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Host suitability of different instars of the whitefly *Bemisia tabaci* 'biotype Q' for *Eretmocerus mundus*

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Abstract. *Eretmocerus mundus* Mercet is a parasitoid of *Bemisia tabaci* (Genn.) indigenous to the Mediterranean and is used commercially for augmentative biological control in Spain and elsewhere. A better understanding of the suitability of different host instars would help optimize production and field application. Incidence of parasitism, development time, survivorship and sex ratio were evaluated when different nymphal instars of the sweetpotato whitefly *Bemisia tabaci* biotype 'Q' were offered for parasitization. Experiments were conducted on sweet pepper at 25 °C, 75% RH and 16:8 (L:D) photoperiod. *E. mundus* oviposited in all nymphal instars of *B. tabaci* except the mature 4th instar or pharate adult (previously designated, 'pupa'). Incidence of parasitism was greatest (33.8 ± 5.1 parasitized nymphs) and development time shortest (14.1 ± 0.1 d) when oviposition occurred under 2nd and 3rd instar nymphs compared to 1st or 4th instars. Survivorship (85%) and offspring sex ratio (39.8% female) did not differ statistically for parasitoids developing in whiteflies that were parasitized as different instars. Although 2nd and 3rd instars were clearly the most favorable host stage for *E. mundus*, its capacity to parasitize and develop on a wide range of host stages is a favorable characteristic for both rearing and field application.

Key words: *Bemisia tabaci*, developmental rate, *Eretmocerus mundus*, host suitability, host instar, parasitism, sex ratio, survivorship

Introduction

Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) is the key pest of vegetables and other horticultural crops in much of the tropics and subtropics. The aphelinid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae), is the most important parasitoid of this pest in protected crops in Southeastern Spain (Rodriguez et al., 1994). Its ability to control *B. tabaci* has been favorably compared to other parasitoids by authors elsewhere including Hafez et al. (1979), Kapadia and Puri (1990), Goolsby et al. (1998) and Greenberg et al. (2002a, b). Furthermore, field studies performed after its introduction

have confirmed the capacity of this parasitoid to control *B. tabaci* (Goolsby et al., 1998; Calvo et al., 2002; Téllez et al., 2002; Urbaneja et al., 2002, 2003; Stansly et al., 2003).

Like other species of *Eretmocerus* spp. (Rose et al., 1995), *E. mundus* is a solitary parasitoid ovipositing externally under the nymphal host. Upon eclosion, the 1st instar larva penetrates the host cuticle, feeds and pupates internally. Additional mortality is inflicted by the adult female through host feeding that occurs preferably on young instars (Gerling and Fried, 2000). *E. mundus* became commercially available in 2002, and there is every indication that its use for augmentative biological control of *B. tabaci* will expand rapidly (Téllez et al., 2002; Urbaneja et al., 2002).

Previous studies on the biology of *E. mundus* were conducted on *B. tabaci* biotype 'B' or unspecified biotypes (Hafez et al., 1979; Tawfik et al., 1979; Foltyn and Gerling, 1985; Kapadia and Puri, 1990; Sharaf and Batta, 1996; Gerling et al., 1998; Jones and Greenberg, 1998; Gerling and Fried, 2000; Greenberg et al., 2002). However, important morphological, biological and behavioral differences have been found between biotypes B and Q, the latter predominating in Spain and other parts of the Mediterranean (Simon et al. 2001; Simon, 2002). In a comparative study, using scanning electron microscopy, Guirao (2002) found some differences in fourth instar nymphal morphology. The size of the wax fringes were bigger in the Q biotype; the B biotype had five pairs of dorsal and three pairs of submarginal setae while the Q biotype had seven and four, respectively. Muñiz and Nombela (2001) obtained the shortest developmental times as well as the lowest developmental thresholds and thermal constant with the biotype Q. Furthermore, biotype Q is more efficient as a vector of the virus TYLCV (Sánchez-Campos et al., 1999). These differences, as well as those among strains of *E. mundus* of different origins, could have important biological consequences. For example, using several *E. mundus* strains collected in different regions of the world, Goolsby et al. (1998) studied their efficacy in controlling *B. tabaci* biotype B and found that the strain collected in the region of Murcia (Spain) was the most efficient.

