Egg-larval opiine parasitoids (Hym., Braconidae) of tephritid fruit fly pests do not attack the flowerhead-feeder *Trupanea dubautiae* (Dipt., Tephritidae)

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Abstract: We investigated the potential impact of three opiine tephritid fruit fly parasitoids: *Fopius arisanus* (Sonan), *Fopius caudatus* (Szépligeti) and *Fopius ceratitivorus* Wharton, on the non-target native Hawaiian tephritid, *Trupanea dubautiae* (Bryan), infesting flowerheads of the endemic Asteraceae shrub *Dubautia raillardioides* Hillebrand. The three species are the only known opiine fruit fly parasitoids that attack host eggs (but occasionally attack first instars). *F. arisanus*, which originated in southeast Asia, is now widely established in Hawaii and elsewhere in the world, while the other two are African species currently in quarantine in Hawaii. In the laboratory, field-collected flowerheads of *D. raillardioides* containing *T. dubautiae* eggs and first instar larvae were exposed to naïve female wasps of each of the three *Fopius* species in the absence (no-choice test) or presence (choice test) of papaya fruit infested with eggs of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), the parasitoids' normal host. All three *Fopius* species visited the papaya fruit much more often than the flowerheads, and displayed normal ovipositional responses (probing and stinging) on the fruit in the choice test. None of the three parasitoid species showed ovipositional responses to flowerheads in either choice or no-choice tests. As a result, not a single *T. dubautiae* egg or larva was attacked by any of the three parasitoids, indicating that these parasitoids of pest tephritids are unable to recognize the microhabitats of flowerhead-feeding tephritids. The results suggest minimal risk of non-target impact in these biological control agents.

Key words: *Fopius arisanus*, *Fopius caudatus*, *Fopius ceratitivorus*, *Trupanea dubautiae*, biological control, non-target test, parasitoids, tephritids

1 Introduction

Concerns regarding non-target impacts of introduced natural enemies have resulted in a tightening of regulations against classical biological control (MESSING, 1999). In particular, classical biological control in Hawaii has been subject to increasing scrutiny and debate because of the highly sensitive island fauna and flora (FUNASAKI et al., 1988; HOWARTH, 1991). Several authors have raised concerns about the impact of introduced fruit fly parasitoids on non-target tephritids in Hawaii (DUAN et al., 1996; DUAN and MESSING, 1997, 1998; DUAN et al., 1998).

More than 30 hymenopteran parasitoids have been introduced into Hawaii for the control of pest tephritids during the last century (WHARTON, 1989). Successful establishment of opiine braconids such as *Fopius arisanus* (Sonan), *Fopius vandenboschi* (Fullaway), *Diachasmimorpha longicaudata* (Ashmead), *Diachasmimorpha tryoni* (Cameron), *Psytalia incisi* (Silvestri), and *Psytalia fletcheri* (Silvestri), has resulted in significant suppression of pest tephritids (WHARTON, 1989). *F. arisanus* is the only extant species attacking host eggs. *Fopius vandenboschi* oviposits preferentially

in early larval instars of hosts (RAMADAN, 2004), while the others prefer to attack older larval hosts (WHARTON, 1989). At present, *F. arisanus* is the dominant parasitoid in Hawaii because of its intrinsic competitive superiority against all extant larval fruit fly parasitoids (WANG et al., 2003).

The relative success of *F. arisanus* in the Hawaiian islands has stimulated exploration for other egg-attacking parasitoids. Egg-attacking fruit fly parasitoids pose several advantages over larval parasitoids as biological control agents. For example, they attack hosts at the earliest stage when they are accessible near the fruit surface. Once fly larvae disperse into fruit tissues, especially in large fruits, larval parasitoids may be unable to locate the hosts. Therefore, introduction of effective egg-larval parasitoids is the preferred approach in Hawaii and elsewhere in the world.

