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Hyperparasitism behaviour of the autoparasitoid *Encarsia tricolor* on two secondary host species

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Abstract Hyperparasitism by virgin female *Encarsia tricolor* was studied by direct observation of its behaviour when contacting two secondary host species (*Encarsia formosa* and *E. tricolor*) at different host stages (first and second larval stage, third larval stage, and pupal stage). The searching and hyperparasitism behavioural sequence of *E. tricolor* was independent of the host stage of the whitefly (*Aleyrodes proletella*), and was similar to several related primary parasitoid species. In experiments with equal numbers of secondary hosts, encounter frequencies were equal for both secondary host species in all developmental stages observed. However, rates of hyperparastism were different according to host stage and host species. Hosts in the late larval stages were most preferred for hyperparasitization and the heterospecific *E. formosa* was more preferred as a secondary host than the conspecific, *E. tricolor*, in particular from the prepupal stage onwards. The window of vulnerability, i.e., the duration of the period in which a secondary host is susceptible to hyperparasitism, was largely determined by the occurrence and rate of melanization after the onset of pupation. The duration of a successful hyperparasitization event was longer than one that failed. Superparasitism occurred only once in all cases. The potential effect of autoparasitoids on biological control programs and the consequences for selection and release of an effective, yet ecologically safe agent are discussed.

Keywords *Encarsia formosa* · Hymenoptera · Aphelinidae · Autoparasitoid · Hyperparasitoid · Behaviour · *Aleyrodes proletella* · Environmental effects

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Introduction

It is commonly believed that the application of biological control is a safe alternative to pesticides, and a wide range of parasitoids has been released successfully as biological control agents (Gurr and

Wratten 2000). Several species of aphelinid parasitoids have been used to help suppress populations of the two most economically important whitefly species, *Trialeurodes vaporariorum* (Westwood) (greenhouse whitefly) and *Bemisia tabaci* (Gennadius) (tobacco whitefly) (both Hemiptera: Aleyrodidae; e.g. Gerling et al. 2001; van Lenteren and Woets 1988; van Lenteren et al. 1996). A number of these parasitoids are members of the genus *Encarsia* (Förster), such as *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae). Whereas the economic benefits are clear, the ecological effects of an introduced species on the indigenous fauna are not. During the past decades, Howarth (1991) and others argued that the import and release of exotic species for biological control might create problems for the indigenous fauna. Recent reviews show that such exotic natural enemies have in some cases caused negative effects on non-target organisms and the environments (Louda et al. 2003; van Lenteren et al. 2006).

With regard to biological control of exotic greenhouse whiteflies in Europe, the introduced species *E. formosa* may encounter native species of whiteflies as well as native parasitoids, like *Encarsia tricolor* (Förster). The possibility of such interactions raises several questions: Will the exotic and indigenous parasitoids coexist, or will one of them lose the competition and will it be displaced? What will the effect be on the dynamics of indigenous whitefly populations? And what kind of effect has the exotic biological control agent on the indigenous ecosystem? These questions are related to the interactions between species of parasitoids at the level of parasitoid behaviour, life history and the interaction between these parasitoids and their hosts (Murdoch 1996).

Another question we are faced with is what the effect could be of the release of an exotic primary parasitoid on the population dynamics and survival of facultative autoparasitoids. This question relates to the rather typical biology of several aphelinid parasitoids (e.g. Hunter and Kelly 1998). Males and females of hymenopteran parasitoid species in the Aphelinidae develop in or on different kinds of hosts, and are therefore called heteronomous hyperparasitoids, more specifically autoparasitoids (Hunter and Woolley 2001). Fertilized female eggs develop as obligate primary parasitoids on their primary hosts, whiteflies or scale insects. On the other hand, unfertilized male eggs develop as secondary parasitoids (hyperparasitoids) on larvae or pupae of their own or other primary parasitoid species. Mated female autoparasitoids may lay both fertilized and unfertilized eggs, but virgin females can only lay unfertilized eggs in secondary hosts (Gerling 1966). It is generally thought that parasitoids with hyperparasitic behaviour are injurious in biological control programs (Luck et al. 1981) and it is standard quarantine procedure to exclude exotic obligate hyperparasitoids from biological control programmes (Sullivan and Völkl 1999). However, several autoparasitoid species have been successfully introduced as biological control agents (Bográn and Heinz 2002), some introductions have, however, resulted in problems, such as those of *Encarsia pergandiella* Howard. The latter species was imported into Italy to control the greenhouse whitefly, but established outside, and can now be found all around the Mediterranean Area (Portugal, Spain, Italy, France, Tunesia) (Loomans and van Lenteren 1999), regionally upsetting successful biological control applications by primary parasitoids in greenhouses (Gabarra et al. 1999, 2003). In New Zealand there have been several instances where *E. pergandiella* has been present in significant numbers on greenhouse tomato crops and although large introductions of *E. formosa* were made weekly, control of the pest was lost (John Thompson, personal communication 2005).

The host range of autoparasitoids is much wider and multitrophic effects are larger than that of primary parasitoids, because of their heteronomous hyperparasitoid behaviour. Therefore, the concern about the direct and indirect ecological effects of autoparasitoids in biological control remains wide open (Rosen 1981). Since autoparasitoids occupy two trophic levels, it is not proper to separate the overall interactions into several two-species interactions (host-parasitoid or primary-hyperparasitoid) (Hassell 2000). We propose to study such relationships from two viewpoints: (1) the effect of two competing parasitoids for one primary host; (2) and the effects of the interactions between a primary and a hyperparasitoid (secondary parasitoid) (Fig. 1; May and Hassell 1981).

In the current study we considered the relationship between an exotic biological control agent (the strictly primary parasitoid *E. formosa*) and a native parasitoid (the facultative autoparasitoid *E. tricolor*)

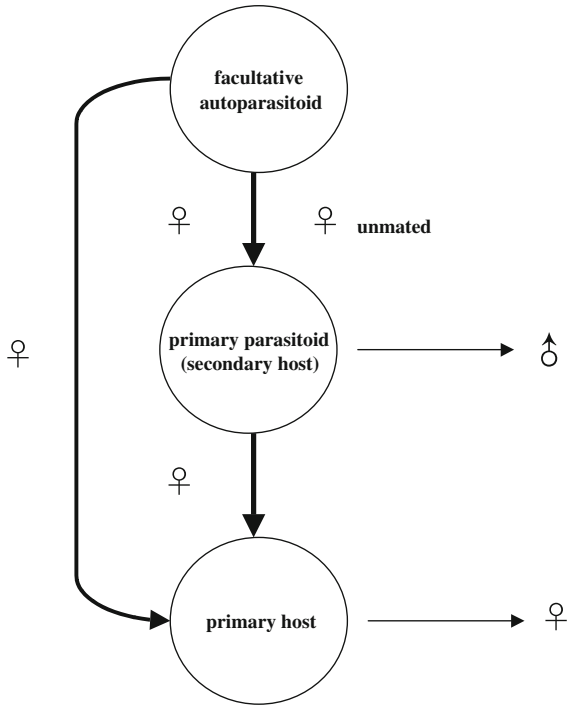

Fig. 1 Diagram to illustrate the relationships of a three-species system, which is containing an autoparasitoid (after May and Hassell 1981)

