ha in 1976, and stabilised during the '76-'84 period. At the beginning of the 80's, commercial solutions for the biological control of aphids, leaf miners and *Trips tabaci* became available and were applied on large greenhouse areas (van Lenteren & Woets, 1988). Simultaneously the use of *E. formosa* increased again. By 1993 it was applied on almost 5000 ha and *P. persimilis* on more than 7000 ha. In 1992, biological control was applied on almost 14.000 ha world-wide in at least 20 out of 35 countries that have a greenhouse industry (van Lenteren, 1992 & 1995). More than 90 percent of this area is dedicated to greenhouse vegetable production and biological control became the rule in tomato, cucumber and sweet pepper in several countries (Maisonneuve, 2001). Recently, biological control has also been spreading to ornamental crops. In gerbera crops in the Netherlands, 180 ha out of 210 ha are under biological control and a similar area of 180 ha out of 900 ha for cut roses (K. De Smet, personal communication, 2000). An area of 575 ha or 11 % of the total area of greenhouse ornamentals was under biological control in The Netherlands in 1999 (Maisonneuve, 2001), increasing to about 700 ha in 2000 (K. De Smet, personal communication, 2000).

While 20 years ago only two natural enemies were mass reared, now about 125 species are commercially available to control a wide range of pests and this number is likely to increase. These natural enemies can be divided in three main groups: parasitoids, predators and entomopathogens. As an example, we discuss the natural enemies used for whitefly control. *E. formosa* is still the most frequently used parasitoid but recently also *Eretmorecus eremicus* and *E. mundus* are used in the Mediterranean basin to control the whitefly complex *T. vaporariorum-Bemisia* spp. The use of a series of other parasitoids belonging to the genus *Encarsia* (6 species), *Amitus* (2 species) and *Eretmocerus* (6 species) is under study or development. Whitefly predators that are commercially applied are the coccinellid *Delphastus pusillus* and the mirid bug *Macrolophus caliginosus*, the former being a specific whitefly predator and applied to a rather restricted area, while the latter also attacks other pests, and therefore, its use has increased significantly in recent years. Several other predators are under evaluation: *Chrysoperla* spp. (2 species), *Dicyphus* spp., *Amblyseius* spp. and *Euseius* spp. Commercially, the whitefly pathogen *Verticillium lecanii* is applied on a limited scale, but several other fungi are under study including *Aschersonia* spp., *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces* spp. (van Lenteren & Martin, 1999).

The biological control systems are becoming more complete and in these systems pesticides are only used as an emergency measure (van Lenteren, 1997 & 2000b).

Although the use of greenhouses is generally conducive to pest development, its use may also favour the application of biological control. In temperate zones, where traditionally greenhouse biological control was developed, greenhouses are mostly made of metal and covered by glass. Before starting a new crop, the greenhouse can be cleansed of remaining pests and diseases from the previous crop. Isolation helps to reduce crop exposure to pest immigration. Sophisticated climate control systems regulate the climate close to optimal set points. This leads not only to better crop yields, but also to more stable relations between pests and natural enemies (Gullino et al., 1999; van Lenteren, 2000a).

In the Mediterranean basin and in subtropical or tropical areas, however, greenhouse structures are mostly made of wood and covered with plastic. This makes cleansing more difficult. The open structure allows pests to easily immigrate into de greenhouse. Climate control is reduced to opening and closing wall curtains causing large diurnal climate variations. The pest spectrum in those areas is generally also broader. This makes the implementation of a biological control system more difficult (Gullino et al., 1999; van Lenteren, 2000a). However, in tropical countries pests are often controlled naturally in greenhouse crops (De Vis & Fuentes, 2001, De Vis & van Lenteren, 1999; Bueno & van Lenteren, 2001).

Notwithstanding the previous mentioned difficulties, biological control increased significantly in the Mediterranean basin and is also spreading to the new greenhouse production areas in Asia, Africa and South America, stimulated by the commercial producers of natural enemies looking for new markets (Bolckmans, 1999). This development goes along with the dramatic increase in the world greenhouse area. In 1980, the world greenhouse area was estimated to be 150.000 ha increasing to 280.000 by 1995 and to 307.000 by 1999 (Gullino et al., 1999). This area is likely to increase significantly in the coming years. The different ecological conditions and pest spectra in those new greenhouse production areas, however, will need research to adapt the currently used biological control systems to the local conditions. For the locally occurring pests, natural enemies have to be found, evaluated and control systems (way, timing, frequency of introduction, dosage, etc.) developed. The same can be said for pests that are introduced into other countries through increased international trade (van Lenteren, 2000b).

**Present situation of greenhouse production in Colombia**

*Area and crops*
The Colombian greenhouse industry is almost completely dedicated to cut flowers with more than 4500 ha in production, making Colombia the second exporter of cut flowers in the world. This industry developed during the past 20-30 years. Recently the flower industry has extended to Ecuador where lower production costs have helped the expansion of the area in greenhouse production from virtually nothing to more than 1200 ha in a decade.

Colombian greenhouse vegetable production was incipient until quite recently when producers began transferring field grown tomato crops to greenhouses. Severe pest and disease outbreaks in field grown tomato crops often cause total loss of the crop before harvest. The use of greenhouses reduces this kind of risk, increases production and the final benefit. Greenhouses are currently used for tomato production in the intermediate climate

4

zone (altitude 1800-2000 m) where traditionally field grown tomatoes are produced, but also in cold climate zones such as the Bogota Plateau (altitude 2650 m). The extension of greenhouse vegetable productions is estimated to be at least 250 ha, compared to about 10 ha five years ago (Harold Ubaque, personal communication). This area is expected to increase significantly in the coming years, as other crops such as lettuce, cucumber, eggplant and sweet pepper are gradually being transferred to greenhouses.

### Greenhouse structures
The greenhouses used in Colombia have an open structure. They have a fixed open ridge, no movable vents in the roof, manual side wall curtains, no heating and polyethylene is used as a covering material. Because of the open structure, the greenhouse climate can hardly be controlled and depends almost entirely on the outside climate, resulting in high day and night variation. Because of the altitude, the climate in greenhouses situated on the Bogota Plateau is rather cool with a mean daily temperature of 14 to 15 °C. Night temperatures vary between 10 and 15 °C and day temperatures between 15 and 25 °C. During the driest months of the year (January, February and August), night temperature can decrease to 0 °C or lower (Cooman et al., 1999).

### Pest control in Colombian greenhouses.
Integrated pest management strategies have been developed and implemented since the 90's in the flower industry in Colombia, but these strategies do not include biological control and depend predominantly on chemical control (Pizano, 1997). Biological control of leafminers has been developed and implemented in *Gypsophyla* sp. by introduction and conservation of *Diglyphus begini* (Jose Ricardo Cure, personal communication). Recently (2000), biological control is under development in a small number of flower farms (Emilio Rojas, Andres Camacho, personal communication). The total area of ornamentals where biological control of insect pests is applied was 8.7 ha in 1999 (Maisonneuve, 2001).

Integrated pest management techniques or biological control are hardly known by vegetable growers, so that pest control is completely based on pesticides. In field grown tomatoes, sprays with several pesticides are applied generally once or twice a week and during a cropping season up to 30 applications are made (Zuluaga et al., 1995). This high frequency of pesticide use has lead to high levels of pesticide resistance, as was recently found by Cardona et al. (2001) for *T. vaporariorum* and *B. tabaci* in annual crops throughout Colombia and Ecuador. When managed properly, greenhouses may reduce pest and disease pressure. However, many of the growers that recently started growing greenhouse tomatoes are used to producing field grown tomatoes in the traditional way, and are not familiar with greenhouse management, leading to bad harvests and pest and disease outbreaks, and high quantities of pesticides are still applied.

The high number of applications in tomato crops in the intermediate climate is also caused by the broad pest spectrum. This includes the greenhouse whitefly (*T. vaporariorum*), leaf miners (*Liriomyza* spp.), aphids (*Macrosiphum* sp., *Aphis gossipii*, *Myzus persicae*, etc.), lepidopteran pests attacking fruits (*Neoleucinodes elegantalis*, *Tuta absoluta*), lepidopteran pests attacking leaves (*Spodoptera* sp., *Plusia* sp., *Trichoplusia* sp.), mites (*Tetranychus urticae*, *Aculops lycopersici*), dipteran pests mining stems and attacking recently pollinated ovaries (*Melanagromyza caucencis*, *M. tomaterae*, *M. socolena*), coleopteran pests attacking leaves (*Epitrix* sp., *Diabrotica* sp., *Systena* sp.) and thrips (*Trips palmi*). On the Bogota

Plateau, however, this range is limited to leaf miners, aphids, *T. absoluta, A. lycopersici* and the greenhouse whitefly (De Vis & Fuentes, 2001), making pest control much easier. *Bemisia* spp. are present in Colombia, but are restricted to the lower altitudes and since greenhouse tomatoes are generally grown at higher altitudes, *T. vaporariorum* is normally the only whitefly found is these production systems. In a survey, Quintero et al. (2001) found that *B. tabaci* was found at altitudes between 50 and 1350 m, *B argentifolii* between 0 and 890 m and *T. vaporariorum* between 600 and 2860 m.

Since 1995, greenhouse tomatoes have been produced at the Horticultural Research Centre of the Jorge Tadeo Lozano University and the greenhouse whitefly *T. vaporariorum* has been the dominant pest. Most other pests have been satisfactorily kept under control by naturally occurring parasitoids. *Therefore, with a biological solution for the control of* T. vaporariorum, *the production of tomatoes without insecticides should be possible in greenhouses on the Bogota Plateau. This is the subject of the present thesis. For the biological control of* T. vaporariorum *two parasitoids were evaluated: the introduced* E. formosa *and the native* Amitus fuscipennis.

### Encarsia formosa and Amitus fuscipennis

*E. formosa* belongs to the family of the Aphelinidae and was described in 1924 by Gahan from species collected in Idaho, USA (Gahan, 1924). The genus *Encarsia* contains more than 200 species (Polaszek et al., 1992), parasitoids of whiteflies and armoured scales (van Lenteren et al., 1997). Commercially, only *E. formosa* is produced to control *T. vaporariorum* and *Bemisa spp.* in greenhouses, but a series of other *Encarsia* species is under study (van Lenteren et al., 1997; van Lenteren & Martin, 1999). *E. formosa* has been introduced in countless countries around the world and was introduced in Colombia for the first time in the 1986. The strain imported in Colombia originated from Koppert in the Netherlands (A. Lopez-Avila, personal communication). Koppert started its rearing with specimens obtained from the UK where it was found in a botanical garden in the 70's (Ravensberg, 1991). Before A. Lopez-Avila introduced it in Colombia, *E. formosa* was reported in the department of Antioquia by Bustillo et al. (1985). The *E. formosa* used in this research originated from Biobest in Belgium (1995), but also parasitoids descending from those imported by A. Lopez-Avila were introduced once in our rearing.

*A. fuscipennis* belongs to the family of the Platygastridae, was described by MacGown & Nebeker in 1978, and re-described by Viggiani in 1991. A total of 19 *Amitus* species have been described (reviewed by Manzano, 2000), all parasitoids of Aleyrodidae. Of these, two species control whiteflies that are greenhouse pests, *A. fuscipennis* is a parasitoid of *T. vaporariorum* and *A. bennetti* of *Bemisia tabaci*. It is not clear, however, if both species have only one species as host. *A. fuscipennis* has been recorded in Mexico, Guatemala, Costa Rica, Dominican Republic and Colombia (MacGown & Nebeker, 1978). In Colombia, it is abundant in the intermediate climate zones where *T. vaporariorum* is also very abundant.

Both *E. formosa* and *A. fuscipennis* are thelytokous, induced by the rickettsia Wolbachia (Zchori-Fein et al., 1992; Manzano, 2000). This characteristic can be advantageous for biological control since it may lead to a higher intrinsic rate of increase and better control at low densities because mating is not necessary and no hosts are 'wasted' to produce males. Additionally, rearing is easier and cheaper (Stouthamer, 1993).

*E. formosa* is synovigenic (Van Keymeulen & Degheele, 1977) while *A. fuscipennis* is pro-ovigenic (Viggiani, 1991; Manzano, 2000). Flanders (1950) originally defined pro-ovigenic parasitoids as those that stop ovigenesis before oviposition starts and synovigenic as those that continue ovigenesis after the beginning of the oviposition throughout the adult life of the female. In post-Flanders papers, pro-ovigenic parasitoids are defined as those that emerge with their full complement of mature or almost mature eggs as opposed to synovigenic parasitoids that emerge with no or only few fully developed eggs and the majority of the eggs mature during adult life (e.g. Jervis & Kidd, 1986; Heimpel & Collier, 1996, Jervis et al., 2001). It seems, however, that not all parasitoids can be classified as strictly pro-ovigenic or strictly synovigenic (Donaldson & Walter, 1988) and recently Jervis et al. (2001), classified parasitoids on a pro-ovigenic-synovigenic scale. At emergence, *E. formosa* females have about 8-10 mature eggs available (van Vianen & van Lenteren, 1986), but time is needed to develop a full batch of about 15 eggs (van Lenteren et al., 1987). *E. formosa* produces yolk containing (i.e. anhydropic) eggs (Van Keymeulen & Degheele, 1977) and feeds on hosts and/or honeydew to obtain the necessary nitrogenous compounds for egg maturation (van Alphen et al., 1976; van Lenteren et al., 1987). Generally, anhydropic eggs do not increase in size during incubation while hydropic (i.e. yolk deficient) eggs can expand 10-20 fold after deposition in the host (Dowell, 1978; Jervis & Kidd, 1986). Pro-ovigenic parasitoids, including *A. fuscipennis*, do not host-feed (Heimpel & Collier, 1996, Jervis et al., 2001, Manzano, 2000), and although no references were found is seems that pro-ovigenic parasitoids produce only hydropic eggs.

Synovigenic species are long-lived while pro-ovigenic species have a short longevity (Flanders, 1950; Donaldson & Walter, 1988). Flanders (1950) suggested that pro-ovigenic species might exert control on high-density host populations, and that large numbers of parasitoids would be needed to search a certain area with low host density because of their short life span. Synovigenic species might be more effective in biological control because of the high longevity and thus better in controlling low host density populations.

## Can *E. formosa* control *T. vaporariorum* in unheated greenhouses on the Bogota Plateau?

*E. formosa* is probably the best known parasitoid and is used world-wide to control *T. vaporariorum* and *Bemisia* spp. in greenhouse crops. More than three decades of continuous research have unravelled the tritrophic system host plant-*T. vaporariorum-E. formosa*. Van Roermund & van Lenteren (1992a & 1992b) summarised *T. vaporariorum* and *E. formosa* demographic data and modelled temperature effects on life history parameters as function of host plant and host stage. Noldus & van Lenteren (1989) and van Lenteren & Noldus (1990) reviewed the ecology of the *E. formosa* and *T. vaporariorum* respectively. Van Roermund & van Lenteren (1995a & 1995b) determined the foraging behaviour of *E. formosa* on tomato leaflets. This gives the following general picture:

*E. formosa* has a higher intrinsic rate of increase than *T. vaporariorum* at temperatures higher than 14 °C. *E. formosa* does not distinguish hosts from a distance. Parasitoids land randomly on leaves or leaflets. Searching on a leaflet is also at random and the walking speed and walking pattern are not influenced by host encounters. Residence time on clean tomato leaflets is 20 minutes and does not change on whitefly infested leaflets where hosts are not discovered. However, residence time increases to 100 minutes when whitefly honeydew is

7

detected. After an encounter with a host, the giving-up time (period spent on the leaf after a host contact) is 20 minutes, leading to higher residence times on infested leaflets than on clean leaflets. Oviposition in an unparasitized host increases giving up time to 40 minutes leading to arrestment on leaves where suitable hosts are found. Host acceptance depends largely on the host stage, but unparasitized hosts of the preferred developmental stage (L 3-4) are accepted in 70% of the encounters. Superparasitism is generally lower than 10 %.

All this knowledge was brought together in a tritrophic model that helped to clarify how the system works for greenhouse tomatoes (van Roermund et al., 1997) and why it can fail under certain conditions (van Lenteren et al., 1996). This indicated that the parasitoid is able to suppress *T. vaporariorum* population below the economic threshold, but that it does not create a stable pest-enemy equilibrium. The model identified a series of parameters of the three trophic levels that influence particularly the outcome of biological control of *T. vaporariorum* by *E. formosa*. It simulated a significant higher reduction of the *T. vaporariorum* population when varying the following input parameters by 25 %:

- *Host plant related parameters*: reduction of leaf initiation rate, reduction of leaflet area.
- *T. vaporariorum related parameters*: decrease of the whitefly development rate, increase of the diameter of the host stage.
- *E. formosa related parameters*: increase in the development rate, increase of the longevity, increase of the number of released adults, increase of the mean flight distance between plants, increase of the width of the searching path, increase of the walking speed, increase of the walking activity, increase of the host acceptance, decrease of the tendency of changing from the lower to the upper leaf side, and increase of the tendency of changing from the upper to the lower leaf side.

Van Lenteren et al. (1996) listed eight conditions under which biological control could fail: when the host is too good for the whitefly, when the host plant is bad for the natural enemy (e.g. leaf hairs), when bad quality *E. formosa* are used, when climate conditions are unsuitable, when there is interference of pesticides, when leaves with immature parasitoids are removed from the crop, when parasitoids are introduced too late, or when the greenhouse is too small. Two of those conditions (high quality host plants and temperature conditions unsuitable) are present together when growing beef tomatoes in unheated greenhouses in the high altitude tropics such as the Bogota Plateau.

With respect to the first condition, studies by van Es (1987) have shown that the greenhouse whitefly population develops better on beef than on round tomato varieties. In this research, the fecundity increased by a factor of 2 or more and adult longevity by a factor of 1.5-1.9 on beef tomato varieties when compared to a round tomato cultivar. Therefore, biological control may become more difficult on this better host plant.

With respect to the second condition, at temperatures lower than or equal to 18 °C, the fecundity and oviposition frequency of *E. formosa* are lower than those of *T. vaporariorum* (van Roermund & van Lenteren, 1992a & 1992b). At 15 °C, its intrinsic rate of increase ($r_m$) is estimated to be only slightly higher than that of *T. vaporariorum* (van Lenteren et al., 1997), suggesting that biological control should be possible under these conditions. However, to be successful in the field, the parasitoid has to be efficient in finding and parasitizing its host. Christochowits et al. (1981) showed that *E. formosa* used in the 70's and the 80's in The Netherlands could fly at temperatures around 17 °C. Van Roermund & van Lenteren (1995a), showed that *E. formosa* did not fly any more at 18 °C and we used parasitoids imported form Europe. Because of the low temperature in greenhouses on the Bogota Plateau, the

parasitoid's searching efficiency could be reduced seriously. On the Bogota plateau, day length varies between 11.7 and 12.3 hours and a reduction in day length of 1 hour increased the simulated *T. vaporariorum* population by 19 % (van Roermund et al., 1997). However, greenhouse experiments in The Netherlands showed that *E. formosa* is able to control whitefly population in greenhouses with a relatively low temperature regime (18°C day and 7°C night) during winter (Hulspas-Jordaan et al., 1987). Although in that experiment day length was shorter and radiation lower in comparison with the Colombian situation, these conditions last only a few months at the beginning of the year. In contrast, unheated greenhouses on the Bogota Plateau have a constant low mean temperature during the whole year. Therefore biological control of *T. vaporariorum* by *E. formosa* is not obvious under these conditions and had to be tested.

## Is *A. fuscipennis* an alternative to *E. formosa*?

In Colombia, *A. fuscipennis* can perform very well in the field without any artificial introduction. It was found parasitizing up to 80 % of *T. vaporariorum* pupae in heavily infested tomato crops in Fusagasuga in the intermediate climate zone, near Bogota in Colombia (De Vis & van Lenteren, 1999). Marqués & Valencia (1991) compared its performance with *E. formosa* on greenhouse chrysanthemum in Colombia with two introductions of 250 adults·m$^{-2}$ at week 7 and 9 after transplant. At week 10 and 12 after transplant, they observed a parasitization of respectively 32 and 24 % by *A. fuscipennis* compared to respectively 9.4 and 15 % by *E. formosa*. *A. fuscipennis* was found for the first time at the Horticultural Research Centre, situated on the Bogota Plateau, parasitizing naturally *T. vaporariorum* in greenhouse tomatoes in 1995. Preliminary experiments in 1996 showed that *A. fuscipennis* could keep *T. vaporariorum* under control for three months (De Vis, unpublished results).

Based on the previous information and according to the selection criteria for parasitoids in greenhouse systems (van Lenteren, 1986, van Lenteren & Woets, 1988), *A. fuscipennis* was deemed a suitable candidate for seasonal inoculative biological control. According to the parasitoid evaluation flow diagram of van Lenteren (1986), the following steps in the evaluation of a new parasitoid are determine its innate capacity for population increase ($r_m$) at the temperature the parasitoid it to be used. Then, if the $r_m$ is higher than that of *T. vaporariorum*, the searching efficiency should be evaluated. According to the previous described model, the most important characteristics that define a parasitoid's searching efficiency are walking speed, walking activity, probability of host acceptance and the arrestment effect when searching on clean and infested leaflets (van Lenteren & van Roermund, 1999). If the searching efficiency is high then greenhouse trials should confirm if the parasitoid is able to effectively control *T. vaporariorum*.

## Is the use of both parasitoids an alternative?

Both *E. formosa* and *A. fuscipennis* are thelytokous and thus primary parasitoids. The use of both species will lead to interspecific competition, rather than intraguild predation. Intraguild predation would occur when the larvae of one parasitoid species would develop at the expense the larvae of another species. This occurs e.g. in heteronomous autoparasitoids

where males are produced hyperparasitically in developing females of another species (Rosenheim et al., 1995).

Competition takes place on two levels: that of the adults searching for hosts and that of the larvae competing within the host (Mills, 1999). So studies related to interspecific competition should evaluate both adult parasitoid behaviour as well as larval competition. The latter is, however, more difficult to study as it occurs within the host.

An important aspect of the foraging behaviour that may influence the outcome of interspecific competition is interspecific host discrimination. Few studies on interspecific host discrimination have been done with whitefly parasitoids and all of them include a heteronomous hyperparasitoid. For these type of parasitoids, difference should be made between primary host discrimination (for the allocation of female eggs) and secondary host discrimination (for the allocation of male eggs) (Artigues et al., 1992). Only 2 studies were found on the interspecific host discrimination for the allocation of primary developing parasitoid eggs in whiteflies. All of the four studied parasitoid species (*Amitus hesperidum & Encarsia opulenta*, Dowell et al., 1981; *Encarsia lutea & Eretmocerus mundus*, Gerling & Foltyn, 1987) did not show host discrimination. Although Dowell et al. (1981) stated that *E. opulenta* showed interspecific host discrimination, their conclusion seems to be erroneous (see Chapter 8, discussion). More studies have been done on the secondary host choice of heteronomous hyperparasitoids. *Encarsia tricolor* (Williams, 1991; Avilla et al., 1991) and *Encarsia transvena* (Hunter & Kelly, 1998), prefer heterospecifics as a secondary host to produce male offspring while *Encarsia pergandiella* does not (Buijs et al., 1981; Pedata & Hunter, 1996).

A second aspect is the outcome of the larval competition of primary parasitoid larvae. Gerling and Foltyn (1987) found that *E. lutea* was superior to *Eretmocerus mundus* at the larval stage, irrespective of the order of the parasitization by the two species. No other studies were found for heterospecific larval competition in whitefly parasitoids.

In two classical biological control programs, two pro-ovigenic *Amitus* species have been used together with other synovigenic parasitoids to control high-density whitefly pest in citrus orchards (Dowell, 1979; DeBach & Rose, 1976). Studies on the population development of the parasitoids showed that in both cases the *Amitus* species became dominant and exerted rapid control on the high-density pest populations. Once the pests were brought to low densities, however, they were displaced by the other parasitoids. Flanders (1969) stated that the short longevity of the pro-ovigenic *Amitus* was the main cause for its displacement by the other parasitoids. Although in our case two thelytokous species will compete (in the previous examples arrhenotokous species were involved), the short longevity of the *Amitus* species may indicate that *A. fuscipennis* will also have a relatively short longevity and that its efficiency in greenhouse tomatoes may greatly depend on that characteristic, rather than on its thelytoky.

## Aim and outline of this thesis

The general objective of this thesis was to contribute to the development of an applicable biological control system for the greenhouse whitefly in tomatoes under the specific greenhouse conditions of the Bogota Plateau in Colombia.

First, we determined longevity, fecundity, oviposition frequency and intrinsic rate of increase of *T. vaporariorum* on the beef tomato cultivar "Boris" under local greenhouse conditions and estimated the possibilities for biological control by *E. formosa* (Chapter 2).

Secondly, the potential of *A. fuscipennis* was evaluated in laboratory experiments. Its life history was determined on tomato at 15, 20, 25 and 30 °C and compared to that of *E. formosa*. With this information we calculated the intrinsic rate of increase in order to confirm whether it was a candidate for biological control (Chapter 3).

To be successful in the field, the parasitoid has, in addition to a sufficient intrinsic rate of population increase, to be efficient in finding and parasitizing its host. Therefore, as a second part in the evaluation, the searching efficiency of *A. fuscipennis* on tomato leaflets was established (Chapter 4).

To evaluate if both parasitoids could be used together, an interaction experiment was done: the host handling and oviposition behaviour of each parasitoid (*E. formosa* and *A. fuscipennis*) was evaluated with respect to *T. vaporariorum* larvae previously parasitized by the other and the outcome of *T. vaporariorum* larvae that were multiparasitized defined. The foraging behaviour, as function of host stage of the two parasitoid species was compared (Chapter 5).

The two parasitoid species were evaluated under the conditions of greenhouses on the Bogota plateau. During three consecutive tomato crops planted in a plastic greenhouse and a glasshouse, the biological control of *T. vaporariorum* by *E. formosa* was tested and recommendations were developed for its use under local conditions (Chapter 6). The use of *A. fuscipennis* with or without *E. formosa* was then tested under the same conditions and recommendations for the use of *A. fuscipennis* were developed (Chapter 7).

In chapter 8 the results are summarised and discussed, and suggestions are made for future research.

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11

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## 2. Longevity, fecundity, oviposition frequency and intrinsic rate of increase of the greenhouse whitefly, *Trialeurodes vaporariorum* on greenhouse tomato in Colombia

### Abstract

Longevity, fecundity, oviposition frequency and intrinsic rate of increase ($r_m$) of the greenhouse whitefly were determined on beef tomato cv. Boris, in an unheated, automated greenhouse with an average temperature of 16 °C and an average RH of 81 %. The mean longevity of females and males was 36.5 and 47.2 days, respectively. The fecundity was 208.5 eggs per female, the oviposition frequency was 5.7 eggs per living female per day, and the intrinsic rate of increase was 0.0645. These values are higher if compared to results of previous research on tomato in general, but it is known that beef tomato cv.'s are better host plants than round tomato cv.'s. When compared to the results of a previous study on beef tomato in The Netherlands, the longevity was shorter, the oviposition frequency was higher and the fecundity was similar. The Colombian whitefly strain shows differences in longevity and oviposition frequency when compared to European whitefly strains. The estimated $r_m$ of *Encarsia formosa*, parasitoid of *T. vaporariorum*, was 0.0974 and is considerably higher than the $r_m$ of the greenhouse whitefly determined under the same experimental conditions. This is a promising indication for biological control of greenhouse whitefly in Colombian greenhouses.

### Introduction

Biological control of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) by *Encarsia formosa* (Gahan) is normal practice on more than 5000 ha in countries with important greenhouse industries (van Lenteren, 1992 & 1995). Decades of research have revealed the relationship between *E. formosa* and its host, as well as the host plant. In most cases, *E. formosa* has a higher progeny production – a higher intrinsic rate of increase ($r_m$) - than *T. vaporariorum*, and can maintain its host well below economic thresholds. Nevertheless, in some cases, biological control is not successful. When host plant quality is excellent for *T. vaporariorum* its $r_m$ increases considerably while the $r_m$ of *E. formosa* remains constant. Low greenhouse temperatures can also have a negative influence on biological control by reducing the $r_m$ of *E. formosa*, and its activity and mobility (van Lenteren et al., 1996).

In Colombia, the production area of greenhouse tomatoes is increasing. The climate of unheated greenhouses on the Bogota plateau is cool with a mean daily temperature of 15 to 16 °C. *T. vaporariorum* is an important pest in this production system. Growers would like to use biological control. However, no data are available on the development of *T. vaporariorum* and its parasitoid *E. formosa* under these specific conditions. The present research forms a first step in the development of a biological control system for *T. vaporariorum* under Colombian greenhouse conditions.

Studies by van Es (1982) have shown that the European strains of the greenhouse whitefly develop better on beef tomato than on round tomato. The fecundity on beef tomato cv. Dombo and Portanto was 216 and 219 respectively, more than double as high as the fecundity on a round tomato cv. Moneydor, which was 90. The longevity on the cv.'s Dombo and Portanto was 57 and 69 days respectively, compared to only 37 days on the cv. Moneydor. The immature mortality and the time required for immature development, however, were similar for both types of tomatoes. Other studies have shown differences in population growth parameters of *T. vaporariorum* strains. An example is the difference between Dutch and Hungarian whitefly strains (Van Lenteren et al., 1989), where Hungarian

15

whiteflies had an immature mortality of 36.9 % compared to 76.7 % for Dutch whiteflies, both on Hungarian sweet pepper. Because of this big difference in immature mortality, the net reproduction rate (Ro, number of females/female) was higher for the Hungarian whitefly strain, although the fecundity was lower. The Ro was 9.7 and 15.5 for the Dutch and Hungarian whitefly strains respectively. Average life span, development time and fecundity were, on the contrary, higher for the Dutch whitefly strain. It is assumed that these differences have developed during the past century as *T. vaporariorum* was introduced for the first time in 1856 on the European continent, when it was accidentally imported into the UK, supposedly from the Americas. It may be well possible that the Colombian strain of *T. vaporariorum* differs from the European strains.

In this paper, we present data on longevity, fecundity, oviposition frequency and the intrinsic rate of increase of a Colombian strain of *T. vaporariorum* under local greenhouse conditions on the tomato cv. Boris.

## Materials and methods

The experiment took place at the Horticultural Research Centre (CIAA) of the Jorge Tadeo Lozano University in Chia, on the Bogota Plateau, at 2600 m above sea level in Colombia.

An unheated greenhouse with automated ventilation and continuous climate monitoring was used for the experiment. The climate computer calculated and saved mean temperature and relative humidity measurements every ten minutes. Based on these data, hourly mean temperature, relative humidity were calculated and the mean and standard deviation of the temperature and relative humidity for each hour of the day, with days as replicates.

Seven-week-old tomato plants of the cv. Boris (Bruinsma Seeds, 's Gravenzande, The Netherlands) were transplanted into two beds on March 6, 1998. At the beginning of the experiment (23/4), the plants measured 77 cm and had 17 leaves. Fertilisation took place based on regular soil analyses. The soil was fertilised prior to transplant and as of 16 April, weekly until the end of the experiment.

Adult whiteflies were removed three times a week with an aspirator to prevent them from ovipositing on the leaves that would be used for the experiment The tomato russet mite, *Vasates lycopersici* was controlled two times with sulphur (Elosal, 4 ml/l). The application was done in two stages. Before spraying, the clip cages in which we kept the whiteflies for the experiment were moved to the plants of one bed and the other bed was spayed. Two days later, the whiteflies were all transferred to clean clip cages and placed on the plants of the previously treated bed and the untreated bed was sprayed.

Tomato leaves containing whitefly pupae from the CIAA's rearing unit were placed in a climatized room at 23 °C on April 22. The following day, whiteflies were collected and separated into pairs, one male and one female. Fifty pairs were mounted on the tomato plants in the greenhouse, using clip cages of two cm in diameter. The cages were mounted on the third expanding leaf of the plant, where the whiteflies would normally be found, one clip cage per plant. Every two days, the clip cages with the whiteflies were moved to a new leaf of the same plant. Before moving, the new leaflet was controlled for the presence of whitefly eggs and if eggs were found, the leaflet was rejected. Mortality was recorded and if a male died before the female, a new male was introduced into the clip cage. The leaflets used the previous 2 days, were detached and brought to the laboratory where the number of deposited eggs were counted using a stereomicroscope. The trial ended on July 18.

The mean and standard deviation of the longevity of males and females was calculated, assuming that an individual died the day before it was found dead in the clip cages. The mean and standard deviation of the number of eggs per introduced female per two days, the number of eggs per living female per two days, and total fecundity were also calculated. The average oviposition frequency was calculated as mean fecundity/mean longevity. The intrinsic rate of increase ($r_m$) was calculated using the two equations of Andrewartha et al (1954):

1. $r_{m1} = (\ln R_{o1})/T_1$

   where $R_{o1}$ is the net reproductive rate (or number of females produced by one female) and $T_1$ the generation time. $R_{o1}$ is calculated as the product of fecundity, immature survival rate and the sex ratio. $T_1$ is calculated as the sum of the immature development time and the time required until 50 % of the eggs are laid.

2. $r_{m2} = (\ln R_{o2})/T_2$

   where $R_{o2}$ is the net reproductive rate and $T_2$ the mean generation time calculated as:

   $R_{o2} = \Sigma L_x M_x$ and $T_2 = \Sigma L_x M_x X / \Sigma L_x M_x$,

   where $L_x$ and $M_x$ are the age-specific survival rate and the age-specific fecundity, respectively (x = age).

## Results

The mean temperature during the trial was $16.0 \pm 5.1$ °C and the mean relative humidity was $81 \pm 13.3$ %. The mean temperature curve (Figure 1A) showed that the mean night temperature was slightly above 10 °C with a small standard deviation. During the day, the temperature reached a platform of almost 23 °C with a higher standard deviation than at night. The mean relative humidity (Figure 1B) was above 90 % during the night showing hardly any variation. During the day, the mean relative humidity showed a minimum of 65 % with a standard deviation up to 12.5 %. During the first two weeks of the experiment and during day 28-35, the temperature was higher than the mean temperature (Figure 1C). The mean daily relative humidity was lower during the first month and oscillated between 74 and 92 %.

The mean longevity of the females was $36.5 \pm 18.2$ days with a minimum of 7 and a maximum of 85 days. The mean longevity of the males was $47.2 \pm 20.0$ days with a minimum of 7 and a maximum of 89 days. The total fecundity was $208.5 \pm 146$ eggs per female with a maximum of 581 and a minimum of 20 eggs. The average oviposition frequency was 5.7 eggs per living female per day.

The first females died after 10 days and the mortality is almost constant until day 56 (2% / day) and then becomes smaller (0.33% / day) (Figure 2A). The number of eggs per introduced female per two days increased fast to a maximum of 15.5 on day 8 after the start of the trial to decline slowly afterwards. The number of eggs per living female reached its maximum at the same day and then declined stepwise (Figure 2B). The standard deviation of the oviposition frequency was most of the time above 7 (Figure 2C).
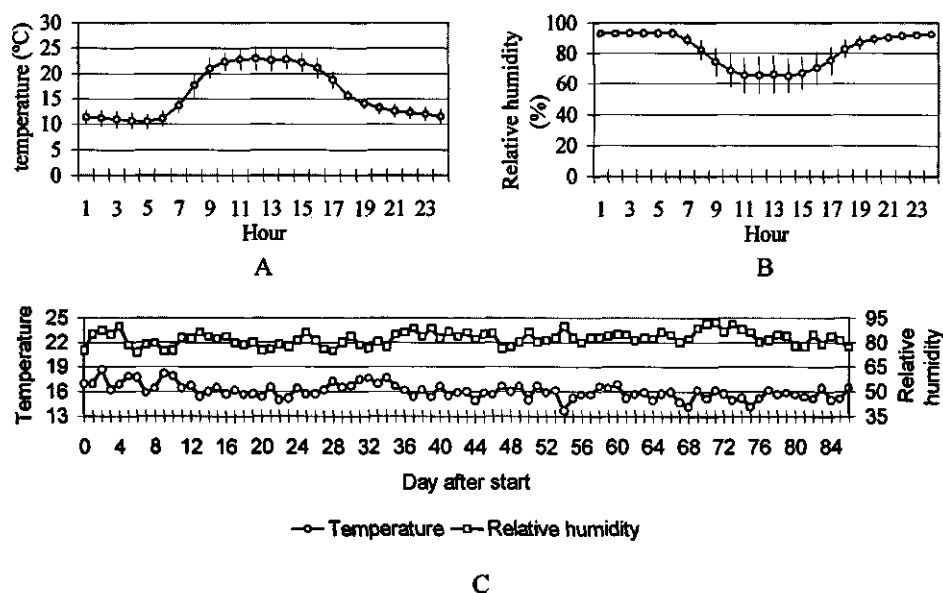
Figure 1. Hourly mean temperature (°C) with standard deviation (graph A), relative humidity (%) with standard deviation (graph B) and mean daily temperature and relative humidity (graph C) in the automated plastic greenhouse conditions of the Horticultural Research Centre.

Sex ratio, immature mortality, and the time required for immature development were not determined in this study. However, Van Es (1982) didn't find differences between immature mortality, sex ratio, and development duration from egg to adult when determined on beef tomato or when determined on a round tomato. Van Roermund & van Lenteren (1992) summarised all data for immature mortality an sex ratio. From their review we conclude that their is also variability in these characteristics, which might be related to whitefly strain differences, but as we could not trace a clear trend between host plant quality, sex ratio and immature mortality, we will use the average data of van Roermund & van Lenteren (1992) for our calculations: 16.7 % for the immature mortality and 0.483 for the sex ratio. The time required for immature development (48.5 days) was calculated based on the hourly temperature data and the equation of van Roermund & van Lenteren (1992). The time until 50 % of the eggs were laid was 18 days. According to the first calculation method, the generation time, $T_1$, was 66.5 days; the net reproduction rate, $R_{o1}$, 84.1; and the intrinsic rate of increase, $r_{m1}$, 0.0666. According to the second equation, the generation time, $T_2$, was 69.9; the net reproduction rate, $R_{o2}$, 91.2; and the intrinsic rate of increase, $r_{m2}$, 0.0645.
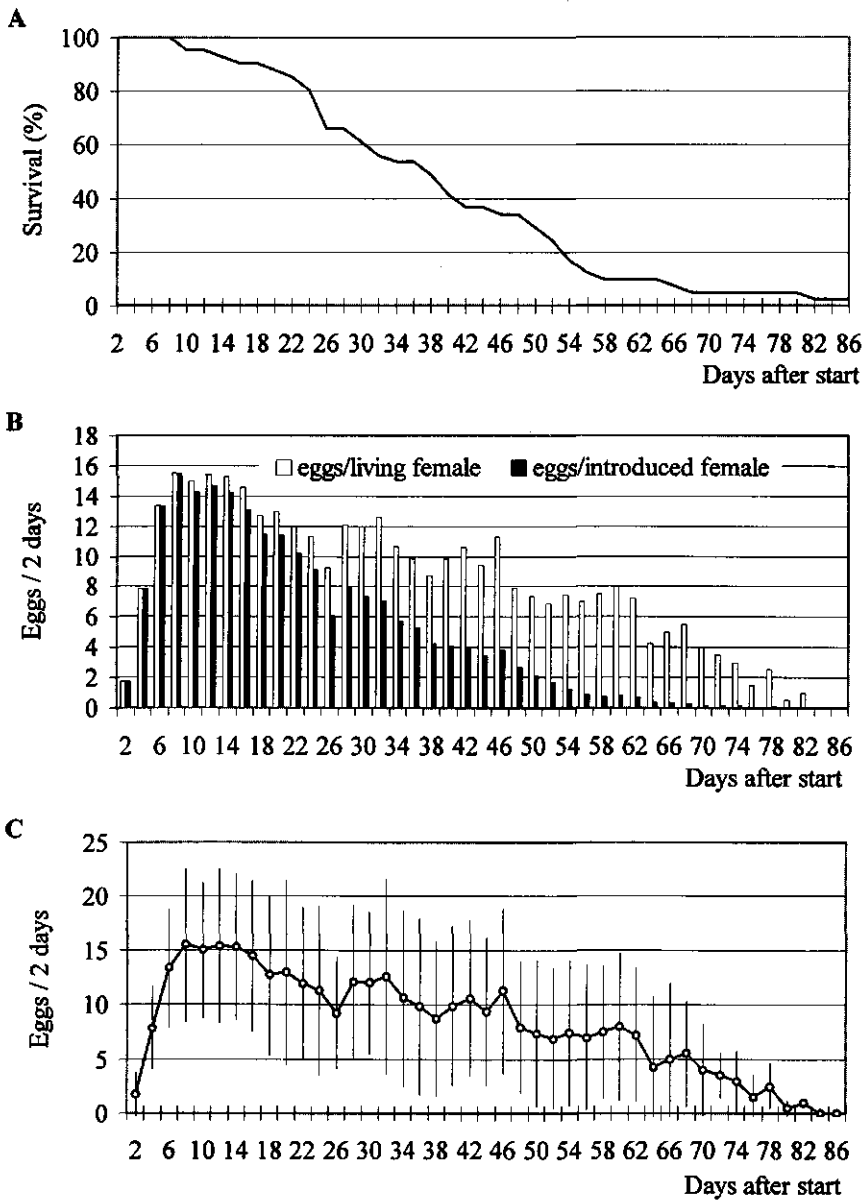
Figure 2. Percentage whitefly females surviving (graph A), the number of eggs per introduced and per living whitefly female per two days (graph B) and the variation of the number of eggs per living whitefly female (graph C) of *T. vaporariorum*.

## Discussion

### Whitefly strain

Longevity, fecundity and oviposition frequency changes with temperature, tomato cultivar and whitefly strain. To evaluate whether a difference between the Dutch and the Colombian strain exists, the results of this experiment were compared with those of van Es (1982) who undertook a similar study on beef tomato cv.'s Dombo and Portanto at 22.5 °C (Table 1). The longevity was 57.4 and 68.6 days on the cv.'s Dombo and Portanto respectively. This is 57 and 87 % more than the longevity of 36.5 days of this trial on the cv. Boris. This difference can not be explained only by the difference in temperature. Both 16 and 22.5 °C are sub-optimal temperatures for *T. vaporariorum* longevity. The optimal temperature for longevity is between 16 and 18 °C and at 16 °C the longevity is higher than at 22.5 °C (van Roermund & van Lenteren, 1992). A second difference between the two trials is the frequency with which the whiteflies were changed to new leaves: van Es (1982) changed whiteflies every week compared to every two days in this experiment. The more frequent manipulation of whiteflies by us could have influenced the longevity negatively. It seems, however, that the difference in longevity is too high to be explained only by temperature and/or manipulation.

Compared to van Es (1982), a shorter longevity but a higher oviposition frequency was found in this study: 5.7 eggs per living female per day compared to 3.7 and 3.2 van Es found on the cv.'s Dombo and Portanto respectively, which is equivalent to 65 and 78 % more. Van Roermund & van Lenteren (1992) found that oviposition frequency reaches its maximum at 22 °C. The trial of van Es (1982) was done at 22.5 °C while this trial was done at 16 °C. As both experiments were done on beef tomato cv.'s, we suppose that this large difference in oviposition frequency are due to a difference in whitefly strain.

Table 1. Longevity, fecundity, oviposition frequency and intrinsic rate of increase ($r_m$) of *T. vaporariorum*.

| Authors, year | Tomato cv. | Type | Temperature (min-max) °C | Longevity days | Fecundity eggs·female$^{-1}$ | Oviposition frequency eggs·female$^{-1}$·day$^{-1}$ | $r_m$ |
|---|---|---|---|---|---|---|---|
| Burnett, 1949 | Bonnie Best | - | 15.0 | 50.5 | 93.6 | 1.9 | |
| Hussey & Gurney, 1957 | - | - | 15.6 | 31.3 | 131.5 | 4.2 | |
| Loyd, 1922 | - | - | 17.3 (6.1-37) | 34.0 | 92.0 | 2.7 [a] | |
| van Es, 1982 | Dombo | Beef | 22.5 (16-24) | 57.4 | 215.6 | 3.7 [a] | 0.0742 [b] |
| | Portanto | Beef | | 68.6 | 219.0 | 3.2 [a] | 0.0744 [b] |
| | Moneydor | Round | | 37.1 | 90.4 | 2.7 [a] | 0.0633 [b] |
| This trial, 1998 | Boris | Beef | 16.0 (5.4-30) | 36.5 | 208.5 | 5.7 | 0.0645-0.0666 |

[a] Recalculated: fecundity/longevity
[b] Estimated

The total fecundity in van Es' and our trial was almost the same because the lower longevity found by us was compensated by a higher oviposition rate. Van Es (1982) found a fecundity of 215 and 219 eggs/female on the cv.'s Dombo and Portanto respectively at 22.5 °C, compared to 209 eggs/female on the cv. Boris in this trial at 16 °C. The temperature for maximum oviposition of *T. vaporariorum* is between 17 and 20 °C (van Roermund & van Lenteren, 1992).

It can be concluded that the whiteflies in this experiment laid a similar number of eggs at a higher rate and in a shorter (life) time compared to the results of van Es (1982). It is, however, difficult to believe that simply temperature and whitefly-handling differences can explain this. Rather, we assume that a difference exists between the Dutch and the Colombian whitefly strain. Further research, using the same tomato cv. the same experimental temperatures and reducing the whitefly manipulation should confirm this.

*Tomato cultivars*
To identify the differences with other, non-beef tomato cv.'s, we compared our results with other trials at low temperature (Burnett, 1949, Hussey et al., 1957 and Loyd, 1922). All previous trials at low temperature found a lower fecundity (Table 1). As no difference between the fecundity of the Dutch and the Colombian whitefly strain was found, we can conclude that the beef tomato cv. Boris is a better host plant than the cv.'s used in the other trials. Van Es (1982) also found this. She found a fecundity of more than double when comparing the beef tomato cv.'s Dombo and Portanto with the round tomato cv. Moneydor.

*Intrinsic rate of increase*
The $r_m$ for *T. vaporariorum* on round tomato cv.'s at 16 °C estimated by van Lenteren et al. (1996) is 0.0663, close to the $r_m$ we calculated. We would expect a higher $r_m$ in this experiment because of the higher fecundity as result of better host plant quality (beef tomato cv.). The estimates of van Lenteren et al. were, however, done for a constant temperature while this experiment was done at varying temperatures and reproduction and development have a non-linear relation with temperature (van Roermund & van Lenteren, 1992). At 16 °C, the $r_m$ estimated for *E. formosa* is 0.0974 (van Lenteren et al., 1996), considerably higher than the $r_m$ we found for *T. vaporariorum* in this study. Considering only the potential population growth, then biological control of *T. vaporariorum* with *E. formosa* under the specific conditions should be possible. However, the commercially available *E. formosa* strains do not disperse very well at temperatures below 18 °C (van Roermund, 1995). Therefore, the next step in our research will be to study the capacity of *E. formosa* to control *T. vaporariorum* under the Colombian greenhouse conditions characterised by a low average temperature (Chapter 6).

**Acknowledgements**

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## 3. Life history of *Amitus fuscipennis* as parasitoid of the greenhouse whitefly *Trialeurodes vaporariorum* on tomato as function of temperature [1]

### Abstract

Life history parameters of *Amitus fuscipennis* MacGown & Nebeker (Hymenoptera: Platygastridae) as parasitoid of *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) were determined at 15, 20, 25 and 30 °C on tomato using three different methods. For each method, immature development, mortality, longevity, fecundity, oviposition frequency and post-oviposition period were determined and temperature dependent relations were estimated. Oviposition frequency was also estimated as function of parasitoid age. Immature development had a maximum of 61 days at 15 °C that decreased to 22 days at 30 °C. Mortality in the grey stage was less than 2% at temperatures lower than 30 °C, where it was 60 %. Longevity fluctuated between 3 and 18 days. Fecundity increased from 338 eggs·female⁻¹ at 15 °C to a maximum of 430 eggs·female⁻¹ at 25 °C and then decreased to 119 eggs·female⁻¹ at 30 °C. Oviposition frequency varied between 3 and 46 eggs·female⁻¹·day⁻¹ and had its maximum on the first day after emergence of the parasitoid. Net reproduction rate, generation time and intrinsic rate of increase were calculated. The intrinsic rate of increase increased from 0.090 at 15 °C to a maximum of 0.233 at 25 °C and then decreased to 0159 at 30 °C. The influence of the methods to determine life history parameters on the results is discussed. The results are compared also with the life history of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) parasitoid of the same host. Advantages and disadvantages of the pro-ovigenic *A. fuscipennis* as compared to the synovigenic *E. formosa* are discussed.

### Introduction

*Amitus fuscipennis* MacGown & Nebeker is a thelytokous parasitoid of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Viggiani, 1991). MacGown & Nebeker described it in 1978 and Viggiani re-described it in 1991. It has been recorded in Mexico, Guatemala, Costa Rica, Dominican Republic and Colombia (MacGown & Nebeker, 1978). In Colombia, it is abundant in the intermediate climate zones together, as *T. vaporariorum*. Research has been done on its life history on bean and chrysanthemum (Medina et al., 1994; Manzano et al., 1999), its use as biological control of *T. vaporariorum* on chrysanthemum (Marqués & Valencia, 1991) and on its mass rearing (García & Monroy, 1995).

The immature development consists of 5 phases: three larval stages, a pre-pupal and a pupal stage. The young immature stages of *T. vaporariorum* are preferred for oviposition (Medina et al., 1994).

The parasitoid performs very well in the field without any artificial introduction. We found it parasitizing 80 % of the *T. vaporariorum* larvae in heavily infested tomato crops in Fusagasuga, near Bogota in Colombia. Marqués & Valencia (1991) compared its performance with *Encarsia formosa* Gahan on chrysanthemum introducing 1500 adults·m⁻² at week 7 and 1500 adults·m⁻² at week 9 after transplant in cages of 6 m2 with 150 plants that were naturally infested with *T. vaporariorum*. At week 10 and 12 after transplant, they observed a parasitization of respectively 32 and 24 % by *A. fuscipennis* compared to respectively 9.4 and 15 % by *E. formosa*. *A. fuscipennis* could therefore be a good alternative for or an addition to

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the biological control of *T. vaporariorum* by *E. formosa* on greenhouse tomatoes in Colombia. In this paper, we present data on the life history of *A. fuscipennis* on tomato at different constant temperatures, and we compare it to *E. formosa* as a first step in the evaluation of this parasitoid for biological control of the greenhouse whitefly on tomato in Colombia.

**Materials and methods**

The experiments took place at the Horticultural Research Centre (CIAA) of the University Jorge Tadeo Lozano in Chia, on the Bogota Plateau, at 2600 m altitude in Colombia.

*Experimernt 1*: Leaflets of seven-week-old tomato plants of the cultivar Boris (Bruinsma Seeds, 's Gravenzande, The Netherlands) were infested, using clip cages with an internal diameter of 20 mm in which 25 whiteflies were introduced. The whiteflies originated from the CIAA's rearing unit where they are reared on tomato. Adult whiteflies were removed four days after infestation and eggs were permitted to develop to first instar larvae in the greenhouse. The infested leaflets were protected from parasitism by small gauze bags. Plants with first instar larvae were then brought to a temperature-controlled room with L12:D12 regime. A standard high-pressure sodium lamp of 400W (WR400 AL, Poot Light Systems, The Netherlands) provided light. Temperature (± 0.5 °C) was controlled by a heater and air conditioner connected to a control unit (Watlow 93, Watlow controls, Winona, Minnesota, USA). Relative humidity was controlled with a humidifier (Herrmidifier 500, Herrmidifier Inc., Lancaster, USA) to 70 ± 10 %. One *A. fuscipennis* female per leaflet was placed in a clip cage on the site where approximately 50 *T. vaporariorum* larvae were located. The *A. fuscipennis* females originated from the CIAA's rearing unit. In a preliminary experiment, it was observed that the *A. fuscipennis* females spent much time preening on the wall of the clip cages, thereby running the risk of getting stuck in the accumulated honeydew. To prevent this, the clip cages were placed on the leaf in a way that also a small part of the leaf area in the clip cage was free of larvae. Every three days the females were moved to new leaflets. This process was maintained until all females were dead. Mortality was registered daily. Leaflets with parasitized larvae were enclosed in small gauze bags for further development. When parasitoids reach the pupal stage the whitefly pupae turn grey. Daily, grey pupae were removed from the leaves and introduced into gelatine capsules, which were observed until emergence of parasitoids.

Mean fecundity, longevity, immature mortality of the grey stage and development times were calculated. The lower development threshold was determined by linear regression of the development time data and the upper lethal temperature was determined by extrapolation of the mortality at 25 and 30 °C. The development rate was calculated as the reciprocal of the development time. The relationship between parameters and temperature was determined using different models:

- Linear: $Y = a + b \cdot X$
- Exponential: $Y = \exp(a + b \cdot X)$
- Weibull (Weibull, 1951): $Y = c \cdot b^{-1} \cdot [(X-a) \cdot b^{-1}]^{c-1} \cdot \exp\{-[(X-a) \cdot b^{-1}]^{c}\} \cdot d$
- Logan (Logan et al., 1976): $Y = a \cdot \{\exp[b \cdot (X-d)] - \exp[b \cdot (e-d) - (e-X) \cdot c^{-1}]\}$

where Y is the estimated parameter, a, b, c, d and e are constants and X is the temperature.

The constants of the Logan and Weibull models were determined using the Marquard-method of the NLIN procedure of the SAS statistical analysis software (SAS Institute Inc., Cary, NC, USA). Calculations were stopped when the difference between the sum of squares of consecutive iterations was less than $10^{-8}$.

Oviposition frequency was calculated as fecundity divided by longevity.

The intrinsic rate of increase ($r_m$), generation time (T), and the net reproduction rate ($R_o$) were calculated using the formulas of Andrewartha & Birch (1954):

$r_m = (\ln R_o) \cdot T^{-1}$ where

$R_o = \Sigma\, L_x \cdot M_x$ and $T = (\Sigma\, L_x \cdot M_x \cdot X) \cdot (\Sigma\, L_x \cdot M_x)^{-1}$ where,

$L_x$ and $M_x$ are the age-specific survival rate and the age-specific fecundity respectively and X is the age. The experiment was undertaken at 15, 20, 25 and 30 °C.

To determine longevity without hosts, 20 females were enclosed in plastic vials, one female per vial. The vials were covered with gauze with a droplet of honey on it as food source.

