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Foraging behaviour of parasitoids of *Bemisia argentifolii* on poinsettia

Krisztina Hudák[1,2], Joop C. van Lenteren[1], Yu Tong Qiu[1], Béla Pénzes[2]
[1]*Department of Entomology, Wageningen University, The Netherlands*
[2]*Department of Entomology, Budapest University of Economic Sciences and Public Administration, Budapest, Hungary*

Abstract

The foraging behaviour of five parasitoids of *Bemisia argentifolii* Bellows and Perring [two strains of *Encarsia formosa* (Gahan) (NL and MD), *Eretmocerus eremicus* Rose and Zolnerowich, *Eretmocerus mundus* Mercet, and *Amitus bennetti* Viggiani and Evans] on leaves of poinsettia was studied in the laboratory. The residence times, searching activities, numbers of encounters with hosts, numbers of ovipositions, and percentage acceptance of the hosts by the different strains and species of parasitoids were determined by direct observation of the behaviour. Mean residence time of all parasitoids that searched on leaves with hosts varied between 3045 seconds (*E. eremicus*) and 5657 seconds (*A. bennetti*). Mean residence time of parasitoids that did not find hosts on these leaves varied between 1915 seconds (*E. eremicus*) and 4709 seconds (*A. bennetti*). At the low host densities offered few hosts were encountered; *E. mundus* found the highest number of hosts. Those parasitoids that encountered a host showed mean residence times that increased from 1.6 fold (*E. formosa* NL) to 2.7 fold (*E. eremicus*). In experiments with clean leaves the residence time varied between 653 seconds (*A. bennetti*) and 1606 seconds (*E. formosa* NL) and when there was honeydew on the upperside of leaves, residence time varied between 2157 seconds (*E. eremicus*) and 5285 seconds (*A. bennetti*). Both contact with honeydew and hosts led to arrestment and, thus, to a higher probability to find hosts. Searching activity (percentage of total time on leaf searching for hosts) varied from 58% (*A. bennetti*) to 84% (*E. mundus*). The success ratio (percentage of hosts accepted for oviposition after encounter) was lowest for *E. formosa* (58%) and highest for *A. bennetti* (100%). Because of its high searching activity, its high host encounter rate and its favourable developmental and reproductive capabilities, *E. mundus* is expected to be the most efficient *Bemisia* parasitoid of the five tested species/strains.

Key words: *Bemisia argentifolii*, *Encarsia formosa*, *Eretmocerus eremicus, Eretmocerus mundus*, *Amitus bennetti*, poinsettia, foraging behaviour, evaluation of natural enemies.

Introduction

The silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring) (Homoptera Aleyrodidae), also referred to as the B-biotype of *Bemisia tabaci,* is a severe pest world-wide. Although it was frequently reported as a pest of field crops like bean and cotton (Brown *et al*., 1995), it caused unprecedented damage initially on poinsettia (*Euphorbia pulcherrima* Willd. ex Koltz) in greenhouses in Florida (Price, 1987). *Bemisia* then spread all over the United States causing damage on a range of field crops, vegetables and ornamental plants. It was accidentally imported into Europe around 1987 (Fransen, 1994) and became a serious pest of crops in greenhouses. The species that recently spread all over the world was first designated as the "B" strain of *B. tabaci*, and later on identified as a new species, *B. argentifolii* (Bellows *et al*., 1994). However, the taxonomy of this pest remains confused and controversial (Naranjo and Ellsworth, 2001), and we refer to Perring (2001) for a recent discussion of the *B. tabaci* species complex. *Bemisia* causes direct feeding damage, vectors a number of devastating plant viruses, reduces the quality of the harvested product due to honeydew excretion, and can be the source of various other problems (Drost *et al*., 1998).

At present, management of *B. argentifolii* depends mainly on chemical control but as this species is resistant to many insecticides, chemical control is difficult and resistance management is of high priority (Costa *et al*., 1993; Cahill *et al*., 1996; Palumbo *et al*., 2001). Also, chemical pesticides are interfering with biological pest control, which is now the main tactic used for pest control in modern greenhouses (van Lenteren, 2000). In addition, chemical control may create problems for human health and the environment, and thus the need of more sustainable and more effective control strategies is urgently required.

