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REARING PHONOTACTIC PARASITOID FLIES [*DIPTERA : TACHINIDAE, ORMIINI, ORMIA* SPP.]

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Females of ormiine tachinids fly to their hosts' calling songs and deposit larvae on the host or nearby. Two species, *Ormia ochracea* (Bigot) and *O. depleta* (Wiedemann), were reared for at least 8 generations, making them the first ormiines to be laboratory-propagated. Both were reared on natural hosts : *Gryllus* spp. field crickets (principally *G. rubens*) for *O. ochracea,* and *Scapteriscus* spp. mole crickets for *O. depleta.* Commercially reared *Acheta domesticus* tested as hosts were less satisfactory. Hosts were parasitized manually or by confinement with flies or planidia (infective larvae). Transparent, cylindrical, sleeved cages were designed to accommodate parasitized hosts and pupae and adults of *O. ochracea.* Cages were joined to allow *O. ochracea* to cycle through its stages with minimum handling and care. Parasitized hosts and pupae of *O. depleta* were held in containers of damp sand ; adults were held in cages developed for *O. ochracea.* Adults of both species were maintained on applesauce, sugar cubes, powdered milk, and water. The life cycle of *O. ochracea* was about 31 days and of *O. depleta* about 36 days, with the principal difference being the time required for planidia to complete development. In *O. ochracea* the adults emerged synchronously but in *O. depleta* males preceded females. In both species sex ratio was generally 1 : 1 and females lived slightly longer than males. *O. depleta* from our laboratory colony have been released for biological control of mole crickets.

KEY-WORDS : *Ormia ochracea, Ormia depleta, Tachinidae,* Florida, rearing, *Scapteriscus.*

Adults of the tachinid tribe ormiini have been found in many parts of the world (**Leonide,** 1969) but until recently have been collected in small numbers and have received little attention. **Sabrosky** (1953) and **Sabrosky & Arnaud** (1965) divided New World species of Ormiini between the genera *Ormia* and *Euphasiopteryx.* Recently **Wood** (1987) and **N. E. Woodley** (pers. com.) have placed all species in *Ormia.*

Ormia adults (fig. 1) are crepuscular and nocturnal parasitoids of crickets and katydids (Orthoptera : *Gryllidae* and *Tettigoniidae*) (**Sabrosky,** 1953 ; **Sabrosky & Arnaud,** 1965). Larviparous females are phonotactic, locating their hosts by orienting to the male hosts' calling songs (**Cade,** 1975 ; **Mangold,** 1978 ; **Burk,** 1982a, b ; **Fowler & Kochalka,** 1985). A sarcophagid, *Colcondamyia auditrix* Shewell, is the only other parasitoid known to orient to the song of its calling host, a cicada (**Soper** *et al.,* 1976). The behavior of *Ormia* females, after finding their hosts, is poorly known. **Fowler** *et al.* (1988) observed that *O. depleta*

Wiedemann females deposited larvae around the calling chamber of male *Scapteriscus vicinus* Scudder. **Townsend** (1911 & 1912) and **Nutting** (1953) found larvae of *Ormia* spp. to be morphologically adapted for seeking out hosts. **Cade** (1975) found that *O. ochracea* (Bigot) deposited larvae on and around a dead *Gryllus* sp. attached to a speaker playing *Gryllus* sp. calls. Regardless of whether larvae are deposited on or near their hosts, a single larvae is capable of killing a host.

