# QUARANTINE EVALUATION OF EXOTIC PARASITOIDS OF THE SWEETPOTATO WHITEFLY, *BEMISIA TABACI* (GENNADIUS)[1]

John Goolsby[2], Jesusa Crisostomo Legaspi[3], Benjamin C. Legaspi, Jr.[4]

## ABSTRACT

Nineteen strains of parasitoids belonging to the genera *Eretmocerus* and *Encarsia* were evaluated as potential biological control agents of the sweetpotato whitefly, *Bemisia tabaci* on melons. Of these strains, three are undescribed species of *Eretmocerus*, two collected from Texas and one from Taiwan. Percentage parasitism and numbers of hosts attacked were measured on melon plants in the greenhouse. Percentage parasitism was found to decline with host numbers and was described by an exponential decay function. Highly significant differences were found in both numbers of hosts attacked and in percentages of parasitism among the different strains, but no strong evidence was found to indicate that *Eretmocerus* species are better parasitoids than *Encarsia* species, or vice versa. The evaluations indicate that a species of *Encarsia* nr. *pergandiella* from Brazil (identification number M94055) appeared to be the most promising parasitoid of *B. tabaci* in a melon crop.

## INTRODUCTION

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Biotype "B") also known as *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae), is an extremely destructive pest of field, ornamental and greenhouse crops throughout the world. *B. tabaci* inhabits a wide geographical range (Cock 1986, 1993), possesses a high fecundity and a short life-cycle (Baumgartner and Yano 1990), attacks a broad range of host plants (Cock 1986, Byrne et al. 1990), and serves as a vector for plant viruses (Brown et al. 1992). The whitefly also produces honeydew that promotes growth of sooty mold which contributes to crop loss. Moreover, populations of *B. tabaci* have displayed resurgence and resistance to conventional insecticides (Dittrich et al. 1990).

In the Lower Rio Grande Valley of Texas, *B. tabaci* infested over 100,000 acres of cotton in 1991, causing an estimated crop loss of about $80 million (Faust 1992). Coupled with losses in fruit and vegetable production, total crop loss in the Lower Rio Grande Valley in 1991 was estimated to exceed $100 million. In the same year, *B. tabaci* in Texas caused $250 million in economic losses and more than 6000 lost jobs (Perring et al. 1993, D. Riley pers. comm.). Additionally, damage in 1991 was estimated at $37 million in Arizona, $120 million and 3400 jobs lost in California, and over $140 million in Florida.

This study used melon as a host plant to evaluate 17 exotic and two native strains of parasitoids as possible biological control agents of *B. tabaci*. Melons were selected as a host because they are the first crop in the spring to harbor a large population of whiteflies. These early populations migrate to other crops and overwhelm attempts at control (J. C. Legaspi, unpubl. data). In addition, native parasitoids have not been found to be effective control

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[1] Homoptera: Aleyrodidae

[2] Mission Biological Control Center, USDA-APHIS-PPQ, PO Box 2140, Moore Air Base, Mission TX 78573

[3] Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596

[4] Joint affiliation: Biological Control of Pests Research Unit, USDA-ARS and Texas Agricultural Experiment Station, 2413 East Highway 83, Weslaco, TX 78596

agents of *B. tabaci* on melons (M. Ciomperlik, Mission Biological Control Center, unpubl. data). Parasitoids may have been hampered by the hirsute leaves of melons. Because of the prohibitive costs of maintaining numerous parasitoid strains, this evaluation is necessary in order to prioritize cultures for rearing and to identify the most promising parasitoids for later field evaluations in melons.

## MATERIALS AND METHODS

The whiteflies used as hosts in this experiment were taken from a colony established from material collected from cotton in Weslaco, TX, in 1994. This whitefly colony was maintained in environmental growth chambers which held fluctuating daily temperature from 24 to 29°C, a 50-70% RH, and a 14:10 L:D photoperiod. The colonies was maintained using hibiscus (*Hibiscus rosa-sinensis*) as a host plant.