on a native host (the cabbage whitefly, *Aleyrodes proletella* L.). The autoparasitoid *E. tricolor* is widely distributed throughout the West Palaearctic region. Females are solitary endoparasitoids of several whitefly species, including *A. proletella*, *Aleurotuba jelinekii* (Frauenfeld) and *T. vaporariorum* (Williams 1995). Males are solitary endoparasitoids of primary parasitoids of whiteflies, such as *E. inaron* (Walker), *E. formosa* and females of its own kind (Avilla and Copland 1987; Williams 1991, 1996). Both the primary female and secondary male have the following developmental stages: egg, first, second and third instar larva, prepupa and pupa. The developmental rate of both the male and female immature stages of the parasitoid is influenced by the host stages which are parasitized (Williams 1995) and the male has a shorter developmental time than the female (Avilla and Copland 1987). Until now, no direct behavioural records of hyperparasitic behaviour in *E. tricolor* have been reported, though these kind of observations assist in evaluating the efficiency of natural enemies in biological control programmes (van Roermund et al. 1996). Direct observations and subsequent comparison of the behavioural strategy towards conspecific and heterospecific secondary hosts may be used also to clarify the issues on the role of autoparasitoids in biological control mentioned above. From both systems mentioned (Avilla and Copland 1987; Williams 1991)—directly or, as in the Avilla's paper, indirectly—a preference emerged of *E. tricolor* females towards heterospecific secondary hosts for male egg oviposition. Behavioural observations will assist in testing the hypothesis that autoparasitoids can discriminate between species of secondary hosts to reduce self-hyperparasitism and henceforth this preference may negatively affect the outcome of a biological control programme. We specifically studied the host preference of the native autoparasitoid *E. tricolor* when offered different secondary host species, and the effect that different life stages of the host may have on this preference.

Materials and methods

Insect and plant rearing

The cabbage whitefly, *Aleyrodes proletella*, was used as primary host. It was cultured on cabbage (Brussels sprouts, *Brassica oleracea gemmifera* cv. Cyrus) in a greenhouse at 21°C and 16L:8D. As primary parasitoids we used *E. formosa* and *E. tricolor* females. *E. formosa* was obtained from a commercial company (EnStrip®, Koppert Biological Systems, The Netherlands) and was reared on cabbage with *A. proletella* in ventilated plastic cages (45 × 30 × 35 cm). *E. tricolor* was collected from cabbage fields (Wageningen, The Netherlands) and also reared in cages on cabbage and cabbage whitefly. During the rearing process, clip cages (20 mm diameter) were used to introduce a certain number of whiteflies or parasitoids to the target leaves.

Host choice tests

Whiteflies were confined to the underside of leaves inside clip cages for 24 h, thus allowing the new generation of whitefly individuals to develop almost synchronously. After 14–15 days, when immature whiteflies were in the late third (L3) to early fourth (L4) nymphal stage, primary parasitoids, *E. formosa* or *E. tricolor*, were introduced in clip cages, and

plants were moved into a climate cell at 25°C. Introduced wasps were removed after 24 h. After some days, when secondary larval parasitoid hosts were present, most unparasitized whiteflies had already emerged, or were in the red-eyed stage and could be easily recognized and removed. It is difficult to recognize parasitoid larvae as such from the outside through the whitefly cuticle when they are still in their early instar stages. By removing unparasitized whiteflies we avoided providing primary whitefly hosts instead of secondary parasitoid hosts to *E. tricolor* during the experiments. Parasitoid immatures (secondary hosts) in the late larval or prepupal stage can easily be recognized by their shape through the transparent whitefly cuticle (primary hosts). The justification of our approach was also proven during dissection, where secondary host larvae were always found.

Choice tests were designed with the two secondary host species offered simultaneously in one arena. The hosts were parasitized whitefly nymphs containing female *E. tricolor* and female *E. formosa* immatures in equal numbers. Twelve hosts of each species in the same stage were provided in one arena to an individual female of *E. tricolor*. A small drop of honey was applied as food source.

Three treatments were performed including three different host stages: first and second instar larvae (L1 and L2), third instar larvae (L3), and prepupa. Between 4 and 12 days after primary parasitoids were introduced, whitefly hosts containing secondary host larvae or prepupae inside were selected and moved to a clean leaflet with a needle and glued with starch. This was done after 4–6 days to obtain L1 and L2 secondary hosts, 8–9 days to obtain L3, and 10–12 days to obtain prepupal hosts. The leaflet was placed on a piece of moist filter paper in a small Petri dish. The size of the leaflets was about 2.5 × 2.0 cm, and the distance between the hosts was about 0.5 cm. Each treatment was repeated 8 or 9 times.

For each treatment, the searching and host-handling behaviour of 48–72 h old *E. tricolor* virgin females was observed under a stereoscope for a maximum period of 2 h. The whole process was recorded by using a handheld computer and The Observer (Noldus IT, Wageningen, The Netherlands) for registration and calculation of behavioural elements. The following behavioural elements we were distinguished: encountering a host, drumming a host, turning on a host, drilling a host with the ovipositor, host feeding, honeydew feeding, walking, standing (including standing still and standing with preening) and jumping (van Lenteren et al. 1980). An observation was aborted when: (1) the wasp spent more than 15 min standing still on the leaflet or left the leaflet immediately after introduction, or (2) the wasp encountered all the provided hosts within the maximum period of 2 h. After the observation period, all hosts were dissected to examine whether parasitoid eggs were present. Parasitoids that left the leaf-surface during the observation period without displaying any searching behaviour, were not considered valid records and were excluded from data analysis.

Data analysis and statistical test

Statistical tests were performed using SPSS 10 (©SPSS Inc. 1989–1999). Results of observations involving different host stages were analyzed using an ANOVA test. A χ^2 test was used to compare the differences between values for the two host types within the same host stage.

Results

Description of searching and parasitization behaviour of *E. tricolor*

The behaviour of virgin *E. tricolor* females can be divided in two parts, searching on the leaf and handling of hosts. When virgin *E. tricolor* females were introduced onto a leaflet, most of them began to search (walking and drumming), using their antennae. They sometimes made short stops during which they either stood still, preened, or fed on the honey. The following sequence of behavioural elements was exhibited when a host was found: encountering a host, drumming a host with antennae, turning on the host, drilling the host with the ovipositor (oviposition posture) or leaving the host. Sometimes host-feeding occurred after drilling a host.