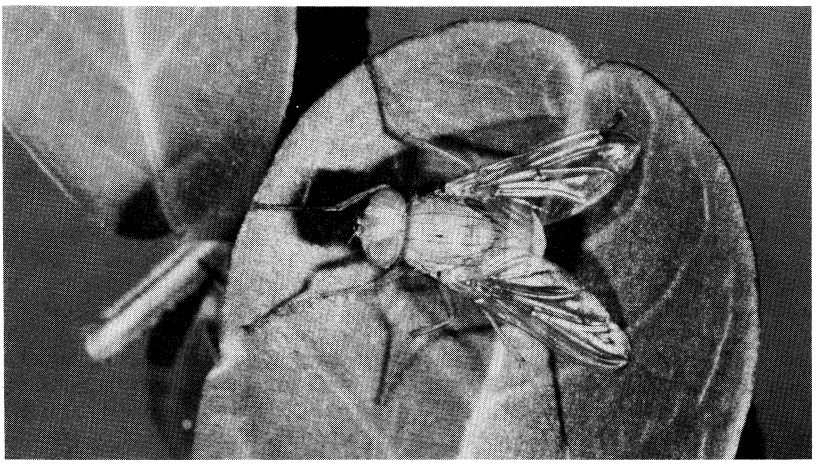

Fig. 1. *Ormia depleta,* female (photo courtesy of **J. L. Castner**).

O. depleta was first identified as a natural parasitoid of *Scapteriscus* sp. mole crickets collected in Brazil (**Wolcott,** 1940). [**Wolcott,** according to **Sabrosky** (1953), misidentified the specimens as *Ormia australis* (Townsend).] More recently **Fowler & Kochalka** (1985), working in Paraguay, found that *O. depleta* was attracted to the call of *Scapteriscus borellei* Giglio-Tos. **Camargo** *et al.* (1986), **Fowler** (1987a, b & 1989), and **Fowler & Garcia** (1987) later showed that natural hosts of *O. depleta* included *S. borellei, S. imitatus* Nickle & Castner, *S. vicinus, Anurogryllus* sp., and *S. abbreviatus* Scudder, a non-calling species. The mole crickets *S. abbreviatus, S. borellei,* and *S. vicinus,* were introduced from South America into the southeastern United States *ca.* 1900 (**Walker & Nickle,** 1981 ; **Nickle & Castner,** 1984). They have since become pest species and in Florida alone cause at least $ 30 million of damage annually (**Walker,** 1984). Rearing methods were developed for *Ormia depleta* because this species was a potential biocontrol agent for mole crickets in the southeastern United States. Methods for rearing a local native species, *O. ochracea,* parasitoids of *Gryllus* spp. field crickets (**Reinhard,** 1922 ; **Cade,** 1975 ; **Walker,** 1986), were developed first and then adapted for rearing the Brazilian species, *O. depleta.*

MATERIALS AND METHODS

O. OCHRACEA

REARING CAGES

A large, clear, cylindrical sleeved cage was designed for holding as many as 200 adults (fig. 2). Flies could be viewed clearly, collected easily, and tended with few escapes. This

cage was made of a rectangular sheet of heavyweight acetate, 64 cm × 96 cm. A sleeve, the upper portion of a hose leg, was glued between the perimeter of a 15 cm diam hole in the center of the acetate sheet and a corresponding acetate ring. Dimensions of the ring were 15 cm inside diam × 18 cm outside diam. The acetate sheet was rolled into a cylinder, 64 cm high × 29 cm diam and held in place with glue or filament tape. Each end of the cylindrical cage fit into the rim of a plastic bucket lid.