Two additional egg-attacking tephritid fruit fly parasitoids from east Africa, *Fopius ceratitivorus* Wharton and *Fopius caudatus* (Szépligeti) have recently been introduced into quarantine in Guatemala (LOPEZ et al., 2003) and via Guatemala into Hawaii

(BOKONON-GANTA et al., 2004). *F. ceratitivorus* is a newly discovered species (WHARTON, 1999). Quarantine tests showed that *F. ceratitivorus* has the potential to contribute to biological control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (LOPEZ et al., 2003; BOKONON-GANTA et al., 2004). *F. caudatus* (= *Opius caudatus* or *Biosteres caudatus*) was recorded as a parasitoid of *C. capitata* (WHARTON, 1999), but little is known about the biology of this species. Information on the potential impacts of the three *Fopius* species on non-target tephritids is also lacking.

There are 33 non-target tephritid flies in Hawaii, including 26 endemic species, and five deliberately introduced and two inadvertently introduced weed control agents (HARDY and DELFINADO, 1980). Among the 26 endemic species, 21 species belong to the genus *Trupanea*, 19 of which are flowerhead feeders (HARDY and DELFINADO, 1980). All the endemic Hawaiian *Trupanea* have similar life history traits and infestation ecology (HARDY and DELFINADO, 1980). *Trupanea dubautiae* (Bryan) is an abundant species infesting flowerheads of the native Asteraceae shrub *Dubautia raillardioides* Hillebrand that grows in mesic forests of Kauai island (DUAN et al., 1996). It is important to protect these native flowerhead feeders from attack by introduced biological control agents because they are important pollinators for rare and endemic plants in Hawaii.

Recently, a series of retrospective studies has investigated the potential non-target impacts of introduced larval fruit fly parasitoids in Hawaii (DUAN et al., 1996; DUAN and MESSING, 1997, 1998; DUAN et al., 1998). To date, field surveys have not discovered any introduced fruit fly parasitoids emerging from endemic flowerhead-feeding tephritids (DUAN et al., 1996). Likewise, laboratory tests did not show that any flowerhead-feeding tephritids were likely to be attacked by larval fruit fly parasitoids, because of the lack of a positive behavioural response to flower microhabitats (DUAN and MESSING, 1997, 1998). However, the larval parasitoids, *D. tryoni* and *D. longicaudata* were found to attack two non-target gall-forming tephritids, *Eutreta xanthochaeta* (Aldrich) and *Procecidochares utilis* (Stone), which are themselves introduced weed biological control agents (DUAN et al., 1996, 1998).

Although a strong body of evidence from previous studies has indicated the safety of opiine braconids in Hawaiian ecosystems, evaluation of non-target impact of any newly introduced opiine parasitoid is still prudent. In this study, we evaluate the potential impact of three *Fopius* parasitoids on the endemic tephritid *T. dubautiae*. Because insect parasitism is the result of a sequence of directed searching behaviours at progressively finer environmental levels, from host habitat location to host location to host acceptance (WANG and KELLER, 2002), we focused on the evaluation of host microhabitat-locating behaviour, i.e. the ecological suitability of non-target tephritid microhabitats to the parasitoids. Such an approach may help us to predict the non-target risk of similar endemic flowerhead-feeding tephritids and to narrow down effective host test range.

2 Materials and Methods

2.1 Test parasitoids and their normal hosts

We used *C. capitata* as the normal host of the three test parasitoids: *F. arisanus*, *F. caudatus*, and *F. ceratitivorus*. All tests were conducted under laboratory conditions with *F. arisanus* at the Kauai Agricultural Research Center (KARC, $23 \pm 2^\circ\text{C}$, 60–80 RH%, 12 L : 12 D), Kauai, and the other two parasitoids at the quarantine facility of the Hawaiian Department of Agriculture (HDOA, $25 \pm 2^\circ\text{C}$, 70–90 RH%, 12 L : 12 D), Honolulu, Hawaii.

Fopius arisanus was provided by the USDA-ARS Pacific Basin Agricultural Research Center (PBARC), Honolulu, Hawaii. Parasitized puparia were shipped to KARC weekly. Colonies of both *F. caudatus* and *F. ceratitivorus* were reared in the quarantine facility of HDOA on *C. capitata* using papaya as host fruit (BOKONON-GANTA et al., 2004). In all tests we used female wasps of each species that had been housed with an approximately equal proportion of males in holding cages ($25 \times 25 \times 25$ cm) for 1–2 weeks after emergence, with water and food provided but deprived of hosts prior to tests. Thus the female wasps used in this study were naïve (without oviposition experience) and sexually mature with an expected high mature egg-load (RAMADAN et al., 1992; WANG and MESSING, 2003a).