No significant differences ($P > 0.1$) were found in any of the durations of the same behavioural element performed by adult females of *E. tricolor* on the two secondary hosts, *E. formosa* or *E. tricolor* (Fig. 2A). Therefore, the further descriptions of the general behaviour, such as time budget and behavioural

sequences, were made by combining the observations for the two secondary hosts.

Durations of behavioural elements (seconds ± s.e.) dealing with a host were calculated from the moment of encountering a host till leaving that host (Fig. 2A) and include antennal rejection of the hosts (i.e. parasitoids left hosts after antennal testing (drumming or drumming and turning)); ovipositor rejection (i.e. parasitoids inserted ovipositors into hosts after antennal testing, but did not lay any egg); host acceptance (i.e. hyperparasitization, parasitoids inserted their ovipositors into hosts, and laid a male egg inside it); and host-feeding after oviposition posture. Hosts that were rejected after antennal drumming were examined by the parasitoid only for a short time (15.0 ± 1.4 s in *E. formosa*, 12.9 ± 0.8 s in *E. tricolor*). The duration in oviposition posture resulting in host rejection was much longer (332.9 ± 44.8 s for *E. formosa*, 281.6 ± 36.4 s for *E. tricolor*). The time to accept a host and laying an egg was still longer (580.8 ± 73.2 s for *E. formosa*, 563.1 ± 120.1 s for *E. tricolor*). When host-feeding occurred, it always lasted longer than 25 min (1516.9 ± 142.1 s for *E. formosa* and 1770.6 s for *E. tricolor*), and was quite different from the duration of feeding on the honey, which took only about 2 min (Fig. 2B).

Visits to the leaflet were generally short and consisted largely of walking, standing and honey feeding. The average duration of walking was 23.0 ± 51.2 s,

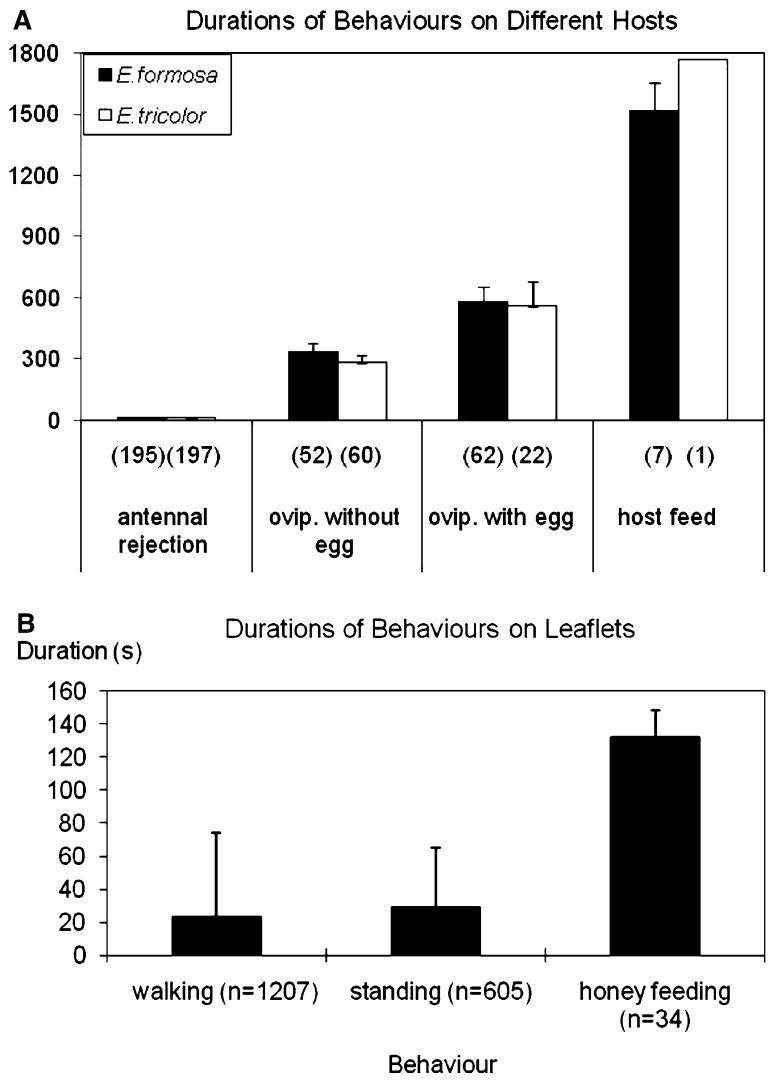

Fig. 2 Average duration (time in seconds ± s.e.) of behavioural elements exhibited by virgin female *E. tricolor* when searching on leaflets and when handling hosts. The figures in brackets under the bars represent the number of times a certain behavioural element was recorded. (**A**) average duration (time in seconds ± s.e.) of behavioural elements (antennal rejection, ovipositor rejection, parasitization and host feeding) on different secondary hosts. (**B**) average duration (time in seconds ± s.e.) of behavioural elements (walking, standing and honey feeding) on leaflets

duration of standing 28.9 ± 36.0 s, and duration of feeding on honey lasts 132.1 ± 16.1 s.

The time budget of *E. tricolor* in this set-up was as follows: a wasp spent on average 66.9% of its time handling hosts, and 33.1% on searching, honey feeding and standing still (Fig. 3). More than half (55.0%) of the total time of handling a host was spent on oviposition behaviour.

A simplified diagram showing the behavioural sequences observed is presented in Fig. 4. Walking was the most frequently observed activity (1207 times), the next in line was standing (605 times) and encountering host (596 times). Drumming always followed upon host encounter. More than half (56.4%) of host encounters and drumming was followed by turning on the host, and 60.7% of this turning was followed by insertion of the ovipositor. After oviposition, females often left the hosts (70.1%) and started walking. However, in 26.0% of the cases they stayed on the host preening for a while before leaving.

