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Development of a methodology to study the intrinsic rate of increase of whitefly parasitoids: design of an oviposition device

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Abstract

The present work contributes to an accurate quantification method of the parameters of the biotic potential of *Encarsia formosa* Gahan (Hymenoptera Aphelinidae), applicable to other species of whitefly parasitoids. The oviposition devices are easy to make and they allow standardisation of a whitefly parasitoid study by daily observations of the main parameters such as fecundity, longevity and host-feeding by adult parasitoids, which are important for the parasitoids mass-production and biological control of whiteflies. Results show that two physical parameters determine the expression of biotic potential: the confinement induces an important decrease of the fecundity and longevity of *E. formosa*; the position of plant leaf with nymphs of *Trialeurodes vaporariorum* (Westwood) (Homoptera Aleyrodidae) that the parasitoid female can explore for oviposition and host-feeding influences on the biotic potential. The heterogeneity observed in the replicates suggests that during their mass-processing the parasitoids suffer abiotic conditions leading to changes in the biotic potential of progeny.

Under strictly constant laboratory conditions of temperature, hygrometry and photoperiod, an identical trend is observed for mother and daughter generations: fecundity and longevity are higher in aired oviposition devices and exploitable host-nymphs on leaf situated on top of device. In these laboratory conditions, results for fecundity and longevity are better, and the same trend is observed for host-feeding of mother and daughter generations.

The above defined standardised methodology will allow comparison of the biotic potential of other species of whitefly parasitoids because it reduces variations of abiotic origin.

Key words: Aleyrodidae, Aphelinidae, *Trialeurodes vaporariorum*, *Encarsia formosa*, adult, oviposition device, intrinsic rate of increase, fecundity, longevity, host-feeding.

Introduction

The traditional integrated control strategy on horticultural and ornamental protected crops, based mainly on the use of beneficial insects, has been changed greatly since the arrival of the biotype "B" of the whitefly *Bemisia tabaci* (Gennadius) (Homoptera Aleyrodidae) in the western part of the Mediterranean basin. Before its introduction, the only injurious whitefly was *Trialeurodes vaporariorum* (Westwood) (Homoptera Aleyrodidae) which can be successfully controlled by the parasitoid *Encarsia formosa* Gahan (Hymenoptera Aphelinidae). Only a few studies pointing out the specific biological features of this parasitoid, such as the major effect of temperature, have been carried out under experimental conditions (Burnett, 1949; Kajita, 1979; Arakawa, 1982). This feature varies according to the population structure of the pest [single nymphal instar (Burnett, 1949; Arakawa, 1982); many nymphal instars (Kajita, 1979)] and with or without feeding on honeydew (Vet and van Lenteren, 1981).

The first change in research orientation occurred with the rise in greenhouse-heating costs. The progressive cost reduction of inputs favoured research oriented towards a better knowledge of intrinsic-rate-of-increase parameters of *E. formosa* under constant low temperatures (van Lenteren and Schaal, 1981), and towards the use of less exigent species such as *Encarsia tricolor* Förster (Hymenoptera Aphelinidae) (Christochowitz *et al.*, 1981). Nevertheless, the large heterogeneity of results obtained as a consequence of the variety of adopted protocols did not lead to a reliable standard methodology for estimating biotic-parameters of a parasitoid species under different abiotic factors. In addition, the results obtained did not provide a valid comparison for the performance of the various beneficial insects to be released.