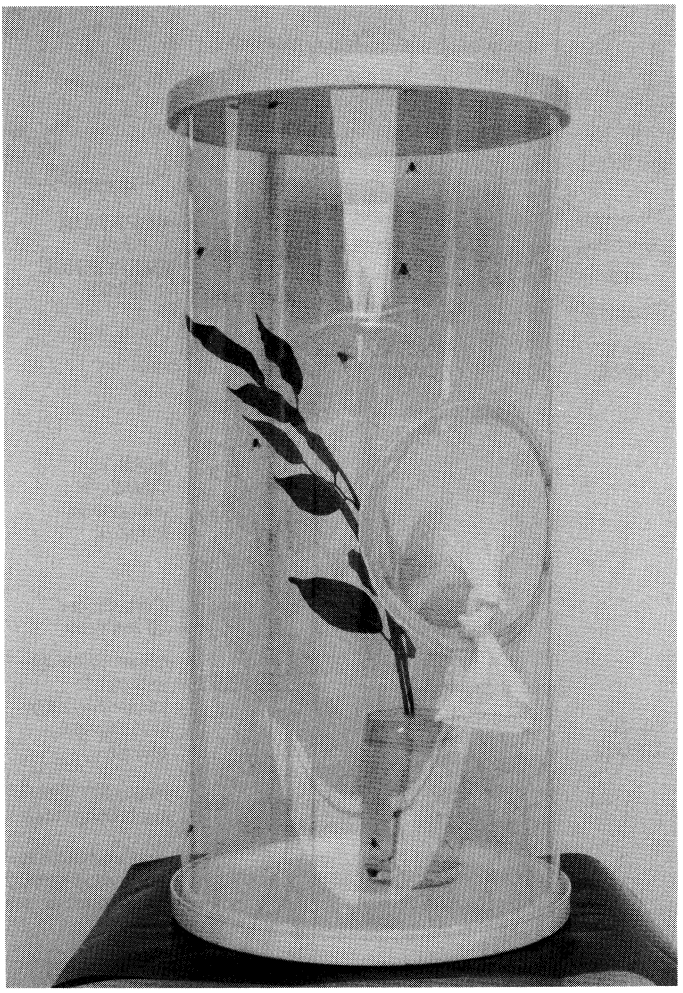

Fig. 2. *Ormia* spp. adult rearing cage.

A parasitism cage (fig. 3A, pt. b) with the same dimensions as the adult cage, and a pupation and emergence cage (fig. 3A, pt. c), 18 cm high × 29 cm diam with a 12 cm diam hole for a sleeve, were also constructed. Lids of cages were modified (fig. 3A, pt. a-c) to enable stages of the parasite to move from one cage to another (fig. 3B).

