

The Bionomics of *Coptera Haywardi* (Ogloblin) (Hymenoptera: Diapriidae) and Other Pupal Parasitoids of Tephritid Fruit Flies (Diptera)

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The endoparasitoid *Coptera haywardi* (Ogloblin) (Diapriidae) was discovered in Mexico attacking the pupae of the Mexican fruit fly, *Anastrepha ludens* (Loew). Typically, parasitoids of Diptera Cychlorrhapha pupae develop as ectoparasitoids and are generalists that attack hosts in a number of families. Aspects of the bionomics of *C. haywardi* were compared to those of two chalcidoid ectoparasitoids, *Dirhinus himalayanus* Westwood and *Spalangia gemina* Boucek. *C. haywardi* developed in three genera of Tephritidae, but not in species of other families. The two species of chalcidoids developed in all the calypterate and acalypterate hosts to which they were exposed. In an olfactometer *C. haywardi* preferred *Anastrepha suspensa* (Loew) pupae, while the chalcidoids preferred the pupae of *Musca domestica* L. This preference in *S. gemina* was diminished in insects that had been reared on *A. suspensa*. *C. haywardi* oviposited in the *A. suspensa* pupae that had been previously parasitized by the braconid *Diachasmimorpha longicaudata* (Ashmead). However, it completed development only in unparasitized pupae. Mortality of the primary parasitoid due to *D. himalayanus* was approximately two-thirds the mortality inflicted on the host fly. *S. gemina* did not discriminate between parasitized and unparasitized pupae of *A. suspensa* and developed in both. *C. haywardi* appears to have a more restricted host range relative to chalcidoid pupal parasitoids and this may be due to its endoparasitic development. © 1998 Academic Press

Key Words: *Anastrepha*; Chalcidae; *Coptera*; Diapriidae; *Dirhinus*; Pteromalidae; *Spalangia*.

INTRODUCTION

Tephritid fruit flies are major pests of fruit crops in the tropics and subtropics and, because of the erection of quarantines, important barriers to trade (e.g., Aluja, 1994). They oviposit in ripening fruits, and mature larvae exit from fallen fruits or, less frequently, drop to the ground from ripe fruits still hanging in trees (e.g.,

Aluja, 1994). Tephritid larvae burrow into the soil to pupate, typically to depths of 1–5 cm and often near or directly beneath their hosts (e.g., Thomas, 1993). While burrowing and later as buried pupae, they are exposed to a number of predators and hymenopteran parasitoids (e.g., Boller, 1966; Sivinski, 1996).

Pupal parasitoids were among the earliest described natural enemies of pestiferous fruit flies. For example, an early exploration for natural enemies of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), returned to Hawaii from West Africa with live specimens of the chalcid *Dirhinus giffardii* Silvestri, the diapriid *Coptera* (= *Psilus* = *Galeus*) *silvestrii* (Kieffer), and the pteromalid *Muscidifurax vorax* Girault (Silvestri, 1914). Additional species that did not survive the voyage included another chalcid, *Dirhinus ehrhorni* Silvestri, *Spalangia afra* Silvestri (Pteromalidae), and *Trichopria capensis* Kieffer (Diapriidae). These same parasitoid genera figured in subsequent expeditions to obtain biological control agents for *C. capitata* and the Oriental fruit fly, *Bactrocera* (= *Dacus*) *dorsalis* (Hendel) (Bess, 1961). By the end of the 1950s, Hawaiian entomologists alone had received, in addition to the above, *Coptera* (= *Psilus*) *magnificus* (Nixon) from Kenya, several Asian *Coptera* spp., a *Trichopria* sp. from India, five species of *Spalangia*, and another pteromalid, the now widespread *Pachycrepoideus vindemiae* (Rondani).

Despite their diversity and long history in biological control, relatively little is known about the behaviors and ecology of pupal parasitoids of Tephritidae, particularly those in the Diapriidae. *Coptera haywardi* (Ogloblin) (Figs. 1a and 1b) is a member of a genus that is rarely collected from other than tephritid puparia (see, however, the comments of Muesebeck, 1980; the discovery of a *Coptera* individual in the muscid *Stomoxys calcitrans* (L.), Hogsette *et al.*, 1994; and the host record for *Coptera muscidorum* Dodd in Nixon, 1930). *C. haywardi* was discovered parasitizing Mexican fruit flies, *Anastrepha ludens* (Loew), infesting citrus in Veracruz State, Mexico (Lopez, 1996).