The parasitoids evaluated in this study represented the greatest geographical and genetic diversity available in culture at the Mission Biological Control Center (MBCC). These whitefly parasitoids were collected from all parts of the world as shown in Table 1. The 19 strains/species of parasitoids of *B. tabaci* are tabulated according to site and country of collection, collectors, host plant on which material was collected, date of the collection and identification of the parasitoid. Pending futher genetic and morphological assessment, the strains/species are classified according to a unique "M" number assigned by MBCC. The parasitoids are species of *Encarsia* and *Eretmocerus*. All parasitoid cultures were reared using the whitefly colonies on hibiscus, and were maintained in similar environmental growth chambers held at the same conditions.

Three of the strains represent new species of *Eretmocerus* (Table 1). Strain M94002 was collected by M. Rose and S. Stauffer [Dept. of Entomology, Texas A&M University (TAMU)] on tomato, and strain M94003 was collected by J. Rodriguez in Mission, Texas, on cabbage. Strain M93058 was collected by C. Moomaw (TAMU) in Taiwan on tomatoes. All three species are currently being described by M. Rose. The *Encarsia* collected in Parbhani, India, (M92018) will be described as a new species by J. Heraty (UC Riverside).

The host plant chosen for the evaluations was melon (*Cucumis melo* var. "Perlita"). The melons were planted in pots (15cm diameter) which were held in a quarantine greenhouse maintained at 27°C and 50-60% RH and 12:12 L:D photoperiod. After four weeks, the melons were infested with about 10,000 adult whiteflies. After exposure for three days, individual leaves were covered with homemade sleeves to prevent further whitefly oviposition. The sleeves consisted of two sheets of organza mesh (≈ 8x8 cm, ≈ 60 mesh size) glued together on all sides, except for one. The remaining unglued side was secured using Velcro® strips. The whitefly immature stages were allowed to develop for 14 days, i.e. to second and third instars, before they were exposed to parasitoids.

Two adult female parasitoids of a given strain were collected from mixed age (1 to 7-days old) cultures, and this constituted a replicate. Each replicate was placed in a sleeve for 48 h, thus allowing parasitism. After the exposure period, the parasitoids were removed from the sleeves. Each of the 19 strains was replicated ten times. The developing parasitoids were held in their respective sleeve for 14-16 days to develop to the pupal stage. After this interval, numbers of parasitized and unparasitized whiteflies were recorded. Parasitism was determined by the presence of an immature parasitoid or characteristic exit hole from the host exuvium. The entire experiment was performed twice.

All statistical analyses were performed using the Systat® Statistical Package, version 5.2 (Wilkinson et al. 1992). Numbers of hosts attacked and percentage parasitism were analyzed by One-Way ANOVA using strain/species as the treatment. Replicates were excluded when the sum of parasitized and unparasitized whiteflies totalled less than ten. In order to test whether *Eretmocerus* spp. or *Encarsia* spp. exhibited greater numbers of hosts attacked or percentage parasitism, the data were divided according to genus and analyzed for effect of species using ANOVA. Data from both trials were also pooled to test for a possible effect of trial number on numbers attacked and percentage parasitism using a Two-Way ANOVA. When ANOVAs were significant, means were separated using Tukey's test corrected for experiment wise error and all error limits were set at $p = 0.05$. The data from both trials were pooled and nonlinear regressions were performed using total numbers of hosts available as predictors for both numbers of hosts attacked and percentage parasitism.