*Experiment 2*: During the three days the *A. fuscipennis* females were permitted to oviposit in experiment 1, honeydew accumulated on the walls of the clip cages, causing high mortality of the *A. fuscipennis* females. Therefore a new method was developed and the experiment was repeated using a whole leaflet, reducing in this way the density of the *T. vaporariorum* larvae. The infestation was done using small gauze bags, enclosing the whole leaflet and with 50 *T. vaporariorum* adults, resulting in approximately 150 whitefly larvae per leaflet. All the larvae were made available to the ovipositing female enclosing the *A. fuscipennis* female and the leaflet in plastic cylinders with an internal diameter of 8 cm and 14 cm long. The experiment was from then on managed as in experiment 1.

*Experiment 3*: Here *A. fuscipennis* females were moved to new leaves every day instead of every three days, in order to determine the immature development time more accurately. Other procedures were identical to experiment 2.

**Results**

*Immature development*

Among the three experiments, differences in immature development were found only at 15 and 30 °C. At 15 °C, a difference of about 7 days was found between the total immature development time in experiment 1 (68.1 days) and experiments 2 and 3 (61.2 and 60.6 days respectively). At 30 °C the total immature development time of experiment 1 was shorter (18.9 days) than that of experiment 2 (20.7 days), which in turn was shorter than that of experiment 3 (21.9 days). At 20 and 25 °C, the results for immature development were similar for all experiments: between 30.6 and 31.2 days at 20 °C and between 23.6 and 23.8 days at 25 °C. The differences found in the total immature development times were caused merely by a difference in the immature development time of the white stage, and not by differences in the grey stage (Tables 1-3). The Duncan grouping of methods (experiments) classified the development time of the white stage at 15 °C of experiment 1 and 3 in the same group, as well as the total development time of experiment 1 and 2 at 20 °C, and the total development time of experiment 1 and 3 at 25 °C. All other development times were classified in different groups.

25

Table 1. Life history parameters of *A. fuscipennis* on *T. vaporariorum*, determined by using clip cages and providing a new leaf with hosts every 3 days

| Parameter | 15 °C n | Mean ± s.d. [1] | 20 °C n | Mean ± s.d. [1] | 25 °C N | Mean ± s.d. [1] | 30 °C n | Mean ± s.d. [1] |
|---|---|---|---|---|---|---|---|---|
| Development time egg-pupa (days) | 600 | $57.1 \pm 2.9$ [a] | 500 | $25.3 \pm 0.7$ [b] | 319 | $18.4 \pm 1.0$ [c] | 199 | $14.6 \pm 0.7$ [d] |
| Development time pupa-adult (days) | 600 | $11.0 \pm 0.5$ [a] | 500 | $5.3 \pm 0.6$ [b] | 319 | $5.2 \pm 0.6$ [c] | 199 | $4.3 \pm 0.4$ [d] |
| Development time egg-adult (days) | 600 | $68.1 \pm 2.9$ [a] | 500 | $30.6 \pm 0.8$ [b] | 319 | $23.6 \pm 1.0$ [c] | 199 | $18.9 \pm 0.7$ [d] |
| Development egg-adult (degree days) | | 429 | | 346 | | 385 | | 402 |
| Mortality in grey stage (%) | | 2.3 | | 2.6 | | 16.5 | | 74.8 |
| Longevity with hosts (days) | 20 | $18.3 \pm 11.6$ [a] | 27 | $10.8 \pm 4.7$ [b] | 27 | $5.2 \pm 3.4$ [c] | 24 | $3.4 \pm 1.5$ [c] |
| Longevity without hosts (days) | 30 | $18.4 \pm 3.9$ [b] | 30 | $25.0 \pm 9.4$ [a] | 20 | $19.0 \pm 2.9$ [b] | 20 | $12.2 \pm 4.0$ [c] |
| Fecundity [2] (eggs·female$^{-1}$) | 20 | $90 \pm 44$ [a] | 27 | $110 \pm 48$ [a] | 27 | $42 \pm 23$ [b] | 24 | $9.9 \pm 9.5$ [c] |
| Oviposition frequency [2] (eggs·female$^{-1}$·day$^{-1}$) | | 4.9 | | 10.2 | | 8.1 | | 2.9 |
| Post-oviposition period (days) | 20 (6) | $3.3 \pm 5.9$ | 27 (6) | $0.5 \pm 1.0$ | 27 | 0 | 24 | 0 |
| $R_0$ = net reproduction rate | 20 | 88 | 27 | 106 | 27 | 35 | 24 | 2.5 |
| T = generation time | 20 | 76.8 | 27 | 35.6 | 27 | 26.8 | 24 | 22.1 |
| $r_m$ = intrinsic rate of increase | 20 | 0.058 | 27 | 0.131 | 27 | 0.133 | 24 | 0.042 |

[1] Separation of means with the Duncan test. Means with the same letter in a row are not significantly different.
[2] Measured indirectly as parasitized pupae that reach the grey stage. Does not take into account mortality in the white stage. Real fecundity and oviposition frequency are therefore higher.

Table 2. Life history parameters of *A. fuscipennis* on *T. vaporariorum*, determined by using cylinders and providing a new leaf with hosts every 3 days

| Parameter | 15 °C | | 20 °C | | 25 °C | | 30 °C | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean ± s.d. [1] | n | Mean ± s.d. [1] | n | Mean ± s.d. [1] | n | Mean ± s.d. [1] |
| Development time egg-pupa (days) | 740 | $50.5 \pm 2.5$ [a] | 525 | $25.5 \pm 1.1$ [b] | 510 | $18.9 \pm 1.2$ [c] | 440 | $16.5 \pm 0.7$ [d] |
| Development time pupa-adult (days) | 740 | $10.7 \pm 0.7$ [a] | 525 | $5.1 \pm 0.4$ [b] | 510 | $4.9 \pm 0.4$ [c] | 440 | $4.2 \pm 0.4$ [d] |
| Development time egg-adult (days) | 740 | $61.2 \pm 2.5$ [a] | 525 | $30.6 \pm 1.1$ [b] | 510 | $23.8 \pm 1.2$ [c] | 440 | $20.7 \pm 0.9$ [d] |
| Development egg-adult (degree days) | | 514 | | 410 | | 437 | | 484 |
| Mortality in grey stage (%) | | 1.6 | | 2.0 | | 1.4 | | 60.7 |
| Longevity with hosts (days) | 20 | $16.7 \pm 7.4$ [a] | 28 | $13.6 \pm 4.7$ [b] | 20 | $10.7 \pm 4.6$ [b] | 20 | $5.2 \pm 2.1$ [c] |
| Fecundity [2] (eggs·female$^{-1}$) | 20 | $245 \pm 105$ [a] | 28 | $270 \pm 67$ [a] | 20 | $313 \pm 82$ [a] | 20 | $62 \pm 29$ [b] |
| Oviposition frequency [2] (eggs·female$^{-1}$·day$^{-1}$) | | 14.7 | | 19.9 | | 29.3 | | 11.9 |
| Post-oviposition period (days) | 20 (5) | $1.3 \pm 2.8$ | 28 (9) | $0.9 \pm 1.4$ | 20 | 0 | 20 | 0 |
| $R_o$ = net reproduction rate | 20 | 241 | 28 | 264 | 20 | 309 | 20 | 24.5 |
| T = generation time | 20 | 69.4 | 28 | 34.5 | 20 | 27.6 | 20 | 23.9 |
| $r_m$ =intrinsic rate of increase | 20 | 0.079 | 28 | 0.162 | 20 | 0.208 | 20 | 0.134 |

[1] Separation of means with the Duncan test. Means with the same letter in a row are not significantly different.
[2] Measured indirectly as parasitized pupae that reach the grey stage. Does not take into account mortality in the white stage. Real fecundity and oviposition frequency are therefore higher.

Table 3. Life history parameters of *A. fuscipennis* on *T. vaporariorum*, determined by using cylinders and providing a new leaf with hosts every day

| Parameter | 15 °C | | 20 °C | | 25 °C | | 30 °C | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean ± s.d.[1] | n | Mean ± s.d.[1] | n | Mean ± s.d.[1] | n | Mean ± s.d.[1] |
| Development time egg-pupa (days) | 3319 | 50.6 ± 0.3 [a] | 3997 | 25.7 ± 0.4 [b] | 3800 | 18.6 ± 0.3 [c] | 477 | 17.9 ± 0.7 [d] |
| Development time pupa-adult (days) | 3319 | 10 ± 0 [a] | 3997 | 5.7 ± 0.4 [b] | 3800 | 5.0 ± 0 [c] | 477 | 4.0 ± 0 [d] |
| Development time egg-adult (days) | 3319 | 60.6 ± 0.3 [a] | 3997 | 31.2 ± 0.2 [b] | 3800 | 23.6 ± 0.3 [c] | 477 | 21.9 ± 0.7 [d] |
| Development egg-adult (degree days) | | 467 | | 398 | | 418 | | 497 |
| Mortality in grey stage (%) | | 1.8 | | 1.7 | | 1.8 | | 60 |
| Longevity with hosts (days) | 10 | 12.1 ± 3.7 [a] | 10 | 9.9 ± 1.2 [ab] | 9 | 9.3 ± 2.9 [b] | 10 | 5.1 ± 1.8 [c] |
| Fecundity [2] (eggs·female$^{-1}$) | 10 | 338 ± 48 [b] | 10 | 407 ± 15 [a] | 9 | 430 ± 74 [a] | 10 | 119 ± 48 [c] |
| Oviposition frequency [2] (eggs·female$^{-1}$·day$^{-1}$) | 10 | 27.9 | 10 | 41.1 | 9 | 46.2 | 10 | 23.3 |
| Post-oviposition period (days) | 10 | 0 | 10 | 0 | 9 | 0 | 10 | 0 |
| $R_o$ = net reproduction rate | 10 | 332 | 10 | 400 | 9 | 419 | 10 | 47.7 |
| T = generation time | 10 | 64.7 | 10 | 34.1 | 9 | 27.1 | 10 | 24.4 |
| $r_m$ =intrinsic rate of increase | 10 | 0.090 | 10 | 0.176 | 9 | 0.233 | 10 | 0.159 |

[1] Separation of means with the Duncan test. Means with the same letter in a row are not significantly different.

[2] Measured indirectly as parasitized pupae that reach the grey stage. Does not take into account mortality in the white stage. Real fecundity and oviposition frequency are therefore higher.

Linear regressions of the total development rate and the development rate of the white stage as function of temperature had coefficients of determination higher than 0.94. Those of the grey stage were between 0.74 and 0.81, depending on the experiment. The lower development thresholds, calculated as the intercept divided by the slope, were lower for the grey stage (between 3.3 and 3.9 °C) than for the white stage (between 7.2 and 9.5 °C). The thresholds for the total development time were in between the values for the white and the grey stage and were 8.7 °C for experiment 1, 6.6 °C for experiment 2 and 7.3 °C for experiment 3 (Table 4).

Table 4. Relationships between the total immature development rate of *A. fuscipennis* and temperature, based on the linear regression $Y = a + b \cdot X$; $T_{lower}$ (calculated as a/b) is the lower threshold and $T_{upper}$ (calculated by extrapolation of the mortality at 25 and 30 °C) is the upper lethal temperature; $r^2$ is the coefficient of determination; n is the number of individuals.

| Stage | Method | n | a | b | $r^2$ | $T_{lower}$ | $T_{upper}$ |
|-------|--------|---|---|---|-------|-------------|-------------|
| White | Clip cages, 3 days | 1618 | -0.0331 | 0.00349 | 0.975 | 9.5 | |
| | Cylinders, 3 days | 2215 | -0.0202 | 0.00282 | 0.951 | 7.2 | |
| | Cylinders, 1 day | 11593 | -0.0243 | 0.00306 | 0.951 | 7.9 | |
| Grey | Clip cages, 3 days | 1618 | -0.0378 | 0.00971 | 0.746 | 3.9 | 31.7 |
| | Cylinders, 3 days | 2215 | -0.0313 | 0.00954 | 0.797 | 3.3 | 33.3 |
| | Cylinders, 1 day | 11593 | -0.0356 | 0.00972 | 0.816 | 3.7 | 33.4 |
| Total | Clip cages, 3 days | 1618 | -0.0227 | 0.00261 | 0.965 | 8.7 | |
| | Cylinders, 3 days | 2215 | -0.0144 | 0.00219 | 0.946 | 6.6 | |
| | Cylinders, 1 day | 11593 | -0.0173 | 0.00236 | 0.956 | 7.3 | |

The immature development time decreased exponentially as temperature increased. The coefficients of determination of the fitted Logan models of the development rate for the white stage and the total development had coefficients of determination higher than 0.93, while for the grey stage this coefficient was between 0.71 and 0.85 according to the experiment. (Table 5 and Figure 1). For the total immature development rate, the coefficients of determination of the Logan models were higher than those of the linear models. For the white and grey stage in the clip cage method and for the grey stage in the cylinder method with new leaves every 3 days, the linear models had higher coefficients of determination than the Logan model (Tables 4 & 5).

Table 5. Relationships between the development rate of the white stage, the grey stage and the total immature development rate of *A. fuscipennis* and temperature, based on the Logan model, $Y = a \cdot \{exp[b \cdot (X-d)]-exp[b \cdot (e-d)-(e-X) \cdot c^{-1}]\}$, where a, b and c are coefficients, $T_{lower}$ and $T_{upper}$ are the lower threshold and upper lethal temperatures, $r^2$ is the coefficient of determination and n the number of individuals.

| Stage | Method | n | $T_{lower}$ | $T_{upper}$ | a | b | c | $r^2$ |
|---|---|---|---|---|---|---|---|---|
| White | Clip cages, 3 days | 1618 | 8.7 | 31.7 | 0.0110 | 0.101 | 1.18 | 0.950 |
| | Cylinders, 3 days | 2215 | 6.6 | 33.3 | 0.0088 | 0.107 | 2.83 | 0.964 |
| | Cylinders, 1 day | 11593 | 7.3 | 33.4 | 0.0622 | 0.170 | 5.69 | 0.981 |
| Grey | Clip cages, 3 days | 1618 | 8.7 | 31.7 | 0.0732 | 0.066 | 1.03 | 0.713 |
| | Cylinders, 3 days | 2215 | 6.6 | 33.3 | 0.0563 | 0.079 | 2.53 | 0.795 |
| | Cylinders, 1 day | 11593 | 7.3 | 33.4 | 0.0718 | 0.061 | 1.62 | 0.854 |
| Total | Clip cages, 3 days | 1618 | 8.7 | 31.7 | 0.0097 | 0.094 | 1.15 | 0.935 |
| | Cylinders, 3 days | 2215 | 6.6 | 33.3 | 0.0078 | 0.101 | 2.73 | 0.955 |
| | Cylinders, 1 day | 11593 | 7.3 | 33.4 | 0.0080 | 0.132 | 4.51 | 0.971 |
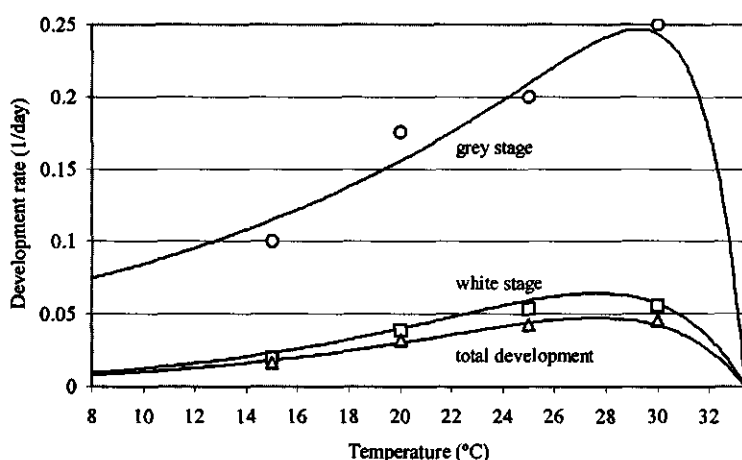


Figure 1. Relationship between the immature development rate of the white stage, the grey stage and the total immature development rate of *A. fuscipennis* and temperature, using cylinders and providing a new leaf with hosts every day. Dots represent mean development rates. For more information see Table 5.
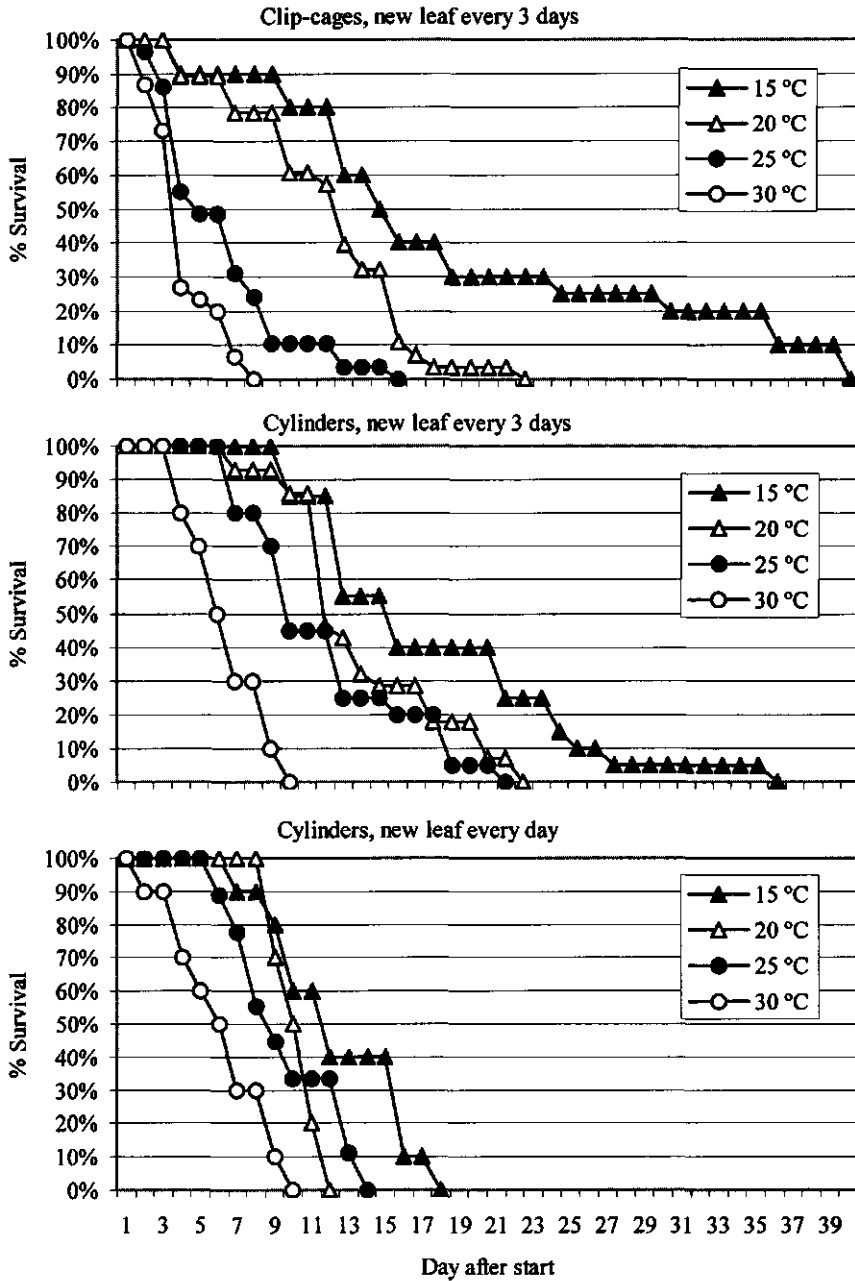
Figure 2. Survival over time of *A. fuscipennis* females in the three experiments.

No clear relation was found between the total development and the degree-days as the figure for each temperature was different. In the three experiments, the minimum degree-days was found at 20 °C. Within each experiment, the difference between the lowest and the highest figure was 24-25 % (Tables 1-3).

*Immature mortality*
Mortality in the white stage was not determined. The mortality in the grey stage of experiment 1 was low at 15 and 20 °C (2.3-2.6 %), but increased to 16.5 % at 25 °C and to even 75% at 30 °C. In experiment 2 and 3, the mortality in the grey stage was equal or lower than 2 % at temperatures equal or lower than 25 °C, and 60 % at 30 °C (Tables 1-3).

*Sex ratio*
No males were observed.

*Longevity*

The mean longevity without hosts had a maximum of 25 days at 20 °C, decreased as temperature increased (12.2 days at 30 °C) and as temperature decreased (18.4 days at 15 °C) (Table 1).
In all experiments, the mean longevity in the presence of the host had a maximum at 15 °C and decreased as temperature increased. Important differences were observed among the experiments. At 15 and 20 °C, the lowest longevity was observed in experiment 3 (12.1 and 9.9 days respectively), while at 25 and 30 °C the lowest longevity was observed in experiment 1 (5.2 and 3.4 days respectively). At 15 °C, the highest longevity of 18.3 days was observed in experiment 1, while at the other temperatures the highest longevity was observed in experiment 2 (Tables 1-3 and Figure 2).
The best coefficients of determination of longevity were obtained with the linear model, although coefficients of determination were very low (Table 6 and Figure 3). The longevity showed a high standard deviation and coefficient of variation (cv. = standard deviation/mean), the latter varying between 0.11 and 0.63. In experiment 1 and 2, the cv. was higher than 0.33 for all temperatures and in experiment 3 it was lower than 0.33 for all temperatures.

Table 6. Relationships between longevity and temperature of *A. fuscipennis*, based on the linear model $Y = a+b \cdot X$, where a is the intercept and b the coefficient; $r^2$ is the coefficient of determination.

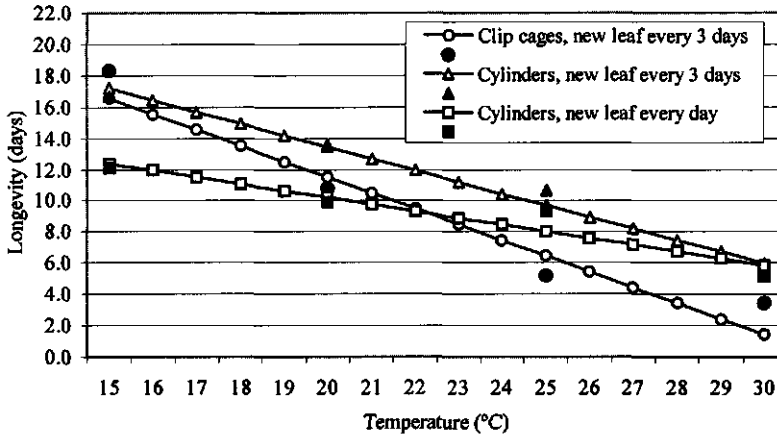| Method | a | b | $r^2$ |
|---|---|---|---|
| Clip cages, new leaf every 3 days | 31.7 | -1.01 | 0.457 |
| Cylinders, new leaf every 3 days | 28.4 | -0.748 | 0.401 |
| Cylinders, new leaf every day | 18.9 | -0.435 | 0.483 |

Figure 3. Relationship between longevity and temperature of *A. fuscipennis*. Black dots represent the corresponding mean longevity of each method. For more details see Table 6.

*Pre-oviposition period*
In experiment 3, all *A. fuscipennis* females began laying eggs at the first day of the experiment and at all temperatures. No pre-oviposition period was observed.

*Fecundity*
The fecundity was the lowest (9.9 eggs·female$^{-1}$ at 30 °C) in experiment 1 at all temperatures and highest (430 eggs·female$^{-1}$ at 25 °C) in experiment 3 at all temperatures. It increased by a factor 3.7 to 12 when comparing experiment 1 and 3. In experiment 1, the maximum fecundity (110 eggs·female$^{-1}$) was observed at 20 °C, while in experiment 2 and 3 the maximum fecundity was observed at 25 °C (313 and 430 eggs·female$^{-1}$ respectively). In all experiments, the fecundity was lowest at 30 °C (Tables 1-3). The fecundity showed a high standard deviation and coefficient of variation, the latter varying between 0.04 and 0.96. The cv. was higher in experiment 1 than in experiment 3. At 30 °C, the cv. was higher compared to the other temperatures. The Weibull model gave the best coefficients of determination, although they were very low (Table 7 and Figure 4).

Table 7. Relationships between fecundity and temperature of *A. fuscipennis*, based on the Weibull model $Y = c \cdot b^{-1} \cdot [(X-a) \cdot b^{-1}]^{c-1} \cdot \exp\{-[(X-a) \cdot b^{-1}]^c\} \cdot d$, where a is the lower threshold temperature of 10 °C and b, c and d are coefficients; $r^2$ is the coefficient of determination.

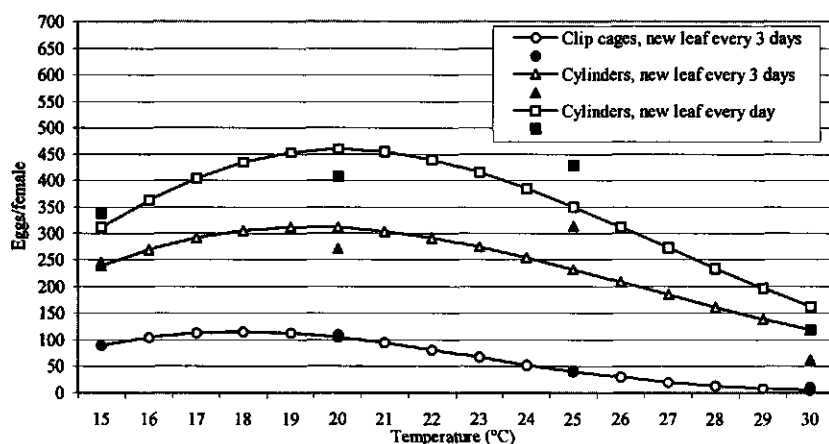| Method | b | c | d | $r^2$ |
|---|---|---|---|---|
| Clip cages, new leaf every 3 days | 10.2 | 2.32 | 1234 | 0.569 |
| Cylinders, new leaf every 3 days | 13.4 | 1.98 | 4902 | 0.392 |
| Cylinders, new leaf every day | 13.4 | 2.16 | 6822 | 0.713 |



Figure 4. Relationship between the total fecundity and temperature of *A. fuscipennis*. Black dots represent the corresponding mean fecundity of each method. For more details see Table 7.

*Oviposition frequency*

At all temperatures, the oviposition frequency was lowest in experiment 1 (5, 10, 8 and 3 eggs·female$^{-1}$·day$^{-1}$ at 15, 20, 25 and 30 °C respectively), while it was highest in experiment 3 (28, 41, 46 and 23 eggs·female$^{-1}$·day$^{-1}$ at 15, 20, 25 and 30 °C respectively). In experiment 1, the maximum oviposition frequency was observed at 20 °C, while in experiment 2 and 3 the maximum fecundity was observed at 25 °C (29 and 46 eggs·female$^{-1}$·day$^{-1}$ respectively). In all experiments, the oviposition frequency was lowest at 30 °C (3, 12 and 23 eggs·female$^{-1}$·day$^{-1}$ in experiment 1, 2 and 3 respectively) (Tables 1-3). The Weibull model best described the oviposition frequency as function of temperature, but only the results of the first 2 oviposition days gave a high coefficient of determination (Table 8 and Figure 5).

Table 8. Relationships between oviposition frequency and temperature at different ages of *A. fuscipennis*, based on the Weibull model $Y = c \cdot b^{-1} \cdot [(X-a) \cdot b^{-1}]^{c-1} \cdot \exp\{-[(X-a) \cdot b^{-1}]^c\} \cdot d$, where a is the lower oviposition threshold temperature and b,c and d are coefficients; based on the data of the cylinder method with a new leaf with hosts every day; $r^2$ is the coefficient of determination.

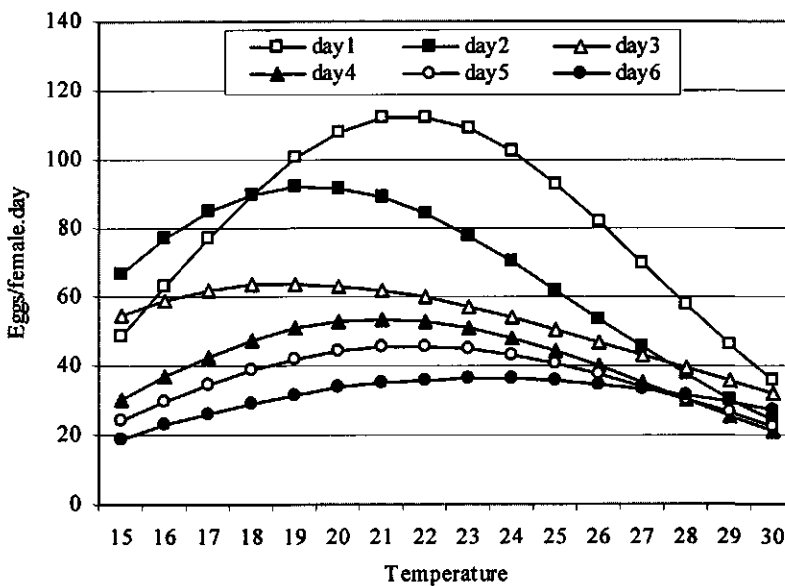| Age (days) | a | b | c | d | $r^2$ |
|---|---|---|---|---|---|
| 1 | 10 | 13.8 | 2.67 | 1459 | 0.871 |
| 2 | 10 | 12.5 | 2.15 | 1281 | 0.871 |
| 3 | 10 | 14.7 | 1.73 | 1191 | 0.332 |
| 4 | 10 | 14.0 | 2.33 | 783 | 0.634 |
| 5 | 10 | 14.9 | 2.31 | 717 | 0.492 |
| 6 | 10 | 17.7 | 2.15 | 716 | 0.165 |



Figure 5. Relationship between oviposition frequency and temperature at different ages of *A. fuscipennis*. For more details see Table 8.

*Change of oviposition frequency during ageing*
The number of eggs laid per living female per three days in experiment 1 showed no clear pattern. In experiment 2, oviposition frequency showed a decreasing pattern from a maximum on the first period of three days at 20 and 25 °C (143 and 174 eggs·female⁻¹·three-days⁻¹ respectively) while at 15 and 30 °C the oviposition frequency showed ups and downs. In
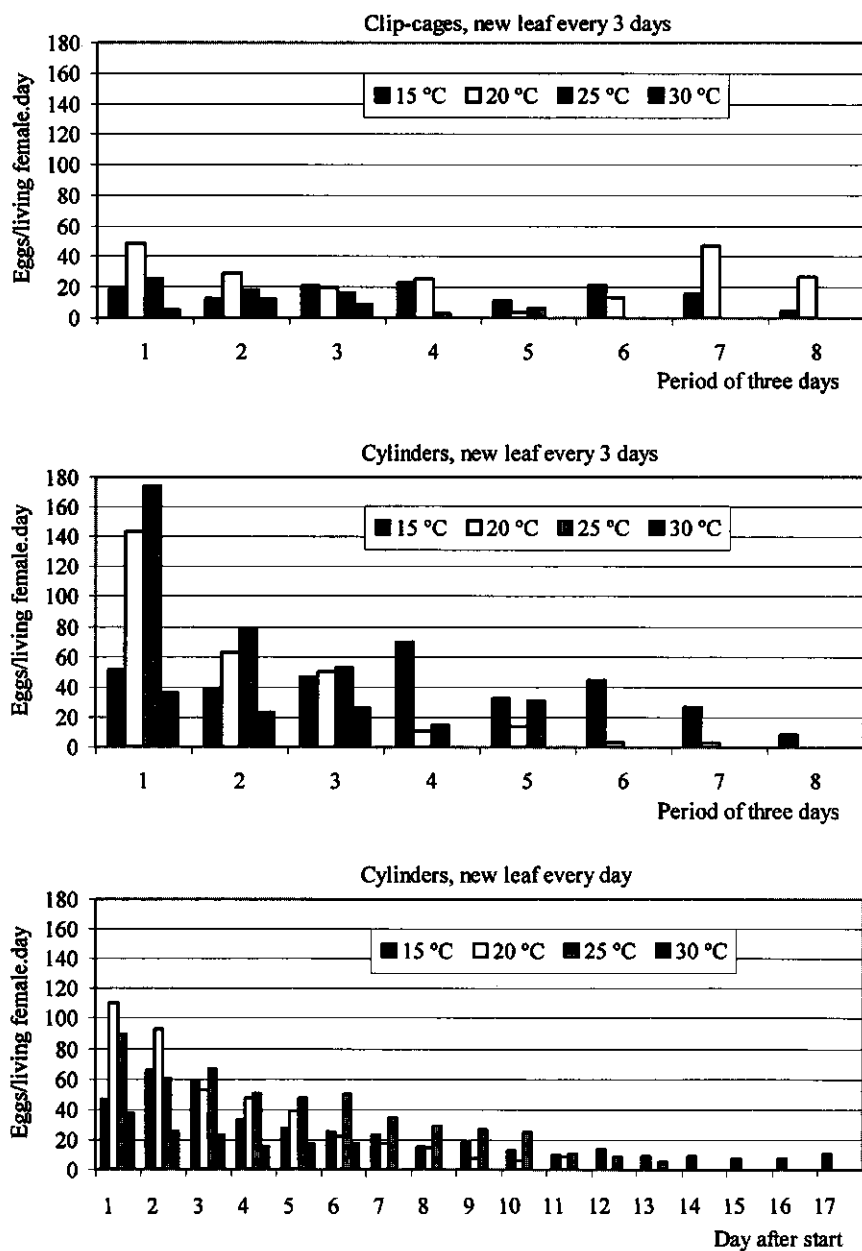
Figure 6. The number of eggs laid per living *A. fuscipennis* female in the three experiments
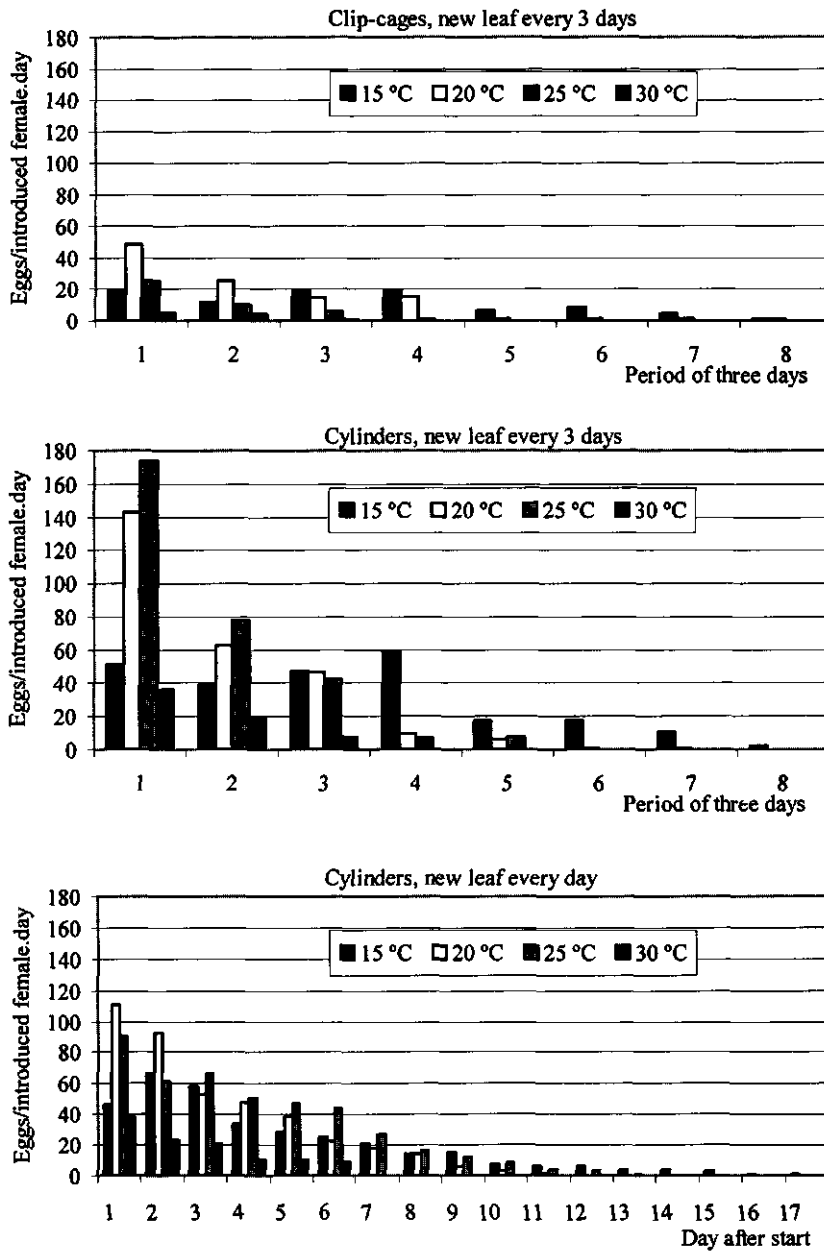
Figure 7. The number of eggs laid per introduced *A. fuscipennis* female in the three experiments

experiment 3, a decreasing pattern was observed at all temperatures, but at 15 °C the oviposition frequency had its maximum (66.1 eggs·female$^{-1}$·day$^{-1}$) on the second day. At 20, 25 and 30 °C, the maximum oviposition frequency was 111, 90 and 38 eggs·female$^{-1}$·day$^{-1}$ respectively (Figure 5). Except for the results of experiment 3 at 15 °C, the number of eggs laid per introduced female declined from a maximum on the first period (or day) more rapidly than the number of eggs laid per living female (Figure 6).

We could describe the oviposition frequency as function of age in experiment 3 with the exponential model. However, the coefficient of determination was only high at 20 °C (Table 9 and Figure 8).

Table 9. Relationships between oviposition frequency and age at different temperatures of *A. fuscipennis*, based on the exponential model $Y = exp(a + b·X)$, according to the data of the cylinder method with a new leaf with hosts every day; $r^2$ is the coefficient of determination.

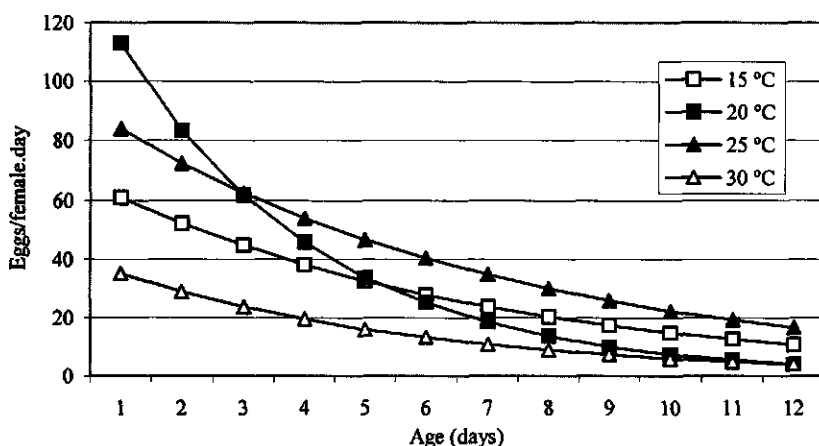| Temperature (°C) | a | b | $r^2$ |
|---|---|---|---|
| 15 | 4.27 | -0.158 | 0.714 |
| 20 | 5.03 | -0.302 | 0.952 |
| 25 | 4.58 | -0.148 | 0.704 |
| 30 | 3.75 | -0.196 | 0.520 |



Figure 8. Relationship between oviposition frequency and age of *A. fuscipennis* at different temperatures. For more details see Table 9.

Net reproduction rate, generation time and intrinsic rate of increase

As no males are produced and at 15 and 20 °C the mortality in the grey stage was very low (less than 2.6 %), the net reproduction rate was only a little lower than the fecundity. At 25 and 30 °C, the mortality in experiment 1 was 15 % higher compared to experiment 2 and 3, and in this experiment the net reproduction rate was significantly lower than the fecundity. The highest net reproduction rate of 419 was observed at 25 °C in experiment 3 and the lowest of 2.5 at 30 °C in experiment 1 (Tables 1-3).

At 15 °C, generation time was lowest in experiment 3, 64.7 days compared to 76.8 days in experiment 1. At 20 and 25 °C, the generation time was similar for all experiments. At 30 °C, the generation time was highest in experiment 3, 24.4 days, compared to 22.1 days in experiment 1 (Tables 1-3).

The intrinsic rate of increase ($r_m$) was the highest at all temperatures in experiment 3 (0.090, 0.176, 0.233 and 0.159 at 15, 20, 25 and 30 °C respectively). It had a maximum in all experiments at 25 °C. In experiment 1, the minimum $r_m$ of 0.042 was at 30 °C while in experiment 2 and 3 the minimum (0.079 and 0.090 respectively) was at 15 °C (Tables 1-3).

**Discussion**

The immature development time at 15 and 30 °C showed a larger variation among experiments than at 20 and 25 °C. The longer development time observed in experiment 1 was possibly due to a temperature gradient in the climate room. In experiment 2, the temperature near the whitefly larvae could have increased a little as an effect of the use of cylinders.

The objective of experiment 3 was to determine the developmental times more precisely. In experiment 2, the females were changed to new leaves every three days so the mean development time could not be determined very carefully. However, the results show that the difference between the methods was very small, indicating that with the method of experiment 2 similar information could be obtained with less work.

Medina et al. (1994) found development times of 42.4, 35.9, 53.7 and 52.1 days on bean and 46.4, 44.6, 66.5 and 53.8 days on chrysanthemum, respectively in greenhouse, laboratory, and two outside conditions. No mean temperatures were given for those sites, but temperature varied between 14.8 and 25.2 in the greenhouse, 16 and 22 in the laboratory, 12.4 and 23.1 outside (site A) and 12.8 and 23.0 outside (site B). These results are within the range of the results of this experiment. Manzano et al. (1999) found on bean at 15 °C and 45 % relative humidity (r.h.) a development time of 51.0 days, 10 to 17 days shorter than our results. However, at the same temperature but at 75 % r.h. (in our experiment 70 ± 10 %), the results were similar to ours, as well as at 25 °C.

The immature mortality in the grey stage in experiment 1 at 25 and 30 °C was higher when compared to the results of experiment 2 and 3. A possible cause for this difference can be the differences in the density of the whitefly larvae on the leaves. In experiment 1, the density was higher, causing competition among the larvae. This could have reduced the quality of the *T. vaporariorum* larvae and could have increased the immature mortality of *A. fuscipennis* in the grey stage. The difference was larger at higher temperatures indicating that the honeydew formation, which was more pronounced at 25 and 30 °C could also have negatively influenced the quality of the *T. vaporariorum* larvae and thus the survival of the grey stage of *A. fuscipennis* as well.

Differences in longevity, fecundity and oviposition frequency were observed between the three experimental methods. The main difference between the clip cage method (experiment 1) and the cylinder method (experiment 2 and 3) was the artificial mortality observed in the clip cages. Because of the limited space, honeydew accumulated on the walls of the clip cages, especially at 25 and 30 °C. *A. fuscipennis* females got stuck in the honeydew and died. This decreased the longevity, fecundity and oviposition frequency. It was impossible to distinguish between artificial and natural death as females that suffered natural death could also fall into the honeydew. In the clip cages, the females were observed mainly cleaning themselves on the wall of the cage, while in the cylinder method they were mostly on the leaves. This may indicate that the females do not like leaves contaminated with honeydew. The behaviour of cleaning on the wall of the clip cages could have reduced the oviposition frequency. The negative effect of the honeydew was not observed at 15 or 20 °C because the production of honeydew was much lower at those temperatures.

The different numbers of whitefly larvae offered to *A. fuscipennis* could have influenced longevity as well. In experiment 1, approximately 50 larvae were offered per 3 days, in experiment 2, approximately 150 larvae per 3 days and in experiment 3, approximately 150 larvae per day. So in experiment 1 the number of whitefly larvae offered was too low to realise maximum oviposition capacity and the females could not deposit their eggs as fast as in experiment 2. In the same way, the females of experiment 2 could not deposit their eggs as fast as in experiment 3.

Medina et al. (1994) found a longevity of 13.8 days on chrysanthemum at temperatures varying between 16.3 and 21.7 °C, which was within the range found in this trial. Without hosts and with honey, Medina et al. (1991) found a longevity of 11.0 days, which was shorter than the longevity we found. Also without host and at $75 \pm 5$ % r.h., Manzano et al. (1999) found longevities of 42.2, 18.1 and 10.1 days at 15, 19 and 25 °C respectively. The first result being longer than our, while the other two are shorter. It is not clear if differences are due to experimental set-ups or rather due to genetic differences.

Oviposition frequency seems to be influenced by egg load and/or age. The oviposition frequency of *A. fuscipennis* had generally a maximum on the first day and declines immediately, despite the availability of the same number of *T. vaporariorum* larvae. This may indicate that the parasitoid increases host selection and attacks a more restricted set of hosts as egg load decreases or age increases. This was already observed and modelled by Mangel (1987).

In experiment 3, no significant difference was found between the fecundity at 20 and 25 °C. Also linear regression excluding the data of 30 °C gave very low coefficients of determination. This independence of fecundity from temperature within certain limits was to be expected with a pro-ovigenic parasitoid. At 30 °C, the fecundity was much lower but we suppose that this was caused mainly by the higher immature mortality as we measured fecundity indirectly by counting grey pupae. The immature mortality in the grey stage was 60 %. If mortality in the white stage were the same, the total fecundity at 30 °C would be 331. At 15 °C, the mobility of the *A. fuscipennis* was much lower and this reduces its oviposition frequency. If the females do not die before all eggs are deposited the fecundity should not be significantly different from the fecundity at other temperatures. However, the fecundity was significantly lower, indicating that not all females were able to deposit all their eggs before they died.

The fecundity found in this trial was considerably higher than that found by Medina et al. (1994). They found a fecundity of 103 eggs per female on chrysanthemum at temperatures varying between 21.7 and 16.3 °C.

The intrinsic rates of increase found by Manzano et al. (1999) on bean were 0.099 at 19 °C and 0.144 at 22 °C on cultivar "Chocho" and 0.085 at 19 °C on cultivar "Ica-Pijao", using clip cages. Our results using the clip cage method are considerably higher at 20 °C than their results at 19 °C. At 22 °C, on the contrary, their result was higher than the $r_m$ we found at 20 °C or at 25 °C. It is difficult to determine the influence of the host plant from these results, referring to the above-discussed problems with the clip cage method, which certainly also influenced negatively the results of Manzano et al. (1999). The $r_m$ values they found are much lower than the values we found with the cylinder method, and it is difficult to believe that the differences in intrinsic rate of increase are only due to the difference in host plant.

When we compare the biological parameters of *A. fuscipennis* with those of *E. formosa* several parameters are similar while other show important differences (Tables 3 &10):

- The upper lethal temperature of *E. formosa* is 38 °C, higher than that found for *A. fuscipennis* in this trial. The lower development threshold was estimated between 6.6 and 8.7 °C for *A. fuscipennis* and higher, 10.5 °C, for *E. formosa*.
- The development time from egg to adult is shorter for *A. fuscipennis* at lower temperatures (15-20 °C) and longer at higher temperatures (25-30 °C) compared with *E. formosa* when parasitizing *T. vaporariorum* L1 larvae. The total development time of *E. formosa* parasitizing *T. vaporariorum* L3 larvae is shorter than that of *A. fuscipennis* parasitizing *T. vaporariorum* L1 larvae at all temperatures.
- The mortality of *Encarsia formosa* parasitizing L1 whitefly larvae is 7.4 % in the black stage (van Roermund & van Lenteren, 1992), higher in comparison with the mortality in the grey stage of *A. fuscipennis*. However, the grey stage of *A. fuscipennis* coincides with the pupal stage while the black stage of *E. formosa* begins before the pupal stage. At 30 °C, the mortality of the grey stage of *A. fuscipennis* is 60 %. No data were found for *E. formosa* at that temperature but the mortality of *E. formosa* was found to be independent of temperature within the range 17-27 °C.
- In the presence of hosts, *E. formosa* has a longer longevity than *A. fuscipennis*. However, the longevity of *A. fuscipennis* at low host densities is not known. In the absence of hosts, longevity is longer for *E. formosa* at 15 and 20 °C, but at higher temperatures longevity it is higher for *A. fuscipennis*.
- The fecundity of *A. fuscipennis* was higher than the fecundity of *E. formosa* except at 30 °C. The lower fecundity at 30 °C could be due to the mortality in the white stage as fecundity in this trial was measured counting *T. vaporariorum* larvae that reached the grey stage.
- The oviposition frequency of *E. formosa* is constant in time until mean longevity has been reached and then it declines (van Roermund & van Lenteren, 1992), while the oviposition frequency of *A. fuscipennis* declines already after the first day (Figures 6 & 7).
- At early adult life, the oviposition frequency of *A. fuscipennis* is much higher than the oviposition frequency of *E. formosa*.
- The intrinsic rate of increase of *A. fuscipennis* is higher compared to that of *E. formosa*, except at 30 °C where the $r_m$ of *E. formosa* is considerably higher. Both parasitoids have an intrinsic rate of increase higher than that of their host, *T. vaporariorum*, but at 30 °C, *E. formosa* has a higher advantage than *A. fuscipennis* (Table 11).

When we consider all the above results we may conclude that *A. fuscipennis* might be a better parasitoid under the following conditions:

- In unheated greenhouses with low greenhouse temperatures. The greenhouses on the Bogota plateau in Colombia have a cold climate with mean temperatures of 15-16 °C, cold nights and short warm periods during the day. Under those conditions, egg production by the synovigenic parasitoid *E. formosa* might be a serious limitation and too few whiteflies might be parasitized (van Roermund, 1995).
- To quickly reduce whiteflies at high-density spots. For such situations, *A. fuscipennis* is an excellent candidate because of its high oviposition frequency early in life.

The previous conclusions are confirmed by the results of Marqués & Valencia (1991). They compared the performance of *A. fuscipennis* with that of *E. formosa* on chrysanthemum. In cages of 6 m², they introduced 1500 adults at week 7 and 1500 adults at week 9 after transplant on 150 plants that were naturally infested by *T. vaporariorum*, and observed a higher parasitization by *A. fuscipennis* than by *E. formosa* at week 10 and 12 after transplant.

Table 10. Life history of *E. formosa* on *T. vaporariorum* larvae at 15, 20, 25 y 30°C.

| Parameter | host stage | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|
| Development time egg-adult (days) | L1 | 63.5 | 35.4 | 21.4 | 15.1 |
| Development time egg-adult (days) | L3 | 42.2 | 26.0 | 17.3 | 13.5 |
| Mortality white stage (%) | L1 | | | 7.4 | |
| Mortality black stage (%) | L3 | | | 3.3 | |
| Total immature mortality (%) | L1 | | | 41.9 | |
| Total immature mortality (%) | L3 | | | 11.8 | |
| Longevity with host (days) | L3 | 38.4 | 24.2 | 15.3 | 9.7 |
| Pre-oviposition period (days) | All stages | - | 0.79 | 0.18 | 0.04 |
| Fecundity (eggs·female⁻¹) | L1-pupa | 63 | 301 | 390 | 178 |
| Oviposition frequency (eggs·female⁻¹·day⁻¹) | All stages | 1.7 | 9.4 | 15.4 | 10.6 |

Source: van Roermund & van Lenteren (1992)

Table 11. Intrinsic rate of increase of *T. vaporariorum*, *E. formosa* and *A. fuscipennis* as function of temperature.

| | Temperature (°C) | | | |
|---|---|---|---|---|
| | 15 | 20 | 25 | 30 |
| *T. vaporariorum* | 0.060 | 0.091 | 0.123 | 0.154 |
| *E. formosa* | 0.085 | 0.149 | 0.213 | 0.277 |
| *A. fuscipennis* (cylinders, new leaf every day) | 0.090 | 0.176 | 0.233 | 0.159 |

Source: *E. formosa* and *T. vaporariorum*: van Roermund (1995); *A. fuscipennis*: this paper.

Possible disadvantages of *A. fuscipennis* are:

- Their short adult life span, especially at low temperatures. However, it is not known what the longevity of the parasitoid would be at low *T. vaporariorum* densities. When the

density of *T. vaporariorum* larvae is low, its longevity might increase. We already found a longer longevity when the number of *T. vaporariorum* larvae were limited compared to high *T. vaporariorum* densities.

- *A. fuscipennis* does not show host-feeding while *E. formosa* kills about 10 % of its hosts by host-feeding. So the host kill rate of *E. formosa* (parasitizing and host feeding) could be higher than that of *A. fuscipennis* (only parasitizing).

In conclusion, *A. fuscipennis* could to be a good candidate for biological control of the greenhouse whitefly. Whether *A. fuscipennis* will be sufficiently efficient in controlling its host in greenhouse and field conditions depends on its host acceptance and searching efficiency (van Roermund, 1995).

## Acknowledgements

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## 4. Foraging of *Amitus fuscipennis* for whitefly (*Trialeurodes vaporariorum*) on tomato leaflets

**Abstract**

The residence time and time allocation of *Amitus fuscipennis* were measured on clean tomato leaflets at various temperatures, on leaflets with honeydew, and on leaflets with 1 or 4 unparasitized L1 larvae or 4 grey pupae of *Trialeurodes vaporariorum*. On clean leaflets, the residence time was about one hour at 20, 25 and 30 °C, and three hours at 15 °C. Residence time did not increase on leaflets with honeydew or on infested leaflets, except for leaflets with four unparasitized L1 larvae where it increased to about one and a half hour. On those leaflets, the Giving up time was also higher than on leaflets with other host types. The percentage time walking (of the total residence time decreased with host handling time) increased from 34 % on clean leaflets to 49 % on leaflets with honeydew, to 60 % on leaflets with grey pupae, and to more than 78 % on leaflets with L1 larvae. The time handling hosts was lower than 5 % for all treatments. Walking speed was 0.9, 1.5 and 1.7 mm/s at 15-16, 19-21 and 25-27 °C respectively and the width of the searching path was 0.8 mm. On leaflets with one L1 host, fewer hosts were encountered (1.95 – 2.35) than on those with four L1 host (6.10 – 6.68), while on leaflets with four grey pupae the number of encounters (2.05) was similar to that of leaflets with one host. The number of hosts that were parasitized was lower on leaflets with one L1 host (0.31-0.33) than on those with four L1 host (1.42-1.65). Host acceptance was 71 % for unparasitized L1 larvae, 33% for recently self-parasitized larvae, 62 % for larvae recently parasitized by a conspecific and 0 % for whitefly pupae containing a parasitoid pupa. Self and conspecific superparasitism was considerable. The minimum time needed for a successful oviposition was 23 s. The results are compared with data of *Encarsia formosa*. On infested leaves, the searching efficiency of *A. fuscipennis* was higher than that of *E. formosa*. This can be explained by its higher walking activity, lower time handling hosts, more rapid walking speed and wider searching path. Conclusions are drawn for the application of the parasitoid in practice.

