Biological Control 53 (2010) 9–17

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Consideration of *Eurytoma sivinskii* Gates and Grissell, a eurytomid (Hymenoptera) with unusual foraging behaviors, as a biological control agent of tephritid (Diptera) fruit flies

J. Mena-Correa [a], J. Sivinski [b], A. Anzures-Dadda [a], R. Ramírez-Romero [a,1], M. Gates [c], M. Aluja [a,*]

[a] Instituto de Ecología, A.C., Apartado Postal 63, 91000 Xalapa, Veracruz, Mexico
[b] Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, P.O. Box 14565, Gainesville, FL 32604, USA
[c] Systematic Entomology Laboratory, PSI, ARS, USDA, c/o Smithsonian Institution, National Museum of Natural History, P.O. Box 37012, Washington, DC 20013-7012, USA

ARTICLE INFO

Article history:
Received 8 March 2008
Accepted 17 March 2009
Available online 24 April 2009

Keywords:
Demography
Eurytomidae
Fruit fly
Parasitoid
Tephritidae
Hyperparasitism

ABSTRACT

A recently discovered Mexican parasitoid species of Eurytomidae (Hymenoptera), *Eurytoma sivinskii* Gates and Grissell, has the unique behavior, for its family, of attacking tephritid fruit fly pupae (*Anastrepha* spp.) on or in the soil. Adults burrowed but did so rarely, thus pupae on the soil surface were significantly more vulnerable than those underground. Females facultatively hyperparasitized other larval-prepupal and pupal parasitoids such as *Opius hirtus* (Braconidae), *Coptera haywardi* (Diapriidae) and *Pachycrepoideus vindemiae* (Pteromalidae). While *E. sivinskii* developed in the pupae of various other *Anastrepha*, including, *A. serpentina* and *A. striata*, it also attacked cyclorraphous Diptera such as *Musca domestica* and a tachinid species. The number of expected female offspring (R_o) was 44.3 when measured as eclosed eggs (i.e., that became larvae) and 34.3 when measured as the number of emerged adults, and the intrinsic rate of natural increase (r_m) was 0.34. This is high relative to other fruit fly parasitoids and suggests that *E. sivinskii* could rapidly exploit a clumped resource. We conclude that the marginal ability of *E. sivinskii* to attack buried pupae and the environmental risks it poses through its broad host range and capacity for hyperparasitism make it a poor candidate for tephritid biological control.

1. Introduction

Insect biological control has come under increased scrutiny and is suspected of having occasionally released inappropriate natural enemies that had a negative effect on local arthropod numbers and diversity (Simberloff and Stiling, 1996; Henneman and Memmott, 2001; Zimmermann et al., 2001; Messing et al., 2005; Messing and Wright, 2006). These types of concerns are best allayed by accruing critical information on potential biological control agents such as host range (danger to non-targets), ability to hyperparasitize, and certain demographic parameters such as intrinsic rate of increase. This was our aim here with the recently described Mexican species *Eurytoma sivinskii* Gates and Grissell (Gates and Grissell, 2004; Gates et al., 2008) which was discovered attacking pupae of the West Indian fruit fly, *Anastrepha obliqua* (Macquart) a species of the exclusively frugivorous, and often pestiferous, tephritid tribe Toxotrypanini (Aluja, 1994). Very little is known on the biology of this species. Recently, Mena-Correa et al. (2008) determined that *E. sivinskii* is ectoparasitic and that the life cycle is completed in 23.1 ± 1 (mean ± SE) days at 27 ± 2 °C. Females are capable of superparasitism, laying eggs primarily in the medial and posterior portions of the host. Independent of where the eggs were placed within the puparium, adults tended to emerge through the middle of it.

Tephritid fruit fly biological control relies heavily on larval/egg-prepupal braconid parasitoids (Purcell, 1998; Ovruski et al., 2000). A number of species have been both classically introduced around the world and considered for mass-release in support of fly-free zones and eradication programs (Sivinski et al., 1996; Montoya et al., 2000). However, it has been proposed that there may be an underappreciated role for augmentation of pupal parasitoids as well, since insects that escape a larval parasitoid might fall victim to a pupal parasitoid later in their development. Mixed mass-releases of sterile Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), braconids and the pteromalid pupal parasitoid *Pachycrepoideus vindemiae* (Rondani) have been popular with Costa Rican agriculturalists (Camacho, 1998). The problem with *P. vindemiae* is that it is an ectoparasitoid able to attack a wide range of hosts, among them pollinators and other beneficials (Guillén et al., 2002, and references therein). Generalism raises questions

* Corresponding author.
E-mail address: martin.aluja@inecol.edu.mx (M. Aluja).
[1] Present address: Departamento de Botánica y Zoología, Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias, km. 15.5 Carr. Guadalajara-Nogales, Zapopan, Jalisco, Mexico.

1049-9644/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2009.03.019

concerning both non-target effects and lack of efficacy as declining target populations cause them to turn to relatively more numerous non-targets (Sivinski et al., 1998). An interesting alternative to this exotic species was discovered in Central Veracruz, Mexico in the late 90s by López et al. (1999): the relatively specialized native diapriid endoparasitoid *Coptera haywardi* (Ogloblin) (Sivinski et al., 1998). For example, while *P. vindemiae* only attacked *Anastrepha ludens* (Loew) pupae on the surface, *C. haywardi* was able to attack pupae that were buried up to 5 cm deep (Guillén et al., 2002). In the case of pupae placed on the surface, parasitism by *C. haywardi* reached 49.5% which was significantly higher than the 19% recorded for *P. vindemiae* (Guillén et al., 2002). In subsequent field-cage tests, *C. haywardi* showed great potential for the control of *C. capitata* in Guatemala attacking 81.8% of pupae naturally buried at depths of 1–5 mm (Baeza-Larios et al., 2002).

Most species of the large and widespread parasitoid genus *Eurytoma* (Hymenoptera: Chalcidoidea: Eurytomidae) attack gall-forming Cynipidae (Hymenoptera) and Diptera (Tephritidae and Cecidomyidae) (DiGuilio, 1997). Among the Tephritidae, flies within *Eurosta* and *Trupanea*, exclusively attacking flower heads, are in turn attacked by eurytomid parasitoids (Horner, 1999; Goeden et al., 1998). Interestingly, *E. sivinskii* instead of attacking pupae in galls or flowerheads, forages for host pupae in and on the soil (Mena-Correa, 2005). Because of its peculiar foraging tactics for economically important hosts such as *A. obliqua*, *A. ludens* (Loew), the Mexican fruit fly. *A. ludens* is a polyphagous fly found throughout Mexico and Central America where it is a major pest of citrus, particularly grapefruit (*Citrus paradisi* Macfadyen), as well as mango (*Mangifera indica* L.) (Aluja et al., 2009a).

We hypothesized that several factors might influence the efficacy of *E. sivinskii* attacking *A. ludens*, and by implication the parasitoid's possible role in biological control. (1) *Location of hosts within the soil:* Frugivorous fruit fly larvae typically leave their host fruits to pupate in the soil at depths of from near the surface to ~5 cm (e.g., Hodgson et al., 1998). The position of pupae in the ground affects both their vulnerability to predators (Aluja et al., 2005) and to parasitoids (Guillén et al., 2002). For example, *C. haywardi*, a tephritid specialist, can parasitize *C. capitata* pupae at depths of at least 15 mm in the field but parasitism decreases significantly with increasing burial depths (Baeza-Larios et al., 2002). (2) *Experience:* It is widely known that experience influences the foraging efficiency of parasitoids (Quicke, 1997). For example, previous exposure to host visual and chemical cues and oviposition substrates increases responsiveness and rates of oviposition by females (Wäckers and Lewis, 1994). (3) *Soil structure:* In addition to depth, the degree of soil compaction and ease of digging can influence access to hosts (Guillén et al., 2002). Substrate particle size and moisture content play a role in the ability of cyclorraphous fly parasitoids to reach host pupae (Smith and Rutz, 1991; Geden, 1999; Rueda and Axtell, 1985a). (4) *Intrinsic rate of increase:* The capacity of *E. sivinskii* for increase, a function of longevity and fecundity, could influence its abundance and distribution relative to other tephritid biological control agents.