The introduction of the biotype "B" of *B. tabaci* and trials of indigenous parasitoids or of exotic beneficial insects justify the use of a methodology allowing the reliable comparison between intrinsic rate of increases of these indigenous and exotic insects or between stocks of the same insect. Starting from 1995 (Brown *et al.*, 1995), several systematic studies characterize the biotypes of *B. tabaci* living in the Mediterranean basin (Guirao *et al.*, 1996; 1997a; 1997b; Chermiti *et al.*, 1997; De Barro *et al.*, 2000; Simón *et al.*, 2003; Bosco *et al.*, 2006; De La Rùa *et al.*, 2006) and checklists of their parasitoids are published for the Mediterranean basin and for other regions of the world (Polaszek *et al.*, 1992; Onillon *et al.*, 1994; Riley and Ciomperlink, 1997; Stansly *et al.*, 1997; Schuster *et al.*, 1998; Benral Vega, 2000a; 2000b; Smith *et al.*, 2000; Viscaret *et al.*, 2000; Lopez-Avila *et al.*, 2001). These checklists revealed a rich and diversified parasitic entomofauna which needs to be carefully characterized in order to estimate their efficacy and to compare their performance. It mainly concerns of *Encarsia pergandiella* Howard, *Encarsia hispida* De Santis, *Encarsia inaron* (Walker), *Encarsia sophia* (Giraud et Dodd) and *Eretmocerus mundus* Mercet.

This paper is a contribution to a standard methodology for comparison of the intrinsic rate of increase of the parasitoids of *B. tabaci* and *T. vaporariorum* in relation to the biotic and abiotic characteristics of the habitat.

Materials and methods

Oviposition devices

These devices are made of two transparent cylindrical plastic boxes, one which fits snug inside the other. The lower part of the device is a cylindrical box, 30 mm in height and 30 mm in diameter. The upper part is a truncated-cone shaped box, 40 mm in height 30 mm in basal diameter and 20 mm in upper diameter. The device is put into a 2 litre-rectangular plastic box, on a wire mesh held at 1 cm above the box bottom on small wooden blocks. A 0.5 cm thick cotton-layer is placed under the mesh and moistened in order to maintain a high level of humidity (figure 1).

Oviposition device types and descriptions

H i g h - m e s h t y p e (H M t y p e)

The bottom of the cylindrical box is cut and replaced with a fine (150 µm) thermo-sealed mesh. A circular central opening (15 mm in diameter) is cut in the top of the truncated-cone shaped box. A plant leaf disc (20 mm in diameter) infested with whitefly nymphs is placed on the opening of the top box. A soft plastic cap with a hole in its centre closed by a fine (150 µm) thermo-sealed mesh is put above the leaf disc. An air flow is maintained and the whitefly nymphs fixed on the underside of the leaf disc can be reached by a parasitoid female introduced into the device. This type of oviposition device simulates the natural conditions where the whitefly nymphs are feeding on the underside of the leaf.

L o w - m e s h t y p e (L M t y p e)

The bottom of the cylindrical box is the same as in the HM type, but the top of the truncated-cone shaped box is closed. The leaf disc infested with whitefly nymphs is placed on the bottom of the cylindrical box. The whitefly nymphs are not in their natural position when a female parasitoid is introduced into the device.

S o l i d - t o p t y p e (S T t y p e)

The bottom of the cylindrical box is solid, preventing an air flow within the oviposition device. The truncated-cone shaped box is the same as in the HM type. The whitefly nymphs attached on the underside of the leaf disc simulates the natural position for a parasitoid female introduced into the device.

S o l i d - b o t t o m t y p e (S B t y p e)

The bottom of the cylindrical box is solid as in the ST type, but it is covered with a piece of wet filter paper where the leaf disc with whitefly nymphs is placed. The top of the truncated-cone shaped box is closed, so that some confinement is maintained. The whitefly nymphs are not in their natural position when a female parasitoid is introduced into the device.

