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ARTIFICIALLY-REARED WHITEFLIES, *BEMISIA ARGENTIFOLII*, (HOMOPTERA:ALEYRODIDAE) AS HOSTS FOR PARASITIC WASPS

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ABSTRACT

Bemisia argentifolii (Powell & Bellows) (Homoptera: Aleyrodidae) (= *B. tabaci* Gennadius B biotype) nymphs reared on an artificial feeding system were successfully parasitized by three species of wasps, *Encarsia formosa* Gahan, *Eretmocerus eremicus* Rose and Zolnerwich, and *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae). *Encarsia formosa* and *Eretmocerus* spp. preferred to parasitize third and fourth instar nymphs including the late fourth instar "red eye" stage. Oviposition rate and developmental time compared favorably to some reports on plants, but successful production of adult wasps was low. This system can be used to elucidate host-parasite interactions without confounding interactions with plants.

Key Words: *Encarsia formosa*, *Eretmocerus eremicus*, *Eretmocerus mundus*, *Bemisia tabaci* B-biotype, parasitoid, biological control, rearing

RESUMEN

Las ninfas de *Bemisia argentifolii* (Powell & Bellows) (Homoptera: Aleyrodidae) (= *B. tabaci* Gennadius B biotype) criadas del sistema artificial de alimentación fueron parasitadas exitosamente por tres especies de avispas, *Encarsia formosa* Gahan, *Eretmocerus eremicus* Rose and Zolnerwich, y *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae). *Encarsia formosa* y *Eretmocerus* spp. preferieron parasitar la tercera y cuatra estadia ninfal incluyendo la estadia cuatra tardia de "ojo rojo". La tasa de oviposición y el tiempo de desarrollo comparaban favorablemente a algunos reportajes sobre plantas, pero la producción exitosa de las avispas adultas fué baja. Se puede usar este sistema para elucidar las interacciones del hospedero-parasito sin complicar las interacciones con las plantas.

Parasitic wasps are important biological control agents for *Bemisia argentifolii* (Powell & Bellows) (Homoptera: Aleyrodidae) (= *B. tabaci* Gennadius B biotype), a major pest of cotton, melon and other crops and a vector of begomovirus. We report here the first successful parasitism of whitefly nymphs reared on a sterile artificial feeder system. Three of the major parasitoid species commercially sold for management of *B. argentifolii* and other whitefly species in field and greenhouse situations were included in these experiments. Use of the artificial rearing system should permit further studies of parasitoid physiology, behavior, and host/parasite interactions without the confounding effects of plant biochemistry. Refinement of this system could potentially lead to commercial production of parasitoids without the requirement for plants.

MATERIALS AND METHODS

Whitefly Feeder System

B. argentifolii nymphs were reared on a sterile artificial feeding system as described by Jancovich et al. (1997) and Davidson et al. (2000). The feeder system, which is comprised of a 2-piece polycarbonate chamber holding a 45 mm, 1.0 μm pore-size Teflon® filter membrane (Micron Separations-Osmonics, Inc., Westboro, MA) was autoclaved before filling with a diet consisting of filter-sterilized 15% sucrose plus 5% Difco® (Detroit, MI) yeast extract solution. Whitefly eggs were harvested from cotton or melon leaves using a WaterPik® dental device and surface-sterilized using 70% ethanol and 10% Roccal II® (Sterling Drug) solution before being placed on the membrane (Davidson et al. 2000). This membrane has a regular geometric pattern on its surface that permitted accurate mapping of each parasitized nymph (Fig. 1). Adult *B. argentifolii* have been produced on this feeder system and diet (Davidson et al. 2000).

Sterilization and Introduction of Parasitoids

Encarsia formosa pupae were harvested from a laboratory colony maintained on *B. argentifolii* on cotton in a greenhouse at Arizona State University or from *B. argentifolii* maintained on sweet potato at the USDA-ARS Beneficial Insects Research Unit, Weslaco, Texas. *Eretmocerus eremicus* and *E. mundus* pupae were obtained from the Weslaco, Texas facility where they were reared