**Introduction**

Biological control of pests on greenhouse grown tomatoes has become a common practice around the world. For biological control of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), the parasitoid *Encarsia formosa* Gahan is traditionally used (van Lenteren, 1992, 1995, 2000). In Colombia, commercial application of biological control in greenhouse tomatoes does not yet exist. In 1997, a research program was started to evaluate and/or adapt the biological control system of greenhouse whitefly with *E. formosa* to the local conditions and to evaluate the native parasitoid *Amitus fuscipennis* MacGown & Nebeker.

Greenhouses on the Bogota plateau (altitude of 2600 m) have a rather cold climate with a mean annual temperature of 14.5-16 °C. This low temperature might limit the use of *E. formosa*, because at temperatures lower than or equal to 18 °C, fecundity and oviposition frequency of *E. formosa* are lower than those of *T. vaporariorum* (van Roermund & van Lenteren, 1992a & 1992b). In addition, the parasitoid does not fly and has a low searching activity at temperatures lower than 18 °C (van Roermund & van Lenteren, 1995b). This could seriously hamper successful biological control.

Therefore, also a locally occurring parasitoid, *A. fuscipennis*, was studied as a candidate of whitefly control. Life history of *A. fuscipennis* as a parasitoid of *T. vaporariorum* (De Vis et al., 2001; Chapter 3) revealed that the intrinsic rate of increase of the parasitoid is substantially higher than that of *T. vaporariorum* and similar to that of *E. formosa* at temperatures from 15 to 30 °C. The two parasitoid species have different, possibly complementary, life history traits. The pro-ovigenic *A. fuscipennis* has a high egg load and

high oviposition frequency (De Vis et al., 2001, Chapter 3) and could therefore prove to be good at reducing whiteflies in high-density spots. The synovigenic *E. formosa* has a high longevity, which it can prolong through oosorbtion (Van Keymeulen & Degheele, 1978; van Lenteren et al., 1987), and the strategy of this species might be better for keeping *T. vaporariorum* for long periods at low density.

However, to be efficient in the field, the parasitoid first has to locate and parasitize hosts before being able to realise its intrinsic rate of increase. Locating a patchily distributed host like whiteflies involves a series of different steps that brings the parasitoid closer the host (Lewis et al., 1990). By using information from the environment, such as chemical stimuli, parasitoids increase their searching efficiency. Chemicals produced by the host plant usually help the parasitoid to find a new habitat and host specific chemicals are often used to detect hosts or host patches from a short distance (Vet & Dicke, 1992). Within a patch individual hosts have to be located, evaluated and accepted or rejected for oviposition or host feeding, and as the patch is depleted, parasitoids have to decide when to leave (Waage, 1979; van Roermund et al., 1994). Furthermore, the foraging behaviour of an individual wasps can be very variable and the origin of this variation can be genotypic, phenotypic or physiological (Lewis et al., 1990).

Manzano (2000) already determined several aspects of the previously mentioned foraging framework for *A. fuscipennis* on bean. She found, as for *E. formosa* (Noldus & van Lenteren, 1989), that both naive and experienced *A. fuscipennis* females were not attracted by clean or infested bean leaves or plants even from a short distance, and landing on a leaf seemed to be a random process. Foraging efficiency within a patch is influenced strongly by walking speed, walking activity, probability of host acceptance and the arrestment effect when searching on clean and infested leaflets (van Lenteren & van Roermund, 1999). Manzano (2000) found that *A. fuscipennis* had a high walking speed and walking activity, and accepted easily hosts resulting in a high percentage parasitism. The parasitoid was arrested by encounters with or ovipositions in hosts and showed area-restricted search after encountering a host on a leaflet, indicating a high searching efficiency in host patches.

The experiment of Manzano, however, were done on bean and host plant characteristics like leaf hairs, leaf or leaflet area, plant architecture etc. influence strongly the foraging behaviour (e.g. van Lenteren, 1995; Sütterlin & van Lenteren, 1999 & 2000). Additionally, to make comparisons with *E. formosa* possible, we had to evaluate the foraging behaviour of *A. fuscipennis* under similar conditions as it was done for *E. formosa*. In this paper, we determine the foraging behaviour and parameters that influence the searching efficiency of *A. fuscipennis* on clean and infested tomato leaflets, as a second step to evaluate the use of *A. fuscipennis* in greenhouse grown tomatoes and to compare its searching efficiency with that of *E. formosa*.

## Materials and methods

Two experiments were done at the Horticultural Research Centre of the University of Bogota Jorge Tadeo Lozano near Bogota using the same methods as described by van Roermund and van Lenteren (1995b).

In a first experiment, the foraging behaviour of *A. fuscipennis* was studied on clean tomato leaflets at 15, 20, 25 and 30 °C, introducing the parasitoid on the upper leaf side, and at 25 °C with introduction on the lower leaf side. These treatments were selected because in

crops with low host density more than 99 % of the parasitoids are searching on clean leaflets (van Roermund et al., 1997) and the greenhouse conditions on the Bogota plateau are very variable (Cooman et al., 1999; Chapter 2, 6 & 7). The side of the leaflet where the parasitoid is introduced can influence the time distribution on upper and lower leaf side and *T. vaporariorum* larvae are only present on the lower leaf side, but host derived cues like honeydew and exuviae can be present on the upper side.

In the second experiment, the behaviour was studied at 25 °C on tomato leaflets with honeydew or infested with various (low) numbers and types of *T. vaporariorum* larvae: (1) leaflets with one unparasitized L1 larva, (2) leaflets with four unparasitized L1 larvae, (3) leaflets with one recently parasitized L1 larva, (4) leaflets with four recently parasitized L1 larvae, and (5) leaflets with four parasitized grey pupae (parasized host with parasitoid in pupal stage). In this experiment, parasitoids were introduced on the lower leaf side. Recent parasitized larvae were parasitized 2-4 hours before the experiment. These treatments were selected because in crops with low host density, the *T. vaporariorum* density in host patches is very low and also to determine the effect of previous host parasitization on the foraging behaviour of *A. fuscipennis*. The temperature was rather high when compared to the greenhouse temperature of greenhouses situated on the Bogota plateau (Cooman et al., 1999; Chapter 2, 6 & 7), but to make comparison with data of *E. formosa* possible we decided for this temperature.

Tomato plants of the variety Boris (Bruinsma Seeds, 's Gravenzande, The Netherlands) with leaflets prepared as indicated above were brought to a climate room with a L12:D12 light regime, a light intensity at the site of the experiment of 28 $\mu$mol$\cdot$m$^{-2}\cdot$s$^{-1}$, a relative humidity of 70 $\pm$ 10 % and a temperature of 15, 20, 25 or 30 $\pm$ 1 °C. A standard high-pressure sodium lamp of 400W (WR400 AL; P.L. Light Systems Canada Inc., Grimsby, Ontario, Canada) provided light; a heater and air conditioner connected to a control unit (Watlow 93, Watlow controls, Winona, Minnesota, USA) controlled temperature; and a humidifier (Herrmidifier 500, Herrmidifier Inc., Lancaster, PA., USA) controlled humidity. Whiteflies and parasitoids originated from the Horticultural Research Centre's rearing unit where they were reared on tomato. To obtain recently emerged (naive) parasitoids, tomato leaflets bearing parasitized whitefly pupae were placed in a petri dish with honey in the climate room at the same temperature of the experiment until emergence.

Before starting an observation, the leaflets were mapped and whitefly larvae were numbered. An observation began from the moment of introduction of a naive parasitoid on the leaflet until it flew or walked away from the leaflet via the petiole. The leaflet area was 23.5 $\pm$ 1.6 cm$^2$. During the observation the parasitoid's position and activity were registered with a PC using the software "The observer 2" (Noldus, Wageningen, The Netherlands). The registered positions were (1) centre of upper leaf side, (2) border of upper leaf side, (3) centre of lower leaf side, (4) border of lower leaf side and (5) petiole. The registered activities were (1) searching while drumming, (2) standing still/eating/preening, (3) handling of each individual larva (4) oviposition posture with respect to each individual larva. A total of 388 observations were made, resulting in 391 hours of observation time.

Additionally an experiment was done at the Laboratory of Entomology at Wageningen University to determine the walking speed of *A. fuscipennis* on clean leaflets using materials as described by Drost et al. (1999). Tomato leaflets were cut to fit in a petri dish and were then placed upside down on 100 % water-saturated polyurethane foam in a petri dish, to maintain the leaflet hydrated during the experiment. The surface tension of the water adhered

the leaflet, including the leaflet borders, to the foam. Temperature was measured with a thermometer with the bulb located on the foam. Since the leaf and thermometer were in contact with the water, the temperature on the surface of the leaf was measured exactly. Observations were done in a large room and the temperature control was not very accurate, causing variation in temperature during and between observations. The observations were made using the software Ethovision (Noldus, Wageningen, The Netherlands). Before starting an observation, the leaflet was scanned by the system and an artificial arena, about 5 mm smaller than the leaflet at all edges was defined. In this way, the edge effect was eliminated, because *A. fuscipennis* females were found to follow the edge during observations as the water barrier prevented them from leaving the leaflet. Afterwards a recently emerged naive female was introduced on the leaflet and the tracks were saved digitally, taking three frames per second. After the observations, the tracks were analysed visually on the monitor one by one and a part of the track during which the parasitoid walked uninterruptedly within the artificial arena was selected for walking speed analysis with the Ethovision software.

The width of the searching path was estimated measuring the distance between the antennae of 20 walking females using a stereo microscope with an ocular graduated ruler.

Mean and standard error of all variables were calculated and a Kruskal-Wallis one-way analysis of variance (Anova) was done to detect significant differences between the mean values of the experiments. By comparison of mean ranks subsets of similar (homogeneous) mean ranks were identified.

## Results

### Residence time and time allocation

Residence times showed a very high variation (Tables 1-3). On clean leaflets (Table 1) the mean residence time was 2554 s at 30 °C and 11320 s at 15 °C, but no significant difference was found. At 15 °C, residence time had a minimum of 270 s and a maximum of 30253 s. The time spent on searching of 826 - 1075 s and on standing still of 1440- 10865 s, corresponding to respectively 32-43 % and 57-69 % of the total time was not significantly different among treatments. Although at 15 °C the mean time standing still was very high (10470 s), a relatively low percentage of the time spent on standing still (68 %) was found because the observations with very high residence times had a percentage time standing still higher than 95 %, while that of those with low residence times was 0 to 27 %. The duration of the searching intervals was shorter at 25 °C and when introducing the parasitoid on the upper leaf side, than in the other treatments. The standing still intervals were longer at 15-20 °C than at 25-30 °C.

On 36 % of the leaflets with one unparasitized larva the host was discovered, as well as on 44 % of the leaflets with one recently parasitized host (difference not significant, $\chi^2$=3.66). On leaflets with four unparasitized and four parasitized L1 hosts, this percentage increased to 55 %, which is significantly higher than for leaflets with 1 larva ($\chi^2$=17.96). On the leaflets with four grey pupae the host was discovered in 43 % of the cases (difference between one L1 and four grey larvae not significant; difference between four L1 and four grey larvae significant, $\chi^2$=8.41).

Table 1. Time spent by *Amitus fuscipennis* females on different activities and at different positions on **clean tomato leaflets** as function of temperature and side of the leaf where the parasitoid was introduced. The standard error of the mean follows mean values; n represents the number of parasitoids tested. For the duration of the different activity intervals, the number of replicates is given between brackets.

| Temperature (°C) | 15 | 20 | 25 | 25 | 30 |
|---|---|---|---|---|---|
| Introduction side | upper | upper | upper | lower | upper |
| n | 10 | 23 | 39 | 43 | 27 |
| Total residence time (s) | 11320 ± 3520 a | 4282 ± 865 a | 4584 ± 872 a | 3051 ± 368 a | 2554 ± 326 a |
| Time searching (s) | 850 ± 254 a | 841 ± 202 a | 1075 ± 207 a | 826 ± 170 a | 890 ± 151 a |
| Time standing still (s) | 10470 ± 3436 a | 3441 ± 798 a | 3510 ± 812 a | 2225 ± 335 a | 1664 ± 277 a |
| Searching (% of total) | 32.0 ± 12.4 a | 31.3 ± 5.5 a | 34.9 ± 4.8 a | 34.2 ± 4.7 a | 43.1 ± 5.2 a |
| Standing still (% of total) | 68.1 ± 12.6 a | 69.4 ± 5.3 a | 65.2 ± 5.4 a | 66.4 ± 5.0 a | 57.0 ± 5.3 a |
| Duration searching intervals (s) | 170 ± 32 (50) a | 164 ± 24 (118) a | 95 ± 8 (439) b | 185 ± 22 (192) a | 156 ± 17 (154) a |
| Duration standing still interval (s) | 2276 ± 599 (46) a | 747 ± 107 (106) a | 313 ± 45 (437) c | 532 ± 74 (180) b | 312 ± 61 (144) bc |
| Time on upper leaf side (s) | 957 ± 634 ab | 606 ± 206 ab | 678 ± 119 a | 324 ± 112 b | 1142 ± 279 a |
| Time on lower leaf side (s) | 10358 ± 3371 a | 3657 ± 848 ab | 3905 ± 854 ab | 2721 ± 358 ab | 1401 ± 269 b |
| Time on upper leaf side (% of total) | 31.0 ± 12.8 ab | 26.1 ± 7.1 a | 34.8 ± 6.0 a | 10.8 ± 3.5 b | 44.5 ± 6.5 a |
| Time on lower leaf side (% of total) | 68.9 ± 12.9 ab | 73.3 ± 7.2 b | 65.2 ± 6.0 b | 89.0 ± 3.5 a | 55.0 ± 6.4 b |
| Number of leaf side changes | 1.9 ± 0.6 a | 3.2 ± 0.6 a | 5.7 ± 1.2 a | 4.4 ± 1.1 a | 6.2 ± 1.3 a |
| Number of parasitoids that never changed leaf sides (%) | 10% | 9% | 15% | 44% | 15% |

* Kruskal-Wallis test ($\alpha=0.05$). Different letters in a row indicate significant differences.

Table 2. Time spent by *Amitus fuscipennis* on different activities and at different positions on tomato leaflets infested with different number and type of whitefly larvae, **when hosts were not discovered**. The standard error of the mean follows mean values; n represents the number of parasitoids tested and for the duration of the different activity intervals, the number of replicates is given between brackets.

| Host type | Not parasitized | Not parasitized | Recently parasitized | Recently parasitized | Grey pupae |
|---|---|---|---|---|---|
| Number of hosts | 1 | 4 | 1 | 4 | 4 |
| n | 35 | 18 | 25 | 17 | 27 |
| Total residence time (s) | 1648 ± 244 a | 3253 ± 806 a | 3056 ± 543 a | 3817 ± 867 a | 2920 ± 369 a |
| Time searching (s) | 1341 ± 222 a | 1772 ± 563 a | 1591 ± 256 a | 2148 ± 643 a | 938 ± 182 a |
| Time standing still (s) | 308 ± 79 b | 1481 ± 425 ab | 1465 ± 470 ab | 1669 ± 555 ab | 1982 ± 315 a |
| Searching (% of total) | 75.1 ± 4.5 a | 57.6 ± 9.0 ab | 65.2 ± 6.5 a | 64.8 ± 8.6 a | 37.8 ± 4.9 b |
| Standing still (% of total) | 24.9 ± 4.5 b | 42.4 ± 9.0 ab | 34.8 ± 6.5 b | 35.2 ± 8.6 b | 62.2 ± 4.9 a |
| Duration searching intervals (s) | 431 ± 78 (109) a | 367 ± 64 (87) a | 355 ± 57 (112) a | 589 ± 125 (62) a | 176 ± 21 (144) a |
| Duration standing still interval (s) | 121 ± 20 (89) a | 329 ± 83 (81) a | 370 ± 69 (99) a | 516 ± 140 (55) a | 391 ± 72 (137) a |
| Time on upper leaf side (s) | 803 ± 152 a | 1169 ± 476 ab | 634 ± 188 ab | 817 ± 391 ab | 212 ± 106 b |
| Time on lower leaf side (s) | 827 ± 162 b | 2074 ± 491 ab | 2407 ± 503 a | 2990 ± 751 a | 2695 ± 332 a |
| Time on upper leaf side (% of total) | 42.5 ± 5.7 a | 27.0 ± 7.5 ab | 18.8 ± 4.6 ab | 23.0 ± 5.7 ab | 7.7 ± 2.1 b |
| Time on lower leaf side (% of total) | 55.4 ± 5.6 b | 72.6 ± 7.6 ab | 80.1 ± 4.6 ab | 75.8 ± 5.8 ab | 91.9 ± 2.1 a |
| Number of leaf side changes | 6.0 ± 1.0 a | 7.4 ± 2.9 a | 5.5 ± 1.0 a | 4.2 ± 1.2 a | 3.4 ± 0.8 a |
| Number of parasitoids that never changed leaf sides (%) | 11% | 39% | 24% | 18% | 19% |

Kruskal-Wallis test ($\alpha$=0.05). Different letters in a row indicate significant differences.

The mean residence time on clean leaflets (at 25 °C when the parasitoid was introduced on the lower leaf side) was $3051 \pm 368$ s. This time was not significantly different from the residence time of $3361 \pm 183$ s on infested leaflets (pooled observations, whether host was discovered or not), that of $2757 \pm 235$ s on infested leaflets when the host was not discovered, and that of $2575 \pm 465$ s on leaflets with honeydew (Kruskal Wallis test, p>0.37 for the three comparisons). However, the residence time on clean leaflets and that on infested leaflets where the host was not discovered, was significantly lower than the residence time of $4077 \pm 271$ s on infested leaflets on which the host was discovered (Kruskal Wallis test, p=0.02 and $p<10^{-4}$ respectively). The time allocation to the different activities showed important differences when comparing the previous mentioned leaflets types. The percentage time spent on searching increased from $34.2 \pm 4.7$ % on clean leaflets to $49.0 \pm 5.1$ % on leaflets with honeydew, $60.8 \pm 3.0$ % on infested leaflets where the host was not found and $78.1 \pm 2.0$ % on infested leaflets where the host was found. As a result, the percentage time standing still decreased from $66 \pm 5.0$ % on clean leaflets to $19.2 \pm 2.1$ % on infested leaflets where the host was discovered (Kruskal Wallis tests on time searching and time standing still, P < 0.002 for the comparisons: clean – infested, clean – infested when host was not discovered, clean – infested host discovered, and infested host not discovered – infested host discovered). The time allocation on leaflets with honeydew was similar to that on clean leaflets (Kruskal Wallis tests on time searching and time standing still; p > 0.08).

On infested leaflets where hosts were not discovered (Table 2), no significant differences were found for the total residence times among treatments. Time allocation and the duration of the searching and standing still intervals were usually not different among treatments. However, on the leaflets with four grey pupae the time and percent time spent standing still was significantly higher and the percent time dedicated to searching significantly lower than in the other treatments, excluding the treatment with four unparasitized L1 larvae. The residence time on infested leaflets when hosts were discovered was higher than that on infested leaflets when the hosts were not discovered (Kruskal–Wallis test, $p<10^{-4}$). However, when looking at the separate treatments (Table 2 & 3), it increased only on those leaflets with unparasitized hosts and not on leaflets with parasitized hosts (Kruskal Wallis test, $p<10^{-4}$ and $p= 4 \cdot 10^{-4}$ for leaflets with one and four unparasitized L1 larvae respectively; for the other treatments the difference was not significant). Differences among the treatments were due to both the number and type of hosts on the leaflets:

With respect to the number of hosts, the residence time of $3508 \pm 362$ s on leaflets with one L1 larva was lower than that of $4846 \pm 419$ s on leaflets with four L1 larvae (Kruskal–Wallis test, p=0.0059). On the latter leaflets, the time spent on searching of $3778 \pm 302$ s was higher than that of $2811 \pm 249$ s on the former leaflets (Kruskal–Wallis test, p=0.0126). Also the time assessing hosts and ovipositing hosts was significantly higher (Kruskal–Wallis test, $p<10^{-4}$), but not the time spent standing still. The time spent manipulating hosts and ovipositing increased with a factor of three to four and the respective percentages increased with a factor 2.

With respect to the type of hosts, the residence time on leaflets with unparasitized larvae of $5069 \pm 413$ s was higher than that of $3313 \pm 350$ s on leaflets with recently parasitized L1 larvae (Kruskal–Wallis test, p=0.0003). Similarly, the giving up time (GUT, time after the last oviposition or encounter) of $2610 \pm 291$ s on leaflets with unparasitized L1 larvae was higher than that of $1464 \pm 153$ s on those with parasitized L1 larvae (Kruskal–Wallis test, $p=4 \cdot 10^{-4}$)

Table 3. Time allocation of *A. fuscipennis* on different activities on tomato leaflets infested with different number and type of whitefly larvae, when hosts were discovered or honeydew was on the leaflet. The standard error of the mean follows mean values; n represents the number of parasitoids tested, and for the duration of the different activity intervals, the number of replicates is given between brackets.

| Host type | Unparasitized | Unparasitized | Recently parasitized | Recently parasitized | Grey pupae | Honeydew |
|---|---|---|---|---|---|---|
| Number of hosts | 1 | 4 | 1 | 4 | 4 | 0 |
| n | 20 | 22 | 20 | 21 | 20 | 21 |
| Total residence time (s) | 4078 ± 542 ab | 5969 ± 560 a | 2938 ± 460 b | 3670 ± 523 ab | 3559 ± 733 b | 2575 ± 465 b |
| Time walking/drumming (s) | 3327 ± 364 ab | 4659 ± 462 a | 2296 ± 306 bcd | 2856 ± 270 abc | 1769 ± 471 cd | 1110 ± 222 d |
| Time standing still (s) | 679 ± 244 a | 1101 ± 260 a | 601 ± 204 a | 650 ± 359 a | 1781 ± 457 a | 1378 ± 310 a |
| Time assessing host (s) | 20 ± 3 bc | 60 ± 7 a | 12 ± 2 c | 47 ± 9 ab | 9 ± 2 c | |
| Time ovipositing (s) | 52 ± 7 ab | 149 ± 17 a | 28 ± 5 bc | 117 ± 21 a | 0 ± 0 c | |
| Time walking/drumming (%) | 84.9 ± 2.6 a | 78.4 ± 3.5 a | 83.2 ± 3.3 a | 83.9 ± 3.3 a | 59.6 ± 6.6 ab | 49.0 ± 5.1 b |
| Time standing still (%) | 12.9 ± 2.6 bc | 17.8 ± 3.6 bc | 14.8 ± 3.5 bc | 11.0 ± 3.4 c | 39.8 ± 6.7 ab | 45.7 ± 5.9 a |
| Time assessing host (%) | 0.7 ± 0.1 ab | 1.1 ± 0.1 ab | 0.6 ± 0.1 ab | 1.5 ± 0.3 a | 0.7 ± 0.2 b | |
| Time ovipositing (%) | 1.5 ± 0.3 a | 2.7 ± 0.3 a | 1.4 ± 0.3 a | 3.6 ± 0.5 a | 0.0 ab | |
| Duration of walking intervals (s) | 465 ± 56 (143) a | 290 ± 29 (353) bc | 469 ± 68 (98) ab | 331 ± 39 (181) bc | 171 ± 23 (205) c | 115 ± 12 (202) d |
| Duration of inactivity intervals (s) | 153 ± 32 (89) bc | 124 ± 23 (195) c | 261 ± 40 (46) a | 240 ± 72 (57) abc | 230 ± 40 (156) ab | 271 ± 49 (107) abc |
| Time until 1st encounter | 987 ± 253 a | 604 ± 181 a | 898 ± 394 a | 1027 ± 329 a | 1005 ± 497 a | |
| Giving-up time | 2186 ± 316 ab | 2996 ± 469 ab | 1665 ± 150 ab | 1273 ± 259 b | 1929 ± 476 ab | |

* Kruskal-Wallis test ($\alpha = 0.05$). Different letters in a row indicate significant differences.

and that of 1929 ± 476 s on those with four grey pupae (Kruskal–Wallis test, p=0.0326). The times spent on the different activities were all significantly higher on leaflets with unparasitized larvae than on those with parasitized L1 larvae. However, the most important difference was found for the time spent on searching: 4025 ± 312 s on leaflets with unparasitized larvae and 2582 ± 206 s on leaflets with parasitized L1 larvae (Kruskal–Wallis test, p=3·10$^{-4}$). The time until the first encounter was similar among treatments.

The percentage of time dedicated to host handling was not higher than 5.1 %. Among treatments, the time spent on host handling (manipulating + ovipositing) varied from 9 s for leaflets with four grey larvae, over 41 s and 72 s for leaflets with one parasitized and unparasitized larva respectively to 164 s and 209 s for leaflets with four parasitized and unparasitized larvae respectively.

On leaflets with honeydew, significantly less time was spent on searching and significantly more time was spent on standing still. On these leaflets a mean of 87 s or 5 % of the total time was spent on feeding on the honeydew.

The walking activity (Table 4) tended to be higher after the last encounter than before the first encounter on leaflets with L1 larvae, but this was only significant for leaflets with one unparasitized L1 host. The overall walking activity varied between 82 and 87 % on leaflets with L1 larvae. On those with four grey pupae it was 30 % lower, but not significantly different.

Table 4. Mean walking activity of *Amitus fuscipennis* (expressed as % of time decreased with host handling time) before the first encounter, after the last encounter and during the total residence time on the tomato leaflets with different number and type of hosts. The standard error of the mean follows mean values; n represents the number of parasitoids tested.

| Host type | Unparasitized | Unparasitized | Recently parasitized | Recently parasitized | Grey pupae |
|---|---|---|---|---|---|
| Number of hosts | 1 | 4 | 1 | 4 | 4 |
| n | 20 | 22 | 20 | 21 | 20 |
| Walking activity before first encounter | 81.9 ± 4.3 b | 81.4 ± 6.0 a | 88.5 ± 4.3 a | 82.9 ± 5.7 a | 79.3 ± 6.6 a |
| Walking activity after the last encounter | 94.7 ± 1.6 a | 84.1 ± 4.6 a | 88.4 ± 3.6 a | 93.3 ± 3.4 a | 66.6 ± 7.4 a |
| p [*] | 0.02 | 0.78 | 0.78 | 0.19 | 0.27 |
| Walking activity during the total residence time [**] | 86.8 ± 2.7 ab | 81.6 ± 3.7 ab | 85.0 ± 3.5 a | 88.5 ± 3.5 b | 59.7 ± 6.8 ab |

[*] Wilcoxon signed rank test for pairwise comparison for the comparison of walking activity before the first and after the last encounter of each column

[**] Kruskal-Wallis test (α=0.05). Different letters in a row indicate significant differences

The residence time was not well correlated with the frequency of host contacts or with the frequency of ovipositions (Table 5). On leaflets with unparasitized larvae the correlation coefficient was higher than on leaflets with parasitized larvae. Linear regression of residence time with number of host contacts or number of ovipositions yielded determination coefficients ($r^2$) of 0.11.

Table 5. Spearman's correlation ($r_s$) between residence time and total number of encounters, number of ovipositions and correlation between number encounter and number of ovipositions of *Amitus fuscipennis* on infested tomato leaflets as function of host type; n represents the number of parasitoids tested.

| Host type | n | Total time vs. encounters | Total time vs. ovipositions | Encounters vs. ovipositions |
|---|---|---|---|---|
| Not parasitized | 95 | 0.626 | 0.649 | 0.959 |
| Recently parasitized | 83 | 0.148 | 0.115 | 0.860 |
| Grey pupae | 47 | - 0.011 | - | - |
| All | 225 | 0.345 | 0.356 | 0.820 |

*Position on the leaflet*
On leaflets with honeydew *A. fuscipennis* spent 54 % of the time on the lower leaf side. This was significantly lower than on clean or infested leaflets, where it was between 72 and 85 %. The time spent on the lower leaf side of infested leaflets when hosts were discovered (3420 ± 241 s) was significantly higher (Kruskal-Wallis test, $P<10^{-4}$) than that on leaflets where the host was not discovered (2049 ± 196 s). The respective percentages of 84.9 ± 1.5% and 73.9 ± 2.6 %, however, were similar (Kruskal-Wallis test, P=0.15).

On clean leaflets (Table 1) and when the parasitoid was introduced on the upper leaf side, no significant differences among treatments were found for the time and percentage of the time spent on the lower or on the upper leaf side; except for the time spent on the lower leaf side at 15 ℃ of 10358 ± 3371 s, which was higher than that of 1401 ± 269 s at 30 ℃. At 25 °C and when introducing the parasitoid on the lower leaf side, the percentage time spent on that side of 89.0 ± 3.5 % was higher than that of 65.2 ± 6.0 % when the parasitoid was introduced on the upper leaf side. However, no difference was found for the time spent on the lower leaf side for both treatments. The number of leaf side changes was very variable and no difference could be found between the treatments. However, when introducing the parasitoid on the lower leaf side, the number of parasitoids that never changed leaf sides was significantly higher than when it was introduced on the upper leaf side.

On infested leaflets where the host was not discovered (Table 2), the distribution of the time on the two leaflet sides was similar among treatments except for that of leaflets with one unparasitized larva, which differed from that of leaflets with four grey pupae. On the latter, more time was spent on the lower leaflet side. The number of leaf side changes was similar among treatments.

Table 6. Time allocation of *A. fuscipennis* on different positions on tomato leaflets infested with different number and type of whitefly larvae, **when hosts were discovered or honeydew was on the leaflet**. The standard error of the mean follows mean values; n represents the number of parasitoids tested.

| Host type | Unparasitized | Unparasitized | Recently parasitized | Recently parasitized | Grey pupae | Honeydew |
|---|---|---|---|---|---|---|
| Number of hosts | 1 | 4 | 1 | 4 | 4 | 0 |
| n | 20 | 22 | 20 | 21 | 20 | 21 |
| Time on upper leaf side (s) | 832 ± 152 ab | 945 ± 192 ab | 584 ± 224 ab | 434 ± 122 ab | 353 ± 136 b | 951 ± 181 a |
| Time on lower leaf side (s) | 3223 ± 499 ab | 4998 ± 537 a | 2338 ± 321 b | 3225 ± 515 ab | 3170 ± 634 b | 1614 ± 337 b |
| Time on upper leaf side (% of total) | 20.5 ± 3.4 ab | 16.1 ± 3.0 b | 14.4 ± 3.9 b | 12.1 ± 2.9 b | 8.9 ± 3.5 b | 45.6 ± 5.9 a |
| Time on lower leaf side(% of total) | 78.8 ± 3.6 ab | 83.4 ± 3.0 a | 84.8 ± 3.8 a | 87.5 ± 3.0 a | 90.3 ± 3.6 a | 54.0 ± 5.9 b |
| Number of leaf side changes | 10.4 ± 2.0 a | 10.6 ± 1.8 a | 7.0 ± 1.8 ab | 4.9 ± 1.0 ab | 3.3 ± 1.0 b | 5.6 ± 1.0 ab |
| Parasitoids never changing leaf side | 5% | 9% | 15% | 19% | 25% | 0% |

Kruskal-Wallis test (α=0.05). Different letters in a row indicate significant differences.

Table 7. Host handling behaviour of *A. fuscipennis* on tomato leaflets infested with different number and type of whitefly larvae, **when hosts were discovered**. The standard error of the mean follows mean values; n is the number of parasitoids tested.

| Host type | Not parasitized | Not parasitized | Recently parasitized | Recently parasitized | Grey pupae |
|---|---|---|---|---|---|
| Number of hosts | 1 | 4 | 1 | 4 | 4 |
| n | 20 | 22 | 20 | 21 | 20 |
| Number of encounters with hosts | 2.00 ± 0.24 b | 6.32 ± 0.66 a | 1.75 ± 0.20 b | 5.57 ± 0.71 a | 2.05 ± 0.26 b |
| Number of assessments | 2.30 ± 0.27 b | 6.68 ± 0.71 a | 1.95 ± 0.22 b | 5.95 ± 0.70 a | 2.05 ± 0.26 b |
| Number of antennal rejections | 0.50 ± 0.15 b | 2.00 ± 0.29 a | 0.95 ± 0.23 ab | 2.24 ± 0.46 a | 2.05 ± 0.26 a |
| Number of ovipositorial rejections | 0.45 ± 0.18 ab | 1.09 ± 0.26 a | 0.25 ± 0.12 ab | 0.48 ± 0.24 ab | 0.00 b |
| Number of ovipositions | 1.35 ± 0.18 ab | 3.59 ± 0.34 a | 0.75 ± 0.12 bc | 3.24 ± 0.41 a | 0.00 c |
| Number of hosts encountered | 1.00 ± 0.00 b | 3.18 ± 0.23 a | 1.00 ± 0.00 b | 3.05 ± 0.19 a | 1.65 ± 0.15 b |
| Number of host parasitized | 0.90 ± 0.07 b | 3.00 ± 0.25 a | 0.70 ± 0.11 bc | 2.57 ± 0.26 a | 0.00 c |
| Self-superparasitism (%) | 33.3 | 16.5 | 6.6 | 19.4 | 0.0 |

Kruskal-Wallis test (α=0.05). Different letters in a row indicate significant differences.

On leaflets where the host was discovered (Table 6), differences were mostly due to the host type. The time spent on the lower leaf side on leaflets with L1 parasitized hosts of 4153 ± 389 s was higher than that of 2792 ± 311 s on leaflets with unparasitized L1 hosts (Kruskal-Wallis test, P=0.0013). The opposite was found for the time spent on the upper leaf side (Kruskal-Wallis test, P=0.0020). The number of leaf side changes was lower and the number of parasitoids that never changed leaf sides was higher on leaflets with recently parasitized L1 host than on leaflets with unparasitized L1 hosts. Leaflets with grey pupae showed similar time distribution as leaflets with recently parasitized L1 larvae, but the number of leaf side changes was lower and the number of parasitoids that never changed form leaf side was higher.

Sometimes the parasitoid walked onto the petiole and returned to the leaf again. In those cases, the observation was not finished. Only when the parasitoid did not return to the leaf was the observation terminated. The time spent on the petiole was very low in all experiments, with a mean of less than 38 s and less than 0.8 % of the total time spent on the leaf.

The parasitoids left the leaflet predominantly by flying away from the upper border or the lower centre or by walking via the petiole (Figure 1). In the experiment with infested leaflets, a higher percentage of the parasitoids left the leaflets by walking via the petiole. In all experiments, the parasitoids hardly ever left the leaf from the upper centre.
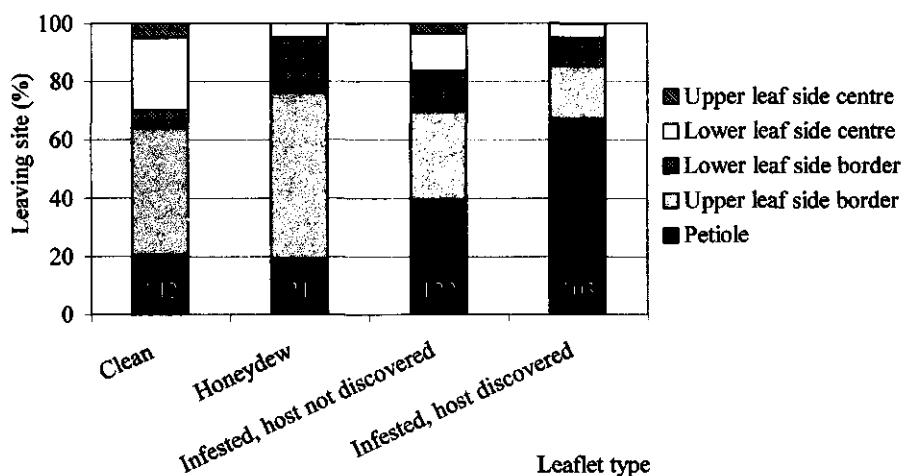


Figure 1. Site from which the parasitoids left on clean leaflets, leaflets with honeydew, infested leaflets where the host was not discovered and infested leaflets where the host was discovered. The number of parasitoids tested is given at the base of each column

*Host encounters, handling and acceptance.*
Once found, the host was assessed and then rejected or the oviposition posture was adopted. After handling the host, walking while drumming was normally resumed. When an

oviposition posture was adopted, the parasitoids could not always introduce the ovipositor successfully into the whitefly larva. *A. fuscipennis* oviposits with its back to the *T. vaporariorum* larva and sometimes irregularities in the leaf surface or obstacles such as leaf veins or hairs impeded introduction of the ovipositor by the parasitoid. In these cases, the host might either be abandoned or be re-assessed followed by adoption of the oviposition posture. Up to three host assessments, each followed by oviposition posture, were observed during several encounters. A total of 21 such complex host handling behaviours were observed, corresponding to 10.7 % of all the encounters on leaflets with one L1 larvae and to 5.1 % on those with four L1 larva. These percentages were the same for leaflets with unparasitized and parasitized L1 larvae. However, the success rate (successful oviposition) of 80.0 % was higher for conspecific parasitized larvae than that of 45.5 % for unparasitized larvae. It was similar for leaflets with one or four larvae.

The number of encounters with hosts (Table 7) was significantly higher, i.e. increased with a factor of 3, on leaflets with four L1 hosts (6.1 – 6.7 encounters per leaflet) compared to those with one L1 host (2.0 – 2.4 encounters per leaflet). On leaflets with four grey pupae the number of encounters (2.1 encounters per leaflet) was similar to the number of encounters on leaflets with only one L1 host.

The shortest oviposition posture resulting in a grey pupa was 22.3 s and all oviposition postures longer than 23.0 s were successful. To calculate the number of successful ovipositions the latter minimum time was used. The number of ovipositions was significantly higher on leaflets with four L1 larvae compared to those with one (Table 7). The number of rejections, ovipositorial rejections and successful ovipositions on leaflets with unparasitized larvae were similar to those on leaflets with parasitized L1 larvae.
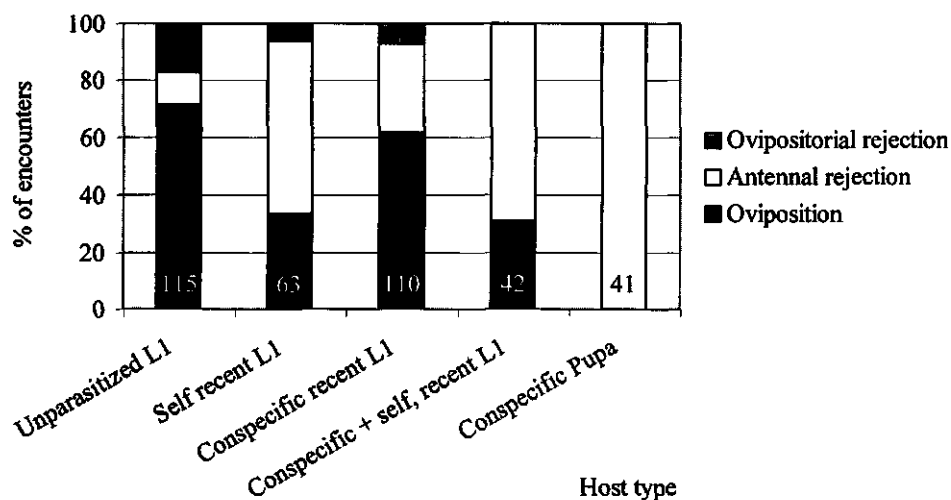
Table 8. Mean duration of host handling behaviours of *A. fuscipennis* on different types of *T. vaporariorum* larvae (unparasitized, self-parasitized or parasitized by a conspecific) at 25 °C. The standard error of the mean follows mean values; the number of replicates is given between brackets.

| Host type | Unpara-sitized | Self recent | Conspecific recent | Conspecific recent and self recent | Conspecific |
|---|---|---|---|---|---|
| Handling behaviour | L1 | L1 | L1 | L1 | grey pupa |
| Assessing host leading to rejection | 13.4 ± 2.2 (16) a | 7.6 ± 1.2 (38) ab | 7.9 ± 0.9 (40) a | 4.5 ± 0.6 (31) b | 4.4 ± 0.6 (41) b |
| Assessing host before oviposition | 8.8 ± 0.6 (82) a | 10.1 ± 1.2 (22) a | 7.8 ± 1.2 (68) b | 9.0 ± 1.9 (14) ab | |
| Assessing host before ovipositorial rejection | 8.1 ± 1.1 (30) a | 6.7 ± 1.3 (4) a | 7.6 ± 1.5 (15) a | | |
| Oviposition | 37.5 ± 1.3 (83) a | 38.0 ± 2.8 (22) a | 34.5 ± 2.0 (68) a | 36.9 ± 5.1 (14) a | |
| Ovipositorial rejection | 10.4 ± 0.9 (30) a | 12.8 ± 3.6 (4) a | 11.5 ± 1.7 (15) a | | |

* Kruskal-Wallis test (α=0.05). Different letters in a row indicate significant differences.

*A. fuscipennis* super-parasitized larvae parasitized by itself or by a conspecific up to three times. This led to five different types of larvae: 1) unparasitized larvae, 2) recently self-parasitized, 3) recently parasitized by a conspecific, 4) recently self-parasitized and by a conspecific, and 5) parasitized by a conspecific with a parasitoid in pupal stage. The mean handling time per handling behaviour changed little as a function of these host types (Table 8). The duration of the antennal rejection of L1 larvae, parasitized by a conspecific and by itself, and of grey pupae was shorter than for the other host types. The duration of the successful ovipositions and ovipositorial rejection was similar for all host types. Host acceptance, however, changed significantly as function of the host type (Figure 2). It decreased from 71.3 % for unparasitized larvae to 33.3 % for larvae that were self-parasitized ($\chi^2$=44.5, highly significant). Furthermore, the percent unsuccessful ovipositions was lower and the percent antennal rejections higher for parasitized larvae than for unparasitized larvae. When comparing unparasitized larvae with larvae recently parasitized by a conspecific, the host acceptance was slightly lower (61.8 %, $\chi^2$=4.85, significant). Acceptance of hosts that were self-parasitized and parasitized by a conspecific was similar to that of hosts that were self-parasitized only. Oviposition postures were never observed on grey pupae, these were always rejected after assessment with the antennae. Acceptance, calculated as the number of hosts encountered (once or more) divided by the number of hosts that were parasitized (once or more), was 94.4 % for unparasitized larvae, compared to 81.9% for larvae recently parasitized by a conspecific ($\chi^2$=24.3, highly significant).



Figure 2. Host handling behaviour of *A. fuscipennis* on different types of *T. vaporariorum* larvae (unparasitized, self-parasitized or parasitized by a conspecific) at 25 °C (% of total encounters). Number of replicates (n) is given at base of each column.

*Width of the searching path and walking speed*

The width of the searching path of *A. fuscipennis* was (mean ± SE) 0.78 ± 0.01 (n=20) mm. The walking speed was (mean ± SE) 0.87 ± 0.05 mm/s (n = 24), 1.5 ± 0.1 mm/ s (n = 32) and 1.7 ± 0.2 mm/s (n = 22) at 15-16 °C, 19-21 °C and 25-27 °C respectively. The walking speed at 15 °C was significantly different from that at 20 and 25 °C (Kruskal Wallis test, p= <0.0001).

A. *fuscipennis* females had a rather straight searching path, with few turns at all temperatures. Because of this straight searching path, many females reached the leaf border very quickly. Then they kept walking near the edge trying to change to the other leaf side or to drink water or plant juices at the leaf edges. At 15-16 °C, they did not fly away. At 19-21 °C, they made short flights, often landing on the foam near the leaflet, and at 25-27 °C the females had long upward flights and landed mostly on the side curtains or the roof of the observation chamber.

Using the previous results, we calculated the encounter rate (RE) of *A. fuscipennis* with L1 larvae and that of *E. formosa* with L3 –L4 larvae at 25 °C using the formula of Skellam(1958):

$$RE = (WI + DM) * WS * ACT * DENS, \qquad (1)$$

where WI = the width of the parasitoid's searching path = 0.55 mm for *E. formosa* (van Roermund & van Lenteren, 1995b) and 0.78 mm for *A. fuscipennis*, DM = the host diameter = 0.20 mm for L1 larvae and 0.52 mm for L4 larvae (van Lenteren et al., 1976a); WS = the walking speed = 0.62 mm/s for *E. formosa* (van Roermund & van Lenteren, 1995b) and 1.67 mm/s for *A. fuscipennis*; ACT = walking activity = 75 %; and DENS = the host density = four hosts on a leaflet of 22 cm$^2$ or $1.82 * 10^{-3}$ host/mm$^2$. The resulting encounter rate of *A. fuscipennis* of 8.8 L1 larvae/hour was higher than that of 3.2 L4 larvae/hour of *E. formosa*.

**Discussion**

At temperatures of 20 °C or higher, *A. fuscipennis* spent considerable more time on clean leaflets (2554-4584 s) than *E. formosa* (1034-1739 s, van Roermund & van Lenteren, 1995b). The walking activity of 31-42 % of *A. fuscipennis* on such leaflets was considerably lower than that of 63 - 76 % of *E. formosa* at 20 °C or higher (van Roermund & van Lenteren, 1995a). At low temperatures (15-18 °C), *E. formosa* hardly moved, reduced its walking activity to 10 %, did not fly and it was therefore impossible to determine its residence time (van Roermund & van Lenteren, 1995b). *A. fuscipennis*, on the contrary, had a mean residence time of 11320 s, still moved, left the leaflets flying and had a walking activity of 32 % at 15 °C. So at low temperatures *A. fuscipennis* seems to be more active, which could favour its use in greenhouses with a low temperature.

On clean leaflets when introduced on the upper leaf side, *A. fuscipennis* spent less time (26-44 %) on the upper leaf side than *E. formosa* (56-72 %, van Roermund & van Lenteren, 1995b). When introduced on the lower leaf side, *A. fuscipennis* spent only 11 % on the upper leaf side compared to 33.5 % for *E. formosa*. Searching on the upper side of the leaflets can give information on the presence of *T. vaporariorum* larvae on the leaflets above by encountering exuviae or honeydew, but the time spent on that leaf side is lost for finding larvae, which are only present on the lower leaf side.

A. *fuscipennis* discovered the host on a similar number of leaflets with recently parasitized L1 larvae, but on fewer leaflets with four grey pupae than *E. formosa*. Van

Roermund & van Lenteren (1995b) found that on 48, 63 and 72 % of the leaflets *E. formosa* encountered the host on leaflets with respectively one recently parasitized L3-4 larvae, four recently parasitized L3-4 larvae and four black pupae respectively. For *A. fuscipennis* these values were respectively 44, 55 and 43 %, but the recently parasitized larvae were of the first instar instead of L3-4 larvae. The lower probability to find the host, due to the smaller host stage, was compensated by the higher walking speed and greater width of the searching path. The low encounter rate of *A. fuscipennis* on leaflets with grey pupae can be explained by the low walking activity of 38 % on such leaflets compared to 65 % on leaflets with recently parasitized larvae.

*E. formosa* increased its residence time on leaflets where the host was found with a factor of 2 to 4, when compared to leaflets where the host was not found. For *A. fuscipennis*, the residence time on leaflets where the host was discovered was also higher than on those where the hosts were not discovered, but the increase was much smaller. Additionally, when looking at the separate treatments, this arrestment response of *A. fuscipennis* was only found on the leaflets with unparasitized hosts. On those leaflets the giving-up time (GUT, time after the last encounter or oviposition) was higher than on leaflets with parasitized host, which explains the higher residence time. Apparently, encounters with and/or oviposition(s) in parasitized hosts reduced the GUT of *A. fuscipennis* and thus the residence time on leaflets with that type of hosts. This was also found for *Leptopilina heterotoma* (van Lenteren, 1991) and contrast with *E. formosa* that increases the GUT and residence time after parasitizing in an unparasitized host (van Roermund et al. 1994).

In contrast to *E. formosa*, *A. fuscipennis* was not arrested on the leaflet by the presence of honeydew, but changed its time allocation: the walking activity and the time spent on the upper leaf side (where the honeydew was present) increased significantly compared to clean leaflets. In comparison, *E. formosa* was arrested on leaflets with honeydew but not on the leaf side where the honeydew was present (van Roermund et al., 1994). Li et al. (1993) also found that honeydew elicited strong host-seeking behaviour of *Amitus longiconis*.

A more important shift in the time allocation was found when hosts were present on the leaflets: again, more time and % time was spent on searching and less time and % time was spent on standing still. The time spent on searching on infested leaflets when the host was not discovered was 61 % compared to 36 % on clean leaflets. It is not clear what could have caused this change in time allocation because those leaflets can be considered as clean. Possibly *A. fuscipennis* detects somehow the presence of hosts, reacts by increasing its searching activity, but if after a certain time no hosts are discovered it leaves the leaf. Van Roermund & van Lenteren (1995b) found a walking activity (% time walking excluding host handling time) of 71.7 % on infested leaflets for *E. formosa*. For *A. fuscipennis* we found a higher walking activity of 82 - 89 % on leaflets with L1 larvae.

The duration of the intervals of walking and standing still found by Manzano (2000) of respectively 49.1 and 10.5 s were lower than those of respectively 171.3-468.5 and 152.7-230.1 s, found on infested leaflets in this study. However, Manzano used leaflets with a higher host density.

On infested leaflets where the host was discovered, the mean total host handling time (manipulating and ovipositing) of *A. fuscipennis* was between 9 and 209 seconds and the % host handling time between 0.7 and 5.1 %. For *E. formosa* this percentage was much higher, varying between 1.8 and 21.8 % (van Roermund & van Lenteren, 1995b). This higher host handling time of *E. formosa* cannot only be explained by the higher number of encounters,

but also by the longer duration of each separate host handling behaviour and additionally *E. formosa* feeds on hosts. The mean duration of a successful oviposition of *A. fuscipennis* on L1 hosts of 34-38 s was shorter than that of 362-654.9 s for *E. formosa* on L3-4 hosts (van Roermund & van Lenteren, 1995b). At 19 °C and on bean leaves, Manzano (2000) found a slightly higher oviposition time of 48.4 s for *A. fuscipennis* on L1 hosts, but this can be explained by the lower temperature. Drost et al. (1999) found that the oviposition time of 39.1 s for *A. bennetti* on *B. tabaci* was independent of the host stage at 25 °C. On L1, L2 and L3 larvae of *A. woglumi*, Dowell et al. (1981) reported oviposition times for *A. hesperidum* of respectively 27.6, 39.3 and 76.5 s (temperature not given), being shorter on L1 larvae and longer on L3 larvae, when compared to our data.

Using infested bean plants, Manzano (2000) found residence times for *A. fuscipennis* of 9882 s, during which the parasitoids visited a mean of 8.9 leaflets with a mean area of 34.3 cm$^2$, leading to a lower mean residence time per leaflet (1110 s) than that found here. However, only one leaflet out three that form a leaf were infested with 4 *T. vaporariorum* L1 larvae, which resulted in a lower host encounter rate. The walking activity on infested plants was 78.3 %, comparable to that of 82-88 % found here. The GUT of 7385 s they found for leaving the plant was much higher than that observed here for tomato leaflets, but possibly during that period more than one leaflet was visited. The number of encounters with hosts of 4.5 found by Manzano (2000) was similar to that we found on leaflets with 4 L1 hosts. Their host density was 33 % lower (4 hosts on three leaflets, a mean of 1.3 per leaflet), but the residence time was 3 times as high resulting in a similar number of encounters. The host handling time of 2.8 % found by Manzano (2000) was similar to that of this study. She also found that 80% of the *A. fuscipennis* females visited petioles and stems. *A. fuscipennis* females spent between 79 and 90 % of the total time on the lower leaf side, which is similar to the 83 % found by Manzano (2000).

Females that left the leaflet by flying did so in 85 % of the times from the leaf edge, which coincides with the 88.3 % found by Manzano (2000). In this study, almost all parasitoids visited the petiole for (a) short period(s) during their stay on a leaflet and on leaflets where the host was discovered, a significantly higher percent of the females left the leaflet by walking via the petiole than on clean leaflets. This can be an adaptation to the clumped distribution of *T. vaporariorum* larvae. When a leaflet is infested, it is likely that nearby leaflets are also infested and the safest and less energy consuming way to travel to those leaflets might be by walking. Flying is supposed to be more risky and energy consuming as wind can blow the flying parasitoid away from the patch. On leaflets, *A. fuscipennis* showed area restricted search (reduction of walking speed and increasing turning rate) when encountering a host (Manzano, 2000). The finding that parasitoids leave infested leaflets by walking to a higher extent than clean leaflets can be interpreted as an area restricted search on a higher level and might increase the parasitoid's searching efficiency.

The time spent on the lower leaf side of infested leaflets was similar for *A. fuscipennis* when compared to *E. formosa*, being near or slightly higher than 80 % for both parasitoids. Additionally, the time until the first encounter we found for *A. fuscipennis* (604 - 1027 s) was in the same range as that found by van Roermund & van Lenteren (1995b) for *E. formosa* (434 - 1345 s).

Time allocation on leaflets with grey pupae was comparable to that on clean leaflets: more time was spent standing still and less time spent searching. This type of larva was never parasitized by *A. fuscipennis*, which prefers L1-L2 larvae for oviposition and does not accept

L4 larvae for oviposition (Manzano, 2000). As the distribution of the different age or type of whitefly larvae goes together with the age of the leaves (Noldus & van Lenteren, 1989), the possibility of finding an L1 host on leaves with L4 larvae or pupae is very low. So the different behaviour of *A. fuscipennis* on leaves containing L4 larvae or pupae could be a result of its host preference. Van Roermund & van Lenteren (1995b) did not find this difference for *E. formosa*.

The number of encounters on infested leaflets with four hosts was about four times that found on leaflets with one host. This is to be expected, as the encounter rate is directly dependent on the host density in equation (1).