Colonies of *C. capitata* were maintained at the KARC and the Fruit Fly Biological Control Laboratory of the University of Hawaii at Manoa, Honolulu from eggs or puparia provided by PBARC. Adult flies (10–12-day-old) were used to obtain *C. capitata* egg-infested papaya fruits for later tests with the three *Fopius* species (for detailed procedures see WANG and MESSING, 2003b). In all tests we used papaya, *Carica papaya* as a host fruit of *C. capitata*. All fruits were purchased from local stores, and were washed and kept in the refrigerator at 7°C for 3–5 days before being used for experiments, to kill any naturally infested fly eggs (HARRIS and BAUTISTA, 1996). One day prior to the tests, individual papaya fruits were exposed to approximately 100 pairs of adult *C. capitata* flies in a cage ($25 \times 25 \times 25$ cm) for 24 h for egg infestation. To facilitate egg-laying by *C. capitata* and standardize the sample for the estimation of host egg density per fruit for later tests, 50 needle punctures were evenly made on each fruit surface before exposure, so that the flies laid eggs into the artificial punctures (WANG and MESSING, 2003b). Each *C. capitata* egg-infested papaya fruit served as a test unit of host and host substrate (microhabitat).

2.2 Collection of *Trupanea dubautiae* and its host plant

All *T. dubautiae* used in this study were collected around Pihea trail at Kokee State Park, Kauai, at approximately 1270 m elevation, where several previous studies used *T. dubautiae* for field surveys and non-target tests with larval fruit fly parasitoids (DUAN et al., 1996; DUAN and MESSING, 1997, 1998). *D. raillardioides* blooms from February to May. Each individual inflorescence consists of 200–700 flowerheads. According to DUAN et al. (1996), the phenology of *T. dubautiae* is closely related to four phenological stages of flowerheads of *D. raillardioides*: pre-blooming stage, at which peduncles of each inflorescence are not fully expanded and petals are not visible; full-blooming stage, at which peduncles of the inflorescence are fully expanded; petal fall stage, at which all petals start falling; and mature stage, at which flowerheads have lost all petals and started to mature seeds. At pre-blooming stage, *D. raillardioides* flowerheads contain mostly *T. dubautiae* eggs and first to second instars, while in the later two stages most flowerheads contain mature larvae or puparia.

Information on the basic reproductive biology of *T. dubautiae* has not been previously reported, so we first conducted preliminary observations on the fly's oviposition behaviour. In February 2004, flowerheads of *D. raillardioides* containing mature larvae and puparia of *T. dubautiae* were collected from the field, and individual inflorescences were plugged into 50-ml vials with the stem (approximately 5 cm) immersed in water, and brought back to the laboratory at KARC. After approximately 2 weeks, adult flies started to emerge. It was observed that newly emerged adult *T. dubautiae* were not reproductively mature. Mating occurred 4–5 weeks post-eclosion, and females started ovipositing 5–6 weeks post-eclosion. Females laid eggs directly inside flowerheads. They walked upon and examined flowerheads while probing and inserting the ovipositor into the flowerheads. Oviposition followed probing and lasted approximately 3–5 min.

Dissection of field-collected flowerheads in the first week of March 2004 showed that each flowerhead contained a mean of 0.8 ± 0.1 ($n = 50$) *T. dubautiae* eggs and 0.3 ± 0.1 ($n = 50$) first instars. Thus, for the later tests we collected flowerheads of *D. raillardioides* at pre-blooming stage during March 2004. Each inflorescence (consisting of 100–200 flowerheads, approximately 8 cm high and 10 cm diameter) was plugged into a 50-ml vial filled with water, and served as a test unit of the non-target host microhabitat.

2.3 Experimental set-up

The test protocol was similar for each parasitoid species. Each test consisted of two treatments: a choice test and a no-choice test. All tests were conducted in screen cages ($25 \times 25 \times 25$ cm) with honey and water provided.