TABLE 1.Parasitoids of Sweetpotato Whitefly, *Bemisia tabaci*, Evaluated as Biological Control Agents

| Strain No. | Collection Site | Collector(s) | host plant | Collection date | Identification |
|---|---|---|---|---|---|
| M92014 | Murcia, Spain | A. Kirk<br>K. Chen<br>R. Sobhian | cotton | 1992 | *Eretmocerus* nr. *mundus* |
| M92018 | Parbhani, India | R. Nguyen | | 1992 | *Encarsia* sp. nov. |
| M92019 | Padappai, India | A. Kirk<br>L. Lacey | eggplant | 1992 | *Eretmocerus* nr. *mundus* |
| M93003 | Murcia, Spain | A. Kirk<br>L. Lacey | lantana | 1992 | *Encarsia transvena* |
| M93005 | Thirumala, India | A. Kirk<br>L. Lacey | | 1992 | *Eretmocerus* nr. *mundus* |
| M93058 | Tainan, Taiwan | C. Moomaw | tomato, poinsettia | 12-4-93 | *Eretmocerus* sp. nov. |
| M93064 | Mazotos, Cyprus | A. Kirk<br>L. Lacey | lantana | 10-4-93 | *Encarsia lutea* |
| M94002 | College Station, TX | M. Rose<br>S. Stauffer | tomato | 1994 | *Eretmocerus* sp. nov. |
| M94003 | Mission, TX | J. Rodriguez | cabbage | 1994 | *Eretmocerus* sp. nov. |
| M94014 | Benguet, Philippines | J. Legaspi,<br>R. Carruthers<br>T. Poprawski | white potatoes | 3-8-94 | *Encarsia transvena* |
| M94016 | Shan-Hua, Taiwan | J. Legaspi,<br>R. Carruthers<br>T. Poprawski | poinsettia | 3-14-94 | *Encarsia transvena* |
| M94017 | Shan-Hua, Taiwan | J. Legaspi,<br>R. Carruthers<br>T. Poprawski | soybean, tomato | 3-14-94 | *Encarsia transvena* |
| M94019 | Shan-Hua, Taiwan | J. Legaspi<br>R. Carruthers<br>T. Poprawski | soybean, tomato | 3-14-94 | *Encarsia transvena* |
| M94023 | Sai Noi Klong Ha Roi, Thailand | A. Kirk<br>L. Lacey | eggplant, melons | 3-9-94 | *Eretmocerus* sp. |
| M94036 | Chiang Mai, Thailand | A. Kirk<br>L. Lacey | *Chromolaena odorata* | 3-25-94 | *Eretmocerus* sp. |
| M94047 | Kuala Lumpur, Malaysia | A. Kirk<br>L. Lacey | *Mussaenda* sp. | 3-28-94 | *Encarsia transvena* |
| M94055 | Sete Lagoas, Brazil | M. Rose | soybean | 2-20-94 | *Encarsia* nr. *pergandiella* |
| M94056 | Sete Lagoas, Brazil | M. Rose | soybean | 2-25-94 | *Encarsia* nr. *hispida* |
| M94120 | Golan Heights, Israel | A. Kirk<br>L. Lacey | melons | 10-94 | *Eretmocerus* nr. *mundus* |

## RESULTS AND DISCUSSION

Statistical analyses will be presented in the following sequence: for each trial, we present the effects of species/strain on numbers attacked and percentage parasitism, followed by the comparison between *Eretmocerus* and *Encarsia* species. Finally, we present the comparisons between trials and the nonlinear regressions using the pooled data.

*Trial 1.* The results of the first trial are summarized in Table 2 which shows mean numbers of hosts attacked (± SE), in descending order, by each strain of parasitoid and mean percentage parasitism (± SE, arc sin transformation). The column for "N" indicates the number of replicates used from the initial ten following the exclusion of replicates with low total whitefly counts.

The numbers of whiteflies attacked were significantly different among the strains of parasitoids ($F = 4.38$; $df = 18, 153$; $r^2 = 0.34$; $P < 0.01$). The highest number of hosts attacked was recorded for strain M94055, a species of *En.* nr. *pergandiella* collected from Brazil. Percentage parasitism was also significantly different among strains of parasitoids ($F = 3.91$; $df = 18, 153$; $r^2 = 0.32$; $P < 0.01$). Using this measure of attack, the best performing strains were M92018 (*Encarsia* from India) and M94019 (*Encarsia* from Taiwan).