In addition to examining behaviors related to the efficacy of *E. sivinskii* in controlling tephritid pests, we considered the possible costs associated with its introduction or augmentation. (1) *Host range and the danger to non-target Diptera:* Broad host ranges are common in ectoparasitoids of dipteran pupae (Sivinski et al., 1998) and pose a threat to non-target flies. Furthermore, parasitoids exhibiting broad host ranges may be less effective as they turn from the increasingly rare targets in populations in the process of suppression. (2) *Hyperparasitism:* Pupal parasitoid development in primary parasitoids may adversely affect overall control by limiting their numbers and population growth. Some species of *Eurytoma* act as hyperparasitoids and have been reported attacking cocoons of the gregarious endoparasitoid *Cotesia glomerata* L. (Hymenoptera: Braconidae) (Tagawa and Fukushila, 1993).

2. Materials and methods

2.1. Insect cultures and experimental conditions

This study was carried out at the Unidad de Entomología Aplicada of the Instituto de Ecología A.C., Xalapa, Veracruz, Mexico. Environmental conditions were fixed at 27 ± 2 °C, 75 ± 5% RH, and a 12:12 h photoperiod.

E. sivinskii were reared on two-d old lab-reared *A. ludens* pupae following the methods described by Aluja et al., (2009b). Our colony was started with individuals collected in 1997 in *A. obliqua* pupae derived from tropical plum (*Spondias mombin* L., Anacardiaceae) growing in Tejería, Veracruz, México (Aluja et al., 2009b). Adult parasitoids were transferred to Plexiglas cages (30 × 30 × 30 cm) at emergence and fed *ad libitum* with water and honey. To prevent oviposition experience, parasitoids were not exposed to host pupae until used for experiments. Twenty-four hours before an experiment began, parasitoids were transferred to smaller Plexiglas cages (23 × 23 × 23 cm) using glass vials. In all cases, after *A. ludens* pupae were exposed to parasitoids, they were removed and placed in plastic cups (200 ml) containing moistened vermiculite and covered with a fine-mesh cloth lid. Water used to dampen the vermiculite contained sodium benzoate (2 g/l) to suppress fungal growth.

2.2. Parasitism related to host-burial depth and female experience

After running a preliminary experiment with two soil types (clay-rich and sandy), we decided to only use the clay-rich soil in the formal experiment as preliminary results indicated that few females dug in search of pupae and that such behavior was not influenced by soil type. We chose the clay-rich soil (50% RH) as this is the most common soil type in the region where *E. sivinskii* was originally discovered. The soil was obtained in a fragment of tropical montane cloud forest that surrounds the grounds on which the Instituto de Ecología, A.C. is build. Soil type was determined by texture analysis with the assistance of the Departamento de Suelos of the Instituto de Ecologia A.C. in Xalapa, Veracruz, Mexico.

The experimental design was a 2 × 5 factorial arrangement of treatments as follows: two female experience levels (naive or with previous oviposition experience and five burial depths of pupae within soil (0, 0.5, 1.0, 2.0 and 4.0 cm). In total, there were 10 treatment combinations, and five replicates for each combination. Host pupae were exposed to female parasitoids in plastic cups (200 ml) containing 5 cm of soil. Twenty *A. ludens* pupae per plastic cup were buried artificially at 0, 0.5, 1.0, 2.0, and 4.0 cm depth. Three days after pupae were buried, each groups of 20 *A. ludens* pupae were exposed to *E. sivinskii* cohorts of 10 naive or experienced females (8 to 11-d old) for 72 h.

Pupae were recovered from the soil following exposure. Ten pupae from each replicate were dissected under a stereo microscope to verify parasitism (presence or absence of *E. sivinskii* eggs). The other 10 pupae were individually placed in glass flasks (5 × 1 cm) containing moistened vermiculite. After 10 d, fly and parasitoid emergences were recorded daily for 20 d.

Rates of parasitism (i.e., percent parasitism) among treatments, in pupae that were dissected or kept until adult parasitoids emerged, were compared using a factorial analysis of variance ANOVA (Zar, 1996), with depth of burial and female experience level (naive vs. experienced) as factors. Data in proportions were arcsin transformed before analysis (Zar, 1996).

2.3. Host breadth

The capacity of *E. sivinskii* to attack a variety of hosts was examined in no-choice and choice experiments. Pupae from three *Anastrepha* species (*A. ludens*, *A. obliqua* and *A. serpentina*), were used as were those of an unidentified tachinid (Diptera: Tachinidae), *Paleaosepsis* sp. (Diptera: Sepsidae) and *Musca domestica* L. (Diptera: Muscidae).

Wild *A. ludens*, *A. obliqua*, *A. serpentina* and *A. striata* puparia originated from infested *Citrus paradisi* Macfadyen, *Manilkara zapota* (L.), *Mangifera indica* L. and *Psidium guajava* L., respectively, that had been shipped from Uruapan, Michoacán, Mexico. Puparia originating from laboratory-reared *A. ludens* stemmed from our colonies. The other three dipterans were collected in a compost pile in Coatepec, Veracruz, Mexico (a mixture of rotten fruit, vegetables and chicken manure).

Infested fruit and compost samples were brought to the laboratory and kept in plastic baskets placed above washbowls containing vermiculite as a pupation medium (Guillén et al., 2002). The vermiculite was checked every two days to recover newly formed pupae, which were then placed in 200 ml plastic cups (6 × 5 cm) containing moistened vermiculite and covered with a fine-mesh cloth lid. All pupae remained in these cups for three–five days and were weighed before use in the experiments. In the case of compost samples, we sorted all pupae by size and shape. Most of the material was used in our experiments, but samples were also kept to identify the adults emerging from the puparia.

No-choice test. Female parasitoids were released in the presence of only one potential host species. Because pupae of each species were obtained at different times, treatments were performed following availability of host pupae. Thus, four sub-treatments were performed (Table 1). For each potential host species, 40 pupae were exposed to cohorts of 10 female and 5 male *E. sivinskii* (nine to 12-d old) for 48 h. The experiment was replicated five times.

Choice test. Female parasitoids were exposed to pupae of the different potential host species offered simultaneously. Pupae were marked with different colored dots (Vinci®, Vinci de México, S.A. de C.V., Mexico) that corresponded to species. Because pupae of all the species tested were not available at the same time, we ran three sets of tests with different combinations of species (details in Table 2). Forty or 45 host pupae (i.e., 10 or 15 pupae per species) were exposed at the same time to cohorts of 10 female *E. sivinskii* and five males (nine to 12-d old) for 48 h. We replicated each test five times. In both the choice and no-choice test pupae were weighed individually to determine any effect of host weight, such as sex-ratio biases, on the rate or nature of parasitism.

Table 1
Mean ± SE percent parasitism by *E. sivinskii* related to female experience and the depth at which *Anastrepha ludens* pupae were buried. In one case pupae were dissected to quantify the number of *E. sivinskii* eggs inside them and in the other, we allowed adults to emerge from parasitized pupae.

Depth (cm)	Experienced	Naive
Dissected pupae		
0	88 ± 4.9	84 ± 4
0.5	0 ± 0	0 ± 0
1.0	0 ± 0	0 ± 0
2.0	0 ± 0	0 ± 0
4.0	2 ± 2	2 ± 2
Emerged adults		
0	86 ± 4	82 ± 5.8
0.5	0 ± 0	0 ± 0
1.0	0 ± 0	0 ± 0
2.0	2 ± 2	0 ± 0
4.0	0 ± 0	0 ± 0

Table 2
Analysis of variance for experiment on parasitism by *E. sivinskii* related to female experience (experienced vs. na) and host-burial depth (response variable was percent parasitism). In one case pupae were dissected to quantify the number of *E. sivinskii* eggs inside them and in the other, we allowed adults to emerge from parasitized pupae.