During the last decades, much work has been done on finding efficient natural enemies of whiteflies, in particular of *B. argentifollii* (for overviews, see Gerling 1990, Gerling and Mayer, 1995, Gerling *et al*., 2001). Despite all these publications related to biological control of *Bemisia*, Naranjo and Ellsworth (2001) conclude that: "biological control of *B. tabaci* by parasitoids, predators and fungi represents a key strategy whose potential has gone largely unrealized in many affected cropping systems throughout the world." Gerling *et al.* (2001), when reviewing parasitoids and predators of *Bemisia*, state: "Although certain natural enemies have proven effective components in *B. tabaci* control, there are still unexplored, potentially valuable species in many areas of the world.", and also: "The listed fauna of *B. tabaci* parasitoids is extensive, but relatively few have been studied or are intentionally used for pest control." It is our own experience that many whitefly biological control projects were opportunistic, terminated prematurely and often even without publishing the research results. As such projects contribute negatively to the image of biological control, it was our philosophy to contribute by long-term, pure scientific and applied research on whiteflies (e.g. van Lenteren and Noldus, 1990) and parasitoids (e.g. van Lenteren *et al.*, 1996).

For the last 25 years our research group has been working on biological control of greenhouse pests, initially mainly on parastioids of greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood), van Lenteren *et al*., 1996), and during the past 15 years also on parastioids of *Bemisia* (van Lenteren *et al*., 1997, Drost *et al*., 2000, Qiu *et al.,* 2003). For *B. tabaci*, Gerling *et al.* (2001) list 34 species of *Encarsia*, 12 species of *Eretmocerus*, one species of *Signiphora* and *Methycus*, and two *Amitus* species. These authors conclude that: "with the exception of *En. formosa* … and despite the frequent use of *Encarsia* species, data on their biological and taxonomic characteristics remain deficient even for commonly used species." Even less is known for the other parasitoid genera, although recently the biology of *Eretmocerus* and *Amitus* has received some attention (see below).

Seasonal inoculative releases of *Encarsia formosa* Gahan (Hymenoptera Aphelinidae) to control greenhouse whitefly, *T. vaporariorum,* have been commercially very successful in greenhouses during the last 30 years (van Lenteren and Woets, 1988, Gerling *et al.*, 2001). However, *B. argentifolii* was found to be a less favourable host for the *E. formosa* strain that was mass produced in the Netherlands (so called NL strain), and control with the *E. formosa* in greenhouses was also not successful at high release rates of 4-7 adult females per plant per week in North America (Hoddle and van Driesche, 1996). A strain of *E. formosa* collected in Maryland, USA (the so called MD strain) was considered to be a more promising agent for *B. argentifolii* control according to laboratory and greenhouse evaluation (Heinz and Parrella, 1994; van Lenteren and Brasch, 1994; Hoddle *et al*.; 1997, van Lenteren *et al*., 1997), so it was included in the current study, together with the Dutch *E. formosa* strain for comparison.

Field experience showed that in North America *Eretmocerus* species were the most abundant parasitoid group (Goolsby *et al*., 1998) on *B. argentifoliii*. Furthermore, inundative releases of *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera Aphelinidae) in a greenhouse were found to reduce the *B. argentifolii* populations substantially (Hoddle *et al*. 1998). In the mean time, it was found that in Europe *Eretmocerus mundus* Mercet (Hymenoptera Aphelinidae) often spontaneously entered greenhouses and attacked *Bemisia*. Therefore, we included *E. eremicus* and *E. mundus* in our study. We also studied the parasitoid *Amitus bennetti* Viggiani and Evans (Hymenoptera Aphelinidae), because it has a rather different reproduction strategy (Drost *et al.,* 1999). *Encarsia* and *Eretmocerus* are synovigenic and exhibit host feeding, whereas *Amitus* is proovigenic. We have recently published information on the developmental rate, immature mortality and reproductive capacity of these 4 species of parasitoids (Drost *et al*., 1998, 1999, 2000, Qiu *et al*., 2003).

This paper is focused on the foraging behaviour of five species/strains of parasitoids. The host-searching efficiency of natural enemies is an important parameter in the evaluation of their potential for biological control of insect pests (van Roermund *et al*., 1997a, b). Because host-densities are typically low under a successful biological control program, the probability of encountering hosts for parasitization is extremely important. Species with a high fecundity but an inefficient host-searching behaviour may seldomly encounter hosts to deposit their eggs at low host densities. This could be compensated by releases of large numbers of parasitoids, but it would dramatically increase costs of control. It is therefore economically more efficient to select for parasitoids with good searching capacities (Drost *et al*., 2000).

We report about direct observations on five parasitoid species/strains of *B. argentifolii*, and for each of them the residence time, number of encounters with hosts, number of ovipositions in hosts, and the percentage acceptance of hosts was determined. Three types of plants were offered to the parasitoids: plants with clean leaves, plants with honeydew on the leaf upperside and plants with whitefly hosts at the underside of the leaves. The results are used to discuss aspects of the biological control efficacy of these parasitoid species.