Host instar suitability is of practical concern in optimizing rearing resources and the success of field application. Foltyn and Gerling (1985) evaluated the incidence of parasitism in and preference for different host instars of an unspecified biotype of *B. tabaci* by *E. mundus*. Effects of host instar on incidence of parasitism, longevity and development time of *E. mundus* on *B. tabaci* biotype 'B' were investigated by Jones and Greenberg (1998). However, these authors did not evaluate survivorship or sex ratio, two parameters of special interest for rearing. The present study was undertaken to evaluate host instar effects on the incidence of parasitism, developmental

time, survivorship and offspring sex ratio of *E. mundus* parasitizing *B. tabaci* biotype 'Q'.

Materials and methods

Test insects. Experimental hosts and parasitoids (*B. tabaci* and *E. mundus*) came from colonies maintained by Koppert Biological Systems S.L. (Águilas Murcia, Spain) originally collected from multiple locations in the provinces of Murcia and Almería. The whitefly biotype used in this system was identified as 'Q' using PCR technology (J.L. Cenís, CIDA La Alberca. Murcia, SP, personal communication).

Plant hosts. *S*weet pepper (*Capsicum annuum* L 'Spiro' (Seminis Ibérica S.A., Almería) was used as a whitefly host. Plants were seeded simultaneously in tray cells and later transplanted into individual 1 liter pots to obtain homogenous and pesticide-free plants. Plants were used when the 4th node leaves were fully expanded.

Whiteflies. Pepper plants (N = 45) were exposed in a 2 m × 4 m × 2.5 m whitefly production cage in an air-conditioned greenhouse maintained at 23 ± 2 °C and 60 ± 5% RH to obtain a uniform cohort of eggs. Plants were removed after 24 h, vacuumed clean of whitefly adults and placed in an identical but whitefly-free cage provided with 8 yellow sticky traps hung at canopy level to capture errant whiteflies and other insects. Whitefly development was monitored daily with a stereoscopic binocular microscope and the test stages were used at the following intervals: 12 d (1st instar) 14 d (2nd instar) 16 d (2nd and newly formed 3rd instars) 18 d (mature 3rd instar or 4th instar) and 20 d (mature 4th instars and pharate adults).

Parasitoids. Pupae of *E. mundus* were obtained from the production facility and held in a climate controlled cabinet at 25 ± 1 °C 75 ± 5% RH and 16:8 L:D. Pupae were sexed under a stereoscopic microscope according to antennal color: dark for males, light for females. One female pupa and two male pupae were then introduced in Petri dish (5.2 cm dia.) and held for a day after emergence to allow for mating before use in experiments.

Experimental Procedure. Freshly cut leaf disks 5.2 cm in diameter were examined under a stereoscopic binocular microscope to ascertain that each held between 30 and 60 nymphs of the appropriate stage. Excess nymphs were removed with a small brush. Leaf discs were then placed in a petri dish of the same diameter on a fine layer of agar of approximately 2 mm thick (2% w/v). A single couple of *E. mundus* was introduced into the petri dish which was held in the climate controlled cabinet for 24 hours. Each treatment was replicated 10 times.

Parasitoids were removed after 24 hours and leaf discs were allowed to continue incubating in the cabinet. Mycetome displacement of parasitized whiteflies could be observed within 7 to 8 days at which time parasitism was evaluated. Apparently unparasitized nymphs were inverted to verify lack of parasitization and to check for non-viable parasitoid eggs that may have remained under the nymphs without emerging. Fertility was calculated as the total number of parasitized nymphs divided by the same plus the number of non-viable eggs. Approximately 2/3 of the parasitized nymphs were held for emergence to estimate developmental time, survivorship (number emerged divided by total number parasitized) and sex as determined by antennal and genital morphology (Rose and Zolnerwhich, 1997).

Analysis. Data were determined to be normal by goodness of fit or transformed to log(x) or cos(x) and again tested for normality. Results were subjected to one way analysis of variance and the Tukey test was used for mean separation at $p < 0.05$ (SPSS, 1999). Fertility was analyzed using the Mann-Whitney test because data could not be normalized nor was there variance homogeneity among samples.

Results

Eretmocerus mundus successfully oviposited under all the nymphal instars of *B. tabaci* with the exception of the 20 d-old 4th nymphal instar (Figure 1). However, number of parasitized nymphs varied significantly with host instar (df = 4, 49; F = 3,851; $p = 0.009$). The most frequently (33.8 $\pm$ 5.1) parasitized host was aged 14 d and consisted of 2nd and 3rd instars (Figure 1). However, parasitization was not significantly greater than that observed for 2nd instar nymphs 12 d old (28.6 $\pm$ 3.4) or 3rd instar nymphs 16 d old (27.5 $\pm$ 7.9). The lowest incidence of parasitism was observed on 1st instar nymphs, i.e. 10 d old (17.7 $\pm$ 3.8) and 3rd and 4th instar nymphs, i.e. 18 d old (17.9 $\pm$ 7.8).