The higher walking speed and greater width of the searching path of *A. fuscipennis* compensate largely the disadvantage when searching for smaller L1 larvae: the calculated encounter rate of *A. fuscipennis* with L1 larvae was more than twice as high than that of *E. formosa* with L4 larvae. The number of encounters of *A. fuscipennis* with L1 larvae in our experiments was similar to that of *E. formosa* with L3-4 larvae. On leaflets with one and four unparasitized L 3-4 larvae, van Roermund & van Lenteren (1995b) found 2.1 and 7.6 encounters per leaflet respectively, compared to respectively 2.0 and 6.2 encounters of *A. fuscipennis* with L1 larvae in this trial. On leaflets with one and four parasitized L 3-4 larvae those values were 4.4 and 6.6 encounters for *E. formosa* and respectively 1.8 and 5.6 encounters with L1 larvae for *A. fuscipennis*. However, the residence time of *E. formosa* was much higher than that of *A. fuscipennis* resulting in higher observed encounter rates for *A. fuscipennis*, at least on leaflets with four larvae. Those rates were 2.4, 5.7 and 4.2 encounters per hour on leaflets with respectively one L1 larvae, four L1 larvae and four grey pupae for *A. fuscipennis* and 2.8, 3.8 and 2.9 encounters per hour on leaflets with respectively one L 3-4 larva, four L 3-4 larvae and four black pupae for *E. formosa*. On leaflets with four L 3-4 larvae, the observed encounter rate was similar to the calculated for *E. formosa*. For *A. fuscipennis*, however, the observed was lower than the calculated. We determined the walking speed on clean leaflets, which could be an overestimation of the walking speed on infested leaflets as Manzano (2000) found that *A. fuscipennis* decreases its walking speed after encountering a host. Manzano also found a lower walking speed of 1.4 mm/s on bean leaves at 24 °C than that of 1.7 mm/s at 25 °C, we found. Additionally, we observed that *A. fuscipennis* walked over L1 larvae without really detecting them. Unfortunately we did not quantify this phenomenon, which was also observed for *Eretmocerus sp.* by Headrick et al. (1995). Because *T. vaporariorum* pupae have a diameter 2.8 times larger than L1 larvae (van Lenteren et al., 1976a), we would expect more encounters on leaves with pupae compared to leaves with L1 larvae, but this was not the case. Although the walking activity of *A. fuscipennis* on leaflets with grey pupae was lower, this cannot totally explain the low observed encounter rate. Also for *E. formosa* this was the case and it is not clear what caused this relatively low encounter rate on leaflets with pupae.

The percent host acceptance was slightly above 70 % for both *A. fuscipennis* and *E. formosa*. But, *A. fuscipennis* accepted 33.3 % and 61.8 % of L1 larvae respectively recently self-parasitized and recently parasitized by a conspecific, compared to respectively 0-10 % and 14.2 % for *E. formosa* (van Lenteren et al.; 1976b; van Roermund & van Lenteren, 1995a). Pupae containing a parasitoid in the pupal stage were not accepted for oviposition by either parasitoid species.

On leaflets with one and four unparasitized hosts the number of ovipositions of 0.8 and 3.2 of *E. formosa* (van Roermund & van Lenteren 1995a) were similar to those of respectively

0.9 and 3.0 for *A. fuscipennis*. However, the number of ovipositions of 0.3 and 0.7 of *E. formosa* (van Roermund & van Lenteren 1995a) on leaflets with respectively one and four recently parasitized larvae were much lower than those 0.8 and 3.2 of *A. fuscipennis*. So superparasitism was much more frequent for *A. fuscipennis* than for *E. formosa*.

Self-superparasitism can be advantageous when the risk of parasitism by conspecifics is high and the presence of more than one egg in a host increases the probability of gaining offspring (van Alphen & Visser, 1990). Supposing that an *A. fuscipennis* female would only forage on leaflets with 1 or 4 unparasitized larvae, neglecting travel time between patches, a longevity of 9.3 days (Chapter 3), 12 hours searching time per day and using mean residence time and host attack data for clean and infested leaflets, we calculated that respectively 52 and 140 *T. vaporariorum* larvae would be parasitized and a total of 77 and 167 eggs would be laid during the parasitoid's life time. Under these conditions *A. fuscipennis* is thus not egg limited as egg load is at least 430 eggs (De Vis et al., 2001; Chapter 3). The number of infested leaflets in a crop with low host density is, however, very low and can be less than 1 % (van Roermund et al., 1997), reducing further the possibility to oviposit. By not ovipositing in self-parasitized host, the parasitoid would have saved respectively 25 and 27 eggs and about 15-16 minutes time. The probability to find another host is this short time span is very low. Self-superparastitism could thus be advantageous for *A. fuscipennis* under these conditions of "egg-abundance" and "host scarcity" if the probability of gaining offspring from self-superparasitized host is higher than 0. The same can be said for conspecific superparasitism. However, to confirm this, the outcome of competing larvae within one host should be defined.

Although *A. fuscipennis* seems to be more efficient than *E. formosa* in finding and parasitizing hosts on infested leaflets, its long residence time on clean leaflets seems to reduce the probability to find infested leaflets. This, together with its short adult life span (De Vis et al., 2001; Chapter 3) will reduce its overall searching efficiency in a crop with low host density. Van Roermund et al. (1997) found that in a crop with low host density, less than 1% of the leaflets were infested with *T. vaporariorum* and that less than 1 % of the parasitoids were searching on infested leaflets. Under these conditions, *E. formosa*, which has a much higher life span than *A. fuscipennis* (Chapter 3) might be more efficient. In crops with a high host density or in high-density spots, on the contrary, *A. fuscipennis*, which has a high egg load and a high oviposition frequency at early adult life (De Vis et al., 2001; Chapter 3), might have an advantage over *E. formosa*. The joint use of both parasitoids could be an interesting option, where *A. fuscipennis* could reduce *T. vaporariorum* in high-density spots while later *E. formosa* could keep *T. vaporariorum* at low levels.

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## 5. Comparison of foraging behaviour, interspecific host discrimination and competition of *Encarsia formosa* and *Amitus fuscipennis*

### Abstract

The foraging behaviour of *Amitus fuscipennis* MacGown & Nebeker and *Encarsia formosa* Gahan was studied on tomato leaflets with 20 *Trialeurodes vaporariorum* (Westwood) larvae in the first or third stage. Ten of the whitefly larvae were previously parasitized and contained a conspecific or a heterospecific parasitoid egg or larva. The host type (host stage and/or previous parasitization) did not influence the foraging behaviour of either parasitoid species. The residence time of *A. fuscipennis* on these tomato leaflets was about 1 hour and twice as much for *E. formosa*. *A. fuscipennis* stood hardly still and fed little, while *E. formosa* showed extensive standing still and feeding. As a results, the time walking while drumming was similar for both parasitoid species. The number of host encounters and ovipositions per leaflet was similar for both parasitoid species, However, the residence time of *A. fuscipennis* was half as long as that of *E. formosa* so the rate of encounters and ovipositions was higher for *A. fuscipennis*. Therefore, *A. fuscipennis* is more efficient in finding and parasitizing hosts under these conditions. The walking activity and host acceptance of the synovigenic *E. formosa* diminished with the number of ovipositions, but not for the pro-ovigenic *A. fuscipennis*. *E. formosa* is egg-limited, while *A. fuscipennis* is time limited because of its short life span and high egg load. Both parasitoid species discriminated well between unparasitized larvae and self-parasitized larvae, but discriminated poorly those larvae parasitized by a conspecific and did not discriminate at all larvae parasitized by a heterospecific. Self superparasitism, conspecific superparasitism and multiparasitism were observed for both parasitoid species. A reliable method was developed to dissect parasitized *T. vaporariorum* larvae using Phloxine-B to stain *A. fuscipennis* eggs, which confirmed super- and multiparasitism. Superparasitism always resulted in emergence of one parasitoid and multiparasitism resulted in a higher emergence of one parasitoid of the species that had parasitized first. The data suggest that *A. fuscipennis* is a good candidate for use in biological control of high density hot spots of *T. vaporariorum* when we consider its high encounter and oviposition rate.

### Introduction

Biological control of pests on greenhouse grown tomatoes has become a common practice around the world. For biological control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), the parasitoid *Encarsia formosa* Gahan is traditionally used (van Lenteren, 1992 & 1995). Recently, the use of *E. formosa* has also been complemented with releases of the predator *Macrolophus caliginosus* and the parasitoid *Eretmocerus eremicus* in Europe (van Lenteren, 2000).

In Colombia, biological control of pests in greenhouse tomatoes is not yet commercially applied. To evaluate and/or adapt the biological control system of greenhouse whitefly with *E. formosa* to the local conditions, and to study the possible use of the native parasitoid *Amitus fuscipennis* MacGown & Nebeker, a research program was started at the Universidad de Bogotá Jorge Tadeo Lozano.

Life history of *A. fuscipennis* as a parasitoid of *T. vaporariorum* was determined on bean (Manzano et al., 2000) and on tomato (De Vis et al., 2001). This revealed that the intrinsic rate of increase of the parasitoid is substantially higher than that of *T. vaporariorum* between 15 and 30 °C, and slightly higher than that of *E. formosa* at temperatures lower than 30 °C. De Vis et al. (in preparation, Chapter 4) found that *A. fuscipennis* had a higher searching efficiency on tomato leaflets infested with *T. vaporariorum* larvae, which, combined with a similar host acceptance, resulted in a higher oviposition rate than that of *E. formosa*. These findings coincide with those of Manzano on bean (2000). These attributes make *A. fuscipennis* a good candidate for biological control of *T. vaporariorum* in greenhouse

tomato and because of its high egg load and high oviposition frequency (De Vis et al., 2001), it could prove to be a good reducer of high density spots in the greenhouse, while *E. formosa* is used to keep the density of T. vaporariorum low. Because of this, the joint use of both parasitoid species in greenhouses might be more efficient than the use of either one of them separately. This situation would be created naturally when releasing *E. formosa* in greenhouses situated in zones where *A. fuscipennis* is present in the environment and can easily immigrate into greenhouses.

However, the simultaneous introduction will lead to competition, which takes place on two levels: that of the adults searching for hosts and that of the larvae competing within the host (Mills, 1999). So studies related to interspecific competition should evaluate both the foraging and host evaluating behaviour of the adult parasitoids as well as larval competition.

An important aspect of the foraging behaviour that may influence the outcome of interspecific competition is interspecific host discrimination. Few studies on interspecific host discrimination have been done with whitefly parasitoids and all of them include a heteronomous hyperparasitoid. Heteronomous hyperparasitoids produce females as primary parasitoids and males as secondary hyperparasitoids of developing larvae of either its own or another species. For these types of parasitoids, difference should be made between primary host discrimination (for the allocation of female eggs) and secondary host discrimination (for the allocation of male eggs) (Artigues et al., 1992). The allocation of a male egg in a heterospecific parasitoid larva is an example of hyperparasitism and can also be considered as intraguild predation (Rosenheim et al., 1995), while the allocation of a female egg in a whitefly larva parasitized by another species is an example of multiparasitism and is rather an example of competition than of intraguild predation. In the latter case, heterospecific larvae have to compete for the same resource, but they will not parasitize each other. In this competition, only one larva can survive because both *E. formosa* and *A. fuscipennis* are solitary parasitoids. Only 2 studies were found on the interspecific host discrimination for the allocation of primary developing parasitoid eggs in whiteflies. All of the four studied parasitoid species (*Amitus hesperidum* & *Encarsia opulenta*, Dowell et al., 1981; *Encarsia lutea* & *Eretmocerus mundus*, Gerling & Foltyn, 1987) did not show host discrimination. Although Dowell et al. (1981) stated that *E. opulenta* showed interspecific host discrimination, their conclusion seems to be erroneous (see discussion). More studies have been done on the secondary host choice of heteronomous hyperparasitoids. *Encarsia tricolor* (Williams, 1991; Avilla et al., 1991) and *Encarsia transvena* (Hunter & Kelly, 1998), prefer heterospecifics as a secondary host to produce male offspring while *Encarsia pergandiella* does not (Buijs et al., 1981; Pedata & Hunter, 1996). If heterospecific host discrimination occurs, then we might also expect an effect on the foraging behaviour of the parasitoids, including residence time, time allocation within host patches and host handling times. No other studies were found where the effect of encounters with hosts parasitized by heterospecific on the residence time or time allocation in a patch was evaluated. In previous research, it was found that encounters with and/or ovipositions in unparasitized host increased the residence time of *A. fuscipennis* (De Vis et al., in preparation; Chapter 4) and *E. formosa* (van Roermund et al, 1994) on tomato leaflets.

A second aspect is the outcome of the larval competition of primary parasitoid larvae. Gerling and Foltyn (1987) found that *E. lutea* was superior to *Eretmocerus mundus* at the larval stage, irrespective of the order of the parasitization by the two species. No other studies were found for heterospecific larval competition in whitefly parasitoids.

Host stage preference may play an important role in this interspecific relationship. *A. fuscipennis* prefers L 1-2 larvae for oviposition (Manzano, 2000; Medina et al., 1994) and *E. formosa* L 3-4 larvae (Nell et al., 1976). The two species might thus exploit different niches in the same crop. As *A. fuscipennis* prefers the young host stages, *E. formosa* would then encounter L 3-4 host larvae that were earlier parasitized by *A. fuscipennis* while *A. fuscipennis* would not encounter L1-2 host previously parasitized by *E. formosa*. Both parasitoid species avoid self- and conspecific superparasitism, although the avoidance is much stronger for *E. formosa* than for *A. fuscipennis* (van Lenteren et al., 1976b; De Vis et al., in preparation, Chapter 4). Therefore, if their own niches would be totally exploited, the parasitoid would then search for the non-preferred host stages and invade the niche of the other species where they would then compete for the same hosts.

In this paper, we study the foraging and host examination behaviour of *E. formosa* and *A. fuscipennis* with respect to *T. vaporariorum* larvae parasitized by the other and to define the outcome of *T. vaporariorum* larvae that were multiparasitized. Additionally, the foraging behaviour of both parasitoid species, as function of host type, was compared.

**Materials and methods**

The experiments were done at the Centro de Investigaciones y Asesorías Agroindustriales (CIAA) of the Universidad de Bogota Jorge Tadeo Lozano, Colombia. In seven experiments, the foraging behaviour of *A. fuscipennis* or *E. formosa* was studied at 25 °C on tomato leaflets bearing 20 *T. vaporariorum* larvae of which 10 were parasitized previously. Previous parasitization could be by the same species as the evaluated species or by the heterospecific. At the moment of the introduction of the evaluated parasitoid, the parasitized larvae could contain a parasitoid egg (2-4 hours between the parasitization by the first parasitoid and the introduction of the evaluated parasitoid) or a parasitoid larva (after parasitization in the L1 stage by the first parasitoid, the *T. vaporariorum* larvae were reared to the L3 stage). At the moment of the introduction of the evaluated parasitoid, the host stage was L1 or L3. The different treatments can be found at the top of Table 1.

Whiteflies and parasitoids originated from the CIAA's rearing unit where they were reared on tomato. To obtain recently emerged (naive) parasitoids, tomato leaflets bearing parasitized whitefly pupae were placed in a petri dish with honey in a climatised room at 25°C until emergence. For *A. fuscipennis*, recently emerged females were used and for *E. formosa*, 1 day old females were used to assure a full batch of eggs (van Vianen & van Lenteren, 1986).

Tomato plants of the variety Boris (Bruinsma Seeds, 's Gravenzande, The Netherlands) with leaflets (leaflet area $29.4 \pm 2.1$ cm$^2$) prepared as described above were brought to a climatised room with a L12:D12 light regime, a light intensity at the site of the experiment of 28 $\mu$mol·m$^{-2}$·s$^{-1}$, a temperature of $25 \pm 1$ °C and a relative humidity of $70 \pm 10$ %. A standard high-pressure sodium lamp of 400W (WR400 AL; P.L. Light Systems Canada Inc., Grimsby, Ontario, Canada) provided light; a heater and air conditioner connected to a control unit (Watlow 93, Watlow controls, Winona, Minnesota, USA) controlled temperature; and a humidifier (Herrmidifier 500, Herrmidifier Inc., Lancaster, PA, USA) controlled humidity. Before beginning observations, the leaflets were mapped and whitefly larvae were numbered. An observation started at the moment of introduction of a naive parasitoid on the leaflet and ended when it flew away or walked from the leaflet via the petiole. The parasitoids were observed using magnifying glasses with an magnification of 6, and the position and activity of

the wasps were registered on a PC using the software "The Observer" (Noldus, Wageningen, The Netherlands). The registered positions were (1) upper leaf side centre, (2) upper leaf side border, (3) lower leaf side centre, (4) lower leaf side border and (5) petiole. The registered activities were (1) walking while drumming, (2) standing still, (3) (host) feeding, (4) preening, (5) host assessing of each individual larva, and (6) oviposition posture with respect to each individual larva. All parasitoids were introduced on the lower leaf side and for every experiment a minimum of 20 repetitions were done. 83 *A. fuscipennis* and 60 *E. formosa* females led to respectively 77.5 and 115.4 hours of observation time.

50 % of the leaflets of each experiment were kept in the climate room until emergence of the parasitoids. The leaflets were each covered with a gauze bag. The other 50 % were detached after the experiment and the larvae were dissected to quantify and identify the parasitoid eggs. The larvae were removed from the leaflets, deposited on a slide in a small droplet of a filtered Phloxine-B solution (10 g/l) and covered with a cover glass. Slides were then examined by microscope. Usually the L1 larvae exploded by just placing the cover glass on them, but if that was not the case, pressure was exercised until they exploded. The small, translucent eggs of *A. fuscipennis* are impossible to distinguish without staining. After some reaction time with the staining solution they could easily be detected. *E. formosa* eggs are larger and can be distinguished readily from *A. fuscipennis* eggs. In experiment 3, *A. fuscipennis* had already developed to larva at the time of observation and for best detection of the larvae, a diluted solution of the Phloxine-B (5 g/l) was used. In experiment 7, the dissections were problematic, because for good detection of the larvae the diluted solution had to be used and for the eggs the concentrated. No reliable method to visualise both larvae and eggs of *A. fuscipennis* could be developed and the results of these dissections were therefore omitted.

To evaluate the possible effect of encounters with hosts parasitized by a heterospecific, residence time, time allocation and host handling times were calculated. The foraging and host handling behaviour of the two parasitoid species were presented in ethograms.

Mean and standard error of all variables were calculated and a Kruskal-Wallis one-way analysis of variance (Anova) was done to detect significant differences between the mean values of the experiments. By comparison of mean ranks subsets of similar (homogeneous) mean ranks were identified. The results of these analyses are mentioned in the tables. To distinguish more clearly between the residence times and time allocation of the two parasitoid species, and the effect of the host stage, the results were pooled per parasitoid and also for host stage within parasitoid and a separate Kruskal Wallis Anova tests was done for the pooled data sets. The results of the calculations with pooled data are given in the text. To test differences in host acceptance (proportion of host encounters), Z-values were calculated with the two proportions ($p_1$ & $p_2$) and the respective number of observations ($n_1$ & $n_2$): $Z = (p_1-p_2) \cdot [p_1 \cdot (1-p_1) \cdot n_1^{-1} + (p_2 \cdot (1-p_2) \cdot n_2^{-1}]^{-\frac{1}{2}}$ with a normal distribution ($\mu=0$ & $\sigma=1$).

## Results

*Residence time and time allocation to different activities*
Visual observation of the data of the different experiments shows that differences found for the residence time and time allocation (Table 1) were mostly due to the parasitoid species and to a lesser extent to the host stage. When comparing data of experiments with the same

parasitoid and host stage, but different type of initial parasitization, no significant differences were found.

The following differences were found when comparing parasitoid species (pooled data): *E. formosa* remained twice as long on the leaflets compared to *A. fuscipennis* with mean residence times of 6925 ± 492 s (n=60) and 3362 ± 151 s (n=83) respectively (Kruskal-Wallis test, $p < 10^{-4}$). However, this higher residence time for *E. formosa* did not lead to a longer time walking while drumming, because this was similar for both parasitoid species: 2463 ± 190 s for *E. formosa* and 2579 ± 121 s for *A. fuscipennis*. As a result, the % time walking while drumming of 77.3 % for *A. fuscipennis* was significantly higher than the 39.9 % of *E. formosa* (Kruskal-Wallis test, $p < 10^{-4}$). *E. formosa* spent much more time standing still, in the oviposition posture and on (host) feeding, respectively 1743 ± 273 s, 1432 ± 111 s and 412 ± 90 s, compared to *A. fuscipennis*, which spent only 33 ± 12, 251 ± 19 and 7 ± 7 s on these activities, resulting in 8.5 % of the total time on the leaflet spent to these tree activities, compared to 47.8 % for *E. formosa* (Kruskal-Wallis test, $p < 10^{-4}$ for the three activities and its respective % of the total time). The time spent on preening and evaluating hosts was double as high for *E. formosa* when compared to *A. fuscipennis* (Kruskal-Wallis test, $p < 7 \cdot 10^{-4}$), leading to a similar percentage of the time dedicated to these activities.

The residence time and host handling time changed as function of the host stage offered for both parasitoid species (pooled data per host stage for each parasitoid). For *E. formosa*, the residence time on leaflets with L3 larvae of 7590 ± 606 s was significantly higher than that of 5597 ± 782 s on leaflets with L1 larvae (Kruskal Wallis test, p= 0.012, n = 20 and 40 respectively). For *A. fuscipennis*, the residence time on leaflets with L1 larvae of 3620 ± 172 s was significantly higher than that of 3084 ± 247 s on leaflets with L3 larvae (Kruskal Wallis test, p=0.027, n = 40 and 43 respectively). So both parasitoid species seem to spend more time on leaves with their preferred host stage. *E. formosa* spent significantly more time in oviposition posture on leaflets with L3 larvae (1677 ± 144 s) than on leaflets with L1 larvae (943 ± 107 s) (Kruskal-Wallis test, p =0.001). For *A. fuscipennis* the time in oviposition posture was not significantly different (236 ± 25 s and 267 ± 30 s on leaflets with L1 and L3 larvae respectively). However, when expressed as % of the total time on the leaf (6.4 ± 0.6 % and 8.7 ± 0.9 % on leaflets with L1 and L3 larvae respectively), the difference was significant (Kruskal-Wallis test, p =0.031). Additionally, for *E. formosa* a higher % time assessing the host was observed on leaflets with L3 larvae compared to those with L1 larvae and for *A. fuscipennis* a larger (%) time preening was observed on leaflets with L1 larvae compared to those with L3 larvae. All other activities did not show significant differences when comparing leaflets with L1 and L3 larvae for both parasitoid species.

No differences between experiments were found for the time until the first encounter and the time after the last encounter/oviposition until abandoning the leaflet (= Giving Up Time, GUT) (Table 1). When comparing between parasitoid species, the time until the first encounter of 508 ± 64 s and the GUT of 845 ± 70 s of *A. fuscipennis*, were not significantly different when compared to the respective times of 881 ± 181 s and 1243 ± 162 s of *E. formosa*. However, when comparing host stages, the time until the first encounter of 1738 ± 430 s on leaflets with L1 larvae was significantly higher than that of 452 ± 122 s on leaflets with L3 larvae for *E. formosa* (Kruskal Wallis test, p= 0.0002). For *A. fuscipennis* no significant difference was found (535 ± 87 s and 479 ± 96 s on leaflets with L1 and L3 larvae respectively). The GUT on leaflets with L1 or L3 larvae was not different for either parasitoid.

Table 1. Time spent (mean ± SE) by *A. fuscipennis* and *E. formosa* on different activities on tomato leaflets infested with 20 *T. vaporariorum* larvae of which 50 % were earlier parasitized by either *A. fuscipennis* or *E. formosa*.

| Evaluated parasitoid – host stage | *E. formosa* –L3 | *E. formosa* –L3 | *E. formosa* –L1 | *A. fuscipennis* –L1 | *A. fuscipennis* –L1 | *A. fuscipennis* –L3 | *A. fuscipennis* –L3 |
|---|---|---|---|---|---|---|---|
| First parasitoid – host stage | *E. formosa* –L3 | *A. fuscipennis* –L1 | *A. fuscipennis* –L1 | *A. fuscipennis* –L1 | *E. formosa* –L1 | *E. formosa* –L3 | *A. fuscipennis* –L1 |
| n | 20 | 20 | 20 | 23 | 20 | 20 | 20 |
| Total residence time (s) | 6441 ± 670 ab | 8739 ± 958 a | 5597 ± 782 abc | 3444 ± 265 cd | 3822 ± 208 bcd | 3306 ± 258 cd | 2863 ± 421 d |
| Time searching (s) | 2177 ± 262 a | 2479 ± 257 a | 2734 ± 438 a | 2704 ± 248 a | 2786 ± 169 a | 2534 ± 206 a | 2273 ± 318 a |
| Time standing still (s) | 1284 ± 405 ab | 2829 ± 558 ab | 1115 ± 355 abc | 27 ± 11 bc | 61 ± 42 c | 15 ± 9 c | 30 ± 24 c |
| Time preening (s) | 681 ± 122 a | 853 ± 173 a | 488 ± 105 ab | 363 ± 59 ab | 597 ± 118 ab | 357 ± 86 ab | 225 ± 64 b |
| Time assessing host (s) | 267 ± 32 a | 232 ± 36 ab | 105 ± 15 bc | 105 ± 24 c | 122 ± 18 abc | 116 ± 18 abc | 85 ± 14 c |
| Time oviposition posture (s) | 1724 ± 180 a | 1629 ± 229 a | 943 ± 107 a | 218 ± 31 b | 256 ± 41 b | 284 ± 41 b | 250 ± 44 b |
| Time feeding (s) | 308 ± 86 a | 717 ± 224 a | 212 ± 99 ab | 25 ± 25 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b |
| Time searching (%) | 37.6 ± 3.8 b | 32.2 ± 2.6 b | 50.0 ± 3.8 b | 77.5 ± 2.5 a | 73.9 ± 3.0 a | 76.9 ± 2.2 a | 80.8 ± 2.1 a |
| Time standing still (%) | 15.9 ± 3.8 ab | 28.0 ± 4.6 a | 15.7 ± 4.3 abc | 1.0 ± 0.5 bc | 1.3 ± 0.9 c | 0.4 ± 0.2 c | 0.4 ± 0.3 c |
| Time preening (%) | 9.7 ± 1.2 a | 9.6 ± 1.4 a | 8.5 ± 1.6 a | 11.4 ± 2.0 a | 14.5 ± 2.5 a | 10.5 ± 2.1 a | 6.6 ± 1.9 a |
| Time assessing host (%) | 4.1 ± 0.4 a | 2.7 ± 0.4 ab | 2.2 ± 0.3 b | 3.3 ± 1.0 ab | 3.4 ± 0.5 ab | 3.6 ± 0.4 ab | 3.3 ± 0.6 ab |
| Time oviposition posture (%) | 28.0 ± 2.2 a | 18.7 ± 1.7 a | 18.8 ± 2.3 ab | 6.0 ± 0.5 c | 6.9 ± 1.1 c | 8.6 ± 1.2 c | 8.9 ± 1.3 bc |
| Time feeding (%) | 4.7 ± 1.3 a | 8.8 ± 2.6 a | 4.8 ± 2.4 ab | 0.8 ± 0.7 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b |
| Time until 1st encounter | 293 ± 75 b | 612 ± 230 ab | 1738 ± 430 a | 427 ± 100 ab | 659 ± 145 ab | 452 ± 151 b | 505 ± 124 ab |
| Time after last encounter or oviposition | 1004 ± 233 a | 1380 ± 303 a | 1344 ± 306 a | 872 ± 136 a | 914 ± 144 a | 909 ± 160 a | 716 ± 126 a |

* Kruskal-Wallis test ($\alpha=0.05$). Different letters in a row indicate significant differences.

Table 2. Walking activity (mean ± SE) of *A. fuscipennis* or *E. formosa* during the total time on the leaflet (overall walking activity), before the first encounter and after the last encounter on tomato leaflets infested with 20 *T. vaporariorum* larvae of which 50 % were earlier parasitized by either *A. fuscipennis* or *E. formosa*.

| n | Evaluated parasitoid – host stage | First parasitoid – host stage | Overall walking activity | Walking activity before 1st encounter | Walking activity after last encounter | p[4] |
|---|---|---|---|---|---|---|
| 20 | *E. formosa* – L3 | *E. formosa* – L3 | 60.5 ± 5.9 cd[1] | 92.0 ± 4.4 a[1] | 54.2 ± 7.4 c[1] | $<10^{-4}$ |
| 20 | *E. formosa* – L3 | *A. fuscipennis* – L3 | 47.9 ± 5.5 d | 88.7 ± 5.3 a | 57.3 ± 7.5 c | $2.8\,10^{-3}$ |
| 20 | *E. formosa* – L1 | *A. fuscipennis* – L1 | 70.7 ± 5.5 cd | 77.7 ± 5.9 a | 72.3 ± 6.7 bc | 0.73 |
| 23 | *A. fuscipennis* – L1 | *A. fuscipennis* – L1 | 86.0 ± 2.3 ab | 83.0 ± 5.0 a | 92.8 ± 2.3 ab | 0.13 |
| 20 | *A. fuscipennis* – L1 | *E. formosa* – L1 | 82.5 ± 3.2 abc | 89.7 ± 4.3 a | 86.6 ± 4.9 ab | 0.13 |
| 20 | *A. fuscipennis* – L3 | *E. formosa* – L3 | 87.8 ± 2.3 ab | 90.2 ± 4.6 a | 91.6 ± 2.8 ab | 0.38 |
| 20 | *A. fuscipennis* – L3 | *A. fuscipennis* – L3 | 92.2 ± 21.1 a | 87.7 ± 4.6 a | 98.6 ± 10 a | $5.9\,10^{-3}$ |
| 60 | *E. formosa* – L1 & L3 | | 59.7 ± 3.4 b[2] | 86.1 ± 3.1 a[2] | 61.3 ± 4.2 b[2] | $<10^{-4}$ |
| 83 | *A. fuscipennis* – L1 & L3 | | 87.1 ± 1.3 a | 87.5 ± 2.3 a | 92.4 ± 1.6 a | 0.24 |
| 20 | *E. formosa* – L1 | | 70.7 ± 5.5 a[3] | 77.7 ± 5.9 b[3] | 72.3 ± 6.7 a[3] | 0.73 |
| 40 | *E. formosa* – L3 | | 54.2 ± 4.1 b | 90.4 ± 3.4 a | 55.8 ± 5.2 b | $<10^{-4}$ |
| 43 | *A. fuscipennis* – L1 | | 84.3 ± 1.9 b[3] | 86.1 ± 3.3 a[3] | 89.9 ± 2.6 a[3] | 0.73 |
| 40 | *A. fuscipennis* – L3 | | 90.0 ± 1.6 a | 88.9 ± 3.2 a | 95.1 ± 1.6 a | 0.19 |

[1] Kruskal Wallis test. Different letter in a column indicate significant differences between experiments
[2] Kruskal Wallis test. Different letter in a column indicate significant differences between parasitoids
[3] Kruskal Wallis test. Different letter in a column indicate significant differences between host stages
[4] Wilcoxon Signed Rank test for pairwise comparison between walking activity before the first and after the last encounter.

No significant difference was found in walking activity before the first encounters of the different experiments (Table 2). When comparing between parasitoid species, the walking activity before the first encounter was similar: 86.1± 3.1 % for *E. formosa* and 87.5 ± 2.3 % for *A. fuscipennis*. The walking activity after the last encounter/oviposition was significantly different between experiments, indicating mostly differences between parasitoid species: that of *E. formosa* was 61.3 ± 4.2 % compared to 92.4 ± 1.6 % for *A. fuscipennis* (Kruskal - Wallis test, $p < 10^{-4}$). The walking activity before the first oviposition was significantly higher than that found after the last encounter/oviposition for *E. formosa* foraging on leaflets with L3 larvae. The opposite was found for *A. fuscipennis* foraging on leaflets with L3 larvae containing a conspecific larva (Table 2). For *E. formosa* (pooled data), the walking activity after the last encounter/oviposition of 61.3 ± 4.2 % was significantly lower than that before the first encounter of 86.1 ± 3,1 % (Kruskal-Wallis test, $p < 10^{-4}$). For *A. fuscipennis*, the walking activity after the last encounter/oviposition of 92.4 ± 1.6 % was similar to that of 87.5 ± 2.3 % before the first encounter. The walking activity of *E. formosa* was more or less constant during the first 5 ovipositions and then decreased (Figure 1), while that of *A. fuscipennis* increased slightly with the number of ovipositions. These tendencies were significant for both parasitoid species.
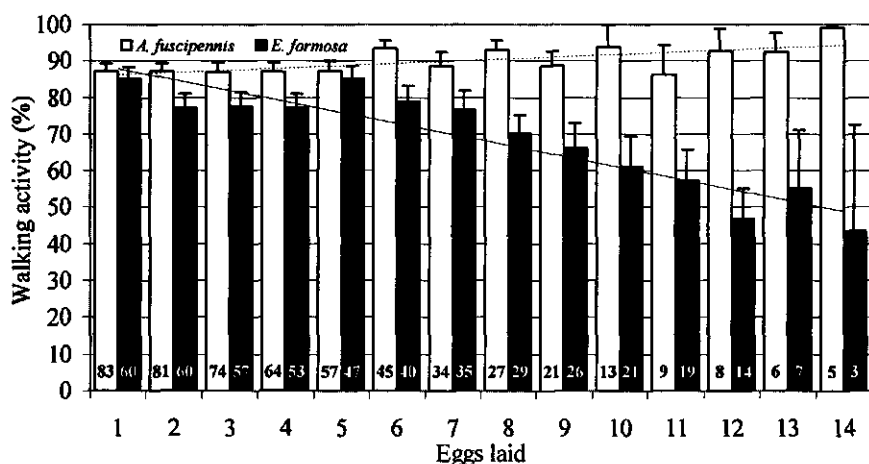


Figure 1. Walking activity of *E. formosa* (n=60 females) and *A. fuscipennis* (n=83) as function of the number of eggs laid (1 egg laid = from beginning of the observations until first egg laid, 2 eggs laid = from the first oviposition until the second, etc.). Bars represent standard error of the mean and the number of replicates is given at the base of the columns. Linear regression of the means gave $Y = 90.9 - 3.0\ X$, $R^2 = 0.84$ and $Y = 85.6 + 0.6\ X$, $R^2 = 0.45$ for *E. formosa* and *A. fuscipennis* respectively.

The residence time correlated poorly with the number of encounters or the number of successful ovipositions. For *A. fuscipennis* (n=83) the Spearman's correlation coefficient of the residence time with the number of encounters and the number of successful ovipositions was 0.58 and 0.57 respectively. For *E. formosa* (n=60), these values were 0.57 and 0.55, respectively. Linear regression of the residence time (in seconds) and the number of successful ovipositions yielded a constant of 2265 and an intercept of 201 for *A. fuscipennis* and for *E. formosa*, 3165 and 538 respectively. The r-square, however, was very low: 0.28 for *A. fuscipennis* and 0.30 for *E. formosa*.

*Sequence and duration of behavioural events*
Ethograms of the search and oviposition behaviour were developed for *E. formosa* and *A. fuscipennis* (Figures 2 and 3). Three basic behavioural sequences were found for both parasitoid species: i) walking while drumming, encountering host, assessing host and walking while drumming ii) the same as previous but after assessing host, oviposition posture is adopted followed by walking while drumming, and iii) walking while drumming, preening and walking while drumming. For *E. formosa*, additionally, a standing still loop, occasionally in combination with preening was observed. *A. fuscipennis* did not host-feed and feeding on honeydew was hardly observed, nor was standing still. In most cases, after a certain activity, walking while drumming was resumed to a lesser extent by *E. formosa* than by *A. fuscipennis* and instead feeding or standing still was observed for *E. formosa*. After an oviposition posture, *E. formosa* showed feeding, preening and standing still, activities hardly shown by *A. fuscipennis* after an oviposition posture.

For 28 *E. formosa* females, 50 encounters with 40 hosts led to 74 feedings on those hosts, which could be either feeding on honeydew present on the host or feeding on the body fluids of the host, as those two activities were not recorded separately. In all cases, the hosts had been parasitized successfully in a previous encounter, except in 5 cases where they were parasitized during the same encounter. In most cases, oviposition had been taken place during the immediately preceding encounter and the host was only left during a short period of walking while drumming and then rediscovered. An ethogram (Figure 4) was made of these 50 encounters, all starting with host encounter and finishing with walking while drumming. The normal route was walking while drumming, encountering host, assessing host, feeding and walking while drumming. In several cases, the feeding was interrupted once or several times by standing still or preening. Also some ovipositions and ovipositorial rejections were observed. The mean duration of the feedings was 229 ± 23.8 s; per encounter, 1.18 feedings were registered with a total mean feeding time of 340 ± 35.5 s per encounter. Of these hosts 15 were dissected and in all host, except one, eggs were found. The other 25 host were reared to emergence: from 19, *E. formosa* emerged, from 5, *A. fuscipennis* and one died.

Differences in the duration of the different activities were mostly due to differences in the parasitoid and/or the host stage (Table 3). When comparing parasitoid species, the most important differences were: *A. fuscipennis* spent fewer (15.1 per observation) but longer (171.2 ± 7.6 s) periods on walking while drumming when compared to *E. formosa* (21.4 periods per observation of 115.4 ± 6.5 s; Kruskal Wallis test, p< $10^{-4}$). It also spent fewer (0.7 per observation) and shorter (45.7 ± 11.3 s) periods on standing still compared to *E. formosa* (5.7 periods per observation of 303 ± 29 s; Kruskal Wallis test, p< $10^{-4}$). Finally, *A. fuscipennis* spent less periods on preening, 4.8 per observation, compared to 9.6 for *E. formosa*, the duration of the preening periods of respectively 80 and 70 s being similar for
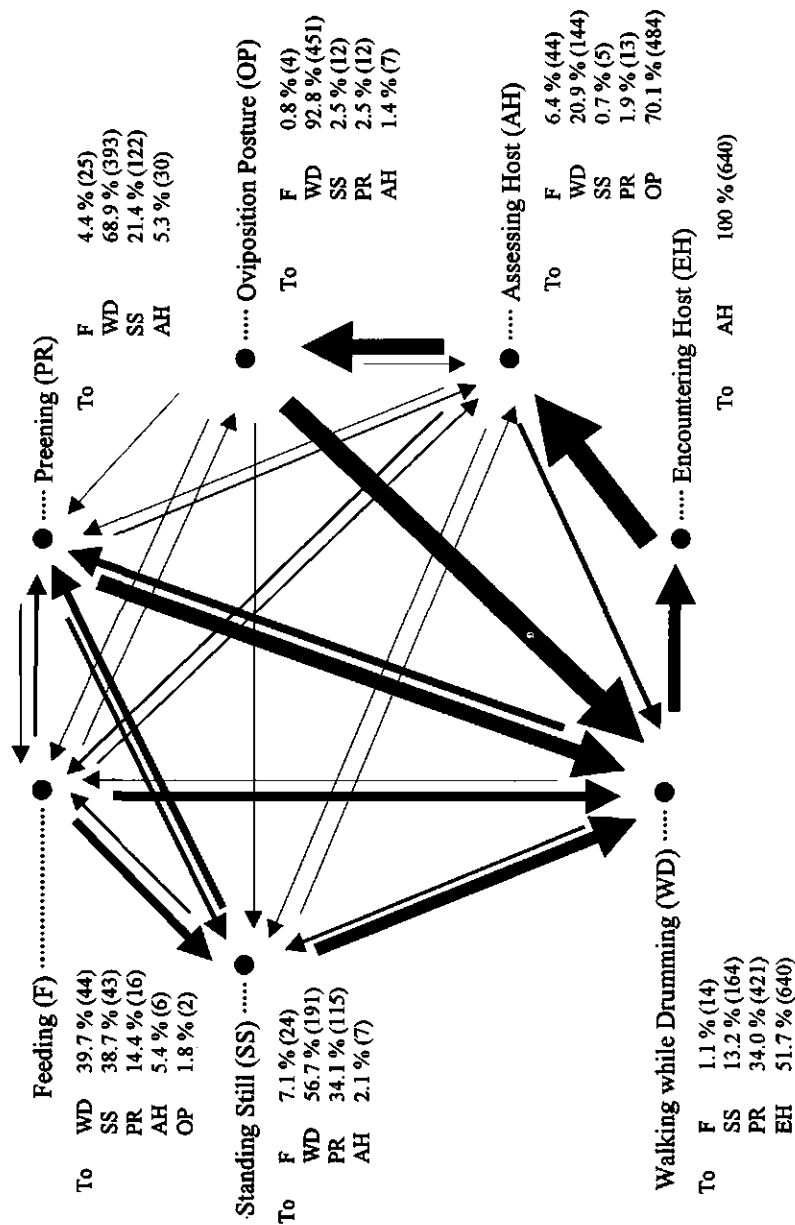
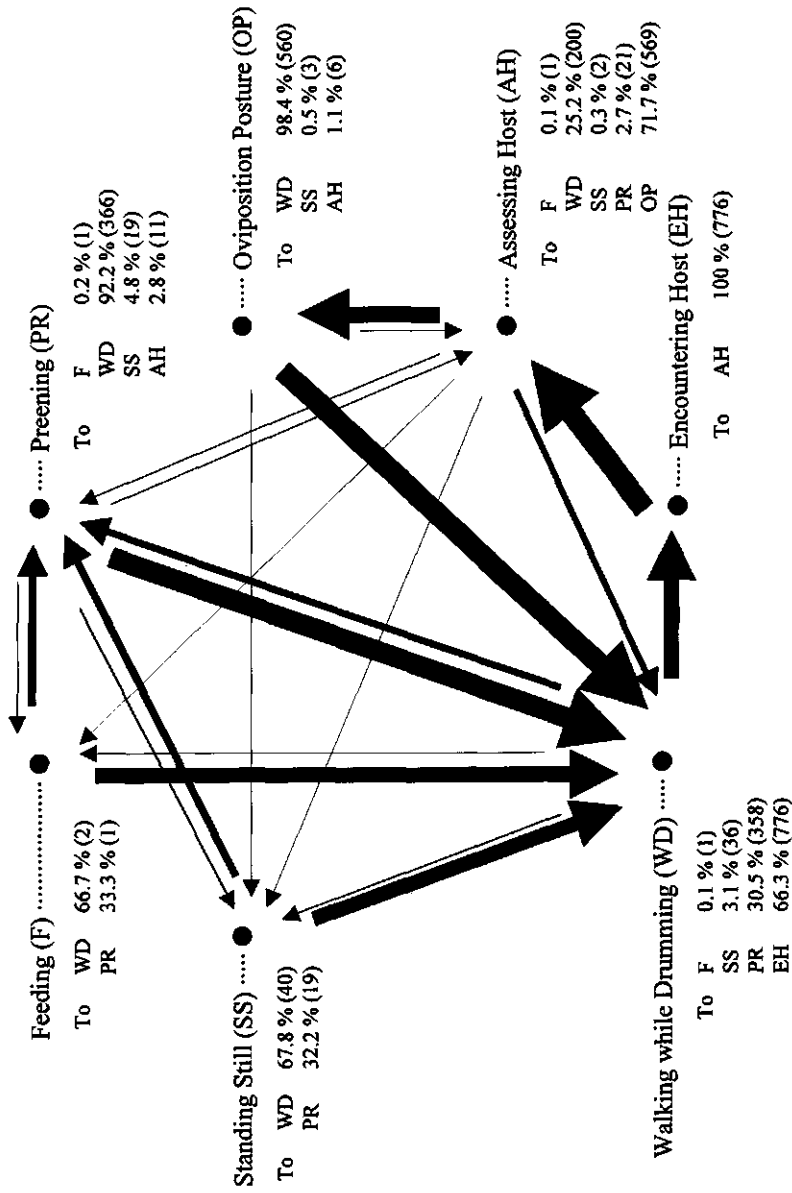Figure 2. Ethogram of searching and oviposition behaviour of *E. formosa*

Figure 3. Ethogram of searching and oviposition behaviour of *A. fuscipennis*
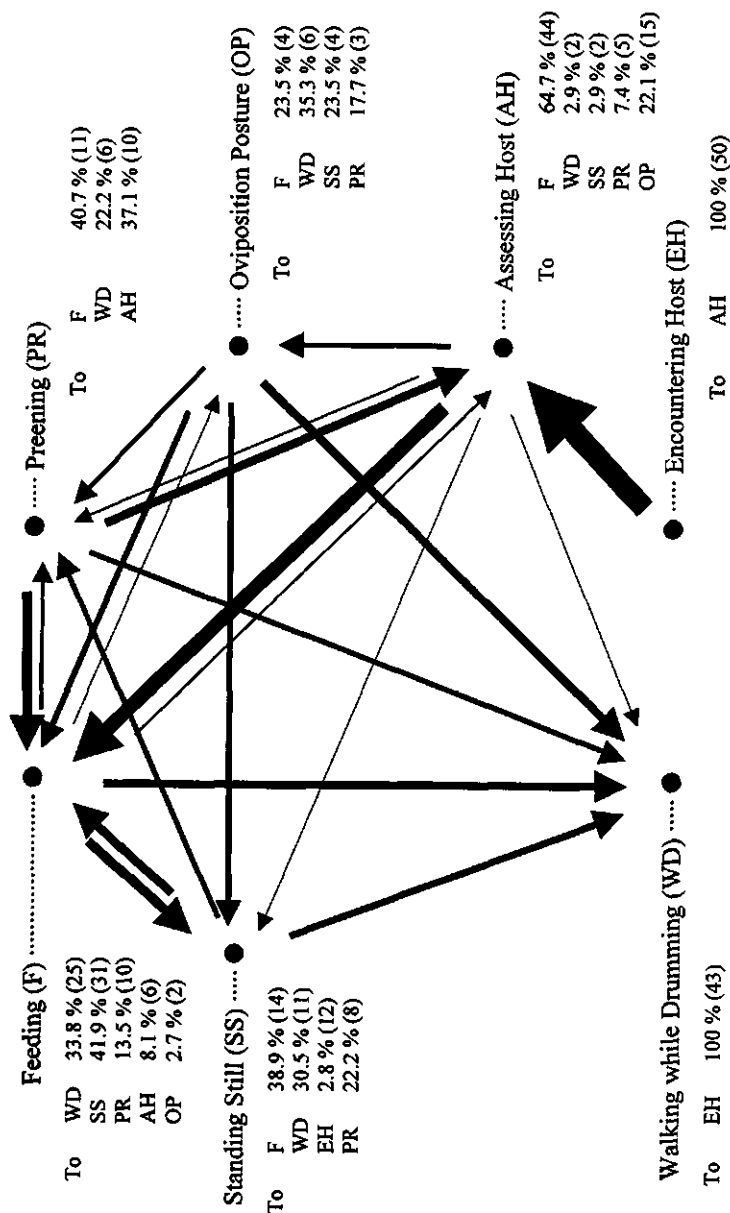
Figure 4. Ethogram of feeding behaviour of *E. formosa* while being on the host

Table 3. Duration (mean ± SE) of the different behavioural events of *A. fuscipennis* and *E. formosa* on different activities on tomato leaflets infested with 20 *T. vaporariorum* larvae of which 50 % were earlier parasitized by either *A. fuscipennis* or *E. formosa*. The number of replicates is given between brackets.

| Evaluated parasitoid – host stage | E. formosa –L3 | E. formosa –L1 | E. formosa –L1 | A. fuscipennis –L1 | A. fuscipennis –L1 | A. fuscipennis –L3 | A. fuscipennis –L3 |
|---|---|---|---|---|---|---|---|
| First parasitoid – host stage | E. formosa –L3 | A. fuscipennis –L1 | A. fuscipennis –L1 | A. fuscipennis –L1 | E. formosa –L1 | E. formosa –L3 | A. fuscipennis –L1 |
| Feeding | 205.2 ± 36.4 (30) a * | 239 ± 30.8 (60) a | 202 ± 44.2 (21) a | 193.6 ± 163.4 (3) a | | | |
| Searching | 84.2 ± 6.6 (517) d | 101.3 ± 9.6 (492) cd | 199.6 ± 20.7 (274) ab | 165.6 ± 11.7 (376) a | 178.6 ± 16.3 (312) abc | 156.4 ± 15.3 (324) bcd | 190.2 ± 19.4 (239) abc |
| Standing still | 184.8 ± 29.5 (139) b | 349.3 ± 42 (163) a | 506.9 ± 136.5 (44) a | 33.3 ± 11.9 (19) c | 151.4 ± 67.1 (8) - | 50.8 ± 20.5 (6) - | 22 ± 6.6 (27) c |
| Preening | 58.4 ± 5.1 (233) c | 71.9 ± 6.1 (237) abc | 91.2 ± 11 (107) ab | 60.5 ± 7.1 (138) bc | 118.2 ± 15.4 (101) a | 69.4 ± 10.3 (103) abc | 79 ± 14.2 (57) abc |

* Kruskal-Wallis test ($\alpha=0.05$). Different letters in a row indicate significant differences.

both parasitoid species. When comparing host stages significant differences were found for almost all activities for *E. formosa*, but not for *A. fuscipennis*. On leaflets with L1 larvae, the intervals dedicated to walking while drumming, standing still and preening were longer while those activity intervals where hosts were involved (host assessing, host feeding, (un)successful oviposition) were shorter. The frequency per observation of the different activity periods was higher on leaflets with L3 larvae. On leaflets with L1 larvae, *E. formosa* spent fewer (13.7 per observation) but longer (199.6 ± 20.7 s) periods on walking while drumming compared to leaflets with L3 larvae (25.2 periods per observation of 92.5 ± 5.8 s; Kruskal Wallis test, $p < 10^{-4}$). Also, on leaflets with L1 larvae it spent fewer (2.2 per observation) and longer (506.9 ± 136.5 s) periods on standing still compared to leaflets with L3 larvae (7.5 periods per observation of 273.6 ± 26.8 s; Kruskal Wallis test, $p = 2.7 \cdot 10^{-3}$). Fewer periods of preening were observed on leaflets with L1 larvae, 5.4 per observation, compared to 11.8 on leaflets with L3 larvae, having a duration of respectively 91.2 ± 11.0 and 65.2 ± 4.0 s (Kruskal Wallis test, $p = 7.5 \cdot 10^{-3}$).

For both parasitoid species, the duration of host assessment as well as the duration of the oviposition postures on hosts parasitized by a heterospecific was equal to that on unparasitized host (Table 4). However, host assessment before rejection of a self-parasitized host was shorter (difference only significant for L3 host). For *E. formosa*, the assessment before an unsuccessful oviposition of a self-parasitized L3 host (8.0 s) was significantly lower than of unparasitized L3 host (32.2 s). For *A. fuscipennis*, the assessment before rejection of an L3 host with a conspecific larva (8.1 s) was significantly lower than that of unparasitized hosts (15.5 s). For both L1 and L3 larvae, the duration of successful oviposition and ovipositorial rejection was generally independent of the type of parasitization for *E. formosa* as well as for *A. fuscipennis*. When comparing host stages, the duration of successful ovipositions on L1 larvae for *A. fuscipennis* of 37 ± 1.6 s was shorter than the 48.4 ± 1.5 s on L3 larvae (Kruskal Wallis test, $p < 10^{-4}$) and the duration of unsuccessful ovipositions of 14.9 ± 0.8 s on L1 larvae was longer than the 12.4 ± 2.8 s on L3 larvae (Kruskal Wallis test, $p = 2. 10^{-4}$). For *E. formosa*, assessing hosts before an oviposition had a mean duration of 17.2 ± 1.3 for L1 larvae compared to 21.3 ± 0.8 s for L3 larvae (Kruskal Wallis test, $p = 1.7 \cdot 10^{-3}$). The respective oviposition times were 175.3 ± 4.6 s and 196 ± 4 s (Kruskal Wallis test, $p = 1.5 \cdot 10^{-3}$).

*Time allocation to different positions on the leaflet and leaving point*

Differences in time allocation to the different positions on the leaflets were mostly not significantly different when comparing among experiments (Table 5). When comparing between parasitoid species the differences were highly significant. *E. formosa* spent more time (6785 ± 876) on the lower leaf side than *A. fuscipennis* (3058 ± 336 s), 97.2 and 92.0 % respectively (Kruskal-Wallis test, $p < 10^{-4}$). Also, *E. formosa* stayed less time on the border of the leaf, 872 ± 113 s or 12.6 %, compared to 956 ± 105 s or 28.7 % for *A. fuscipennis* (Kruskal-Wallis test, $p = 0.0025$ for the time on the border and $p < 10^{-4}$ for its percentage).

The number of times the parasitoids changed leaf sides was not significantly different among experiments (Table 5). Comparing parasitoid species, *E. formosa* changed leaf sides less frequently than *A. fuscipennis*: 0.56 ± 0.15 and 2.54 ± 0.39 times respectively (Kruskal-Wallis test, $p < 10^{-2}$). Of the *E. formosa* females, 73.3 % never changed leaf sides compared to 45.8 % for *A. fuscipennis*. Comparing leaflets with different types of larvae within experiments with the same parasitoid species, no differences were found between the number

Table 4. Duration (mean ± SE) of host handling behaviours of *A. fuscipennis* and *E. formosa* on L1 an L3 host with different types of parasitization (containing no egg, an own egg, a conspecific egg or larva or a heterospecific egg or larva). The number of replicates is given between brackets.

| Parasitoid | Host stage | Parasitization | Assessing before successful oviposition | Assessing before unsuccessful oviposition | Assessing before rejection | Successful oviposition | Unsuccessful oviposition |
|---|---|---|---|---|---|---|---|
| *A. fuscipennis* | L1 | Not | 12.9 ± 3.8 (87) a | 5.4 ± 0.5 (18) ab | 17 ± 3.9 (41) a | 38 ± 3.4 (87) a | 13.1 ± 1.7 (18) a |
| | | Own egg [2] | 8.1 ± 1.3 (33) a | 6.3 ± 2.4 (14) b | 12.5 ± 3.5 (23) a | 37.9 ± 4.1 (33) a | 15.3 ± 1.6 (14) a |
| | | Consp. egg | 7.1 ± 0.7 (62) a | 8.7 ± 1.7 (18) ab | 10.7 ± 3.1 (18) a | 33.8 ± 1.2 (62) a | 16.2 ± 1.6 (18) a |
| | | E.f. egg | 9.7 ± 1.1 (62) a | 17 ± 3.4 (22) a | 17 ± 4.3 (28) a | 38 ± 3 (62) a | 14.7 ± 1.4 (22) a |
| | L3 | Not | 10.1 ± 0.8 (92) a | 11.1 ± 3.3 (20) a | 15.5 ± 2 (45) a | 48.5 ± 2.4 (92) a | 10.6 ± 1.5 (20) a |
| | | Own egg [2] | 8.2 ± 1.4 (28) a | 8.2 ± 3.9 (3) a | 12.1 ± 3.2 (21) b | 49 ± 3.3 (28) a | 4.2 ± 0.7 (3) a |
| | | A.f. larva | 9.8 ± 1.5 (41) a | 9.3 ± 1.8 (7) a | 8.1 ± 1.6 (25) b | 50.4 ± 3.9 (41) a | 7.9 ± 1.9 (7) a |
| | | E.f. egg | 9.7 ± 1.4 (50) a | 11 ± 4.2 (7) a | 16.6 ± 3.5 (22) ab | 47.6 ± 2.7 (50) a | 10.3 ± 1.3 (7) a |
| *E. formosa* | L1 | Not | 17.3 ± 1.8 (39) a | 15.2 ± 6.5 (3) a | 4.5 ± 2.6 (2) ab | 177.1 ± 7.1 (39) a | 44.4 ± 28.8 (3) a |
| | | Own egg [2] | 23.2 ± 12.9 (4) a | 5.4 ± 3.4 (6) a | 3.7 ± 0.7 (22) b | 181.6 ± 12 (4) a | 61 ± 20.4 (6) a |
| | | A.f. egg | 16.7 ± 1.8 (58) a | 18.2 ± 4.4 (7) a | 10.6 ± 2.3 (6) a | 173.7 ± 6.4 (58) a | 92.4 ± 14.7 (7) a |
| | L3 | Not | 20.9 ± 1 (113) a | 32.2 ± 10.3 (11) a | 18.1 ± 8.2 (28) a | 202.1 ± 7.3 (114)ab | 59.4 ± 13.7 (11) a |
| | | Own egg [2] | 23.1 ± 2.8 (52) a | 8.0 ± 1.9 (19) b | 7.0 ± 0.9 (91) b | 176.4 ± 6.8 (51) b | 80 ± 7.9 (19) a |
| | | A.f. larva | 18.9 ± 1.4 (75) a | 22.7 ± 7.5 (5) ab | 13.7 ± 3.3 (24) ab | 200.5 ± 11.1 (75)ab | 89.2 ± 15 (5) a |
| | | Consp. egg | 22.4 ± 1.4 (78) a | 21.9 ± 4.3 (11) ab | 19.2 ± 7.1 (30) a | 195.6 ± 5.1 (78) a | 81.2 ± 15.8 (11) a |

[1] Kruskal-Wallis test ($\alpha$=0.05). Different letters in a column (for the specific host stage and parasitoid) indicate significant differences.
[2] Additionally to the their own eggs, these hosts could also contain a conspecific egg or larva or a heterospecific egg or larva. These host classes were merged because of the low number for the separate classes.