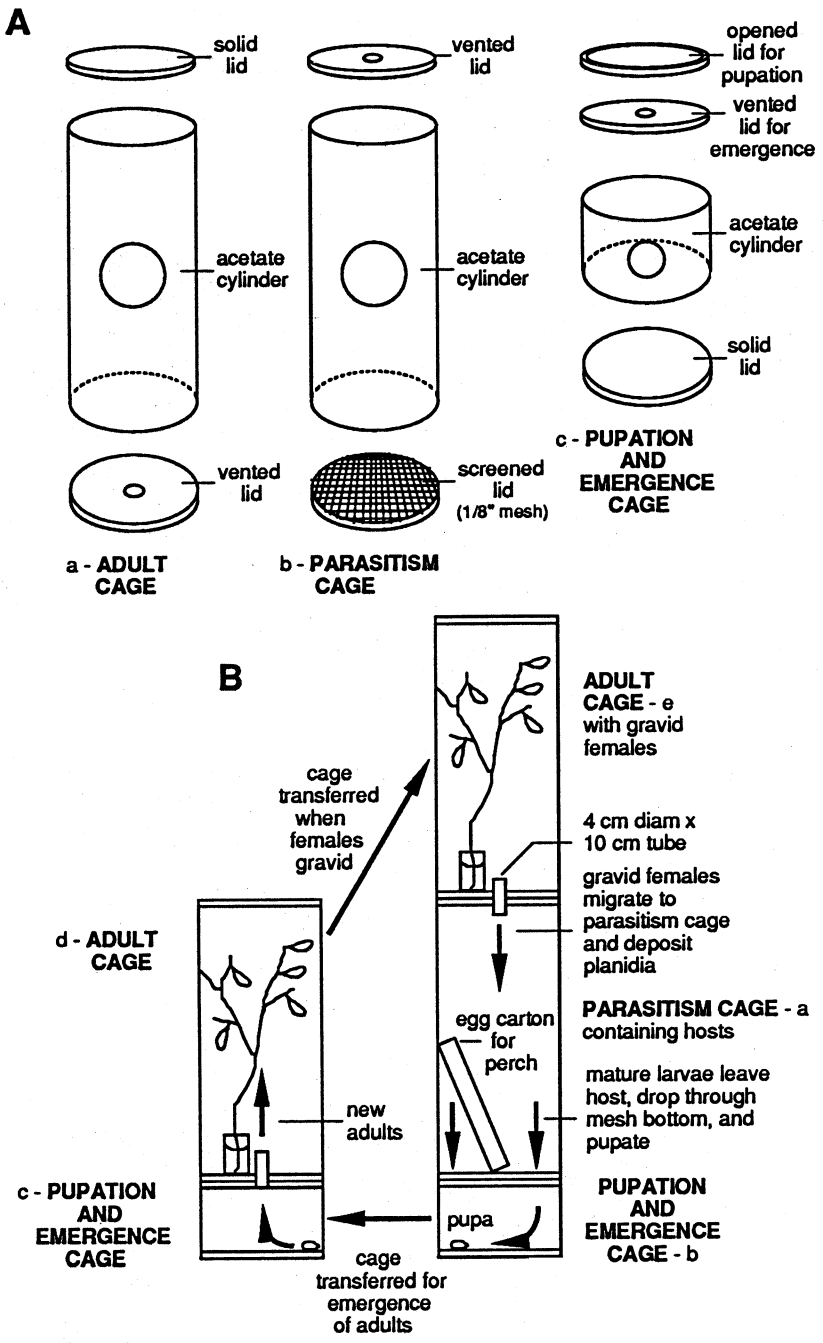

Fig. 3. A. Exploded views of *O. ochracea* cages. B. Operation of cages showing flow of life stages. (For clarity, sleeves are omitted ; cage openings are omitted from B.)

SPECIES OF HOSTS USED AND ORIGIN OF PARASITOID

G. rubens Scudder, either field-collected (**Walker,** 1986) or lab-reared (**Wineriter & Walker,** 1988), were used most often as hosts ; *G. firmus* Scudder and *G. ovisopis* Walker were used occasionally. The origin of fly stock was Alachua Co., FL. using methods devised by **Walker** (1986 & 1989).

PARASITISM OF HOSTS

Hosts were parasitized by confining 1 to 25 gravid flies with as many as many as 200 hosts in the parasitism cage. The parasitism cage (fig. 3A, pt. b) was provisioned with Purina cricket chow, a slice of apple for flies and crickets, water dispensed through a cotton wick, damp sand for cricket oviposition, and pieces of egg carton for cricket perches.

PROVIDING FOR PUPAE

Following confinement of flies with hosts, the parasitism cage was placed on top of the pupation and emergence cage (fig. 3B, pt. a & b). About 7 days later, mature larvae emerged from moribund or dead crickets in the parasitism cage, crawled through the mesh bottom of the cage (fig. 3A, pt. b) and dropped into the pupation and emergence cage where they pupated (fig. 3B, pt. b). Cage wastes also fell through the screened bottom, but did not seem to affect mature larvae or pupae.

PROVIDING FOR ADULT EMERGENCE

After pupation was complete, the parasitism cage was removed, and the top lid of the pupation and emergence cage was changed from a lid with the entire center removed (opened lid) to a lid vented with a 4 cm diam opening in the center (fig. 3A, pt. c). Then the adult cage (fig. 3A, pt. a) was placed on top of the pupation and emergence cage (fig. 3B, pt. c & d). The 2 cages were connected by pushing a tube through the vented bottom lid of the adult cage until it was flush with the opening of the vented top lid of the pupation and emergence cage (fig. 3B, pt. c & d). The adult cage contained flat pieces of paper toweling as bedding ; a leafed branch for possible mating and perching sites ; food comprised of Mott's natural apple sauce, a piece of sugarcube, and powdered milk ; and water dispensed through a cotton wick.