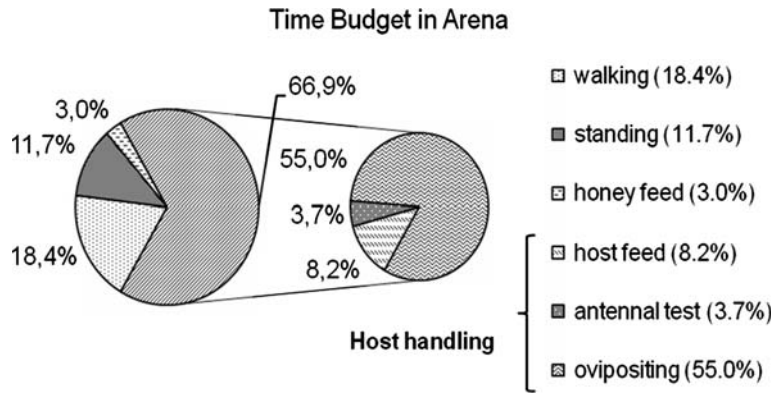

Fig. 3 Time budget of *E. tricolor* when exposed to hosts on part of a leaf in a Petri dish. Total time left, host handling on the right

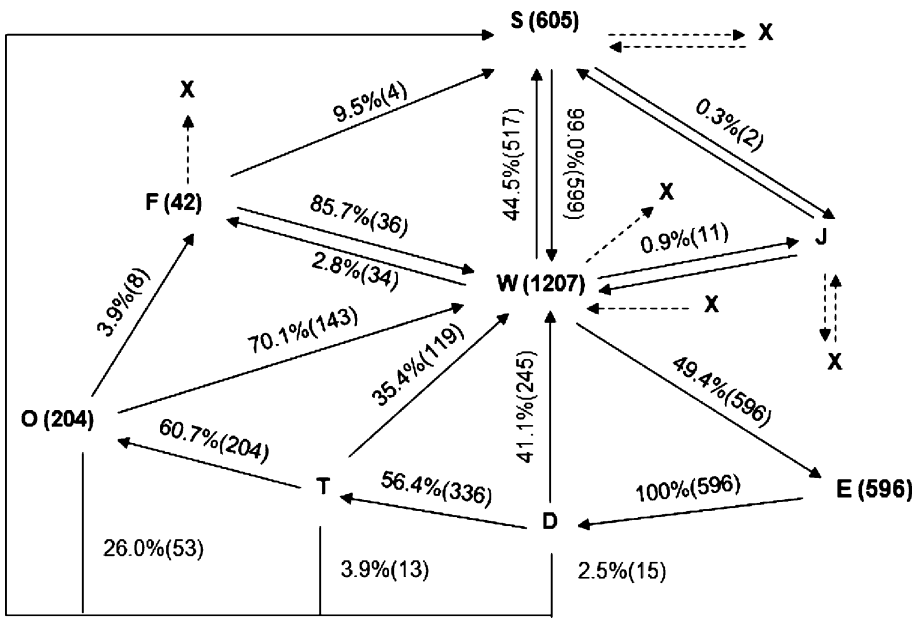

Fig. 4 Diagram of behavioural sequences of virgin *E. tricolor* females as observed in a Petri dish when exposed to equal number of heterospecific and conspecific secondary hosts ($n = 25$): W = walking; S = standing (still or preening); F = feeding (on host or honey); E = host encounter; D = drumming; T = turning on host; O = oviposition posture; J = jumping; X = start/terminate/out of arena. Numbers show the percentages of behavioural elements involved, between brackets are the absolute numbers of behavioural elements observed

In a few cases (3.9%), hosts were used for host-feeding after finishing the oviposition posture: wasps retracted their ovipositor, turned on the host to locate the hole they had made, and started feeding with their heads bent down. We observed that the sequence of drilling, turning and feeding on the host was repeated often, probably to widen the hole or to make a new one.

Effect of the host stage and host species on hyperparasitism behaviour

(1) Parasitism percentages in different host stages and host species

The percentage of hosts encountered, ovipositor drilling (excluding host-feeding), male egg deposition and host feeding on each type of host are shown in Fig. 5 and Table 1. All these percentages were calculated based on the number of hosts provided as 100%.

Parasitoids encountered secondary hosts in similar numbers. Thus the percentages of hosts encountered were the same for the two host species and all stages, and varied between 72.2 ± 5.6% and 85.9 ± 8.0% (Table 1). Host stage clearly affected the parasitoids' acceptance behaviour. When the encountered secondary hosts were *E. formosa* in the L3 and prepupal stages, the host drilling percentages averaged 83.3 and 77.1% respectively, and these percentages are significantly higher than the percentages of host drilling in stage L1/L2 (38.9%).

When *E. tricolor* was the secondary host, the host drilling percentage in the L3 stage was significantly higher than the percentages found for the other two stages. Subsequently, a larger percentage of hosts were found hyperparasitized in stage L3 (37.5 ± 8.3%) when compared to the early larval (L1/L2) and prepupal stages, in which only about 2% of the hosts were hyperparasitized. When the hosts were *E. formosa*, a low percentage of hosts (14.8 ± 4.3%) in early larval (L1/L2) stages were hyperparasitized, a higher percentage in the prepupal stage, and the highest percentage in L3. Thus, we conclude that L3 of both primary host species is the most preferred stage for hyperparasitization.

The percentages of hyperparasitism of *E. formosa* were always higher than those of *E. tricolor*. Significant differences were found for all host stages (Fig. 5). This means that the heterospecific secondary hosts are more preferred than the conspecific ones.

The average absolute number of eggs laid by an individual *E. tricolor* female was strongly dependent on the host stage offered. When exposed to an array of 24 early larval stages during 2 hours 1.0 ± 0.3 eggs were found, while in third larval stages 6.3 ± 0.8 and in prepupa 2.7 ± 0.3 eggs were found. Superparasitism occurred only once in all experiments.

(2) Time budgets on hosts of different stages and species

Table 2 shows the time budget of *E. tricolor* females when dealing with hosts of different stages. The total observation time of wasps on leaflets with the different host species and stages was the same, and the time budgets mentioned in Table 2 are all based on this total time. A clear difference in relative time distributed amongst the different behavioural elements can be seen when referring to different host stages. On all hosts, most time was spent on drilling. The longest drilling times were found on the L3 stage (*E. formosa* 47.0 ± 3.1%, *E. tricolor* 29.4 ± 2.7%) compared to the other two stages. The fact that the longest drilling times were found on the L3 stage was consistent with the highest number of hosts drilled and highest number of oviposition postures observed on this stage (Table 1).

When hosts were in the prepupal stage, wasps spent more time examining the host through antennal drumming than in the other two stages. Host-feeding only occurred in the early larval stages (L1/L2).