Table 5. Time spent (mean ± SE) by *A. fuscipennis* or *E. formosa* (evaluated parasitoid) at different positions on tomato leaflets infested with 20 *T. vaporariorum* larvae of which 50 % were parasitized by *A. fuscipennis* or *E. formosa* (first parasitoid). The number of times the parasitoids changed leaf sides, the number of parasitoids that never changed leaf sides and the position of the parasitoids at the moment of abandoning the leaflet (expressed as % of the number of observations of an experiment).

| Evaluated parasitoid – host stage | *E. formosa* –L3 | *E. formosa* –L3 | *E. formosa* –L1 | *A. fuscipennis* –L1 | *A. fuscipennis* –L1 | *A. fuscipennis* –L3 | *A. fuscipennis* –L3 |
|---|---|---|---|---|---|---|---|
| First parasitoid – host stage | *E. formosa* –L3 | *A. fuscipennis* –L1 | *A. fuscipennis* –L1 | *A. fuscipennis* –L1 | *E. formosa* –L1 | *E. formosa* –L3 | *A. fuscipennis* –L1 |
| n | 20 | 20 | 20 | 23 | 20 | 20 | 20 |
| Time on upper leaf side (s) | 74 ± 43 a | 67 ± 34 a | 205 ± 124 a | 184 ± 53 a | 249 ± 99 a | 310 ± 102 a | 343 ± 142 a |
| Time on lower leaf side (s) | 6347 ± 678 ab | 8625 ± 951 a | 5383 ± 805 abc | 3213 ± 267 cd | 3559 ± 213 bcd | 2961 ± 236 cd | 2473 ± 284 d |
| Time on petiole (s) | 20 ± 8 a | 46 ± 32 a | 9 ± 6 a | 47 ± 13 a | 15 ± 6 a | 34 ± 10 a | 32 ± 28 a |
| Time on leaf border (s) | 738 ± 219 ab | 542 ± 192 b | 1335 ± 421 ab | 1125 ± 163 a | 848 ± 118 ab | 937 ± 171 ab | 891 ± 172 ab |
| Time on upper leaf side (%) | 1.3 ± 0.8 a | 0.6 ± 0.3 a | 4.6 ± 2.8 a | 6.3 ± 2.0 a | 6.4 ± 2.4 a | 8.4 ± 2.5 a | 7.3 ± 2.5 a |
| Time on lower leaf side (%) | 98.2 ± 0.9 ab | 98.2 ± 1.0 a | 95.2 ± 2.8 ab | 92.1 ± 2.0 b | 93.2 ± 2.4 ab | 90.48 ± 2.6 ab | 92.2 ± 2.6 ab |
| Time on petiole (%) | 0.5 ± 0.2 ab | 1.2 ± 1.0 b | 0.2 ± 0.2 ab | 1.6 ± 0.5 a | 0.4 ± 0.2 ab | 1.2 ± 0.4 ab | 0.5 ± 0.3 ab |
| Time on leaf border (%) | 10.7 ± 2.0 bc | 6.7 ± 2.2 c | 20.5 ± 4.4 abc | 33.8 ± 4.2 a | 22.9 ± 3.2 ab | 27.6 ± 3.9 a | 30.0 ± 3.9 a |
| Number of leaf side changes | 0.45 ± 0.17 a | 0.55 ± 0.32 a | 0.70 ± 0.26 a | 2.91 ± 0.85 a | 2.60 ± 0.62 a | 3.05 ± 0.90 a | 1.55 ± 0.69 a |
| Parasitoids that never changed leaf sides (%) | 70 | 80 | 70 | 48 | 40 | 30 | 65 |
| Lower border | 25.0 | 60.0 | 65.0 | 26.1 | 45.0 | 25.0 | 15.0 |
| Lower centre | 25.0 | 15.0 | 0.0 | 4.3 | 0.0 | 0.0 | 5.0 |
| Upper border | 0.0 | 0.0 | 0.0 | 4.3 | 0.0 | 5.0 | 10.0 |
| Upper centre | 5.0 | 0.0 | 0.0 | 0.0 | 5.0 | 0.0 | 0.0 |
| Petiole | 45.0 | 25.0 | 35.0 | 65.2 | 50.0 | 70.0 | 70.0 |

Kruskal-Wallis test (α=0.05). Different letters in a row indicate significant differences.

of times the parasitoids changed leaf sides, nor between the number of parasitoids that never changed leaf sides.

*A. fuscipennis* left the leaflet in most cases (63.9%) by walking away via the petiole and by flying away from the lower border of the leaf (27.7 %). *E. formosa* left the leaflet to a lesser extent by walking via the petiole (35.0 %), but more frequently by flying away from the lower border (50 % ) or the lower centre (13.3 %) (Table 5).

*Host encounters and acceptance.*
Differences found between the number of encounters with hosts and the number of ovipositions between the experiments (Table 6) were related to the parasitoid species and to the host stage. The number of host encounters with hosts per leaflet of *E. formosa* $11.4 \pm 0.9$ was not significantly higher than that of $9.48 \pm 0.63$ of *A. fuscipennis* (Kruskal-Wallis test, p = 0.21). However, the number of encounters per hour residence time of $10.43 \pm 0.54$ of *A. fuscipennis* was significantly higher than that of $6.38 \pm 0.41$ of *E. formosa* (Kruskal-Wallis test, $p = < 10^{-4}$). Several hosts were encountered more than once, so, the number of different hosts encountered was about two thirds of the number of encounters with hosts: $7.12 \pm 0.5$ and $6.24 \pm 0.35$ for *E. formosa* and *A. fuscipennis* respectively (Kruskal-Wallis test, p = 0.13). No significant differences were found between the number of encounters when comparing leaflets with L1 larvae and leaflets with L3 larvae for *A. fuscipennis*. For *E. formosa*, however, the differences were highly significant. The number of encounters with hosts on leaflets with L3 larvae of $13.43 \pm 1.15$ was significantly higher than that of $7.35 \pm 0.87$ on leaflets with L1 larvae (Kruskal-Wallis test, $p = 7 \cdot 10^{-4}$). Also, the number of encounters per hour residence time of $6.89 \pm 48$ on leaflets with L3 larvae was significantly higher than that of $5.35 \pm 0.73$ on leaflets with L1 larvae (Kruskal-Wallis test, p = 0.042). The number of hosts encountered was $8.03 \pm 0.62$ and $5.3 \pm 0.72$ on leaflets with L3 and L1 larvae respectively (Kruskal-Wallis test, $p = 9 \cdot 10^{-3}$).

The number of ovipositions per leaflet of $6.98 \pm 0.5$ of *E. formosa* was significantly higher than that of $5.47 \pm 0.41$ of *A. fuscipennis* (Kruskal-Wallis test, p = 0.016). However, the number of ovipositions per hour residence time of $5.82 \pm 0.36$ of *A. fuscipennis* was significantly higher than that of $3.97 \pm 0.26$ of *E. formosa* (Kruskal-Wallis test, $p = 4 \cdot 10^{-4}$). The number of ovipositions was higher than the number of hosts parasitized, the last being $6.07 \pm 0.42$ and $4.75 \pm 0.32$ for *E. formosa* and *A. fuscipennis* respectively (Kruskal-Wallis test, p = 0.016). Self-superparasitism was similar for both parasitoid species: 13.7 and 13.0 % for *E. formosa* and *A. fuscipennis* respectively.

No significant differences were found between the number of ovipositions when comparing leaflets with L1 larvae and leaflets with L3 larvae for *A. fuscipennis*. For *E. formosa*, however, the differences were significant: the number of ovipositions on leaflets with L3 larvae of $7.95 \pm 0.64$ was significantly higher than that of $5.05 \pm 0.62$ on leaflets with L1 larvae (Kruskal-Wallis test, p = 0.01); the number of ovipositions per hour residence time of $4.14 \pm 0.31$ on leaflets with L3 larvae was not significantly higher than that of $3.64 \pm 0.47$ on leaflets with L1 larvae (Kruskal-Wallis test, p = 0.26); the number of hosts parasitized was $6.68 \pm 0.52$ and $4.85 \pm 0.63$ on leaflets with L3 and L1 larvae respectively (Kruskal-Wallis test, p = 0.03); and self-superparasitism was 16.8 and 4.0 % on leaflets with L3 and L1 larvae respectively.

The acceptance of *E. formosa* of L1 and L3 *T. vaporariorum* larvae parasitized by itself is significantly lower than that of unparasitized larvae. Hosts that contained a conspecific egg

Table 6. Encounters with hosts and ovipositions (mean ± SE) of *A. fuscipennis* or *E. formosa* (evaluated parasitoid) on tomato leaflets infested with 20 whitefly larvae of which 50 % were parasitized by *A. fuscipennis* or *E. formosa* (first parasitoid).

| Evaluated parasitoid – host stage | *E. formosa* – L3 | *E. formosa* – L3 | *E. formosa* – L1 | *A. fuscipennis* – L1 | *A. fuscipennis* – L1 | *A. fuscipennis* – L3 | *A. fuscipennis* – L3 |
|---|---|---|---|---|---|---|---|
| First parasitoid – host stage | *E. formosa* – L3 | *A. fuscipennis* – L1 | *A. fuscipennis* – L1 | *A. fuscipennis* – L1 | *E. formosa* – L1 | *E. formosa* – L3 | *A. fuscipennis* – L1 |
| n | 20 | 20 | 20 | 23 | 20 | 20 | 20 |
| Number of encounters with hosts / hour of residence time | 8.19 ± 0.67 ab | 5.60 ± 0.58 b | 5.35 ± 0.73 b | 9.53 ± 0.77 a | 9.94 ± 0.97 a | 11.39 ± 1.31 a | 10.97 ± 1.28 a |
| Number of encounters with hosts / leaflet | 14.20 ± 1.57 a | 12.65 ± 1.72 ab | 7.35 ± 0.87 b | 9.74 ± 1.36 ab | 10.10 ± 0.96 ab | 10.30 ± 1.54 ab | 7.75 ± 1.05 b |
| Number of hosts encountered / leaflet | 8.25 ± 0.83 a | 7.80 ± 0.93 a | 5.30 ± 0.72 a | 6.52 ± 0.77 a | 6.80 ± 0.6 a | 6.15 ± 0.67 a | 5.45 ± 0.71 a |
| Acceptance (% of encounters) of hosts not parasitized by 1st parasitoid [**] | 84.0 | 64.9 | 88.6 | 56.3 | 64.4 | 55.8 | 62.9 |
| Acceptance (% of encounters) of hosts parasitized by 1st parasitoid [**] | 66.1 | 70.5 | 81.7 | 63.3 | 55.6 | 63.3 | 54.8 |
| Number of ovipositions / hour of residence time | 5.04 ± 0.43 ab | 3.23 ± 0.36 b | 3.64 ± 0.47 b | 5.55 ± 0.56 ab | 5.56 ± 0.65 ab | 6.62 ± 0.77 a | 5.61 ± 0.94 ab |
| Number of ovipositions / leaflet | 8.65 ± 0.92 a | 7.25 ± 0.89 ab | 5.05 ± 0.62 ab | 5.70 ± 0.92 ab | 5.65 ± 0.62 ab | 6.05 ± 0.80 ab | 4.45 ± 0.86 b |
| Number of hosts parasitized / leaflet | 7.10 ± 0.74 a | 6.25 ± 0.75 ab | 4.85 ± 0.63 ab | 4.83 ± 0.66 ab | 5.00 ± 0.52 ab | 5.15 ± 0.61 ab | 4 ± 0.74 b |
| Self-superparasitism (%) | 18.4% | 15.0% | 4.0% | 14.6% | 11.4% | 14.9% | 10.1% |

[*] Kruskal-Wallis test ($\alpha=0.05$). Different letters in a row indicate significant differences.
[**] Only hosts which were not self-parasitized were taken into account.

or an *A. fuscipennis* egg or larva on top of their own, were accepted equally when compared to hosts with their egg only. The acceptance of L3 unparasitized hosts of 74.3 % was not significantly higher than that of L3 larvae parasitized by a conspecific of 66.1 % (Z=1.47, p = 0.071). Hosts containing an *A. fuscipennis* egg or larva were accepted at the same rate as unparasitized larvae (Table 7).

For *A. fuscipennis*, the acceptance of L1 unparasitized hosts of 59.6 % was equal to that of hosts with a conspecific egg of 63.3 %. The acceptance of self-parasitized larvae of 39.3 % was significantly lower than the previous two host types. All other comparisons between the acceptance of the different L1 host classes were not significant. When parasitizing L3 larvae no significant differences were found between the acceptance of *T. vaporariorum* larvae of the different parasitization classes: hosts containing its own egg, a conspecific larva or an *E. formosa* egg were accepted at the same rate as unparasitized larvae (Table 8).

Neither parasitoid species changed its host evaluation behaviour when comparing unparasitized larvae and larvae parasitized by a conspecific or a heterospecific. To evaluate the percentage of host acceptance as function of the number of eggs laid, the acceptance data of the larvae not previously self-parasitized (including unparasitized, parasitized by a conspecific and parasitized by a heterospecific) were pooled. The host acceptance of *E. formosa* decreased with number of ovipositions, while for *A. fuscipennis* it increased (Figure 5), although a lot of variation was observed. For larvae that were self-parasitized the acceptance results were much lower than for larvae that were not self-parasitized. Furthermore, no reduction in the host acceptance with increasing number of ovipositions was found for this type of larvae, because the number of replicates was low and the variation was high.
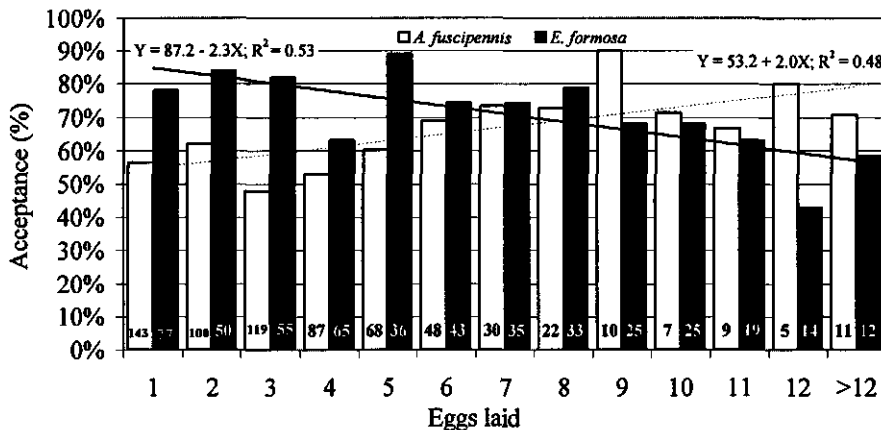


Figure 5. Acceptance of not self-parasitized *T. vaporariorum* larvae by *E. formosa* (n=60 females) and *A. fuscipennis* (n=83) as function of the number of eggs laid (1 egg laid = from the beginning of the observations until the first oviposition; 2 eggs laid = from the first oviposition until the second, etc.). The number of encounters is given at the base of the columns.

Table 7. Percentage of unsuccessful ovipositions (Unsuc.), antennal rejections (Rejec.) and successful ovipositions or acceptances (Succ.) of encounters (n) of *E. formosa* with whitefly larvae of different stages and different previous parasitization (containing its own egg, a conspecific egg, an *A. fuscipennis* (*A.f.*) egg or larva or combinations); and values of the Z statistic and significance level for comparisons between the percentage of successful ovipositions or host acceptance of different parasitization classes.

| Stage | Parasitization | n | Unsuc. (%) | Rejec. (%) | Succ. (%) | Values of Z and significance level[1] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Unparasitized | Own egg | *A.f.* egg | | |
| L1 | Unparasitized | 44 | 6.8 | 4.6 | 88.6 | | | | | |
| | Own egg | 15 | 26.7 | 66.6 | 6.7 | 10.22*** | | | | |
| | *A.f.* egg | 71 | 9.9 | 8.4 | 81.7 | 1.05 n.s. | 9.49*** | | | |
| | Own + *A.f.* egg | 17 | 11.8 | 70.6 | 17.6 | 6.82*** | 1.13 n.s. | 6.2*** | | |
| | | | | | | Unparasitized | Own egg | Consp. egg | Own and consp. egg | *A.f.* larva |
| L3 | Unparasitized | 152 | 7.3 | 18.4 | 74.3 | | | | | |
| | Own egg | 57 | 10.5 | 56.2 | 33.3 | 5.71*** | | | | |
| | Consp. egg | 118 | 8.5 | 25.4 | 66.1 | 1.47 n.s. | 4.3*** | | | |
| | Own + consp. egg | 67 | 12.0 | 53.7 | 34.3 | 5.89*** | 0.14 n.s. | 4.38*** | | |
| | *A.f.* larva | 104 | 4.8 | 23.1 | 72.1 | 0.39 n.s. | 5.35*** | 0.97 n.s. | 5.19*** | |
| | Own egg + *A.f.* larva | 39 | 12.8 | 59.0 | 28.2 | 5.75*** | 0.6 n.s. | 4.5*** | 0.66 n.s. | 5.2*** |

[1] Test for the comparison of two proportions ($p_1$ and $p_2$), $Z = (p_1-p_2) \cdot [p_1 \cdot (1-p_1) \cdot n_1^{-1} + p_2 \cdot (1-p_2) \cdot n_2^{-1}]^{-\frac{1}{2}}$ with a normal distribution ($\mu=0$ & $\sigma=1$). N.s., *, ** and ***, means that the proportions are not significantly different or significantly different at the 5, 1 and 0.1 % level respectively.

Table 8. Percentage of unsuccessful ovipositions (Unsuc.), antennal rejections (Rejec.) and successful ovipositions or acceptances (Succ.) of encounters (n) of *A. fuscipennis* with whitefly larvae of different stages and different previous parasitization (unparasitized, containing its own egg, a conspecific egg or larva, an *E. formosa* (*E.f.*)egg or combinations); and values of the Z statistic and significance level for comparisons between the percentage of successful ovipositions or host acceptance of different parasitization classes.

| Stage | Parasitization | n | Unsuc. (%) | Rejec. (%) | Succ. (%) | Values of Z and significance level [1] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Unparasitized | Own egg | Consp. egg | Own and consp. egg | *E. f.* egg |
| L1 | Unparasitized | 146 | 12.3 | 28.1 | 59.6 | | | | | |
| | Own egg | 28 | 25.0 | 35.7 | 39.3 | 2.01* | | | | |
| | Conspecific egg | 98 | 18.4 | 18.4 | 63.2 | 0.58 n.s. | 2.3* | | | |
| | Own + conspecific egg | 24 | 12.5 | 29.2 | 58.3 | 0.12 n.s. | 1.7 n.s. | 0.44 n.s. | | |
| | *E. formosa* egg | 112 | 19.6 | 25.0 | 55.4 | 0.68 n.s. | 1.46 n.s. | 1.17 n.s. | 0.27 n.s. | |
| | Own + *E. formosa* egg | 18 | 22.2 | 33.3 | 44.5 | 1.22 n.s. | 0.41 n.s. | 1.48 n.s. | 0.90 n.s. | 0.86 n.s. |
| | | | | | | Unparasitized | Own egg | Consp. larva | Own egg and consp. larva | *E. f.* egg |
| L3 | Unparasitized | 157 | 12.7 | 28.7 | 58.6 | | | | | |
| | Own egg | 25 | 4.0 | 32.0 | 64.0 | 0.52 n.s. | | | | |
| | Conspecific larva | 73 | 11.0 | 34.2 | 54.8 | 0.54 n.s. | 0.82 n.s. | | | |
| | Own egg and conspecific larva | 9 | 0.0 | 55.6 | 44.4 | 0.83 n.s. | 1.12 n.s. | 0.59 n.s. | | |
| | *E. formosa* egg | 79 | 8.9 | 27.8 | 63.3 | 0.70 n.s. | 0.04 n.s. | 1.07 n.s. | 1.08 n.s. | |
| | Own and *E. formosa* egg | 18 | 11.0 | 44.5 | 44.5 | 1.15 n.s. | 1.52 n.s. | 0.79 n.s. | <0.01 n.s. | 1.46 n.s. |

[1] Test for the comparison of two proportions ($p_1$ and $p_2$). $Z = (p_1-p_2)[p_1 \cdot (1-p_1) \cdot n_1^{-1} + (p_2 \cdot (1-p_2) \cdot n_2^{-1})]^{-\frac{1}{2}}$ with a normal distribution $\mu=0$ & $\sigma=1$). N.s., *, ** and ***, means that the proportions are not significantly different, significantly different at the 5, 1 and 0.1 % level respectively.

*Dissections*
The results of the dissection can be found in Table 9. For experiments 1 to 6, a total of 709 parasitized larvae were dissected, containing an expected total of 911 parasitoid eggs, 429 *A. fuscipennis* eggs and 482 *E. formosa* eggs. Of these, 9 *E. formosa* eggs (1.9 %) in 9 larvae and 18 *A. fuscipennis* eggs (4.2 %) in 17 larvae were not found. The duration of the ovipositions corresponding to the eggs that were not found were considerably higher than the times that were used to distinguish between a successful and unsuccessful ovipositions (23 s for *A. fuscipennis* and 120 s for *E. formosa*). Moreover, in most of the cases the duration of the oviposition postures corresponding to an egg that was not found was longer than the shortest oviposition posture leading to a successful oviposition of the corresponding female.

For *A. fuscipennis*, of all oviposition postures lower than 23 s, only one of 22.7 s was successful. This was the shortest successful oviposition in an L1 larva while that in an L3 larva was 24.1 s. For *E. formosa*, of all oviposition postures with a duration lower than 120 s, only one of 118.9 s in an L1 larva was successful. The shortest successful oviposition in an L3 larva was 120 s.

*Emergence*
The emergence of 970 parasitized larvae was registered, of which 255 were superparasitized or multiparasitized. All superparasitized larvae lead to the emergence of one parasitoid. Three larvae parasitized by *A. fuscipennis* and two by *E. formosa* died.

In all the experiments with multiparasitism, the parasitoid of the species that had parasitized first emerged more frequently (69.7-83.3 %) than the parasitoid of the species that parasitized second. From 72.4 % of multiparasitized L1 larvae, and where *A. fuscipennis* was the first parasitoid, *A. fuscipennis* emerged. Similarly, from 69.7 % of multiparasitized L1 larvae, and where *E. formosa* was the first in parasitoid, *E. formosa* emerged. In comparison, when *E. formosa* parasitized L3 larvae that were parasitized by *A. fuscipennis* in the L1 stage, the percent emergence of *A. fuscipennis* did not increase (72.1 %). However, when *A. fuscipennis* parasitized L3 larvae that were recently parasitized by *E. formosa* the emergence of *E. formosa* of 83.3 % was higher (Figure 6).

**Discussion**

Residence times for *E. formosa* on leaflets with L3 larvae were similar to those found by van Roermund & van Lenteren (1995 b). As a higher host density was used in this study, the time registered until the first encounter on leaflets with L3 larvae (452 s) was shorter than the time they found (702 – 1345 s) on leaflets with 1 or 4 hosts and where the parasitoid was not introduced near the hosts. However, the time until the first encounter registered here was higher that what they found (164 s) on leaflets with 77-200 black pupae. The GUT of 1243s for *E. formosa* was rather low compared with the median GUT of 2401 s found by van Roermund et al. (1994) on leaflets where oviposition in unparasitized hosts took place. Sütterlin & van Lenteren (1999) found a higher GUT and assigned it to egg depletion. However, the parasitoids in this study were also egg depleted and nevertheless they left the leaflet rather fast. So, it is not clear why the GUT registered here is lower.

Table 9. Frequency of *T. vaporariorum* larvae with different number of eggs or larvae of *A. fuscipennis* (*A.f.*) and/or *E. formosa* (*E.f.*) found during dissections of *T. vaporariorum* larvae with different number of observed parasitizations by the evaluated parasitoid (Par. 2) and the first parasitoid (Par. 1) for the different experiments. Bold values and underlined values indicate 'missing' *E. formosa* and *A. fuscipennis* eggs respectively.

| Evaluated parasitoid – host stage / First parasitoid – host stage | Par. 1 | Par. 2 | n | Number of whitefly larvae where the following number of parasitoid eggs were found in dissections | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 1 *E.f.* | 2 *E.f.* | 3 *E.f.* | 1 *A.f.* | 2 *A.f.* | 1 *E.f.* 1 *A.f.* | 1 *E.f.* 2 *A.f.* |
| *E. formosa* – L3 / *E. formosa* – L3, n = 129 | 0 | 1 | 25 | | 25 | | | | | | |
| | 0 | 2 | 3 | | | 3 | | | | | |
| | 0 | 3 | 1 | | | | 1 | | | | |
| | 1 | 0 | 60 | | 60 | | | | | | |
| | 1 | 1 | 31 | | | 31 | | | | | |
| | 1 | 2 | 8 | | | **5** | 3 | | | | |
| | 1 | 3 | 1 | | | | **1** | | | | |
| *E. formosa* – L3 / *A. fuscipennis* – L1, n = 117 | 0 | 1 | 16 | **1** | 15 | | | | | | |
| | 0 | 2 | 1 | | 1 | | | | | | |
| | 1 | 0 | 69 | _1_ | | | | 68 | | | |
| | 1 | 1 | 30 | | _1_ | | | | | 29 | |
| | 1 | 2 | 1 | | | | | | | | **1** |
| *E. formosa* – L1 / *A. fuscipennis* – L1, n = 121 | 0 | 1 | 20 | | 20 | | | | | | |
| | 0 | 2 | 1 | | | 1 | | | | | |
| | 1 | 0 | 70 | | | | | 70 | | | |
| | 1 | 1 | 30 | | | | | | | 30 | |
| *A. fuscipennis* – L1 / *A. fuscipennis* – L1, n = 100 | 0 | 1 | 8 | | | | | 8 | | | |
| | 0 | 2 | 2 | | | | | | 2 | | |
| | 1 | 0 | 65 | _2_ | | | | 63 | | | |
| | 1 | 1 | 25 | _1_ | | | | _4_ | 20 | | |
| *A. fuscipennis* – L1 / *E. formosa* – L1, n = 121 | 0 | 1 | 17 | | | | | 17 | | | |
| | 0 | 2 | 4 | | | | | _1_ | 3 | | |
| | 1 | 0 | 73 | | 73 | | | | | | |
| | 1 | 1 | 23 | | | | | | | 23 | |
| | 1 | 2 | 4 | | | | | | | _3_ | 1 |
| *A. fuscipennis* – L3 / *E. formosa* – L3, n = 121 | 0 | 1 | 18 | | | | | 18 | | | |
| | 0 | 2 | 3 | | | | | _2_ | 1 | | |
| | 1 | 0 | 79 | | 79 | | | | | | |
| | 1 | 1 | 20 | | _1_ | | | | | 19 | |
| | 1 | 2 | 1 | | | | | | | _1_ | |

Figure 6. Parasitoid emergence from *T. vaporariorum* larvae of different stages (L1 or L3) containing a parasitoid egg or larva of a conspecific or a heterospecific and that were superparasitized or multiparasitized by *E. formosa* (E.f.) or *A. fuscipennis* (A.f.). The number of replicates is given at the base of each column.

In our experiments, leaflets with L1 larvae resulted in shorter residence times, a longer time until the first encounter, and fewer encounters and ovipositions as compared to leaflets with L3 larvae. Also, the total time spent on oviposition posture, the time needed to assess the host and to lay an egg were shorter. On the contrary, the duration of the searching intervals, the periods standing still and those spent on preening were longer. The GUT was similar for leaflets with L1 and L3 larvae. The differences were probably caused by the lower encounter rate as the host diameter of L1 larvae is about 51 % of that of L3 larvae (van Lenteren et al., 1976a). This increased the periods between encounters and the possibility that the GUT is attained, causing those leaflets to be abandoned faster. More time walking while drumming was spent on leaflets with L1 larvae. As fewer eggs on those leaflets were laid, the walking activity did not decrease so much as a result of egg depletion (van Roermund & van Lenteren, 1995 b, Sütterlin & van Lenteren 1999). This explains why no difference was found between the walking activity before the first and that after the last oviposition on leaflets with L1 larvae. On leaflets with L3 larvae, van Roermund & van Lenteren (1995 b) did not find a decrease in walking activity, but the number of ovipositions in their experiment was lower than in this study and egg depletion negligible. In other experiment, however, they found a reduction of the walking activity with increasing number of eggs laid, but the decrease found here was less than that found by them (van Roermund & van Lenteren, 1995 b) or that found by Sütterlin & van Lenteren (1999). In our observations, the walking activity was more or less constant until the $6^{th}$-$7^{th}$ oviposition, while they already found a reduction from $2^{nd}$ or the $3^{rd}$ oviposition.

For *E. formosa*, oviposition in L1 hosts (175.3 s) was faster than in L3 hosts (196.3 s) and shorter than that of 362 s found by van Roermund & van Lenteren (1995 b). Sütterlin & van Lenteren (1999) found mean oviposition times of 453 s on gerbera and 727 s on tomato.

Van Roermund & van Lenteren (1995 b) report unpublished data of other authors in their appendix: 216 s on cucumber and 209 on tomato and cucumber without mentioning the host stage. The data here are comparable with the latter two but are considerably lower than the former two. Assessing L1 hosts was generally faster than L3 hosts. So, total host handling time depends on the host stage.

For *A. fuscipennis* on infested bean plants, Manzano (2000) found residence times of 9882 s, during which the parasitoids visited a mean of 8.9 leaflets with a mean area of 34.3 cm$^2$, leading to a lower mean residence time per leaflet (1110 s) than that found here. However, only one leaflet out of three that form a leaf were infested with 4 *T. vaporariorum* L1 larvae, which resulted in a lower host encounter rate than here. The walking activity on infested plants was 78.3, comparable to that of 84.3 found here. The GUT of 7385 s they found for leaving the plant was much higher than that observed here for tomato leaflets, but possibly during that period more than one leaflet was visited. The number of encounters with hosts observed in this study was double as high as that found by Manzano (2000) and this also resulted in a higher host handling time of 11.0 % compared to 2.8 % found by Manzano (2000). She also found that 80% of the *A. fuscipennis* visited petioles and stems. In this study, almost all parasitoids visited the petiole for (a) short period(s) during its stay on a leaflet and 65 % of the females left the leaflet by walking away via the petiole. Females that left the leaflet by flying, did that in 90 % of the times from the leaf edge, which coincides with the 88.3 % found by Manzano (2000). *A. fuscipennis* females spent 92 % of the total time on the lower leaf side, which is higher than the 83 % found by Manzano (2000). However, in their case, only 1 out of 3 leaflets was infested and they found that the time spent on the lower leaf side of uninfested plants was lower than on infested plants.

The residence time of *A. fuscipennis* was half as long compared to that of *E. formosa*, however, the time spent walking while drumming was equal. The time spent on all other activities was much shorter, particularly that spent standing still. Host handling, including evaluation, rejection, oviposition or ovipositorial rejection were much faster and the total time dedicated to these activities was only 22 % of the time that *E. formosa* spent on these activities. Additionally, *A. fuscipennis* presented hardly any feeding and no host feeding at all. Manzano (2000) also found this on bean leaves. The periods dedicated to walking while drumming were longer and the period dedicated to standing still were much shorter as well as the time needed for an oviposition. The duration of the host assessment was in the same range as that of *E. formosa*.

For *E. formosa*, van Lenteren et al. (1980) measured the foraging behaviour much more in detail and they did not present a general ethogram so it is difficult to compare the results of this study with theirs, but in general we found comparable behaviour sequences.

The behavioural sequences and duration found for *A. fuscipennis* were quite different when compared with data of Manzano (2000), who observed the behaviour of 15 females at 19 °C during 1 hour on bean leaflets with about 30 (estimated) hosts of all stages. The percentage of the number of intervals walking while drumming and preening as percentage of the total number of activities was similar to that of Manzano (2000), but that of standing still was significantly higher. This resulted in a significantly lower percentage of the host handling activities including encountering host, assessing host and oviposition postures. Presumably this higher frequency and time dedicated to standing still was a result of the lower temperature in the experiments of Manzano (2000). Another remarkable difference with the data of Manzano (2000) is that after ovipositions the hosts were drummed again in 38.9 % of the

times. We found this in only 1.1 % of the times. The duration of the intervals of walking, standing still and preening found by Manzano (2000) were respectively 49.1, 10.5 and 17.9 compared to 171.0, 46.0 and 80.0 s, found in this study, while for the other activities the duration was comparable. The distribution of the behavioural events we found for the different behaviours coincide generally with those presented by Joyce et al. (1999) for *A. bennetti* on cotton leaves infested with an unknown number of *B. tabaci* larvae of all stages at 27 °C. For *A. bennetti* feeding was also hardly found and standing still was not found at all. The duration of the searching and preening intervals was 18.3 and 17.1 s respectively, again much shorter than our results. They found drumming hosts after oviposition posture in 13.3 % of the times, which is higher than our results but lower than those of Manzano (2000). For *A. bennetti*, preening was found after all activities, including after oviposition. Preening followed almost always after oviposition for *A. hesperidum* on *A. woglumi* on citrus (Dowell et al., 1981). Neither we, nor Manzano (2000), found preening after oviposition.

Successful ovipositions of L1, L2 and L3 larvae had a duration of respectively 48.4, 82.1 and 68.3 s at 19 C on bean (Manzano, 2000), which is much higher than our values, possibly due to the lower temperature. Drost et al. (1999) found that the oviposition time of 39.1 s for *A. bennetti* on *B. tabaci* was independent of the host stage at 25 °C. On L1, L2 and L3 larvae of *A. woglumi*, Dowell et al. (1981) reported oviposition times for *A. hesperidum* of respectively 27.6, 39.3 and 76.5 s (temperature not given), being shorter on L1 larvae and longer on L3 larvae, when compared to our data.

The number of encounters with hosts of *A. fuscipennis* was slightly lower than that of *E. formosa* but in about 50 % of the time, so that the number of encounters per hour of residence time was 63 % higher than that of *E. formosa*. The same was found for the number of ovipositions. However, during one visit, *E. formosa* parasitized 30 % of the hosts present on the leaflet compared to 24 % for *A. fuscipennis*. The host stage had almost no influence on the residence time and foraging results of *A. fuscipennis*. Only a slightly higher residence time was found on leaflets with L3 larvae when compared to leaflets with L1 larvae, but time allocation was equal. The number of encounters and ovipositions was similar on leaflets with L1 and L3 larvae. This could be a result of the area-restricted search and reduction of the walking speed of *A. fuscipennis* after encountering a host (Manzano, 2000). *E. formosa* searches at random and walking speed and searching pattern are not influenced by host encounters (van Lenteren et al., 1976a; Drost et al., 2000).

Walking activity and host acceptance are influenced by the egg load for the synovigenic *E. formosa* (van Roermund & van Lenteren, 1995a, Sütterlin & van Lenteren, 1999). The maximum egg load of *E. formosa* is about 9 eggs (van Vianen & van Lenteren, 1986), so in our experiments egg depletion occurred for *E. formosa* females, explaining the reduction in walking activity and host acceptance we observed. The mean number of ovipositions of *A. fuscipennis* was 5.5 in our experiments, reducing the egg load by 1.2 % as fecundity at 25 °C was 430 eggs per females (De Vis et al., 2001). So the egg load was hardly influenced by the ovipositions that were made during the observations and neither the walking activity, nor the host acceptance changed with the number of ovipositions made during the observations.

*A. fuscipennis* left the leaflet in 64 % of the cases by walking away via the petiole and to a lesser extent by flying away. Travelling via the petioles or stems of a tomato plant is a dangerous operation for a parasitoid as glandular trichomes can be a mortal trap. In greenhouse experiments with *A. fuscipennis*, we observed many parasitoids stuck in the those trichomes, which could reduce the efficiency of this parasitoid species in the field. *E. formosa*

females, however, left the leaflets by flying in most of the cases, avoiding in this way the risk of travelling on the 'dangerous' stems.

Host acceptance of *E. formosa* seems to be a very complicated matter and results of experiments are variable. Up to now, it has been demonstrated that it depends on host stage (Nell et al., 1976; van Lenteren et al., 1976b), previous parasitization of the hosts (van Lenteren et al., 1976b; van Roermund & van Lenteren 1995a; this paper), parasitoid experience (van Roermund & van Lenteren 1995a), egg load or the number of ovipositions in a time span of a few hours (van Roermund & van Lenteren 1995a; Sütterlin & van Lenteren 1999; this paper), and possibly also host plant, as results on different host plants are not always similar. Additionally, the origin of the parasitoids is not always the same. Ovicide (the killing of eggs within the whitefly host with the ovipositor) was first reported for *E. formosa* by Arakawa (1987) but recently the importance was documented by Netting and Hunter (2000) and may also influence strongly the host acceptance of parasitized hosts. The experimental set-ups of the different authors were very variable so comparison of the results is difficult. Nevertheless, some similarities and differences were found with previous studies. In our study, *E. formosa* discriminated clearly larvae parasitized by itself, discriminated poorly larvae parasitized by a conspecific and discriminated not at all larvae parasitized by *A. fuscipennis*. A clear effect of the host stage on the host acceptance was found. The acceptance of unparasitized L3 larvae (74 %) was similar to that found by van Roermund & van Lenteren (1995a) on L3/L4 larvae, however, that of parasitized larvae we found was higher. They found acceptances for naive females of 0 and 14.2 % on L3/L4 hosts recently self-parasitized and recently parasitized by a conspecific respectively, compared to, respectively, 33.3 and 66.1 % we found on L3 hosts. Also, Nell et al. (1976), using experienced females on leaves with all host stages, found acceptances of less than 10 % for recently self-parasitized larvae. The acceptance of self-parasitized larvae was lower for L1 larvae when compared to L3 larvae. Possibly the detection of the parasitism is easier on the smaller L1 larvae than on the larger L3 larvae. The low acceptance rate found in previous studies was not necessary due to experience, as van Roermund & van Lenteren (1995 b) found a higher acceptance for experienced females than for naive females on larvae recently parasitized by a conspecific. Nell et al. (1976) found an acceptance of 7 and 35 % for L1 and L3 unparasitized hosts respectively compared to 88.6 and 74.3 % we found for the same host stages respectively. However, their experiments were done on leaves with all host stages and with experienced females. The higher acceptances of parasitized larvae we found could be caused by ovicide (Netting & Hunter, 2000). We were unaware of the importance of ovicide in *E. formosa* and one of the explanations of the high acceptance rate could be that conspecific eggs were killed during oviposition. In our rearing unit, we always worked with high densities of *E. formosa* adults in the parasitization chambers and females presenting ovicide might be selected rather quickly in such conditions as their fitness is higher than females that do not present this behaviour. Discrimination of self-parasitized hosts was also found for *E. deserti* (Gerling et al., 1987), *E. tricolor* (Artigues et al., 1992) and discrimination of host parasitized by a conspecific was also found for *A. hesperidum* (Dowell et al., 1981), *E. lutea* (Gerling & Foltyn, 1987) but not for *E. opulenta* (Dowell et al., 1981).

In our experiment, interspecific host discrimination was absent for both *E. formosa* and *A. fuscipennis*. Hosts containing an egg or a larva of *A. fuscipennis* were accepted at the same rate as unparasitized hosts by *E. formosa* and hosts containing an *E. formosa* egg were accepted at the same rate as unparasitized host by *A. fuscipennis*. Furthermore, the duration of

the assessment of host parasitized by a heterospecific was equal to that of unparasitized hosts. No other studies on interspecific host discrimination were found for *E. formosa* or *A. fuscipennis*, but it has been studied for the parasitoid pairs *Encarsia lutea* (Masi) - *Eretmocerus mundus* (Mercet) on *Bemisia tabaci* (Gennadius) (Gerling and Foltyn, 1987) and *Encarsia opulenta* (Silvestri) - *Amitus hesperidum* Silvestri on *Aleurocanthus woglumi* (Ashby) (Dowell et al., 1981) and for *E. pergandiella* parasitizing on *T. vaporariorum* host parasitized by *E. formosa* (Buijs et al., 1981). None of these parasitoid species showed interspecific host discrimination. Although Dowell et al. (1981) report interspecific host discrimination for *E. opulenta*, they drew their conclusion not on the basis of encounters but of number of larvae that were multiparasitized on leaves with both unparasitized larvae and larvae parasitized by *A. hesperidum*. Additionally, redoing the calculus of the $\chi^2$-test they made to detect a difference between the observed and expected numbers of multiparasitized and parasitized larvae gave a result of 2.86 (they found 6.98), which was not significant at the 5 % level. So, on the basis of their data, no interspecific host discrimination can be assigned to *E. opulenta*.

Although we can not be sure that the feeding when the parasitoid was on the host was on body fluids or on honeydew present on the host, it is striking that feeding on the host was observed only on those previously self-parasitized, and that only one of the hosts died. Nell et al. (1976) observed that host feeding of *E. formosa* on *T. vaporariorum* larvae led to death of those hosts in many cases. They also observed that host feeding never occurred on parasitized hosts and that hosts used for feeding were never parasitized afterwards. The same authors (van Lenteren et al., 1980) mention frequent feeding on honeydew when the wasp was on the host. Based on these arguments we think that the feedings we observed were on honeydew present on the host and that no host feeding occurred during our experiments. However, the possibility exists that it was on the host's haemolymph, emanated after the ovipositions, which would then have been consumed during a later visit. Feeding on ovipositional wounds also was frequently observed for *E. opulenta* on *A. woglumi*. It is not clear, however, if in this case the hosts selected for host-feeding survived (Dowell et al., 1981). The duration of the feedings was in the range of those found by van Lenteren et al. (1980) but shorter than those found by van Roermund & van Lenteren (1995 b) and Sütterlin & van Lenteren (1999).

For the cases in which an oviposition posture longer than the minimum limits was observed but no egg was found, three explications can be given: 1) the egg was not present and those oviposition postures correspond to ovipositorial rejection, 2) the egg was damaged during the dissection, and 3) the egg was hidden by the intestines. In the case of *E. formosa*, we suppose that the third option is not valid since *E. formosa* eggs are big and easy to distinguish. In one case, we saw that an *E. formosa* egg exploded during dissection so option 2 is possible, although we rather think that the eggs were not present and that these observations correspond to ovipositorial rejections. Van Roermund & van Lenteren (1995a) and Sütterlin & van Lenteren (1999) also stated that the 120-second criterion is not accurate to distinguish between oviposition and ovipositorial rejection. The number of ovipositorial rejections of more than 120 s (9) was rather small when compared to the number of those of less than 120 s (61). We found that 9.3 % of the oviposition postures (n = 754) resulted in ovipositorial rejection, while Sütterlin & van Lenteren (1999), found 31.4 % (n = 51). However, they used larvae glued with honeydew on a leaf and this procedure could have influenced host quality.

For the 18 *A. fuscipennis* eggs that were not found, the situation was somewhat different. Sometimes the intestines of the *T. vaporariorum* larvae, which also stained pink, did not spread very well and we suspect that in some cases the *A. fuscipennis* egg was hidden. So we are not so sure that these 18 cases correspond to ovipositorial rejection. This poor spreading of the intestines happened more frequently when the droplet of the staining solution was too small. The best results were obtained with droplets of about 40 μl.

Oviposition postures were marked as successful when its duration was higher than 23 s for *A. fuscipennis* s and 120 s for *E. formosa*. These values proved to be accurate as a lower limit. One oviposition duration of 118.9 s was successful for *E. formosa* and one of 22.7 s was successful for *A. fuscipennis*, but also one of 118.9 s was unsuccessful for *E. formosa* and one of 22.7 and two of 22.9 s were unsuccessful for *A. fuscipennis*. So, a small overlapping area exists where a successful oviposition would be marked erroneously as unsuccessful when using these limits. Based on the data these lower limits can be used for both L1 and L3 larvae. When also used as upper limit, a small error was made as for only 1.4 % of the *E. formosa* ovipositions postures and 4.5 % of the *A. fuscipennis* postures no eggs were found. This was also confirmed by the emergence data.

With the current data we can not conclude that one of the two parasitoid species is superior to the other when competing within the host body since multiparasitism always ended in a similar emergence of the first parasitoid. More experiments should be done to evaluate, for example, the outcome of L3 larvae parasitized first by *A. fuscipennis* and then by *E. formosa*, and L1 larvae parasitized first by *E. formosa* and then by *A. fuscipennis* in the L3 stage. It would also be interesting to dissect multiparasitized larvae during their development to clarify at what time and which mechanism eliminates one of the two eggs or larvae. *A. fuscipennis* eggs are smaller than *E. formosa* eggs. At the moment, it is not clear whether this difference is a competitive advantage for *E. formosa*.

Initially we hypothesised that the parasitoid species would exploit different niches in the same crop because of their different host stage preferences (*A. fuscipennis* would search for L1-L2 larvae and *E. formosa* for L3-L4 larvae). We did not find evidence for this hypothesis as both *E. formosa* and *A. fuscipennis* displayed a similar foraging behaviour and host acceptance on leaflets with preferred host stages and on leaflets with non-preferred host stages. When used together, both parasitoid species will possibly compete on the same leaves, leading to multiparasitism. When using only one parasitoid, superparasitism will occur but surely to a lower extent than multiparasitism when both are used, as both parasitoid species accept larvae parasitized by a conspecific to a lower extent than those parasitized by a heterospecific. But we used naïve females and possibly experienced females could develop a different behaviour towards non-preferred host stages or hosts parasitized by a heterospecific.

Based on these data it is difficult to predict what the outcome would be when both parasitoids are introduced into the greenhouse: similar, better or poorer control. In order to be able to answer this question, experiments on a greenhouse scale are necessary (see next chapters).

## Acknowledgements

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# 6. Biological control of *Trialeurodes vaporariorum* by *Encarsia formosa* on tomato in unheated greenhouses in the high altitude tropics

## Abstract

During three consecutive production cycles, biological control of *Trialeurodes vaporariorum* by *Encarsia formosa* was tested on a beef tomato crop in a glasshouse and a plastic greenhouse on the Bogota Plateau in Colombia. Population development of *T. vaporariorum* and parasitism by *E. formosa* were followed during 26-28 weeks. The mean temperature in the plastic greenhouse was around 16 °C and around 17 °C in the glasshouse. *E. formosa* was introduced at a rate of 3 adults per m$^2$ and per week in the 1997 production cycle, and respectively 3 and 5 pupae per m$^2$ and per week in 1998 and 1999, from the start of the experiment until reaching a total of about 66 pupae. In 1997, the adult whitefly population increased exponentially to a peak of 76 adults per plant in the plastic greenhouse. At that time, the whitefly population in the glasshouse reached a peak of only 12 adults per plant. The percent parasitism fluctuated between 42 and 82 % in the glasshouse and between 28 and 47 % in the plastic greenhouse. In 1998, the *T. vaporariorum* population remained out of control in both greenhouses and reached a peak of respectively 80 and 53 *T. vaporariorum* adults per plant in the plastic greenhouse and the glasshouse. Parasitism fluctuated between 55 and 97 % in the glasshouse and between 32 and 84 % in the plastic greenhouse. In 1999, biological control was successful in both greenhouses. Most of the time, *T. vaporariorum* populations were lower than 1.2 adults per plant and parasitism by *E. formosa* was 80 % or higher. We suppose that the higher temperature is the main reason for better parasitism in the glasshouse when compared to the plastic greenhouse. The successful results of 1999 show that biological control is possible under the short day and low temperature conditions of greenhouses situated in the high altitude tropics such as the Bogota Plateau. Recommendations are given for the application of *E. formosa* based on the results of these experiments.

## Introduction

Protected cultivation of tomatoes is a recent development in Colombia, generally replacing field-grown tomatoes. The high risk due to pests and diseases, causing in some cases total loss of the crop in the field, is one of the reasons that induced the change to greenhouse production. The use of greenhouses reduces pest and disease risks, and increases production and profits. Greenhouses are currently used for tomato production in the intermediate climate zone (altitude 1800-2000 m) where traditionally field-grown tomatoes are cultivated, but also in cold climate zones such as the Bogota Plateau (altitude 2665 m). One of the advantages of tomatoes production on the Bogota Plateau is the reduced pest spectrum. From 1995 on, greenhouse tomatoes have been produced at the Horticultural Research Centre of the University of Bogota Jorge Tadeo Lozano, where the most important pest has been the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood). Leafminers and aphids were generally kept at low densities by naturally occurring parasitoids (De Vis & van Lenteren, 1999).

Biological control of pests on greenhouse grown tomatoes has become a common practice around the world. For biological control of greenhouse whitefly, the parasitoid *Encarsia formosa* Gahan is traditionally used (van Lenteren, 1992 & 1995). Recently, *E. formosa* has also been complemented with the predator *Macrolophus caliginosus* and the parasitoid *Eretmocerus eremicus* in Europe (van Lenteren, 2000). Modelling work of the tritrophic tomato-*T. vaporariorum*-*E. formosa* system in greenhouses showed that under certain conditions biological control does not work. Two of these conditions playing a role are (1) high quality host plants for *T. vaporariorum*, and (2) unsuitable temperature conditions

(van Lenteren et al., 1996). These conditions occur simultaneously when growing beef tomatoes in unheated greenhouses in the high altitude tropics such as the Bogota Plateau.

Concerning high host plant quality, studies by van Es (1987) have shown that the greenhouse whitefly population develops better on beef than on round tomato varieties. The fecundity increased by a factor 2 or more and adult longevity by a factor of 1.5-1.9 on beef tomato varieties when compared to a round tomato cultivar.

Related to temperature, we can say that the greenhouse climate on the Bogota Plateau is rather cool with mean temperatures of 15-16 °C, day temperatures of 18-22 °C and night temperatures of 5-12 °C. This is lower than the mean temperature in the climatised greenhouses in Western Europe. Pre-1979 data suggested that the intrinsic rate of increase ($r_m$) of *E. formosa* was lower than that of *T. vaporariorum* at temperatures lower than 20 °C. However, new data showed that it was already higher than that of *T. vaporariorum* from 12 °C on (van Lenteren & Hulspas-Jordaan, 1983). This was later confirmed by the modelling work of van Roermund & van Lenteren (1997). Therefore, biological control should be possible under the climatic conditions of greenhouses on the Bogota Plateau. However, other factors such as the parasitoid's searching behaviour might be limited at these low temperatures. Madueke (1979) showed that *E. formosa* did not fly and showed hardly any activity at temperatures lower than 21 °C. However, Christochowits et al. (1981) showed that *E. formosa* could still fly at temperatures around 17 °C and van der Laan et al. (1982) showed that *E. formosa* could migrate at temperatures as low as 13°C. But later, Van Roermund & van Lenteren (1995) showed that *E. formosa* did no longer fly at 18 °C or lower temperatures. This fact could seriously lower the parasitoid's dispersal over the greenhouse and prevent whitefly patches from being found and parasitized.

Greenhouse experiments in The Netherlands showed that *E. formosa* is able to control whitefly population in greenhouses with a relatively low temperature regime (18°C day and 7°C night) during winter in Holland (Hulspas-Jordaan et al., 1987). Although in that experiment day length was shorter and radiation lower in comparison with the Colombian situation, such conditions last only a few months at the beginning of the year in The Netherlands. In contrast, unheated greenhouses on the Bogota Plateau have a constant low mean temperature during the whole year.

Therefore, the success of *E. formosa* as biological control agent of *T. vaporariorum* could not easily be predicted when growing beef tomatoes in greenhouses situated on the Bogota Plateau. However, evaluation of the potential growth of *T. vaporariorum* under the local greenhouse conditions and on the beef tomato variety Boris showed that biological control by *E. formosa* should be possible (De Vis & van Lenteren, in preparation; Chapter 2). In this paper we evaluate the biological control capacity of *E. formosa* under the Bogota Plateau greenhouse conditions and on a high quality tomato cultivar, and formulate recommendations for its use.

**Material and methods**

Three tomato production cycles were conducted at the Horticultural Research Centre (CIAA) of the Jorge Tadeo Lozano University, 20 km north of Bogota at 2665 metres altitude in two types of unheated greenhouses. The first was a plastic greenhouse of the standard Colombian design (fixed open ridge and manual wall curtains) equipped with a thermal screen. The second was a "Dutch-Venlo" type glasshouse with automated roof ventilation, a thermal

screen (except in 1997) and was controlled by a climate computer (Midi-Clima, Van Vliet, The Netherlands). This computer also recorded temperature in both greenhouses using NTC temperature sensors installed in a ventilated box. Relative humidity was calculated using the temperature data of a dry and a wet sensor installed in the same box. The boxes were installed in the centre of each greenhouse at plant height and every ten minutes the mean value of the previous 10 minutes was stored. Tomato plants of the variety. Boris (Bruinsma Seeds, 's Gravenzande, The Netherlands) were transplanted in both greenhouses during three consecutive production cycles: 1) 6-week old plants transplanted on May 23, 1997 and crop finished on January 15, 1998; 2) 7-week old plants transplanted on May 22, 1998 and crop finished on January 8, 1999; and 3) 10-week old plants transplanted on January 25, 1999 and crop finished on August 12, 1999. At the beginning of the experiments, when the first *E. formosa* were introduced, the plants were 10 weeks (4 weeks after transplant) in 1997, 14 weeks (7 weeks after transplant) in 1998 and 11 weeks (1 week after transplant) in 1999. At that moment, a natural infestation of *T. vaporariorum* adults was already present in all experimental greenhouses. These adults may have survived from previous crops. No important immigration of *T. vaporariorum* adults could have taken place at that time as the outside climate conditions are too cold for *T. vaporariorum* population build-up. In adjacent greenhouses either *T. vaporariorum* was controlled chemically to low densities, or the crops in those greenhouse were not suitable for *T. vaporariorum* development.