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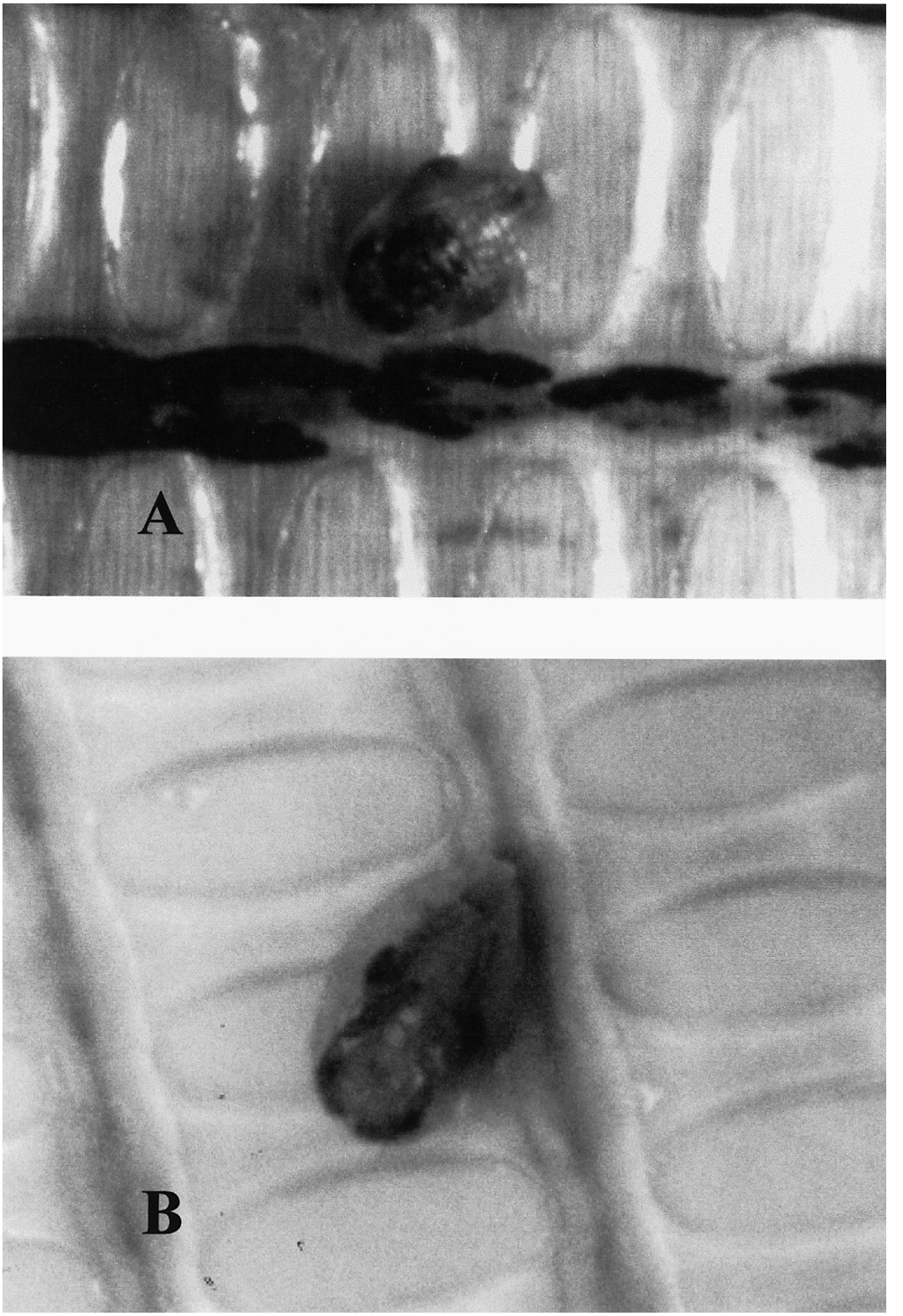


Fig. 1. A. *E. formosa*-parasitized whitefly nymph on feeder membrane. B. *E. mundus*-parasitized nymph on feeder membrane.

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on a *B. argentifolii* colony on sweet potato. *E. eremicus* originated from a culture established by Koppert, Inc., California; *E. mundus* was originally collected near Murcia, Spain. Wasp pupae removed individually from leaves using a fine-pointed metal needle were surface-sterilized by a brief rinse in 70% ethanol. They were then placed in 10% Roccal® (Sterling Drug, Montvale, NJ) solution for two minutes, followed by a rinse in sterile water. Surface-sterilized pupae were placed in a sterile petri dish with a small drop of sterile honey, and held in a growth chamber at 28°C, 14/10 L/D.