Upon emergence, adults moved upward from the pupation and emergence cage through the connecting tube to the adult cage. To facilitate this process, the acetate cylinder of the pupation and emergence cage was wrapped in black plastic to exclude light. The few flies that did not move into the adult cage were transferred manually via the sleeves. After emergence was complete the tube connecting the 2 cages was capped to prevent reverse movement.

MAINTAINING AND MONITORING ADULT FLIES

Adult flies were given fresh food and water at least every other day. Paper towel strips were changed weekly and the cage cleaned. About 2 weeks after adult emergence, males were sometimes removed to make more space for females. (Females were presumed to have had ample opportunity to mate.) Cages were monitored routinely for females gravid with planidia by searching the cage for flies with dark, opaque abdomens — i.e., with planidia showing through.

STARTING A SECOND GENERATION

When several females had become gravid, the parasitism cage was set up again, and the adult cage was placed on the parasitism cage. The 2 cages were connected by pushing the connecting tube already in the bottom lid of the adult cage through the top lid of the parasitism cage (fig. 3B, pt. a & e). Gravid flies moved downward through the tube to mingle with and parasitize hosts. Thus, a 2nd generation was started, and the stages cycled through the cages as before.

LIFE HISTORY IN THE LABORATORY

After this rearing method was worked out, data were collected on the life cycle in the laboratory. The duration of the life stages, the pattern of adult emergence, and approximate numbers of planidia/female were determined. All life stages of *O. ochracea* were reared at 16L : 8D (fluorescent light) and 25 °C.

O. DEPLETA

REARING CAGES

Only adult *O. depleta* could be reared in the same type of cage as *O. ochracea* adults. Hosts and *O. depleta* pupae required different housing, explained below.

SPECIES OF HOSTS USED AND ORIGIN OF PARASITOID

Field-collected (**Walker,** 1982) or lab-reared (**Lenczewski & Walker,** unpublished) *Scapteriscus* spp. mole crickets, were used as hosts. The origin of fly stock was Piracicaba, Sao Paulo, Brazil, using methods devised by **Fowler** (1987b & 1988) and **Walker** (1989).

PARASITISM OF HOSTS

Hosts could not be confined easily or efficiently with gravid flies as in *O. ochracea.* Therefore, larvae were placed on hosts or in containers holding hosts.

Planidia were removed from gravid flies and 1 to 4 planidia placed on the soft connective tissue behind and underneath the posterior of a host pronotum. If planidia and hosts were abundant, planidia were placed on the damp sand or side of the vials housing hosts ; rate of parasitism was slightly lower using this method. Once infected, each host was placed in a 60 ml snap-cap, plastic vial (4 cm diam × 6 cm ht), containing 3 cm of damp sand, a 3 mm cube of apple, and a pinch of cricket chow. Vials were capped with lids having a 2 mm hole for ventilation and laid on their sides in trays to provide more floor space for crickets.

PROVIDING FOR PUPAE

After 12 days, contents of vials were removed and searched for pupae. Pupae collected were placed in wells, 1.8 cm diam × 0.5 cm deep, made in a container of damp sand (fig. 4A). No more than 3 pupae were placed in each well. Positioning of the pupae horizontally with the anal spiracles upward (fig. 4B & 4C) was critical. When positioned differently, wings of emerging adults did not expand properly, and their ptilina remained distended. When all pupae were in wells, they were covered with about 5 cm of damp loose sand and the container capped.