Discussion

Searching and parasitization behaviour of *E. tricolor*

Searching and hyperparasitism behaviours exhibited by virgin *E. tricolor* females were similar to behaviours observed for other, primary whitefly parasitoid species such as *E. formosa* (van Lenteren et al. 1980) and *Amitus fuscipennis* MacGown and Nebeker (Manzano et al. 2002). Our observations on *E. tricolor* show that the behavioural duration of accepting hosts (oviposition) was much longer than that of rejecting a

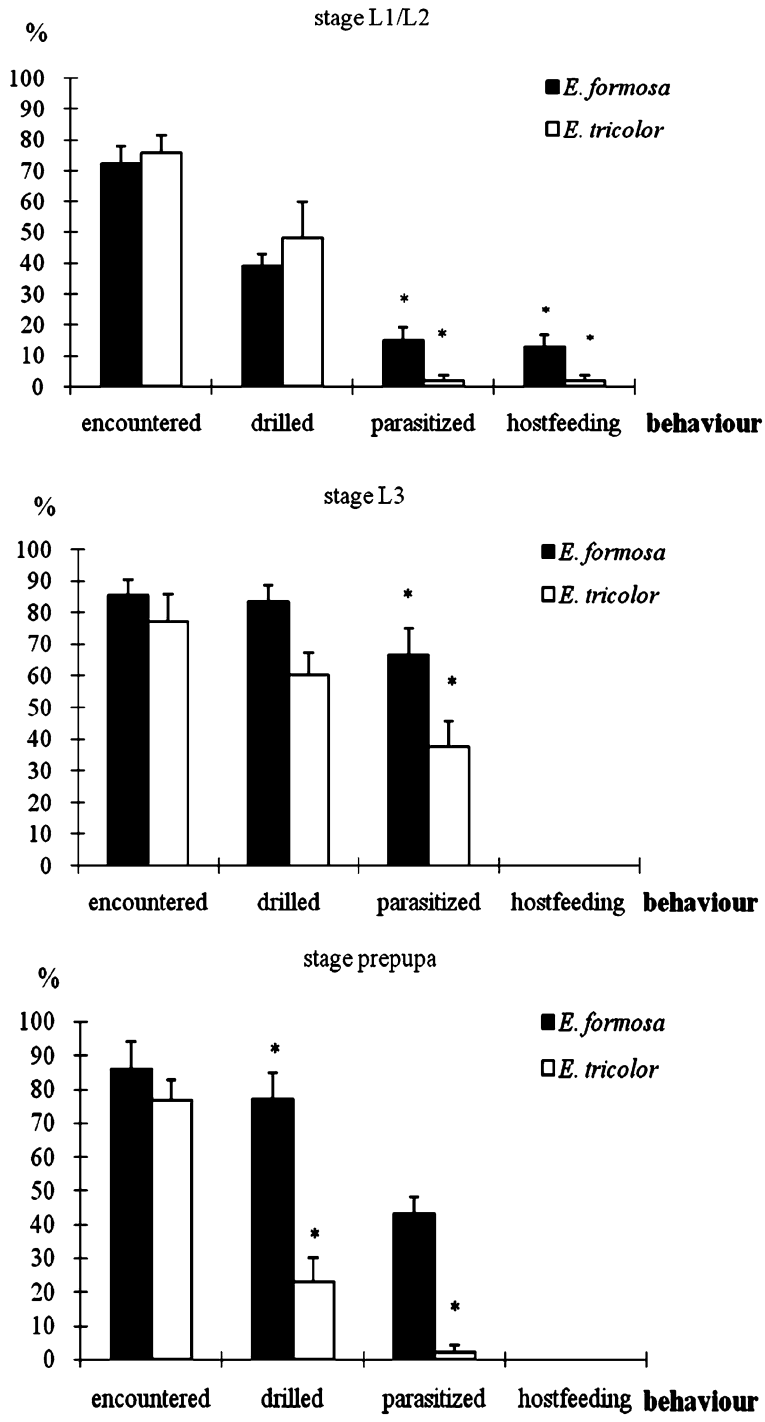

Fig. 5 Percentages (±s.e.) of host encountered, ovipositor drilled, male egg laid and host fed found for different host species and stages (number of host provided for each stage/species was taken as 100%). Values between species within the same host stage are significantly different when followed by an asterisk ($P < 0.05$, χ^2)

host after ovipositor probing. This was also observed for *E. formosa* (van Lenteren et al. 1980), and the time involved in oviposition attitude can thus be used to determine if oviposition has occurred or not. The time needed by *E. tricolor* to lay a female egg was found to be about 200–300 s (Williams 1995), which is much shorter than the time needed for laying a male egg (hyperparasitism, more than 560 s, Fig. 2).

Table 1 Average percentage (±s.e.) of hosts encountered, drilled, parasitized (male egg deposition) and host-fed by virgin females of *E. tricolor*, when two host species were provided simultaneously (12–12) in different stages

Host stage	*N*	% Hosts encountered		% Hosts drilled		% Hosts parasitized		% Host fed upon	
		E. formosa	*E. tricolor*	*E. formosa*	*E. tricolor*	*E. formosa*	*E. tricolor*	*E. formosa*	*E. tricolor*
L1/L2	9	72.2 ± 5.6 a	75.9 ± 5.6 a	38.9 ± 3.9 a	48.1 ± 11.9 a	14.8 ± 4.3 a	1.9 ± 1.9 a	13.0 ± 3.7 a	1.9 ± 1.9 a
L3	8	85.4 ± 4.9 a	77.1 ± 8.9 a	83.3 ± 5.5 b	60.4 ± 7.0 a	66.7 ± 8.3 b	37.5 ± 8.2 b	0 b	0 a
Prepupa	8	85.9 ± 8.0 a	76.6 ± 6.3 a	77.1 ± 7.9 b	22.9 ± 7.0 b	43.2 ± 4.7 c	2.1 ± 2.1 a	0 b	0 a

Values are significantly different when followed by different letters in the same column ($P < 0.05$, ANOVA; χ^2 test used as post hoc test)

The same was found for other autoparasitoids, such as *E. pergandiella* or *Encarsia* spp., which both spent more time to lay hyperparasitic eggs than primary ones (Buijs et al. 1981; Kajita 1989). This might be explained by the fact that more effort has to be paid by autoparasitoids to deposit a male egg inside the secondary hosts, as two layers of cuticles have to be penetrated, instead of only one as in primary hosts. A longer time may also be caused by a more time consuming host selection process to locate the secondary host inside the primary host.

Superparasitism occurred only once during all observations. This indicates that virgin *E. tricolor* females are able to discriminate between secondary hosts hyperparasitized by themselves, and avoid self-superparasitism, similar to what is found for many other primary parasitoids (van Lenteren et al. 1976b; van Lenteren 1981; Nuffio and Papaj 2001). However, Hunter (1989) and Pedata and Hunter (1996) reported that another autoparasitoid *E. pergandiella* does not discriminate between hosts with and without an egg.

Host-feeding only occurred during the early larval secondary host stages. Remarkably, the heterospecific secondary host species *E. formosa* was largely preferred over the conspecific secondary host (seven times out of eight), but this needs to be substantiated as the number of observations was low.