In the choice test, we placed a fruit test unit and a flowerhead test unit inside a cage. The fruit was placed on a vial on a stand at about the same height as the flowerheads in the middle of the cage approximately 10 cm apart. Twenty female wasps of either species were transferred from their holding cages into a 35-ml plastic vial using an aspirator, which was placed upright in the middle between the fruit and the flowerhead test units. In the no-choice test, 20 females were released into a cage but only the flowerhead test unit was provided in the cage. Tests for each parasitoid species were replicated five times, each using a new cohort of parasitoids.

Each test started at 09:30 h and finished after 24 h exposure the following day. During the first day, two observation sessions (one in the morning and the other in the afternoon) were made, each consisting of 2 h. At 15 min

intervals we checked each test unit once and recorded the total number of the wasps on the fruit and flowerheads. The detailed host-location behaviour of *F. arisanus* has been described (WANG and MESSING, 2003c). Preliminary observation showed that the host-location behaviour of the other two *Fopius* was similar to *F. arisanus* (X. G. WANG et al., unpubl. data). Thus, during each sampling, the number of wasps that were displaying either probing (the wasp raises her abdomen, bends her ovipositor, and drills the ovipositor inside the substrate, or pushes her ovipositor back and forth while drilling) or stinging the host (the wasp keeps her body motionless while she inserts her ovipositor into the substrate) was also recorded. The morning session started 30 min after the release (i.e. from 10:00 to 12:00 h) when most of the wasps had left the vial; the afternoon session was conducted from 14:00 to 16:00 h. During each observation session, papaya fruit and flowerhead positions were exchanged after the first 1 h observation. In total, there were nine samplings for each observation session.

After the 24 h exposure, the papaya fruit and flowerheads were collected from the cage. Ten sub-samples of egg clusters were arbitrarily removed from each side of the papaya fruit by carefully cutting out fruit tissue around a puncture, and all eggs in each puncture were dissected under a stereomicroscope to determine the number of parasitoid eggs laid inside each host egg. Whenever the egg density was higher than 20 in a given puncture, we randomly dissected 20 eggs to estimate the total number of parasitoid ovipositions in the puncture. The actual number of ovipositions by each of the parasitoids during the 24 h exposure may be slightly underestimated, because some flies might not have laid eggs into the artificial punctures.

Fifteen flowerheads were randomly selected and dissected to record the number of *T. dubautiae* eggs and first instar larvae. *F. arisanus* was occasionally reported to attack a few first instar host larvae (NEWELL and RATHBURN, 1951), although it mainly attacks host eggs. Likewise, *F. ceratitivor* rarely attacks the first instar of *C. capitata* (BOKONON-GANTA et al., 2004). However, some *T. dubautiae* eggs might have hatched to first instars during the 24 h exposure. Thus, all *T. dubautiae* eggs and first instars in the flowerheads were dissected to determine the presence or absence of the parasitoid eggs.

2.4 Data analysis

The data were analysed to determine if each parasitoid species preferred the normal host microhabitat rather than the non-target microhabitat in choice tests, and if each

Table 1. Behavioural responses of three *Fopius* egg-larval parasitoids to *Dubautia raillardioides* flowerheads containing *Trupanea dubautiae* eggs and first instars in the absence (no-choice test) or presence (choice test) of papaya fruit infested with *Ceratitis capitata* eggs (normal host)

Test parasitoids	Choice test ¹				No-choice test ¹	
	Frequency of visits ² to		Frequency of probes on		Frequency of visits to flowerheads	Frequency of probes on flowerheads
	Papaya fruit	Flowerheads	Papaya fruit	Flowerheads		
<i>Fopius arisanus</i>	39.0 $\pm$ 2.8 a	4.7 $\pm$ 1.7 b	26.2 $\pm$ 4.2	0	6.1 $\pm$ 1.8	0
<i>Fopius caudatus</i>	32.2 $\pm$ 2.4 a	1.8 $\pm$ 0.3 b	14.8 $\pm$ 1.0	0	4.4 $\pm$ 1.5	0
<i>Fopius ceratitivor</i>	13.3 $\pm$ 2.6 a	1.9 $\pm$ 0.2 b	7.7 $\pm$ 2.3	0	2.6 $\pm$ 0.3	0