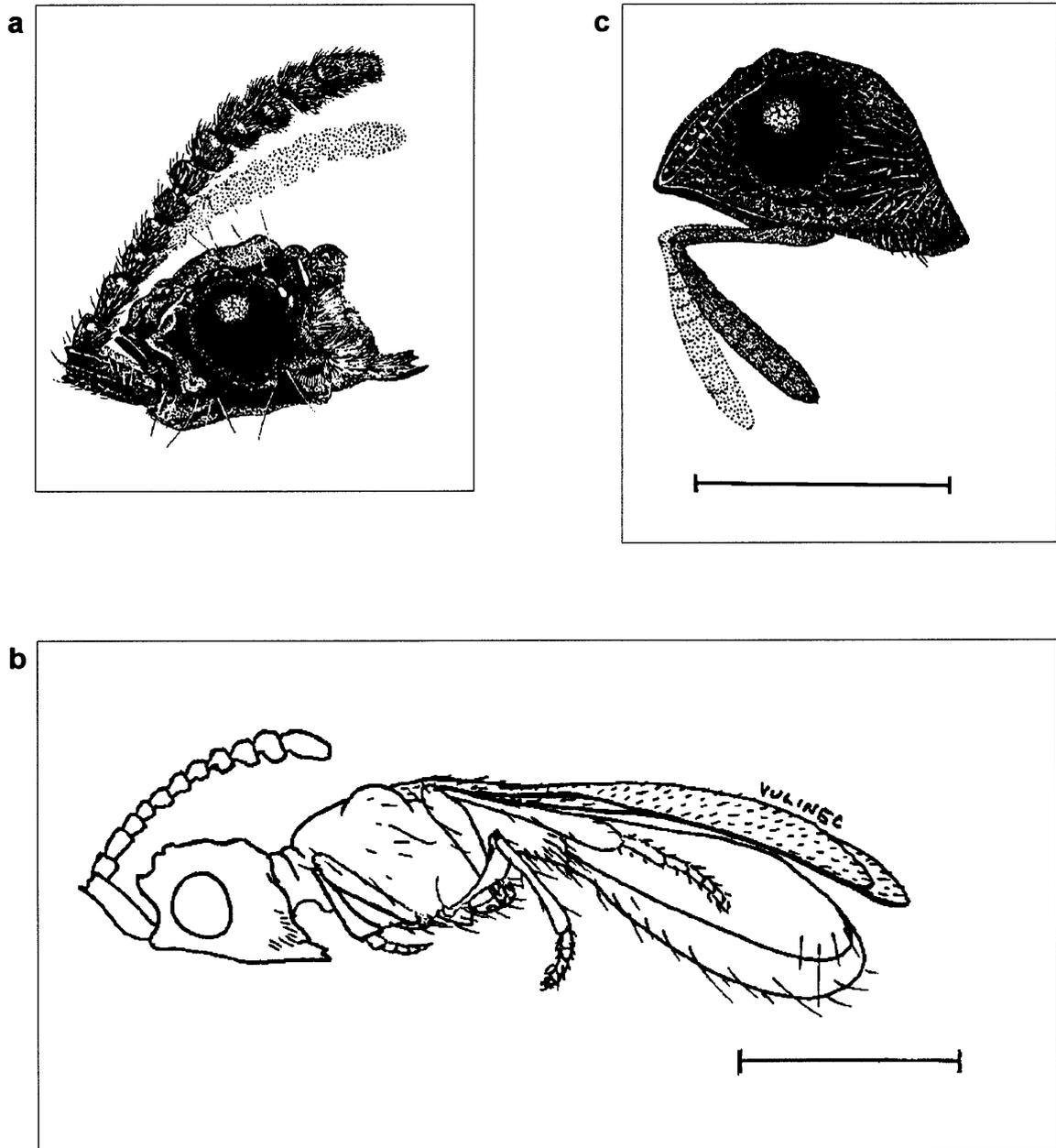


FIG. 1. (a) The head of the diapiiid *Coptera haywardi* and (b) a lateral view of *C. haywardi*; (c) The head of the chalcid *Dirhinus himalayanus*. All lines represent 1 mm. The apparent convergence in the hypognathus morphology of the heads may be due to similar adaptations for digging in soil to locate the pupae of flies.

Like its congener, *C. silvestri*, but unlike many chalcidoid pupal parasitoids, *C. haywardi* is an endoparasitoid (see Silvestri, 1914). It is not presently clear whether *C. haywardi* should be further classified as a koinobiont or idiobiont. Pupal parasitoids are typically characterized as idiobionts because the parasitoid develops in a host whose growth (resource accumulation) is completed (cf. Godfray, 1994). However, *C. haywardi* may encounter the same complex body-cavity environment, including host immune responses, faced by a

typical koinobiont. Following parasitism by *C. haywardi*, the fruit fly–host pupa’s circulatory system can be observed through the cuticle to function in an apparently normal manner. All other things being equal, endoparasitoid–koinobionts are often more specialized and display narrower host ranges than ectoparasitoid–idiobionts, due to their more intimate relationships with their hosts (e.g., Hawkins, 1994).

We compare and contrast the bionomics of *C. haywardi* with two species of chalcidoid ectoparasitic pupal

parasitoids, *Dirhinus himalayanus* Westwood (Chalcidae) and *Spalangia gemina* Boucek (Pteromalidae), whose development in Tephritidae has not been previously examined.

D. himalayanus belongs to the chalcid subfamily Dirhininae whose members commonly attack the pupae of tephritids (Grissell and Schauff, 1990), but which are not restricted to fruit fly hosts. *D. himalayanus* has been widely collected from muscoid flies in excrement and vertebrate cadavers in India, Nepal, and Pakistan (Boucek & Narendran, 1981). It was also once reared from a tachinid parasitoid of the arctiid *H. cunea* (Drury). In the laboratory, females will dig to prodigious depths in order to locate pupae (>30 cm; J. Sivinski, personal observation). Mating, oviposition, and certain life history characteristics of *D. himalayanus* have been described by Bai (1990; referred to as *D. pachycerus*). The peculiar horned head and powerful legs may be an aid to excavation (Fig. 1c).

S. gemina is widely distributed in the south Asian and South American tropics (Boucek, 1963). Although often collected from tephritids, including *B. dorsalis*, there are also several records of it attacking tachinids developing in Lepidoptera (Boucek, 1963).

Specifically we examined *C. haywardi* and the chalcid parasitoids for the following: (1) their ability to develop in distantly related calypterate and acalypterate Cyclorrhapha; (2) their propensity to hyperparasitize the braconid *Diachasmimorpha longicaudata* (Ashmead) as it develops in the Caribbean fruit fly, *Anastrepha suspensa* (Loew); and (3) the effect of rearing the parasitoids on either *A. suspensa* or house fly (*Musca domestica* L.), on subsequent adult responses to pupae of the original and the alternative host species (i.e., might host range be narrowed by experience; e.g., Turlings *et al.*, 1992). In the process we describe certain oviposition behaviors in *C. haywardi* and the chalcid, *D. himalayanus*.

METHODS

Parasitoid cultures. Cultures of *D. himalayanus* and *S. gemina* were begun with individuals from colonies maintained for 7 years (~45 generations) and 5 years (~36 generations) respectively on *M. domestica* pupae by the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida. At the time of the experiments, colonies of both species maintained on *A. suspensa* had completed four to six generations. The culture of *C. haywardi* was maintained on *A. suspensa* and was derived from a colony previously kept for ca. 1 year on pupae of *A. ludens*, at the Instituto de Ecología, Xalapa, Veracruz, Mexico. Original collections were made in the villages of Tejeria (latitude 19° 22' north, longitude 96° 53' west, and an altitude of 680 m) and Monte Blanco (latitude 19° 23'

north, longitude 96° 56' West, and an altitude of 1000 m). All the parasitoid species used in experiments had been maintained on *A. suspensa* for at least three generations. *A. suspensa* pupae were obtained from a colony maintained for 9 years (~150 generations) at the Florida Division of Plant Industry, Gainesville, Florida.

Parasitoids were provided with the pupae of either *A. suspensa* or *M. domestica* in 200-ml cardboard containers placed inside 2-liter plastic jars. These were held in environmental chambers at 29°C and 70% relative humidity. Photoperiod was 12 h L:12 h D. Adults were provided with sucrose (sugar cube), honey, and water. Voucher specimens are in the personal collections of J. Sivinski and M. Aluja and the institutional collection at the Instituto de Ecología, Xalapa, Veracruz, Mexico.

