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Parasitization of melon fly (Diptera: Tephritidae) by *Fopius arisanus* and *Psyttalia fletcheri* (Hymenoptera: Braconidae) and the effect of fruit substrates on host preference by parasitoids

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Abstract

Fopius arisanus and *Psyttalia fletcheri* are egg and larval parasitoids, respectively, of tephritid fruit flies including the melon fly, *Bactrocera cucurbitae*. We investigated the consequences of parasitization when eggs or larvae of melon flies were exposed to *F. arisanus* or *P. fletcheri* alone, or to both in succession. In addition, we evaluated the effect of fruit substrates on host preference by gravid parasitoids. *F. arisanus* parasitized 45% of melon fly eggs in the cohort after a 24-h exposure period. The proportion of parasitized hosts increased 2-fold when melon fly larvae were exposed to *P. fletcheri* alone, or when host immatures were exposed sequentially to both parasitoids. While *P. fletcheri* could readily develop on melon fly, *F. arisanus* rarely produced progeny. Life table calculations indicated that parasitization by *F. arisanus* resulted in host kills of 38, 40, and 47% in egg, larval, and pupal stages of the melon fly, respectively, while parasitization by *P. fletcheri* resulted in larval and pupal mortalities of 24 and 79%, respectively. Sequential exposure of melon fly eggs to *F. arisanus*, then larvae to *P. fletcheri*, resulted in host kills of 52, 56, and 91% during the egg, larval and pupal stages, respectively. Parasitization by *F. arisanus*, *P. fletcheri*, or both, suppressed melon fly development by 2-, 5-, and 12-fold, respectively. Given a choice of fruit types, *F. arisanus* and *P. fletcheri* preferred melon fly in zucchini squash, *Cucurbita pepo*, over those in Japanese eggplant, *Solanum melongena*, Chinese bitter melon, *Momordica charantia*, Japanese cucumber, *Cucumis sativus*, and tomato, *Lycopersicon esculentum*. This study provides basic information needed for future work on single or multispecies releases of parasitoids for biological control of the melon fly.
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Keywords: Tephritidae; Melon fruit fly; *Bactrocera cucurbitae*; *Fopius arisanus*; *Psyttalia fletcheri*; Parasitoids; Life table; Host preference

1. Introduction

The melon fruit fly, *Bactrocera cucurbitae* (Coquillett), is a perennial pest of cultivated and feral bitter melon, *Momordica charantia* L., and a wide variety of other cucurbitaceous crops (Harris and Lee, 1989). In the absence of vegetables, *B. cucurbitae* occasionally infests less preferred fruit hosts such as papaya, *Carica papaya* L. (Liquido, 1991). After it was first discovered in Hawaii in 1896 (Back and Pemberton, 1917), attempts were undertaken to utilize natural enemies for biological control of the melon fly. Hence, a number of parasitoids, including *Fopius* (=*Biosteres*) *arisanus* (Sonan) (=*Opius oophilus* Fullaway) and *Psyttalia* (=*Opius*) *fletcheri* (Silvestri) were introduced into Hawaii (van Zwalunwenberg, 1947). These parasitoids became successfully established throughout the major island chain resulting in substantial reductions of fruit fly populations (Bess et al., 1961; Haramoto and Bess, 1970; Stark et al., 1991; Vargas et al., 1993; Wong and Ramadan, 1987).

Psyttalia fletcheri is a strict solitary parasitoid of melon fly larvae (Willard, 1920). *F. arisanus*, on the other hand, is primarily an egg parasitoid but is thought to also parasitize newly hatched or very young fruit fly larvae (Haramoto, 1957). Both parasitoids lay their eggs and complete development within the host. Subse-

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quently, adults emerge from the host puparia, usually a few days after eclosion of fruit flies from unparasitized puparia. Interestingly, despite a predilection for tephritid fruit fly eggs (Harris and Bautista, 1996; Vargas and Nishida, 1985), *F. arisanus* rarely develop fully on melon fly (Nishida and Haramoto, 1953).