¹ Each test was replicated five times. All values are mean $\pm$ SE per hour per replicate based on a total of 18 observations per replicate.
² Frequency of visits refers to the number of wasps observed on the substrate per hour, while frequency of probes refers to the number of wasps that were probing in the substrate or stinging the hosts. Values followed by a different letter within the same row are significantly different (Student's *t*-test, JMP SAS).

parasitoid species could successfully parasitize the non-target hosts in either choice or no-choice tests. Because the tests with *F. arisanus* were conducted under different laboratory conditions than the other two parasitoid species, we did not intend to compare the behavioural responses among the three parasitoids, although the data are presented in the same tables. Thus, all comparisons were conducted within the same species with respect to their response to different test units.

For each treatment we first calculated the total number of wasps that were observed visiting or searching (i.e. displaying probing or stinging behaviour) on each test unit based on a total of 18 samples (nine from each session), and then calculated the mean frequency of wasp visits (i.e. on the test unit) and probing per hour (a total of 4 h) per replicate. This estimate reflects the relative amount of time spent by the parasitoids on the different test units and the degree of the parasitoids' behavioural responses to both microhabitats. The frequency of wasp visits or probes on the two different test units in the choice test was compared using Student's *t*-test (JMP SAS 4.01, Gray, NC).

The length of exposure time may influence a parasitoid's behavioural response. To determine if the frequency of wasp visits to different test units increased or decreased over the observational periods in both the morning and afternoon session, we analysed the relationship between the number of wasp visits (*Y*) and observational order over a morning or afternoon session (*X*) using linear regression (JMP SAS 4.01, Gray, NC).

3 Results

In the choice tests, all three *Fopius* parasitoid species visited *C. capitata* egg-infested papaya fruit significantly more often than flowerheads containing *T. dubautiae* eggs and larvae (*F. arisanus*: $t_8 = -18.1$, $P < 0.01$; *F. caudatus*: $t_8 = -12.5$, $P < 0.01$; *F. ceratitivorius*: $t_8 = -4.4$, $P < 0.01$) (table 1). Even when only flowerheads containing *T. dubautiae* eggs and larvae were provided in the no-choice tests, the observed frequency of wasp visits to flowerheads was also very low for all of the three tested species (table 1).

When female wasps of all three species visited *C. capitata* egg-infested papaya fruit, they repeatedly displayed probing behaviour before locating potential host oviposition sites. In contrast, not a single wasp of any of the three species was observed to display normal probing behaviour on flowerheads containing *T. dubautiae* eggs and larvae, in either choice or no-choice tests (table 1). All wasps that landed on the flowerheads appeared to be only resting (the wasp remains motionless on the flowerhead with its antennae and ovipositor stretched out laterally). Each parasitoid species successfully parasitized *C. capitata* eggs on papaya fruit during the 24 h exposure (table 2). However, not one of the *T. dubautiae* eggs or first instars on the flowerheads was parasitized by any of the three parasitoids in either choice or no-choice tests (table 2).

The dynamic patterns of wasp visits to the normal host microhabitats were different among the three *Fopius* parasitoid species in the choice tests (fig. 1). Increased frequency of wasp visits by *F. arisanus* were observed following the release during the morning

Table 2. Ovipositional responses of three *Fopius* egg-larval parasitoids to *Dubautia raillardioides* flowerheads containing *Trupanea dubautiae* eggs and first instars in the absence (no-choice test) or presence (choice test) of papaya fruit infested with *Ceratitis capitata* eggs (normal host)