Host specificity and oviposition behavior. Parasitoids, provided with food and water and kept in the previously described containers, were presented with 1-day-old pupae and kept with these pupae until the emergence of host adults. This assured that the parasitoids were present during any vulnerable periods in host development. Methods of presenting hosts varied with species. Groups of 25–200 parasitoids were provided with 20–100 hosts per female of *A. suspensa*, *M. domestica*, *Stomoxys calcitrans* (L.) (Muscidae), *Hydrotaea aenescens* (Wiedemann) (Muscidae), *Phormia regina* (Meigen) (Calliphoridae), and *Drosophila virilis* Sturtevant (Drosophilidae). Twenty-five pupae of the tachinid *Ormia depleta* Wied. obtained from Dr. H. Frank, University of Florida, were exposed to 10 female and 5 male *C. haywardi* in a 9-cm-diameter petri dish lined with damp paper. Forty pupae of the papaya fruit fly, *Toxotrypana curvicauda* (Gerstaecker), a close relative of *Anastrepha* (Foote, 1993), were obtained from papayas collected in the field near Xalapa, Veracruz, Mexico. These were presented in lots of 10 to four groups of 15 female and 5 male *C. haywardi*. In two instances, 10 pupae of *A. ludens* were mixed with those of the *T. curvicauda*. The pupae were exposed for 3 days and then held under ambient conditions at the Instituto de Ecología. Unemerged pupae were dissected to determine the fate of the host. *C. haywardi* were provided with an excess (>100 hosts/female) of *C. capitata* pupae at the USDA-APHIS/MOSCAMED insect rearing facility in Guatemala City, Guatemala.

In an additional examination of host range, six females of *C. haywardi* were individually observed in the presence of both *A. suspensa* and *M. domestica* in order to determine if their behaviors toward the different pupae were similar. A female was first placed in a 6-cm petri dish containing a layer of clean sand and 10 partially buried 1- to 2-day-old *M. domestica* pupae. The parasitoid's actions in relation to the pupae (antennation, orientation, and oviposition) were noted over a period of ca. 20 min and the parasitoid was then subsequently transferred to an identical petri dish

containing 10 *A. suspensa* pupae (2–12 days in age) where her behaviors were again recorded.

Mean behavioral durations (time spent foraging for and mounting hosts) were compared through Student's *t* test (SAS Institute, 1988).

Oviposition behaviors of *C. haywardi* and *D. himalayanus* (i.e., location of oviposition in *A. suspensa* pupae and oviposition duration) were observed under a microscope in manner described above. There were 57 observed instances of *C. haywardi* attacking pupae of various ages and 42 of *D. himalayanus*. Means were compared through Student's *t* test and relationships between variables determined by correlation (SAS Institute, 1988).

Heterospecific hyperparasitism. Approximately 50 randomly chosen parasitoids of undetermined sex were placed in 250-ml cardboard cups whose lids had been replaced with fine-mesh screening (due to limited numbers, 15 individuals of *C. haywardi* were used in each cup). Water and sucrose were provided. One-day-old pupae of *A. suspensa* that had been exposed as larvae to the braconid *D. longicaudata* were added in lots of 10–50 ml, depending on the species of parasitoid and the availability of pupae, and left for 1 week. An identical set of cups, but ones containing host flies and primary parasitoids alone, was prepared in the same manner. These were used to determine the emergence of the host and primary parasitoid in the absence of the pupal parasitoid. Mortalities of the host fly and the primary parasitoid due to the pupal parasitoid were estimated by the differences between the emergence of adult hosts and primary parasitoids in cups that did and did not contain the pupal parasitoid. There were 15 temporally distinct replicates of five containers of pupae exposed to *C. haywardi* and 5 unexposed. There were three replicates of five containers of pupae exposed and unexposed to *D. himalayanus*. Parasitized pupae were obtained from the Division of Plant Industry, Gainesville, Florida. Typically, batches of such pupae were 30–50% parasitized. After 1 week the adult parasitoids were removed and the pupae were held under the conditions described above.

Mean percentage of parasitisms were compared by Student's *t* test and Wilcoxon's paired *T* test, and contingency data were compared by Fisher's exact test (SAS Institute, 1988; Zar, 1974).

In addition, parasitized and unparasitized pupae of *A. suspensa* were simultaneously exposed to *C. haywardi* and observations made on the oviposition behaviors of the parasitoid. This was done to determine if the parasitoid discriminated between parasitized and unparasitized pupae and what were the outcomes of ovipositions in the different types of pupae. Parasitized pupae were taken from lots exposed to *D. longicaudata* and recognized by the presence of oviposition scars on the puparia. A layer of vermiculite was placed in a 2.0-cm dish. Five parasitized and five unparasitized

pupae, 3–5 days in age, were partially embedded in the vermiculite. The positions of the two forms of pupae in the dish were alternated between observations. A female *C. haywardi* (7–11 days of age) was placed in the container and observed under a microscope. Time prior to oviposition, location of the oviposition in the puparium, and time to complete oviposition were recorded. Following oviposition the pupae were held for 30 days and those that did not yield an adult insect were dissected to determine their contents. Mean behavioral durations were examined for differences by Student's *t* test and relationships between variables by correlation (SAS Institute, 1989).

Response to host pupae. A bioassay device was used to determine if the parasitoids used distance cues, presumably chemical, to locate host pupae and whether they had a preference for either the pupae of *A. suspensa* or *M. domestica*. The device consisted of a 1-liter plastic container, 18 × 15 × 5 cm, whose lid had been replaced with fine-mesh screen. At opposite ends of the chamber, small funnels were inserted into the floor. These funnels were 4 cm across the top with an aperture of 3 mm. Funnels emptied into collecting jars whose bottoms were replaced with fine-mesh screen. When the olfactometer was placed on blocks above the surface of a table, air flow from the top of the large container through the bottom of the collecting jars, and vice versa, was possible. Fifteen milliliters of either *M. domestica* or *A. suspensa* pupae (~40 pupae/ml) were placed in each collecting jar. Approximately 15 pupal parasitoids were then transferred to the large container, after which they could go down the funnels toward either species of pupae or remain in the main chamber. After 24 h the collecting jars were removed and the insects they held counted and sexed. Insects used in the tests had been reared on either *A. suspensa* or *M. domestica*. They had eclosed in the presence of pupae of the same host species and thus had contact with a particular fly species both as larvae and as adults. Sixteen replicates were performed for each species, 8 for each colony reared on the different hosts (6 replicates in the case of *C. haywardi*). Because *C. haywardi* could not be reared on the pupae of *M. domestica* there was no investigation of the effect of rearing-host species on adult foraging. The locations of the collecting jars holding a particular species of pupae were alternated. Data on the attraction of male parasitoids to pupae was kept and analyzed separately. Multiple mean responses were compared by ANOVA with subsequent use of the Waller–Duncan *K*-ratio *t* test. Pairs of means were analyzed by Student's *t* test (SAS Institute, 1988).