There is variation in fruit fly parasitization by *F. arisanus* or *P. fletcheri* in different fruit substrates. The oriental fruit fly, *Bactrocera dorsalis* (Hendel), infesting guavas, *Psidium guajava* L., and false kamani, *Terminalia catappa* L., had higher rates of *F. arisanus* parasitization in the field than did oriental fruit fly infesting citrus fruits (Haramoto, 1957; Harris et al., 1988; Vargas et al., 1993). Bautista and Harris (1996) demonstrated that *F. arisanus* discriminated among fruit types infested with eggs of *B. dorsalis* or Mediterranean fruit fly, *Ceratitis capitata* (Weidemann). They found that ripe coffee berries, *Coffea arabica* L., were preferred for parasitization regardless of the species of fruit fly eggs inoculated in the fruits. On the other hand, *P. fletcheri* preferred melon fly larvae infesting fruits of wild apple, *Momordica balsamina* L., over those in cucumber, *Cucumis sativus* L. (Nishida, 1955; Willard, 1920). Moreover, *P. fletcheri* was not only attracted to fruits but also to other parts of the wild apple plant regardless of the presence of melon fly larvae (Nishida, 1956). In addition, natural fruit odors (Messing and Jang, 1992) as well as highly attractive volatiles given off by infested fruits (Leyva et al., 1991) seemed to influence the host preferences of fruit fly parasitoids.

Fopius arisanus and *P. fletcheri* were initially established in our laboratory in the mid-1980s from parasitoid collections obtained on Oahu, Hawaii. *F. arisanus* was recovered from parasitized puparia of the oriental fruit fly, *B. dorsalis* (Hendel), infesting papaya, *C. papaya* L., in Waikane (Harris and Okamoto, 1991) while *P. fletcheri* was collected from melon fly infesting zucchini squash, *Cucurbita pepo* L., in Waimanalo (Wong and Ramadan, 1992). Subsequently, rearing methods were developed that facilitated mass production of these parasitoids (Bautista et al., 1999, 2000; Wong and Ramadan, 1992). Recently, an Integrated Pest Management (IPM) program was undertaken in Hawaii for area-wide suppression of tephritid fruit flies. A package of fruit fly control technologies was implemented in Kamuela, on the island of Hawaii, with augmentative biological control as a component strategy. Targeting melon fly initially, the larval parasitoid *P. fletcheri* was used for augmentative releases in the demonstration site. Commencing in April 2002, weekly releases of *P. fletcheri* were undertaken to achieve a high parasitoid to host ratio in order to suppress the melon fly population. However, as we progress in our IPM program, releases of the egg parasitoid *F. arisanus* concurrently with *P. fletcheri* may be an option in our biocontrol strategy. Although no serious adversity has yet been documented from multispecies releases of parasitoids (van den Bosch, 1971), the outcome of a biological control program is predicated on how different parasitoids might interact with one another (Ehler and Hall, 1982; Zwolfer et al., 1976). To date, no information is available on consequences of interaction between *F. arisanus* and *P. fletcheri*.

This study was undertaken to quantify the impact of *F. arisanus*, *P. fletcheri* or both on survival and mortality of the melon fly with life table schedules, determine effects of multiparasitism on progeny survival of parasitoids, and evaluate the influence of fruit substrates on host preference of parasitoids.

2. Materials and methods

2.1. Source of parasitoids and host fruit fly

Fopius arisanus and *P. fletcheri*, currently into the 188th and 234th generation, respectively, were obtained from laboratory colonies that had been in propagation since the 1980s. *F. arisanus* and *P. fletcheri* were propagated on the oriental fruit fly, *B. dorsalis* (Hendel) and the melon fly, *B. cucurbitae* (Coq.), respectively, according to mass-production methods developed earlier (Bautista et al., 1999, 2000; Wong and Ramadan, 1992). The eggs and larvae of *B. cucurbitae* were obtained from colonized fruit flies propagated as larvae on a semi-synthetic wheat diet formulation (Spencer and Fujita, 1998; Tanaka et al., 1969; Vargas, 1989).

Unless otherwise noted, experiments were conducted in the laboratory with temperatures of 21–24 °C (mean = 22.4 ± 1.2 °C), 55–73% (mean = 63 ± 5%) relative humidity and 12D:12L photo period. Spun honey (Sioux Honey, Sioux City, IA) and water, accessed from a wet cotton dental wick (Tidi Products, Rialto, CA) inserted into a 9-cm diameter water-filled plastic reservoir, were provided to parasitoids.

2.2. Test A. Parasitization as host mortality factor

To quantify the mortality of *B. cucurbitae* in each stage of development, 3 patterns of fruit fly exposure to *F. arisanus* and *P. fletcheri* were undertaken. Assays consisted of exposing melon fly eggs and/or larvae to *F. arisanus* or *P. fletcheri*, respectively, or to both parasitoids in succession, i.e., eggs first to *F. arisanus*, then larvae that developed from same cohort to *P. fletcheri*. Each parasitoid was given access to host immatures for 24 h to provide gravid parasitoids sufficient time to encounter more hosts. Female parasitoids with no egg-laying experience (=naive) were used in each test. During the experiment, fruit fly eggs or larvae were sampled to determine the level of parasitization. Hence, the number of host eggs that was used in each treatment was

in excess of 2000 to compensate for hosts that were subsequently sampled. Test was replicated 5 times.