Female wasps were chilled until immobile and moved to whitefly feeders 1-3 d after emergence. One wasp was confined to each chamber under a sterile slide for 24 or 48 h at 28°C, 14/10 L/D. Any chambers that became contaminated by fungi were discarded. *E. formosa* were not mated as they are uniparental parasitoids. *Eretmocerus* spp. females were exposed to males that emerged from the same group of surface-sterilized pupae but mating status was not determined. Mating is not necessary for egg production by *E. formosa*, *E. mundus or E. eremicus* (Gerling 1966; Vet & van Lenteren 1981).

Estimation of Parasitism

On this feeder system, 25-50% of *B. argentifolii* nymphs develop at least to the third instar by 14 d after egg application (Davidson et al. 2000). *E. formosa* females were added to feeders 19.5 d (±1.5 d) after whitefly egg application. Female *E. mundus* and *E. eremicus* were applied to feeders 17.5 d (±1.5 d) after whitefly egg application. Whitefly nymphs were counted by stage within two d after wasp application. The "red eye" (pre-adult) stage was counted as a separate category from early fourth instar. Development of nymphs on feeders was not synchronous; all stages were present at the time of introduction of wasps.

Whitefly nymphs were observed every three d for 23 d (±5 d) after exposure to a wasp. Nymphs with evidence of *E. formosa* host-feeding or stings (circular, brown to black holes in the host's dorsal surface) were counted, host instar was recorded, and the location of the host and stage of the parasite were mapped on a computer-scanned, magnified image of the feeder membrane. Twenty feeders exposed to *E. formosa* for 24 h and 7 feeders exposed for 48 h remained uncontaminated by fungus for the full experimental period.

Eretmocerus eremicus and *E. mundus* are initially ectoparasitoids, leaving no distinctive marking on the host (Gerling 1966; Powell & Bellows 1992). Changes in the location of the mycetomes, and color and shape of the whitefly nymph were used as indicators of parasitism. *Eretmocerus*-parasitized nymphs were also dissected 30 d after wasp exposure to confirm parasitism.

Data Analysis

Normality could not be obtained through transformation; therefore, nonparametric statistics were used in all analyses. Kruskal-Wallis nonparametric ANOVA (SAS version 8.0; SAS Institute, Inc., Cary, NC) was applied to raw data to determine significant differences among: number of available whitefly nymphs within each stage; number of nymphs stung by stage; and number of successful wasp eggs by host stage. Where significant differences were found, pairwise comparisons were performed using the Nemenyi test, a variation of the Tukey test (Zar 1996). Because sample sizes were not the same for 24 and 48 h experiments, these data could not be grouped for nonparametric analysis. Statistical analyses were performed on data from *E. formosa* only, as the numbers exposed to *Eretmocerus* spp. were too small for analysis.

Whitefly stages, *E. formosa* stings, and "successful" *E. formosa* eggs in each stage (those that produced recognizable larvae) were pooled within each time period and averaged using Excel (Microsoft 2000; Microsoft Corp., Redmond, WA). Averages and standard deviations for successful eggs in each stage were divided by the average total number of successful eggs on the feeders to find the proportions displayed in Figure 4.

Results

E. formosa

Significant differences were found among the numbers of available whitefly nymphs within each stage exposed to *E. formosa* for 24 h (χ^2_4 = 35.233, P = 0.0001) or 48 h (χ^2_4 = 21.688, P = 0.0002). Pairwise comparison using rank sums showed no significant differences between the numbers of first, second, third or fourth instar whitefly nymphs available for parasitism by *E. formosa* for either 24 or 48 h; however, significantly fewer "red-eye" stage forms were available at both time periods (Fig. 2).

Significant differences were found among the numbers of whitefly nymphs stung by stage at 24 h (χ^2_4 = 48.78, P < 0.0001) and 48 h (χ^2_4 = 20.68, P = 0.0004). Pairwise comparison using rank sums showed no significant differences between the numbers of first, second, and "red eye" nymphs stung. However, significantly more 3rd and 4th instar nymphs were stung (Fig. 3).