RESULTS

Host specificity. *C. haywardi* developed only in tephritid pupae (Table 1). When known numbers of pupae

TABLE 1

The Success (+) or Failure (–) of Pupal Parasitoids to Develop in Various Species of Hosts

Hosts	Parasitoids		
	<i>Coptera haywardi</i> (Diapriidae)	<i>Dirhinus himalayanus</i> (Chalcidae)	<i>Spalangia gemina</i> (Pteromalidae)
<i>Anastrepha suspensa</i> (Tephritidae)	+	+	+
<i>Anastrepha ludens</i> (Tephritidae)	+		
<i>Toxotrypana curvicauda</i> (Tephritidae)	+		
<i>Ceratitis capitata</i> (Tephritidae)	+		
<i>Musca domestica</i> (Muscidae)	–	+	+
<i>Stomoxys calcitrans</i> (Muscidae)	–	+	
<i>Hydrotaea anescens</i> (Muscidae)	–	+	
<i>Phormia regina</i> (Calliphoridae)	–	+	
<i>Ormyia depleta</i> (Tachinidae)	–		
<i>Drosophila virilis</i> (Drosophilidae)	–		

Note. Cross hatching means a particular fly was not presented to a particular parasitoid.

of *A. suspensa* and *M. domestica* were presented to a cohort of females parasitism was 10–11 and 0% respectively. None of the six females that sequentially examined pupae of *M. domestica* and *A. suspensa* oviposited in a *M. domestica* pupa, but all subsequently penetrated the puparium of an *A. suspensa* pupa. While all females examined multiple *M. domestica* puparia during the ca. 20-min-long observation period, these examinations consisted for the most part of brief antennations. On one occasion, a female remained mounted on a *M. domestica* puparium for 5 min. During that time she partially exerted her ovipositor, but did not touch the puparium with it. The time spent by a parasitoid before touching her ovipositor to a *A. suspensa* puparium ranged from 2–10 min. *C. haywardi* developed in 5 of 40 *T. curvicauda* (Papaya fruit fly) pupae (12.5%). However, a normal emergence occurred in only one instance. In the other four cases, dissection revealed fully developed, but dead, adults that presumably had not been able to penetrate the host's relatively large and heavy puparium (see Hagley *et al.*, 1993). Twenty *A. ludens* pupae which had been simultaneously exposed to the same parasitoid cohorts as half of the *T. curvicauda* pupae were 70% parasitized. A colony of several hundred *C. haywardi* was maintained for more than four generations on the pupae of *C. capitata*.

S. gemina was reared on both *A. suspensa* and *M.*

domestica pupae (Table 1). *D. himalayanus* successfully developed on these an additional two species of Muscidae and a species of Calliphoridae (Table 1).

Oviposition behaviors. The age of *A. suspensa* pupae influenced the outcome of ovipositions (or insertions of the ovipositor that resembled oviposition) by *C. haywardi* and *D. himalayanus* (Fig. 2). When host pupae were either 2 or 3 days old, successful parasitism by *C. haywardi* was significantly higher in 3-day-old pupae ($\chi^2 = 5.0$; $P < 0.05$). *D. himalayanus* was significantly more likely to develop in pupae 4 to 5 days of age compared to pupae 1 to 3 days of age ($\chi^2 = 6.3$; $P < 0.05$). In both parasitoid species, ovipositions that resulted in emergence of an adult parasitoid were significantly longer than those that resulted in the survival of the host or the failure of any adult insect to emerge (Table 2). *D. himalayanus*, but not *C. haywardi*, was observed to feed from wounds in the host pupae (31 of 42 observations included adult feeding). There were differences in oviposition sites between the two parasitoids (Fig. 3a). *D. himalayanus* performed 36% of its ovipositions in the posterior two segments of pupae, compared to only 4% by *C. haywardi* in that location. In general, ovipositions in the more frequently used segments were more likely to result in the successful development of the offspring (Fig. 3b).

Response to pupae. The term "response" is used to indicate recovery from a collection jar in the bioassay device. Female *C. haywardi* were significantly more responsive to pupae of *A. suspensa* than to those of *M. domestica* (Fig. 4).

Female *D. himalayanus* that had been reared on both *A. suspensa* and *M. domestica* were more likely to respond to *M. domestica* pupae (Fig. 4). The mean proportion of females responding to *M. domestica*, as opposed to *A. suspensa*, was not affected by the species of host on which they were reared [raised on *M. domestica* and responding to *M. domestica*, 0.65 (SE = 0.07); raised on *M. domestica* and responding to *A. suspensa*, 0.54 (SE = 0.06); raised on *A. suspensa* and responding to *M. domestica*, 0.25 (SE = 0.07); raised on *A. suspensa* and responding to *A. suspensa*, 0.34 (SE = 0.06)]. There was no difference in male response to the two types of pupae (Table 3). In summary, females, but not males were more likely to respond to *M. domestica* pupae. Males were no more likely to respond to pupae than they were to remain in the bioassay device. There was no statistically significant evidence that experience modified the preference for either species of host.

Female *S. gemina* reared on *M. domestica* were more likely to respond to *M. domestica* than to *A. suspensa* (Fig. 4). There was no preference for either host among females reared on *A. suspensa*. Males reared on either host displayed no preference (Table 3). The proportion of females reared on *M. domestica* and responding to *M. domestica* was greater than the proportion of females

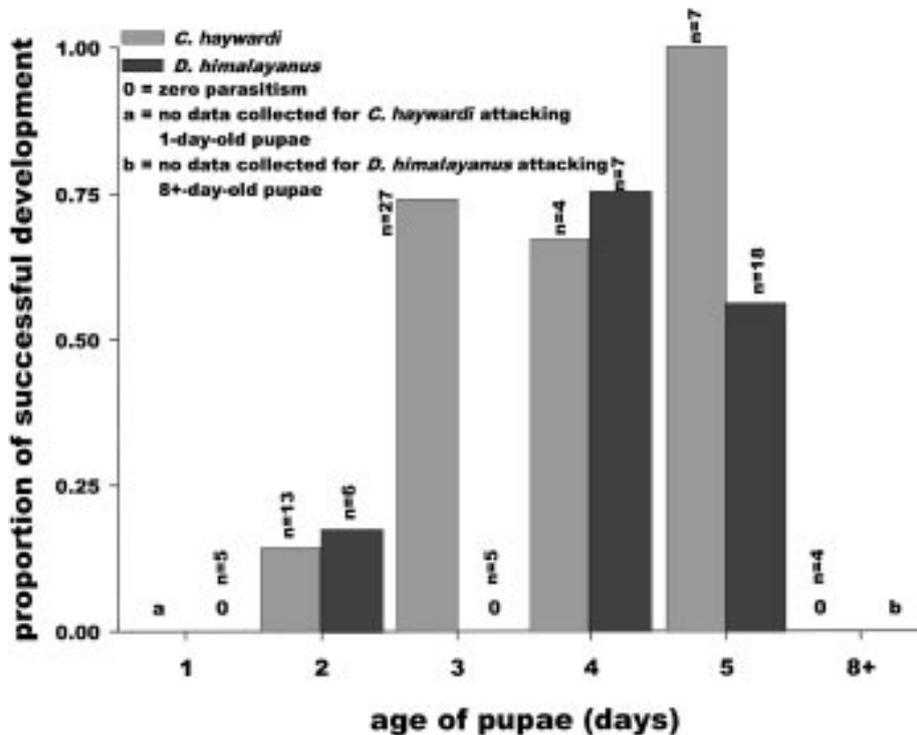


FIG. 2. The proportions of ovipositions by *Coptera haywardi* in *Anastrepha suspensa* pupae of various ages that resulted in the development of an adult parasitoid.