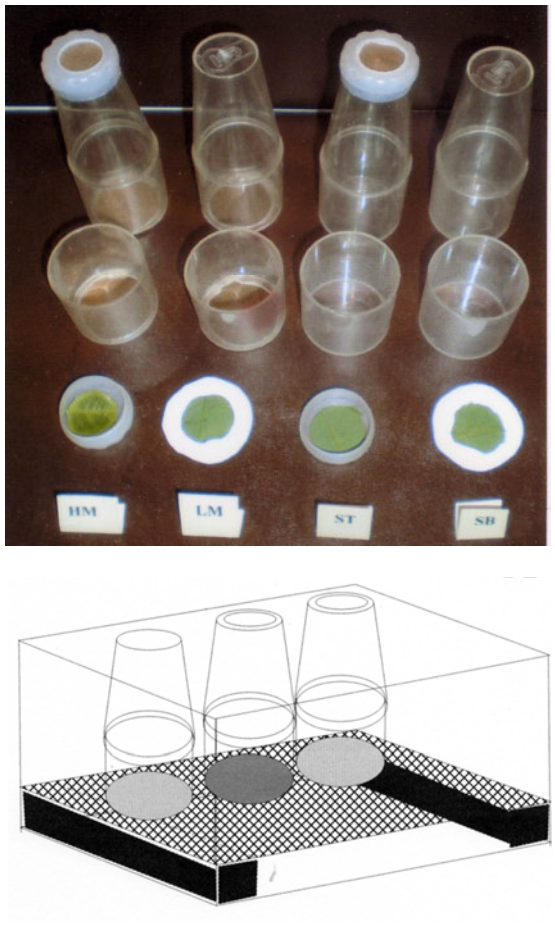

Figure 1. Step-by-step construction of oviposition devices: a) HM type (high mesh type), LM type (low mesh type), ST type (solid top type) and SB (solid bottom type); b) schematic view.

Host plants

Two species of plants were used for rearing nymphs of *T. vaporariorum*. In a first experiment, *Nicotiana glauca* Graham (Solanaceae) was grown in an insect-proof area of a greenhouse-under natural conditions. In a second experiment, the host plant was *Ageratum conyzoides* L. (Asteraceae). The plants infested with whiteflies were stored in controlled conditions within a climatic room (22 ± 0.5 °C, 70 ± 5% RH, photoperiod 16:8 L:D).

Whiteflies

T. vaporariorum reared for 10 years on *N. glauca* under greenhouse conditions (18 - 30 °C and 16:8 L:D during spring and summer, 12 - 22 °C and 8:16 L:D during autumn and winter) was used to determine a standard oviposition device for the study of whitefly parasitoids.

Parasitoids

Specimens of *E. formosa* used in both experiments had different origins. In the first experiment, specimens were supplied by a French private firm all year round. In the second experiment, *E. formosa* was reared in a climatic room (22 ± 0.5 °C, 70 ± 5% RH, 16:8 L:D) at the Biological Control Station of Antibes (Alpes-Maritimes, France),

ensuring a constant and homogeneous production of the parasitoid during the experiment. The parasitoid females used for the experiment were less than 12 hours old and they were emerging from old black pupae of *T. vaporariorum* individually put into gelatine capsules.

Experimental protocol

Three plants of *N. glauca* or *A. conyzoides* were placed twice a week in the whitefly rearing area of a greenhouse, to ensure a regular supply of third-instar nymphs of *T. vaporariorum*. Tobacco leaf discs of 1.75 cm^2 were collected daily by using a hollow punch (18 mm of diameter).The central part of the discs were cleaned with a fine brush to leave only 20 third-instar nymphs on the leaf disc.

Three replicates were conducted, each one including the four different oviposition devices (HM, LM, ST, SB). For each replicate, there were eight devices for each oviposition type for a total of 32 oviposition devices per replicate. One female of *E. formosa* was introduced into each oviposition device.

The devices were inspected daily to check the rate of mortality of the parasitoid and the leaf discs were replaced. Then each oviposition device was placed back in the climatic room at 22 ± 0.5 °C. The removed leaf discs were placed in a partitioned box (10 x 16 cm), on the bottom which was covered with a layer of agar and stored at 9 °C to slow development of the parasitoid. The characteristics of each parasitoid female and type of oviposition device were recorded. Then an amount of leaf discs equal to the number of surviving parasitoid females were prepared for the following day and stored at 9 °C on the bottom of a plastic box which was covered with wet filter paper.

