Physiological Entomology (2007) **32**, 313–321 DOI: 10.1111/j.1365-3032.2007.00575.x

The host- and microhabitat olfactory location by *Fopius arisanus* suggests a broad potential host range

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Abstract. The identification of infochemicals for parasitoid females is a critical issue in applied and fundamental parasitoid research. The olfactory location of host and its microhabitat by *Fopius arisanus* (Sonan, 1932) (Hymenoptera: Braconidae), an egg-pupal parasitoid of fruit flies (Diptera: Tephritidae), is investigated. Potential sources of volatiles are placed in opaque traps and tested in field cages, under seminatural conditions. *Fopius arisanus* females respond positively to synomones from mango leaves and from fruits belonging to many botanical families, including the nonhost plant strawberry. They prefer fruits infested by Tephritidae to uninfested ones but do not discriminate between fresh and old infestations. *Fopius arisanus* females are attracted by the odours of faeces of the tephritid fly *Bactrocera zonata*. They exhibit remote detection of a volatile kairomone coating the egg mass of all tested Tephritidae species but absent in the egg mass of the Muscidae *Stomoxys calcitrans*. All these infochemicals are volatile but only those emanating from fruit and from faeces are attractants perceived before landing. The relationships between this apparent generalist behaviour and the dietary specialization of *F. arisanus* are discussed, according to its ecology and behaviour in its natural environment.

Key words. Behavioural ecology, Braconidae, host selection, infochemicals, insect, polyphagous parasitoid, kairomone, synomone, Tephritidae, tritrophic interactions.

Introduction

Microhabitat location describes the behaviour of a parasitoid, flying through the host habitat, perceiving a landing site, flying towards this site and landing on it, or rejecting it. During this process, parasitoids are known to respond to visual and volatile infochemicals (Vinson, 1991). These volatiles may be emitted by the plant, the host population, the host, or their interaction (Quicke, 1997; Vinson, 1998). The accurate characterization of these infochemicals is important for applied parasitoid research because they play a large part in determining the insect behaviour and ecology. The subject of the present investigation is *Fopius arisanus* (Sonan, 1932) (Braconidae: Opiinae), a solitary egg-pupal parasitoid of Tephritidae. After its introduction in Hawaii in 1946, this species became the predominant parasitoid of tephritid flies there (Haramoto & Bess, 1970; Wong *et al.*, 1984), and contributed significantly to the regulation of *Bactrocera dorsalis* and *Ceratitis capitata* populations (Bess *et al.*, 1961; Vargas *et al.*, 1993). To date, this parasitoid is known to develop on approximately 40 tephritid species and, moreover, to oviposit even in nonhost tephritid species such as *Bactrocera cucurbitae* (Coquillett) (Snowball & Lukins, 1964; Wharton & Gilstrap, 1983; Harris & Bautista, 1996; Chinajariyawong *et al.*, 2000; Lawrence *et al.*, 2000; Quimio & Walter, 2001; Calvitti *et al.*, 2002; Zenil *et al.*, 2004; Carmichael *et al.*, 2005; Rousse *et al.*, 2006).

Wang & Messing (2003) describe the behaviour of *F. arisanus* when foraging for host eggs after landing on the fruit (i.e. the host location). In two studies on colour attraction for *B. dorsalis*, Vargas *et al.* (1991) and Cornelius *et al.* (1999) show that *F. arisanus* responds to coloured sticky traps. Furthermore, Liquido (1991), Harris & Bautista (1996), Bautista & Harris (1996), and Bautista *et al.* (2004) investigate the relationships between host plant odour and parasitism rate, and Altuzar *et al.* (2004) describe its response to fruit odours using a wind tunnel.