reared on *A. suspensa* and responding to *M. domestica* (Fig. 5). The response of females reared on *A. suspensa* to *A. suspensa* pupae compared to those reared on *M. domestica* was significant at 0.06 level. In summary, the behavior of females, but not males, was modified by rearing. Those parasitoids that had experience with *M. domestica* were more likely to respond to *M. domestica* pupae than those reared on *A. suspensa*.

Heterospecific hyperparasitism. Parasitism by *C. haywardi* of *A. suspensa* pupae previously parasitized by the braconid *D. longicaudata* was less than that of *A. suspensa* pupae that had not been previously parasitized by *D. longicaudata*. The ratio of *A. suspensa* adults completing development to *D. longicaudata* adults completing development was significantly less

in pupae that had been exposed to *C. haywardi*; i.e., mortality had been concentrated in the unparasitized pupae (Wilcoxon paired *T* test; $n = 15$; $P < 0.01$). Furthermore, the mean difference in the proportions of *A. suspensa* eclosing from pupae exposed and unexposed to *C. haywardi* was similar to the percentage of parasitism by *C. haywardi* compared to a mean 11% (SE = 3.0) decline in the proportion of *A. suspensa* adults relative to *D. longicaudata* adults. There was a significant correlation between parasitism by *C. haywardi* and the difference in the proportions of adult *A. suspensa* relative to *D. longicaudata* eclosing in the containers of pupae exposed and unexposed to *C. haywardi* ($r = 0.56$; $P < 0.03$).

Where individual *C. haywardi* females were simultaneously presented with unparasitized pupae of *A. suspensa* and pupae parasitized by *D. longicaudata*, there was no statistically significant preference for one form of pupae or the other (15 of 22 ovipositions in parasitized pupae, $\chi^2 = 2.9$, $P > 0.05$), neither was there a difference in the time females spent searching before ovipositing in one of the two forms of pupae [mean parasitized, 191.9 s (SE = 28.6), vs mean unparasitized, 101.4 s (SE = 38.5); $t = 1.8$; $df = 20$; $P = 0.08$]. Oviposition in the two forms of pupae took similar lengths of time (mean parasitized, 28.3 min (SE = 4.0), vs mean unparasitized, 27.2 min (SE = 4.8); $t = 0.17$;

TABLE 2

Comparisons of Oviposition Durations by *C. haywardi* and *D. himalayanus* That Resulted in Either the Development of a Parasitoid or the Survival of the Host/Failure of Any Adult Insects to Emerge

Parasitoid	Condition	N	Mean	SD	df	t	P
<i>Coptera haywardi</i>	Parasitized	30	24.8	11.8	36.5	2.7	0.01
	Unparasitized	24	17.5	7.3			
<i>Dirhinus himalayanus</i>	Parasitized	17	30.5	4.5	40	10	0.0001
	Unparasitized	25	16.9	4.2			

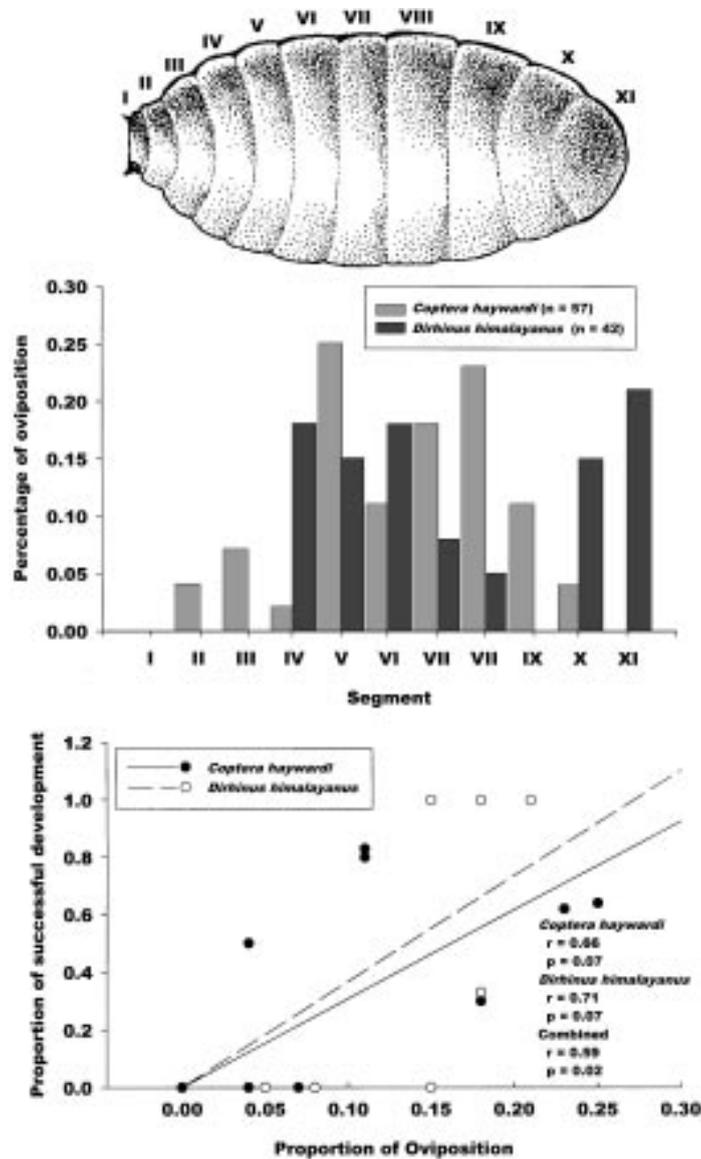


FIG. 3. (a) The locations (sections of the puparium that correspond to the segments of the third larval instar) of ovipositions in pupae of the *Anastrepha suspensa* by the parasitoids *Coptera haywardi* and *Dirhinus himalayanus*. (b) The relationships between the propensities of parasitoids to insert their ovipositors into particular segments and the proportion of ovipositions into those segments that subsequently result in the successful development of an adult parasitoid.