Materials and Methods

Origin and rearing procedures of the insects

The *B. argentifolii* population used in the present study originated from a population that entered the Netherlands on poinsettia cuttings from California in 1989. Whitefly was reared on poinsettia plants in a greenhouse at 26± 1°C and 70% RH with a 16L: 8D h photoperiod. *E. formosa* (NL) probably originated from a population discovered in England in 1926. Parasitoids were delivered weekly by a Dutch commercial company as black pupae on paper cards. The *E. formosa* (MD) population was started from wasps initially found attacking *T. vaporariorum,* but subsequently reared on *B. argentifolii* with poinsettia as the host plant at USDA-ARS laboratory Beltsville, Maryland (Bentz, 1993). We refer to these two *E. formosa* populations as strains because they show many differences in their biology (see e.g. Qiu *et al.*, 2003). A starting colony of *A. bennetti* was obtained from a population on *B. argentifolii* on cotton, kept at the University of California, Riverside, USA, by T.S. Bellows and B. Orr. *E. eremicus* parasitoids were obtained from the same company as *E. formosa* (NL). The species was reared on *T. vaporariorum* on tobacco and originated from Arizona, USA. Pupae were delivered weekly by a Dutch commercial company in bottles with fine vermiculite (Drost *et al*., 2000). *E. mundus* was taken from a stock originally obtained from an Italian commercial company, that reared the parasitoids on *B. tabaci* (unknown biotype), on courgette. Colonies of *E. formosa* (MD) and *E. mundus* were established using poinsettia plants with third and/or fourth instar nymphs of *B. argentifolii*; for *A. bennetti* first and/or second instar nymphs were used. Parasitized pupae were left to emerge in sleeve cages containing a poinsettia plant with whitefly nymphs. Parasitized pupae with *E. formosa* (MD) and *E. mundus* were collected after 18 days and those with *A. bennetti* were collected after 28 days and kept in a glass Petri-dish or vial with a drop of honey until emergence (Drost *et al.*, 2000). All parasitoids were reared at 25 ± 1°C and 60% RH with a 16L: 8D h photoperiod.

Experimental set-up

Poinsettia (*E. pulcherrima* cv. Goldfinger) plants were used as host plants. Every day 2-3 leaves were infested with *B. argentifolii* adults, by using small clip-cages. The infested plants were kept in a separate cage without whitefly adults in a greenhouse compartment. In this way there was a continuous supply of leaves with desired nymphal stages to be used for experiments: 10-11 day old pupae of *B. argentifolii* for *Amitus*, 12-13 day old pupae for *Eretmocerus* species, and 13-15 day old pupae for *Encarsia* strains. Before observation, the leaf with the desired nymphal stage was removed from the plant and kept in a small plastic vial with water. Only four nymphs were left on the underside of the leaf, the others were removed to assure low host density.

Parasitoids were left to emerge in glass Petri-dishes or vials in the presence of honey; inexperienced female parasitoids of 1-2 days of age for *Encarsia* strains and up to 1 day of age for *Amitus* and *Eretmocerus* species were used for observations. All parasitoids were used only once in an experiment (Drost *et al.,* 2000). Nineteen females of *A. bennetti*, 42 of *E. formosa* NL, 40 of *E. formosa* MD, 37 of *Er. eremicus* and 28 of *Er. mundus* were tested. Moreover, 25 females of each parasitoid species/strains were tested on clean leaves, and another 25 leaves with only honeydew on the leaf upperside. To obtain leaves with honeydew, clean leaves were put in a vial and then placed for two hours below whitefly infested poinsettia plants. The average percentage coverage of the leaves with honeydew was about 30%.

The experimental set-up consisted of the vial with the test leaf positioned horizontally among five uninfested poinsettia plants to imitate the light conditions of a crop and to provide the parasitoid with ample opportunity to hop or fly to other leaves (van Roermund and van Lenteren, 1995). Each experiment started when a female parasitoid was released on the upper side of the test leaf and started walking (van Roermund and van Lenteren, 1995) and finished after two hours of observation, or when the parasitoid left the leaf or stood still for more than half an hour. In the experiment with hosts on the leaves, females were also released at the underside of the leaves (see results). Parasitoids were continuously observed through two stereo microscopes (one for each leaf side). Although we speak about ovipositions throughout this paper, we did not dissect the hosts after the oviposition attitude was observed, so all ovipositions are supposed ovipositions. From our earlier work, we know that more than 90% of the oviposition attitudes result in actual egg laying (e.g. van Lenteren *et al.*, 1980; Drost *et al.*, 1999; Qiu *et al.*, 2003)

To analyse the foraging behaviour of parasitoids, all behavioural elements and positions on the leaf were recorded and analysed using the Observer 3.0 (Windows version) of Noldus Technology (Wageningen, the Netherlands). All observations were carried out in a climate chamber at 25°C.