Effects of host stages on hyperparasitism

Hyperparasitizing *E. tricolor* females encountered both species of secondary hosts in similar numbers, regardless the stages offered (Table 1). This indicates that host selection does not occur before a host has been drummed, similar to those reported by van Lenteren et al. (1976a) for the primary parasitoid *E. formosa*. Our observations that the L3 stage is the most preferred stage for hyperparasitization for *E. tricolor* is similar to information provided for *E. tricolor* by Avilla and Copland (1987). These authors found that more males emerged from late larvae to pupae than from early instar larvae (3–4 days), and suggested that either a low oviposition rate or low male survivorship occurred on young secondary hosts. Because we have made direct observations, we can explain their results and conclude that the higher number of males is largely the result of a difference in acceptance of the secondary host, and not the result of an increase in mortality. This may be an evolutionary adaptation of autoparasitoids to decrease the risk of egg-depletion in less suitable hosts. Old larval stages may be more suitable because larger hosts provide better resource for the parasitoids' larvae to develop. For example, Liu and Stansly (1996) and Jones and Greenberg (1999) propose that parasitoids may immediately use host resources in late stages, thereby maximizing the intrinsic rate of increase (r) through decreased generation time, increased fecundity, or both.

A limited egg-load may be a factor influencing a female's searching intensity, oviposition rate and host acceptance (Minkenberg et al. 1992), but this could not have been of influence in our study as of all stages a surplus of hosts was offered. In our experimental conditions an egg-limited parasitoid, as is the case for *E. tricolor,* should maximize the quality of hosts to be parasitized and a long time is expected to select it. The time spent on oviposition was always the most time consuming behavioural element during the maximum observation time of 2 h, as is reflected by the total time in oviposition (Fig 3; Table 2) and the percentages of hosts drilled (Table 1). Yet *E. tricolor* refrained from egg-laying in young secondary hosts, and preferred heterospecific over conspecific hosts in all stages. In

Table 2 Time budget (average percentages of behavioural elements ± s.e.) of virgin female *E. tricolor* in experimental arenas with hosts of different stages and species

Stage	*N*	Time in arena (s)	% Walking time	% Standing time	% Honey feeding	% Antennal testing		% Ovipositing time		% Host feeding	
						E. formosa	*E. tricolor*	*E. formosa*	*E. tricolor*	*E. formosa*	*E. tricolor*
L1/L2	9	6185.2 ± 456.7 a	20.2 ± 2.6 a	11.3 ± 2.7 a	3.6 ± 1.0	1.7 ± 0.3	1.3 ± 0.5	21.3 ± 4.6	19.5 ± 4.4	20.6 ± 5.7	2.9 ± 2.9
L3	8	6016.6 ± 431.5 a	11.1 ± 1.1 b	6.4 ± 1.8 ab	3.4 ± 1.7	1.5 ± 0.4	1.2 ± 0.4	47.0 ± 3.1	29.4 ± 2.7	0	0
Prepupa	8	5822.1 ± 382.5 a	25.4 ± 2.8 a	16.8 ± 3.1 ac	2.3 ± 0.8	3.2 ± 0.8	3.3 ± 0.8	39.8 ± 3.3	9.3 ± 4.1	0	0

Values followed by different letters in the same column are significantly different ($P < 0.05$, ANOVA; χ^2 test used as post hoc test)

addition, the total numbers of eggs laid by *E. tricolor* in our experiments was less than the maximum daily fecundity reported for this species (Williams 1995; Sengonça et al. 2001). An experiment performed by Burger et al. (2006) with *E. formosa* shows similar results in that the time spent on oviposition occupied a large proportion of a wasps' life time. This may indicate that wasps spent more effort on reproduction when hosts are better for parasitism than on host-feeding. When oviposition and host-feeding are considered as a trade-off between reproduction and survival, i.e. current and future reproduction (Jervis and Kidd 1986; Heimpel and Collier 1996), then our results indicate that reproduction prevails over feeding under our experimental conditions.

Effect of host species on hyperparasitism

Several other studies on selection of secondary hosts by *Encarsia* autoparasitoids have been reported (Avilla et al. 1991; Williams 1991; Bográn and Heinz 2002; Pedata and Hunter 1996). In these studies either a preference for heterospecific hosts or no preference was found, but never a preference for conspecific hosts. The results obtained by Avilla et al. (1991) and Williams (1991) indicated that in that case *E. tricolor* preferred heterospecific (*E. formosa* on *T. vaporariorum; E. inaron* (Walker) on *A. proletella*) secondary hosts over conspecific ones. No obvious preference, however, was found for *E. pergandiella* when given a choice between a combination of the conspecific host and a heterospecific host, such as *E. formosa* (Buijs et al. 1981; Pedata and Hunter 1996) and *E. hispida* (as *E. meritoria*) (Pedata and Hunter 1996) on *Trialeurodes vapoariorum*. Bográn and Heinz (2002) showed on the other hand that *E. pergandiella* favoured heterospecific hosts over conspecific hosts (*E. formosa* or *Eretmocerus mundus* Mercet) in the presence of *B. tabaci* as a primary host, but not when a third parasitoid species was present.

What is the mechanism behind the lack or presence of a certain host preference? On one hand, female *E. tricolor* may be able to discriminate between heterospecific and conspecific pupae as put forward by Avilla et al. (1991), on the other hand other autoparasitoid species such as *E. pergandiella* may not (Pedata et al. 2002). Our observations showed that *E. tricolor* could easily discriminate different host species in the prepupal stage through

antennal examination, but females had to insert their ovipositor to discriminate between immature stages of the hosts (Tables 1, 2).

Immatures of the autoparasitoid may have specific mechanisms, such as physical defence (Gerling 1990) and a different life-history strategy, to reduce vulnerability and protect female parasitoid larva from being self-hyperparasitized. Melanized pupae occur in a number of species within the genus *Encarsia*, such as *E. tricolor*, *E. inaron*, *E. hispida* and *E. sophia* (Avilla et al. 1991; Williams 1991; Pedata and Hunter 1996; Hunter et al. 2002). Examining the secondary host choice by the autoparasitoid *E. pergandiella* proved that the melanized pupal sheath in *E. hispida* deterred parasitism (Pedata and Hunter 1996). Immature *Eretmocerus eremicus* Rose & Zolnerowich were vulnerable to parasitism by *E. sophia* for twice the time as were conspecific immature (Hunter et al. 2002). However, the nature of the possible defence of a melanized pupae against hyperparasitism is still unclear. During our experiment, the presence of a melanized pupal sheath of *E. tricolor* occurred soon after the prepupal stage (within one day), but in *E. formosa* this did not occur. Therefore, two hypotheses are put forward by us to explain the preference for the heterospecific host *E. formosa* over the conspecific host by *E. tricolor*: (1) in the process of melanization, the cuticle of the immature, pupal *E. tricolor* female becomes harder and more difficult to penetrate and this physical change leads to a low attack rate (physical defence), (2) the immature *E. tricolor* female has a relative higher developmental speed, and this life-history strategy leaves a shorter time period (vulnerability window) during which it is susceptible to hyperparasitism compared to a heterospecific host like *E. formosa*. Our direct observations indicate that melanization in pupae of *E. tricolor* prevents them from being hyperparasitized by conspecific females. Results from other direct observations (Loomans et al. unpublished) indicate that a combination of these two factors—the presence of melanization and the rate of this process—depend on the whitefly species involved and define the time window of pupal vulnerability to a subsequent autoparasitoid attack. A larger window of vulnerability would largely explain the apparent preference of *E. tricolor* and *E. sophia* (referred to as *E. transvena*) for heterospecific secondary hosts as found for *E. inaron* (Williams 1995) and *E. eremicus* (Hunter and Kelly 1998; Briggs and Collier 2001; Hunter et al. 2002), respectively.