$df = 20$; $P = 0.87$). The consequences of oviposition in parasitized and unparasitized pupae did differ. None of the 15 pupae previously parasitized by *D. longicaudata* yielded an adult parasitoid. Only unparasitized pupae yielded *C. haywardi* (4 of the 7 pupae oviposited into yielded a parasitoid; Fisher's exact test; $P < 0.01$).

D. himalayanus appeared to avoid hyperparasitism though it could develop in pupae attacked by *D. longicaudata*; i.e., the mean mortalities inflicted on unparasitized pupae were higher than those on parasitized pupae. The overall parasitism was high, 1 adult parasitoid/64 pupae resulting in 93% parasitism of *A. suspensa* and 64% parasitism of *D. longicaudata*.

DISCUSSION

Ectoparasitic pupal parasitoids of Diptera often develop in a broad range of hosts. For example, *S. endius* Walker is widely released to control muscoid flies in poultry and livestock operations. It also attacks *A. suspensa* in nature (Baranowski *et al.*, 1993). A partial list of hosts includes one genus of Calliphoridae, six genera of Muscidae, three genera of Sarcophagidae, two genera of Ottidae, and three genera of Tephritidae (Rueda and Axtell, 1985). Both of the examined chalcids, *D. himalayanus* and *S. gemina*, displayed similar extensive host ranges that included species from

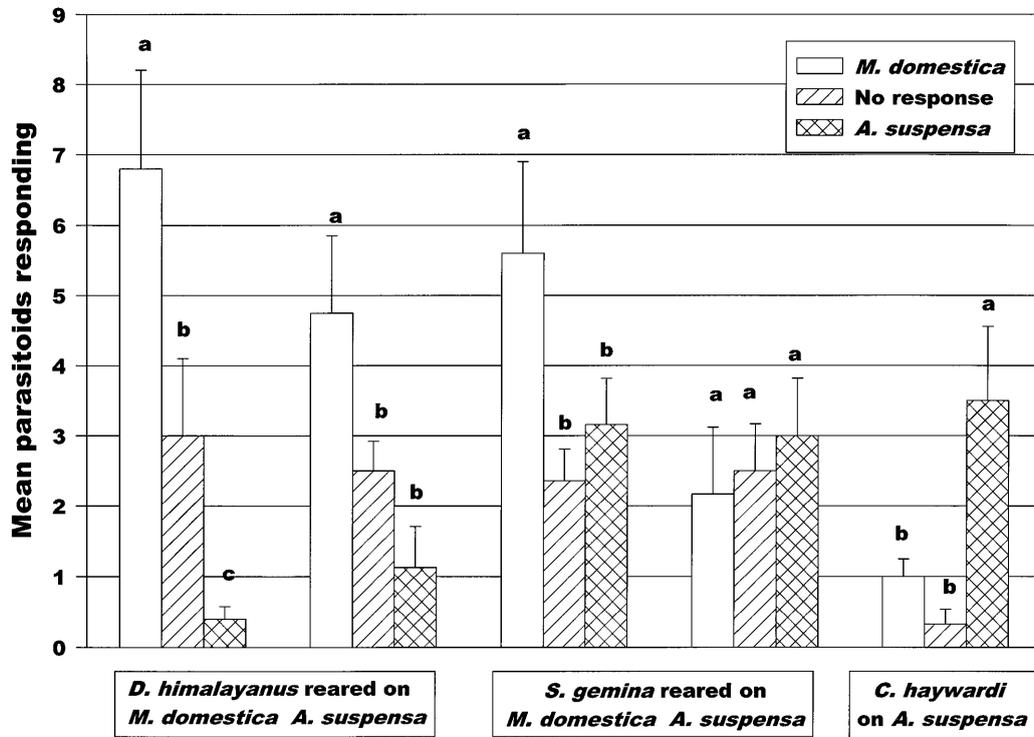


FIG. 4. The responses of female parasitoids in a bioassay device to the pupae of either the *Musca domestica* or the *Anastrepha suspensa*. Insects were raised either on *M. domestica* or on *A. suspensa*. No response refers to insects that did not move into collection chambers. Lines over bars represent standard errors and means sharing a letter are not significantly different (ANOVA/Waller-Duncan K-ratio *t* test).

both calypteratre and acalypterate families. The host range of *C. haywardi*, perhaps restricted to Tephritidae, may be narrower than those of other pupal parasitoids because it develops as an endoparasitoid. Inside its host, it may encounter an environment similar to that typically encountered by a koinobiont; an environment where the host can mount a immune system defense against an internal threat. Parasitoid adaptations to deal with specific internal morphologies and physiologies may lead to greater specialization (e.g., Godfray, 1994).

Generalist parasitoids sometimes learn to respond to cues from previous experiences with hosts in order to

focus their foraging on the most available prey (e.g., Turlings *et al.*, 1992). Only *S. gemina* had a significantly modified response to host pupae due to experience. Females raised on *A. suspensa* were not as attracted to the pupae of *M. domestica* as were those reared on *M. domestica*. Of the three parasitoid species examined in the olfactometer, only *C. haywardi* chose the pupae of the *A. suspensa* over those of *M. domestica*, further evidence of its specialization on Tephritidae.

A second aspect of host range is the ability to develop in previously parasitized hosts. *C. haywardi* oviposited indiscriminately in unparasitized pupae and those parasitized by the braconid, *D. longicaudata*. However,

TABLE 3

The Mean Numbers of Males of Various Parasitoid Species Responding to the Pupae of Either *Musca domestica* or *Anastrepha suspensa* or Remaining in a Bioassay Device

	Rearing host	Responding to <i>M. domestica</i>	SE	Responding to <i>A. suspensa</i>	SD	No response	SE
<i>D. himalayanus</i>	<i>M. domestica</i>	2.50	1.10	1.00	0.27	2.00	0.60
	<i>A. suspensa</i>	1.75	0.31	1.88	0.67	2.50	0.91
<i>S. gemina</i>	<i>M. domestica</i>	1.00	0.36	0.63	0.24	1.45	0.31
	<i>A. suspensa</i>	3.00	0.93	3.00	0.87	2.00	0.60
<i>C. haywardi</i>	<i>A. suspensa</i>	1.70	0.92	3.70	1.60	0.33	0.21

Note. Individuals of species other than *Coptera haywardi* were raised on either *M. domestica* or *A. suspensa* and tested separately to determine if rearing affected host preference. There was no significant preference for one or the other species or host in any of the species (ANOVA/Waller-Duncan K-ratio *t* test).