Source	*df*	*F*	*P*
Dissected pupae			
Experience	1	0.008	0.928
Depth	1	16.032	0.000
Experience × depth	1	0.001	0.923
Emerged adults			
Experience		0.019	0.888
Depth		17.468	0.000
Experience × depth	1	0.007	0.934

Rates of parasitism among treatments were compared using a one-way ANOVA (Zar, 1996) with host species as the independent factor. When significant differences were found, a Tukey's test was performed (Zar, 1996). Data in proportions were arc-sin transformed before analysis (Zar, 1996). The pupal weight and rates of parasitism were tested for correlation using the *r* Spearman test (Zar, 1996).

2.4. Determination of hyperparasitism

Additional parasitoid species reared at the Unidad de Entomología Experimental of the Instituto de Ecología A.C., Xalapa, Veracruz, Mexico (Aluja et al., 2009b) were used to determine the potential of *E. sivinskii* to hyperparasitize. These target parasitoids displayed different types of parasitism (endo- and ectoparasitoids of larvae and pupae), which served to detect any different vulnerabilities to hyperparasitism. Parasitoid species used were: *Opius hirtus* Fisher (endoparasitoid of third instar *A. ludens* larvae), *C. haywardi* (endoparasitoid of *A. ludens* pupae) and *P. vindemiae* (ectoparasitoid of *A. ludens* pupae). Parasitism by *O. hirtus* was accomplished by exposing third instar host larvae to female parasitoids for six–eight hours (details in Aluja et al., 2009b). Parasitism by *C. haywardi* and *P. vindemiae* was accomplished by exposing three-d old pupae to female parasitoids for 24 h (details in Guillén et al., 2002). In the case of *O. hirtus*, we exposed third instar *A. ludens* larvae inside our rearing cages (details in Aluja et al., 2009b). The number of primary parasitoids in these cages fluctuated between 100 and 200 individuals of both sexes. One hundred milliliter of pupae (ca. 2700 individuals) placed on top of a plastic lid were exposed to the two pupal primary parasitoids (*C. haywardi* and *P. vindemiae*) while 30 ml of larvae (ca. 860 individuals) were exposed to the primary larval-prepupal parasitoid (*O. hirtus*). In the latter case the larvae were mixed with guava pulp placed on a plastic lid tightly covered with organdi cloth ("sandwich method" described in Aluja et al., 2009b).

Parasitized hosts were identified by changes on the surface of the puparium. After a few days, the endoparasitoids (*O. hirtus* and *C. haywardi*) caused the host to assume a wrinkled surface with pearly-white tones which became consistently more obvious as the parasitoid's body became distinguishable through the host puparium. The ectoparasitoid (i.e., *P. vindemiae*) was clearly visible through the host puparium.

Twenty pupae of similar ages and previously parasitized by these primary parasitoids were exposed on the surface of a Petri dish to *E. sivinskii* cohorts of 10 females and five males that were six to 10 d of age. *Opius hirtus*, *C. haywardi*, and *P. vindemiae* emerged as adults approximately 16, 30, and 28 d, respectively, after oviposition (preliminary observations). Thus, the following groups were exposed to *E. sivinskii* cohorts of 10 females and five males (one cohort/replicate): (a) 100 pupae (20 per replicate) for

each of the following pupal ages (measured as days elapsed after oviposition of the primary parasitoid had occurred: 1 to 15-d after parasitism by *O. hirtus* (total of 1500 pupae [=15 ages × 5 replicates × 20 pupae/replicate]). We note that among the one-d pupae stemming from larvae parasitized by *O. hirtus*, some pre-pupae were found. (b) 100 pupae (20 per replicate) for each of the following periods (days) after oviposition of the primary parasitoid had occurred: 1 to 30-d after parasitism by *C. haywardi* (total of 2000 pupae [=20 ages × 5 replicates × 20 pupae/replicate]). We note that in this case, pupae at the time of exposure to *E. sivinskii* were 5 to 34-d old since they were exposed at age 3 d to the primary parasitoid; (c) 100 pupae (20 per replicate) for each of the following periods (days) after oviposition of the primary parasitoid had occurred: 1 to 12-d after parasitism by *P. vindemiae* (total of 1200 pupae [=12 ages × 5 replicates × 20 pupae/replicate]). We note that in this case, pupae at the time of exposure to *E. sivinskii* were 5 to 16-d old since they were exposed at age 3 d to the primary parasitoid (as was the case with *C. haywardi*). In addition, for each group exposed to *E. sivinskii*, 20 host pupae/replicate not exposed to *E. sivinskii* were separated as control groups. The exposure time to *E. sivinskii* cohorts was 24 h and five replicates were performed. The percent parasitism/hyperparasitism and sex-ratio of emerging *E. sivinskii* were recorded.

The rates of hyperparasitism (*E. sivinskii* emergences/fly emergences + primary parasitoid emergences + *E. sivinskii* emergences), the efficiency of primary parasitoid (fly emergences/fly emergences + primary parasitoid emergences), and host quality estimated by the primary parasitoid emergences (primary parasitoid emergences/fly emergences + primary parasitoid emergences) (Begon et al., 2006; Sullivan and Völkl, 1999) for each treatment (i.e., days elapsed after oviposition of the primary parasitoid had occurred) were compared via an ANOVA, with arc-sin transformed data. Parasitoid sex-ratios were compared against a 1:1 ratio using a chi-square goodness-of-fit test. Finally, for each set of experiments, parasitoid sex-ratios were compared among treatments within parasitoid species using a chi-square test (Zar, 1996).

2.5. Demographic parameters of E. sivinskii

To obtain parasitoids of known age and history, hosts were exposed daily to one-d old *E. sivinskii* females. Pupae were recovered after 24 h and placed in closed plastic vials (200 ml) containing moistened vermiculite (~5 g). When adults emerged, they were transferred to Plexiglas cages to form experimental cohorts. Because male emergence preceded female emergence, each cohort consisted of 15 recently emerged females and 15 two-d old males. Adults had *ad libitum* access to diluted honey (70% honey, 30% water) (Miel Carlota®, Hérdez S.A. de C.V., Cuernavaca, Morelos, Mexico) and water. We saturated pieces of cotton with the honey/water solution and placed them in 10 cm Petri dishes in the bottom of every cage. Water was administered similarly.

This study examined longevity, fertility and fecundity of insects in three treatments: (a) parasitoids without host exposure (i.e., without direct investment in reproduction), which were observed until all died to be able to estimate longevity; (b) parasitoids with daily exposure to 45 host pupae (3 to 5-d old), which were observed until adult parasitoids emerged from them to calculate parameter R_o for the adult stage (pupa-adult); (c) parasitoids with daily exposure to 45 host pupae (3 to 5-d old), which were dissected to quantify the number of eggs laid by females and their eclosion to calculate parameter R_o based on the number of eclosed eggs (i.e., those hatching into larvae). Dissections were performed five–eight days after parasitoid exposure because during this period it was possible to observe the egg chorion or eclosed larva (Mena-Correa, 2005). There were five replicates for each treatment. A total of 12,200 host pupae were exposed to female parasitoids in treatment "b".