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Nevertheless, the nature of cues guiding females of *F. arisanus* before landing is poorly known. The above mentioned studies show that *F. arisanus* females respond to various fruit odours and that their resulting parasitism rate is influenced by the fruit species or the fruit maturation. The present study analyses, step by step, the response of the parasitoid to volatile infochemical cues. It describes the response to volatiles emitted by the host habitat, the host microhabitat, the host population and the host itself. Dukas & Duan (2000) show that the host microhabitat location behaviour of *F. arisanus* is plastic and can be modified by associative learning, to increase its fitness. This stresses the importance of using naive females. The present study highlights the innate responses of *F. arisanus* females to olfactory stimuli from the host microhabitat olfactory stimuli. It is hoped that this will provide some cues to assess the potential host range and the behaviour of this parasitoid in a tritrophic context.

Materials and methods

Insects

A colony of *F. arisanus* was established in December 2003 in the CIRAD Réunion Entomology Laboratory from a batch of parasitized pupae of *B. dorsalis* obtained from USDA-ARS Hawaii (E. J. Harris). Emerging adults were subsequently offered *B. zonata* eggs for 5–20 generations. The main colony was reared in a 110 × 60 × 60 cm plastic screened cage under an LD 12 : 12 h photoperiod at 25 ± 2 °C and 70 ± 20% RH. The adults were given free access to water on a moistened sponge and to a mixture of honey/agar 15% (1 : 1).

From their emergence to the experiment, the females used in the subsequent bioassays were reared with males in a room free from any fly odour (i.e. conditions similar to the rearing room), and were assumed to be mated.

Experiments

Three sets of choice experiments were carried out. The first set (experiments 1–8; Table 1) aimed to screen various potential sources of attractive cues. In the second set (experiments 9–18; Table 2), the specificity of these infochemical sources was investigated. Finally, the third set (experiments 19–22; Table 3) was designed to assess the range of perception of these volatile infochemicals (i.e. to distinguish between attractant and arrestant volatiles). All experiments were carried out in cylindrical mesh-screened field cages (height 2.5 m, diameter 3 m; Synthetic Industries, Gainesville, Florida). For lexical convention, the fruit tree was considered as habitat and the fruit itself as microhabitat. A fruit is designated as host or nonhost food plant depending on wether it is reported to be attacked by tephritid flies or not (White & Elson-Harris, 1992).

Experiments 1–18: procedure

Except when otherwise specified, five 1.5–2-m tall potted mango trees (*Mangifera indica*) were placed in each cage to create semi natural conditions. The volatile sources were put in black painted traps (Tephritrap, Sorygar, Spain), which were hung 1.5 m above the ground on an H-shaped base and regularly arranged along a 1 m radius circle. The traps on the circumference were placed randomly at the start of the experiments and then rotated 90° clockwise every 1 h to minimize the influence of trap position. The control trap was placed in the middle of this circle, except for the experiment using only two traps where it was placed on the circumference, opposite to the other. Half a dispenser of dichlorvos (approximately 0.5 g, C-72, Biosystèmes, France) was put in each trap to quickly kill every entering wasp. Pre-trials were conducted to ensure these dispensers had no repellent effect.

One hundred sexually mature (6–15-day-old) and naive *F. arisanus* females were released in the cage at the start of the experiment. The traps and their contents were handled with latex gloves. Each experiment lasted 4 h and was replicated six times.

Experiments 1–8: screening for eliciting infochemicals

Experiment 1: infochemicals from host habitat. This experiment was carried out to assess the attractiveness of leaves from host and nonhost food plants. The potted mango trees were removed from the cage during this experiment. Three traps were used. The first trap contained 2–3 g of mango leaf, the second trap the same weight of *Ficus* sp. leaves, and the third trap was empty (control).

Experiments 2–4: infochemicals from microhabitat. In these experiments, the response of the parasitoid to uninfested and tephritid infested fruits was assessed. The fruits were pierced artificially (punched by 40 holes with a needle) to increase the volatile emanations and elicit the fly infestation when necessary. In experiment 2, an empty trap was compared with a trap containing an uninfested orange. In experiment 3, an empty trap was compared with a trap containing an uninfested orange and another one containing a freshly infested orange. This infested orange was first pierced artificially and left for 1 h in the main rearing cage of *B. zonata* (containing 10 000–20 000 flies) just before the experiment.