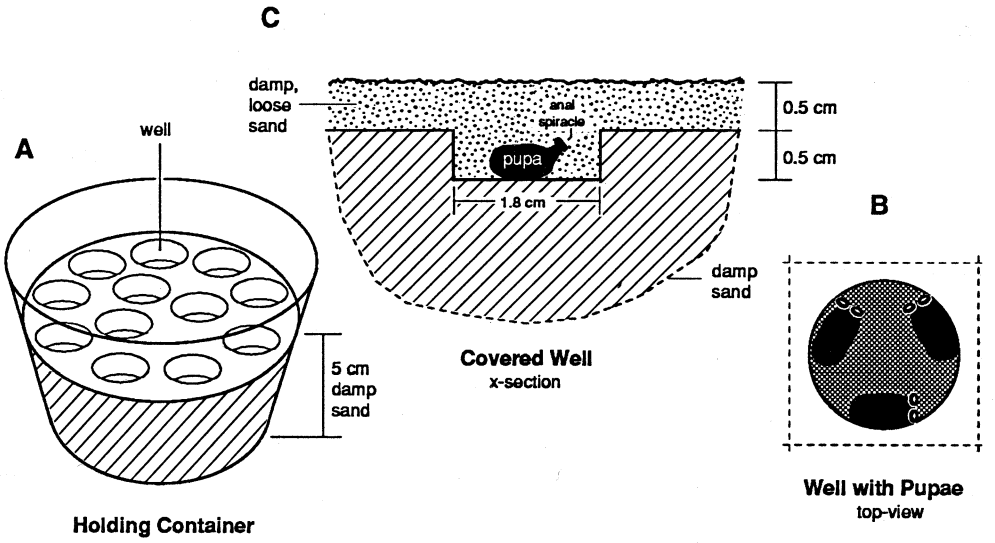

Fig. 4. Holding *O. depleta* pupae for emergence. A. Holding container. B. Top view of well showing placement of pupae. C. Cross-section of well showing orientation of pupae.

Providing for adult emergence

Containers holding pupae were placed on the floor of a cage like that used for *O. ochracea* adults, and the lids removed a day or 2 before expected emergence. If sand became dry during the incubation or emergence period it was remoistened. The cage was provisioned for emergence in the same manner as in *O. ochracea*.

Maintaining and monitoring adult flies

Adult *O. depleta* were cared for like adult *O. ochracea*.

Life history in the laboratory

Once rearing techniques were worked out and propagation under way, *O. depleta* from our laboratory colony were released in Alachua and Manatee Counties, Florida. The duration of the life stages and the pattern of adult emergence of *O. depleta* in the laboratory were determined from progeny of a female trapped at the Alachua site. Estimates of numbers of planidia/female were made from lab-reared females. Life stages of *O. depleta* were reared under natural photoperiod at room temperature, usually 23-25 °C. The natural photoperiod was extended to 12L : 12D in the winter months by the addition of incandescent light in the morning.

RESULTS AND DISCUSSION

O. OCHRACEA AND *O. DEPLETA*

Rearing

Lab-propagated colonies of *O. ochracea* and *O. depleta* were established, and they persisted for at least 8 generations. One colony of *O. ochracea* lasted 10 generations before

being terminated. Methods for rearing *O. ochracea* were simpler as the hosts, *Gryllus* spp., live above ground in nature and live fairly harmoniously when caged in groups ; these characteristics made group parasitism by confinement of hosts with gravid flies possible. In addition, pupae of *O. ochracea* required no special holding conditions. Consequently, parasitized hosts, pupae and adult tachinids could be held in similar cages. The cage design allowed stages of the parasitoid to move from one cage to another with a minimum of handling and care, i.e. the parasitoid did most of the work.

Methods for rearing *O. ochracea* adults were used for rearing *O. depleta* adults but other stages of *O. depleta* required more tedious methods than *O. ochracea* due to differences in host habitat and special requirements for holding pupae. *O. depleta* hosts, *Scapteriscus* spp., live underground and do not fare well when held in groups. Also, some hosts were found to evade parasitism when held in groups and to prey on tachinid pupae. Therefore, hosts were parasitized by placing larvae on hosts or sand in containers of hosts and subsequently housing hosts individually. When mature larvae emerged from hosts, they pupated in the sand rather than on the surface. As a result, *O. depleta* pupae had to be manually harvested from host containers and reburied in damp sand. If pupae were not reburied in damp sand, fewer adults emerged. Orientation of pupae in sand was also critical for proper development of teneral adults.