2.3. Pattern 1 (designated as Fa)

Using the moistened tip of a camel's hair brush, newly laid eggs (1–3 h old) of *B. cucurbitae* were counted in sets of 2025 then seeded on gelled agar inside a modified oviposition dish (Bautista et al., 1999). The dish was exposed to 500 females of *F. arisanus* (8–10-d-old) in a manner described previously (Bautista et al., 1999). Subsequently, the agar with the host eggs was scooped from the oviposition dish with a spatula. A cohort of 25 eggs was sampled and dissected individually under a stereoscope to check for parasitization. The agar with the remaining eggs was placed on ≈800 g of wheat diet (Tanaka et al., 1969) inside a mesh-covered plastic container (24 × 13 × 5 cm) where the host larvae developed. Matured larvae were separated from the wheat diet by emptying the rearing substrate into a mesh sieve and then shooting it with a stream of water. The larvae were placed in a petri dish half-filled with water, tallied, then transferred to a plastic container provisioned with vermiculite (Strong-lite, Pine Bluff, AR) where the host larvae pupated. Nine to 10 days later, pupae were sifted from the pupating medium using a mesh sieve (1 mm^2). The pupae were held in a mesh-covered plastic container until eclosion of fruit flies and parasitoids. Emerged parasitoids and fruit flies as well as uneclosed (dead) puparia were counted.

2.4. Pattern 2 (designated as Pf)

Test hosts were prepared in the same manner described above except that fruit fly eggs were allowed to develop to 4-d-old larvae (late 2nd instars) before they were exposed to *P. fletcheri*. The host larvae were recovered from the rearing substrate, counted, then combined with freshly mixed wheat diet and packed inside a modified oviposition dish (Bautista et al., 2000). Two dishes, containing 450–500 larvae each, were exposed simultaneously to 500 females of *P. fletcheri* (5–6-d-old) in a cubical screen cage (26 × 26 × 26 cm) according to methods described previously (Bautista et al., 2000). Subsequently, the contents of the 2 dishes were retrieved, pooled, and then placed in a rearing cup from which 25 larvae were picked at random for dissection. As in the preceding test, the remaining larvae were processed until pupation, and then held in a plastic container until eclosion of parasitoids and fruit flies. Data on insect emergence and dead puparia were recorded.

2.5. Pattern 3 (designated as Fa + Pf)

The rearing procedures described in preceding sections (Patterns 1 and 2) were followed to facilitate sequential exposure of melon fly to the 2 parasitoids. Accordingly, host eggs were exposed first to *F. arisanus* and thereafter as 4-d-old larvae to *P. fletcheri*. A set of 25 larvae was later sampled for dissection. Host larvae that contained the immatures of a parasitoid species or those found with immatures of both parasitoids were recorded to quantify the levels of single or multiparasitism. As in preceding tests, remaining hosts were processed and reared until emergence of fruit flies and parasitoids. Eclosed parasitoids were identified by species according to taxonomic keys by Beardsley (1961) and Wharton and Gilstrap (1983).

2.6. Unexposed host (Control)

For purposes of comparison, cohorts of melon fly eggs that were left unexposed to parasitoids were reared concurrently with above treatments. An equal number of melon fly eggs (2025) was seeded on gelled agar and reared as larvae on the same wheat diet formulation. When larvae matured, 25 individuals were likewise sampled then discarded. Remaining hosts were processed and reared until pupation and emergence of fruit flies. Emerged flies and dead pupae were recorded.

2.7. Life table statistics

The survival and mortality data obtained in preceding tests were used to generate life table entries for the melon fly. Calculation of life table entries was corrected with average natural egg hatch rates determined from host cohorts sampled prior to the tests. Formulas by Morris and Miller (1954) and Vargas and Nishida (1980) were used in the calculations. Percent of host kill by either parasitoid or both was calculated using the formula $(X - Y) \div X$ by Abbott (1925), where X is the proportion of hosts surviving in the control, Y is the proportion of hosts that survived in parasitized treatments, and $(X - Y)$ is the proportion of hosts assumed killed or parasitized by either *F. arisanus*, *P. fletcheri*, or both.