Four leaf discs with 20 third-instar whitefly nymphs were placed in the oviposition devices and stored in the same conditions of temperature as above, but without parasitoid females. Leaf discs were inspected daily to verify that accidental parasitism or predation was prevented.

The leaf discs were observed daily, under a binocular microscope, in order to determine the number of whitefly nymphs that were parasitised by the females of *E. formosa*. Each dead or live nymph was removed from the leaf disc by using a fine pin and placed in a water drop on a concave slide and dissected in order to count the number of parasitoid eggs. The biological instar of the whitefly nymphs containing parasitoid eggs was recorded.

Statistical analysis

The data obtained were submitted to an analysis of variance and to a multivariate analysis of variance (SAS procedure: ANOVA and MANOVA). Means were then separated using a post-hoc test (SAS procedure: DUNCAN) when the analysis of variance indicated significant results.

Results

E. formosa from a commercial insectary

Two parameters of the intrinsic rate of increase of the parasitoid females were evaluated in this experiment: the total fecundity and the longevity of the parasitoid. The analysis of variance revealed a significant effect of mesh, of the position of the leaf disc and of the replicates on the fecundity and longevity of *E. formosa* females coming from a commercial insectary (table 1).

Fecundity of *E. formosa* females

The highest mean total fecundity was observed with the oviposition device type HM in the second replicate when 126.7 eggs were laid (σ = 58.3) (table 2). The lowest mean total fecundity was recorded with the device type LS (10.9 eggs laid; σ = 12.2) in the third replicate.

The highest mean fecundity (101.9 eggs) (table 2) was recorded with the oviposition device HM. The fecundity was much lower with the devices LM (56.5 eggs), ST (48.5 eggs) and SB (34.2 eggs).The oviposition devices provided with mesh seem to ensure the highest levels of fecundity of *E. formosa* females. This is confirmed by the significantly higher fecundity (table 3) of the females tested with this type of oviposition device (79.2 eggs) compared to the levels observed (41.4 eggs) for females inside devices with solid tops or solid bottoms.

Similar results were collected from *E. formosa* females tested in the oviposition devices where the leaf discs bearing *T. vaporariorum* nymphs were placed on the top of the device (mean fecundity 75.2 eggs) while mean fecundity was lower (45.4 eggs) when the nymphs were placed at the lower part of the device.

Observing the «mesh» factor more carefully, it is evident that the mean fecundity of *E. formosa* females gradually decreased from the first to the third replicate (104.2 - 88.8 and 44.6 eggs) for the females placed inside the devices with mesh (HM and LM). The same trend is recorded for the fecundity of the females tested inside solid oviposition devices (61.2 - 48.1 and 14.9 eggs).

The «position» factor causes a similar response with decreasing fecundity rates from the first to the third replicate (79.9 - 34.1 - 22.2) with low leaf discs (LM and LS). However, in the oviposition devices offering the leaf disc to the parasitoid in a high position (HM and HS), the highest fecundity rate was recorded in the second replicate (102.8 eggs), while it was lower in the first and in the third ones (respectively 85.5 and 37.3 eggs).

Table 1. Results of the analysis of variance (ANOVA) on the levels of fecundity and longevity of *E. formosa* females obtained from a commercial insectary and maintained in 4 different types of oviposition device (values significant at $\alpha \leq 0.05$ are marked in bold).

Sources of variability	Total fecundity	Total longevity
Mesh	**0.0002**	**0.0004**
Position	**0.0028**	**0.0136**
Replicate	**0.0001**	**0.0003**
Mesh * position	0.1125	0.2665
Mesh * replicate	0.8374	0.7410
Position * replicate	**0.0195**	**0.0381**
Mesh * position * replicate	0.4742	0.9391

Table 2. Total fecundity and longevity of *E. formosa* females from a commercial nursery under different factors tested at 22 °C (H: high; L: low; M: mesh; S: solid). The highest values are marked in bold.