Fourth instars, including the "red eye" stage, received proportionally the most stings (68%). An average of 11.8 (±8.5) total whitefly nymphs per feeder were stung by each *E. formosa* female in 24 h, and 19.7 (±14.8) were stung in 48 h. Only one *E. formosa* out of 27 failed to sting whitefly nymphs during confinement on a feeder (for 48 h). First or second instar whitefly nymphs were

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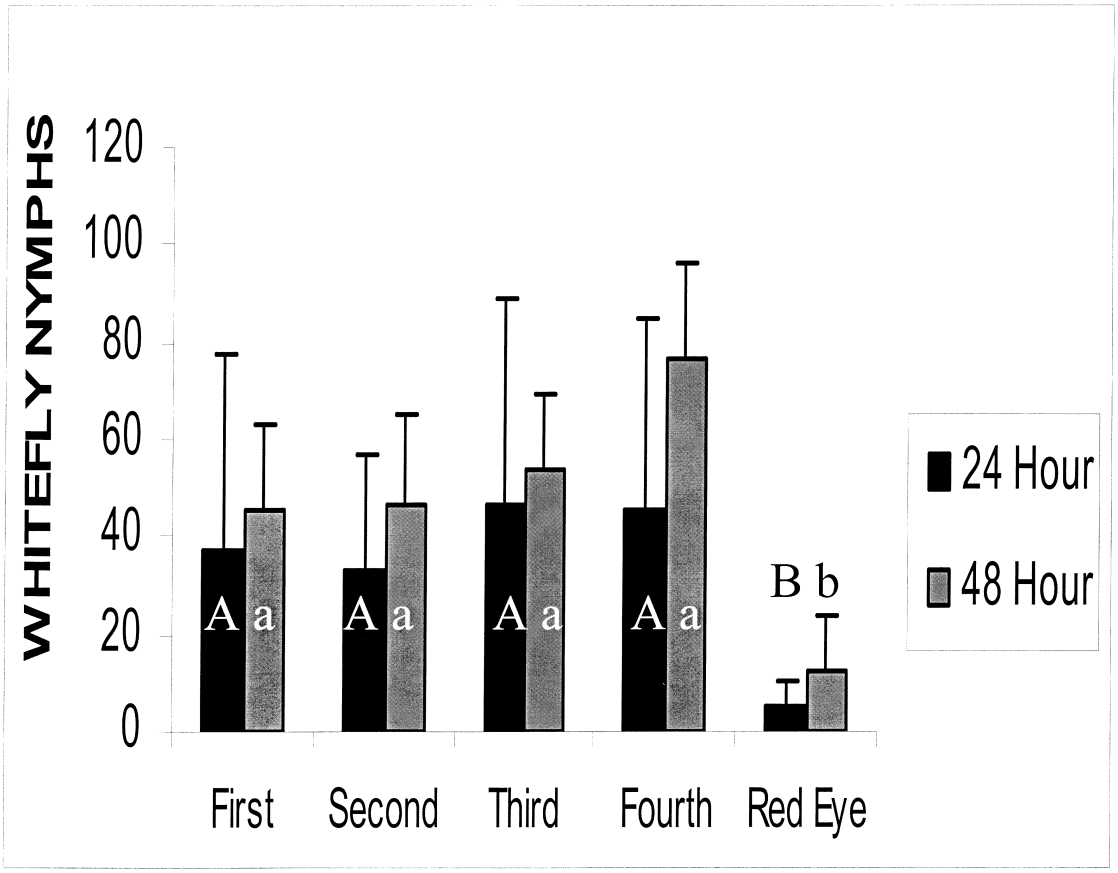


Fig. 2. Total average whitefly nymphs per feeder unit within each stage available for parasitism by *E. formosa* (24 h: $\chi^2_4 = 35.233$, P = 0.0001; 48 h: $\chi^2_4 = 21.688$, P = 0.0002).

rarely stung by *E. formosa*, representing less than 2% of total stings.

Significant differences were found among the number of successful wasp eggs, i.e., eggs that developed into a larva or pupa, by host instar for 24 h ($\chi^2_4 = 26.48$, P < 0.00001) and for 48 h exposure to a wasp ($\chi^2_4 = 10.29$, P = 0.0358). Pairwise comparison showed significantly more 4th instars were hosts to successful eggs than any other instar during 24 h exposure to a wasp. Due to small sample size for 48 h exposure, pairwise comparison showed no significance.