Statistical analyses

Data of the experiments were analysed statistically in SPSS by the Kruskall-Wallis test followed by a distribution-free multiple comparison test. In all cases, we used α=0,05.

Results

Residence time, searching activity and position on the leaf

For all parasitoid species/strains the total mean residence time (= time spent on the leaf after introduction) was shortest on clean leaves, intermediate on leaves with honeydew and longest on leaves with hosts, except for *E. formosa* NL where the residence time on leaves with honeydew is longer than on leaves with hosts (table 1 and figure 1). Both honeydew and host presence strongly increased residence times. Of the five species/strains, *A bennetti* spent the shortest time on clean leaves (about 650 seconds), *E. formosa* MD and *E. mundus* showed intermediate residence times (about 1100 seconds), while *E. formosa* NL and *E. eremicus* stayed longest (about 1600 seconds). The presence of honeydew changes this picture drastically: now *A bennetti* and *E. formosa* NL stayed longest (about 5000 seconds), *E. formosa* MD was intermediate, and *E. eremicus* and *E. mundus* showed the shortest residence times. When hosts are present, residence time of *A. bennetti* was the longest (about 5600 seconds), *E. formosa* MD and *E. mundus* were intermediate (about 4300 seconds) and *E. formosa* NL and *E. eremicus* were the shortest.

Comparison of residence times on leaves with hosts is difficult as the number of females contacting at least one host differed a lot between different species and strains. When the parasitoids did not discover any of the hosts, the residence time was much lower than in the case of encounters. When hosts were encountered the residence time increased 1.6-1.8 fold, except for *E. eremicus* where it increased much stronger, i.e.2.7 fold (figure 2). There is a significant difference in residence times between cases when hosts were discovered or not. The difference in residence time between species is not significant for all the species/strains (figure 2) The long residence times found in cases where hosts were discovered were not caused by parasitoids spending more time standing still, preening or handling hosts, but by longer searching times.

Parasitoids of all species/strains spent most of their time searching (=walking while drumming and drumming) the leaf surface. The searching activity, expressed as the percentage of the total time spent searching on the leaf excluding host handling time, was above 58% for all the five species. Searching activity on leaves with hosts was highest for *E. formosa* NL and *E. mundus* (above 80%), intermediate for *E. formosa* MD (almost 80%), and lowest for *A. bennetti* and *E. eremicus* (between 59 and 70%) (figure 3). Host handling time took only 1-16% of the total residence time (table 1). The host handling time was longest for the *E. formosa* strains (11 and 16% for MD and NL, respectively) and was much shorter for the other species (1-4%). The

Table 1. Data on host searching behaviour of five species/strains of whitefly parasitoids on poinsettia leaves.

A. Poinsettia leaves without hosts and honeydew (clean leaves)

Species /strains	No. of females tested	Mean residence time (SE)	%Time searching (searching activity)	% Time not searching
Amitus bennetti	25	653.7 (130.69)	58	42
Encarsia formosa NL	25	1606.4 (261.49)	63	37
Encarsia formosa MD	25	1059.3 (232.51)	62	38
Eretmocerus eremicus	25	1564.5 (275.67)	59	41
Eretmocerus mundus	25	1193.4 (387.67)	74	26

B. Poinsettia leaves with honeydew on the leaf upper side B

Species /strains	No. of females tested	Mean residence time (SE)	% Time searching (searching activity)	% Time not searching	% Time feeding on honeydew
Amitus bennetti	25	5285.0 (422.69)	65	33	2
Encarsia formosa NL	25	4796.0 (281.17)	61	38	1
Encarsia formosa MD	25	3530.2 (533.46)	61	31	8
Eretmocerus eremicus	25	2157.7 (183.57)	69	29	2
Eretmocerus mundus	25	2403.5 (206.68)	80	18	2

C. Poinsettia leaves with hosts on the leaf underside C

Species /strains	No. of females tested	Mean residence time (SE) for all tested females	Mean residence time (SE,n) when no host contact was made	Mean residence time (SE,n) when host contact was made	% Time searching (searching activity)	% Time handling host	% Time non search/ non handle	% Host acceptance
Amitus bennetti	19	5657.2 (588.4)	4709.6 (674.45;15)	8065.98 (429.33;4)	59	1	40	100
Encarsia formosa NL	42	3488.4 (329.47)	2962.4 (346.3;30)	4803.48 (636.84;12)	82	16	2	58
Encarsia formosa MD	40	4447.8 (406.39)	3564.5 (477.02;27)	6282.48 (460.44;13)	78	11	11	79
Eretmocerus eremicus	37	3045.6 (354.80)	1915.1 (258.96;24)	5132.69 (527.22;13)	66	1	33	77
Eretmocerus mundus	28	4247.7 (415.66)	3226.6 (404.39;18)	6085.76 (558.02;10)	84	4	12	73

handling times were, of course, influenced by the number of encounters with hosts (table 1). Percentage time feeding on leaves with honeydew ranged between 1 and 8% (table 1).