Factors	Levels			n	Fecundity (eggs)		Longevity (days)	
					mean	σ	mean	σ
Mesh (M/S) * position (H/L)	M	L		24	56.5	54.1	9.1	6.8
	M	H		24	**101.9**	72.2	**13.1**	7.4
	S	L		24	34.2	43.1	6.3	4.8
	S	H		24	48.5	35.8	7.8	3.9
Mesh * replicate	M	1		16	**104.2**	82.4	**13.1**	8.6
	M	2		16	88.8	63.7	**13.1**	7.4
	M	3		16	44.6	35.2	7.2	3.6
	S	1		16	61.2	41.2	8.8	4.4
	S	2		16	48.1	44.1	8.1	4.8
	S	3		16	14.9	11.2	4.3	2.2
Position * replicate	L	1		16	79.9	62.3	10.9	7.6
	L	2		16	34.1	36.5	7.2	5.1
	L	3		16	22.2	23.2	5.	3.1
	H	1		16	85.5	74.7	11.0	6.8
	H	2		16	**102.8**	55.1	**13.9**	6.4
	H	3		16	37.3	34.3	6.5	3.4
Mesh * position * replicate	M	L	1	8	85.2	72.8	12.1	9.0
	M	L	2	8	50.9	44.9	9.1	6.3
	M	L	3	8	33.5	26.8	6.1	3.3
	M	H	1	8	123.1	91.8	14.0	8.7
	M	H	2	8	**126.7**	58.3	**17.0**	6.6
	M	H	3	8	55.7	40.8	8.4	3.8
	S	L	1	8	74.6	54.2	9.6	6.1
	S	L	2	8	17.2	13.8	5.2	3.0
	S	L	3	8	10.9	12.2	4.0	2.8
	S	H	1	8	47.9	16.8	8.0	1.5
	S	H	2	8	78.9	42.4	10.9	4.7
	S	H	3	8	18.9	9.1	4.6	1.6

Table 3. Effect of fecundity and longevity on the intrinsic rate of increase of *E. formosa* females in different types of oviposition devices.

Factors	Category	Fecundity (eggs) mean	Longevity (days) mean
Presence of mesh	Mesh	79.2 a	11.1 a
	Solid	41.4 b	7.1 b
Position of the leaf disc	High	75.2 a	10.5 a
	Low	45.4 b	7.7 b
Replicate	1	82.7 a	10.9 a
	2	68.4 a	10.6 a
	3	29.7 b	5.8 b

Duncan's multiple range test ($p < 0.05$).

An uncontrolled factor seems therefore to influence the results of the 3 replicates which were conducted using the specimens regularly sent by a commercial insectary.

E. formosa females longevity

The highest overall longevity of *E. formosa* adult females was observed in the oviposition device type HM (good air flow and natural exposure of the whitefly nymphs to the parasitoid) in the second replicate (17.0 days - σ = 6.7). Taking into consideration the mean longevity values of *E. formosa* females belonging to the same series, it appears evident that the oviposition device type HM ensures the highest longevity reaching 13.1 days (table 2). This is then followed by the type LM scoring 9.1 days, HS 7.8 days and 6.3 days with the device type LS. In general the oviposition devices with a mesh ensure the highest longevity levels of *E. formosa* females. This positive effect is confirmed by the significantly higher total longevity (table 3) of the females tested in oviposition devices with mesh (11.1 days) as compared to the females kept in the oviposition devices with a solid bottom or top (7.1 days). The same result was obtained with the females tested in the devices with leaf discs placed in the upper part for which a mean longevity of 10.5 days was recorded, while the females getting the host in the lower part of the device survived on average only 7.7 days.