In experiment 4, three treatments were compared. The first trap (control) contained an uninfested mango whereas the second trap contained a mango, placed for 1 h immediately before the experiment, in a cage containing 200, 35–45-day-old females of *B. zonata*. The third trap contained a mango treated similarly but 6 days earlier. All mangos were picked at the same time, when mature (i.e. green), and were half ripe at the time of the experiment. After collection and infestation of one-third of them, they were kept at 25 ± 2 °C and 70 ± 20% RH for 6 days.

Experiments 5–6: infochemicals from host population. These two experiments were conducted to determine whether *F. arisanus* females respond to frass/faeces deposited by the adult hosts on the substrate they visit.

Table 1. Response to infochemicals by the female of *Fopius arisanus* (experiments 1–8): comparisons of mean captures.

Number	Experiment	Trap content	Mean ± SE percent of capture	*F*	d.f.	*P*	Mean captures[a]	Tukey's HSD
1	Leaves	Control	21 ± 4	5.85	2,12	$< 10^{-2}$	A	33.8
		Ficus leaves	29 ± 6				AB	
		Mango leaves	50 ± 8				B	
2	Uninfested fruit	Control	12 ± 4	29.64	1,10	$< 10^{-2}$	A	32.5
		Uninfested orange	88 ± 4				B	
3	Fresh infestation	Control	4 ± 2	45.69	2,12	$< 10^{-5}$	A	38.6
		Uninfested orange	28 ± 7				B	
		Infested orange	68 ± 6				C	
4	Old infestation	Uninfested mango	16 ± 4	15.13	2,12	$< 10^{-3}$	A	45.4
		Infested mango	36 ± 2				B	
		Decaying mango	48 ± 5				B	
5	Faeces	Control	4 ± 4	241.65	1,10	$< 10^{-7}$	A	6.8
		Faeces	96 ± 4				B	
6	Faeces comparison	Control	5 ± 4	6.70	4,20	10^{-3}	A	26.4
		Young males	24 ± 6				B	
		Young females	35 ± 3				B	
		Mature males	17 ± 3				B	
		Mature females	19 ± 5				B	
7	*Bactrocera zonata* eggs	Control	15 ± 5	32.29	2,12	$< 10^{-4}$	A	33.4
		Washed eggs	17 ± 4				A	
		Unwashed eggs	68 ± 5				B	
8	Kairomone isolation	Control	17 ± 4	96.18	1,10	$< 10^{-5}$	A	18.6
		Egg macerate	83 ± 4				B	

One hundred females are initially released in the cage. Depicted are the repartitions of the captured females after 4 h.

[a] Mean number of captured females per replication.

In experiment 5, a trap containing a clean square of filter paper (length 20 cm) was compared with a trap containing a similar square of paper marked by *B. zonata* females. To achieve this, the paper was enclosed in a small plastic box (200 mL) with 30 mature (35–45-day-old) females of *B. zonata* for 24 h just before the experiment.

Experiment 6 compared five treatments. One contained a clean square of filter paper, the four other contained papers visited by males or females. For both sexes, either sexually immature (0–5-day-old) or mature (35–45-day-old) adults were used.

Experiments 7–8: infochemicals from host egg mass. These two experiments aimed to demonstrate the response to any volatile kairomone located on the host egg mass. In experiment 7, an empty trap was compared with two other traps, each containing 250 mg of 0–24-h *B. zonata* eggs. In one trap, eggs were washed with distilled water whereas they were kept unwashed in the other.

Two treatments were compared in experiment 8. The first trap contained an open Petri dish with a sponge of the same diameter inside (diameter 9 cm). The sponge was first sterilized in an autoclave then was moistened by an aqueous solution obtained by macerating 500 mg of 0–24-h *B. zonata* eggs in 200 mL of distilled water for 5 min and then by removing the eggs by filtering. The second trap contained a similarly treated Petri dish and a sponge moistened with 200 mL of distilled water (control).