Following Begon et al. (2006), we constructed a life table for each treatment (a–c) with the number of surviving insects (s_x), the age-specific survival (l_x), mortality likelihood (d_x), the daily rate of mortality (q_x), the mean lifespan (L_x) and the expected lifespan (e_x). Moreover, a fecundity table for treatments b and c was prepared including the total offspring (F_x) (reproductive output of the entire population), the age-specific fecundity (m_x) (the fecundity per surviving individual), original female offspring per day ($l_x m_x$), cohort generation time (T_c) (x $l_x m_x / l_x m_x$) and the expected female offspring (R_o) (mean number of offspring produced by an individual over the course of its life) = $\sum l_x m_x$). Finally, for treatment "c", the intrinsic rates of natural increase (r_m) values were calculated according to the Lotka equation (Birch, 1948): $\Sigma e^{-rmx} l_x m_x = 1$, where x is the age, l_x the age-specific survival and m_x the age-specific fecundity. The finite rate of increase ($\lambda = e^{rm}$) and the doubling time (DT = ln $2/r_m$) were evaluated according to DeLoach (1974). We calculated s_x as well as F_x based on the mean of five simultaneous replicates with cohorts of 15 females per replicate. R_o for adult emergence was calculated with data from treatment "b", whereas R_o for eclosed eggs was evaluated with data from treatment "c". The x from "Days" varies according to treatments.

In treatment "b", emergence rates were compared using a one-way ANOVA with the female parasitoid age as the independent factor. The rates of emerged adults were similarly compared using a one-way ANOVA. Data in proportions were arc-sin transformed before analysis (Zar, 1996). All analyses were performed using Statistica® software. When indicated, parasitism rates (i.e., percent parasitism) (No. of adult parasitoid emerged/No. of emerged flies + No. adult parasitoid emerged) were estimated.

3. Results

3.1. Parasitism related to host-burial depth and female experience

Eurytoma sivinskii parasitized significantly more pupae located on the soil surface than buried pupae (Tables 1 and 2). Female experience did not significantly affect parasitism rates (Table 2). A single pupae of 100 buried at 4 cm exposed to naïve *E. sivinskii* females ended up being parasitized. In the case of experienced females they parasitized pupae buried at depths of 2 and 4 cm, respectively (one pupae in each case) (Table 1). These results indicate that females are able to dig in search of pupae but that such an event is extremely rare as females preferred to attack pupae on the soil surface.

3.2. Host breadth

The rates of parasitism were significantly different among host species, according to the experimental conditions and sub-treatments observed.

No-choice tests. (1) Test comparing parasitism on *M. domestica*, *Palaeosepsis* sp. and the tachinid pupae: significant differences in the rates of parasitism were observed among species ($F = 7.21$; df = 2; $P = 0.009$), with the highest rate being observed on *M. domestica* and no development at all recorded on *Palaeosepsis* sp. (Table 3). A positive correlation was found between the rates of parasitism and pupal weight ($R = 0.604$; $P = 0.017$).

(2) Test comparing parasitism on *M. domestica*, *A. obliqua*, *A. striata* and laboratory-reared *A. ludens* pupae: significant differences in the rates of parasitism among species were observed ($F = 15.138$; df = 3; $P < 0.001$). The lowest rate was observed on *M. domestica*, *A. obliqua*, the fly species on which *E. sivinskii* was originally collected in the field, exhibited the highest rate (Table 3).

Table 3
The capacity of *E. sivinskii* to attack a variety of hosts (i.e., host breath) as examined in a no-choice experiment. Mean (±SE) percent parasitism values in different sub-treatments are provided.

Sub-treatment	Fly species	*N*	Parasitism (%)
One[a]	sp. Tachinid	5	10.26 ± 5.3 a
	Palaeosepsis sp.	5	0.00 ± 0.0 b
	M. domestica	5	69.56 ± 17.9 c
Two[b]	*M. domestica*	5	18.67 ± 9.0 a
	A. obliqua	5	99.38 ± 0.6 b
	A. striata	5	96.66 ± 1.3 b
	A. ludens laboratory	5	87.13 ± 7.5 b
Three[c]	*M. domestica*	5	98.15 ± 1.2 a
	A. serpentina	5	97.69 ± 1.7 a
	A. striata	5	98.18 ± 1.8 a
	A. ludens wild	5	48.20 ± 12.0 b
	A. ludens laboratory	5	91.56 ± 6.3 a
Four[d]	*A. ludens* wild	5	22.00 ± 13.5 a
	A. ludens laboratory	5	93.94 ± 4.0 b

[a] Significant differences in the rates of parasitism were observed among species ($F = 7.21$; 2 df; $P = 0.009$).
[b] Significant differences in the rates of parasitism among species were found ($F = 15.138$; 3 df; $P < 0.001$).
[c] Significant differences in the rates of parasitism were observed among species ($F = 6.166$; 4 df; $P = 0.0007$).
[d] Significant differences in the rates of parasitism were observed between species ($F = 28.571$; 1 df; $P = 0.0007$).

Table 4
The capacity of *E. sivinskii* to attack a variety of hosts (i.e., host breath) as examined in a choice experiment. Mean (±SE) percent parasitism values in different sub-treatments are provided.

Sub-treatment	Fly species	*N*	Parasitism (%)
One[a]	sp. Tachinid	5	2.50 ± 2.5 a
	Palaeosepsis sp.	5	0.00 ± 0.0 b
	M. domestica	5	73.33 ± 11.3 c
Two[b]	*M. domestica*	5	92.00 ± 8.0 a
	A. obliqua	5	89.84 ± 5.2 a
	A. serpentina	5	89.92 ± 2.5 a
	A. striata	5	83.78 ± 5.1 a
Three[c]	*A. ludens* wild	5	45.67 ± 7.1 a
	A. obliqua	5	66.67 ± 21.1 a
	A. serpentina	5	37.97 ± 16.6 a
	A. striata	5	38.00 ± 21.1 a

[a] Significant differences in the rates of parasitism were observed among species ($F = 16.179$; 2 df; $P = 0.0004$).
[b] No significant differences in the rates of parasitism among species were found ($F = 0.808$; 3 df; $P = 0.507$).
[c] No significant differences in the rates of parasitism were observed among species ($F = 1.113$; 3 df; $P = 0.373$).

No correlation was observed between parasitism rates and pupal weights ($R = 0.297$; $P = 0.203$).

(3) Test comparing parasitism on *M. domestica*, *A. serpentina*, *A. striata*, wild *A. ludens* and laboratory-reared *A. ludens* pupae: significant differences in the rates of parasitism were found among species ($F = 6.166$; df = 4; $P = 0.0007$) with wild *A. ludens* exhibiting the lowest rates (Table 3). A significant correlation between the rates of parasitism and pupal weights was observed ($R = -0.415$; $P = 0.039$).

(4) Test comparing parasitism on wild *A. ludens* and laboratory-reared *A. ludens* pupae: a significant difference in parasitism rates was observed between laboratory-reared and wild *A. ludens* ($F = 28.571$; df = 1; $P = 0.0007$), the former exhibiting a higher rate of parasitism (Table 3). No significant correlation was found between parasitism rates and pupal weight ($R = 0.265$; $P = 0.460$).

Choice tests. (1) Test simultaneously comparing parasitism on *M. domestica*, *Palaeosepsis* sp. and tachinid pupae: significant differences in the rates of parasitism were observed among host species ($F = 16.179$; df = 2; $P = 0.0004$), with the highest rate of parasitism observed in *M. domestica* (Table 4). A significant correlation between parasitism rate and pupal weight was observed ($R = 0.808$; $P = 0.0003$).

(2) Test simultaneously comparing parasitism on *M. domestica*, *A. obliqua*, *A. serpentina* and *A. striata* pupae: No significant differences in the rates of parasitism were observed among host species ($F = 0.808$; df = 3; $P = 0.507$) (Table 4). No significant correlation was observed between parasitism rate and pupal weight ($R = -0.394$; $P = 0.085$).