The «mesh» factor gives uniform longevity levels for *E. formosa* females in replicates 1 and 2 (respectively 13.1 and 13.1 days in the devices with mesh; 8.8 and 8.1 days in those with solid bottom). A strong decrease in the longevity is recorded in the 3rd replicate. The «position» factor doesn't have such a uniform effect in replicates 1 and 2 (respectively 11.0 and 13.9 days in the devices with the leaf disc placed in the upper part). How-

Table 4. Results of the analysis of variance (ANOVA) on the fecundity and longevity data for *E. formosa* females, reared under constant temperature in climatic chamber, and tested in 4 different types of oviposition device (values significant at $\alpha \leq 0.05$ are marked in bold).

Sources of variability	Total fecundity	Total longevity	Total parasitisation
Mesh	**0.0001**	**0.0001**	**0.0001**
Position	**0.0309**	0.2952	0.1365
Replicate	0.3144	0.3025	0.2615
Mesh * position	0.6451	0.6792	0.2640
Mesh * replicate	0.8770	0.5501	0.1281
Position * replicate	0.7174	0.9808	0.4325
Mesh * position * replicate	0.5807	0.7402	0.0919

Table 5. Effect of fecundity, longevity and parasitisation on the intrinsic rate of increase of *E. formosa* females reared and tested at 22 °C in different types of oviposition devices.

Factors	Levels	Fecundity mean	Longevity mean	Parasitisation mean
Presence of mesh	Mesh	191.0 a	27.0 a	41.5 a
	Solid	68.0 b	13.8 b	13.8 b
Position of the leaf disc	High	144.5 a	21.4 a	30.3 a
	Low	114.4 b	19.4 a	25.0 a
Replicate	1	144.1 a	22.6 a	31.7 a
	2	124.4 a	19.3 a	25.4 a
	3	119.9 a	19.3 a	25.8 a

Duncan's multiple range test ($p < 0.05$).

ever, the strong difference with the third replicate makes it impossible to draw final conclusions on the advantage of one type of oviposition device as compared to the others.

It is likely that *E. formosa* females coming from a commercial insectary all year round were subjected to fluctuating climatic conditions.

E. formosa reared under controlled conditions

In this experiment, during the whole production cycle of the parasitoid, the climatic conditions (temperature, relative humidity and photoperiod) were maintained constant.

The under reported analysis of variance showed that only two sources of variation are involved (table 4).
The presence of the mesh, which allows ventilation and has an effect on the three parameters of the intrinsic rate of increase (total fecundity, total longevity and total predation) and the location of the samples which only influences the total fecundity of *E. formosa* females. These results were confirmed by the Duncan's test used to separate the means (table 5).

Fecundity of *E. formosa* females

The highest mean total fecundity was recorded (table 6) in the oviposition device type HM (good air flow and natural exposure of the whitefly nymphs to the parasitoid) in the first replicate with 206.6 eggs laid (σ = 82.9). The lowest mean total fecundity was observed with the device type LS with 35.1 eggs (σ = 12.9) in the first replicate.

No significant difference was found among the repli-

Table 6. Total fecundity, longevity and parasitisation of *E. formosa* females reared under different factors tested at 22°C (H: high; L: low; M: mesh; S: solid). The highest values are marked in bold.

Factors	Levels		n	Fecundity		Longevity		Parasitisation	
				mean	σ	mean	σ	mean	σ
Mesh (M/S) * Position (H/L)	M	L	24	179.1	94.4	26.4	13.3	40.8	27.5
	M	H	24	**202.8**	79.9	**27.7**	11.3	**42.1**	20.3
	S	L	24	49.8	27.9	12.3	5.2	9.2	5.5
	S	H	24	86.2	35.2	15.2	4.8	18.5	7.6
Mesh * replicate	M	1	16	186.4	78.4	24.7	9.1	36.2	18.6
	M	2	16	**202.8**	94.5	**30.6**	14.4	**50.6**	29.3
	M	3	16	183.7	92.6	25.9	12.5	37.6	22.3
	H	1	16	53.5	22.9	13.9	4.5	15.4	6.2
	H	2	16	85.3	48.5	14.6	6.0	12.9	9.8
	H	3	16	65.1	26.9	12.8	4.9	13.2	8.0
Position * replicate	L	1	16	99.1	82.5	18.1	9.5	24.0	18.2
	L	2	16	127.2	114.0	21.5	15.7	31.4	36.9
	L	3	16	116.9	89.3	18.6	11.2	19.6	15.5
	H	1	16	140.7	91.4	20.6	8.5	27.6	15.5
	H	2	16	**160.9**	71.1	**23.6**	11.5	**32.1**	18.7
	H	3	16	131.9	93.3	20.1	11.9	31.2	23.8