$E.$ *formosa* was introduced at a rate of 3 adults per $m^2$ per week and during 22 weeks in the 1997 trial, 3 pupae per $m^2$ per week and during 22 weeks in the 1998 trial, and 5 pupae per $m^2$ per week and during 13 weeks in the 1999 trial. A total of 63-70 parasitoids per $m^2$ was introduced in all the trials. In the first experiment, newly hatched parasitoids were introduced in the late afternoon (4.30 – 5.30 p.m.), while for the other two experiments, pieces of leaflets with about 70 black parasitized *T. vaporariorum* pupae were equally distributed over the greenhouses. They were fastened to the base of the lower leaves of the tomato plants. Mean emergence of the introduced pupae was 95 % or higher. Parasitoids originated from the CIAA´s rearing unit where they were reared on *T. vaporariorum* on tomato plants.

Whitefly adults were counted weekly on the 8 upper leaves of a stratified sample of 10% of the plants: within a row, of every ten consecutive plants, one was selected at random. Eggenkamp-Rotteveel Mansveld et al. (1978) found that in a large greenhouse a stratified random sampling alone was not suitable to reliably estimate the total whitefly population. Therefore, we assessed the spatial distribution by making a census of whiteflies every five weeks.

Parasitism was assessed by weekly sampling of the parasitized and non-parasitized pupae on a stratified sample of 10 % of the plants, one leaf per plant. Only leaves where some black pupae had already hatched were selected. On those leaves most of the non-parasitized pupae had already hatched. The number of parasitized pupae that were not yet black and thus counted as non-parasitized pupae was in this way reduced to a minimum.

During the 1997 trial, plants with more than 100 whitefly adults were sprayed with buprofezin and thiociclam in week 20 after the start of the experiment and in the 1998 cycle in week 12 of the experiment, to bring populations down to levels manageable with biological control. In week 19 of the experiment of the 1998-production cycle, all plants in the plastic greenhouse were sprayed with the same products.

Powdery mildew and the diseases caused by *Botrytis* and *Alternaria* were controlled with fungicides compatible with *E. formosa* (fenarimol, diclofluanid, iprodione). Leafminers

and aphids were very satisfactorily kept at low densities by naturally occurring parasitoids during the three production cycles. The tomato russet mite, *Aculops lycopersici*, appeared at the beginning of the 1997 trial and was controlled mainly by application of the acaricides propargite, tetradifon, amitraz and hexythiazox on affected plants. Control was not very effective and by week 22 after transplant an application with propargite and tetradifon to the whole greenhouse was necessary. The mite appeared again during the 1998 experiment, in week 2 after transplant, when tetradifon and hexythiazox were applied. This could not prevent it from becoming a serious pest and 4-6 applications with fenbutatinoxide had to be applied on the whole crop and throughout the production cycle. After this production cycle, the greenhouse was disinfected with bleach solution as to eradicate the pest and during the first two weeks of the 1999 production cycle fenbutatinoxide was applied preventively twice to the new plants and greenhouse structures. The pest did not reappear during the rest of this production cycle. The total number of applications on the whole crop was 3 in 1997, 6-7 in 1998 and 3-4 in 1999.

**Results**

The mean temperature (days as replicates) was significantly different among production cycles and greenhouses (Figure 1A). The mean temperature in the glasshouse was always 1 to 1.5 °C higher than in the plastic greenhouse. The temperature was lowest during the 1997 trial and highest during the 1998 trial. The mean relative humidity (days as replicates) was equal among the three trials in the plastic greenhouse, where it was higher than in the glasshouse. In the glasshouse, the relative humidity was higher during the first two production cycles than in the last one (Figure 1B). The mean daily temperature curve of both greenhouses showed a small but consistent difference. Mean hourly night temperatures were between 10 and 15 °C and day temperatures between 15 and 25 °C for both greenhouses (Figure 1C, showing the data of 1998).

At the start of the experiments, the whitefly population was similar in 1997 and 1998, but significantly lower in 1999: 0.47 and 0.57 whitefly adults per plant in the glasshouse and the plastic greenhouse respectively in 1997, compared to 0.51 and 0.42 in 1998 and 0.023 and 0.034 in 1999.

In 1997, from week 3 on, the population started to increase in both greenhouses (Figure 2), but at a higher rate in the glasshouse compared to the plastic greenhouse. In week 7, the population reached a first peak in the glasshouse after which it decreased and became stable at a level of about 3 whiteflies per plant. In the plastic greenhouse, however, the population increase slowed down only slightly at that time. Between week 10 and 16, parasitism was near 80 % in the glasshouse compared to about 40 % in the plastic greenhouse (Figure 3). As of week 16, the populations started to grow faster again in both greenhouses until reaching a peak in week 19. This increase coincided with a decrease in the parasitism in both greenhouses. From week 20 onwards, the whitefly population declined in both greenhouses as a result of a chemical control applied to plants with more than 100 whitefly adults. Only 2.8 % of the plants were sprayed in the glasshouse, but in the plastic greenhouse the infestation was so high that all plants had to be sprayed. Parasitism continued declining during the rest of the production cycle. The total number of black and white pupae per sampled leaf increased exponentially to a maximum of 34 in the plastic greenhouse in week 20. At that time, a mean of 6 pupae per leaf was found in the glasshouse and the maximum for the glasshouse of 8.4

pupae per leaf was found in week 22. The number of pupae per leaf was always higher in the plastic greenhouse (Figure 4).
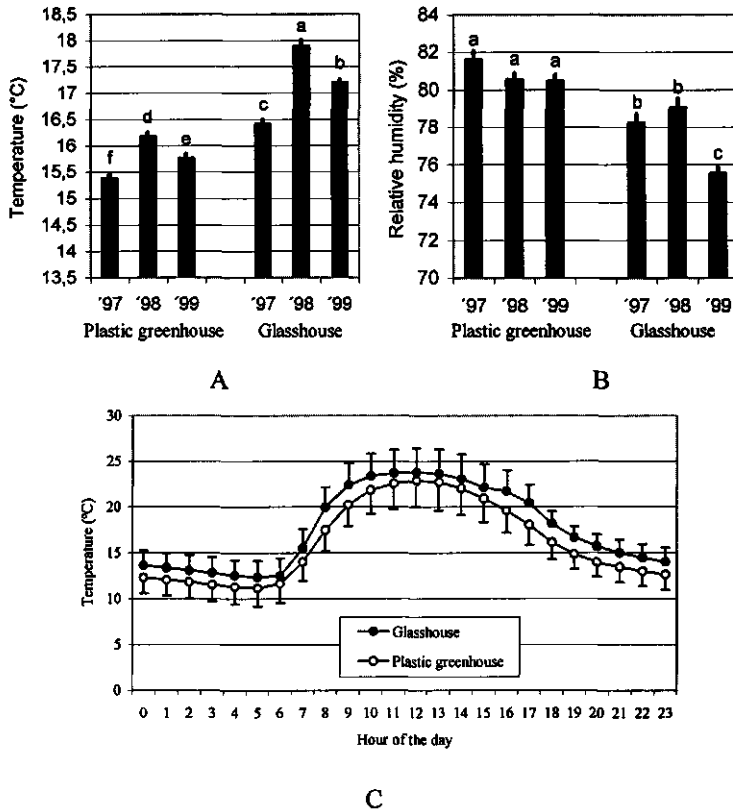


A

B

C

Figure 1. Mean temperature (Graph A) and relative humidity (Graph B) of the greenhouses during the three production cycles. Means with the same letter are not significantly different ($\alpha=0.05$). Mean temperature curve (Graph C) of both greenhouses during the 1998 production cycle.

In 1998, the *T. vaporariorum* adult population increased faster than in 1997 in both greenhouses. In the glasshouse, it passed that of the plastic greenhouse in week 11 (Figure 2). At that time, plants with more than 100 adults were sprayed; 22% of the plants in the glasshouse were sprayed compared to 5 % in the plastic greenhouse. This caused an important decline in the *T. vaporariorum* adult population of the glasshouse, but not in the plastic greenhouse. However, parasitism in the glasshouse, which had been below 80 % during the
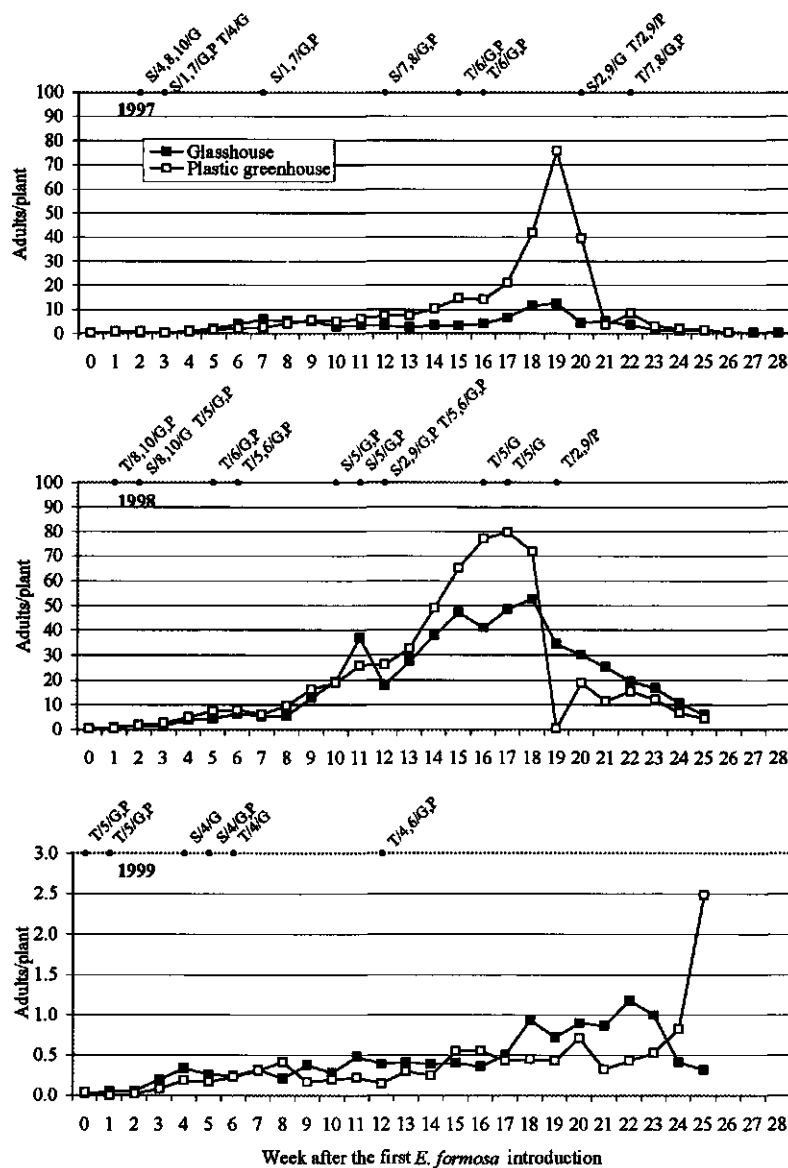
Figure 2. Evolution of the number of adult whiteflies per plant in the glasshouse and in the plastic greenhouse during the three consecutive production cycles. The chemical treatments are specified above the graphs: S=spot treatment; T=total crop treatment; G=glasshouse; P=plastic; 1=amitraz; 2=buprofezin; 3=dichlofluanid; 4=fenarimol; 5=fenbutatinhydroxide; 6=iprodione; 7=propargite; 8=tetradifon; 9=thiocyclam hydrogen oxalate; 10=hexythiazox.
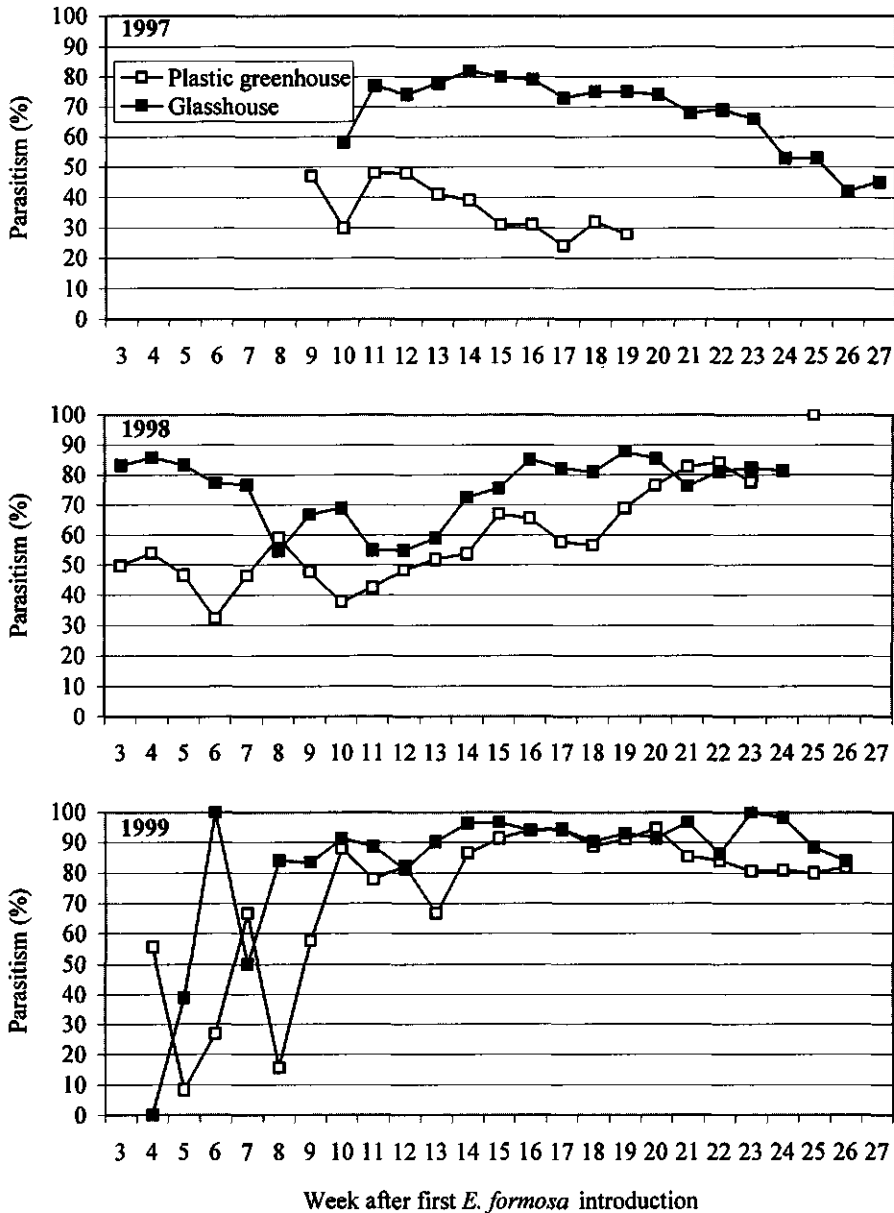
Figure 3. Evolution of the percent parasitism in the plastic greenhouse and in the glasshouse during the three consecutive production cycles.
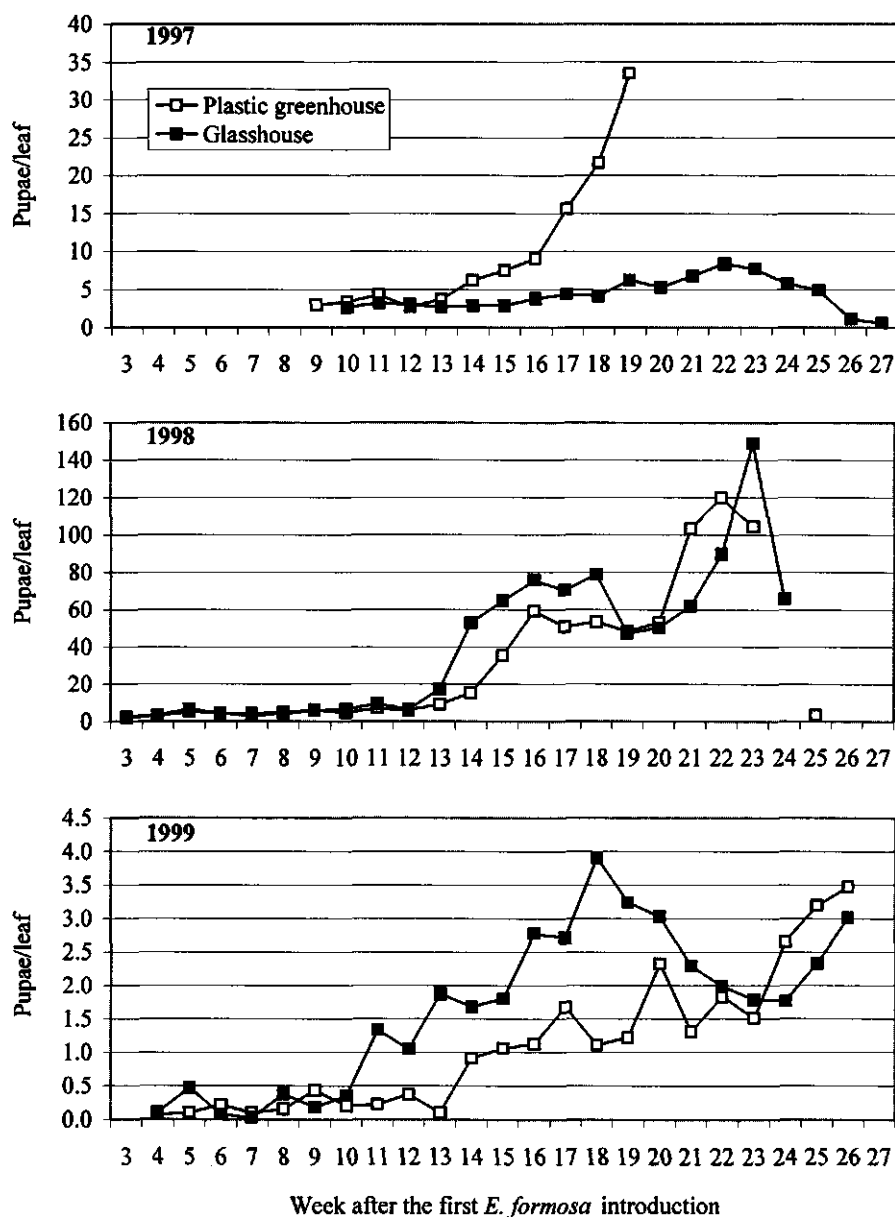
Figure 4. Evolution of total (black+white) number of pupae per leaf in the plastic greenhouse and in the glasshouse during the three production cycles.

previous five weeks, increased and by week 16 it was 80% (Figure 3). During weeks 15-18 the population of *T. vaporariorum* adults did not increase very much in the glasshouse and reached a maximum of 53 adults per plant by week 18. At that point, the population had already reached 80 adults per plant in the plastic greenhouse. Because of the high percent parasitism (more than 80 %) and the stable number of whiteflies, the glasshouse was not sprayed at that time, while the plastic greenhouse was sprayed completely because of the lower level of parasitism (60%) and the higher adult whitefly population. Parasitism remained below 60 % in the plastic greenhouse most of the time, but after the chemical control it increased gradually to more than 80 % by week 21. The whitefly population remained below 20 adults per plant. In 1998, the population peaked 3 weeks earlier than in 1997. Also the number of pupae per leaf increased earlier in 1998. In 1997, the total number of black and white pupae in the plastic greenhouse was 35 in week 19, while this level was already reached by week 15 in 1998. By week 15 more than 60 pupae per leaf were found in the glasshouse. In 1998, the number of pupae per leaf was almost always higher in the glasshouse than in the plastic greenhouse and also higher than the number of pupae per leaf found in both greenhouses in 1997 (Figure 4).

In 1999 the population of whiteflies was very low during the whole cycle and did not pass 1.2 adults per plant in the glasshouse and 2.5 adults per plant in the plastic greenhouse. At the end of this production cycle, the adult population was increasing in the plastic greenhouse, while in the glasshouse it was decreasing (Figure 1). Parasitism was above 80 % in both greenhouses, but this level was attained more quickly in the glasshouse (week 6) than in the plastic greenhouse (week 10). It was almost always higher in the glasshouse than in the plastic greenhouse (Figure 3). The total number of black and white pupae per leaf was very low in comparison with the previous experiments and was higher in the glasshouse than in the plastic greenhouse most of the time (Figure 4).

To visualise the spatial distribution, greenhouses were divided in plots of 4 (2x2) plants and the mean number of whiteflies per plant of the plot was calculated based on the results of the whitefly census done every five weeks. According to four density classes, two-dimensional maps were constructed. The distribution maps of the 1997 experiment (Figure 5 & 6) show that the initial whitefly population had a rather random distribution. For the first three sampling dates, no significant differences can be distinguished between the two greenhouses. In both greenhouses, more uninfested squares were observed at the first sampling (week 1-2). These gradually disappeared and by week 10 all squares were infested and the first squares with a mean of more than 10 adult whiteflies per plant appeared. In the plastic greenhouse, a considerable increase in adults could be observed in the eastern part of the greenhouse when comparing the maps of weeks 10 and 15. By week 20, many squares with a mean of more than 100 whiteflies appear in that greenhouse, while in the glasshouse only two squares with a mean of more than 100 whiteflies were found. Following the chemical control of week 20, the population declined and the whitefly density and distribution of week 25 was similar to that of weeks 1-2, but in the plastic greenhouse a slightly higher population was found in the northeast corner. In both greenhouses, the initial random distribution developed to a more aggregated distribution. Patches separated by whitefly free zones did not develop, although plants without whiteflies were frequently observed alongside
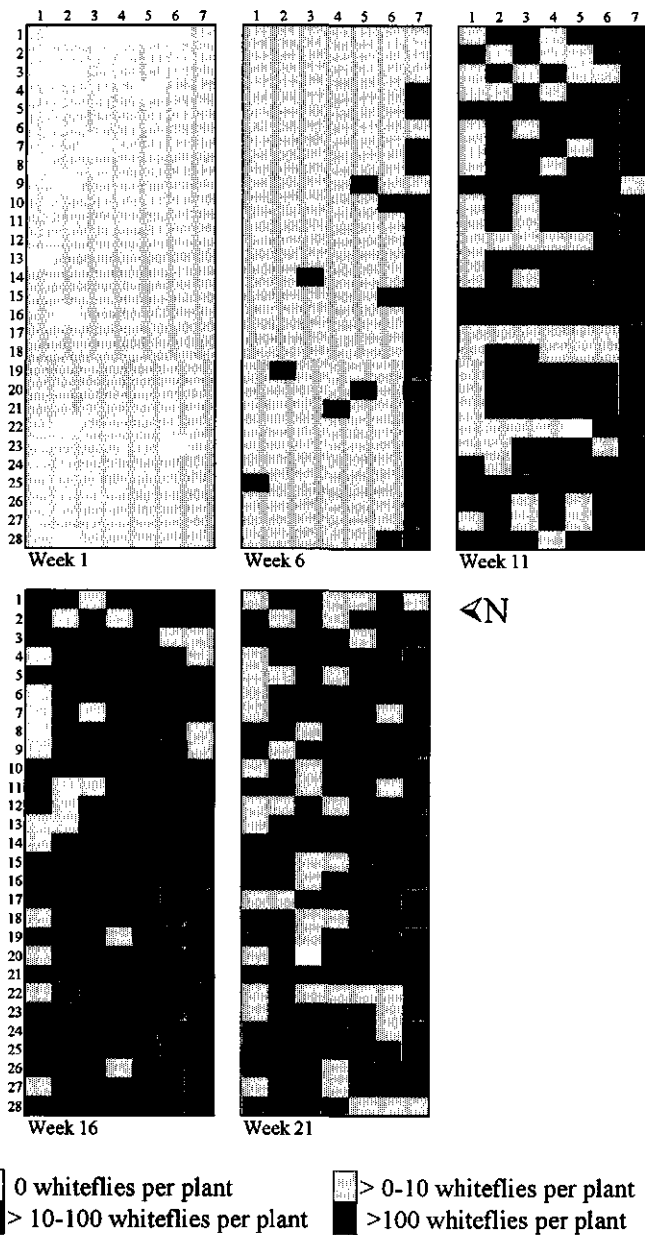
Figure 7. Spatial distribution of adult whiteflies in the glasshouse during the 1998 trial. Every square represents four plants and the grey scale indicates the mean number of whitefly adults on the 8 upper leaves of the four plants. The week number indicates the number of weeks after the first *E. formosa* introduction.
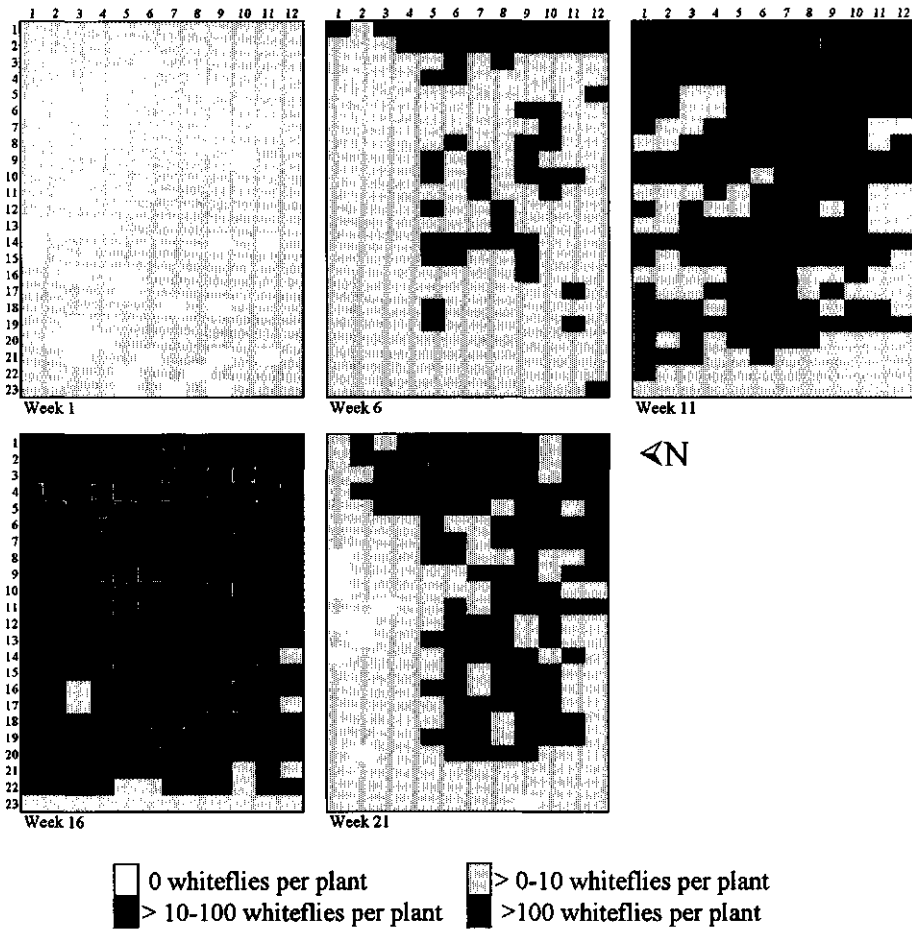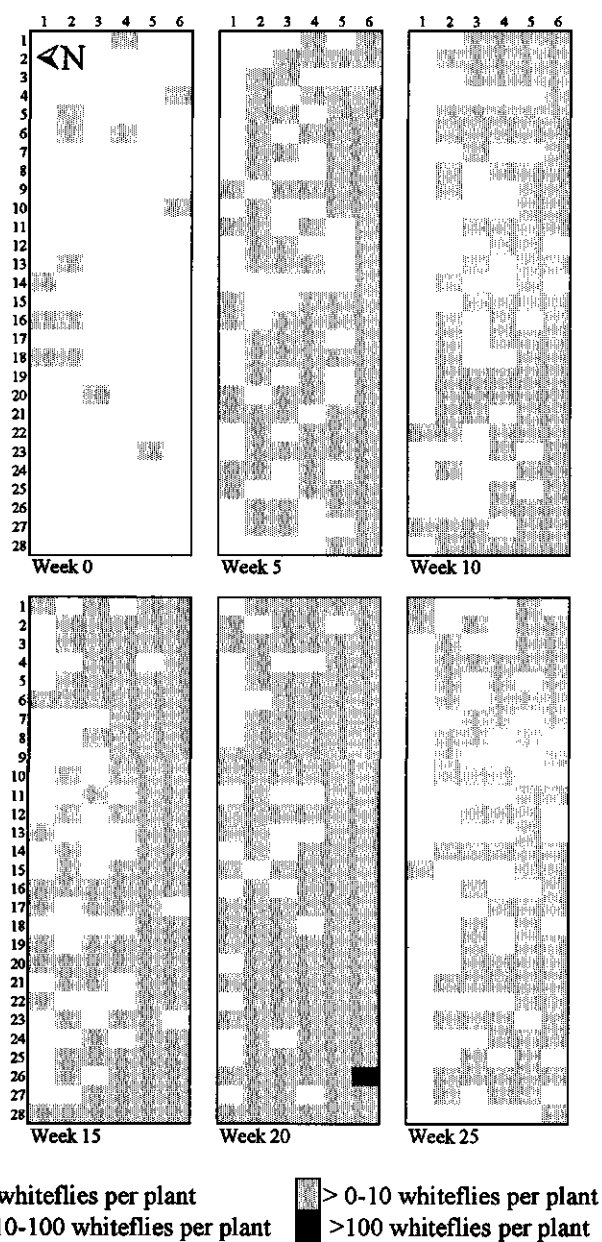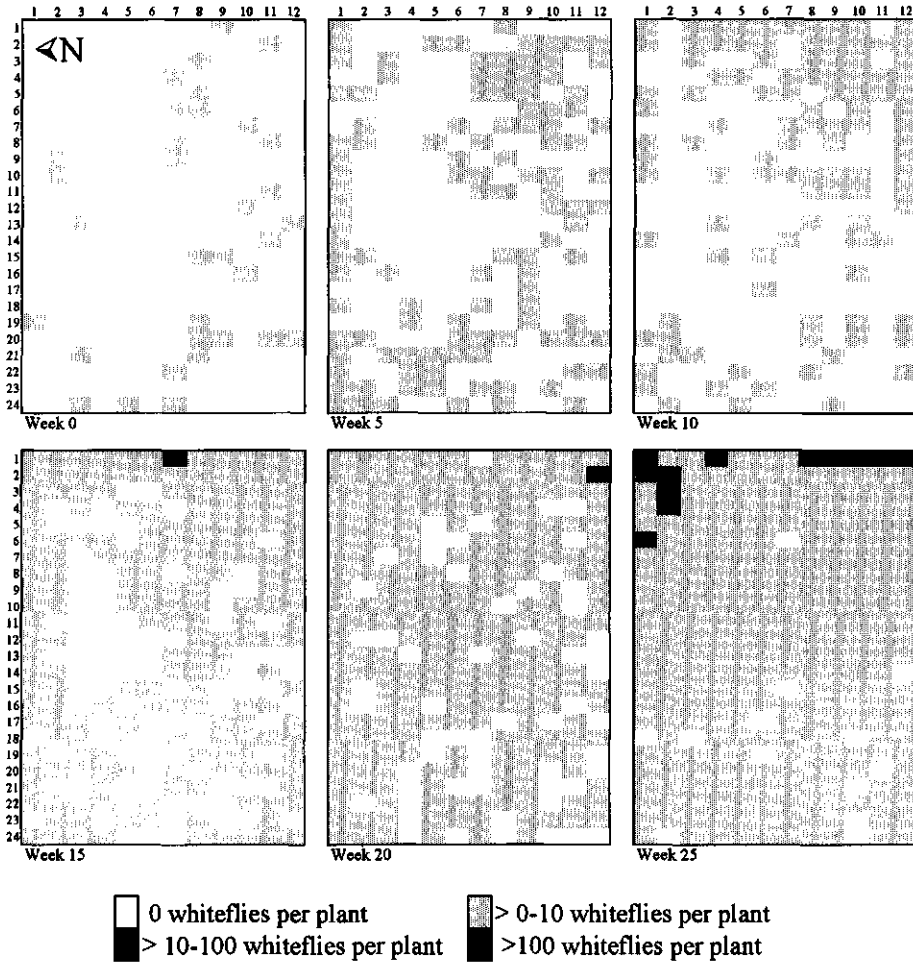
Figure 8. Spatial distribution of adult whiteflies in the plastic greenhouse during the 1998 trial. Every square represents four plants and the grey scale indicates the mean number of whitefly adults on the 8 upper leaves of the four plants. The week number indicates the number of weeks after the first *E. formosa* introduction.

Figure 9. Spatial distribution of adult whiteflies in the glasshouse during the 1999 trial. Every square represents four plants and the grey scale indicates the mean number of whitefly adults on the 8 upper leaves of the four plants. The week number indicates the number of weeks after the first *E. formosa* introduction.

Figure 10. Spatial distribution of adult whiteflies in the plastic greenhouse during the 1999 trial. Every square represents four plants and the grey scale indicates the mean number of whitefly adults on the 8 upper leaves of the four plants. The week number indicates the number of weeks after the first *E. formosa* introduction.

plants that were heavily infested. In 1998 (Figure 7 & 8) and 1999 (Figure 9 & 10), the distribution was similar to that of 1997 in both greenhouses, but with higher levels in 1998 and lower levels in 1999. The whitefly population was always higher along the southern edge of the glasshouse and the eastern edge of the plastic greenhouse.

## Discussion

No data on adult whitefly or parasitoid populations per plant were found in the literature, but the results for the percent parasitism of Hulspas-Jordaan et al. (1987) and those of Eggenkamp-Rotteveel Mansveld et al. (1982) are similar to those we found for the glasshouse in 1997. Those of the plastic greenhouse, however, were much lower. The mean daily temperature of the experiment of Hulspas-Jordaan et al. (1987) is not known. They used a temperature set point of 18 °C during the day and 7 °C at night. We calculated that this would lead, depending on the day length, to a mean day temperature of 11.6 °C at the beginning and 13.1 °C at the end of the experiment. This is much lower than the temperature in our plastic greenhouse. If they had successful biological control under those conditions, we would expect successful control in our plastic greenhouse. However, in our experiment the night temperature was in most cases between the threshold temperatures of *E. formosa* (11.4 °C) and *T. vaporariorum* (7.5 °C), while in the experiment of Hulspas-Jordaan et al. (1987), the night temperature was below the threshold for both insects. The experiment of Hulspas-Jordaan et al. (1987) can therefore be considered as an experiment that was done at 18 °C with intermittent zero development periods at 7 °C. Under these conditions, *E. formosa* has a clear advantage over *T. vaporariorum*. During the experiment of Eggenkamp-Rotteveel Mansveld et al. (1982), the mean temperature increased from 14.2-15.7 °C in week 1 to 18.6-22.1 °C in week 16, with maximum temperatures of more than 25 °C during all the weeks. So in this experiment the temperature conditions were also more favourable than in our experiments.

Hulspas-Jordaan et al. (1987) measured weekly the number of new empty white and new full black pupae that appeared in the crop. The sum of these two types of pupae may approach the sum of black and white pupae we measured, however, we counted only on one leaf per sampled plant. They measured a maximum of 2800 pupae on week 12 of their experiment. Based on the estimated temperature of 13.1 °C at that time and the leaf initiation rate equation of Jones et al. (1991), we calculated that leaf initiation rate would be 1.5 leaves per week for the experiment of Hulspas-Jordaan et al. (1987). The total number of new pupae per leaf can then be calculated as the number of new pupae in the whole greenhouse divided by the number of plants (175) and the number of new leaves that are formed per week (1.5), which gives us 10.7 pupae per leaf. We found a maximum of 8.4 pupae per leaf in the 1997 glasshouse experiment, similar to the maximum value found by Hulspas-Jordaan et al. (1987).

Based on the previous considerations, a better temperature management in the plastic greenhouse could possibly improve the biological control to the same level as in the glasshouse. In 1998, a higher temperature was attained by managing the greenhouse vents and, additionally, *E. formosa* was introduced as pupae in 1998 to avoid any negative effects of dealing with adults. The parasitoids seemed to work well at the beginning of the 1998 experiment as the percent parasitism measured during week 3 to 5 in the glasshouse was above 80 %. This caused a short slowing down of the *T. vaporariorum* adult population increase during weeks 6-8. In the plastic greenhouse, this slowing down was also observed,

although parasitism was only about 50 %. So, the slightly higher temperature of 1998, compared to 1997, did not increase the initial parasitism much in the plastic greenhouse. However, at the end of the production cycle it increased to 80 %, showing that a high percentage of parasitism was possible in the plastic greenhouse. *T. vaporariorum* population got out of control in both greenhouses, supposedly caused by the sprayings against the tomato russet mite. Although in most of the cases the products should be compatible (Table 1), many *E. formosa* adults were found dead after these applications. We suppose that this was more caused by the physical effect of applying water with high pressure to the plants than by the chemical products themselves. We therefore propose to make additional parasitoid introductions after full crop applications with pesticides, and particularly during the introduction phase.

Table 1. Pesticides used during the trials and their compatibility with natural enemies. The value indicates the toxicity of the respective product according the IOBC rating system, where 1 = harmless or less than 25 % mortality; 2 = slightly harmful, between 25 and 50 % mortality; 3 = moderately harmful between 50 and 75 % mortality and 4 = very harmful, more than 75 % mortality. The persistence of the product, in weeks, is given between brackets. When more than one figure is given, toxicity for the different natural enemies was different. A question mark indicates that no data are available.

| Active ingredient | Trade name | E. formosa | | Other | Predators |
|---|---|---|---|---|---|
| | | Adult | Pupa | parasitoids | |
| Amitraz | Mitac | 4 (2-4) | 4 | 1, 2 (0) | 1, 2, 3, 4 (0, 3) |
| Buprofezin | Oportune | 2 (0.5) | 1 | 1 (0) | 1, 2 (1) |
| Dichlofluanid | Euparen | 1 (1) | 4 | 1, 2 (0) | 1, 2, (0) |
| Fenarimol | Rubigan | 1 (0) | 1 | 1 (0) | 1 (0) |
| Fenbutatinhydroxide | Torque | 1 (0) | 1 | 1 (0) | 1 (0) |
| Hexythiazox | Nissorun | 1 (0) | 2 | 1 (0) | 1 (0) |
| Iprodione | Rovral | 1 (0) | 1 | 1 (0) | 1 (0) |
| Propargite | Omite | 3 (1) | 3 | 1,2,3,4 (0, ?) | 1,2,3,4 (0, ?) |
| Tetradifon | Tedion | 1 (0.5) | 2 | 1 (0) | 1,2 (0) |
| Thiocyclam hydrogen-oxalate | Evisect | 1 (0) | 4 | 1,2,3,4 (?) | 1,2,3,4 (0, 1, 2) |

Source: http//www.koppert.nl; Side effect database.

The introduction scheme of Hulspas-Jordaan et al. (1987) consisted of 4 introductions with a total of 20.6 *E. formosa* adults per $m^2$ in a period of 6 weeks (week 2 after transplant, 5.15 adults; week 4, 5.15 adults; week 6, 5.15 adults and week 8, 5.15 adults). Eggenkamp-Rotteveel Mansveld et al. (1982) introduced 27.5 *E. formosa* pupae per $m^2$ in the same 6-week period (week 4 after transplant, 2 pupae; week 6, 5.9 pupae; week 8, 12.6 pupae and week 10, 7 pupae). In our experiments, 3 *E. formosa* adults (1997) or pupae (1998) per $m^2$ were introduced weekly until reaching a total of 66 per $m^2$. During the first 6 weeks we

introduced 18 *E. formosa* per m². As we worked with a beef tomato variety, which is a better host plant for *T. vaporariorum*, we supposed that a higher number of *E. formosa* should be introduced in to obtain control (De Vis & van Lenteren, in preparation). For the 1999 production cycle we decided to introduce 5 pupae per m² per week, so as to reach a total of 30 after 6 weeks.

The initial whitefly population in the 1997 and 1998 production cycles was relatively high: between 0.42 and 0.57 adults per plant. Hulspas-Jordaan et al. (1987) started with 0.42 whitefly adults per plants, but Eggenkamp-Rotteveel et al. (1982) started with only 0.0067 adults per plant, and Woets (1978) found that the initial whitefly infestation on commercial farms reached only 0.001 to 0.01 adults per plant. Because of the lower initial introductions during the 1997 production cycle, the relatively high initial *T. vaporariorum* infestation and the high leaf area, a lot of immature stages could have escaped parasitism at the beginning of the experiment, resulting in a low parasitism and a high adult whitefly population increase. The disinfection of the greenhouses to control the tomato russet mite reduced the initial whitefly infestation of 0.42-0.57 adults per plant in 1997-1998 to only 0.023-0.034 adults per plant in 1999. This, together with the higher *E. formosa* introduction rate, resulted in a much higher ratio of parasitoids per whitefly in 1999 compared to 1997-1998.

In 1999, the biological control of *T. vaporariorum* was successful in both greenhouses. The low number of pesticide applications resulted in a less negative effect on *E. formosa*. The mean temperature in the plastic greenhouse in 1999 was only 0.4 °C higher than in 1997. This indicates that not the temperature but rather the ratio of parasitoids per whitefly defined by the initial whitefly population, and that the introduction rate limited biological control in the plastic greenhouse in 1997. Nevertheless, the small but continuous and significantly higher temperature in the glasshouse (Figure 1) created a stronger advantage of *E. formosa* over *T. vaporariorum* in the glasshouse than in the plastic greenhouse. With increasing temperature, the increase in fecundity, oviposition frequency and the decrease of immature development and pre-oviposition period (in the range of 15-25 °C) is higher for *E. formosa* than for *T. vaporariorum* (van Roermund & van Lenteren, 1992a & 1992b). The threshold temperatures for egg maturation and immature development of *E. formosa* and *T. vaporariorum* are 14.4 °C and 7.5 °C respectively (van Roermund & van Lenteren 1992a & 1992b). More hours below the *E. formosa* threshold were accumulated during the trial in the plastic greenhouse than in the glasshouse. Additionally, searching efficiency parameters such as walking speed and walking activity of *E. formosa* increase with increasing temperature (van Roermund & van Lenteren, 1995). This may lead to more encounters and parasitizations in the glasshouse. Therefore, temperature can explain partly the higher parasitism found during the three production cycles in the glasshouse when compared to the plastic greenhouse.

The experiments of Hulspas-Jordaan et al. (1987), covered a period of 18 weeks, corresponding to about 2 whitefly generations and those of Eggenkamp-Rotteveel Mansveld et al. (1982) covered 16 weeks, corresponding to about 2.9 generations, and these authors concluded that biological control was successful. All our trials were also successful during the first 16 production weeks, but afterwards *T. vaporariorum* population got out of control in the first two of them. In order to be able to conclude if biological control is efficient, greenhouse experiments should therefore cover the total length of the commercial crop cycles. Our experiments lasted between 26 and 32 weeks, covering 3.6 to 4.7 whitefly generations, which is similar to the current duration of commercial tomato crops in Colombia. Our additional observations of the natural control of aphids and leaf miners (i.e. control without costs !)

make of biological control a very attractive alternative. We can recommend the use of biological control of *T. vaporariorum* by *E. formosa* in unheated greenhouse conditions on the Bogota Plateau, and based on our experiments and data from the literature, we propose the following approach for its successful implementation:

- Start a new crop with a clean greenhouse and clean plants so as to avoid population build-up at the beginning of the crop.
- Introduce *E. formosa* as pupae to avoid negative effects of dealing with adults.
- On high quality post plants, introduce 5 pupae *E. formosa* per week and per m$^2$ and during 13 weeks from the first observation of *T. vaporariorum* adults.
- On low quality host plants, this high dosage can possibly be reduced to 4-5 introductions of 5 pupae per m$^2$ at fortnightly intervals, which is commercially used in Europe (Eggenkamp-Rotteveel Mansveld et al., 1982).
- Manage the vents to increase greenhouse temperature.
- Avoid spraying, even with compatible products. If applications have to be made, only apply spot treatments, and if full crop applications are necessary during the introduction phase, increase the introductions to replace the killed *E. formosa* adults.

## Acknowledgements

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## 7. *Amitus fuscipennis*, an alternative to the biological control of *Trialeurodes vaporariorum* by *Encarsia formosa*?

### Abstract

Biological control of *Trialeurodes vaporariorum* by *Amitus fuscipennis* with or without *Encarsia formosa* was tested in a glasshouse and a plastic greenhouse on the Bogota Plateau in Colombia. During two production cycles, population development of *T. vaporariorum* and parasitism by *E. formosa* and/or *A. fuscipennis* were followed. The mean temperature in the plastic greenhouse was around 16 °C and around 17 °C in the glasshouse. During 13 weeks, a total of 66 parasitoid pupae were introduced in the greenhouses at a rate of 5 *A. fuscipennis* pupae per m$^2$ and per week in the 1999 production cycle, and 2.5 pupae of both *E. formosa* and *A. fuscipennis* per m$^2$ and per week in 2000. In the 1999 production cycle, control was obtained for 5 months in the plastic greenhouse and 3 months in the glasshouse, after which the *T. vaporariorum* adult population increased to a maximum of nearly 50 adults per plant, which required chemical control. Parasitism that was initially higher than 80 %, then decreased to 56 % in the plastic greenhouse and to 20 % in the glasshouse. In the 2000 production cycle, biological control was successful in both greenhouses. *T. vaporariorum* populations were generally lower than 1.2 adults per plant and parasitism, caused mainly by *E. formosa*, was 90 % or higher most of the time. Therefore, *E. formosa* is recommended to keep *T. vaporariorum* populations at low levels in unheated greenhouses, situated in the high altitude tropics such as the Bogota plateau. When high *T. vaporariorum* populations are to be expected or control of high-density spots is required, *A. fuscipennis* can be an appropriate and beneficial addition to the control by *E. formosa*.

### Introduction

Protected cultivation of tomatoes is a recent development in Colombia, generally replacing field-grown tomatoes. The high risk due to pests and diseases, causing in some cases total loss of the crop in the field, is one of the reasons that induced the change to greenhouse production. The use of greenhouses reduces pest and disease risks, and increases production and profits. Greenhouses are currently used for tomato production in the intermediate climate zone (altitude 1800-2000 m) where traditionally field-grown tomatoes are cultivated, but also in cold climate zones such as the Bogota Plateau (altitude 2665 m). One of the advantages of the production of greenhouse tomatoes on the Bogota Plateau, is the reduced pest spectrum. From 1995 on, greenhouse tomatoes have been produced at the Horticultural Research Centre of the University of Bogota Jorge Tadeo Lozano, where the most important pest has been the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood). Leafminers and aphids were generally kept at low densities by naturally occurring parasitoids (De Vis & van Lenteren, 1999).
Biological control of pests on greenhouse grown tomatoes has become a common practice around the world. For biological control of the greenhouse whitefly, the parasitoid *Encarsia formosa* Gahan is traditionally used (van Lenteren, 1992, 1995, 2000). The greenhouse climate on the Bogota Plateau, however, is rather cool with mean temperatures of 15-16 °C, day temperatures of 18-22 °C and night temperatures of 5-12 °C. This is lower than the mean temperature in the climatised greenhouses in Western Europe. At 15 °C, the intrinsic rate of increase ($r_m$) of *E. formosa* is estimated to be slightly higher than that of *T. vaporariorum* (van Lenteren et al., 1996), suggesting that biological control should be possible under these conditions. But, van Roermund & van Lenteren (1995) showed that the *E. formosa* strain used in the Netherlands at that time did not fly at 18° C or lower temperatures, which seriously lowers the parasitoid's dispersal and prevent whitefly patches from being found and

119

parasitized. Nevertheless, recent greenhouse trials showed that biological control of *T. vaporariorum* by *E. formosa* was possible in the specific greenhouse conditions on the Bogota plateau (De Vis & van Lenteren, in preparation; Chapter 6).

A possible alternative to the biological control by *E. formosa* is the native parasitoid *Amitus fuscipennis* MacGown & Nebeker. It can be found abundantly on field-grown tomato crops, parasitizing naturally up to 80 % of the *T. vaporariorum* pupae (De Vis & van Lenteren, 1999). Its life history as a parasitoid of *T. vaporariorum* was determined on bean (Manzano et al., 2000) and on tomato (De Vis et al., 2001; Chapter 3). This revealed that the intrinsic rate of increase of the parasitoid is substantially higher than that of *T. vaporariorum* between 15 and 30 °C, and slightly higher than that of *E. formosa* at temperatures lower than 30 °C. De Vis et al. (in preparation-2; Chapter 4) found that *A. fuscipennis* had a higher searching efficiency resulting in a higher oviposition rate on tomato leaflets infested with *T. vaporariorum* larvae than that found by van Roermund & van Lenteren (1995) for *E. formosa*. These findings coincide with those of Manzano (2000) on bean. However, *A. fuscipennis* had a longer residence time on clean leaflets and a shorter longevity than *E. formosa* and this might reduce its efficacy as parasitoid in crops with a low host density.

Interaction experiments between *E. formosa* and *A. fuscipennis* showed that both parasitoid species treated *T. vaporariorum* larvae parasitized by the other species in the same way as unparasitized larvae. Therefore, multiparasitism was observed frequently and in 70-80 % of the cases, multiparasitized whitefly larvae resulted in the emergence of a parasitoid of the species that parasitized first. Thus, neither species was observed to be superior to the other when internal competition for host possession occurred (De Vis et al., in preparation-1; Chapter 5).

The two parasitoid species have a similar intrinsic rate of increase but different, possibly complementary, life history traits. The pro-ovigenic *A. fuscipennis* has a high egg load and high oviposition frequency (De Vis et al., 2001, Chapter 3) and could therefore prove to be good at reducing whiteflies in high-density spots. The synovigenic *E. formosa* can prolong its longevity through oosorbtion (Van Keymeulen & Degheele, 1978; van Lenteren et al., 1987) resulting in a longer adult life. When hosts are continuously present, it can oviposit a a daily amount of about 10-16 eggs during a period of 30 days or more. The life history strategy of E. formosa could thus be better to keep *T. vaporariorum* at low density during long periods. As a result of these different life history strategies, the joint use of both parasitoids in greenhouses might be more efficient than the use of just one of them.

In the experiments described in this paper, we evaluated the use of *A. fuscipennis* with and without *E. formosa* as biological control agent for the greenhouse whitefly on tomato under the specific greenhouse conditions of the Bogota Plateau.

## Material and methods

Two tomato production cycles were conducted at the Horticultural Research Centre (CIAA) of the Jorge Tadeo Lozano University, 20 km north of Bogota in two types of unheated greenhouses at 2665 metres altitude. The first was a plastic greenhouse of the standard Colombian design (fixed open ridge and manual wall curtains) equipped with a thermal screen. The second was a "Dutch-Venlo" type glasshouse with automated roof ventilation, a thermal screen and was controlled by a climate computer (Midi-Clima, Van Vliet, The Netherlands). This computer also recorded temperature in both greenhouses using NTC

sensors installed in a ventilated box. Relative humidity was calculated using the temperature data of a dry and a wet sensor installed in the same box. The boxes were installed in the centre of each greenhouse at plant height and every ten minutes the mean value of the previous 10 minutes was stored. Tomato plants of the variety. Boris (Bruinsma Seeds, 's Gravenzande, The Netherlands) were transplanted in both greenhouses during two consecutive production cycles: 1) 7-week old plants transplanted on September 6, 1999 and crop finished on March 12, 2000; and 2) 6-week old plants transplanted on March 30, 2000 and crop finished on October 15, 2000. The experiments began during the week of transplant in 1999 one week after transplant in 2000. At that time, a natural infestation of *T. vaporariorum* adults was present in all experimental greenhouses. These adults may have survived from previous crops. No important immigration of *T. vaporariorum* adults could have taken place during the crops as the outside climate conditions are too cool for *T. vaporariorum* population build-up. In the adjacent greenhouses, either *T. vaporariorum* was controlled chemically to low densities or the crops in those greenhouses were not suitable for *T. vaporariorum* development

A. *fuscipennis* was introduced during 13 weeks and at a rate of 5 pupae per m$^2$ per week during the 1999 trial and both *A. fuscipennis* and *E. formosa* at a rate of 2.5 pupae per m$^2$ per week in 2000. A total of 65 parasitoids per m$^2$ were introduced in both trials. Pieces of leaflets with about 70 parasitized *T. vaporariorum* pupae were equally distributed throughout the greenhouses. They were fastened to the base of the lower leaves of the tomato plants. Mean emergence of the introduced pupae was 95 % or higher. Parasitoids originated from the CIAA´s rearing unit where they were reared on tomato plants.

Whitefly adults were counted weekly on the 8 upper leaves of a stratified sample of 10% of the plants: within a row, of every ten consecutive plants, one was selected at random. Eggenkamp-Rotteveel Mansveld et al. (1978) found that in a large greenhouse a stratified random sampling alone was not suitable to reliably estimate the total whitefly population. Therefore, we assessed the spatial distribution by making a census of whiteflies every five weeks.

Parasitism was assessed by weekly sampling of the parasitized and non-parasitized pupae on a stratified sample of 10 % of the plants, one leaf per plant. Only leaves where some black pupae had already hatched were selected. On those leaves most of the non-parasitized pupae had already hatched. The number of parasitized pupae that were not yet black and thus counted as non-parasitized pupae was in this way reduced to a minimum.

During the 1999 trial, plants with more than 100 whitefly adults were sprayed with buprofezin and thiociclam in the glasshouse in week 18 of the experiment. Powdery mildew and the disease caused by *Botrytis* were controlled with fenarimol and iprodione. Leafminers and aphids were very satisfactorily kept at low densities by naturally occurring parasitoids during the three production cycles. The tomato russet mite, *Aculops lycopersici*, was controlled mainly by spot treatments with fenbutatinoxide and occasionally a full crop treatment was necessary. All pesticides were supposed to be compatible with the application of *E. formosa* (Table 1).