Experiments 9–18: specificity of perceived infochemicals

Experiments 9–14: fruit attractiveness. Six experiments were carried out to assess the attractiveness of six fruit species from different families for the females of *F. arisanus*. In each experiment, a trap containing 25–30 g of sliced fruit was compared with an empty trap. The tested fruits were guava *Psidium guajava*, Myrtaceae (experiment 9), zucchini *Cucurbita pepo*, Cucurbitaceae (experiment 10), mango *M. indica*, Anacardiaceae (experiment 11), tomato *Lycopersicon esculentum*, Solanaceae (experiment 12), Indian almond *Terminalia catappa*, Combretaceae (experiment 13) and strawberry *Fragaria vesca*, Rosaceae (experiment 14). These fruits were chosen because they are nonhosts, hosts for ecologically different tephritid species, or preferred hosts for *B. zonata* in La Réunion (see below).

Experiments 15–16: comparisons of host and nonhost food fruits. These two experiments compared the attractiveness of the nonhost food fruit strawberry with that of three other fruits and a control (empty trap). In experiment 15, three fruits that are hosts of ecologically different tephritid species present in La Réunion were used: tomato (attacked by the oligophagous *Neoceratitis cyanescens*), zucchini (attacked by oligophagous tephritids specialized on Cucurbitaceae) and orange (attacked by polyphagous tephritids).

For experiment 16, three fruits were chosen among the preferred hosts of *B. zonata* in La Réunion: mango, Indian almond and guava.

Table 2. Specificity of the response (experiments 9–18): comparisons of mean captures.

Number	Experiment	Trap content	Mean ± SE percent of capture	*F*	d.f.	*P*	Mean captures[a]	Tukey HSD
9	Fruits in no-choice	Control	28 ± 6	23.72	1,10	$< 10^{-2}$	A	28.6
		Guava	72 ± 6				B	
10		Control	7 ± 3	158.60	1,10	$< 10^{-5}$	A	27.8
		Zucchini	93 ± 3				B	
11		Control	14 ± 5	60.50	1,10	$< 10^{-4}$	A	35.8
		Mango	86 ± 5				B	
12		Control	26 ± 3	81.34	1,10	$< 10^{-4}$	A	35.6
		Tomato	74 ± 3				B	
13		Control	12 ± 4	48.11	1,10	$< 10^{-3}$	A	21.7
		Indian almond	88 ± 4				B	
14		Control	7 ± 3	206.11	1,10	$< 10^{-6}$	A	29.4
		Strawberry	93 ± 3				B	
15	Fruits comparisons	Control	2 ± 1	22.64	4,20	$< 10^{-6}$	A	43.2
		Tomato	8 ± 2				A	
		Zucchini	13 ± 4				A	
		Orange	29 ± 6				B	
		Strawberry	48 ± 5				B	
16		Control	2 ± 1	13.46	4,20	$< 10^{-4}$	A	44.8
		Guava	16 ± 5				BC	
		Mango	16 ± 5				BC	
		Indian almond	23 ± 2				C	
		Strawberry	43 ± 2				C	
17	Tephritidae eggs	Control	5 ± 1	15.38	4,20	$< 10^{-5}$	A	46.8
		Ceratitis catoirii	13 ± 3				B	
		Ceratitis rosa	17 ± 4				B	
		Ceratitis capitata	19 ± 4				B	
		Bactrocera zonata	46 ± 7				C	
18	Muscidae eggs	Control	42 ± 10	1.31	1,10	0.28	A	5.2
		Stomoxys calcitrans	58 ± 10				A	

One hundred females are initially released in the cage. Depicted are the repartitions of the captured females after 4 h.

[a] Mean number of captured females per replication.