cates (table 4). The highest fecundity levels were recorded with the oviposition devices type HM where the females laid 209.6 eggs in the first replicate, 206.4 and 192.5 respectively in the second (R2) and third (R3) ones and with the devices type LM where the females laid 163.1 eggs in the first replicate, 199.2 in the second and 174.9 in the third one. The lowest fecundity levels were observed in the series HS with 77.9 eggs (R1), 115.5 eggs (R2) and 71.2 eggs (R3) and LS with 35.1 eggs (R1), 55.2 eggs (R2) and 59.0 eggs (R3).

In the oviposition devices with a mesh on the bottom, *E. formosa* females showed the highest mean fecundity (202.8 eggs in the second replicate) while the lowest fecundity (53.5 eggs) was observed in the devices with a solid bottom (table 6). In the oviposition devices with the leaf discs placed on the upper part, the fecundity of *E. formosa* females is the highest (160.9 eggs in the second replicate) while it is the lowest (99.1 eggs) in the devices with the leaf discs placed in the lower part (R1). The highest mean fecundity in the 3 replicates is 202.8 eggs in the device type HM and the lowest one is 49.8 eggs observed in the device type LS.

E. formosa females longevity

The highest mean longevity of *E. formosa* females was observed using the oviposition device type LM (good air flow and leaf disc placed in the lower part) in the second replicate (30.7 days - σ = 17.2). The lowest mean longevity was observed for the females tested in the device type LS in the first replicate (12.1 days - σ = 4.8). The highest mean individual longevity was 59 days observed on a female tested in a device type LM in the second replicate. The lowest longevity (6 days) was observed in a device type LS in the last 2 replicates (R2 and R3).

No significant differences were found among the replicates. The highest longevity levels were recorded with the devices HM where the females survived 25.4 days in the first replicate, 30.4 days in the second and 27.3 days in the third one and with the devices type LG with 24 days in the first replicate, 30.7 in the second and 24.5 in the third one. The lowest longevity was observed in the series LS with 12.1 days (R1), 12.2 days (R2) and 12.6 days (R3).

In the oviposition devices with mesh on the bottom, *E. formosa* females showed the highest longevity (30.6 days in the second replicate), while the lowest longevity (12.8 days in the third replicate) was recorded in the devices with a solid bottom. The highest longevity of the females is recorded in the devices with the leaf discs placed in the upper part (23.6 days in the second replicate) while the lowest value (18.1 days) was performed by the females tested in the devices types LM and LS (replicate 1). The highest mean longevity in the 3 replicates of the same series was obtained with the device type HM (27.7 days) and the lowest one was observed in the device type LS (12.3 days).

Parasitisation rates by *E. formosa* females

The highest total mean parasitisation rate was obtained by *E. formosa* females of the LM series in the second replicate (50.6 parasitized nymphs; σ = 29.3. (table 6). The lowest parasitisation rate was recorded in the trials with oviposition devices type LS in the second replicate (5.6 parasitized nymphs; σ = 5.2). The highest parasitisation per single female was recorded in the second replicate (123 parasitized nymphs).

No significant differences were found among the 3 replicates. The highest numbers of parasitized nymphs were recorded in the tests carried out with the devices type HM (36.4 parasitized nymphs in the first replicate, 44 in the second replicate and 46 in the third one) and in the trials with the devices type LM (36 parasitized nymphs in the first replicate, 57.1 in the second and 29.2 in the third). The lowest parasitisation levels were observed with the devices type LS (12 parasitized nymphs in the first replicate, 5.6 in the second and 10 in the third one).