**Results**

The mean temperature and relative humidity (days as replicates) was significantly different among production cycles and greenhouses, except for the relative humidity, which was similar in the glasshouse and plastic greenhouse in the 1999 trial (Figure 1A & 1B). The

temperature was higher in the glasshouse than in the plastic greenhouse. The relative humidity was higher in the plastic greenhouse than in the glasshouse in the second experiment but not in the first. The temperature was lower and relative humidity higher during the 1999 than in the 2000 trial. The mean daily temperature curve of both greenhouses showed a small but consistent difference. Mean hourly night temperatures were between 10 and 15 °C and day temperatures between 15 and 25 °C for both greenhouses (Figure 1C, showing the data of 1999).
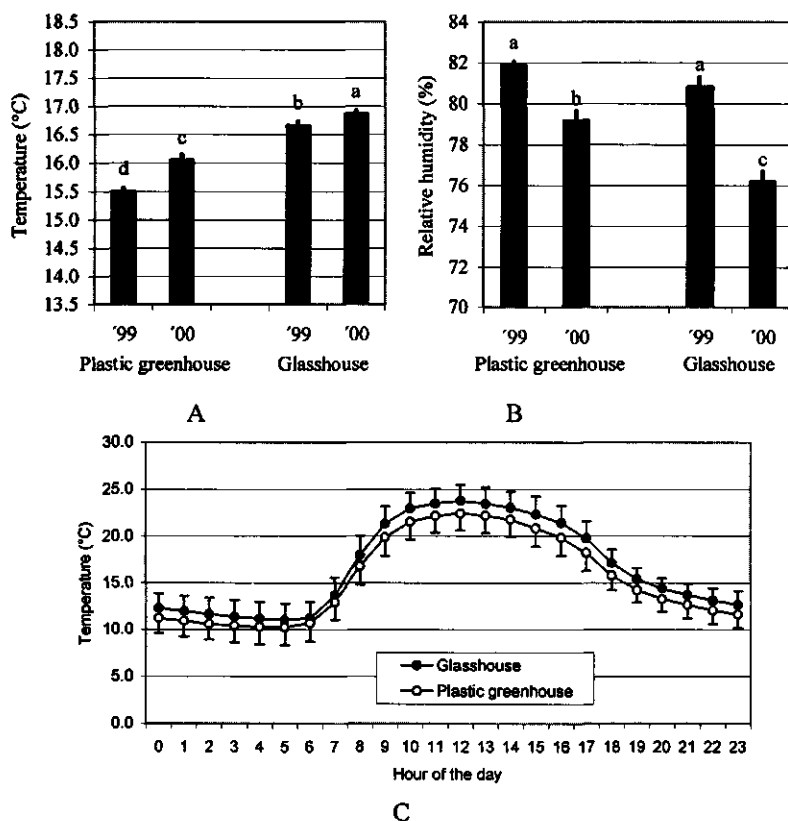


Figure 1. Mean temperature (Graph A) and relative humidity (Graph B) of the greenhouses during the three production cycles. Means with the same letter are not significantly different ($\alpha$=0.05). Mean temperature curve (Graph C) of both greenhouses during the 1999 production cycle.

At the beginning of the experiment, the whitefly population was lower in the 1999 than in the 2000 experiment. In 1999, 0.0016 and 0.0063 whitefly adults per plant were found in the glasshouse and the plastic greenhouse respectively, compared to 0.075 and 0.16 adults per plant in 2000.

The *T. vaporariorum* adult population increased steadily in both greenhouses in 1999, but with a higher rate in the glasshouse. In the glasshouse, it reached 10 adults per plant by week 15, while this level was only reached by week 21 in the plastic greenhouse. After week 15 the difference between the two greenhouses increased, the population in the glasshouse being 2 to 4 times higher than in the plastic greenhouse. But by week 23, the population in the plastic greenhouse increased exponentially, reaching that of the glasshouse by week 24. The maximum *T. vaporariorum* adult population was nearly 50 adults per plant in both greenhouses (Figure 2). At the beginning, parasitism was more than 80 % in both greenhouse, but by week 13 it fell below this level in the glasshouse and by week 17 in the plastic greenhouse. It reached a minimum of 20 % in the glasshouse by week 18 and a minimum of
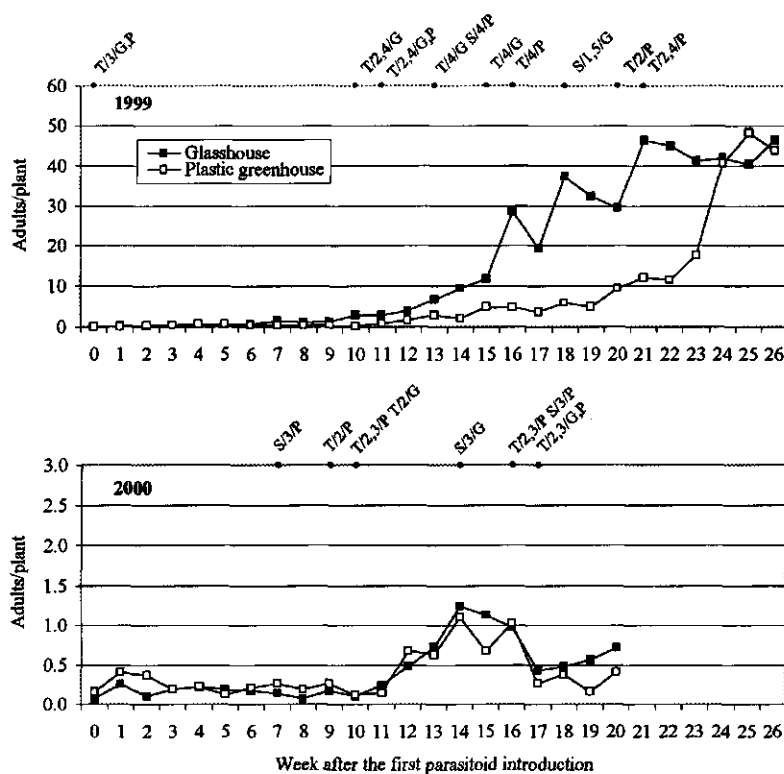


Figure 2. Evolution of the number of adult whiteflies per plant in the glasshouse and in the plastic greenhouse during the two production cycles. The chemical treatments are specified above the graphs: S=spot treatment; T=total crop treatment; G=glasshouse; P=plastic greenhouse; 1=buprofezin; 2=fenarimol; 3=fenbutatinoxide; 4=iprodione; 5=thiocyclam hydrogen oxalate.

48 % by week 24 in the plastic greenhouse. After reaching this minimum it started to increase again to 70 % by week 24 in the glasshouse (Figure 3). Although *E. formosa* was not introduced in this trial, parasitism by this parasitoid was observed from the beginning of the experiment. Most of the time it caused between 10 and 20 % of the total parasitism in the glasshouse, but from week 22 this increased to more than 50 % by the end of the experiment. In the plastic greenhouse, parasitism caused by *E. formosa* was less than 10 % up to week 17 of the experiment, and between 15 and 20 % for the rest of the experiment (Figure 4). The number of pupae per leaf increased slower in the plastic greenhouse than in the glasshouse. In the glasshouse, the level of 20 pupae per leaf (sum of parasitized and unparasitized pupae) was reached by week 20, but only by week 23 in the plastic greenhouse. A maximum of more than 120 pupae per leaf was found by week 24 in the glasshouse and more than 60 per leaf in the plastic greenhouse by week 25 (Figure 5).
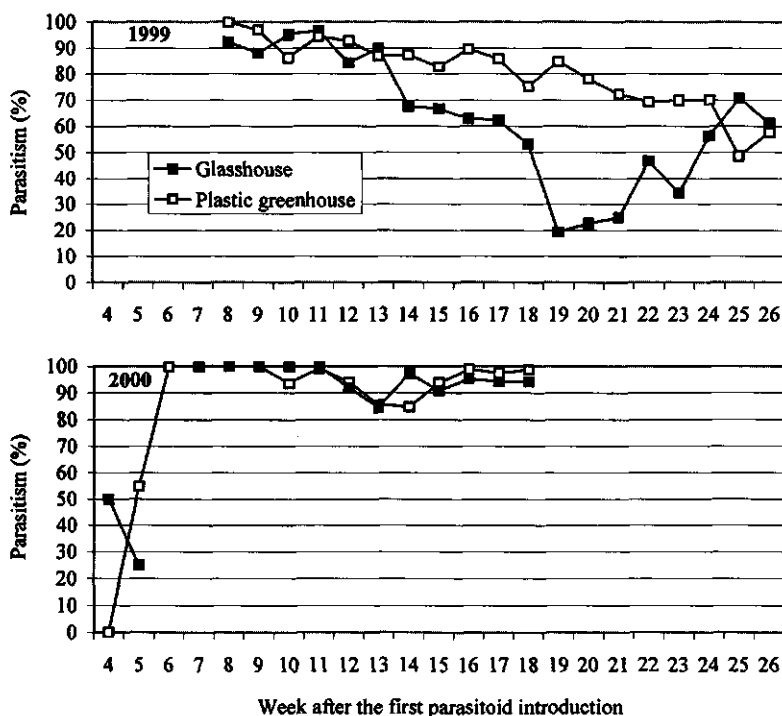


Figure 3. Evolution of the percent parasitism in the plastic greenhouse and in the glasshouse during the two production cycles.

In 2000, the *T. vaporariorum* population was under control in both greenhouses during the whole experiment. Most of the time it was below 0.5 adults per plant with a small increase in the population, up to 1.2 adults per plant during weeks 12 to 16 of the experiment (Figure 2). Parasitism was generally above 90 %, with a small decrease during weeks 12-15, when it was still higher than 80 % (Figure 3). Parasitism was caused mainly by *E. formosa*. In the glasshouse, this parasitoid caused more than 80 % of the total parasitism and from week 13 on, even 90 % or more. In the plastic greenhouse, parasitism by *E. formosa* was lower at the beginning (51 and 67 % in week 6 and 7 respectively), but by week 12 it was also above 90 % and this level was maintained most of the time (Figure 4). Less than 3 pupae per leaf (sum of parasitized and unparasitized pupae) were found in both greenhouses during the whole trial (Figure 5).

To visualise the spatial distribution, greenhouses were divided in plots of 4 (2x2) plants and the mean number of whiteflies per plant of the plot was calculated based on the results of the whitely census done every five weeks. According to four density classes, two-dimensional maps were constructed. The distribution maps of the 1999 experiment (Figure 6 and 7) show the very low initial infestation. Only one and seven infested squares were found in the glasshouse and the plastic greenhouse respectively. However, five weeks later, more than 50 % of the squares were infested in both greenhouses. By week ten, the number of infested
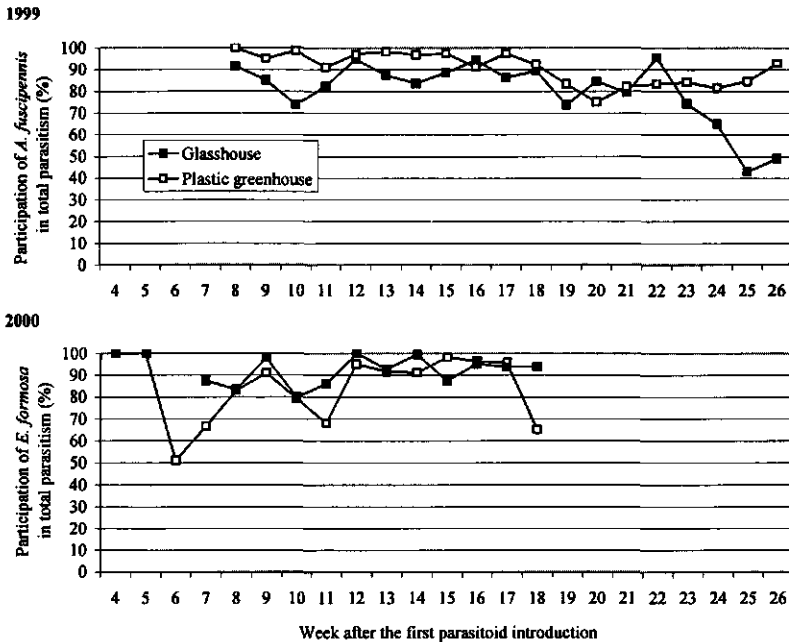


Figure 4. Participation of *A. fuscipennis* (1999) and *E. formosa* (2000) in total parasitism in the plastic greenhouse and in the glasshouse.

squares had decreased in the plastic greenhouse to 48 % while in the plastic greenhouse only 3 uninfested squares were found and the first square with more than 10 *T. vaporariorum* adults per plant was found. From week 15 on, all squares were infested in both greenhouses and by week 25, several squares with more than 100 adults appeared. In the plastic greenhouse, the population was higher against the northern wall in weeks 5 and 15 and the eastern wall in week 20. In the glasshouse, the southern part had the highest *T. vaporariorum* population. In 2000, the number of infested squares at the beginning was higher than in 1999 (Figure 8 and 9). The number of infested squares in week 5 and 10 was lower than or similar to that in week 0 in both greenhouses. More infested squares were found in week 15 and 20 but the infestation level was lower than 10 adults per plant, except for one square in the glasshouse in week 15, and one in the plastic greenhouse in week 20. Patches separated by whitefly free zones did not develop, although plants without whiteflies were frequently observed alongside plants that were heavily infested.
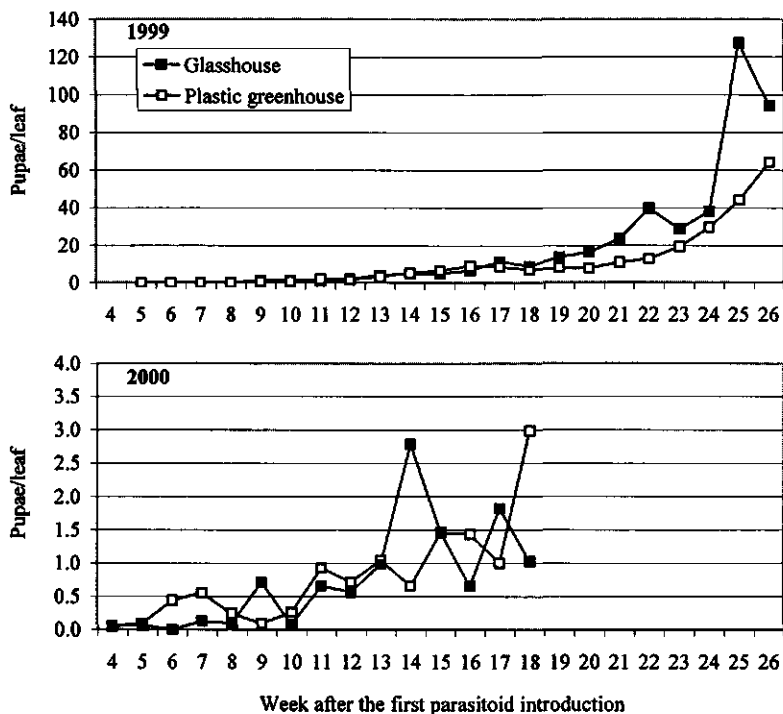


Figure 5. Evolution of total number of parasitized and unparasitized pupae per leaf in the plastic greenhouse and in the glasshouse during the two production cycles.
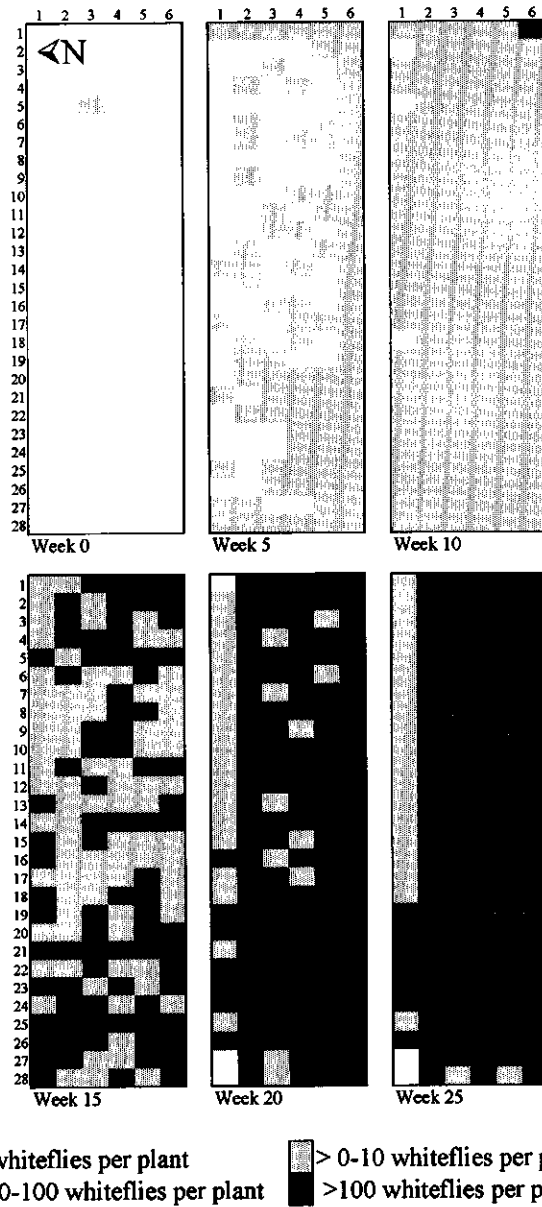
Figure 6. Spatial distribution of adult whiteflies in the 1999 glasshouse trial. Each square represents four plants and the grey scale indicates the mean number of whitefly adults on the 8 upper leaves of the four plants. The week number indicates the number of weeks after the first parasitoid introduction.
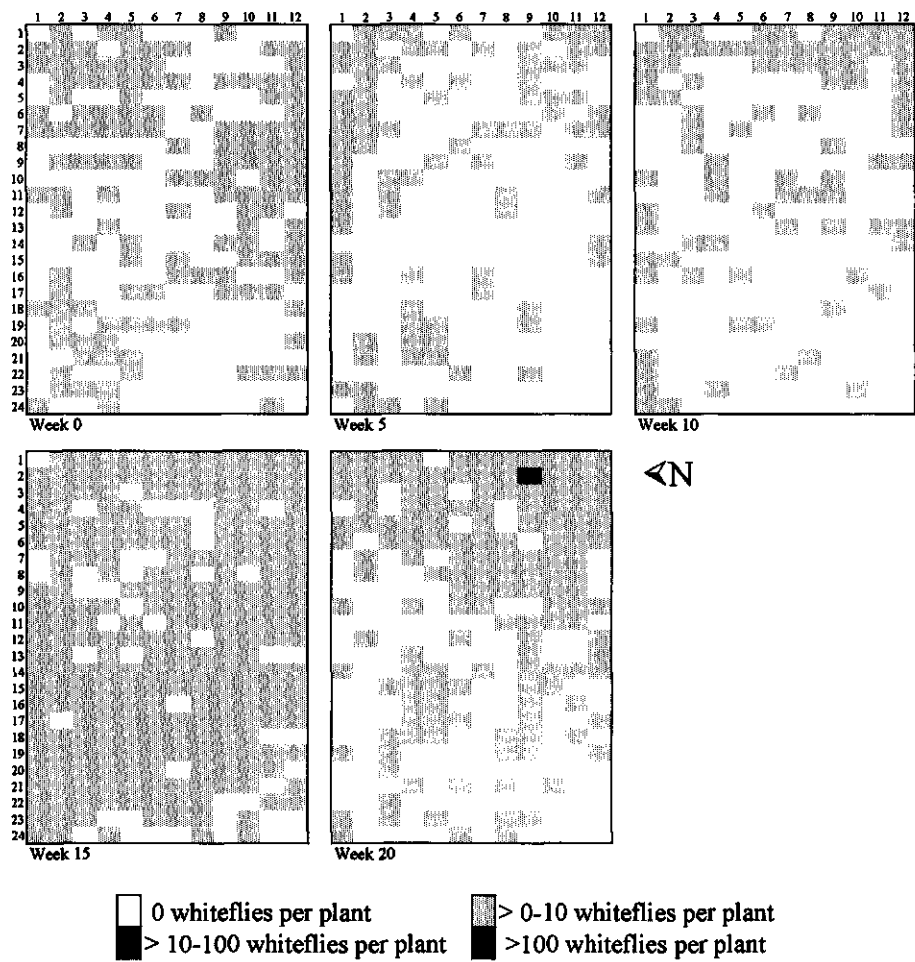
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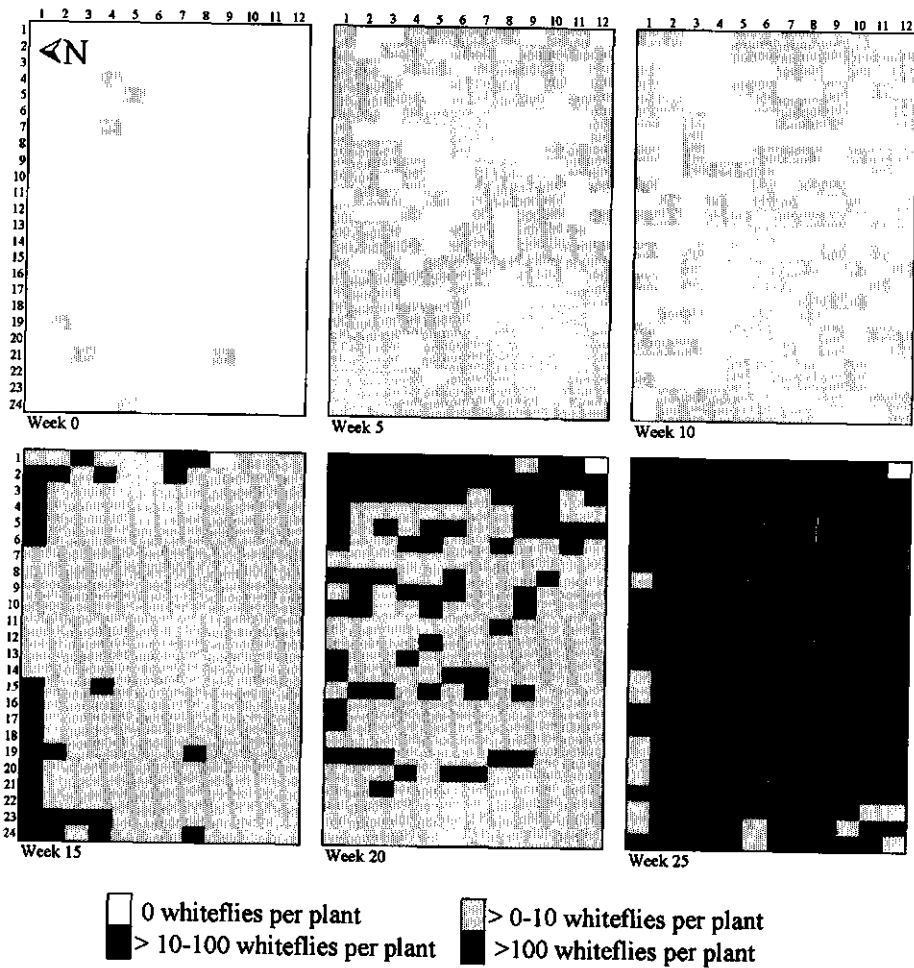
Figure 7. Spatial distribution of adult whiteflies in the 1999 plastic greenhouse trial. Each square represents four plants and the grey scale indicates the mean number of whitefly adults on the 8 upper leaves of the four plants. The week number indicates the number of weeks after the first parasitoid introduction.
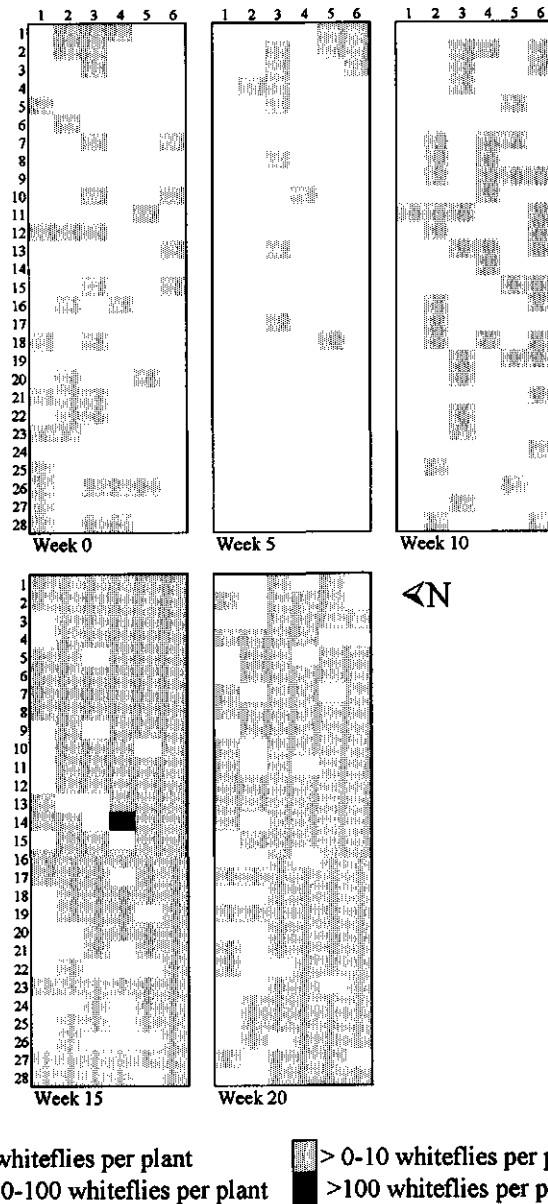
Figure 8. Spatial distribution of adult whiteflies in the glasshouse during the 2000 trial. Every square represents four plants and the grey scale indicates the mean number of whitefly adults on the 8 upper leaves of the four plants. The week number indicates the number of weeks after the first parasitoid introduction.

Figure 9. Spatial distribution of adult whiteflies in the plastic greenhouse during the 2000 trial. Every square represents four plants and the grey scale indicates the mean number of whitefly adults on the 8 upper leaves of the four plants. The week number indicates the number of weeks after the first parasitoid introduction.

**Discussion**

In 1999, the *T. vaporariorum* adult population could not be kept under biological control in either greenhouse. In the plastic greenhouse, it remained below economic injury levels until week 19. The total immature development time of *A. fuscipennis*, calculated with the equation presented in De Vis et al. (2001, Chapter 3) and the hourly temperature data of the 1999 plastic greenhouse, was 48.1 days. Therefore, parasitoids that emerged in week 19 were the result of whiteflies that had been parasitized in week 12. As the *T. vaporariorum* population started to increase by week 19, parasitization in week 12 was apparently not sufficient. To estimate the number of emerging parasitoids per plant we multiplied the number of parasitized pupae per leaf by the estimated leaf initiation rate of 1.9 leaves per week (Jones et al., 1991), resulting in 2.8, 5.5, 8.8, 10.4 and 15.4 emerging parasitoids per plant for weeks 12 to 16. During those weeks, the measured *T. vaporariorum* population had increased from 1.6 to 5.0 adults per plant. The same calculations can be made for the glasshouse, where the *T. vaporariorum* population was under control for the first three months. Here, the total immature development time of *A. fuscipennis* was calculated to be 42.9 days. Therefore, pupae emerging in week 14 were parasitized in week 8. Calculated parasitoid emergence increased from 2.3 to 6.5 adults per week and per plant from week 9 to week 13. Together with the emergence of the introduced parasitoid pupae, the total emergence was estimated to increase from 4.3 to 8.5 parasitoids per week and per plant. Between weeks 8 to 13 the *T. vaporariorum* population was between 1.1 and 6.6 adults per plant. Thus, in the two situations the estimated adult wasp population was larger than the *T. vaporariorum* adult population. Additionally, longevity of *A. fuscipennis* at 15 °C was 18.1 days (De Vis et al., 2001; Chapter 3), so wasps of at least two weeks could have accumulated in the crop. Despite these high estimated numbers of wasps, *T. vaporariorum* got out of control. There may be several explanations for this result:

1. During the weeks previous to the *T. vaporariorum* increase, chemical treatments were applied. This could have hampered emergence or killed adult *A. fuscipennis* resulting in a higher *T. vaporariorum* population in the next generation. During weeks 10 to 16 a total of four full crop sprayings were done in the glasshouse compared to only two in the plastic greenhouse. This can also explain why the *T. vaporariorum* population started to increase earlier in the glasshouse than in the plastic greenhouse. In previous trials, De Vis & van Lenteren (in preparation; Chapter 6) found that sprayings could kill a lot of *E. formosa* adults. It is not clear, however, if sprayings or the chemical products we used affect *A. fuscipennis* more than *E. formosa* (Table 1). At the moment, no data are available for the side effects of pesticides on *A. fuscipennis* adults or immatures.

2. The wasps may not have found the larvae in the extensive crop that had more than 20 leaf layers with up to 20 leaflets per leaf. *A. fuscipennis* females have an egg load of at least 430 eggs at emergence (De Vis et al., 2001; Chapter 3) and in the above section we estimated that the wasp population was larger than the *T. vaporariorum* population. Because fecundity of *T. vaporariorum* is lower than that of *A. fuscipennis*, the parasitoids were surely not egg limited. Rather, they were time limited in this crop with many leaves and leaflets. Still, with this high parasitoid-host ratio, we would expect a higher level of parasitism. Therefore, we suppose that the sprayings killed a lot of *A. fuscipennis* adults.

3. During the last experiment many *A. fuscipennis* adults were found stuck to the glandular trichomes on the stems of the tomato plants, suffering in this way premature death.

However, we did not quantify this mortality and thus cannot estimate the impact of this mortality on the result of biological control. In earlier work, we observed that *A. fuscipennis* females leave leaflets in most cases by walking via the petiole instead of flying (De Vis et al., in preparation-1&2; Chapter 4&5) and Manzano (2000) also observed that parasitoids move between leaflets by walking via petioles and stems. It seems that the glandular trichomes, which are present abundantly on tomato stems, are a mortal trap for these walking wasps. On tomato, this was also found for the predatory mite *Phytoseiulus persimilis* (Nihoul, 1994).

Table 1. Pesticides used during the trials and their compatibility with natural enemies. The value indicates the toxicity of the respective product according to the IOBC rating system where 1 = harmless or less than 25 % mortality; 2 = slightly harmful, between 25 and 50 % mortality; 3 = moderately harmful, between 50 and 75 % mortality and 4 = very harmful, more than 75 % mortality. The persistence of the product, in weeks, is given between brackets. When more than one figure is given, toxicity for the different natural enemies was different. A question mark indicates that no data are available.

| Active ingredient | Trade name | E. formosa | | Other parasitoids | Predators |
|---|---|---|---|---|---|
| | | Adult | Pupa | | |
| Buprofezin | Oportune | 2 (0.5) | 1 | 1 (0) | 1, 2 (1) |
| Fenarimol | Rubigan | 1 (0) | 1 | 1 (0) | 1 (0) |
| Fenbutatinhydroxide | Torque | 1 (0) | 1 | 1 (0) | 1 (0) |
| Iprodione | Rovral | 1 (0) | 1 | 1 (0) | 1 (0) |
| Thiocyclam hydrogen - oxalate | Evisect | 1 (0) | 4 | 1,2,3,4 (?) | 1,2,3,4 (0, 1, 2) |

Source: http//www.koppert.nl, Side effect database.

Because of the potential negative effect of the sprayings, it is not clear whether the pro-ovigenic *A. fuscipennis* is capable of maintaining a *T. vaporariorum* population at low levels during the whole production cycle. Previous experiments where only *E. formosa* was used as biological control agent (De Vis & van Lenteren, in preparation; Chapter 6) showed that this parasitoid was able to maintain *T. vaporariorum* at low levels during a complete growing season of about 6 months and under the same growing conditions. However, when the number of full crop sprayings was high, *T. vaporariorum* population got also out of control with this parasitoid.

*E. formosa* caused more than 80 % of the *T. vaporariorum* parasitism in the 2000 trials. This predominance demonstrates that *E. formosa* is more efficient in controlling *T. vaporariorum* at low densities than *A. fuscipennis*. The searching efficiency of *A. fuscipennis* on infested leaflets is higher than that of *E. formosa* (De Vis et al., in preparation-1&2, Chapter 4&5), but in crops with a low host density, parasitoids are searching most of the time on clean leaflets (van Roermund & van Lenteren, 1997). *E. formosa* stays only about half an hour on clean leaflets (van Roermund & van Lenteren, 1995) compared to about one hour for *A. fuscipennis* (De Vis et al., in preparation- 1, Chapter 4). So, at low host densities, *E.*

*formosa* spends less time searching on the many empty leaves than *A. fuscipennis*. Furthermore, the longevity of *E. formosa* in the presence of hosts and at 15 °C was 31 days (Burnett, 1949) increasing to 99.3 days in the absence of hosts but with a sugar diet (Vet & van Lenteren, 1981). For *A. fuscipennis* longevity was about 18 days under both conditions (De Vis et al., 2001; Chapter 3). Thus, because of the short residence time and the higher longevity, *E. formosa* will visit a considerable higher number of leaflets during its total life span than *A. fuscipennis*. This explains the higher parasitization efficiency of *E. formosa* when compared to *A. fuscipennis* in crops with low host density.

Examples of classical biological control programs where pro-ovigenic *Amitus* species were combined with synovigenic species seem to confirm our findings. *Aleurocanthus woglumi*, the citrus blackfly was successfully controlled in Mexico and the USA by introducing 4 species of parasitoids: at the start of the introductions and when host density was very high, the pro-ovigenic *Amitus hesperidum* was the dominant parasitoid, and aphelinids like *Encarsia opulenta* were hardly found. When host densities became low as a result of *A. hesperidum* parasitism, *E. opulenta* became the dominant parasitoid (Flanders, 1969; Thompson et al., 1987; Dowell et al., 1981; Nguyen & Hamon, 1994). The woolly whitefly, *Aleurothrixus floccosus* was controlled in California by the introduction of an array of different parasitoid species and biotypes, including *Amitus spiniferus* and *Cales noacki*. Also here, initially *A. spiniferus* reduced the high infestation levels within one year after the introduction and then *C. noacki* became the dominant parasitoid, which kept the population at low levels (DeBach & Rose, 1976). In these examples, the *Amitus* species disappeared almost completely after some years (Tsai and Steinberg, 1991; Thompson et al., 1987; DeBach & Rose, 1976).

Examples where two synovigenic parasitoids are used show that this can lead to better control than the use of just one. *E. formosa* and *Eretmocerus eremicus* are currently introduced together to control *T. vaporariorum* and *Bemisia* sp. in greenhouse tomatoes and ornamental crops. In this system, *E. formosa* is supposed to be more efficient at lower temperatures and lower host densities while *E. eremicus* is thought to be more efficient at higher temperatures and higher host densities (Koppert Biological Systems, M. Klein Beekman, personal communication). Further, Heinz and Nelson (1996) showed that the use of *Encarsia pergandiella* or *E. formosa* alone lead to poorer *Bemisia argentifolii* control than when both species were used.

Our greenhouse data do not provide evidence that the use of both *E. formosa* and *A. fuscipennis* leads to a better control than the use of one, however, the examples of *A. spiniferus* and *A. hesperidum* indicate that it might be a good strategy to introduce both *A. fuscipennis* and *E. formosa* when many whiteflies are present. Also Drost et al. (1999) suggested that *Amitus bennetti* might be a good parasitoid to quickly reduce high *B. argentifolii* populations while other parasitoids could then be used to keep populations low. The high control potential of *A. bennetti* was confirmed by Joyce & Bellows (2000) who found a percent parasitism of 31 %, 40 days after releasing 300 *A. bennetti* in cages where 1500 *B. argentifolii* were released on 6 bean plants.

In conclusion, it is recommended to introduce both *E. formosa* and *A. fuscipennis* when the initial *T. vaporariorum* population is high or immigrations of whitefly are to be expected. When the initial *T. vaporariorum* population is low it would be better to introduce only *E. formosa* and if a high-density spot develops or unexpected immigrations occur, *A. fuscipennis* can then still be introduced to reduce the *T. vaporariorum* population quickly. *E. formosa* can

also be used in zones where *A. fuscipennis* is naturally present, as our data showed that control was successful when both species were used. To answer the question of the title of this article, we can say that *A. fuscipennis* is not an alternative, but a promising addition to the biological control of *T. vaporariorum*.

## Acknowledgements

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The appearance of pesticide resistance in several key pest in greenhouse production systems in the 60's and 70's provided the reason for renewed interest in biological control and more specifically in *Encarsia formosa*, parasitoid of the greenhouse whitefly, which had already been used in the 30's as a biological control agent (van Lenteren & Woets, 1988). Since then, biological control systems for most greenhouse pests have been developed and are applied in many countries that have a greenhouse industry (van Lenteren, 1992 & 1995). In several countries, biological control is nowadays the standard in greenhouse vegetables and is also increasingly used in ornamentals.

In the Colombian flower industry, biological control is under research and applied on a small scale, but it is hardly known by growers of greenhouse vegetables. Pest management is still completely based on pesticides leading to widespread resistance (Cardona et al., 2001). This urges for biological control systems adapted to the local conditions and pest spectra. These pest spectra are related to the altitude, being much broader in the intermediate climate zone than in the cold climate zone such as the Bogota Plateau, two areas where greenhouse production of tomatoes occurs. In tomato crops at the Horticultural Research Centre, situated on the Bogota Plateau, the greenhouse whitefly, *T. vaporariorum*, has been the dominant pest in tomato crops, while most other pests were controlled naturally. The development of a biological control system for *T. vaporariorum* was the subject of this thesis. Two parasitoids were evaluated: the introduced *E. formosa* and the native *A. fuscipennis*. *E. formosa* is a widely used synovigenic and thelytokous parasitoid of *T. vaporariorum*, which has been extensively studied. *A. fuscipennis* is also thelytokous but, in contrast to *E. formosa*, it is pro-ovigenic. Little was known of *A. fuscipennis* at the start of this project so basic research had to be undertaken before we could approve it as a biological control candidate and test it in greenhouses. The evaluation of *E. formosa* could be done immediately in the greenhouse.

With the research described in this thesis we hoped to be able to answer the following questions:

- Is *E. formosa* capable of controlling *T. vaporariorum* under the local greenhouse conditions?
- Is *A. fuscipennis* an alternative to *E. formosa*?
- Does the use of both parasitoids lead to better control?

In addition, we tried to adapt the individual based simulation model on the tritrophic relationship between tomato, whitefly and Encarsia formosa (van Roermund et al., 1997a), to the greenhouse situation in Bogota. This model might help us in designing specific strategies for whitefly control in Colombia.

**Is *E. formosa* capable of controlling *T. vaporariorum* under the local greenhouse conditions?**

In Chapter 2, we evaluated the potential growth of *T. vaporariorum* under the local greenhouse conditions and on the beef tomato variety Boris. This revealed that biological control by *E. formosa* should be possible. Greenhouse tests confirmed this, however, only in one out of three consecutive production cycles, biological control of *E. formosa* by *T. vaporariorum* was completely successful (Chapter 6). To assure success in the specific

137

conditions of unheated greenhouses on the Bogota Plateau the following recommendations should be taken into account:

- Start a new crop with a greenhouse and plants with low pest levels, so as to avoid fast population build-up at the beginning of the crop. The best results were obtained when the initial *T. vaporariorum* population was lower than 0.01 adults per plant. However, when the initial population was as high as 0.5-0.6 adults per plant, biological control was also possible.
- Introduce *E. formosa* as pupae to avoid negative effects of dealing with adults.
- For beef tomato varieties, introduce weekly 5 pupae per m$^2$ during 13 weeks to a total of 65 from the first observation of *T. vaporariorum* adults. This dosage is three times higher than that commercially used in Europe (Eggenkamp-Rotteveel Mansveld et al, 1982) so as to compensate for the better host plant quality of the beef tomato variety. It is possible that it can be reduced.
- For round tomato varieties, which are more resistant to *T. vaporariorum* (van Es, 1982) the dosage can be decreased. At the start of this research, the beef tomato variety Boris was grown on several farms and was therefore selected for all the trials. However, it is currently replaced by other varieties on commercial farms. A recent resistance evaluation showed that these varieties are much more resistant to *T. vaporariorum* than the variety Boris (Fuentes et al., 2001), and greenhouse trials introducing only 25 pupae per m$^2$ have been successful (L.E. Fuentes, unpublished results).
- Manage the vents to increase greenhouse temperature. In the glasshouse, which had a higher temperature than the plastic greenhouse, parasitism was always higher. Although other factors than temperature could have changed, from calculations with the temperature data it is clear that *E. formosa* profits more from the higher temperature for its population development than *T. vaporariorum*. Additionally, *E. formosa* increases its searching efficiency at higher temperatures.
- Avoid spraying, even with products that are said to be compatible with parasitoid use. It was found that pesticide applications killed a lot of adult wasps, even when using compatible products and this was possibly the cause of failure of biological control in the 1998 greenhouse experiment. If applications have to be made, only apply spot treatments, and if full field applications are necessary during the parasitoid introduction phase, increase the introductions to replace the killed *E. formosa* adults, in order to replace the killed adults.

Our greenhouse experiments showed that biological control of *T. vaporariorum* by *E. formosa* is possible during a long cropping season in greenhouse with a mean temperature of 15-16 °C and a photoperiod of 12 hours. A short photoperiod can influence negatively biological control as Vet et al. (1981) showed that *E. formosa* is inactive during night. Work of Hulspas-Jordaan et al. (1987) indicated that successful control in greenhouses with low temperature was possible for a short period during the European winter. Our results confirm that this is also possible for a longer period, although proper temperature management with short periods of increased temperature might be important to improve parasitoid redispersal for obtaining successful control.

### Is *A. fuscipennis* an alternative to *E. formosa*?

*Reproductive potential*
When defining the life history of *A. fuscipennis* (Chapter 3), it was found that the use of clip cages (Vet & van Lenteren, 1981; Manzano et al., 2000) that enclose a circle of the leaflet with a diameter of 2 cm influenced negatively the survival and oviposition results when compared to the method using cylinders that enclosed an entire leaflet. Therefore, it is better to use the cylinder method for the evaluation of whitefly parasitoids as to avoid underestimating their real potential.

The life history data of *A. fuscipennis* –using the cylinder method- (Chapter 3) showed that the reproductive potential of *A. fuscipennis* is considerably higher than that of *T. vaporariorum* (van Roermund et al., 1992a). In Table 1, we compare the life history of *A. fuscipennis* with *E. formosa* and *T. vaporariorum*. The reproductive potential of *A. fuscipennis* is similar to that of *E. formosa*, but this potential is realised by a high oviposition frequency during a short life span. Several life history traits suggest that *A. fuscipennis* could be more efficient at low temperatures than *E. formosa*. When comparing *A. fuscipennis* with *E. formosa* at 20 °C or lower (van Roermund & van Lenteren, 1992b), we found that for *A. fuscipennis* the development time from egg to adult in L1 hosts was shorter, the lower development threshold was lower, the fecundity was higher, the oviposition frequency at early adult life was higher, and the intrinsic rate of increase was slightly higher (Table 1). This might be an advantage of *A. fuscipennis* with respect to *E. formosa* when used in greenhouses with low temperature like those situated on the Bogota Plateau.

Table 1. Comparison of life history of E. formosa, A. fuscipennis and T. vaporariorum

| | *A. fuscipennis* vs *E. formosa* | *A. fuscipennis* vs. *T. vaporariorum* |
| --- | --- | --- |
| Development egg–adult in L1 host | shorter at temperatures < 23 °C | longer |
| Development egg-adult in preferred host | longer | longer |
| Lower development threshold | lower | similar |
| Upper development threshold | lower | similar |
| Mortality in pupal stage | lower | similar |
| Adult life span | lower | lower |
| Pre-oviposition period | lower (absent in *A.f.*) | lower (absent in *A.f.*) |
| Fecundity | higher at temperatures < 23 °C, similar at temp ≥ 23 °C | higher |
| Oviposition frequency | higher | higher |
| Intrinsic rate of increase ($r_m$) | higher, except at 30 °C | higher |

The estimated intrinsic rate of increase of *T. vaporariorum* on the variety Boris in our local greenhouse conditions was considerably lower than that of *A. fuscipennis* at 15 °C (Chapter 2). According to the previous data *A. fuscipennis* is a potential candidate for biological control of *T. vaporariorum*. However, to be efficient in the field, the parasitoid first has to locate and parasitize hosts before being able to realise its intrinsic rate of increase.

*Searching efficiency*

Manzano (2000) showed that, as *E. formosa*, *A. fuscipennis* does not detect infested host plants from a distance and landing on a leaflets or plant seems to be random process.

For *E. formosa* the residence time on a clean leaflets is about 20 minutes compared to about 1 hour for *A. fuscipennis*. This time increases significantly for *E. formosa* when hosts or honeydew are encountered. However, for *A. fuscipennis* it remains similar except on leaflets with unparasitized L1 hosts, where it increased only slightly. But, the presence or encounters with hosts or honeydew influenced strongly the searching activity of *A. fuscipennis*. For *A. fuscipennis*, leaflet types can be classified by increasing searching activity as follows: clean leaflets = leaflets infested with grey pupae and pupae not found > leaflets with honeydew > leaflets infested with grey pupae and pupae discovered > leaflets with L1 host and host not discovered > leaflets with L1 host and host discovered. This may indicate that *A. fuscipennis* detects the presence of larvae by some cue, otherwise we would expect the search activity on leaves with L1 larvae that were not discovered to be the same as that on clean leaves. This cue might not be present on leaflets that bear only pupae that have stopped feeding, as searching activity on leaflets with pupae was similar to that of clean leaflets. In contrast, the walking activity of *E. formosa* was not influenced by presence or encounters with hosts or honeydew and was equal among all types of leaflets mentioned above. The Giving Up Time (GUT, time after the last encounter with hosts, equals residence time on leaflets where no host encounters occur) of *E. formosa* on clean leaflets or infested leaflets where the host was not discovered was 20 minutes and doubled by the oviposition in an unparasitized host. For *A. fuscipennis*, the GUT on leaflets with unparasitized hosts was similar to that of clean leaflets, or infested leaflets where the host was not discovered. In contrast, it was lower on leaflets with recently parasitized hosts or leaflets with grey pupae, indicating that encounter/ovipositions in parasitized host may lead to a decrease in GUT and thus in residence time. Further analysis using the proportional hazards model is needed to elucidate this point of the foraging behaviour of *A. fuscipennis*, as events that increase GUT and other that decrease GUT may occur simultaneously. Nevertheless, it is clear that patch-leaving decisions are taken in a different way by the two parasitoids.

On infested leaflets, the searching efficiency of *A. fuscipennis* was significantly higher than that of *E. formosa*. Several aspects of the searching behaviour lead to this higher efficiency: (1) *E. formosa* searches at random and the walking pattern or speed is not altered by encounters with hosts. *A. fuscipennis*, on the contrary, presents initially random search but after encountering a host it reduces its walking speed and increases turning, resulting in an area restricted search (Manzano, 2000, Chapter 4). Additionally, (2) *A. fuscipennis* has a significantly higher walking speed than *E. formosa*, (3) a wider searching path, (4) a higher walking activity and (5) a shorter host handling time (Chapter 5).

For the overall searching efficiency in a crop, however, the picture is not so clear. *A. fuscipennis* stays three times longer on clean leaflets than *E. formosa* and this seems to be a waste of time. On the other hand, this higher residence time might also increase the host discovery on leaflets with low host density. Van Roermund et al. (1997b) estimated that only on 15 % of the leaflets with one L3 host, *E. formosa* discovered the host, while *A. fuscipennis* found the host on 33-44 % of leaflets with one L1 host. Indications of area restricted search on a higher scale than the leaflet was found for *A. fuscipennis*, as on infested leaflets more parasitoids left the leaflets by walking than on clean leaflets and this might increase its searching efficiency on an infested plant. *A. fuscipennis* spends considerably less time on host

evaluation and oviposition. Host acceptance is similar for unparasitized hosts of the preferred host stage for both parasitoids, but self- and conspecific superparastitism are considerably higher for *A. fuscipennis* than for *E. formosa*. However, in canopies with low host densities, van Roermund et al. (1997a) found that less than 1 % of the parasitoids were searching on infested leaflets. In this case, we expect *A. fuscipennis* to be time limited rather than egg limited so superparasitism might not influence the overall control capacity under these conditions. Rather, the shorter longevity of *A. fuscipennis* compared to *E. formosa* might lead to a lower control capacity of *A. fuscipennis* in crops with a low host density. Additionally, *A. fuscipennis* does not show host feeding while *E. formosa* kills about 10 % of its hosts by host-feeding (van Alphen et al., 1976). Finally, the previous conclusions were drawn in the supposition that the foraging behaviour of *A. fuscipennis* is fixed, as it was found for *E. formosa* (van Roermund & van Lenteren, 1995a), and does not change as a result of experience (learning).

Greenhouse experiments confirmed that *A. fuscipennis* was not capable of maintaining *T. vaporariorum* at low densities during a complete cropping season. Although chemical control and the mortal effect of glandular trichomes interfered in this experiment, the short life span of *A. fuscipennis* may have been an important factor as already argued above (Chapter 7). Based on the results, we can thus not recommended *A. fuscipennis* for control of *T. vaporariorum* at low host density. At higher host density, *A. fuscipennis* might be a better candidate than *E. formosa*. Experiments done by Marqués & Valencia (1991) showed that at high host densities, *A. fuscipennis* is more efficient than *E. formosa*. They compared the performance of *A. fuscipennis* with that of *E. formosa* on chrysanthemum by introducing 1500 parasitoids twice on 150 plants that were naturally infested by *T. vaporariorum*, and observed a higher parasitization by *A. fuscipennis* than by *E. formosa*. However, more experiments are needed to confirm the previous proposition. In table 2, the foraging behaviour of *A. fuscipennis* is compared to that of *E. formosa* (van Roermund & van Lenteren, 1995a; van Roermund & van Lenteren, 1995b; van Roermund et al. 1994)

To understand better this tritrophic system, the existing model for *E. formosa* (van Roermund et al., 1997a) could be adapted to *A. fuscipennis*. Life history parameters of *A. fuscipennis* as function of temperature are modelled (Chapter 3) and analysis of the time budgets and foraging behaviour of *A. fuscipennis* on tomato leaflets with the Proportional Hazards Model (Cox, 1972) may elucidate the factors that determine the foraging decisions of the parasitoid (Chapter 4 & 5). However, more information might be needed on the foraging behaviour as function of experience, egg load, host quality, host density and leaflet area. Together with our data the complete behaviour can then be modelled and the results introduced in the tritrophic model. Also, general conclusions can then be drawn on the efficiency of the two different searching strategies of both parasitoid species under different host density conditions.

Manzano (Manzano et al., 2000; Manzano, 2000) evaluated *A. fuscipennis* for the use of *T. vaporariorum* in field grown bean crops in the intermediate climate zones in Colombia and concluded that *A. fuscipennis* might be an effective parasitoid. Our results strongly support that proposition. In the intermediate climate zones, migration of whiteflies from nearby crops or wild vegetation would cause all leaflets of the plants to be infested and under these conditions *A. fuscipennis* is likely to display its total potential.

Table 2. Comparison of foraging behaviour of *A. fuscipennis* with *E. formosa*

| | *A. fuscipennis* vs.*E. formosa* |
|---|---|
| **Patch location** | |
| Random landing | same |
| No volatiles used for patch detection | same |
| **Residence or patch time** | |
| GUT [*] on clean leaflet | higher if temperature > 18 °C |
| Temperature effect on GUT | lower |
| GUT increases by honeydew | no vs. yes |
| GUT does not increase on infested leaflets, where no hosts are found | same |
| GUT increases by oviposition in unparasitized hosts | no (possibly) vs. yes |
| GUT decreases by oviposition in parasitized hosts | yes (possibly) vs. no |
| Patch time increases after encounters with/ovipositions in unparasitized hosts | yes, effect stronger for *E. formosa* |
| Patch time increases after encounters with/ovipositions in parasitized hosts | no vs. yes |
| **Position on leaflet** | |
| Time on upper leaf side equal to time on lower leaf side on clean leaflets | no vs. yes |
| Time on lower leaf side increases by host encounter | no vs. yes |
| Leaving by flying | lower |
| Leaving by walking | higher |
| **Searching efficiency** | |
| Searching on leaf | area restricted vs. random |
| Walking speed | higher |
| Walking speed increases with temperature | same |
| Width of the walking path | Wider |
| Walking activity on clean leaflets | lower |
| Walking activity influenced by honeydew, host presence, host encounter | yes vs. no |
| Walking activity on infested leaflets | lower |
| Walking activity decreases with egg load | unknown vs. yes |
| Host detection on leaves with low host density | higher |
| Encounter rate with hosts | higher |
| **Host handling** | |
| Acceptance of unparasitized host of preferred host stage | same |
| Conspecific superparasitism | higher |
| Self-superparastitism | higher |
| Rejection of hosts containing parasitoid pupa | Same and 100% |
| Host-feeding | no vs. yes |
| Influence of experience on host acceptance | unknown vs no |
| Host handling time | lower |

[*] Giving up time (GUT) is the time that the parasitoid spends on a leaflet after the last host encounter. On clean leaflets GUT equals residence time.

**Does the use of both parasitoids lead to better control?**

The introduction of two thelytokous parasitoids leads to competition that takes place on two levels, that of the adults searching for a common host and that of the parasitoid eggs or larvae within the host. We evaluated both aspects in a foraging and interaction experiment (Chapter 5). This showed that neither parasitoid species changed their behaviour towards *T. vaporariorum* larvae parasitized by the other species as compared to that of unparasitized larvae. Therefore, multiparasitism was observed frequently. In 70-80 % of the cases, multiparasitized larvae resulted in a parasitoid of the species that parasitized first. Neither species was observed to be superior to the other when competing in the same larva (Chapter 5). The two parasitoid species have a similar intrinsic rate of increase but different, possibly complementary, life history traits. The pro-ovigenic *A. fuscipennis* has a high egg load and high oviposition frequency (Chapter 3) and could therefore prove to be a good reducer of high-density spots. The synovigenic *E. formosa* has a longer adult life, which it can prolong through oosorbtion (Van Keymeulen & Degheele, 1978; van Lenteren et al., 1987) and this could be better to keep *T. vaporariorum* at low density. Therefore, the simultaneous use of both parasitoids in greenhouses might be more efficient than the use of just one of them.

We did, however, not obtain better biological control results when using both *A. fuscipennis* and *E. formosa* (Chapter 7) than when using *E. formosa* alone (Chapter 6). The control was significantly better when using *A. fuscipennis* alone (Chapter 7), however, in the experiment where only *A. fuscipennis* was used, the results may have been influenced by pesticide sprayings and parasitoid mortality due to glandular trichomes. Thus, the data do not support the proposition that the use of 2 parasitoids with a different life history is better, and more research is needed to confirm it. Two examples of classical biological control describe a similar system. The control of *Aleurocanthus woglumi* was principally exercised by the pro-ovigenic *Amitus hesperidum* at high host densities, but once reduced, the synovigenic *Encarsia opulenta* became the dominant parasitoid (Flanders, 1969; Thompson et al, 1987; Dowell et al, 1981; Nguyen & Hamon, 1994). Likewise, initially *Amitus spiniferus* reduced the high infestation levels of *Aleurothrixus floccosus* within one year after the introduction and then was displaced by *Cales noacki* who kept the population at low levels (DeBach & Rose, 1976). Similarly, we found that synovigenic *E. formosa* was the dominant parasitoid in the low host density situation of the 2000 experiment. In the classical biological control examples, the *Amitus* species disappeared almost completely after some years (Tsai and Steinberg, 1991; Thompson et al, 1987; De Bach & Rose, 1976). This lead us to the conclusion that in low host density crops it may be better to introduce only *E. formosa*.