Eleven of the 20 *E. formosa* females that remained on feeders for 24 h deposited successful eggs. On average, these females laid 2.7 (±1.9) successful eggs per wasp in 24 h, which represented 12.5% of stings produced by these females. Four of the seven feeders that received *E. formosa* females for 48 h produced successful eggs. These wasps produced an average of 6.3 (±2.9) successful eggs each, representing 18.2% of their stings. The remaining 3 females, placed on feeders for 48 h, did not produce detectable larvae or pupae. The majority of "successful" eggs (leading to a detectable wasp larva, pupa or adult) produced during either 24 or 48 h were laid in fourth instar or "red eye" stage (Fig. 4).

Development of offspring to eclosion was observed on 4 feeders (14.5% of "successful" eggs) within twenty-eight d after exposure to female *E. formosa*. Two percent of stung whitefly nymphs produced adult female *E. formosa*, 9% of the wasps remained as pupae, 12% remained in larval stages, and 77% of stings did not develop apparent larvae. Metamorphosis to pupae was first observed 16.7 d (±3.2 d) and eclosion of adult wasps was first observed 24.8 d (±3.9 d) after exposure to wasps. Eighty-nine percent of *E. formosa* that developed to pupa or adult were produced in fourth instar hosts; the remainder were produced in third instar hosts.

Eretmocerus spp.

Eretmocerus spp. were unable to penetrate beneath the host when Parafilm® membranes were initially used on the whitefly feeder system (Jancovich et al. 1997), but successfully deposited eggs

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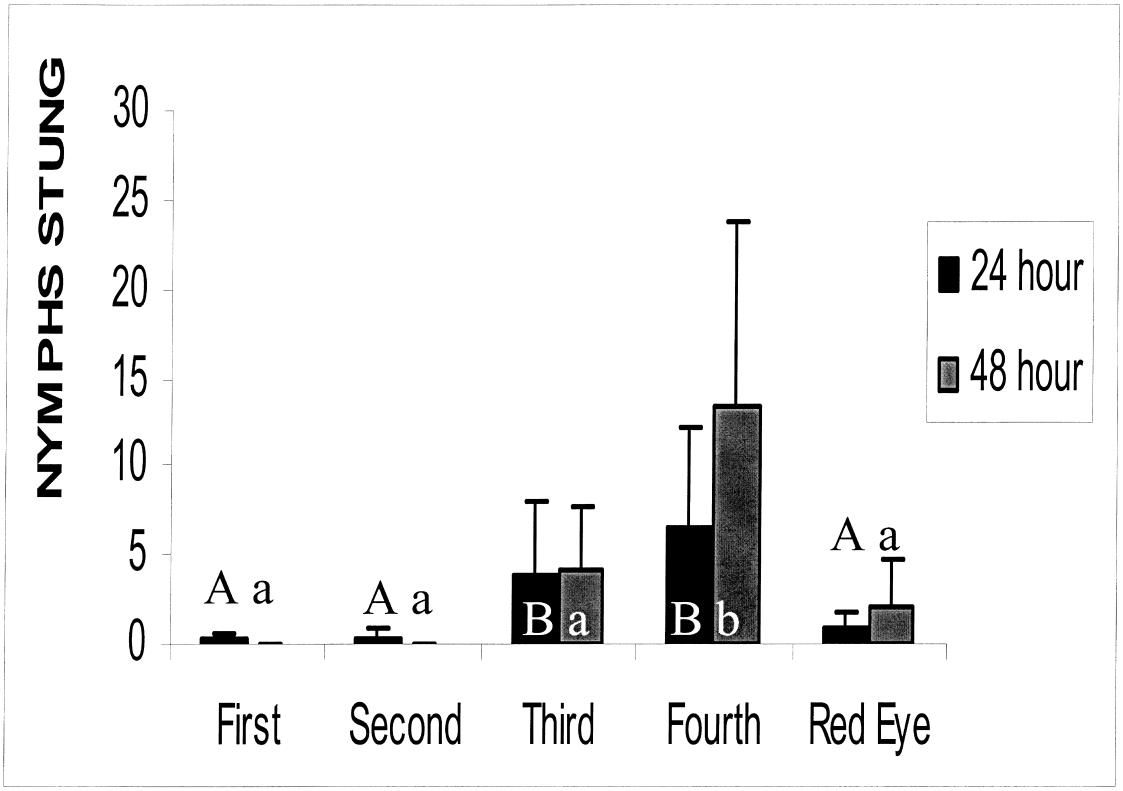