Numbers of hosts attacked and percentage parasitism were grouped according to genus to test whether higher attack rates could be found for either *Eretmocerus* spp. or *Encarsia.* spp. The numbers of hosts attacked were not significantly affected by genus ($F = 3.83$; $df = 1, 170$; $r^2 = 0.022$; $P > 0.05$). Percentage parasitism likewise was not found to be affected by parasitoid genus ($F = 2.55$; $df = 1, 170$; $r^2 = 0.015$; $P > 0.05$).

*Trial 2.* Results of the second trial are shown in Table 3. In the second trial, strain M94017 was eliminated completely from the analyses because of insufficient data caused by low whitefly oviposition. Numbers attacked were again significantly affected by strain of parasitoid ($F = 9.39$; $df = 17, 161$; $r^2 = 0.49$; $P < 0.01$). The highest numbers of hosts attacked were found for strain M92014 (*Er. mundus* from Spain) followed by M94055 (*Encarsia* from Brazil), the strain which exhibited the highest numbers attacked in the first trial. As in the first trial, percentage parasitism was again significantly affected by parasitoid strain ($F = 4.28$; $df = 17, 161$; $r^2 = 0.31$; $P < 0.01$). The clear best performer was again the *Encarsia* from Brazil (M94055), which was significantly different from all other strains.

In the test for differences between the two genera, the number of hosts attacked was found to be significantly affected by genus ($F = 14.1$; $df = 1, 177$; $r^2 = 0.074$; $P < 0.05$). The *Eretmocerus* strains attacked a mean of 22.3 hosts (SE 2.2, n = 90) compared to a mean of 12.4 (SE 1.4, n = 89) for the *Encarsia* strains. However, percentage parasitism was again not found to be affected by parasitoid genus ($F = 1.3$; $df = 1, 177$; $r^2 = 0.007$; $P > 0.05$).

*Comparison between trials.* In the Two-Way ANOVA for parasitoid strain and trial number, the strain eliminated from Trial 2 due to low numbers of usable replicates (M94017) was also eliminated from Trial 1, as required in the statistical analysis. The test on strain and trial number showed that numbers of whiteflies attacked was again significantly affected by the strain of parasitoid ($F = 8.1$; $df = 17, 305$; $r^2 = 0.42$; $P < 0.01$) and trial number ($F = 5.2$; $df = 1, 305$; $r^2 = 0.42$; $P < 0.05$). Fewer hosts were attacked in the first trial (AVE 13.4, SE 1.5, n = 162) compared to the second (AVE 17.4, SE 1.4, n = 179) ($F = 4.0$, $df = 1, 339$; $r^2 = 0.012$, $P < 0.05$). The difference in numbers attacked may be due to the greater numbers of whiteflies present in the second trial. The mean number of whiteflies per trial was calculated by pooling total numbers of parasitized and unparasitized whiteflies across the different strains. In the first trial, mean number of whiteflies was 178.0 (SE 11.9, n = 172), which increased significantly in the second trial to 348.6 (SE 17.9, n = 179) ($t = 7.9$, $df = 308$, $P < 0.01$).

In the analysis of percentage parasitism, parasitoid strain again proved to be a highly significant factor ($F = 3.6$; $df = 17, 305$; $r^2 = 0.3$; $P < 0.01$). The effect of trial number on percentage parasitism was insignificant ($F = 1.5$; $df = 1, 305$; $r^2 = 0.31$; $P > 0.05$). Percentage parasitism was 15.4 (SE 1.3, n = 162) in the first trial and 14.0 (SE 0.9, n = 179) in the second. The general conclusion from the joint analysis between the two trials is that the effect of the parasitoid strain on both numbers of hosts attacked and percentage parasitism is highly significant. No difference in percentage parasitism was found between the two trials.