(3) Test comparing parasitism on wild *A. ludens*, *A. obliqua*, *A. serpentina* and *A. striata* pupae: No significant differences were observed among species ($F = 1.113$; df = 3; $P = 0.373$). Furthermore, we found no significant correlation between parasitism rate and pupal weight ($R = 0.248$; $P = 0.292$).

3.3. Capacity for hyperparasitism

Eurytoma sivinskii was able to hyperparasitize pupae previously parasitized by *O. hirtus* (larval-prepupal parasitoid), *C. haywardi* and *P. vindemiae* (both pupal parasitoids). When *E. sivinskii* attacked pupae previously parasitized by *O. hirtus*, there was a mean rate of hyperparasitism (over all pupal ages) of 69.71 (±13.9) (Table 4). The highest rates of hyperparasitism were observed six-d after oviposition of the primary parasitoid had occurred (which in the case of *O. hirtus* corresponds to the actual age of pupae [i.e., six d-old]) ($F = 5.077$; df = 14; $P < 0.001$), and the lowest rates of hyperparasitism were in young (one to four-d after oviposition of the primary parasitoid had occurred) and old (14 and 15-d after oviposition of the primary parasitoid had occurred) pupae (Fig. 1). Overall (i.e., lumping all pupae together independent of age [i.e., days after oviposition of the primary parasitoid had occurred]), the sex-ratio of hyperparasitizing *E. sivinskii* was significantly female-biased ($\chi^2 = 64.888$; df = 1; $P < 0.001$). The control group (not exposed to *E. sivinski*) exhibited a mean rate of parasitism by *O. hirtus* of 52.85 (±2.19).

Eurytoma sivinskii hyperparasitized pupae previously parasitized by *C. haywardi* at a mean rate of 17.32 (±11.5) (over all pupal ages), with significantly higher rates observed six to 10-d after oviposition of the primary parasitoid had occurred (i.e., 10 to 14-d old pupae as the latter were 3-d old when exposed to the primary parasitoid) ($F = 3.574$; df = 30; $P < 0.001$) (Fig. 1). The lowest rates of hyperparasitism were obtained in older pre-parasitized pupae (i.e., 14 to 30-d after oviposition of the primary parasitoid had occurred [=18 to 34 d-old pupae]). Overall (i.e., lumping all pupae together independent of days elapsed after oviposition of the primary parasitoid had occurred), the sex-ratio of hyperparasitizing *E. sivinskii* was significantly female-biased ($\chi^2 = 27.776$; df = 1; $P < 0.001$). The control group (not exposed to *E. sivinski*) exhibited a mean rate of parasitism by *C. haywardi* of 73.84 (±19.20).

When attacking the primary parasitoid *P. vindemiae*, *E. sivinskii* attained rates of parasitism from day one to eleven after oviposition of the primary parasitoid had occurred (five to 15 d-old pupae as the latter were 3-d old when exposed to the primary parasitoid) that were not significantly different (Fig. 1). The mean rate of hyperparasitism during this period was 71.95 (±22.33) (over all host ages). Significantly higher rates of hyperparasitism were observed one to 11-d after oviposition of the primary parasitoid had occurred (five to 15 d-old pupae) than on older pupae (14.85 ± 18.43 day after oviposition of the primary parasitoid had occurred [=18 to 22 d-old pupae]) ($F = 5.730$; df = 14; $P < 0.001$ As was the case with the previous two experiments, the overall (i.e., lumping all pupae together independent of host age) sex-ratio of

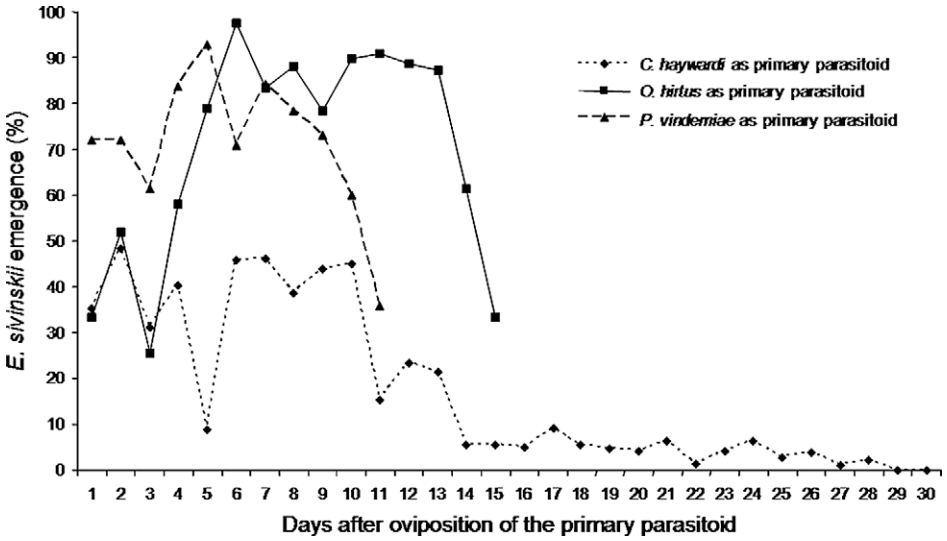

Fig. 1. *Eurytoma sivinskii* emergence in relation to the days elapsed after oviposition of the primary parasitoid had occurred in *Anastrepha ludens* pupae. Pupae were first exposed to the primary parasitoids *Opius hirtus* (attacks third-instar larvae), *Coptera haywardi* and *Pachycrepoideus vindemiae* (both attack pupae) and then to *E. sivinskii*. Age of *A. ludens* pupae corresponds to the days after oviposition of the primary parasitoid had occurred in the case of *O. hirtus* as the larvae attacked pupated the same day. In the case of the primary pupal parasitoids they were offered three-d old *A. ludens* pupae so by the time *E. sivinskii* attacked the same pupae, the latter were five-d old.

hyperparasitizing *E. sivinskii* was significantly female-biased ($\chi^2 = 12.242$; df = 1; $P < 0.001$). The control group (not exposed to *E. sivinski*) exhibited a mean rate of parasitism by *P. vindemiae* of 72.22 (±17.55).

3.4. Demographic parameters of E. sivinskii

Treatment a (i.e., without host exposure): female expected lifespan (L_x) was 51 d, whereas male L_x was 30 d. Curves for male and female age-specific survival (l_x) are shown in Fig. 2A. Maximum female longevity was of 86 d and that of males was of 62 d.

Treatment b (i.e., with host exposure): female L_x was 24.9 d and male L_x was 28.3 d. Curves for male and female age-specific survival (l_x) are shown in Fig. 2B. Maximum female longevity was 77 d and that of males 58 d. Total mean offspring/cohort (F_x) was 425 individuals and the expected female offspring (R_o) was 28.3 adults. Age-specific fecundity (m_x) is shown in Fig. 3A.