2.8. Test B. Effect of fruit substrates on host preference by parasitoids

Five fruit types were assayed to determine the host preference of *F. arisanus* and *P. fletcheri* for *B. cucurbitae* eggs or larvae, respectively. Test fruits included green zucchini squash, *C. pepo* L.; Japanese eggplant (long purple), *Solanum melongena* L.; Chinese bitter melon, *M. charantia* L.; Japanese cucumber, *C. sativus* L.; and garden tomato, *Lycopersicon esculentum* Miller. Assays were conducted outside the laboratory using an outdoor screen cage (183 × 152 × 152 cm). Ambient temperatures of 26–34 °C (mean = 30 ± 4 °C) and relative humidities of 41–67% (mean = 50.7 ± 9%) were

recorded during the tests. The fruits were arranged inside the cage in a completely randomized design (CRD) determined by drawn lots.

2.9. Experimental procedure

Four whole fruits of each type were inoculated with freshly laid eggs (1–3 h old) of *B. cucurbitae*. Each fruit was punctured with 10 holes using the blunt end of a camel brush (no. 10/128) into which 30 eggs were inserted in each hole for a total of 300 eggs per fruit. Each fruit was enclosed with garden netting (1.5×1.5 cm mesh) that was shaped into a bag by securing the open ends of the net with masking tape (Harris and Bautista, 1994). A piece of stiff wire, with one end bent into a hook, was inserted into the net to facilitate hanging of fruits inside the cage.

The newly inoculated fruits were suspended inside the outdoor cage into which 1000 *F. arisanus* females (8–10-d-old) were released. One hour later, and also before the test was terminated after a total exposure time of 5 h, the number of parasitoids on the fruit of each type was counted. Parasitoid counts were pooled per fruit type then averaged between the initial (1st) and final (2nd) tallies since no significant differences were found between observations. Counts included females that were probing the fruit surface or those attempting to oviposit or in the act of egg-laying. In order to determine the rate of parasitization, 4 sets of 100 eggs each per fruit were sampled from each type, and then dissected individually under a stereoscope. Mean counts of host-searching parasitoids and percentage parasitization in each fruit type were generated from 5 replications.

Fruits intended for exposure to *P. fletcheri* were inoculated with melon fly eggs according to procedures described above. Inoculated fruits were placed in a 28.5×36-cm wooden tray (with mesh screen bottom) that fit snugly inside a fiber glass container which served as holding box. The tray was lined with pieces of paper towel that absorbed the juice given off by the fruits as a result of deterioration from fruit fly larval feeding. The holding box with the fruits was held in a room (temperature: 26–27 °C; relative humidity: 55–60 %) where host eggs hatched and larvae developed to 2nd instars (4-d-old). Subsequently, the deteriorating fruits were suspended inside the outdoor cage following a CRD design. A cohort of 1000 *P. fletcheri* females (5–6-d-old) were released into the cage and allowed access to infested fruits for 5 h. As in the preceding procedure, the initial and final tally of parasitoids associated with each fruit type were recorded then averaged since variations between observations were not significant. At the end of the test, fruits were retrieved then 25 larvae were sampled from each fruit for a total of 100 larvae per fruit type. With a pair of forceps, the larvae were dissected individually under a stereoscope to determine parasitization. Data on mean parasitoid counts and percent larval parasitization were generated from 5 replications.

2.10. Data analysis

Percentages were transformed to arcsine square root of proportion and actual counts to square root $X + 0.5$ to homogenize variance before data analysis. Transformed data were analyzed by 1-way ANOVA. Means were separated by Tukey–Kramer Honestly Significant Difference (HSD) method at $P = 0.05$. Untransformed means (±SEM) were used in presentation of results.

3. Results

3.1. Test A. Parasitization as host mortality factor

A life table for melon fly (Table 1), with parasitization by *F. arisanus*, *P. fletcheri*, or both, as mortality factors, was generated from replicated trials ($N = 5$).

Of 2000 melon fly eggs exposed initially to *F. arisanus* (*Fa*), <20% survived to the pupal stage. In *Pf*, where half as many host eggs completed development to late 2nd instars (1072 larvae developed from 2000 eggs used) before they were exposed to *P. fletcheri*, only 9% of these larvae successfully pupated. Sequential exposure of host eggs then larvae to both parasitoids (treatment $Fa + Pf$) reduced pupal yield of melon fly to as little as 4%. On the other hand, >40% of the host eggs left unexposed to parasitoids (Control) developed into pupae. Our life table calculations by Abbott (1925) indicated that the proportionate kills exerted by the test parasitoids on the host pupae corresponded to 47% in *Fa*, 79% in *Pf*, and 91% in $Fa + Pf$.