However, other examples indicate that the combined use of two parasitoid species could lead to a more stable system. Heinz and Nelson (1996) showed that the use of both *Encarsia pergandiella* and *E. formosa* lead to significantly lower *B. argentifolii* levels than the use of just one of them. Also, *E. formosa* and *Eretmocerus eremicus* are commercially used together to control *T. vaporariorum* and *Bemisia* in greenhouse tomatoes and ornamental crops (M. Klein Beekman, personal communication). In these cases, however, two synovigenic parasitoids are used.

In conclusion, it is recommended to introduce only *E. formosa* when the initial *T. vaporariorum* population is low and no immigrations are to be expected. If a high-density spot develops *A. fuscipennis* can then still be introduced to reduce the *T. vaporariorum* population quickly in that spot. *E. formosa* can also be used in zones where *A. fuscipennis* is

143

naturally present as our data showed that *A. fuscipennis* is not deleterious to the control of *T. vaporariorum* by *E. formosa* (Chapter 6 & 7). When the initial whitefly density is high or immigrations are to be expected, the introduction of both parasitoids is recommended. The previous mentioned examples of classical biological control and this research (Chapters 3 to 7) support the more general thesis that pro-ovigenic species can be used better to reduce high host density populations while synovigenic species are better in keeping populations at low levels.

**The use of the tritrophic tomato-*T. vaporariorum-E. formosa* model for evaluation of different management strategies**

An additional objective was to evaluate the effect of different management strategies such as temperature conditions, tomato varieties or introduction strategies on the biological control of *T. vaporariorum* by *E. formosa* using the tritrophic model of van Roermund & van Lenteren (1997a) (hereafter referred to as the model). The model consists of several submodels that simulate the plant growth, pest and parasitoid population development (state variable model using boxcar-train procedures), the spatial distribution of whiteflies and parasitoids (by moving from leaflet to leaflet) and the parasitoid foraging behaviour (Stochastic Monte Carlo simulation model). Input data for the models were drawn from the extensive research done on *T. vaporariorum* and *E. formosa* on tomato. However, the model could not be adjusted to our specific situation and will need major adaptations before it can be used. In a brief overview, we list the difficulties that were encountered. This research was done together with Dr. Y. Tricault at the laboratory of Entomology at Wageningen University.

*The plant growth submodel*
This submodel is based on the leaf initiation rate equation of the tomato growth model TOMGRO (Jones et al., 1991). Leaves consisted of 7 leaflets of 22 cm$^2$. This submodel could not be adjusted to the plant development of the variety Boris growing in the specific conditions of the greenhouses on the Bogota Plateau. The leaf initiation rate equation was adapted to the Colombian situation (provided by Alexander Cooman, personal communication). However, the leaf area could not be described by a fixed number of leaflets of a fixed area. For the Dutch greenhouse conditions, which are controlled by sophisticated climate management systems leading to relative stable growing conditions, the leaf area could be estimated in this way. Under the Colombian greenhouse conditions, leaf area varied extremely with time after transplant and this was caused by (1) variation in number of leaflets per leaf, and (2) variation in leaflet area. A normal tomato leaf consists of seven leaflets, 3 pairs along the petiole and one terminal leaflet. Under Colombian conditions, another 2 to 4 leaflet pairs developed between the main leaflet pairs on the main petiole on leaves that were formed before the fruit growth became important, and, additionally, the main leaflets often split up in two to three leaflets. This leads to leaves with many leaflets (up to 20 or more) of a variable size (up to 130 cm$^2$). The frequency distribution of leaflet size of these large leaves followed an exponential distribution. Afterwards, when vegetative growth and reproductive growth reached equilibrium after the first trusses are harvested, the leaves consisted of seven leaflets that were very small. We ran the model using the glasshouse data of 1997 (Chapter 6) simulating plants with 25 leaf layers, each leaf consisting of 18 leaflets of 65 cm$^2$. In this way, the total leaf area was adjusted to the measured leaf area (Alexander Cooman, personal

communication). In the model simulations, the resulting estimated *T. vaporariorum* population started to increase exponentially after 100 days. However, running the model with leaves consisting of 7 leaflets of 40 cm$^2$ resulted in *T. vaporariorum* populations in the same range as observed in the Colombian greenhouse. Therefore, we propose to adapt the plant growth submodel by making the number of leaflets and leaflet size dependent of leaf layer. However, as we found that frequency distribution of leaflet area within a leaf layer followed an exponential distribution, this might be an approximation of reality that is too rough, and a more detailed model of the leaflet area development might be necessary. This change will affect also the other submodels and considerably increase the simulation time of the model. However, a detailed description of leaflet area is important because the model was very sensitive to leaflet area (*T. vaporariorum* suppression increased by almost 60 % by reducing leaflet area by 25 %) and simplifications may lead to serious errors. We suppose that the plant growth submodel is the most important submodel that needs adjustment for our situation.

*Pest and parasitoid population development model*
We used life history data of Burnett (1949) for *T. vaporariorum*, which resembled those we found in Chapter 1. For *E. formosa* the input data were the same as those used by van Roermund & van Lenteren (1997a). One possible difficulty with our data was that we only counted *T. vaporariorum* adults and not the larvae at the beginning of the greenhouse trials leading to an underestimate of the initial whitefly population. The initial density is very important for the correct simulation of further development of the population.

*Spatial dispersal and distribution submodel*
In the model, the landing of the whiteflies on a new leaflet within the leaf is a random process (the choice of the leaf is not random, young upper leaves are preferred). This might change when leaflet area varies considerably within the leaf. Larger leaflets might be selected more frequently than smaller ones. Also the vertical dispersal of *E. formosa* is at random in the model. Again, *E. formosa* might land more frequently on large leaflets than on small ones. Additionally, we had a crop with about 25 leaf layers. It is doubtful that *E. formosa* females emerging on the lowest leaf layer in the crops will land with the same probability on the highest leaf layer as on the lowest leaf layer. Therefore, the random vertical dispersal of *E. formosa* should be re-evaluated.
    In the model, upward movement of whitefly takes place every time a new leaf was fully developed: the whiteflies present on leaf layer 4 move to the newly developed leaf. Because of the lower temperature in the Colombian greenhouses, the leaf initiation rate is considerably lower than in the Dutch situation. For our conditions the model simulated that whiteflies remained on the same leaf up to 12 days. This should be confirmed by observations in the greenhouse. However, the many activities in the crop like pruning, fastening the top of the plants, pollinating, harvesting, monitoring, etc. might increase the adult whitefly movement. This would then create a less clustered *T. vaporariorum* distribution and the model showed that lower aggregation leads to higher *T. vaporariorum* populations (van Roermund & van Lenteren, 1997a)

*Parasitoid foraging behaviour submodel*

Sütterlin & van Lenteren (1999) found that the Giving Up Time (= time that the parasitoid stays on a leaflet after the last encounter with a host, GUT) on *Gerbera jamesonii* leaves was at least 4 times as high as that on tomato leaflets. Although this could have been a host plant effect, we assume that the increased leaf area was the main cause for this. Because of the presence of large leaflets in our crops, GUT should be determined as function of leaflet area.

Foraging behaviour of *E. formosa* was strongly temperature dependent (van Roermund & van Lenteren, 1995a). As a result of the impossibility to reach an overall good fit of the simulation results with the observed data, we studied the foraging behaviour of *E. formosa* at low temperatures. Surprisingly, the preliminary results indicate that the *E. formosa* strain we used displays its full foraging behaviour at 15 °C (Edison Torrado, personal communication, 2001), while the data of van Roermund & van Lenteren (1995a) showed almost complete inactivity at temperatures equal or lower than 18 °C. Rearing of *E. formosa* at low temperatures in Colombia, may have selected for females that are more active at lower temperature.

At the greenhouse level and for the Dutch situation, the model simulated well the whitefly and parasitoids population, but it remains unclear if the simulated spatial distribution or the distribution over the leaflets coincide with reality. Data on the simulated distribution in space or on the leaflet scale can not be visualised easily and it might be important to evaluate if the model does not simulate unreal or impossible situations at those levels. Adaptation of the model to our conditions can make it more universal, and might increase its applicability to the growing conditions in other regions where greenhouse tomatoes are grown.

## Epilogue

The objective of this research was to understand the relationship between *A. fuscipennis*, *E. formosa*, whitefly and tomatoes under Colombian conditions, and to apply the data to the local needs of the CIAA and UJTL (applied research) and growers (biological solutions for their pest problems). During this research project, both the greenhouses and the laboratories have been visited by more than 5000 persons during the weekly guided visits at the CIAA, including growers, agronomists, scientists, students, or just interested people (Rebecca Lee, personal communication). Short courses for growers have been organised, both at the CIAA and in tomato production zones like Valle de Tenza. As a result several growers have set up trials on their farms and obtained successful biological control in greenhouses on the Bogota plateau, and promising initial biological control results have been found in greenhouses in the intermediate climate zone (In Fusagasuga and Valle de Tenza). Additionally, the experience gained at the CIAA was published in a book with practical guidelines for the production of greenhouse tomatoes under the Colombian conditions (Lee & Escobar, 2001), which includes a chapter dedicated to integrated pest management with emphasis on biological control (De Vis & Fuentes, 2001).

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## Summary

In Colombia, biological control of pests in greenhouse crops is only applied on a very limited scale in ornamentals and as yet non-existent in greenhouse vegetables. Greenhouse production of vegetables – mostly tomatoes- is a recent development, as a result of the high losses of field production due to pests and diseases. Pest spectra in those production systems vary greatly with altitude, being much broader in the intermediate climate zones (altitude 1800-2000) than in the cold climate zones such as the Bogota Plateau (altitude 2660 m). The most important pest in greenhouses situated on the Bogota Plateau is the greenhouse whitefly, *Trialeurodes vaporariorum*. In greenhouses where experimentally no pesticides are applied, aphid and leafminer pests are controlled beneath economic damage thresholds by naturally occurring parasitoids, with the exception of greenhouse whitefly. Therefore, with a biological control system for *T. vaporariorum*, tomato production without insecticides should be possible. In this thesis I evaluate two natural enemies for the biological control of *T. vaporariorum*: the introduced parasitoid *Encarsia formosa* and the native parasitoid *Amitus fuscipennis*.

In chapter 1, the factors that promoted the change from chemical control to integrated pest management are discussed. For the biological control of *T. vaporariorum* in the specific conditions of greenhouses on the Bogota plateau, three questions were formulated: (1) Is *E. formosa* capable of controlling *T. vaporariorum* under the local greenhouse conditions? (2) Is the native parasitoid *A. fuscipennis* an alternative to *E. formosa*? (3) Does the use of both parasitoids lead to better control?

To answer the first question we evaluated the longevity, fecundity, oviposition frequency and intrinsic rate of increase of *T. vaporariorum* under the local greenhouse conditions on the beef tomato variety Boris (Chapter 2). At an average temperature of 16 °C and a mean relative humidity of 81 %, the longevity of females and males was 36.5 and 47.2 days, respectively. The fecundity was 208.5 eggs per female, the oviposition frequency was 5.7 eggs per living female per day, and the intrinsic rate of increase ($r_m$) was 0.0645. These values are higher if compared to results obtained at the same temperature of previous research on tomato in general, but it is known that beef tomato varieties are better host plants for whitefly than round tomato varieties. When compared to the results of a previous study on beef tomato in The Netherlands, in my experiments the longevity was shorter, the oviposition frequency was higher and the fecundity was similar. The estimated $r_m$ of *E. formosa* under the same experimental conditions, was 0.0974 and is considerably higher than that of *T. vaporariorum*, indicating that biological control should be possible.

Subsequently, biological control of *T. vaporariorum* by *E. formosa* was tested during three consecutive production cycles, on a beef tomato crop in a glasshouse and a plastic greenhouse (Chapter 6). Population development of *T. vaporariorum* and parasitism by *E. formosa* were followed during 26-28 weeks. The mean temperature was around 16 °C in the plastic greenhouse and around 17 °C in the glasshouse. *E. formosa* was introduced at a rate of 3 adults per m$^2$ and per week in the 1997 production cycle, and respectively 3 and 5 pupae per m$^2$ and per week in 1998 and 1999, from the start of the experiments until reaching a total of about 66 released parasitoids per m2 . In 1997, the adult whitefly population increased

exponentially to a peak of 76 adults per plant in the plastic greenhouse. At that time, the whitefly population in the glasshouse reached a peak of only 12 adults per plant. The percent parasitism fluctuated between 42 and 82 % in the glasshouse and between 28 and 47 % in the plastic greenhouse. In 1998, the *T. vaporariorum* population remained out of control in both greenhouses and reached a peak of respectively 80 and 53 *T. vaporariorum* adults per plant in the plastic greenhouse and the glasshouse. Parasitism fluctuated between 55 and 97 % in the glasshouse and between 32 and 84 % in the plastic greenhouse. In 1999, biological control was successful in both greenhouses. Most of the time, *T. vaporariorum* populations were lower than 1.2 adults per plant and parasitism by *E. formosa* was 80 % or higher. We suppose that the higher temperature is the main reason for better parasitism in the glasshouse when compared to the plastic greenhouse. The successful results of 1999 show that biological control of *T. vaporariorum* by *E. formosa* is possible under the short day and low temperature conditions of greenhouses situated in the high altitude tropics such as the Bogota Plateau.

Little was know of *A. fuscipennis* when this research was started, so to answer the second question (Is the native parasitoid *A. fuscipennis* an alternative to *E. formosa*?), basic research on the life history and searching efficiency of this parasitoid was needed, before greenhouse tests could be done. Life history parameters of *A. fuscipennis* as parasitoid of *T. vaporariorum* were determined at 15, 20, 25 and 30 °C on tomato (Chapter 3). Immature development, mortality, longevity, fecundity, oviposition frequency and post-oviposition period were determined and temperature dependent relations were estimated. Immature development had a maximum of 61 days at 15 °C and it decreased to 22 days at 30 °C. Mortality of the parasitoids in the pupal stage (= grey stage) was less than 2% at temperatures lower than 30 °C, and at 30 °C it was 60 %. Longevity fluctuated between 18 days (at 15 °C) and 3 days (at 30 °C). Fecundity increased from 338 eggs·female$^{-1}$ at 15 °C to a maximum of 430 eggs·female$^{-1}$ at 25 °C and then decreased to 119 eggs·female$^{-1}$ at 30 °C. Oviposition frequency varied between 3 and 46 eggs·female$^{-1}$·day$^{-1}$ and had its maximum on the first day after emergence of the parasitoid, except at 15 °C where the maximum was reached at the second day after emergence. The $r_m$ increased from 0.090 at 15 °C to a maximum of 0.233 at 25 °C and then decreased to 0159 at 30 °C. The $r_m$ of *A. fuscipennis* is significantly higher than that of *T. vaporariorum* and even higher than *E. formosa* at temperatures lower than 30 °C, and A. fuscipennis is therefore a potential candidate for the biological control of the greenhouse whitefly.

However to be able to control *T. vaporariorum* in the greenhouse, the parasitoid has to find and parasitize its host efficiently. Therefore, as a second step in the evaluation of *A. fuscipennis*, the residence time and time allocation of this parasitoid were measured on clean tomato leaflets at various temperatures, on leaflets with honeydew, and on leaflets with 1 or 4 unparasitized L1 larvae or 4 grey pupae of *T. vaporariorum* (Chapter 4). On clean leaflets, the residence time was about one hour at 20, 25 and 30 °C, and three hours at 15 °C. Residence time did not increase on leaflets with honeydew or on infested leaflets, except for leaflets with four unparasitized L1 larvae where it increased to about one and a half hour. On those leaflets, the Giving Up Time (time spent on the leaflet after the last encounter with a host) was also higher than on leaflets with other host types. The percentage time walking (of the total residence time minus the host handling time) increased from 34 % on clean leaflets to 49 % on leaflets with honeydew, to 60 % on leaflets with grey pupae, and to more than 78 % on leaflets with L1 larvae. The time handling hosts was lower than 5 % for all treatments. Walking speed was 0.9, 1.5 and 1.7 mm/s at 15-16, 19-21 and 25-27 °C respectively and the

width of the searching path was 0.8 mm. On leaflets with one L1 host, fewer hosts were encountered (1.95 – 2.35) than on those with four L1 host (6.10 – 6.68), while on leaflets with four grey pupae the number of encounters (2.05) was similar to that of leaflets with one host. The number of hosts that were parasitized was lower on leaflets with one L1 host (0.31-0.33) than on those with four L1 host (1.42-1.65). Host acceptance was: 71 % for unparasitized L1 larvae, 33% for recently self-parasitized larvae, 62 % for larvae recently parasitized by a conspecific and 0 % for whitefly pupae containing a parasitoid pupa. Self and conspecific superparasitism was considerable. The minimum time needed for a successful oviposition was 23 s. On infested leaves, the searching efficiency of *A. fuscipennis* was higher than that of *E. formosa*, explained by its higher walking activity, lower time handling hosts, more rapid walking speed and wider searching path. For the overall searching efficiency in a crop, however, the picture is not so clear. *A. fuscipennis* stays three times longer on clean leaflets than *E. formosa*. This higher residence time does increase host discovery on leaflets with low host density, but leads to extensive search periods on clean leaves.

The biological control capacity of *A. fuscipennis* was finally tested in two greenhouses with a mean temperature of 16-17 °C (Chapter 7). During 13 weeks, a total of 66 parasitoid pupae were introduced per m2 in the greenhouses, at a rate of 5 *A. fuscipennis* pupae per m$^2$. Biological control was obtained during 5 months in the plastic greenhouse and 3 months in the glasshouse. After these periods, the *T. vaporariorum* adult populations increased to a maximum of nearly 50 adults per plant in both greenhouses. Parasitism was initially higher than 80 %, but then decreased to 56 % in the plastic greenhouse and to 20 % in the glasshouse. Although pesticide sprays and mortality of *A. fuscipennis* adults due to glandular trichomes may have influenced these results, it is supposed that the short longevity of *A. fuscipennis* prevents it from developing its high reproductive potential in crops with low host densities. The use of *A. fuscipennis* alone for the biological control of *T. vaporariorum* in greenhouse tomatoes on the Bogota Plateau is therefore not recommended.

To answer the 3$^{rd}$ question (Does the use of both parasitoids lead to better control?), first a laboratory experiment was set-up to evaluate the foraging behaviour of *A. fuscipennis* and *E. formosa* on tomato leaflets with 20 *T. vaporariorum* larvae in the first or third larval stage (Chapter 5). Ten of the whitefly larvae were previously parasitized and contained a conspecific or a heterospecific parasitoid egg or larva. The host type (host stage and/or previous parasitization) did not influence the foraging behaviour of either parasitoid species. Both parasitoid species discriminated well between unparasitized larvae and self-parasitized larvae, but discriminated poorly those larvae parasitized by a conspecific and did not discriminate at all larvae parasitized by a heterospecific. Self-superparasitism, conspecific superparasitism and multiparasitism were observed for both parasitoid species. Superparasitism always resulted in the emergence of one parasitoid and multiparasitism resulted in a higher emergence of the parasitoid of the species that had parasitized first. No superiority, when competing within the same host, was found for either parasitoid species. Based on these data it is difficult to predict what the outcome would be when both parasitoids are introduced into the greenhouse: similar, better or poorer control. In order to be able to answer this question, once more experiments on a greenhouse scale were done.

Biological control of *T. vaporariorum* by the simultaneous introduction of *A. fuscipennis* and *E. formosa* was tested in two greenhouses with a mean temperature of 16-17 °C (Chapter 7). During 13 weeks, a total of 66 parasitoid pupae per m2 were introduced in the greenhouses at a rate of 2.5 pupae of both *E. formosa* and *A. fuscipennis* per m$^2$ and per week.

Biological control was successful in both greenhouses. *T. vaporariorum* populations were generally lower than 1.2 adults per plant and parasitism, caused mainly by *E. formosa*, was 90 % or higher most of the time. Therefore, *E. formosa* is recommended to keep *T. vaporariorum* populations at low levels in unheated greenhouses, situated in the high altitude tropics such as the Bogota plateau. Additional to the use of *E. formosa*, releases of *A. fuscipennis* seem useful in those cases where high whitefly populations are expected, or when high-density spots need to be controlled, because of the high oviposition frequency of the latter parasitoid.

In Chapter 8 the results are summarised and discussed, and suggestions are made for future research.

**Samenvatting**

Biologische controle van plagen in kassen wordt in Colombia op een zeer beperkte schaal toegepast in de snijbloementeelt en is momenteel onbestaand in de groenteteelt. De productie van groenten in plastic kassen is een zeer recente ontwikkeling om opbrengstverliezen in openluchtteelten, veroorzaakt door ziekten en plagen, te beperken. Het plagenspectrum is sterk afhankelijk van de hoogte en is veel breder in warmere klimaatzones (hoogte 1800-2000 m), waar traditioneel tomaten in volle grond worden geteeld, dan in de koude klimaatzones zoals op de hoogvlakte van Bogota (hoogte 2660 m). In kassen op de hoogvlakte van Bogota waar experimenteel geen pesticiden worden toegepast, worden de meeste plagen zoals luizen en mineervliegen beneden de schadegrens gehouden door natuurlijk voorkomende sluipwespen. Een uitzondering hierop is de kaswittevlieg, *Trialeurodes vaporariorum*. Als een systeem voor de biologisch controle van kaswittevlieg kan ontwikkeld worden, dan is de productie van tomaten zonder gebruik te maken van insecticiden mogelijk. Om dit doel te bereiken, werden twee natuurlijke vijanden van de kaswittevlieg geëvalueerd: de geïntroduceerde sluipwesp *Encarsia formosa* en de inheemse soort, *Amitus fuscipennis*.

*E. formosa* behoort tot de familie van de Aphelinidae en werd beschreven door Gahan in 1924 uitgaande van specimens uit Idaho, USA. Het genus *Encarsia* bevat meer dan 200 soorten waarvan enkel *E. formosa* commercieel geproduceerd wordt om wittevliegen in kassen te bestrijden. *E. formosa* wordt wereldwijd gebruikt en werd voor het eerst in Colombia geïntroduceerd in 1986. *A. fuscipennis* behoort tot de familie van de Platygastridae en werd beschreven door MacGown & Nebeker in 1978. Er zijn 19 *Amitus* soorten beschreven en twee daarvan parasiteren wittevliegen die kasplagen zijn: *A. fuscipennis* parasiteert *T. vaporariorum* en *A. bennetti* parasiteert *Bemisia tabaci*. *A. fuscipennis* komt voor in het noorden van Zuid-Amerika en Centraal Amerika. In Colombia komt deze sluipwesp overvloedig voor in warme klimaatzones samen met zijn gastheer, *T. vaporariorum*. Beide sluipwespen vermenigvuldigen zich door thelytoke parthenogenese. *E. formosa* is synovigeen terwijl *A. fuscipennis* pro-ovigeen is.

In hoofdstuk 1 werden de factoren die bijdroegen tot de omschakeling van chemische naar geïntegreerde plaagbestrijding in het algemeen en specifiek voor kasteelten bediscussieerd. Toegespitst op dit project werden de mogelijkheden voor biologische bestrijding van *T. vaporariorum* met de eerder genoemde sluipwespen onderzocht. Daarbij werden drie vragen geformuleerd: (1) Is *E. formosa* in staat om wittevlieg te controleren onder de locale klimaatomstandigheden? (2) Is de inheemse sluipwesp *A. fuscipennis* een alternatief voor *E. formosa*? (3) Leidt het simultaan gebruik van beide sluipwespen tot een betere controle?

Om de eerste vraag te beantwoorden, werden de levensduurte, de fecunditeit, de eilegfrequentie en de intrinsieke groeisnelheid ($r_m$) van *T. vaporariorum* onder de locale kasomstandigheden op de vleestomaatvarieteit Boris bepaald (Hoofdstuk 2). Bij een gemiddelde temperatuur van 16 °C en een gemiddelde relatieve luchtvochtigheid van 81 %, was de levensduurte van wijfjes en mannetjes respectievelijk 36.5 en 47.2 dagen. De fecunditeit was 209.5 eitjes per wijfje, de eilegfrequentie was 5.7 eitjes per levend wijfje per dag en de $r_m$ was 0.0645. Deze waarden zijn hoger dan die van vroeger onderzoek bij dezelfde

temperatuur op tomaat in het algemeen. Het is echter bekend dat vleestomaatvariëteiten betere waardplanten zijn voor wittevlieg dan variëteiten van ronde tomaten. In vergelijking met gegevens van een vroegere studie op andere vleestomaatvariëteiten in Nederland, was de levensduurte in de huidige studie korter, de eilegfrequentie hoger en fecunditeit gelijk. De $r_m$ van *E. formosa*, geschat voor de proefomstandigheden van dit onderzoek, was hoger dan die van *T. vaporariorum*. Dit geeft aan dat biologische controle van *T. vaporariorum* met *E. formosa* in kassen op de hoogvlakte van Bogota mogelijk zou moeten zijn.

Vervolgens werd biologische controle van *T. vaporariorum* met *E. formosa* gedurende drie opeenvolgende productiecyclussen van vleestomaat getest in een glazen kas en een plastic kas (Hoofdstuk 6). De populatieontwikkeling van *T. vaporariorum* adulten en de parasitering door *E. formosa* werden gevolgd gedurende 26 tot 28 weken. Wekelijks werden 3 volwassen sluipwespen per m$^2$ geïntroduceerd in de proef van 1997 en respectievelijk 3 en 5 poppen per m$^2$ in 1998 en 1999, van de start van de experimenten tot een totaal van ongeveer 66 geïntroduceerde parasitoiden werd bereikt. De gemiddelde temperatuur was ongeveer 16 °C in de plastic kas en ongeveer 17 °C in de glazen kas. In 1997 steeg de populatie van volwassen wittevliegen exponentieel tot een piek van 76 per plant in de plastic kas. Op dat moment bereikte de volwassen wittevliegpopulatie in de glazen kas slechts een piek van 12 per plant. Het parasiteringspercentage fluctueerde tussen 42 en 82 % in de glazen kas en tussen 28 en 47 % in de plastic kas. In 1998 kon de *T. vaporariorum* populatie niet onder controle worden gehouden en bereikte een piek van respectievelijk 80 en 53 volwassen *T. vaporariorum* per plant in de plastic kas en de glazen kas. Het parasiteringspercentage fluctueerde tussen 55 en 97 % in de glazen kas en tussen 32 en 84 % in de plastic kas. In 1999 was de biologische controle succesvol in beide kassen. Meestal waren de volwassen *T. vaporariorum* populaties lager dan 1.2 per plant en het parasiteringspercentage was 80 % of hoger. Verondersteld wordt dat de hogere temperatuur de belangrijkste reden is voor het hoger parasiteringspercentage in de glazen kas in vergelijking met de plastic kas. De succesvolle resultaten van 1999 tonen aan dat biologische controle van *T. vaporariorum* met *E. formosa* mogelijk is onder de korte dag en lage temperatuur omstandigheden van kassen gesitueerd op grote hoogte in de tropen zoals de hoogvlakte van Bogota.

Weinig was geweten van *A. fuscipennis* bij de aanvang van dit onderzoek. Om de tweede vraag te beantwoorden (Is de inheemse sluipwesp *A. fuscipennis* een alternatief voor *E. formosa*?) was eerst basisonderzoek betreffende de levenscyclus en de zoekefficiëntie nodig vooraleer deze parasitoide in kasomstandigheden kon worden getest. De demografische parameters van *A. fuscipennis* als parasitoide van *T. vaporariorum* op tomaat werden bepaald bij 15, 20, 25 en 30 °C (Hoofdstuk 3). De ontwikkelingduur van onvolwassen stadia, de mortaliteit, levensduurte, fecunditeit, eilegfrequentie en post-ovipositie periode werden bepaald. Tevens werden temperatuursafhankelijke relaties geschat voor elk van deze parameters. De ontwikkelingsduur van ei tot adult had een maximum van 61 dagen bij 15 °C en daalde tot 22 dagen bij 30 °C. De mortaliteit van de parasitoiden in het popstadium (grijze stadium) was minder dan 2% bij temperaturen lager dan 30 °C, en bij 30 °C was deze 60 %. De levensduurte van volwassen wijfjes fluctueerde tussen 3 dagen (bij 30 °C) en 18 dagen (bij 15 °C). De fecunditeit steeg van 338 eitjes·wijfje$^{-1}$ bij 15 °C tot een maximum van 430 eitjes·wijfje$^{-1}$ bij 25 °C, en daalde dan tot 119 eitjes·wijfje$^{-1}$ bij 30 °C. De eilegfrequentie varieerde tussen 3 en 46 eitjes·wijfje$^{-1}$·dag$^{-1}$ en had een maximum op de eerste dag na het uitkomen van de parasitoide, behalve bij 15 °C waar het maximum bereikt werd op de tweede dag na het uitkomen. De $r_m$ steeg van 0.090 bij 15 °C tot een maximum van 0.233 bij 25 °C en

daalde dan tot 0.159 bij 30 °C. De $r_m$ van *A. fuscipennis* is significant hoger dan deze van *T. vaporariorum* en zelfs hoger dan deze van *E. formosa* bij temperaturen lager dan 30 °C. *A. fuscipennis* is daarom een potentiële kandidaat voor biologische controle van de *T. vaporariorum*. Echter, om succesvol *T. vaporariorum* te controleren in kassen, moet de parasitoïde op een efficiënte wijze zijn gastheer vinden en parasiteren.

Als tweede stap in de evaluatie van *A. fuscipennis*, werd de verblijftijd en de tijdsbesteding van deze parasitoïde bepaald op schone tomatenblaadjes bij verschillende temperaturen, op blaadjes met honingdauw, en op blaadjes met 1 of 4 ongeparasiteerde L1 *T. vaporariorum* larven of 4 grijze poppen (Hoofdstuk 4). Op schone blaadjes was de verblijftijd ongeveer één uur bij 20, 25 en 30 °C, en drie uur bij 15 °C. De verblijftijd verhoogde niet op blaadjes met honingdauw of op besmette blaadjes, uitgezonderd op blaadjes met vier ongeparasiteerde L1 larven waar deze steeg tot ongeveer anderhalf uur. Op die blaadjes was de opgeeftijd (verblijftijd op het blaadje na de laatste ontmoeting met een gastheer) ook hoger dan op blaadjes met andere gastheertypes. De zoekactiviteit (tijd besteed aan zoeken, uitgedrukt als percentage van de totale verblijftijd min de tijd besteed aan gastheerbehandeling) steeg van 34 % op schone blaadjes tot 49 % op blaadjes met honingdauw, tot 60 % op blaadjes met grijze poppen, en tot meer dan 78 % op blaadjes met L1 larven. De gastheerbehandelingstijd was lager dan 5 %. De loopsnelheid was respectievelijk 0.9, 1.5 en 1.7 mm/s bij 15-16, 19-21 en 25-27 °C en de breedte van het zoekpad was 0.8 mm. Op blaadjes met één L1 larve, vonden minder ontmoetingen met gastheren plaats (1.95 – 2.35) dan op die met vier L1 larven (6.10 – 6.68), terwijl op blaadjes met vier grijze poppen het aantal ontmoetingen (2.05) vergelijkbaar was met dat op blaadjes met één gastheer. Het aantal gastheren dat geparasiteerd werd was lager op blaadjes met één L1 gastheer (0.31-0.33) dan op die met vier L1 gastheren (1.42-1.65). Het percentage aanvaarding van de verschillende gastheertypes (= parasiteren van een gastheer van een bepaald type na een ontmoeting) was: 71 % voor ongeparasiteerde L1 larven, 33 % voor recent, zelf-geparasiteerde larven, 62 % voor L1 larven die recent geparasiteerd werden door een andere sluipwesp van dezelfde soort, en 0 % voor grijze wittevliegpoppen. Superparasitisme van wittevlieglarven die een eigen eitje bevatten (= zelf-superparasitisme) of een eitje bevatten van een andere sluipwesp van dezelfde soort (conspecifiek superparasitisme) was aanzienlijk. De minimale tijd nodig voor een succesvolle ovipositie was 23 s. Op besmette blaadjes was de zoekefficiëntie van *A. fuscipennis* hoger dan deze van *E. formosa*. Dit wordt verklaard door de hogere loopsnelheid, de kortere gastheerbehandelingstijd, de hogere loopsnelheid en het breder zoekpad. Het is echter niet duidelijk of de globale zoekefficiëntie van *A. fuscipennis* in een gewas hoger is dan die van *E. formosa*. *A. fuscipennis* blijft driemaal langer op schone blaadjes dan *E. formosa*. Deze langere verblijftijd verhoogt het ontdekken van gastheren op blaadjes met lage gastheerdichtheden maar leidt tot lange zoekperiodes op blaadjes zonder gastheren.

De biologische controlecapaciteit van *A. fuscipennis* door werd daarna getest in twee kassen met een gemiddelde temperatuur van 16-17 °C (Hoofdstuk 7). Vanaf de start van de experimenten en gedurende 13 weken werden wekelijks 5 *A. fuscipennis* poppen per m$^2$ geïntroduceerd. Biologische controle werd verkregen gedurende 5 maanden in de plastic kas en gedurende 3 maanden in de glazen kas, waarna de volwassen *T. vaporariorum* populatie steeg tot een maximum van bijna 50 per plant in beide kassen. Het parasiteringspercentage was initieel hoger dan 80 % maar daalde dan tot 56 % in de plastic kas en tot 20 % in de glazen kas. Verondersteld wordt dat *A. fuscipennis* door zijn korte levensduur zijn volledig

reproductief potentieel niet aan de dag kan leggen in deze omstandigheden en niet geschikt is om *T. vaporariorum* op een laag niveau te houden. Dit is echter niet met zekerheid te stellen daar de resultaten negatief beïnvloed werden door toepassing van pesticiden en de onnatuurlijke mortaliteit van *A. fuscipennis* wijfjes veroorzaakt door klierhaartjes op de tomatenstengels. Het gebruik van enkel *A. fuscipennis* voor biologische controle van *T. vaporariorum* in kastomaten op de hoogvlakte van Bogota wordt daarom niet aangeraden.

Om de derde vraag (Leidt het simultaan gebruik van beide sluipwespen tot een betere controle?) te beantwoorden werd eerst het foerageergedrag van *A. fuscipennis* en *E. formosa* op tomatenblaadjes met 20 *T. vaporariorum* larven in het eerste of derde larvaal stadium bepaald (Hoofdstuk 5). Tien van deze larven werden vooraf geparasiteerd en bevatten een conspecifiek (van de dezelfde parasitoide soort) of heterospecifiek (van de andere parasitoide soort) eitje of larve. Het type gastheer (bepaald door gastheerstadium en/of voorafgaande parasitering) beïnvloedde het foerageergedrag van geen van beide parasitoiden. Beide parasitoiden discrimineerden goed tussen ongeparasiteerde larven en zelf-geparasiteerde larven, discrimineerden in beperkte mate larven geparasiteerde door een conspecifiek wijfje en discrimineerden larven die geparasiteerd waren door een heterospecifiek wijfje in het geheel niet. Zelf-superparasitisme, conspecifiek superparasitisme en multiparasitisme werden geobserveerd voor beide parasitoiden. Superparasitisme resulteerde altijd in het uitkomen van één parasitoide. Multiparasitisme resulteerde in de meeste gevallen in het uitkomen van een parasitoide van de soort die eerst parasiteerde. Geen van beide parasitoiden vertoonde een competitief voordeel wanneer larven van beide soorten in dezelfde wittevlieg larve aanwezig waren. Gebaseerd op deze gegevens is het moeilijk om uit te maken wat het resultaat zou zijn op biologische controle als beide sluipwespen tegelijkertijd geïntroduceerd worden: gelijkaardige, betere of minder goede controle. Om dit te bepalen werden opnieuw kasproeven opgezet.

Biologische controle van *T. vaporariorum* door de simultaanintroductie van *A. fuscipennis* en *E. formosa* werd getest in twee kassen met een gemiddelde temperatuur van 16-17 °C (Hoofdstuk 7). Vanaf de start van de experimenten en gedurende 13 weken werden wekelijks 2.5 poppen van *E. formosa* en 2.5 poppen van *A. fuscipennis* per m$^2$ geïntroduceerd. Biologische controle was succesvol in beide kassen. De *T. vaporariorum* populaties waren over het algemeen lager dan 1.2 volwassen *T. vaporariorum* per plant en het parasiteringspercentage was meestal 90 % of hoger. Het parasitisme werd vooral veroorzaakt door *E. formosa* en in beperkte mate door *A. fuscipennis*. Daarom, wordt *E. formosa* aangeraden om *T. vaporariorum* populaties op lage niveaus te houden in onverwarmde kassen gesitueerd op grote hoogte in de tropen, zoals op de hoogvlakte van Bogota. Wanneer hoge *T. vaporariorum* populaties verwacht worden of voor de controle van plekken met hoge wittevliegdensiteit wordt, naast het gebruik van *E. formosa*, ook de introductie van *A. fuscipennis* aangeraden omwille van de hoge eilegfrequentie van deze laatste sluipwesp.

In Hoofdstuk 8 worden de resultaten samengevat en bediscussieerd, en suggesties gemaakt voor verder onderzoek.

En Colombia, el control biológico de plagas en cultivos bajo invernadero se aplica solamente en escala limitada en ornamentales y hasta ahora no existe en cultivos de hortalizas. La producción de hortalizas bajo invernadero es un desarrollo reciente y busca disminuir las pérdidas debido a plagas y enfermedades que ocurren en la producción al aire libre. El espectro de plagas en estos sistemas de producción varía con la altura, siendo más amplio en clima intermedio (altura 1800-2000 m.s.n.m.) que en clima frío como el de Sabana de Bogotá (altura 2660 m.s.n.m.). En cultivos experimentales ubicados en la Sabana de Bogotá en los cuales no se aplican insecticidas, la mayoría de las plagas se controla por debajo del umbral económico de daño por parasitóides que ocurren naturalmente, con excepción de la mosca blanca de los invernaderos, *Trialeurodes vaporariorum*. Por lo tanto, con un sistema de control biológico para *T. vaporariorum*, la producción de tomate sin el uso de insecticidas se vuelve posible. En esta tesis se evaluaron dos parasitóides para el control biológico de *T. vaporariorum*: el parasitóide introducido *Encarsia formosa* y el nativo *Amitus fuscipennis*.

*E. formosa* pertenece a la familia Aphelinidae y fue descrito por Gahan en 1924 con base en especímenes de Idaho, EEUU. El genero *Encarsia* contiene más de 200 especies, de las cuales solo *E. formosa* se produce comercialmente para controlar moscas blancas en invernaderos. *E. formosa* es usada mundialmente y fue introducida en Colombia por primera vez en 1986. *A. fuscipennis* pertenece a la familia Platygastridae y fue descrito por MacGown & Nebeker en 1978. Hay 19 especies de *Amitus* descritas, dos de las cuales parasitan mosca blancas que son plagas en invernaderos: *A. fuscipennis* parasita *T. vaporariorum* y *A. bennetti* parasita *Bemisia tabaci*. Se ha encontrado *A. fuscipennis* en el norte de América del Sur y en América Central. En Colombia se puede encontrar abundantemente en clima media junto con su hospedero, *T. vaporariorum*. Ambos parasitoides se reproducen por telitoquía, pero *E. formosa* es sinovigénico mientras *A. fuscipennis* es proovigénico.

En el capitulo 1, se revisaron los factores que promovieron el cambio de control químico al Manejo Integrado de Plagas. Para el control biológico de *T. vaporariorum* en las condiciones específicas de los invernaderos en la Sabana de Bogotá, tres preguntas fueron formuladas y discutidas: (1) Es *E. formosa* capaz de controlar *T. vaporariorum* bajo las condiciones de invernadero en la Sabana de Bogotá? (2) Es el parasitoide nativo *A. fuscipennis* una alternativa para *E. formosa*? (3) Lleva el uso de ambos parasitóides a un mejor control?

Para responder la primera pregunta se evaluaron la longevidad, fecundidad, frecuencia de oviposición y tasa intrínseca de crecimiento de *T. vaporariorum* bajo condiciones de invernadero en la variedad de tomate de mesa Boris (Capitulo 2). Con temperatura promedio de 16 °C y humedad relativa promedio de 81 %, la longevidad de hembras y machos fue 36.5 y 47.2 días respectivamente. La fecundidad fue 208.5 huevos por hembra, la frecuencia de oviposición fue 5.7 huevos por hembra viva por día, y la tasa intrínseca de crecimiento ($r_m$) fue 0.0645. Estos valores son superiores a los resultados de investigaciones anteriores desarrollados a la misma temperatura pero con otras variedades de tomate. Esto confirma que variedades de tomate de mesa son mejores plantas hospederas para *T. vaporariorum* que

variedades de tomate redondo. Comparado con los resultados de un estudio anterior desarrollado en Holanda en variedades de tomate de mesa, la longevidad observada en el presente trabajo fue inferior, la frecuencia de oviposición superior y la fecundidad similar. La tasa intrínseca de crecimiento de *E. formosa*, estimada para las mismas condiciones experimentales, fue 0.0974, lo que es superior a la de *T. vaporariorum*. Eso indica que el control biológico de *T. vaporariorum* con *E. formosa* debería ser posible en invernaderos en la Sabana de Bogotá.

Posteriormente, se evaluó el control biológico de *T. vaporariorum* con *E. formosa* durante tres ciclos de producción consecutivos en tomate de mesa en un invernadero de vidrio y en uno de plástico (Capitulo 6). El desarrollo de la población de adultos de *T. vaporariorum* y el parasitismo por *E. formosa* fueron seguidos durante 26-28 semanas. El promedio de la temperatura fue cerca de 16 °C en el invernadero de plástico y cerca de 17 °C en el invernadero de vidrio. Se introdujo *E. formosa* a una tasa de 3 adultos por m$^2$ por semana en 1997, y respectivamente 3 y 5 pupas por m$^2$ por semana en 1998 y 1999, desde el inicio de los experimentos hasta llegar a un total de cerca 66 parasitoides introducidos. En 1997, la población de adultos de mosca blanca incrementó en el invernadero de plástico exponencialmente hasta llegar a un pico de 76 adultos por planta. En este momento, la población de mosca blanca en el invernadero de vidrio llegó a un pico de solamente 12 adultos por planta. El porcentaje de parasitismo fluctuó entre 42 y 82 % en el invernadero de vidrio y entre 28 y 47 % en el invernadero de plástico. En 1998, la población de *T. vaporariorum* salió fuera de control en ambos invernaderos y llegó a un máximo de 80 y 53 adultos por planta en el invernadero de plástico y de vidrio respectivamente. El porcentaje de parasitismo fluctuó entre 55 y 97 % en el invernadero de vidrio y entre 32 y 84 % en el invernadero de plástico. En 1999, el control biológico fue exitoso en ambos invernaderos. Durante la mayor parte del tiempo, las poblaciones de *T. vaporariorum* fueron inferiores a 1.2 adultos por planta y el porcentaje de parasitismo fue igual o superior a 80 %. Se supone que la mayor temperatura en el invernadero de vidrio fue la razón principal del mayor parasitismo encontrado en este invernadero comparado con el del invernadero de plástico. Los resultados exitosos de 1999 muestran que control biológico de *T. vaporariorum* con *E. formosa* es posible en las condiciones de invernaderos situados en los trópicos altos como la Sabana de Bogotá, caracterizados por días cortos y temperaturas bajas.

Poco se conocía sobre *A. fuscipennis* cuando se inició esta investigación, por lo tanto, para responder la segunda pregunta (Es el parasitoide nativo *A. fuscipennis* una alternativa para *E. formosa*?), se necesitaba de estudios básicos acerca del ciclo de vida y de la eficiencia de búsqueda de este parasitóide, antes de probarlo en invernadero. Los parámetros poblacionales de *A. fuscipennis* como parasitóide de *T. vaporariorum* fueron determinados en tomate a 15, 20, 25 y 30 °C (Capitulo 3). Se determinaron la duración del desarrollo de huevo a adulto, la mortalidad, la longevidad, la fecundidad, la frecuencia de oviposición y el período de postoviposición fueron y se estimaron relaciones en función de la temperatura para cada uno de estos parámetros. La duración del desarrollo de huevo a adulto tuvo un máximo de 61 días a 15 °C y se redujo a 22 días a 30 °C. La mortalidad en el estado pupal del parasitoide (estado gris) fue menos de 2% a temperaturas inferiores a 30 °C, y fue 60 % a 30 °C. La longevidad fluctuó entre 3 (30 °C) y 18 días (15 °C). La fecundidad incrementó de 338 huevos·hembra$^{-1}$ a 15 °C a un máximo de 430 huevos·hembra$^{-1}$ a 25 °C, y después se redujo a 119 huevos·hembra$^{-1}$ a 30 °C. La frecuencia de oviposición varió entre 3 y 46 huevos·hembra$^{-1}$·día$^{-1}$ y tuvo su máximo en el primer día después de la emergencia del parasitóide, excepto a

15 °C, donde el máximo fue en el segundo día después de la emergencia. La $r_m$ incrementó de 0.090 a 15 °C a un máximo de 0.233 a 25 °C, y después se redujo a 0159 a 30 °C. La $r_m$ de *A. fuscipennis* es superior a la de *T. vaporariorum* e incluso a la de *E. formosa* a temperaturas inferiores a 30 °C. Por lo tanto, *A. fuscipennis* es un candidato potencial para el control biológico de la mosca blanca de los invernaderos.

Sin embargo, para poder controlar *T. vaporariorum* en el invernadero, el parasitóide debe encontrar y parasitar de manera eficiente su hospedero. Por lo tanto, como un segundo paso en su evaluación, se estudió el tiempo de estadía y la distribución del tiempo en las diferentes actividades de *A. fuscipennis* en foliolos de tomate limpios a varias temperaturas, en foliolos con miel de rocío, y en foliolos con 1 o 4 larvas L1 sin parasitar o 4 pupas grises de *T. vaporariorum* (Capitulo 4). En foliolos limpios, el tiempo de estadía fue cerca de una hora a 20, 25 y 30 °C, y tres horas a 15 °C. El tiempo de estadía no se incrementó en foliolos con miel de rocío o en foliolos infestados, excepto para foliolos con cuatro larvas L1 sin parasitar, donde se incrementó para aproximadamente hora y media. En estos foliolos, el tiempo de abandono (tiempo en el foliolo después del último encuentro con un hospedero) fue también superior al de foliolos con otros tipos de hospederos. La actividad de búsqueda (tiempo que el parasitoide está buscando, expresado como porcentaje del tiempo total de estadía, menos el tiempo de manejo de hospederos) se incrementó de 34 % en foliolos limpios, a 49 % en foliolos con miel de rocío, a 60 % en foliolos con pupas grises, y a más de 78 % en foliolos con larvas L1. El tiempo de manejo de hospederos fue inferior a 5 % en todos los tratamientos. La velocidad de locomoción fue 0.9, 1.5 y 1.7 mm/s a 15-16, 19-21 y 25-27 °C, respectivamente, y el ancho del camino de búsqueda fue 0.8 mm. En foliolos con una larva L1, menos hospederos fueron encontrados (1.95 – 2.35) que en estos con cuatro larvas L1 (6.10 – 6.68), mientras en foliolos con cuatro pupas grises, el número de encuentros (2.05) fue similar al observado en foliolos con un solo hospedero. El número de hospederos parasitados en foliolos con una larva L1 (0.31-0.33) fue inferior al encontrado en foliolos con cuatro larvas L1 (1.42-1.65). La aceptación de los diferentes tipos de larvas por parte de las hembras fue: 71 % para larvas L1 sin parasitar, 33% para larvas L1 recientemente parasitadas por si misma, 62 % para larvas recientemente parasitadas por un conespecífico y 0 % para pupas de mosca blanca que contenían una pupa del parasitóide. El superparasitismo propio y conespecífico fue considerable. El tiempo mínimo de una oviposición exitosa fue 23 s. En foliolos infestados, la eficiencia de búsqueda de *A. fuscipennis* fue superior a la de *E. formosa*. Esto se explica por su superior actividad de búsqueda, inferior tiempo de manejo de hospederos, superior velocidad de locomoción y más amplio camino de búsqueda. Sin embargo, no es tan claro si la eficiencia de búsqueda global dentro de un cultivo de *A. fuscipennis* es superior a la de *E. formosa*. El tiempo de estadía en foliolos limpios es tres veces mayor que el de *E. formosa*, lo que por un lado aumenta el descubrimiento de hospederos en foliolos con densidades bajas de mosca blanca pero por otro lado lleva a largos periodos de búsqueda en foliolos limpios.

Por lo tanto, se necesitaba probar el control biológico de *T. vaporariorum* con *A. fuscipennis* en invernadero. Esto se hizo en dos invernaderos con una temperatura promedio de 16-17 °C (Capitulo 7). Durante 13 semanas se introdujeron en ambos invernaderos 5 pupas del parasitoide por $m^2$ por semana. Se obtuvo control biológico durante 5 meses en el invernadero de plástico y 3 meses en el invernadero de vidrio. Después de estos periodos, las poblaciones de adultos de *T. vaporariorum* se incrementaron a un máximo de cerca de 50 adultos por planta en ambos invernaderos. El porcentaje de parasitismo fue inicialmente

159

superior a 80 %, pero después se redujo a 56 % en el invernadero de plástico y a 20 % en el invernadero de vidrio. Aunque la aplicación de pesticidas y la mortalidad no natural de adultos de *A. fuscipennis* causado por la presencia trichomas glandulares en los tallos de las plantas de tomate influenciaron negativamente los resultados, se supone que la corta longevidad de *A. fuscipennis* impidió el parasitoide de desarrollar su alto potencial reproductivo en cultivos con baja densidad de hospedero. Por lo tanto, no se recomienda el uso de solo *A. fuscipennis* para mantener *T. vaporariorum* a niveles bajos en cultivos de tomate en la Sabana de Bogotá.

Para responder la tercera pregunta (Lleva el uso de ambos parasitóides a un mejor control?), primero se hizo un experimento en laboratorio para evaluar el comportamiento de búsqueda de *A. fuscipennis* y *E. formosa* en foliolos de tomate con 20 *T. vaporariorum* larvas L1 o L3 (Capitulo 5). Diez de estas larvas de mosca blanca fueron previamente parasitadas y contenían un huevo o una larva de un parasitóide conespecífico o heteroespecífico. El tipo de hospedero (determinado por el estado del hospedero o parasitación previa) no influenció el comportamiento de búsqueda de ninguno de las dos especies de parasitóides. Hembras de ambas especies discriminaron bien larvas no parasitadas y larvas parasitadas por ella misma, pero discriminaron poco larvas parasitadas por un parasitoide conespecífico y no discriminaron larvas parasitadas por un parasitoide heteroespecífico. Superparasitismo propio, superparasitismo conespecífico y multiparasitismo fueron observados para ambas especies de parasitoides. Superparasitismo siempre resultó en emergencia de un individuo, y multiparasitismo resultó en la mayoría de las veces en emergencia de un individuo de la especie de parasitóide que parasitó primero. No se encontró superioridad para ninguno de las dos especies de parasitóides cuando compiten dentro del mismo hospedero. Con base en estos datos es difícil prever el resultado del control biológico cuando ambas especies son introducidas simultáneamente. Para responder esta pregunta, se hicieron nuevamente experimentos en invernaderos.

Se evaluó el control biológico de *T. vaporariorum* con la introducción simultánea de *A. fuscipennis* y *E. formosa* en dos invernaderos con una temperatura promedio de 16-17 °C (Capitulo 7). Durante 13 semanas se introdujeron 2.5 pupas de *E. formosa* y 2.5 pupas de *A. fuscipennis* por m$^2$ por semana. El control biológico fue exitoso en ambos invernaderos. Las poblaciones de *T. vaporariorum* fueron generalmente inferiores a 1.2 adultos por planta y el porcentaje de parasitismo, causado sobre todo por *E. formosa*, fue igual o superior a 90 % durante la mayor parte del tiempo. Por lo tanto, se recomienda el uso de *E. formosa* para mantener poblaciones de *T. vaporariorum* a niveles bajos en invernaderos situados en los trópicos altos como la Sabana de Bogotá. Cuando se esperan altas poblaciones de *T. vaporariorum* o para controlar focos, se recomienda, adicional al uso de *E. formosa*, introducir también *A. fuscipennis* por la alta frequencia de oviposición de este último parasitoide.

En el Capitulo 8 se resumieron y discutieron los resultados, y se hicieron sugerencias para futuros investigaciones.

161

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Piracicaba, Oktober 2001

## Publications

### Related to this thesis

De Vis, R., L.E. Fuentes & J.C. van Lenteren, 1999. Development of biological control of *Trialeurodes vaporariorum* with *Encarsia formosa* and *Amitus fuscipennis* on greenhouse tomato in Colombia. Bulletin IOBC-WPRS 22(1): 267-270.

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Raf M.J. De Vis was born in Aalst, Belgium on January 11, 1965. He graduated in agronomy at the Catholic University of Leuven, Belgium in 1989. From 1989 to 1991, he worked as a consultant for the glasshouse cut flower and ornamental plant industry in Belgium and France. During the same time he assisted research at the Research Centre for Ornamentals (Proefcentrum Sierteelt, PVS) in Destelbergen, Belgium. From 1991 to 1996 he worked for the Catholic University of Leuven as a co-ordinator and researcher at the Horticultural Research Centre (Centro de Investigaciones y Asesorias Agroindustriales, CIAA) of the Jorge Tadeo Lozano University, Colombia. During this time he co-ordinated research in vegetables, cut flowers and fruits. Afterwards, he worked another four years for the Jorge Tadeo Lozano University, as co-director of the CIAA and co-ordinator of the Program of Integrated Pest and Disease Management, with emphasis on biological control.

On the front cover:
Pupae of *T. vaporariorum*: white pupae unparasitized, black pupae parasitized by *E. formosa* and grey pupae parasitized by *A. fuscipennis*, and adults of the respective insects (Photos R. De Vis, E. Torrado and L. Fuentes).

On the back cover:
View of the Horticultural Research Centre (Centro de Investigations y Asesorías Agroindustriales) of the University of Bogota Jorge Tadeo Lozano (Photo Harold Ubaque).

Cover by Piet Kostense