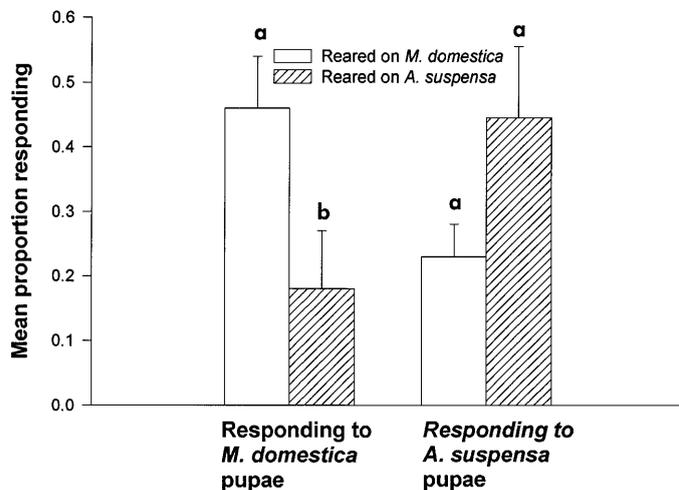


FIG. 5. The mean proportions of *Spalangia gemina* responding to either the species of pupae on which they were reared or to those of the alternative host. Means sharing letters are not significantly different (*t* test).

only ovipositions in unparasitized pupae resulted in the development of *C. haywardi*. This failure to develop is consistent with the lesser proportion of *A. suspensa* relative to *D. longicaudata* in lots of pupae exposed to *C. haywardi* compared to those that remained unexposed. *D. longicaudata* is originally from Malaysia, and though widespread and abundant in Mexico, it has only a brief history of coexistence with *C. haywardi* (Sivinski *et al.*, 1997). Hyperparasitism of a native braconid, such as the common *Doryctobracon areolatus* (Szepieligi), may be more successful.

There is a propensity for hyperparasitism by the African *Coptera* species brought to Hawaii, where large numbers of fruit fly natural enemies have been imported and considered for release (Clausen *et al.*, 1965). As pupal parasitoids accumulated in Hawaii, there was concern that they might hyperparasitize the braconid parasitoids that had emerged as the principal agents of biological control. Dresner (1954) examined the issue with three cultures (species?) of *Coptera* (= *Psilus*). All were capable of acting as hyperparasitoids. However, two of the three cultures appeared to avoid, or be less able to develop in, *B. dorsalis* pupae previously parasitized by *Biosteres* (= *Opius*) *vandenboschi* (Fullaway). Pemberton and Willard (1918) made more detailed observations of *C. silvestrii*. They found it to freely attack and develop in an array of braconids.

For many years, the risks of hyperparasitism and typically low levels of recovery after classical biological control releases limited enthusiasm concerning pupal parasitoids. However, recent developments in augmented parasitoid releases to control fruit fly pests have suggested a new role for pupal parasitoids (see Wong *et al.*, 1991; Sivinski *et al.*, 1996; Kazimirova and Vallo, 1992; Purcell, 1997). Large fruits can serve as refugia for tephritid larvae from many braconids who

are unable to reach larvae with their ovipositors (e.g., Sivinski, 1991; Gut and Brunner, 1994). When a substantial proportion of the host fruits are large, an expensive mass-rearing and release program could be compromised. However, pupal parasitoids (and egg parasitoids as well) are unaffected by large fruits.

Generalism, such as displayed by *D. himalayanus* and *S. gemina*, may result in parasitism of harmless or beneficial host species. In addition, augmented releases of generalists might fail to focus attacks on pests. A decline in the target species would result in increasing parasitism of nontarget species. This sort of undesirable parasitism might be a particular problem when flexible host searching behavior is modified by experience. Such modifications might cause a common nontarget host to be attacked at a greater than random rate. This is not to argue that available pupal parasitoids are inherently too generalized to ever be safely applied. For one thing, little is known of their abilities to disperse from agricultural areas. If dispersal is limited, they may pose a minimal threat to surrounding fly faunas.

However, a specialized tephritid pupal parasitoid might be an advantageous addition to augmented releases. *C. haywardi* has certain useful characteristics. While not restricted to any particular tephritid pest species, its host range appears to be narrower than that of the chalcidoid ectoparasitoids, and there is no indication at present of its being able to hyperparasitize Braconidae. It may also be possible to effectively mass rear. At this time, percentage of parasitism in colonies tends to be low, but this may improve. *Coptera occidentalis* (Muesbeck), a parasitoid recovered from North American *Rhagoletis* spp., parasitizes up to 80% of its factitious *C. capitata* hosts in the laboratory (Kazimirova and Vallo, 1993).

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REFERENCES

- Aluja, M. 1994. Bionomics and management of *Anastrepha*. *Annu. Rev. Entomol.* **39**, 155-178.
- Bai, G. M. 1990. Host-parasite relationship of *D. pachycerus* Masi (Hymenoptera: Chalcidae), with particular reference to its house fly control potential. In "Biocontrol of Arthropods Affecting Live-