Table 2 shows the other variables that were quantified to further elucidate the potential consequences of single or multiple parasitization of melon fly. While *F. arisanus* alone parasitized only half as many host eggs in *Fa*, parasitization of melon fly larvae by *P. fletcheri* alone or sequential attacks of melon fly eggs by *F. arisanus* and then larvae by *P. fletcheri* resulted in significantly higher rates of parasitization (between 90 and 93%) in *Pf* or $Fa + Pf$, respectively ($F = 8.54$; $df = 2, 8$; $P = 0.02$). In treatment $Fa + Pf$, at least one-third of the host larvae were multiparasitized (Fig. 1). Interestingly, the latent mortality of fruit fly pupae (dead puparia from unknown causes) was significantly different among the treatments ($F = 33.47$; $df = 3, 19$; $F < 0.001$). Although *Fa* had the lowest percentage of uneclosed puparia compared to those of *Pf* or $Fa + Pf$, the mortality rates in parasitized treatments were all significantly higher than what was obtained in the control (5%). Our findings further indicated that although melon fly is a natural host of *P. fletcheri*, this was not the case with the egg parasitoid *F. arisanus* (Table 2). Where host eggs

Table 1
Life table generated for melon fruit fly, *B. cucurbitae*, at 22.4 ± 1.2 °C and 63 ± 5% relative humidity with parasitization by *F. arisanus* and *P. fletcheri* as host mortality factor ($N = 5$)

Age interval (host stage) X	Number alive at beginning of X lx				Number dying during X dx				dx as percent of lx $100qx$				Cumulative percent surviving $100rx$				Percent kill exerted on host by parasitoids		
	Unexposed (Control)	Exposed			Unexposed (Control)	Exposed			Unexposed (Control)	Exposed			Unexposed (Control)	Exposed					
		Fa[a]	*Pf*	*Fa + Pf*		*Fa*	*Pf*	*Fa + Pf*		*Fa*	*Pf*	*Fa + Pf*		*Fa*	*Pf*	*Fa + Pf*	*Fa*	*Pf*	*Fa + Pf*
Egg	2000	2000	2000	2000	838	1283	928	1447	42	64	46	72	58	36	54	28	38	–	52
Larva	1162	717	1072	553	267	174	401	161	23	24	37	29	45	27	34	20	40	24	56
Pupa	895	543	671	392	43	91	489	320	5	17	73	82	43	23	9	4	47	79	91
Adult	852	452	182	72															

[a] *Fa* and *Pf* are abbreviations for *Fopius arisanus* and *Psyttalia fletcheri*, respectively.

were exposed to *F. arisanus* alone (*Fa*), or where *F. arisanus* was used in combination with *P. fletcheri* ($Fa + Pf$), very few progeny of *F. arisanus* were subsequently recovered ($F = 15.06$; $df = 3, 19$; $P < 0.001$). On the other hand, *P. fletcheri*, produced as many as 231 and 160 offspring in treatments *Pf* and $Fa + Pf$, respectively.

3.2. Test B. Effect of fruit substrates on host preference by parasitoids

The five fruit types inoculated with melon fly eggs, were equally as attractive to the host searching *F. arisanus* ($F = 0.41$; $df = 4, 24$; $P = 0.80$) (Table 3). Within an hour after the test commenced, about half of 1000 *F. arisanus* released into the outdoor cage had landed on the fruits and began probing for the hosts. During the test, we observed that although bitter melon, eggplant, and zucchini squash were generally attractive to *F. arisanus*, they were not necessarily more enticing than either cucumber or tomato. Nevertheless, although the mean number of fruit-responding females was not significantly different among fruit types, this observation did not translate into similar levels of parasitization across the treatments. Instead, we found that the host eggs in zucchini were more heavily parasitized than those in the other fruit types ($F = 9.79$; $df = 4, 24$; $F = 0.0001$). The proportion of melon fly eggs that was parasitized in zucchini was 2–5 times higher than that for the other assayed fruit types.

Psyttalia fletcheri was generally attracted to all five fruit types that had deteriorated as a result of feeding by melon fly larvae (Table 3). Nevertheless, *P. fletcheri* preferred certain fruit types over others when simultaneously presented hosts in an outdoor cage ($F = 15.81$; $df = 4, 14$; $P = 0.0003$). Cucumber was as attractive as zucchini while tomato and eggplant were the least preferred and statistically indistinguishable. Again, our results on the level of melon fly parasitization were not consistent with the mean number of female *P. fletcheri* visiting each fruit type (Table 3). We found that the host larvae in zucchini were heavily parasitized compared to those infesting the other fruit types ($F = 9.17$; $df = 4, 14$; $P = 0.0002$). Although cucumber was as attractive as zucchini to the host searching females, the host larvae infesting the former were not as intensely parasitized as the latter. In fact, the proportion of parasitized larvae in cucumber was 4–5 times lower than that in zucchini and similar to that in the other fruit types.