Treatment c (i.e., with host exposure and dissection): female L_x was 23.3 d and male L_x 28.1 d. Curves for male and female age-specific survival (l_x) are shown in Fig. 2C. Maximum female longevity was 47 d and that of males was 45 d. Mean egg production per cohort (F_x) was 665 eggs. Mean larvae per cohort (F_x) was 514.2 with

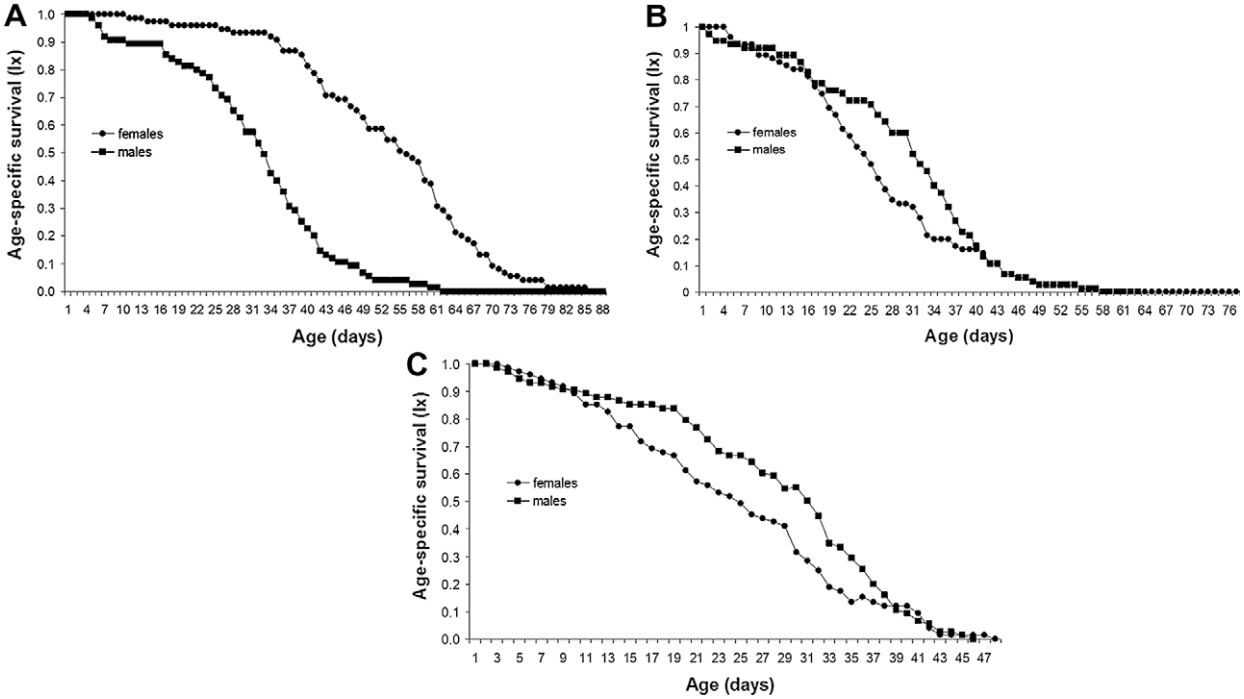

Fig. 2. Demographic parameters for *E. sivinskii* attacking *Ananstrepha ludens* pupae. Age-specific survival (l_x) of males and females for treatment a (A) [i.e., without host exposure], treatment b (B) [i.e., with host exposure], and treatment c (C) [i.e., with host exposure and dissection].

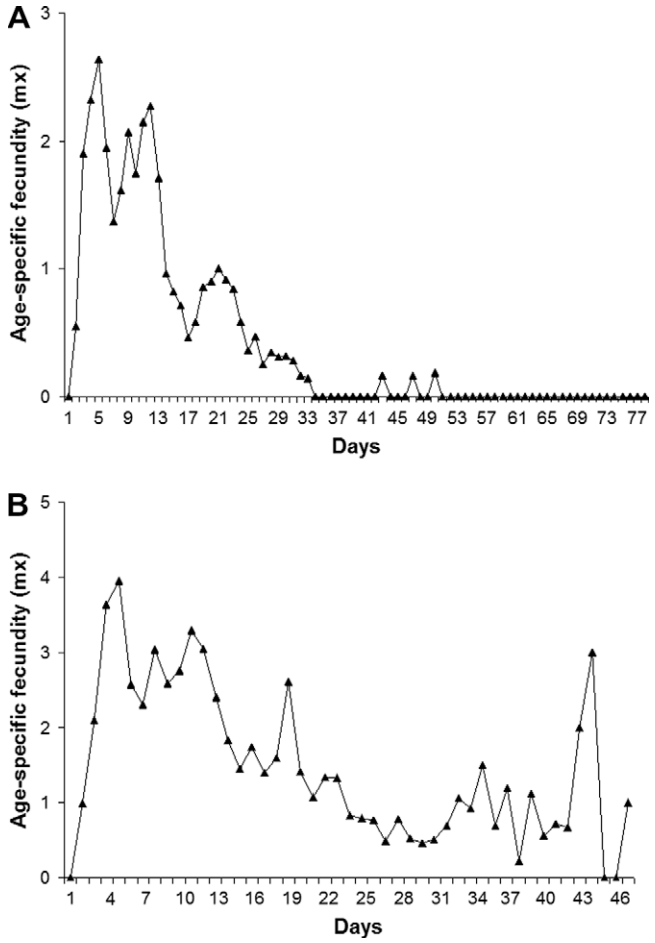

Fig. 3. Demographic parameters for *E. sivinski* attacking *Anastrepha ludens* pupae. Age-specific fecundity (m_x) for treatment b (A) [i.e., with host exposure], and treatment c (B) [i.e., with host exposure and dissection].

an R_o of 44.3 for eclosed eggs (i.e., that became larvae) and 34.3 when measured as the number of eclosed adults (i.e., pupae were observed until all adults emerged from them). The intrinsic rate of natural increase (r_m) was 0.34, with a finite rate of increase (λ) of 1.40 and a doubling time (DT) of 2.05. Age-specific fecundity (m_x) is shown in Fig. 3B.

Recently emerged females did not parasitize hosts. Maximum egg production was recorded in 2 to 15-d old females ($F = 23.936$; df = 47; $P < 0.0001$). In 98.7% of the recorded cases ($N = 975$), only one adult emerged from pupae. However in 13 pupae (1.3% of total) exposed to nine to 13-d old females, two adults emerged.

4. Discussion

Although attacking puparia buried beneath a substrate has been previously noted within the Chalcidoidea (Rueda and Axtell, 1985b; Sivinski et al., 1998), *E. sivinskii* seems to be the first recorded eurytomid capable of digging through soil to parasitize a host. We note however that parasitism occurred almost exclusively on the surface and in the few cases where parasitism was observed in buried pupae, rates declined sharply with burial depth as is also true also of another burrowing tephritid parasitoid, the diapriid *C. haywardi* (Baeza-Larios et al., 2002). Given that *Anastrepha* spp. pupae regularly occur at depths of 2–5 cm (Hodgson et al., 1998), it would seem that most would be beyond the reach of *E. sivinskii*, which in turn would limit its impact as an agent of population control. Furthermore, our experiments did not conclusively reveal how *E. sivinskii* locates either host patches or underground pupae. Additional studies are thus needed to determine for example the influence of potential kairomones or cues from the fruit on which fruit fly larvae are developing.

Parasitism rates are affected by a variety of circumstances and even in the laboratory, host density, age, host and form of exposure can make comparisons among species difficult. Given this caveat, mean pupal parasitism rates in our laboratories in other generalist ectoparasitoid species can range as high as 93% (e.g., *Dirhinus himalayanus* Westwood; Sivinski et al., 1998). In our study, parasitism by *E. sivinskii* reached 99.38 ± 0.6, 98.18 ± 1.8, and 98.15 ± 1.2 in the cases of *A. obliqua*, *A. striata* and *M. domestica*. In this respect, we discovered a striking, and highly significant difference in parasitism, when comparing lab-reared *A. ludens* and wild specimens of the same species (e.g., 93.94 ± 4.0 vs. 22.0 ± 13.5). On the one hand, it could be that residual odors from the grapefruit from which these pupae originated (larvae developed in grapefruit and when exiting the fruit to pupate, were also in contact with rotting fruit and juices oozing from these fruit) were partially repellent to the host-seeking females. On the other, learning could be possibly involved with chemical cues associated with the diet in which lab *A. ludens* were reared exerting a positive effect on *E. sivinskii* females. Finally, it is also possible that pupae from lab-reared larvae contain more nutrients than those derived from grapefruit. At any rate, and with one exception (*Palaeosepsis* sp.), *E. sivinski* was able to inflict heavy mortality on most species exposed to its parasitic activity.