Implications for biological control and non-target effects

In this study, two host species were provided simultaneously and our results suggest that the preference to hyperparasitize by *E. tricolor* was directed to the hetero-specific host *E. formosa*. In addition, as shown by similar results of our host-ratio experiment (Huang et al. 2002), selection of the secondary host for male egg deposition greatly affects the sex ratio. If the autoparasitioid *E. tricolor* is used in combination with *E. formosa* for biological control of whitefly, its behaviour to attack primary parasitoids, may negatively influence the effectiveness of control as predicted by Mills and Gutierrez (1996) in their theoretical model. Data from several cage and field-cage experiments, designed to evaluate the impact of single versus multiple introductions, strongly suggest the occurrence of interspecific competition among primary parasitoids and autoparasitoids. Recent papers give a substantial contribute to the debate on the implications of autoparasitoids for biological control both from empirical (Hunter et al. 2002; Bográn et al. 2002) and theoretical (Schreiber et al. 2001; Briggs and Collier 2001) point of view. All these, and other papers point out that, although autoparasitoids often interact in complex ways with primary parasitoids—often competitively displaced, sometimes co-existent—the final outcome of biological control may not be necessarily disrupted (Heinz and Nelson 1996; Giorgini and Viggiani 2000). Most of these studies and models, however, largely include impacts within agroecosystems (pest–plant–parasitoids) and much less within the natural environment. The outcome may depend, however, upon other biological characteristics, in addition to the relative suitability of the primary, whitefly host to both autoparasitoid and primary parasitoid.

From the point of safety of exotic biological control agents for the indigenous entomofauna, the results of this study can be viewed upon from two viewpoints. When an exotic autoparasitoid released for the control of whitefly pests in greenhouses escapes and establishes outdoors, it might have serious non-target effects. When an exotic, or native,

autoparasitoid that is established outdoors, subsequently invades an agricultural environment like greenhouses, where e.g. *E. formosa* is effectively controlling the whitefly population, it might seriously hamper biological control as shown by Del Bene and Landi (1991) for *E. tricolor* and by e.g. Gabarra et al. (1999, 2003) for *E. pergandiella*. In such greenhouse conditions where many immatures of primary parasitoid species are available that can serve as secondary hosts, and/or large number of unmated wasps are present, autoparasitoids might produce large numbers of male offspring (Williams 1977) and thus disrupt biocontrol. On the other hand when an exotic biological control agent, such as *E. formosa*, escapes from the greenhouse into the natural environment, it may encounter native autoparasitoid species, like *E. tricolor* and be strongly reduced in numbers. The behavioural and biological traits of *E. tricolor* may thus reduce the risk of an exotic primary species like *E. formosa,* or other primary parasitoids naturally invading new habitats (*E. hispida, E. protransvena, E. inaron*) from spreading into a natural environment. There is some support for this hypothesis, because surveys made by Kajita (2000) in Japan suggest that *E. formosa,* when settled outside greenhouses in which it had been released, was frequently attacked by native autoparasitoid species such as *E. japonica* and *E. sophia* (as *E. transvena*). This interesting hypothesis of diminished risks in the field when the exotic primary exotic parasitoids used in greenhouses are attacked by native autoparasitoids certainly justifies further study.

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References

Avilla J, Copland MJW (1987) Effects of host stage on the development of the facultative autoparasitoid *Encarsia tricolor* (Hymenoptera: Aphelinidae). Ann Appl Biol 110:381–389

Avilla J, Anadón J, Sarasúa MJ, Albajes R (1991) Egg allocation of the autoparasitoid *Encarsia tricolor* at different relative densities of the primary host (*Trialeurodes vaporariorum*) and two secondary hosts (*Encarsia formosa* and *E. tricolor*). Ent Exp Appl 59:219–227

Bográn CE, Heinz KM (2002) Host selection by the heteronomous hyperparasitoid *Encarsia pergandiella*: multiple-choice tests using *Bemisia argentifolii* as primary host. Ent Exp Appl 103:11–21

Bográn CE, Heinz KM, Ciomperlik M (2002) Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. Ecology 83:653–668

Briggs CJ, Collier TR (2001) Autoparasitism, interference, and parasitoid-pest population dynamics. Theor Popul Biol 60:33–57

Buijs MJ, Pirovano I, van Lenteren JC (1981) *Encarsia pergandiella*, a possible biological control agent for the greenhouse whitefly, *Trialeurodes vaporariorum*: A study on intra- and interspecific host selection. Med Fac Landbouww RU Gent 46(2):465–471

Burger JMS, Huang Y, Hemerik L, van Lenteren JC, Vet LEM (2006) Flexible use of patch-leaving mechanisms in a parasitoid wasp. J Insect Behav 19:155–170

Del Bene G, Landi S (1991) Biological pest control in glasshouse ornamental crops in Tuscany. Bull IOBC/WPRS 14(5):13–21

Gabarra R, Arnó J, Alomar O (1999) Naturally occurring populations of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in tomato greenhouses. Bull OILB/WPRS 22:85–88

Gabarra R, Batllori M, Albajes R (2003) *Encarsia formosa* and *Encarsia pergandiella*: addition or substraction. Bull IOBC/WPRS 26(10):33–38

Gerling D (1990) Whiteflies: their bionomics pest status and management. Intercept, Andover, UK

Gerling D (1966) Studies with whitefly parasites of Southern California. I. *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae). Can Entomol 98:707–724

Gerling D, Alomar O, Arnó J (2001) Biological control of *Bemisia* using predators and parasitoids. Crop Prot 20:779–799

Giorgini M, Viggiani G (2000) A compared evaluation of *Encarsia formosa* Gahan and *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) as biological control agents of *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) on tomato under greenhouse in south Italy. Bull IOBC/WPRS 23(1):109–116

Gurr G, Wratten S (eds) (2000) Measures of success in biological control. Kluwer, Dordrecht, The Netherlands 448 pp

Hassell MP (2000) The spatial and temporal effect of host-parasitoid interactions. Oxford, UK

Heimpel GE, Collier TR (1996) The evolution of host-feeding behaviour in insect parasitoids. Biol Rev 71:373–400

Heinz KM, Nelson JM (1996) Interspecific interactions among natural enemies of *Bemisia* in an inundative biological control program. Biol Control 6:384–393

Howarth FG (1991) Environmental impacts of classical biological control. Annu Rev Entomol 36:485–509