On leaves where hosts were offered the percentage of the total residence time not spent on searching or handling hosts varied strongly between species/strains. *A. bennetti* and *E. eremicus* spent more than 30% of time on none searching behaviour (table 1). On clean leaves the percentage of time not searching or handling was higher for all species/strains and ranged between 26 and 42 % (table 1). On leaves with honeydew, percentage of time not searching was similar as on clean leaves without (table 1). The shortest average times searching before the first encounter were found for *E. formosa* NL and *E. mundus* (around 2000 seconds), while the times for other species were considerably higher (*E. eremicus* about 2700 seconds, *E. formosa* MD about 3200 seconds and *A. bennetti* about 3400 seconds) (figure 4).

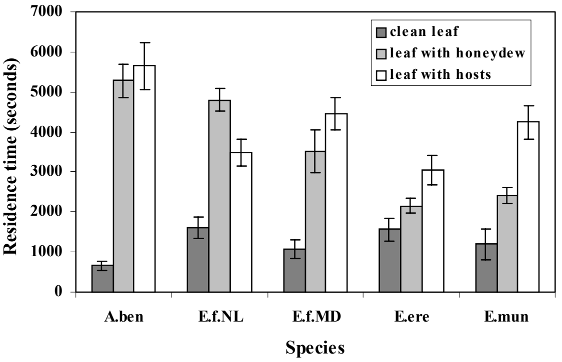

Figure 1. Residence time (in seconds, with standard error) of 5 species/strains of whitefly parasitoids on poinsettia leaves. A.ben: *A. bennetti,* E.f.NL: *E. formosa* NL, E.f.MD: *E. formosa* MD, E.ere: *E. eremicus,* E.mun: *E. mundus.*

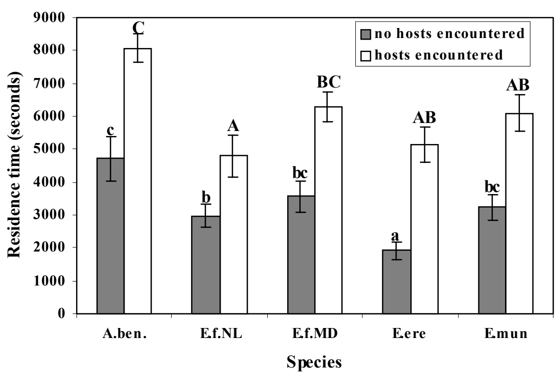

Figure 2. Residence time (in seconds, with standard error) of 5 species/strains of whitefly parasitoids on poinsettia leaves with *B. argentifolii* nymphs. Letters indicate significant difference in residence time. See figure 1 for explanation of species name abbreviations.

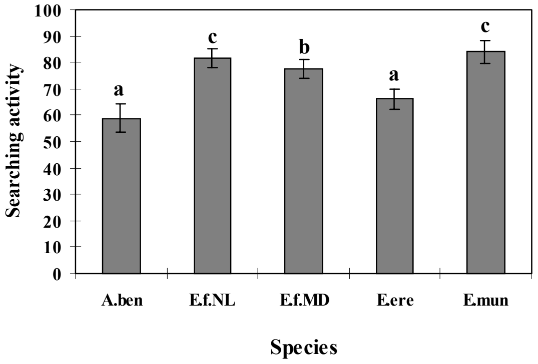

Figure 3. Searching activity (percentage of total time on leaf searching for hosts, with standard error) of 5 species/strains of whitefly parasitoids on poinsettia leaves with *B. argentifolii* nymphs. Letters indicate significant difference in searching activity. See figure 1 for explanation of species name abbreviations.

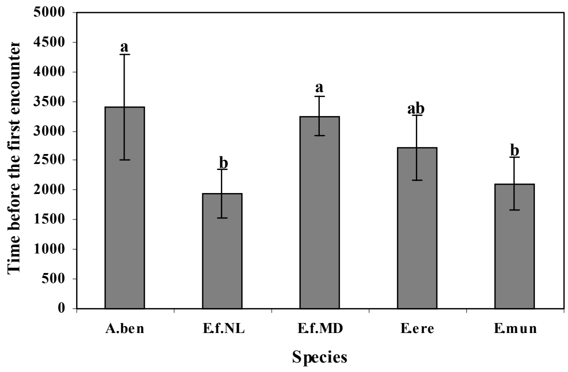

Figure 4. Searching time (in seconds, with standard error) before the first host encounter of 5 species/strains of whitefly parasitoids on poinsettia leaves with *B. argentifolii* nymphs. Letters indicate significant difference in searching time before first encounter. See figure 1 for explanation of species name abbreviations.