4. Discussion

The survival and host mortality schedules presented in the life table indicated that parasitization by either or

Table 2
Percent parasitization and pupal mortality of melon fly, *B. cucurbitae* (Coq.), and progeny yields of *F. arisanus* and *P. fletcheri* resulting from exposure of fruit fly eggs or larvae to either parasitoid alone, respectively, or to both in sequence ($N = 5$)

Treatment	Sequence of host exposure to parasitoids	Percent ± SEM host parasitization[a]	Percent ± SEM unemerged puparia[a]	Mean ± SEM emerged parasitoids[a]
Fopius arisanus (*Fa*)	Alone	44.5 ± 6 a	17.5 ± 3.7 a	2 ± 0.9 a
Psyttalia fletcheri (*Pf*)	Alone	90.2 ± 6 b	35.6 ± 12.8 b	231 ± 48 b
Fopius arisanus	First			2.4 ± 1.5 a
+ *Psyttalia fletcheri* (*Fa* + *Pf*)	Second	93.3 ± 9.4 b	48.3 ± 7.3 b	160 ± 33 b
Melon fly	(Unexposed)	—	4.5 ± 2.3 c	—

[a] Means (±SEM) within a column followed by same letter are not significantly different [$P > 0.05$, Tukey's Honestly Significant Difference method (HSD)].

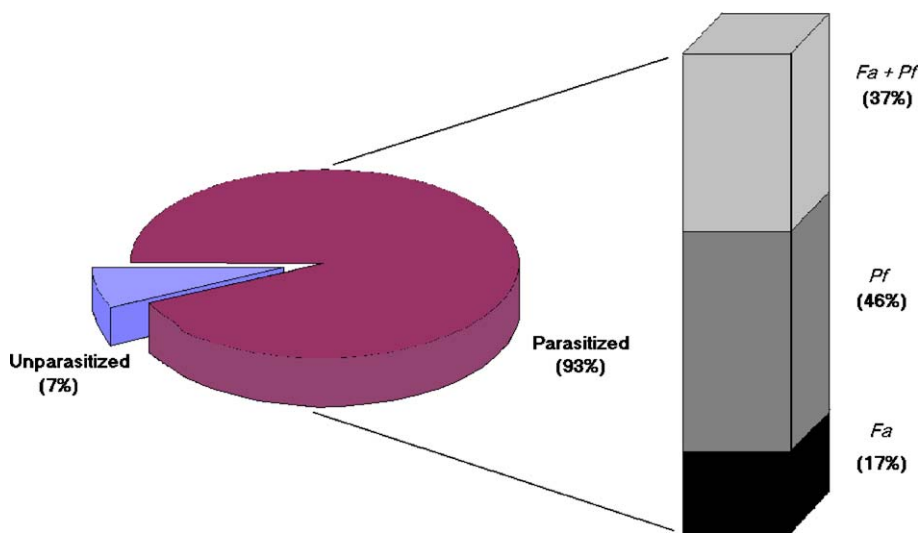

Fig. 1. Species composition of parasitoid immatures in multiparasitized *B. cucurbitae* larvae (treatment *Fa* + *Pf*). *Fa* and *Pf* are abbreviations for *F. arisanus* and *P. fletcheri*, respectively. Labels adjacent to bar graph indicate the species and proportions of parasitoid immatures dissected in parasitized fruit fly larvae.

Table 3
Effect of fruit types on host-searching parasitoids and subsequent parasitization of the melon fly, *B. cucurbitae* (Coq.) by the egg parasitoid *F. arisanus* and larval parasitoid *P. fletcheri* ($N = 5$)

Fruit types	Mean female parasitoids per fruit type[a]		Mean percent parasitization of melon fly[a]	
	F. arisanus	*P. fletcheri*	*F. arisanus*	*P. fletcheri*
Zucchini	38 ± 17 a	55 ± 4 a	58 ± 3 a	32 ± 3 a
Bitter melon	41 ± 23 a	18 ± 4 bc	19 ± 5 b	10 ± 9 b
Cucumber	27 ± 8 a	28 ± 3 ab	19 ± 7 b	7 ± 2 b
Eggplant	39 ± 11 a	9 ± 8 c	26 ± 10 b	4 ± 3 b
Tomato	27 ± 7 a	6 ± 2 c	10 ± 7 b	2 ± 1 b

[a] Means (±SEM) within a column followed by the same letter are not significantly different $P > 0.05$, Tukey's Honestly Significant Difference method (HSD).