Life expectancies and reproductive capacity are the basis for demographic parameters that determine the population rate of increase. Such values can give insights into how rapidly a parasitoid can increase relative to its host and how economically it might be reared as an augmentative biological agent. Survivorship and expected lifespan of females that never oviposited were nearly twice those of ovipositing females. Females reproducing continuously had the same or lower lifespan as males.

Related to the above, but viewed from the perspective of risks to non-targets and to other parasitoids, our results conclusively show that *E. sivinskii* was able to develop in puparia of several dipteran species, and choice experiments revealed no significant preference among *Anastrepha* species or between *Anastrepha* and *M. domestica*. Such a broad host range is typical of chalcidoid pupal ectoparasitoids (Sivinski et al., 1998). *Eurytoma sivinskii* also successfully hyperparasitized several parasitoid species, although we were unable to determine if this hyperparasitism is direct (i.e., by direct oviposition in or on the primary parasitoid) or indirect (i.e., by attacking the primary parasitoid's phytophagous host). The largest degree of hyperparasitism by *E. sivinskii* was observed in middle-aged pupae (six to 13 d-old) originally parasitized by the larval-prepupal parasitoid *Opius hirtus* and the least degree in *C. haywardi* possibly indicating an ability to fend of the attack of the secondary parasitoid. In the case of the pupal parasitoids *C. haywardi* and *P. vindemiae*, *E. sivinskii* preferentially hyperparasitized young pupae (10–14 and five to 15 d-old, respectively). In sum, a clear tendency emerges indicating that *E. sivinskii* females do not like to parasitize old pupae of any type and that by parasitizing younger hosts the proportion of females in the progeny is increased. As similar pattern was reported by (Tagawa and Fukushila, 1993) working with the hyperparasitoid, *Eurytoma* sp. (Eurytomidae) on cocoons of the primary parasitoid, *Cotesia* (=*Apanteles*) *glomerata* (Braconidae).

The intrinsic rate of increase (r_m) for *E. sivinskii* (0.34) was higher than the r_m calculated for other parasitoid species such as the braconids *F. arisanus* (0.12), *D. longicaudata* (0.12) and *F. vandenboschi* (0.08), the first two of which are widely mass-reared for augmentative release (Bautista et al., 2001; Vargas et al., 2002). This is

somewhat surprising, since all other things being equal, an egg-prepupal parasitoid like *F. arisanus*, might be adapted to have more oviposition opportunities than a pupal parasitoid exploiting the same host population; (i.e., mortality afflicting hosts during development will continuously shrink their numbers) (Price, 1975). Perhaps the greater host range of *E. sivinskii* leads to higher encounter rates with a larger variety of individually rare host species. However, the high rate of increase may make it particularly well suited to exploit patchy windfalls such as concentrations of shallowly buried tephritid pupae available for limited periods of time under fruit trees. The latter, added to the ability to lay considerable amounts of eggs and survive up to 86 d, would allow females to successfully exploit host patches over extended periods of time.

Before ending, we would like to note that in this study, in 975 recorded cases (98.7% of total), only one adult emerged from pupae. Two adults emerged in 13 pupae (1.3% of total). This contrasts with results recently published by Mena-Correa et al. (2008), indicating that even though females were capable of superparasitism, laying between 1 and 8 eggs per host (mean ± SE, 2.59 ± 1.56), invariably only one adult parasitoid emerged. We believe this can be explained by the fact that here we exposed close to 1000 pupae in the related experiment and in the Mena-Correa et al. (2008) experiments fewer than hundred pupae were exposed to *E. sivinskii* females. That is, as the phenomenon is very rare, large numbers of pupae need to be exposed to detect it.

In conclusion, the marginal efficacy and considerable environmental shortcomings of *E. sivinskii* make it a poor candidate for tephritid biological control. *E. sivinskii* is an ectoparasitoid which is best able to attack tephritid pupae on the soil surface, and has only a limited capacity to attack the deeply buried pupae typical of pestiferous fruit flies (Hodgson et al., 1998). In addition, the ability of *E. sivinskii* to attack non tephritids, including tachinid parasitoids, and to hyperparasitize valuable braconid primary parasitoids means that there would be environmental and agricultural risks associated with its mass release.

Acknowledgments

We gratefully acknowledge the help of Alberto Mata, Armando Torres Anaya, Cecilia Martínez Arcos, Melissa Galicia, Jovita Martínez Tlapa and Sandy Méndez Trejo (all Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico [INECOL]), who helped rear all the parasitoids used during this study. Larissa Guillén, Andrea Birke, Martín Pale and Guadalupe Córdova (also INECOL) helped organize experiments, provided needed materials and provided critical advice along the way. We thank several anonymous expert taxonomists at the Systematic Entomology Laboratory, USDA-ARS for identifying the tachinid, *Paleaosepsis* sp. and *M. domestica* specimens. Financial support for this study was principally furnished by the Mexican Campaña Nacional contra las Moscas de la Fruta (Secretaría de Agricultura, Ganadería, Desarrollo Rural y Pesca – Instituto Interamericano de Cooperación para la Agricultura (SAGARPA-IICA). Additional funds were provided by the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) and INECOL. Given that *E. sivinskii* was discovered while conducting studies financed by the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO; Proyect Number FB325/H296/96) and the Consejo Nacional de Ciencia y Tecnología (CONACyT)/ Fondo del Sistema de Investigación del Golfo de México (SIGOLFO, Project Number SIG96001/ 96–01-003-V), we also gratefully acknowledge their financial support. Information reported here forms part of the undergraduate thesis of JMC (Universidad Nacional Autónoma de México [UNAM]), directed by MA. M.A. also acknowledges support from CONACyT through a Sabbatical Year Fellowship (Ref. 79449) and thanks Benno Graf and Jörg Samietz (Forschungsanstalt Agroscope Changins-Wädenswil ACW), for providing ideal working conditions to finish writing/editing this paper.

References

Aluja, M., 1994. Bionomics and management of *Anastrepha*. Annual Review of Entomology 39, 155–178.

Aluja, M., Sivinski, J., Rull, J., Hodgson, P.J., 2005. Behavior and predation of fruit fly larvae (*Anastrepha* spp.) (Diptera: Tephritidae) after exiting fruit in four types of habitats in tropical Veracruz, Mexico. Environmental Entomology 34, 1507–1516.

Aluja, M., Rull, J., Pérez-Staples, D., Díaz-Fleischer, F., Sivinski, J., 2009a. Random mating among *Anastrepha ludens* (Diptera: Tephritidae) adults of geographically distant and ecologically distinct populations in Mexico. Bulletin of Entomological Research 99, 207–214.

Aluja, M., Sivinski, J., Ovruski, S., Guillén, L., Cancino, J., López, M., Torres-Anaya, A., Gallegos-Chan, G., Ruíz, L., 2009. Colonization and domestication of seven species of native new world hymenopterous larval-prepupal and pupal fruit fly (Diptera: Tephritidae) parasitoids. Biocontrol Science and Technology doi:10.1080/09583150802377373.

Baeza-Larios, G., Sivinski, J., Holler, T., Aluja, M., 2002. The ability of *Coptera haywardi* (Ogloblin) (Hymenoptera: Diapriidae) to locate and attack the pupae of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), under seminatural conditions. Biological Control 23, 213–218.

Bautista, R.C., Harris, E.J., Vargas, R.I., 2001. The fruit fly parasitoid *Fopius arisanus*: reproductive attributes of pre-released females and the use of added sugar as a potential food supplement in the field. Entomologia Experimentalis et Applicata 101, 247–255.

Begon, M., Townsend, C.R., Harper, J., 2006. Ecology: From Individuals to Ecosystems, fourrd ed. Blackwell Science, Malden, USA.

Birch, L.C., 1948. The intrinsic rate of natural increase of an insect population. Journal of Animal Ecology 17, 15–26.