Fig. 3. Whitefly nymphs per feeder unit within each stage that were stung by *E. formosa* (24 h: $\chi^2_4 = 48.78$, P > 0.0001; 48 h: $\chi^2_4 = 20.6848$, P = 0.0004).

under nymphs when the Teflon® membrane with texture was used. All *Eretmocerus* spp. larvae and pupae developed in fourth instar whitefly larvae. Host feeding was not detected in nymphs exposed to *Eretmocerus* spp. females, as this species host-feeds by probing the vasiform orifice of the host and does not produce melanized spots on the dorsal surface of the host (McAuslane & Nguyen 1996).

Eleven *E. mundus* females were confined individually to whitefly feeders for 24 h and 11 for 48 h. Seven *E. mundus* deposited successful eggs that produced larvae during 48 h exposure to nymphs, but none were produced during 24 h exposure. By 28 d after exposure to *E. mundus*, twelve wasp pupae and 7 larvae were observed. Seven *E. eremicus* were confined individually to feeders for 24 h and 7 for 48 h. Three wasp pupae resulted from eggs oviposited by females confined for 24 h (two from the same female), and none by those confined for 48 h. No adult *E. mundus* or *E. eremicus* wasps were produced from whiteflies reared on the artificial feeder system.

DISCUSSION

E. formosa parasitism of *B. argentifolii* on artificial feeders is comparable in some respects to that observed on plants. The "successful" eggs that developed into larvae were generally laid in 3rd instar nymphs or higher, as is also found on plants (e.g., Nechols & Tauber 1977; Kidd & Jervis 1991; Hoelmer 1996; Hoddel et al. 1998; Jones & Greenberg 1999). The preference of *E. formosa* for hosts that are 3rd instar and older is reflected in the number stung within each instar (Fig. 3) in comparison to the number of nymphs available (Fig. 2). Alternatively, whitefly nymphs that had not progressed to third or fourth instar by ca. 20 d may have been recognized by the wasps as less desirable hosts. If eggs were laid in first or second instar hosts, the wasp larvae may not have developed sufficiently to be detected as a "successful" parasitism. *E. formosa* females laid about 3 "successful" eggs per female per day on *B. argentifolii* on feeders, which compares favorably with oviposition rates reported by Heinz (1996) on *B. argentifolii* (1.3-7.4 eggs/d) but is less than the 8-10 eggs/d observed at similar temperatures by Enkegaard (1994) on plant-fed *B. tabaci* and Vet & Van Lenteren (1981) on *Trialeurodes vaporariorum*. The time to emergence of adult *E. formosa* on *B. argentifolii* reared on feeders, 19-27 d, is less than the range reported by Vet & Van Lenteren (1981) for *E. formosa* reared on