Experiments 17–18: comparisons of egg masses of a tephritid community. In these two experiments, the attractiveness for females of *F. arisanus* of egg masses of different fly species was compared. The egg masses were 250 mg of 0–24 h unwashed eggs. In experiment 17, an empty trap was compared with four other traps containing eggs of *B. zonata*, *C. capitata*, *C. rosa* and *C. catoirii*. These four species were chosen because their ecology shows some similarities: they are polyphagous and have a partially shared host plant range. Consequently, in the field, they may be encountered by the parasitoid on the same fruit. In experiment 18, the empty control trap was compared with a trap containing eggs of the haematophagous fly *Stomoxys calcitrans* (Diptera: Muscidae).

Experiments 19–22: range of perception of infochemicals

These experiments were carried out to determine whether attractive volatiles were perceived in flight by the parasitoid and then induced landing on the source, or whether they were perceived only after landing. The observations took place in a field cage similar to those described above. The sources of olfactory stimuli were put in two yellow Tephritrap, spaced 1 m apart, hung on a 1.5 m long metallic rod, 1.5 m above the ground. Pre-trials showed that yellow was unattractive for naive females of *F. arisanus*. The rod was placed perpendicularly to the axis given by the direction of the sun at the time of the experiment, and two potted mango trees were placed behind it. Two hundred females (same conditions of rearing as described above) were released into the cage 15mn before the start of the experiment (i.e. before hanging the traps). An observer sat 2 m away in front of the rod, and recorded the number of females present on each trap every 3 min. Those females were then collected with a hand-aspirator and removed from the experiment. For the last experiment (experiment 22), the females landing on each trap were counted but not collected (experiment 22-1) and the females that entered each trap were counted and removed every 3 min (experiment 22-2). Each experiment lasted 36 min. The traps were permutated every 9 min. Each experiment was replicated three times.

Four pairs of traps were tested: empty/uninfested orange (experiment 19), uninfested orange/infested orange (experiment 20), clean filter paper/filter paper previously visited by

Table 3. Range of perception of infochemicals (experiments 19–22): comparisons of mean attractions.

Number	Experiment	Trap content	Mean ± SE attraction	*t*	d.f.	*P*
19	Uninfested fruit	Control	2.3 ± 0.4	–5.53	35	$< 10^{-5}$
		Uninfested orange	7.1 ± 0.9			
20	Infested fruit	Uninfested orange	4.4 ± 0.4	–2.00	35	0.05
		Infested orange	5.6 ± 0.5			
21	Faeces	Control	1.9 ± 0.4	–2.87	35	$< 10^{-2}$
		Faeces	3.3 ± 0.4			
22-1	*Bactrocera zonata* (landed)	Control	2.3 ± 0.5	–1.57	35	0.12
		Eggs	3.0 ± 0.4			
22-2	*Bactrocera zonata* eggs (Entered)	Control	0.4 ± 0.1	–3.71	35	$< 10^{-3}$
		Eggs	1.4 ± 0.2			

Two hundred females are initially released in the cage and observed continuously for 36mn. The two traps are checked every 3mn. The mean captures are the average number of females landed on (or entered in) each trap at each recording event.

[a] Mean number of captured females per replication.

mature females of *B. zonata* (experiment 21), and empty/unwashed eggs of *B. zonata* (experiment 22) (see above for the preparation of volatile sources).

Statistical analysis

All analyses were performed using the R software (R Development Core Team, 2006).

For experiments 1–18, the trap content effect was assessed. As climatic variations between cages and days induced a large variance in the number of parasitoids caught, the percentages of total replication captures in each trap was analysed, after root arcsine transformation and corrections for extreme values (0% and 100%) (Fischer & Yates, 1982), in a two-way analysis of variance (R function aov) without interaction. The main factors (i.e. trap content and replication), were assumed to be fixed effects. Comparisons of means were performed using Tukey's test (function Tukey's HSD). The hypothesis of the independence of counts within an experiment was considered to be reasonably met because of a large number of untrapped parasitoids left at the end of each experiment (Tables 1–3).

In experiments 19–22, the variance was weaker. Paired Student's *t*-tests were performed to compare the data of coupled landing observations (function *t*-test).