Test Parasitoids	Papaya fruit ²			Choice test ¹			No-choice test ¹		
				Flowerheads ³			Flowerheads ³		
	<i>C. capitata</i> eggs per cluster	Eggs laid per wasp		<i>T. dubautiae</i> eggs per flowerhead	<i>T. dubautiae</i> larvae per flowerhead	Eggs laid per wasp	<i>T. dubautiae</i> eggs per flowerhead	<i>T. dubautiae</i> larvae per flowerhead	Eggs laid per wasp
<i>Fopius arisanus</i>	13.7 ± 2.1	17.5 ± 2.4		0.9 ± 0.2	0.2 ± 0.1	0	0.7 ± 0.2	0.4 ± 0.1	0
<i>Fopius caudatus</i>	23.4 ± 2.2	10.5 ± 2.5		0.4 ± 0.1	1.0 ± 0.1	0	0.4 ± 0.1	1.0 ± 0.1	0
<i>Fopius ceratitivorius</i>	22.2 ± 6.0	9.8 ± 4.1		0.6 ± 0.1	1.1 ± 0.1	0	0.6 ± 0.1	1.0 ± 0.1	0

¹ Each test was replicated five times. All values are mean ± SE.

² *C. capitata* egg density per cluster (puncture) or number of eggs laid per female wasp was estimated based on the dissection of 10 randomly selected punctures following a 24 h exposure.

³ Number of *T. dubautiae* eggs or larvae (only first instars were counted) was estimated based on the dissection of 15 randomly selected flowerheads following a 24 h exposure.

Fig. 1. Mean ($\pm$ SE) number of wasps of *Fopius arisanus*, *Fopius caudatus* and *Fopius ceratitivorius* observed on papaya fruit infested with *Ceratitis capitata* eggs (normal tephritid host) or *Dubautia raillardioides* flowerheads containing *Trupanea dubautiae* eggs and young larvae (non-target tephritid) in a choice test. For each test, 20 female wasps were released into a cage holding both test substrates ($n = 5$ replicates). The morning observation session started 30 min following the release and was conducted from 10:00 to 12:00 h, while the afternoon session lasted from 14:00 to 16:00 h. At 15 min intervals the numbers of wasps on both substrates was counted once

session ($Y = 2.4 + 0.99X$, $r^2 = 0.64$, $P < 0.001$), and the number of wasp visits reached a peak at the end of the morning observation session, and kept consistently high in the afternoon ($Y = 9.9 - 0.07X$, $r^2 = 0.01$, $P = 0.55$). A similar trend was observed for *F. caudatus* (morning session: $Y = 5.09 + 0.24X$, $r^2 = 0.11$, $P = 0.04$; afternoon session: $Y = 8.55 - 0.16X$, $r^2 = 0.03$, $P = 0.25$). In *F. ceratitivorius*, only a few wasps were observed visiting papaya fruit in the morning and there was no significant increase over the entire morning session ($Y = 1.74 + 0.09X$, $r^2 = 0.04$, $P = 0.19$), until the afternoon when more and more wasps were observed to visit the fruit ($Y = 0.7 + 0.61X$, $r^2 = 0.30$, $P < 0.001$).

Overall, the frequency of wasp visits to flowerheads was always low (less than two females per observation) in both choice and no choice tests (figs 1 and 2). There was no significant increase or decrease in the frequency

Fig. 2. Mean ($\pm$ SE) number of wasps of *Fopius arisanus*, *Fopius caudatus* and *Fopius ceratitivorius* observed on *Dubautia raillardioides* flowerheads containing *Trupanea dubautiae* eggs and young larvae (non-target tephritid) in a no-choice test. For each test, 20 female wasps were released into a cage holding the test substrates ($n = 5$ replicates). All observations were consistent with the choice test (see description in fig. 1)

of visits to the flowerheads over the observational periods by any of the three species (all $P > 0.05$).

4 Discussion

Our results show that all three egg-attacking parasitoids, *F. arisanus*, *F. caudatus* and *F. ceratitivorius*, lack normal ovipositional responses to *T. dubautiae* in its native host plant, *D. raillardioides* flowerheads. This is consistent with several previous studies in which all tested larval fruit fly parasitoids, including *D. longicaudata*, *D. tryoni*, *P. fletcheri*, and *Tetrastichus giffardianus* Silvestri, also lack ovipositional responses to *T. dubautiae* larvae-infesting *D. raillardioides* flowerheads (DUAN and MESSING, 1997, 1998). Although all the females used in this study were naïve and sexually mature, they did not show increasing behavioural responses to non-target host microhabitats over time (figs 1 and 2). As a result, none of the three *Fopius* species was able to locate *T. dubautiae*.