both parasitoids resulted in mortality of *B. cucurbitae* in each stage of pre-imago development. In particular, the levels of host pupal mortalities attributed to the test parasitoids indicated how each species, alone or in combination, resulted in a substantial reduction of potential flies that could have otherwise developed into reproducing adults. Among parasitized treatments, *F. arisanus* in combination with *P. fletcheri* (*Fa* + *Pf*) had exerted the highest pupal kill (>90%), an improvement of ≈12 and 44% over those obtained from parasitization by *P. fletcheri* (*Pf*) or *F. arisanus* (*Fa*) alone, respectively. Apparently, because *F. arisanus* and *P. fletcheri* had inherent abilities to exploit different life stages of the host, their concerted action resulted in a compounding effect that proved costly to cohort survival of developing flies. Consequently, one cannot overlook the potential benefit that may accrue from concurrent releases of both parasitoids for suppression of melon fly. We do not have

field data to ascertain if our findings would hold true in natural habitats but the multispecies approach of parasitoid release cannot be overlooked as an alternative to existing single-species strategies (Huffaker et al., 1972). There may be economic constraints associated with the costs of rearing *F. arisanus* and *P. fletcheri*, but recent advances have resulted in their propagation at minimal costs with fewer personnel-hr and less space requirements (Bautista et al., 1999, 2000). Hopefully, such developments could help alleviate concerns about the costs of parasitoid production.

Gravid *F. arisanus* does not discriminate among tephritid fruit fly eggs, hence it would readily oviposit inside the eggs of melon fly if presented the opportunity (Harris and Bautista, 1996). The proportion of host eggs that did not survive after exposure to *F. arisanus* ($d_x = 64\%$ in life table) was >20% higher than the natural mortality that occurred in the unexposed eggs (Control). This finding substantiated an earlier speculation about the 'hidden' mortality associated with oviposition puncture by *F. arisanus* that cause direct kill of fruit fly eggs (Bautista and Harris, 1997; Bess et al., 1961; Harris and Bautista, 2001). Among several factors mentioned were injury to host eggs that may result from trauma caused by the drilling movements of ovipositors through the chorion (Lawrence et al., 2000), introduction of toxic substances or microbial pathogen into the oviposition puncture, or corking-off of lesions (Haramoto, 1957; Kaya and Nishida, 1968; Newell and Rathburn, 1951). In view of *F. arisanus*' inherent ability to kill fruit fly eggs or effect mortality during latent stages of host development (Harris and Bautista, 2001; Nishida and Haramoto, 1953), one cannot underestimate the value of this parasitoid when utilized in a biological control program against melon fly. We must emphasize that the actual effectiveness of *F. arisanus* against melon fly is underestimated because calculations of field parasitization are based largely on the number of parasitoids that eclosed from fruit fly puparia rather than the actual number of host eggs destroyed or parasitized (Bautista and Harris, 1996; Kaya and Nishida, 1968).

Unlike *P. fletcheri* that attacks larvae and breeds freely on *B. cucurbitae* (Wong and Ramadan, 1992), *F. arisanus* rarely developed or reproduced on melon fly (Nishida and Haramoto, 1953). Although our dissection data showed that *F. arisanus* parasitized about half of melon fly eggs exposed in *Fa*, only 2 total progeny successfully developed into adults. Likewise, in treatment $Fa + Pf$ where at least one-third of the host larvae were multiparasitized, the presence of *P. fletcheri* did not improve nor favor progeny development of *F. arisanus* in the same manner that was reported for *Tetrastichus giffardianus* (Silv.), a eulophid parasitoid of Mediterranean fruit fly, *C. capitata* Weidemann (Pemberton and Willard, 1918). These workers found that *T. giffardianus* completed development on melon fly only when host larva was likewise parasitized by *P. fletcheri*. Although only a few *F. arisanus* reached the adult stage, these individuals may have expressed some tolerance to the immune response of the host larvae (Nishida and Haramoto, 1953). Still, it may be difficult, if not impossible, to rear or establish a laboratory colony of *F. arisanus* on melon fly (E.J.H., unpublished data).