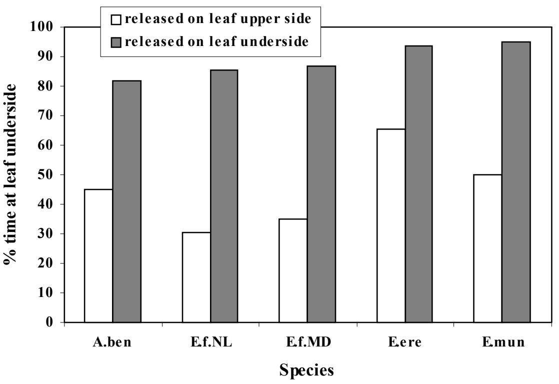

Figure 5. Percentage of residence time spent at the leaf underside by 5 species/strains of whitefly parasitoids on poinsettia leaves with *B. argentifolii* nymphs. See figure 1 for explanation of species name abbreviations.

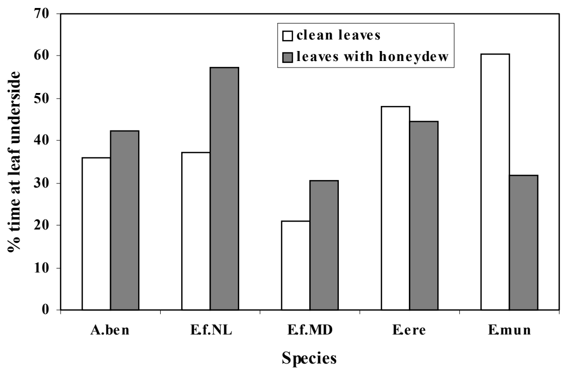

Figure 6. Percentage of residence time spent at the leaf underside by 5 species/strains of whitefly parasitoids on poinsettia leaves when released on the leaf upper side. See figure 1 for explanation of species name abbreviations.

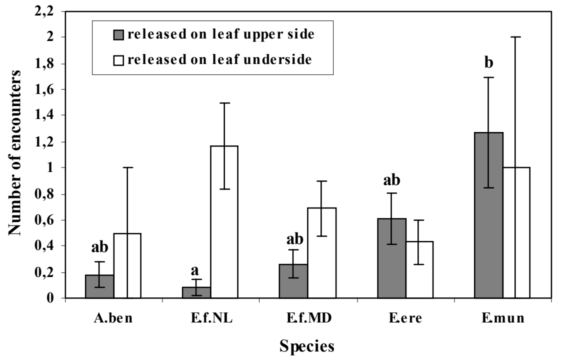

Figure 7. Mean number of host encounters per female per observation (with standard error) in 5 species/strains of whitefly parasitoids on poinsettia leaves. Letters indicate significant difference in number of encounters. In case of releases at the underside no significant differences in number of encounters were found. See figure 1 for explanation of species name abbreviations.

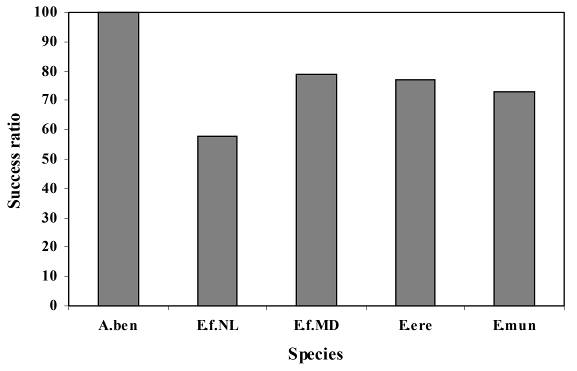

Figure 8. Success ratio (percentage of hosts accepted for oviposition after encounter) of 5 species/strains of whitefly parasitoids on poinsettia leaves with *B. argentifolii* nymphs. See figure 1 for explanation of species name abbreviations.

Parasitoids change from one leaf side to another while searching. In the experiments where hosts were present and when females were released on the upper leaf side, parasitoids spent less than half of their residence time on the underside, except *E. eremicus* which spent two third of its residence time on the underside (figure 5, white columns). When released on the underside of the leaf, parasitoids of all species/strains spent more than 80% of the residence time on the underside (figure 5, gray columns). On clean leaves most time was spent on the leaf upper side, with the exception of *E. mundus,* which spent more time on the leaf underside (figure 6). On leaves with honeydew at the leaf upperside, three species/strains (*A. bennetti*, *E. formosa* NL and MD) spent more time on the leaf underside than on the clean leaves, *E. eremicus* spent a similar percentage of time at the underside, and only *E. mundus* spent less time at the underside.