Camacho, H., 1998. Using sterile insects and parasitism in the integrated fruit fly management project in Costa Rica. In: Proceedings of the Fifth International Symposium on Fruit Flies of Economic Importance. Penang, Malaysia, pp. 158–196.

DeLoach, C.J., 1974. Rate of increase of populations of cabbage, green peach and turnip aphids at constant temperatures. Annals of the Entomological Society of America 67, 332–339.

DiGuilio, J., 1997. Eurytomidae. In: Gibson, G., Huber, J., Wooley, J. (Eds.), Annotated Key to the Genera of Nearctic Chalcidoidea (Hymenoptera). NRC Research Press, Ottawa, pp. 477–495.

Gates, M.W., Grissell, E.E., 2004. A new species of *Eurytoma* (Hymenoptera: Eurytomidae) attacking the mango fruit fly, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae). In: Rajmohana, K., Sudheer, K., Girish-Kumar, P., Santhosh, S. (Eds.), Perspectives on Biosystematics and Biodiversity. University of Calicut, Kerala, India, pp. 147–159.

Gates, M., Mena-Correa, J., Sivinski, J., Ramírez-Romero, R., Córdova-García, G., Aluja, M., 2008. Description of the immature stages of *Eurytoma sivinskii* Gates and Grissell (Hymenoptera: Eurytomidae), an ectoparasitoid of *Anastrepha* (Diptera: Tephritidae) pupae. Entomological News 119, 354–360.

Geden, C., 1999. Host location by House Fly (Diptera: Muscidae) parasitoids in poultry manure at different moisture levels and host densities. Environmental Entomology 28, 755–760.

Goeden, R., Teerink, J., Headrick, D., 1998. Life history and descriptions of immature stages of *Trupanea jonesi* Curran (Diptera: Tephritidae) on native Asteraceae in southern California. Proceedings of the Entomological Society of Washington 100, 126–140.

Guillén, L., Aluja, M., Equihua, M., Sivinski, J., 2002. Performance of two fruit fly (Diptera: Tephritidae) pupal parasitoids (*Coptera haywardi* [Hymenoptera: Diapriidae] and *Pachycrepoideus vindemiae* [Hymenoptera: Pteromalidae]) under different environmental soil conditions. Biological Control 23, 219–227.

Henneman, M.L., Memmott, J., 2001. Infiltration of a Hawaiian community by introduced biological control agents. Science 293, 1314–1316.

Hodgson, P.J., Sivinski, J., Quintero, G., Aluja, M., 1998. Depth of pupation and survival of fruit fly (*Anastrepha* spp.: Tephritidae) pupae in a range of agricultural habitats. Environmental Entomology 27, 1310–1314.

Horner, J., 1999. The influence of oviposition phenology on survival in host races of *Eurosta solidaginis*. Entomologia Experimentalis et Applicata 93, 121–129.

López, M., Aluja, M., Sivinski, J., 1999. Hymenopterous larval-pupal and pupal parasitoids of *Anastrepha flies* (Diptera: Tephritidae) in Mexico. Biological Control 15, 119–129.

Mena-Correa, J., 2005. Biología Básica de *Eurytoma sivinskii* (Hymenoptera: Chalcidoidea: Eurytomidae). Thesis. Universidad Nacional Autónoma de México, México, DF, México.

Mena-Correa, J., Sivinski, J., Gates, M., Ramírez-Romero, R., Aluja, M., 2008. Some aspects of the basic biology of *Eurytoma sivinskii* Gates and Grissell, an unusual eurytomid (Hymenoptera) parasitoid of fruit fly (Diptera: Tephritidae) pupae. Florida Entomologist 91, 598–603.

Messing, R.H., Wright, M.G., 2006. Biological control of invasive species: solution or pollution? Frontiers in Ecology and the Environment 4, 132–140.

Messing, R., Roitberg, B., Brodeur, J., 2005. Measuring and predicting indirect impacts of biological control: competition, displacement, and secondary interactions. In: Bigler, F., Kuhlmann, U., Babendreier, D. (Eds.), Environmental Impact of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment. CABI Publishing, Wallingford, UK, pp. 64–77.

Montoya, P., Liedo, P., Benrey, B., Cancino, J., Barrera, J., Sivinski, J., Aluja, M., 2000. Biological control of *Anastrepha* spp. (Diptera: Tephritidae) through augmentative release of *Diachasmimorpha longicaudtata* (Ashmead) (Hymenoptera: Braconidae). Biological Control 18, 216–224.

Ovruski, S., Aluja, M., Sivinski, J., Wharton, R., 2000. Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. Integrated Pest Management Reviews 5, 81–107.

Price, P., 1975. Reproductive strategies for parasitoides. In: Price, P. (Ed.), Evolutionary Strategies of Parasitic Insects and Mites. Plenum Press, New York, USA, pp. 87–111.

Purcell, M., 1998. Contribution of biological control to integrated pest management of tephritid fruit flies in tropics and subtropics. Integrated Pest Management Reviews 3, 63–83.

Quicke, D.L.J., 1997. Parasitic Wasps, first ed. Chapman and Hall, London, UK.

Rueda, L.M., Axtell, R.C., 1985a. Effect of depth of house fly pupae in poultry manure on parasitism by six species of Pteromalidae (Hymenoptera). Journal of Entomological Science 20, 444–449.

Rueda, L.M., Axtell, R.C., 1985b. Guide to common species of pupal parasites (Hymenoptera: Pteromalidae) of the house fly and other muscoid flies associated with poultry and livestock manure. Technical Bulletin, North Carolina Agricultural Research Service 278, 88.

Simberloff, D., Stiling, P., 1996. How risky is biological control? Ecology 77, 1965–1974.

Sivinski, J., Calkins, C., Baranowski, R., Harris, D., Brambila, J., Diaz, J., Burns, R., Holler, T., Dodson, G., 1996. Suppression of a Caribbean fruit fly (*Anastrapha suspensa* (Loew)) (Diptera: Tephritidae) population through augmented releases of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biological Control 6, 177–185.

Sivinski, J., Vulinec, K., Menezes, E., Aluja, M., 1998. The bionomics of *Coptera haywardi* (Ogloblin) (Hymenoptera: Diapriidae) and other pupal parasitoids of tephritid fruit flies (Diptera). Biological Control 11, 193–202.

Smith, L., Rutz, D.A., 1991. The influence of light and moisture gradients on the attack rate of parasitoid foraging for hosts in a laboratory arena (Hymenoptera: Pteromalidae). Journal of Insect Behavior 4, 195–208.

Sullivan, D.J., Völkl, W., 1999. Hyperparasitism: multitrophic ecology and behavior. Annual Review of Entomology 44, 291–315.

Tagawa, J., Fukushila, H., 1993. Effects of host age and cocoon position on attack rate by the hyperparasitoid, *Eurytoma* sp. [Hym.: Eurytomidae] on cocoons of the parasitoid, *Cotesia* (=*Apanteles*) *glomerata* [Hym.: Braconidae]. Entomophaga 38, 69–77.

Vargas, R.I., Ramadan, M., Hussain, T., Mochizuki, N., Bautista, R.C., Stark, J.D., 2002. Comparative demography of six fruit fly (Diptera: Tephritidae) parasitoids (Hymenoptera: Braconidae). Biological Control 25, 30–40.

Wäckers, F.L., Lewis, W.J., 1994. Olfactory and visual learning and their combined influence on host site location by the parasitoid *Microplitis croceipes* (Cresson). Biological Control 4, 105–112.

Zar, J., 1996. Biostatistical Analysis, third ed. Prentice Hall, Upper Saddle River, N.J.

Zimmermann, H.G., Moran, V.C., Hoffmann, J.H., 2001. The renowned cactus moth, *Cactoblastis cactorum*: its natural history and threat to native Opuntia floras in Mexico and the United States of America. Diversity and Distribution 6, 259–269.