Host stage effects on developmental time were statistically significant (df = 4, 681; F: 53.039; $p < 0.0001$). Development was most rapid (14.1 $\pm$ 0.1 d) in the 14 d old 2nd instars and slowest (16.8 $\pm$ 0.2 d) in 10 d-old 1st instars (Table 1).

Mean egg fertility was estimated at 99.2 $\pm$ 0.5% with no significant effect of host stage (U = 31.0, $p = 0.28$). Survivorship of larvae was estimated at 99.8 $\pm$ 0.2% and of pupae at 85.3 $\pm$ 1.5% for a total of 84.2 $\pm$ 1.3% with no significant differences observed among host instars (F = 2.5, $p = 0.06$; F = 0.64, $p = 0.63$ and F = 0.39; $p = 0.81$ respectively, df = 4.47 for all). Sex ratio averaged 38.8 $\pm$ 1.0 or 58.5 $\pm$ 1.0% female with no statistical differences between treatments (df = 4, 43; F: 0.246; $p = 0.910$).

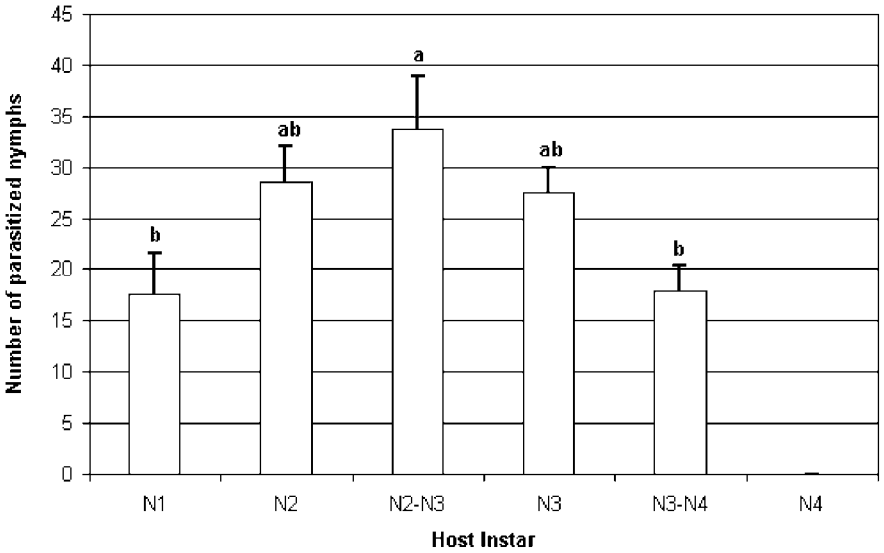

Figure 1. Number of parasitized nymphal instars of the sweetpotato whitefly *Bemisia tabaci* biotype 'Q' (X ± ES) by *Eretmocerus mundus*. Bars topped by the same letter represent means that are not statistically different (ANOVA, $p < 0.05$).

Table 1. Developmental time (mean d ± SE) of *Eretmocerus mundus* on different nymphal instars of *Bemisia tabaci* biotype 'Q' (n = number of replicates)

Days after oviposition (host intars present)	Egg – pupa mean ± SE (n) d	Pupa – adult mean ± SE (n) d	Egg – adult mean ± SE (n) d
10 (1st)	12.0 ± 0.1 (97) c	4.8 ± 0.1 (84) a	16.8 ± 0.2 (83) c
12 (2nd)	10.6 ± 0.1 (177) b	4.7 ± 0.2 (156) a	15.3 ± 0.1 (156) b
14 (2nd and 3rd)	9.5 ± 0.1 (227) a	4.7 ± 0.1 (198) a	14.1 ± 0.1 (198) a
16 (3rd)	9.9 ± 0.1 (177) a	4.4 ± 0.2 (149) a	14.3 ± 0.1 (149) a
18 (3rd and 4th)	9.6 ± 0.2 (108) a	5.0 ± 0.1 (85) a	14.6 ± 0.1 (85) ab
20 (4th)	–	–	–

Means followed by the same letter within the same column were not statistically different (ANOVA, $p < 0.05$).