Some of the medium of rearing *O. depleta* may be eliminated by suspending larvae in solution and dispensing larvae with a pipet on the host or host substrate as done by **Gnatt** *et al.* (1974) with larvae of the tachinid *Lixophaga diatraeae* (Townsend). Another option is finding a suitable alternate laboratory host which lives above ground and can be held in groups, thereby allowing group parasitism as in *O. ochracea.* We tested commercially available fish bait crickets, *Acheta domesticus* (L.), as hosts for both *O. ochracea* and *O. depleta.* Pupae and adult tachinids of both species were obtained from crickets living long enough for larvae to develop. However, high mortality of controls obfuscated results and further testing is required.

While we were able to establish colonies of both species, initial efforts failed because few or no females produced planidia. We overcame this problem by holding large numbers of flies together rather than in small groups or pairs. However, even in larger groups, usually no more than 20-30 % of females produced planidia. Why more than half of the females failed to produce planidia remains an important question in rearing. One possibility is that low numbers of females were mating. In a recent study, examination of spermathecae of 11 one-month-old females, exposed to males for at least 2 weeks after emergence, showed only one female to contain sperm. Moreover, while this female contained hundreds of sperm, she had only one planidium.

These results indicate many females are failing to mate. The low proportion of mating could be due to unnatural mating conditions in the cage. *Ormia* spp. may normally mate at an encounter site high above the ground and with a high male-to-female ratio (**Lederhouse** *et al.,* 1976 ; **Burk,** 1982b).

Life history in the laboratory

The life cycle of *O. ochracea* was about 31 days, from oocyte to adult, while that of *O. depleta* was 36 days. Development times of life stages were similar except that *O. depleta* females required about one more week to develop planidia than *O. ochracea* (table 1). Most gravid females examined contained eggs and immature larvae as well as planidia, suggesting that females deposit larvae over many days. Numbers of planidia/female at time of examination ranged from 65 to 517 for *O. ochracea,* mean 219 $\pm$ 38 (s.e.), N = 11 field-collected females, and for *O. depleta,* 70 to 310, mean = 187 $\pm$ 45 (s.e.), N = 6 lab-reared females.

TABLE 1

Life history of O. ochracea *and* O. depleta *in the laboratory.*
O. ochracea *reared at 25 °C and 16L : 8D.* O. depleta *reared at about 23-25 °C, natural photoperiod supplemented, when necessary, with incandescent light at dawn to increase daylength to 12 h*

Stage	Time Spent in Each Stage (days)	
	O. ochracea	*O. depleta*
Oocyte to 1st planidium		
♂♂		
N	53	3
range	11-25	16-36
median	19-21	—
Larva in host		
All		
N	136	—
x ± s.e.	7.4 ± 0.06	—
range	7-9	—
♂♂		
N	—	40
x ± s.e.	—	8.3 ± 0.07
range	—	8-9
♀♀		
N	—	43
x ± s.e.	—	8.2 ± 0.06
range	—	8-9
Pupa		
♂♂		
N	42	35
x ± s.e.	12.3 ± 0.07	11.1 ± 0.05
range	12-13	11-12
♀♀		
N	49	43
x ± s.e.	12.4 ± 0.10	11.8 ± 0.07
range	12-15	11-13
Adult		
♂♂		
N	41	30
x ± s.e.	14.9 ± 1.56	14.0 ± 2.5
range	2-46	2-46
median	14	6
♀♀		
N	36	34
x ± s.e.	22.8 ± 2.55	17.8 ± 2.7
range	0-58	2-51
median	17.5	8