- stock and Poultry" (D. A. Rutz and R. S. Patterson, Eds.), pp. 11–27. Westview Press, Boulder, CO.
- Baranowski, R., Glenn, H., and Sivinski, J. 1993. Biological control of the Caribbean fruit fly (Diptera: Tephritidae). *Fla. Entomol.* **76**, 245–251.
- Bess, H. A. 1961. Fruit fly parasites and their activities in Hawaii. *Proc. Haw. Entomol. Soc.* **17**, 367–378.
- Boller, E. 1966. Der einfluss naturlicher reduktionsfaktoren auf die kirschenfliegen *Rhagoletis cerasi* L. in der nordwestschweiz, unter besonderer berucksichtigung des puppenstadiums. *Schweiz. Landw. Forschung* **5**, 154–210.
- Boucek, Z. 1963. A taxonomic study in *Spalangia* latr. (Hymenoptera, Chalcidoidea). *Acta Entomol. Mus. Nat. Prauge* **35**, 429–512.
- Boucek, Z., and Narendran, T. 1981. Indian chalcid wasps (Hymenoptera) of the genus *Dirhinus* parasitic on synanthropic and other Diptera. *Syst. Entomol.* **6**, 229–251.
- Clausen, C. P., Clancy, D. W., and Chock, Q. C. 1965. "Biological Control of the Oriental Fruit Fly (*Dacus dorsalis* Hendel) and Other Fruit Flies in Hawaii," USDA Technical Bulletin 1322.
- Dresner, E. 1954. Observations on the biology and habits of pupal parasites of the Oriental fruit fly. *Proc. Haw. Entomol. Soc.* **15**, 299–310.
- Eskafi, F., and Kolbe, M. 1990. Predation on larval and pupal *Ceratitidis capitata* (Diptera: Tephritidae) by the ant *Solenopsis geminata* (Hymenoptera: Formicidae) and other predators in Guatemala. *Environ. Entomol.* **19**, 148–153.
- Foote, R. H., Blanc, F. L., and Norrbom, A. L. 1993. "Handbook of the Fruit Flies of America North of Mexico." Cornell Univ. Press, Ithaca.
- Godray, H. C. J. 1994. "Parasitoids: Behavioral and Evolutionary Ecology." Princeton Univ. Press, Princeton, NJ.
- Grissell, E., and Schauff, M. 1990. "A Handbook of the Families of Nearctic Chalcidoidea (Hymenoptera)." Entomol. Soc. Wash. Pub., Washington, DC.
- Gut, L., and Brunner, J. 1994. Parasitism of the apple maggot, *Rhagoletis pomonella*, infesting hawthorns in Washington. *Entomophaga* **39**, 41–49.
- Hagley, E. A. C., Biggs, A. R., Timbers, G. E., and Coutu-Sundy, J. 1993. Effect of the age of the puparium of the apple maggot, *Rhagoletis pomonella* (Walsh) on parasitism by *Phygadeuon wiesmanni* Sachtl. (Hymenoptera: Ichneumonidae). *Can. Entomol.* **125**, 721–724.
- Hawkins, B. A. 1994. "Pattern and Process in Host-Parasitoid Interactions." Cambridge Univ. Press, Cambridge, UK.
- Hogsette, J. A., Farkas, R., and Coler, R. R. 1994. Hymenopteran pupal parasites recovered from house fly and stable fly (Diptera: Muscidae) pupae collected on livestock and poultry facilities in northern and central Hungary. *Environ. Entomol.* **23**, 778–781.
- Kazimirova, M., and Vallo, 1992. Influences of larval density of Mediterranean fruit fly (*Ceratitidis capitata*, Diptera, Tephritidae) on parasitism by a pupal parasitoid, *Coptera occidentalis* (Hymenoptera, Proctotrupoidea, Diapriidae). *Acta Entomol. Bohemoslov.* **89**, 179–185.
- Krombein, K., Hurd, P., Smith, D., and Banks, B. 1979. "Catalog of Hymenoptera in America North of Mexico." Smithsonian Inst. Press, Washington, DC.
- Lopez, M. O. 1996. "Patrones de Parasitismo en Moscas de la Fruta del Genero *Anastrepha* (Diptera: Tephritidae), en Frutos Nativos (*Spondias mombin* L. y *Psidium guajava* L.) y Exotocos (*Magifera indica* L. y *Citrus sinensis* L.). Ingeniero Thesis, Univ. of Veracruz, Xalapa, Veracruz, Mexico.
- Muesebeck, C. 1980. "The Nearctic Parasitic Wasps of the Genera *Psilus* and *Coptera* Say (Hymenoptera. Proctotrupoidea, Diapriidae)." USDA Technical Bulletin 1617.
- Nixon, G. E. J. 1930. The Ethiopian representatives of the genus *Galesus* (Proctotrupoidea), with descriptions of new species. *Ann. Mag. Nat. Hist. Ser.* **10**, 399–414.
- Pemberton, C. E., and Willard, H. F. 1918. A contribution to the biology of fruit fly parasites in Hawaii. *J. Agric. Res.* **15**, 419–465.
- Purcell, M. 1997. Contribution of biological control to integrated pest management of tephritid fruit flies in sub-tropical and tropical regions. *Integr. Pest Manag. Rev.* in press.
- Rueda, L., and Axtell, R. 1985. "Guide to Common Species of Pupal Parasites (Hymenoptera: Pteromalidae) of House Fly and Other Muscid Flies Associated with Poultry and Livestock Manure. North Carolina Agricultural Research Series Technical Bulletin 278.
- SAS Institute, 1988. "SAS/STAT User's Guide." Cary, NC.
- Silvestri, F. 1914. "Report of an Expedition to Africa in Search of Natural Enemies of Fruit Flies (Trypaneidae). Territory of Hawaii Board of Agriculture and Forestry Bulletin 3.
- Sivinski, J. 1991. The influence of host fruit morphology on parasitism rates in the Caribbean fruit fly (*Anastrepha suspensa* (Loew)). *Entomophaga* **29**, 571–574.
- Sivinski, J. 1996. The past and potential of biological control of fruit flies. In "Fruit Fly Pests—A World Assessment of their Biology and Management" (B. McPherson and G. Steck, Eds.), pp. 369–375. St. Lucie Press, Delray Beach, FL.
- Sivinski, J., Calkins, C. O., Baranowski, R., Harris, D., Brambila, J., Daiz, J., Burns, R., Holler, T., and Dodson, G. 1996. Suppression of a Caribbean fruit fly (*Anastrepha suspensa* (Loew) Diptera: Tephritidae) population through augmented releases of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). *Biol. Contr.* **6**, 177–185.
- Sivinski, J. M., Aluja, and M. Lopez. 1997. The spatial and temporal distributions of parasitoids of Mexican *Anastrepha* species (Diptera: Tephritidae) within the canopies of fruit trees. *Ann. Entomol. Soc. Am.* **90**, 604–618.
- Thomas, D. B. 1993. Survivorship of the pupal stages of the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae) in an agricultural and nonagricultural situation. *J. Entomol. Sci.* **28**, 350–362.
- Thomas, D. B. 1995. Predation on the soil inhabiting stages of the Mexican fruit fly. *Southwest Entomol.* **20**, 68–71.
- Turlings, T., Wackers, F., Vet, L., Lewis, W., and Tumlinson, J. 1992. Learning of host location cues by hymenopterous parasitoids. In "Insect Learning: Ecological and Evolutionary Perspectives" (A. Lewis and D. Papaj, Eds.), pp. 51–78. Chapman and Hall, New York.
- Wong, A., and Wong, T. 1988. Predation of the Mediterranean fruit fly and the Oriental fruit fly (Diptera: Tephritidae) by the fire ant (Hymenoptera: Formicidae) in Hawaii. *Proc. Haw. Entomol. Soc.* **28**, 169–177.
- Wong, T., McInnis, D., Nishimoto, J., Ota, A., and Chang, V. 1984. Predation of the Mediterranean fruit fly (Diptera: Tephritidae) by the Argentine ant (Hymenoptera: Formicidae) in Hawaii. *J. Econ. Entomol.* **77**, 1454–1458.
- Wong, T., Ramadan, M., McInnis, D., Mochizuki, N., Nishito, J., and Herr, J. 1991. Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) to suppress a Mediterranean fruit fly population in Kula, Maui, Hawaii. *Biol. Contr.* **1**, 2–7.
- Zar, J. H. 1974. "Biostatistical Analysis." Prentice-Hall, Englewood Cliffs, NJ.