Discussion

We observed *E. mundus* successfully parasitizing all the nymphal instars of *B. tabaci* biotype 'Q' with the exception of 20 d 4th instars. Jones and Greenberg (1998) working with *B. tabaci* biotype 'B' observed parasitization of all instars, although incidence was greatly reduced in 'late 4th instars or so called red-eye nymphs. These authors reported percentage para-

sitism as greatest in 2nd instars whereas we found greater incidence in the mixture of late 2nd and early 3rd instars. Foltyn and Gerling (1985) found that *E. mundus* preferred 3rd instars of an unspecified biotype of *B. tabaci* when given a choice of instars. Most rejections of unaccepted hosts occurred after antennal drumming and before probing with the ovipositor. No one has yet reported simultaneous choice and no-choice tests with *E. mundus* to determine whether preference corresponds to suitability for parasitisization, as appears to be the case of *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) (Liu and Stansly, 1996).

Developmental time was longest when first instars were parasitized as compared to all other instars tested (Table 1). Again, we observed a favorable response to the most suitable late 2nd early 3rd instar stage. This seems somewhat at variance with Jones and Greenberg (1998) who observed most rapid development on the 2nd and early 4th instars with 3rd instars intermediate. There is certainly agreement that longest development of this species (Foltyn and Gerling,1985; Jones and Greenberg, 1998) and related species of *Eretmocerus* sp. (Gerling, 1966; Mcauslane and Nguyen, 1996; Donnell and Hunter 2002; Hu et al., in press) and *Encarsia pergandiella* (Liu and Stansly, 1996b) is seen when oviposition occurs in the 1st instar.

Foltyn and Gerling (1985) stated that *E. mundus* eggs hatched only under 4th instar nymphs such that the period between oviposition and hatching ranged between 4 and 10 d depending on host age. However, it now appears that the parasitoid hatches and the first instar, prepenetration, waits for the host to develop to instar 4 before penetrating and molting to the second instar (D. Gerling, personal communication).

In 1966, Gerling reported for a different species of *Eretmocerus* that there was a delay in development only after the parasitoid larva had penetrated the young *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) nymph and molted to the 2nd instar. Parasitoid development did not resume until the host had molted to an adult. Hu et al. (2002) demonstrated that no matter which instar was parasitized, the parasitoid *Encarsia formosa* Gahan never molted to its last instar until the host, *T. vaporariorum*, had reached Stage 5 of its last instar, a stage in which the whitefly has initiated the nymphal adult molt and adult development. Similar results were observed when *B. tabaci* (Biotype B) was parasitized by *E. formosa* (Hu et al., in press).

We determined that there was no effect of host stage on preimaginal survivorship, and that most mortality occurred in the pupal stage. Gerling and Fried (2000) reported parasitoid pupal mortality of between 74% and 84% depending upon whether pupae were placed in gelatin capsules or left on leaves. Greenberg et al. (2002b) observed 84.9% emergence of *E. mundus*

from visibly parasitized nymphs, presumably identified by displacement of the mycetomes. These two previously published values agree with our results.

Based on the literature, a male-skewed sex ratio in *E. mundus* is not the norm. Gerling and Fried (2000) observed changes in progeny sex ratio of *E. mundus* based on the age of the ovipositing adult female. For eggs laid on days 1, 6 and 10, respectively, 53%, 67% and 18% were female. Greenberg et al. (2002) observed approximately 53% females resulting from oviposition under 2nd instar nymphs with no difference between *B. tabaci* and *T. vaporariorum*. Sex ratio from field collections in India (Kapadia and Puri, 1999), while always favoring females, varied with season, from 55% female in August to 75% in January. This tendency seems to run counter to that observed in the laboratory by Sharaf and Batta (1985) who reported 60.6% females emerging from *B. tabaci* at 25 °C but only 41.7% females at 14 °C. Emergence of field-collected *E. mundus* tested on *B. tabaci* biotype 'Q' on tomato in greenhouses of Murcia and Almeria (Spain) provinces during fall 2001 was 58% female (Stansly et al., 2003). Thus, it would appear that the innate tendency of *E. mundus* is toward a female-biased sex ratio unless conditions are in some way sub-optimal.

Our results again demonstrate the plasticity of *E. mundus* to adapt to different environmental conditions, in this case host age, that contribute to its value as a biological control agent of *B. tabaci*. Furthermore, this information contributes to a greater understanding of the *B. tabaci-E. mundus* relationship, and could be useful in mass rearing and field release protocols.

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