Although *F. arisanus* and several introduced larval fruit fly parasitoids are widely established in Hawaii, none of the numerous field surveys conducted in Hawaii (FUNASAKI et al., 1988; DUAN et al., 1996)

discovered introduced fruit fly parasitoids emerging from *T. dubautiae* or any other endemic tephritid. To date, only *D. longicaudata* and *D. tryoni* were found to attack the gall-forming tephritids, *E. xanthochaeta* and *P. utilis* in the field (DUAN et al., 1996, 1998). In laboratory tests female *D. longicaudata*, *D. tryoni* and *T. giffardianus* also showed ovipositional responses to the Hamakua pamakani gall fly, *Procecidochares alani* L. (an introduced weed biological control agent), but field surveys did not find any introduced fruit fly parasitoids associated with this gall fly (Purcell et al., 1997). Recently, a test in quarantine also showed that *F. ceratitivorus* is unlikely to attack *P. alani* (BOKONON-GANTA et al., 2004).

Visual (fruit shape, size and colour) and olfactory stimuli emitted by ripening and host-infested fruits have been shown to play an important role in host microhabitat recognition of opiine braconids attacking fruit-inhabiting tephritids (VARGAS et al., 1991; MESSING and JANG, 1992; HARRIS and BAUTISTA, 1996). Among the three *Fopius* parasitoids, the host-searching behaviour of *F. arisanus* is well documented. Physical appearance of host fruits is an important cue for host habitat location by female *F. arisanus*, with light colours (yellow and white) more attractive than other colours (VARGAS et al., 1991). Host fruit species, size, shape and ripeness all have been shown to influence parasitism by *F. arisanus* (VARGAS et al., 1991; HARRIS and BAUTISTA, 1996). Other stimuli important to the parasitoid's attraction to potential host microhabitats are the presence of host-associated cues (e.g. host oviposition scars and adult fly residues of *C. capitata*) (WANG and MESSING, 2003c). Thus, host microhabitat selection plays a central role in the process of host selection in opiine fruit fly parasitoids. DUAN and MESSING (1997) suggest that the fruit-like spheroid shape of a gall may be similar enough to a small fruit to elicit host-searching behaviour in some larval fruit parasitoids. However, flowerheads of Asteraceae shrubs have no resemblance at all to the host fruits of frugivorous tephritids.

Several authors have emphasized the importance of ecological host range for non-target testing of parasitoids (e.g. DUAN and MESSING, 1997; MESSING, 2001). However, most non-target host range tests focus on host acceptance and suitability, i.e. the last steps in the hierarchy leading to parasitism, rather than commencing with locating host habitats. For example, many non-target host tests for weed biological control focus on the evaluation of introduced agents on taxonomically or phylogenetically related species to the target host plants [using centrifugal phylogeny as a basis for non-target test (MESSING, 2001)]. This method may prove effective for non-target test of weed biocontrol agents, but may be unrealistic in parasitoids (MESSING, 1999, 2001). It has been shown that when non-target tephritids were removed from their natural microhabitats and placed on an artificial substrate of target fruit flies, some larval fruit fly parasitoids can attack the non-target hosts (e.g. DUAN and MESSING, 1998). Obviously, this is artificial, and ecologically unrealistic. The physiologically suitable non-target tephritid flies are not ecologically suitable, simply because the wasps are

unable to recognize the microhabitat (DUAN and MESSING, 1997, 1998). Host specificity of insect parasitoids arises from behavioural and physiological adaptations that limit the range of organisms that they will attack and utilize as hosts. Behavioural adaptation precedes physiological adaptation in the evolution of host specificity (FUTUYMA and MORENO, 1988), thus understanding the behavioural basis of host specificity or behavioural evaluation in host range tests is critical. This study confirms the importance of understanding host habitat location behaviour for non-target tests in fruit fly parasitoids that narrow down effective host test range.

Because of the lack of ovipositional response by the three *Fopius* species to the *T. dubautiae*-infesting flowerhead feeders of Asteraceae plants, we conclude that endemic flowerhead feeders are at minimal risk from introduced egg-attacking fruit fly parasitoids.

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