TABLE 2. First Trial: Evaluation of Parasitoids against *B. tabaci* in Greenhouse melons

| Strain number | Number attacked | Tukey's HSD | Percentage Parasitism | Tukey's HSD | N |
|---|---|---|---|---|---|
| M94055 *Encarsia* (Brazil) | 41.7 ± 11.1 | a | 19.1 ± 3.0 | a,b,c | 10 |
| M94019 *Encarsia* (Taiwan) | 32.2 ± 4.3 | a,b | 35.1 ± 7.6 | a | 6 |
| M94056 *Encarsia* (Brazil) | 26.4 ± 5.6 | a,b,c | 27.9 ± 5.3 | a,b | 9 |
| M92014 *Eretmocerus* (Spain) | 24.4 ± 10.7 | a,b,c | 23.8 ± 7.3 | a,b,c | 8 |
| M93064 *Encarsia* (Cyprus) | 16.6 ± 6.1 | b,c | 22.6 ± 5.5 | a,b,c | 10 |
| M94023 *Eretmocerus* (Thailand) | 14.1 ± 4.0 | b,c | 17.1 ± 4.2 | a,b,c | 8 |
| M94036 *Eretmocerus* (Thailand) | 13.9 ± 6.0 | b,c | 13.2 ± 3.3 | a,b,c | 9 |
| M92018 *Encarsia* (India) | 13.7 ± 2.7 | b,c | 30.7 ± 6.1 | a | 10 |
| M94120 *Eretmocerus* (Israel) | 11.9 ± 5.3 | b,c | 12.5 ± 4.0 | a,b,c | 10 |
| M94014 *Encarsia* (Philippines) | 10.8 ± 2.9 | b,c | 16.0 ± 3.8 | a,b,c | 8 |
| M93005 *Eretmocerus* (India) | 9.2 ± 4.7 | b,c | 11.3 ± 3.9 | a,b,c | 9 |
| M93003 *Encarsia* (Spain) | 7.7 ± 4.7 | b,c | 5.5 ± 2.9 | b,c | 10 |
| M92019 *Eretmocerus* (India) | 6.3 ± 2.7 | b,c | 13.6 ± 5.3 | a,b,c | 10 |
| M94002 *Eretmocerus* (Texas) | 4.4 ± 2.8 | b,c | 11.5 ± 6.2 | a,b,c | 10 |
| M94016 *Encarsia* (Taiwan) | 4.3 ± 1.9 | b,c | 8.0 ± 4.0 | b,c | 9 |
| M94003 *Eretmocerus* (Texas) | 4.0 ± 3.7 | b,c | 6.3 ± 5.1 | b,c | 10 |
| M94047 *Encarsia* (Malaysia) | 3.4 ± 2.3 | c | 4.6 ± 3.1 | c | 10 |
| M94017 *Encarsia* (Taiwan) | 1.6 ± 1.5 | c | 2.3 ± 1.7 | c | 10 |
| M93058 *Eretmocerus* (Taiwan) | 0.5 ± 0.3 | c | 2.6 ± 1.8 | c | 6 |

TABLE 3. Second Trial: Evaluation of parasitoids against *B. tabaci* in greenhouse melons