Encounters, ovipositions and host acceptance

The average number of host encounters and ovipositions in hosts was rather low. This phenomenon may be explained by the release method. Initially, parasitoids that were released on the upper leaf side were not very successful in finding hosts, so later parasitoids were released on the underside of the leaf. The difference in results between the two methods is obvious (figure 7) especially with *E. formosa* (NL) where the total number of encounters was 14.6 times higher when parasitoids were released at the leaf underside. *E. mundus* was the only species which had a relatively high number of host encounters even when released at the upper side of leaves, and this species also had the highest number of encounters when released at the leaf underside.

During the experiment not all of the encounters finished with oviposition. Sometimes the parasitoid examined a host and rejected it for oviposition, or it just seemed to "ignore" it and walked over it without any examination. To quantify host acceptance, we calculated the success ratio, i.e. the percentage of hosts accepted for oviposition after encounter (van Roermund and van Lenteren, 1995). *A. bennetti* accepted all hosts encountered (success ratio of 100%). *E. formosa* MD, *E. eremicus* and *E. mundus* showed a lower, similar success ratio of between 75 and 79%. Only *E. formosa* NL showed a ratio below 60%. (figure 8, table 1).

Discussion and conclusions

Residence time, searching activity and position on the leaf

Like in most other reports concerning searching behaviour of natural enemies, residence times, times spent searching or handling hosts, and times spent not searching show great variability (e.g. van Roermund and van Lenteren, 1995; Drost *et al.*, 2000; de Vis *et al.*, 2003). With only one exception, total residence times were shortest on clean leaves, intermediate on leaves with honeydew and longest on leaves with hosts for all parasitoid species/strains tested. This is, of course, not surprising, because on leaves with hosts parasitoids can oviposite, and ovipositions were known to result in arrestment behaviour for several of the tested parasitoids (van Lenteren *et al.*, 1996; Drost *et al.*, 2000; de Vis *et al.*, 2003). Also the arrestment effect of honeydew on whitefly parasitoids had been found earlier (e.g. van Lenteren *et al.*, 1996). The finding that parasitoids which did not find a host still search considerably longer on leaves with hosts than on leaves without hosts might seem surprising but can be explained by the fact that (some of) these females might have encountered exuvia of hosts or honeydew (van Vianen and van Lenteren, unpublished data).

Parasitoids spent from 60 to more than 80% of time searching the leaf surface. The values for searching activity that we found in the current experiments on poinsettia leaves with *B. argentifolii* as host are similar to searching activities found earlier for *E. eremicus* (75%) and *E. mundus* (60% - 75%) with the same host and host plant (Ardeh, Drost and van Lenteren, unpublished data). Also comparable percentages searching activity were found for *E. formosa* NL when searching for *T. vaporariorum* on tomato (75%; van Roermund *et al.*, 1997a, b), on gerbera (60-75%, Sütterlin and van Lenteren, 1999) and on various other host plants (van Roermund and van Lenteren, 1995). It is known that searching activity may reduce drastically in *E. formosa* after a female has laid 4 or more eggs (van Roermund and van Lenteren, 1995; Sütterlin and van Lenteren, 1999), but in the current experiments *Encarsia* females oviposited usually not more than twice.

For all parasitoid species/strains tested, host handling time made up a small percentage of total residence time, which is again in line with earlier findings (e.g. van Roermund and van Lenteren, 1995). Percentage time not spent searching ranged between 2 and 42%. For each species/strain it was generally highest on clean leaves, and similar on leaves with honeydew or leaves with hosts. Also these percentages are similar to what others found (van Roermund and van Lenteren, 1995; van Roermund *et al.*, 1997a, b; Sütterlin and van Lenteren, 1999).

Time spent on the upper or underside of the leaves depended on the site of release (more time was spent on the upper side if parasitoids were released at the upper side) and the type of leaf offered (more time was spent on the underside of leaves when hosts were present). Van Roermund *et al.* (1994) and van Roermund and van Lenteren (1995) found that the division of landings by *E. formosa* on upper or underside of tomato leaves with *T. vaporariorum* is 50-50, that encounters with hosts on the underside of leaves leads to arrestment on the underside and, thus, to higher percentages of time spent at the underside. Sütterlin *et al.* (2000) found that *E. formosa* females landed for 25% on the upper and for 75% on the undersurface of Gerbera leaves with *T. vaporariorum*, and also in this case time spent at the underside increased due to parasitoid arrestment after finding a host.

Encounters, ovipositions and host acceptance

At the low host density purposefully offered in order to test the search capacity of parasitoids at pest densities that can be tolerated in greenhouses, *E. mundus* had the highest encounter frequency with hosts and the highest number of ovipositions; the other species/strains encountered fewer hosts. Due to differences in experimental set-up it is difficult to compare these data with those of other authors.