The total parasitisation performed by the parasitoid females, tested with or without mesh on the bottom of the oviposition device, was higher (50.6 parasitized nymphs) in the second replicate with devices with mesh and lower (12.9 parasitized nymphs) in the first replicate with devices with a solid bottom. The females tested in oviposition devices where the leaf disc was placed in the upper part showed the highest total parasitisation activity (32.1 parasitized nymphs in the second replicate), while the lowest levels (19.6 parasitized nymphs) were performed by the females tested having the host placed in the lower part of the device.

Discussion

The results pointed out several important elements in the search for a rigorous methodology which is essential in defining the characteristics of the intrinsic rate of increase of *E. formosa* developing on its host *T. vaporariorum*. And this in relation to the abiotic conditions of the environment as well as in comparing the performance of parasitoids (*E. formosa*, *E. pergandiella*, *E. hispida*, *E. sophia*, *Eretmocerus mundus* and *Eretmocerus eremicus* Rose et Zolnerowich) which could potentially be used as biocontrol agents of *B. tabaci*.

In order to define the rules of use of the oviposition device, it is necessary to strongly reduce the risk of interference with parameters external to the tested device. During the first experiment, the influence of the qualitative and quantitative composition of the whitefly nymphs population and its daily variations on the fecundity and longevity of the parasitoid was eliminated by offering the parasitoid female a constant number of hosts at the same biological instar (3rd nymphal instars of *T. vaporariorum*). The regular availability of the parasitoid, produced by a commercial nursery, would seem to guarantee in the time the quality of the beneficial insect.

The variability observed in the three replicates resulted from a significant variability of the quality of the parasitoids produced by the commercial nursery. Likely the parasitoid assimilated, throughout the chain of production, the climatic conditions (thermoperiod, photoperiod) to which it was submitted through the first experiment where during 8 months, females of *E. formosa* needs to feed on nymphs. The parasitoid produced in a climatic

room and used in the second experiment was maintained under constant and reliable conditions of temperature, humidity and photoperiod during the experiment. This technique allowed the production of regular amounts of *E. formosa* pupae of constant quality.

For the host-plants *N. glauca* presents an excellent resistance to dehydration and a very good adaptability to high infestations of whiteflies. *A. conyzoides* is easy to grow, rustic and supports also high infestations of whiteflies.

Characteristics of a standard oviposition device could be defined by good ventilation (mesh) and the position of the leaf disc bearing nymphal instars of whitefly preferred by the parasitoid female. These two characteristics have a strong influence on the fecundity and the longevity of *E. formosa* females and on their parasitical activity on the 3rd instar nymphs of *T. vaporariorum* The mean fecundity of a single *E. formosa* female produced at 22 °C on 3rd instar *T. vaporariorum* nymphs (202.8 eggs) is constant among the 3 replicates and much higher than the level reported in the literature under close temperatures (Kajita, 1981; Yoldas, 2001).

The longevity of *E. formosa* females in this study (27.7 days on average at 22 °C), is largely above the survival levels reported in the literature which are below 21.5 days at 21 °C (Burnett, 1949) and even lower. Only the presence of honeydew allows the parasitoids to survive longer (48.4 days at 21 °C) (Vet and van Lenteren, 1981).

Determining the most effective beneficial insects for biological control of *T. vaporariorum* and *B. tabaci* requires rigorous methodologies of study in order to determine differences in the intrinsic rate of increase in relation to the limits that the parasitoid will find in its environment (temperature, humidity, photoperiod). Knowledge of the actual characteristics of the parasitoid established by a standard type of oviposition device should indicate the thermal limits of activity of the beneficial insect. A standard type of oviposition device should allow the comparison of the performances of the different species and biotypes of *Encarsia* and *Eretmocerus* in relation to the host biodiversity, both in their response to the abiotic factors and in their permanent interaction in the framework of interspecific and intraspecific competition.

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