| Strain number | Number attacked | Tukey's HSD | Percentage Parasitism | Tukey's HSD | N |
|---|---|---|---|---|---|
| M92014 *Eretmocerus* (Spain) | 55.0 ± 9.2 | a | 16.9 ± 2.0 | b | 10 |
| M94055 *Encarsia* (Brazil) | 37.6 ± 4.0 | a,b | 37.5 ± 6.7 | a | 10 |
| M92019 *Eretmocerus* (India) | 29.1 ± 4.4 | b,c | 16.4 ± 2.1 | b | 10 |
| M94120 *Eretmocerus* (Israel) | 26.8 ± 7.1 | b,c,d | 16.2 ± 4.0 | b | 10 |
| M93058 *Eretmocerus* (Taiwan) | 25.9 ± 6.6 | b,c,d | 15.3 ± 3.0 | b | 10 |
| M94036 *Eretmocerus* (Thailand) | 20.7 ± 2.9 | b,c,d,e | 14.4 ± 1.3 | b | 10 |
| M94016 *Encarsia* (Taiwan) | 13.8 ± 4.2 | c,d,e | 13.7 ± 2.5 | b | 10 |
| M94002 *Eretmocerus* (Texas) | 13.6 ± 3.4 | c,d,e | 16.5 ± 2.5 | b | 10 |
| M93005 *Eretmocerus* (India) | 12.9 ± 4.1 | c,d,e | 16.4 ± 6.0 | b | 10 |
| M93003 *Encarsia* (Spain) | 12.5 ± 3.0 | c,d,e | 11.9 ± 2.4 | b | 10 |
| M94047 *Encarsia* (Malaysia) | 10.9 ± 3.3 | c,d,e | 10.0 ± 2.7 | b | 10 |
| M94056 *Encarsia* (Brazil) | 10.0 ± 2.8 | c,d,e | 12.3 ± 3.9 | b | 10 |
| M93064 *Encarsia* (Cyprus) | 9.3 ± 3.0 | c,d,e | 8.4 ± 3.0 | b | 9 |
| M92018 *Encarsia* (India) | 8.6 ± 1.9 | c,d,e | 8.5 ± 1.4 | b | 10 |
| M94003 *Eretmocerus* (Texas) | 8.4 ± 2.9 | c,d,e | 9.4 ± 2.3 | b | 10 |
| M94023 *Eretmocerus* (Thailand) | 8.2 ± 3.1 | c,d,e | 13.4 ± 4.4 | b | 10 |
| M94014 *Encarsia* (Philippines) | 5.8 ± 1.4 | d,e | 9.0 ± 1.7 | b | 10 |
| M94019 *Encarsia* (Taiwan) | 3.0 ± 0.9 | e | 5.0 ± 1.3 | b | 10 |

*Nonlinear regressions.* Using the pooled data from both trials, a nonlinear regression was performed on the total number of hosts as a predictor for numbers attacked. The form of equation chosen was a Type II functional response (Hassell 1978):

$$N_a = N_t\left[1 - \exp\left\{-\frac{a'TP_t}{1 + a'T_hN_t}\right\}\right]$$

where $N_a$ is the number of hosts attacked, $N_t$ is the number of hosts available, a' is the searching efficiency, $P_t$ is number of parasitoids searching, T is the searching time and $T_h$ is the handling time required to attack the host. $N_t$ was estimated by using the total of parasitized and unparasitized hosts; $P_t$ was set to two parasitoids and T was set to two days. A significant fit was found ($F = 46.3$; $df = 2, 170$; $r^2 = 0.35$; $P < 0.01$) which yielded an estimate of 0.0793 for a' and 0.19 for $T_h$. The numbers of hosts attacked and the disc equation estimate are shown in Fig. 1A.

A simple exponential decay function was used to estimate total number of hosts as a predictor for percentage parasitism (arc sin transformed). The equation used was:

$$Y = c \exp(\alpha N_t)$$

where $N_t$ again is total hosts available estimated by using the sum of parasitized and unparasitized hosts, and c and a are constants. The regression yielded estimates of 23.35 for c and -0.0033 for a ($F = 84.7$; $df = 2, 170$; $r^2 = 0.5$; $P < 0.01$). Arc sin percentage parasitism and the exponential decay equation are shown together in Fig. 1B.

Although the experimental protocol was identical for both trials, it appears that the number of hosts available was higher in the second. Host density may have a significant effect on attack rates as measured using both number of hosts attacked and percentage parasitism. Higher host densities may result in higher numbers of hosts attacked but lower percentages of parasitism. Because host densities cannot be held constant across different trials, the interpretation of results across trials must be made with caution.