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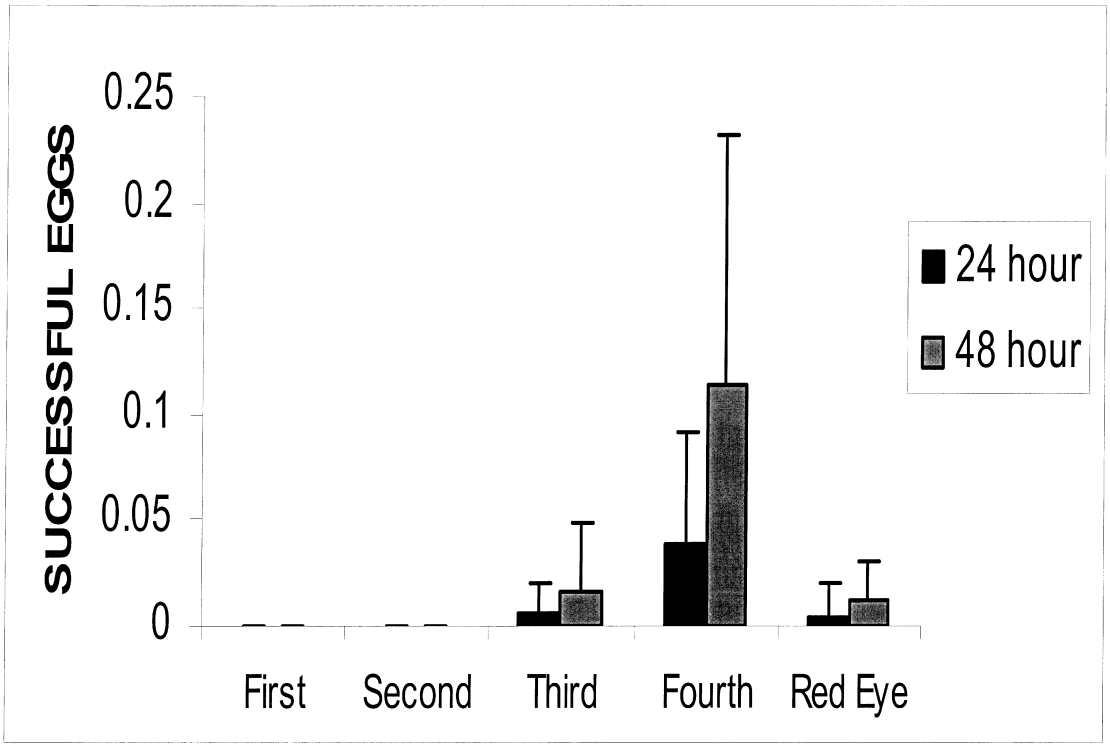


Fig. 4. Proportion of successful *E. formosa* eggs (forming detectable larva or pupa) by host stage.

T. vaporariorum on plants (28-38 d for females and 31-35 d for males).

Wasp production on the feeder system was far less efficient in producing live adult daughter wasps than that recorded on nymphs on plants. The percentage of host punctures that did not lead to development of larvae (77%) is far higher than on plants, where around 30-40% of punctures have been attributed to host feeding (Enkegaard 1994; reviewed by Heinz 1996). On the feeders, wasp females were confined to a much smaller area (2 × 3 cm) and more dense host population (ave. 185 nymphs/feeder) than on the leaf, which probably contributed to a high level of host feeding. Gerling (1966) observed host feeding by protein-starved *E. formosa* females, leading to death of the hosts. Since *E. formosa* females were held without a protein supply until confined to feeders, it is probable that they were protein-starved. *Encarsia formosa* is not a highly efficient parasitoid of *B. argentifolii*, *B. tabaci*, or *T. vaporariorum* on the plant. Parasitism rates from 2-3% to 30% have been reported (Bethke et al. 1991; Henter & Van Lenteren 1996; Hoddle & Van Driesche 1999).

Eretmocerus spp. were less successful on the feeder system than *E. formosa*, although parasitism was observed. Because the feeder system was subject to fungal contamination and greatly reduced survival of whitefly hosts after more than ca. 30 d, our experiments may have been terminated before *Eretmocerus* spp. larvae could complete development.

Our study has demonstrated that artificially-reared *B. argentifolii* nymphs are acceptable hosts for both *E. formosa* and *Eretmocerus* spp. parasitoids. However, successful production of adult wasps from these feeders is far below that observed on *Bemisia* spp. reared on plants, and is not currently practical for commercial use. Nevertheless, the feeder system provides a method for investigating important factors that impact the ability of these parasitoids to control *B. argentifolii* in greenhouse or field situations. For example, this system enables investigations on the influence of the host plant chemicals on parasite efficiency. Although surface characteristics of the leaf have been shown to affect *E. formosa* searching behavior (De Barro et al. 2000; reviewed by Hoddle et al. 1998), much less is known about nutritional and chemical factors involved in host-plant differences in *E. formosa* parasitism efficiency (Van Lenteren et al. 1987; Bentz et al. 1996; reviewed in Hoelmer 1996 & Hoddle et al. 1997). In other experiments, we have used this system to investigate the effects of feeding the antibiotic tetracycline to *E. formosa* females, which is reported to eliminate the *Wolbachia* symbionts

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(Hunter 1999). Male *E. formosa* offspring were produced, demonstrating that the artificial feeder system may be useful for studies of the wasp symbionts, as it has been for studies of the whitefly symbionts (Davidson et al., unpubl.).

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