The onset of production of planidia in females varied considerably in both species while emergence of mature larvae from hosts and development time of pupae were nearly synchronous in both species. Emergence of adults occurred early in the morning before 10 00 h for both species and spanned a 3-4 day period. In *O. ochracea,* there was no difference in emergence pattern between sexes (N = 42 ♂♂, 49 ♀♀ ; χ^2, $P > 0.25$), but in *O. depleta,* emergence pattern was significantly different (N = 35 ♂♂, 43 ♀♀ ; χ^2, $P < 0.001$) ; ♂♂ generally preceded ♀♀. Sex ratio was usually 1 : 1 for both species.

Adults of both species lived from 1 day to as long as 7 or 8 weeks. Mortality generally occurred at a slow constant rate. Females on average lived longer than males. Adults were most active at dawn and dusk except for feeding.

In summary, we developed rearing methods for *Ormia* spp. Methods for rearing *O. ochracea* are highly refined ; methods for *O. depleta* are more tedious. Both methods produced ample numbers of flies. *O. depleta* currently is being reared for release in selected areas of Florida where mole crickets are important pests. Previous to this study, few *Ormia* spp. had been observed or collected, except for recent sticky and sound-trapping of females (**Fowler & Kochalka,** 1985 ; **Walker,** 1986 ; **Fowler & Garcia,** 1987 ; **Fowler,** 1987a, 1987b & 1988). Now all stages and both sexes of these unique phonotactic flies are available for further studies.

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RÉSUMÉ

Elevage de diptères parasitoïdes phonotactiques [*Dipt. : Tachinidae, Ormiini, Ormia* spp.]

Les femelles des tachinides Ormiini volent aux chants de leurs hôtes et elles posent leurs larves sur l'hôte ou tout proche de lui. Nous avons élevé 2 espèces pour la 1re fois ; *Ormia ochracea* (Bigot) et *O. depleta* Wiedemann, durant 10 et 8 générations respectivement. L'élevage était réalisé sur hôtes naturels : grillons des champs (*Gryllus* spp., principalement *G. rubens*) pour *O. ochracea,* et courtilières du genre *Scapteriscus* pour *O. depleta.* Les grillons domestiques [*Acheta domesticus* (L.)], achetés dans le commerce, convenaient moins. Des femelles gravides des diptères ont été attrapées aux pièges utilisant le chant des hôtes. Les hôtes ont été parasités à la main, ou par confinement avec les diptères femelles ou les larves infectantes. Des cages cylindriques, transparentes, et avec un manchon de tissu, ont été construites pour recevoir des hôtes parasités, des nymphes et des adultes d'*O. ochracea.* La réunion de 2 cages a permis le développement du cycle complet d'*O. ochracea* avec un minimum de soins et de manipulations. Les hôtes parasités et les nymphes d'*O. depleta* ont été enfermés dans des boîtes de sable humidifié ; les adultes ont été enfermés dans des cages du même type que celles utilisées pour *O. ochracea.* Les adultes des 2 espèces ont été alimentés avec de la sauce aux pommes, des morceaux de sucre, du lait en poudre et de l'eau. Une génération d'*O. ochracea* dure environ 31 jours et une d'*O. depleta,* environ 36 jours ; la différence principale est la durée de développement des larves infectantes. La durée de développement des ♂♂ et des ♀♀ d'*O. ochracea* est identique, mais chez *O. depleta* les ♂♂ se développent plus rapidement que les ♀♀. Le rapport des sexes est 1 : 1 généralement chez les 2 espèces, et les ♀♀ vivent un peu plus longtemps. Des individus d'*O. depleta* de notre élevage ont été lâchés comme agents de lutte biologique contre les courtilières du genre *Scapteriscus.*

MOTS CLÉS : *Ormia ochracea, Ormia depleta, Tachinidae,* Floride, élevage, *Scapteriscus.*

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