In summary, the method of evaluating the performance of the parasitoids produced reasonably consistent results as similar results were obtained in both trials. The data indicate highly significant differences in both numbers of hosts attacked and in percentages of parasitism among the different strains, but no strong evidence was found to indicate that *Eretmocerus* species are better parasitoids of *B. tabaci* than *Encarsia* species, or vice versa. Obviously, a limited set of experiments such as those described are insufficient to select a single "best" strain from among those tested and laboratory evaluations such as those described may be poor indicators of success in the field. Any general conclusions must take into consideration the experimental conditions used because other strains may perform better using different host plants or temperature conditions, for example. Performance may also be affected by interspecific competition, especially against the local parasitoid complex. Furthermore, we were unable to measure host feeding which is clearly an important factor in determining the efficacy of a parasitoid of *B. tabaci.* With these limitations, the evaluations indicate that the *Encarsia* nr. *pergandiella* from Brazil (M94055) appears to be the most promising parasitoid of *B. tabaci* in melons. This strain and others which performed well in these evaluations merit further consideration using different crops and experimental conditions and may prove effective in mass release field trials.

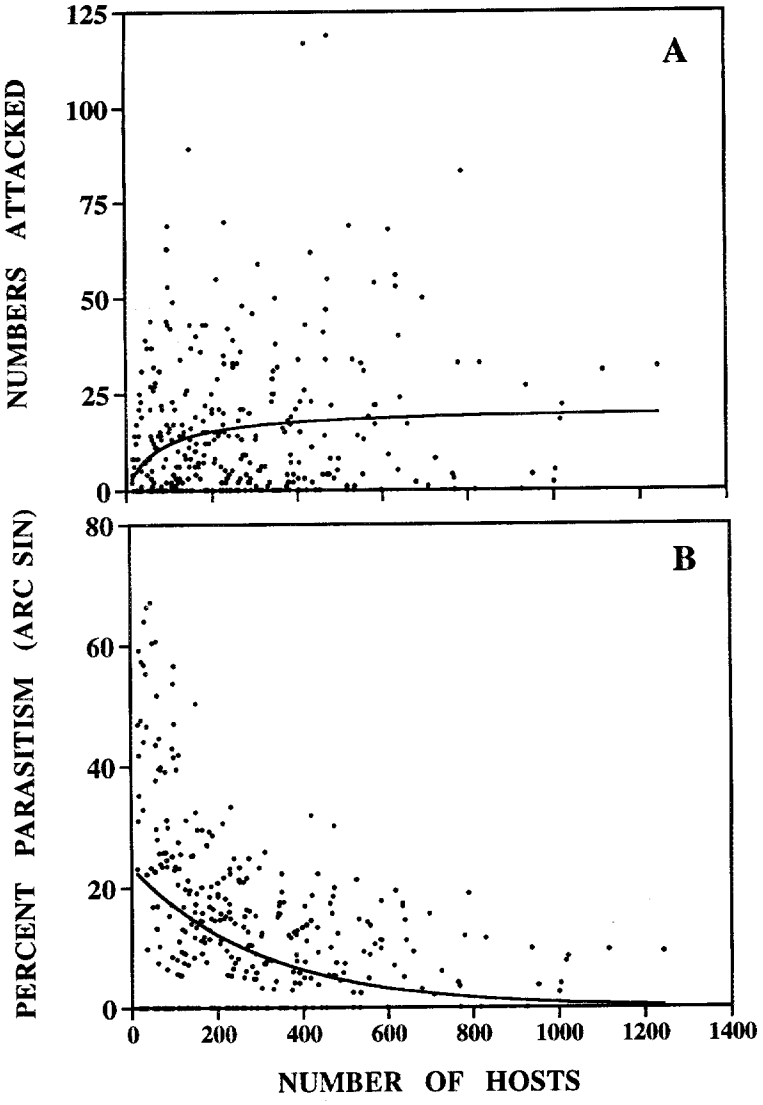


FIG 1. Nonlinear regressions of host numbers as predictors of number of hosts attacked and percentage parasitism (arc sin transformation). A) Number of hosts attacked is described by a Type II functional response equation where a' = 0.0793, Th = 0.19, T = 2, Pt = 2 ($F = 46.3$; df = 2, 170; $r^2 = 0.35$; $P < 0.01$). B) Percentage parasitism was described using an exponential decay function: $Y = 23.35 \exp(-0.0033 N_t)$ ($F = 84.7$; df = 2, 170; $r^2 = 0.5$; $P < 0.01$).

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