The success ratio (percentage of hosts accepted for oviposition after encounter) was maximal (100%) for *A. bennetti*. For the other parasitoids values were found similar or higher than what was found earlier by other authors for *E. formosa* (van Lenteren *et al.*, 1980; van Roermund and van Lenteren, 1995; Sütterlin and van Lenteren, 1999).

Putting the puzzle together

Our earlier results (Vet and van Lenteren, 1981; Drost *et al.*, 1998, 1999, 2000) show that *E. formosa* MD has the shortest development time, the longest life-span in the presence of hosts, and the highest fecundity at temperatures lower than 20ºC, which indicates that this parasitoid might be a candidate for *Bemisia* control at low temperature conditions. At temperatures higher than 20ºC, the *Eretmocerus* species are performing better. But it is not only developmental time, life-span and fecundity that determines the success of a parasitoid.

Searching efficiency at low host densities is at least of same importance, because in order to realize its reproductive potential a parasitoid will first have to find hosts.

Our earlier work on the searching behaviour of these 5 parastoid species/strains (Drost *et al.*, 2000) showed that *A. bennetti* walks fastest and straightest, the *Eretmocerus* species have an intermediate walking speed and the *Encarsia* strains walk slowest. *A. bennetti,* the species that walks significantly faster than the other species/strains before host encounter does, however, not find hosts faster. *A. bennetti* is significantly smaller than the *Eretmocerus* species and the size of its preferred host stage (first instar nymphs) is smaller than the larger instars preferred by the other species/strains, and this explains the similar encounter rates (Drost *et al.*, 2000). Encounter and oviposition in a host nymph reduces the walking speed significantly for *A. bennetti* and *E. eremicus;* for the other species/strains the walking speed remains the same (Drost *et al.*, 2000). In all species/strains hosts are encountered randomly.

The results described in this paper provide the following new information: residence times in combination with the percentage of searching time result in similar times spent actively searching for hosts for the 5 species/strains of parasitoids. *E. mundus* encounters most hosts and accept about 70% of the hosts encountered, the other species/strains encounter fewer hosts per unit of time with similar percentages of acceptance (*E. formosa* NL and MD, *E. eremicus*), while *A. bennetti* encounters the smallest number of hosts but accepts them all for oviposition.

Based on all these data combined, we hypothesize that *E. mundus* will perform best at low host densities under warm conditions. This speculation needs to be tested and quantified in individual based simulation models developed earlier by our group as well as in commercial greenhouses (van Roermund *et al.*, 1997a, b), because of the as yet qualitative interpretations of complex data on searching and life histories of these parasitoids.

Now, what is the current practice of biological control of *B. argentifolii* in Europe? In Mediterranean Europe, biological control of *B. tabaci* in greenhouses is currently applied by mixed releases of two species of parasitoids: either *E. formosa* with *E. eremicus* (both parasitoids attack *Bemisia* and *Trialeurodes*) or *E. formosa* with *E. mundus* (*Encarsia* attacks both species of whitefly, *E. mundus* only attacks *Bemisia*) (Gerling *et al.*, 2001). Manzaroli *et al.* (1997) obtained good results when applying *E. formosa* and *E. mundus* in Italy. Also, a mixture of *E. formosa* and *E. eremicus* has successfully been applied on a large scale for several years, e.g. on 500 ha of tomato and 1000 ha of pepper in Spain (Gerling *et al.*, 2001). The use of a mix of species is based on several reasons: (1) The *Eretmocerus* species are excellent parasitoids of *B. argentifolii*, and (2) they are effective at relatively high temperatures; (3) *E. formosa* is an excellent parasitiod of *T. vaporariorum*, which often occurs together with *B. argentifolii* in Mediterranean Europe, and (4) this species is effective at relatively low temperatures. The data presented in this paper, in combination with other data of our group (Qiu et al., 2003; de Vis *et al.*, 2003) and the practical biological control results obtained in Mediterranean support countries (Gerling *et al.*, 2001), support the approach of mixed species releases.

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Authors' addresses: Krisztina Hudák (corresponding author e-mail: hudak@omega.kee.hu) Department of Entomology, Wageningen University, P.O. Box 8031, 6700EH Wageningen, The Netherlands; Department of Entomology, Budapest University of Economic Sciences and Public Administration, 1118 Budapest, Ménesi út 44, Hungary; Joop van Lenteren, Yu Tong Qiu, Department of Entomology, Wageningen University, P.O. Box 8031, 6700EH Wageningen, The Netherlands; Béla Pénzes, Department of Entomology, Budapest University of Economic Sciences and Public Administration, 1118 Budapest, Ménesi út 44, Hungary.

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