Huang Y, Loomans A, Bukovinszkiné-Kiss G, van Lenteren JC (2002) Heteronomous hyperparasitoids for biological control of whiteflies: for better or for worse? Exp Appl Entomol N.E.S 13:131–136

Hunter MS (1989) Sex allocation and egg distribution of an autoparasitoid, *Encarsia pergandiella* (Hymenoptera: Aphelinidae). Ecol Entomol 14:57–67

Hunter MS, Kelly SE (1998) Hyperparasitism by an exotic autoparasitoid: secondary host selection and the window of vulnerability of conspecific and native heterospecific hosts. Ent Exp Appl 89:249–259

Hunter MS, Woolley JB (2001) Evolution and behavioral ecology of heteronomous aphelinid parasitoids. Annu Rev Entomol 46:251–290

Hunter M, Kelly S, Collier T (2002) Does an autoparasitoid disrupt host suppression provided by a primary parasitoid? Ecology 83:1459–1469

Jervis MA, Kidd NAC (1986) Host-feeding strategies in hymenopteran parasitoids. Biol Rev 61:395–434

Jones WA, Greenberg SM (1999) Host instar suitability of *Bemisia argentifolii* (Homoptera: Aleyrodidae) for the parasitoid *Encarsia pergandiella* (Hymenoptera: Aphelinidae). J Agric Urban Entomol 16:49–57

Kajita H (1989) Mating and oviposition of three *Encarsia* species (Hymenoptera: Aphelinidae) on the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). Appl Entomol Zool 24:11–19

Kajita H (2000) Geographical distribution and species composition of parasitoids (Hymenoptera: Chalcidoidea) of *Trialeurodes vaporariorum* and *Bemisia tabaci*-complex (Homoptera: Aleyrodidae) in Japan. Appl Entomol Zool 35:155–162

Liu T-X, Stansly PA (1996) Pupal orientation and emergence of some aphelinid parasitoids (Hymenoptera) of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Ann Entomol Soc Am 89:385–390

Loomans AJM, van Lenteren JC (1999) Evaluating environmental effects of *Encarsia* species (Hymenoptera: Aphelinidae) introduced for whitefly control into Europe. Bull IOBC/WPRS 22(1):153–156

Louda SM, Pemberton RW, Johnson MT, Follett PA (2003) Non-target effects: the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annu Rev Entomol 48:365–396

Luck RF, Messenger PS, Barbieri JF (1981) The influence of hyperparasitism on the performance of biological control agents. In: Rosen D (ed) The role of hyperparasitism in biological control: a symposium. Agricultural Sciences Publications, Berkeley, California, USA, pp 34–43

Manzano MR, van Lenteren JC, Cardona C (2002) Searching and oviposition behaviour of *Amitus fuscipennis*, a parasitoid of the greenhouse whitefly. J Appl Entomol 126:528–533

May RM, Hassell MP (1981) The dynamics of multiparasitoid-host interactions. Am Nat 117:234–261

Mills NJ, Gutierrez AP (1996) Prospective modelling in biological control: an analysis of the dynamics of heteronomous hyperparasitism in a cotton-whitefly-parasitoid system. J Appl Ecol 33:1379–1394

Minkenberg OPJM, Tatar M, Rosenheim JA (1992) Egg load as a major source of variability in insect foraging and oviposition behavior. Oikos 65:135–142

Murdoch WM (1996) Theory for biological control: recent developments. Ecology 77:2001–2013

Nuffio CR, Papaj DR (2001) Host marking behavior in phytophagous insects and parasitoids. Ent Exp Appl 99: 273–293

Pedata PA, Hunter MS (1996) Secondary host choice by the autoparasitoid *Encarsia pergandiella*. Ent Exp Appl 81:207–214

Pedata PA, Giorgini M, Guerrieri E (2002) Interspecific host discrimination and within-host competition between *Encarsia formosa* and *E. pergandiella* (Hymenoptera: Aphelinidae), two endoparasitoids of whiteflies (Hemiptera: Aleyrodidae). Bull Ent Res 92:521–528

Rosen D (1981) The role of hyperparasitism in biological control: a symposium. Agricultural Sciences Publications, Berkeley, California, USA

Schreiber SJ, Mills NJ, Gutierrez AP (2001) Host-limited dynamics of autoparasitoids. J Theor Biol 212:141–153

Sengonça C, Wang X-Q, Liu B (2001) Development, longevity and parasitization of white fly parasitoid, *Encarsia tricolor* Forster (Hym., Aphelinidae), at different temperatures. Z Pflkrankh Pflsch 108:298–304

Sullivan DJ, Völkl (1999) Hyperparasitism: multitrophic ecology and behavior. Annu Rev Entomol 44:291–315

van Lenteren JC (1981) Host discrimination by parasitoids. In: Nordlund DA, Jones RL, Lewis WJ (eds) Semiochemicals: their role in pest control. Wiley, New York, pp 153–179

van Lenteren JC, Woets J (1988) Biological control and integrated pest control in greenhouses. Annu Rev Entomol 33:239–269

van Lenteren JC, Nell HW, Sevenster-van der Lelie LA, Woets J (1976a) The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), I: host finding by the parasite. Ent Exp Appl 20:123–130

van Lenteren JC, Nell HW, Sevenster-van der Lelie LA (1976b) The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), III: Discrimination between parasitized and unparasitized hosts by the parasite. Z Ang Entomol 81:377–380

van Lenteren JC, Nell HW, Sevenster-van der Lelie LA (1980) The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), IV: Oviposition behaviour of the parasite, with aspects of host selection, host discrimination and host feeding. Z Ang Entomol 89:442–454

van Lenteren JC, van Roermund HJW, Sütterlin S (1996) Biological control of greenhouse whitefly (*Trialeurodes vaporariorum*) with the parasitoid *Encarsia formosa*: how does it work? Biol Control 6:1–10

van Lenteren JC, Bale J, Bigler F, Hokkanenand HMT, Loomans AJM (2006) Assessing the risks of biological control agents of arthropod pests. Annu Rev Entomol 51:609–634

van Roermund HJW, van Lenteren JC, Rabbinge R (1996) Biological control of greenhouse whitefly with the parasitoid *Encarsia formosa* on tomato: an individual-based simulation approach. Biol Control 9:25–47

Williams JR (1977) Some features of sex-linked hyperparasitism in Aphelinidae (Hymenoptera). Entomophaga 22:345–350

Williams T (1991) Host selection and sex ratio in a heteronomous hyperparasitoid. Ecol Entomol 16:377–386

Williams T (1995) The biology of *Encarsia tricolor*: an autoparasitoid of whitefly. Biol Control 5:209–217

Williams T (1996) Invasion and displacement of experimental populations of a conventional parasitoid by a heteronomous hyperparasitoid. Biocontrol Sci Technol 6:603–618