We encountered 50–70% parasitized larvae that contained encapsulated eggs or larvae of *F. arisanus*. The same level of encapsulation was observed in the combination treatment $Fa + Pf$ where 17 and <40% of larval hosts either harbored the immatures of *F. arisanus* alone or those of *F. arisanus* and *P. fletcheri*, respectively. In all these cases, the eggs or larvae of *F. arisanus* were either partly covered or completely enveloped with host tissues (phagocytosed) but nonetheless floating freely inside the host body cavity. Our findings substantiated the work of other investigators who reported that *B. cucurbitae* was resistant to invasion by *F. arisanus* (Nishida and Haramoto, 1953). It is not understood why *F. arisanus* could not circumvent the physiological barrier expressed by the melon fly. Lawrence et al. (2000), in their quest to establish a colony of *F. arisanus* on the Caribbean fly, *Anastrepha suspensa* (Loew), discovered calyx cells in *F. arisanus* ovaries that secrete protein-laden fluids which were introduced into the host eggs during parasitization. While similar structures and secretions in other braconid parasitoids inhibit encapsulation of parasitoid eggs by the host (Webb and Luckhart, 1996), the physiological role of the calyx fluid in *F. arisanus*, if any, remains unknown. The inherent resistance manifested by melon fly against *F. arisanus* is not a unique event. The melon fly had expressed the same phenomenon in response to other parasitoids, including, *Tetrastichoides* sp. and *Apodesma* sp. (Kotinsky, 1909), *Biosteres vandenboschi*, *Psyttalia incisi*, and *Diachasmimorpha longicaudata* (Nishida and Haramoto, 1953). Moreover, similar attempts to rear parasitoids of the Mediterranean fruit fly, *C. capitata*, namely, *Dichasmimorpha tryoni* Cam., *D. fullawayi* Silv., *Opius humilis* Silv. and *T. giffardianus* Silv., on melon fly were likewise unsuccessful (Pemberton and Willard, 1918).

Despite differences of fruit size and skin color (Leyva et al., 1991), *F. arisanus* sought all the fruits indiscriminately and did not seem to favor certain fruit types while searching for the host eggs. Gravid females landed, examined, and probed the test fruits at random for the presence of potential hosts, hence the frenzy of egg-laying activities associated with their visit. This includes probing fruit surface using antennae as feelers; bending the abdomen at an angle to orient ovipositor over egg inoculation holes; insertion of ovipositor accompanied by pumping movements, etc.

In the case of *P. fletcheri*, however, the host-searching females preferred certain fruit types over others. Except that all the fruits had deteriorated as a result of voracious

feeding by larvae, zucchini squash, and cucumber attracted more *P. fletcheri* than either tomato or eggplant. Decaying fruits are highly attractive to fruit fly larval parasitoids (Greany et al., 1977b), hence the choice made by *P. fletcheri* in favor of zucchini squash and cucumber may have resulted from more enticing chemical volatiles that were given off by these fruits (Leyva et al., 1991). Nevertheless, the non-random pattern of host search displayed by *F. arisanus* or the order of preference exhibited by *P. fletcheri* among fruit types did not necessarily correspond with the intensity of parasitization inflicted on the hosts. Among the 5 fruit types assayed, *F. arisanus* and *P. fletcheri* heavily parasitized melon fly eggs or larvae that occurred only in zucchini squash, respectively. This observation indicated that the mere presence of *F. arisanus* or *P. fletcheri* (at times accompanied by egg-laying movements) on a fruit did not necessarily constitute successful parasitization of the hosts (Bautista and Harris, 1996). Apparently, a gravid female may alight, explore, and probe for the hosts in a fruit but would hesitate to lay an egg if potential hosts were determined unsuitable for oviposition. Considering that sense organs (chemo- and mechano-receptors) are present in the ovipositor, it is not surprising that a female parasitoid is able to discriminate between or among hosts (Greany et al., 1977a) or orient to them acoustically, as may be the case with larval parasitoids (Lawrence, 1981; Nishida, 1956). Interestingly, the choice made by *F. arisanus* and *P. fletcheri* resulted in higher host parasitization in zucchini alone, one of the most susceptible fruit substrates and highly preferred by melon fly (J. DeFrank, unpublished data). Parasitoid preference for certain fruits may not be fully understood but it could be an important factor to consider during releases of parasitoids in the natural habitat.

Our study demonstrated that (i) parasitization by *F. arisanus* or *P. fletcheri* alone could effectively suppress and substantially reduce development of melon fly; (ii) level of control exerted on developing flies was considerably higher than anticipated when both parasitoids sequentially attacked the host immatures in concert; (iii) direct kill or latent host mortality associated with female *F. arisanus* oviposition had a substantial impact on melon fly development, although this parasitoid produced very few progeny on melon fly; and (iv) a preference for hosts infesting certain fruit types could play a definitive role in enhancing the effectiveness of parasitoids when released in a diverse habitat. This information should serve as a basis for the use of *F. arisanus* and *P. fletcheri* to suppress melon fly populations.

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