Results

Experiments 1–8: screening for eliciting infochemicals (Table 1)

Mango leaves were found to be attractive for the females of *F. arisanus*. The attractiveness of *Ficus* sp. leaves was not significantly different from that of mango leaves or control. The uninfested orange was also attractive. However, the infested orange was significantly more attractive than the uninfested one, and the decaying mango fruit, containing only larvae, was as attractive as a recently infested fruit. The filter paper visited by mature females of *B. zonata* became attractive for females of *F. arisanus*, and this was true whatever the sex or the sexual maturity of the flies. Finally, the presence of an egg mass of *B. zonata* was attractive for females of *F. arisanus*.

Experiments 9–18: specificity of perceived infochemicals (Table 2)

All tested fruits, including the nonhost food fruit strawberry, were attractive for the females of *F. arisanus* in 'no-choice situations'. The two choice experiments showed that this attraction was dependant on the fruit species. In both cases, the strawberry belonged to the most attractive fruits. The egg masses of all tested tephritid were shown to be attractive. However, females of *F. arisanus* showed a significant preference for the egg mass of *B. zonata*. The eggs of *S. calcitrans* were not attractive for the females.

Experiments 19–22: range of perception of infochemicals (Table 3)

The volatiles emitted by the fruit and the fly faeces were perceived during flight by female *F. arisanus* and induced their landing on the trap that contained them. Before landing, females were also able to distinguish between the infested fruit and the non-infested one, and preferred the former. By contrast, the odour of *B. zonata* eggs was not perceived during flight because landing occurred randomly on both traps. However, volatiles from eggs were perceived after landing and induced significantly more catches in the trap containing the eggs than in the empty trap.

Discussion

Females of *F. arisanus* respond positively to synomones from the host habitat and microhabitat. It is assumed that they search for their host because they have been fed during their rearing, although a response to potential sugar source cannot be excluded, for example in the response to volatiles from fruits. Altuzar *et al.* (2004) demonstrate such a response of *F. arisanus* females to guava and orange synomones in wind tunnel assays. Other opiine parasitoids of Tephritidae respond to synomones from host food fruits: *Psyttalia fletcheri* females respond to volatiles from zucchini (Messing *et al.*, 1996) and *Diachasmimorpha longicaudata* to volatiles from various fruits (Leyva *et al.*, 1991). A positive innate response to host food plant odours is thought to be more common in parasitoids of monophagous hosts (Kaiser & Cardé, 1991; Kester & Barbosa, 1991, 1994; Van Baaren & Nénon, 1996). However, this kind of attraction, although greatly depending on experience (Udayagiri & Jones, 1993; Couty *et al.*, 1999), can be found innately in parasitoids of polyphagous hosts too (Campan *et al.*, 2002; Jang, 1997).

Curiously, in *F. arisanus*, this innate response is unselective and might lead to nonhost food plants. Attraction of parasitoids to nonhost food plants is uncommon but has already been observed. Perfecto & Vet (2003) note that naive *Cotesia glomerata* females are attracted by volatiles from potato (*Solanum tuberosum*), although this plant is not attacked by its favourite host *Pieris rapae*. Although *C. glomerata* has a narrow host range, *P. rapae* is polyphagous and feeds on several plant families. *Cotesia glomerata* is therefore assumed by Perfecto & Vet (2003) to respond to some generic green leaf volatiles. Similarly, *Macrocentrus grandii*, parasitoid of polyphagous Pyralidae, responds to generic volatiles (Udayagiri & Jones, 1992). Indeed, most juicy fruits are potentially attacked by one or more tephritid species (White & Elson-Harris, 1992) and the risk of failure for a naive female attracted by any juicy fruit is therefore poor. *Fopius arisanus* females could respond innately to a generic volatile or mixture of volatiles, shared by many if not all juicy fruits. The observed preferences of the parasitoid may correspond to variations in concentrations of volatiles between fruit species. In terms of cost and benefits, it might be less costly for a polyphagous parasitoid of tephritid flies to respond globally to all types of juicy fruits rather than innately discriminate among many chemical fruit signatures of which only a few are not food plants for tephritids. However, there are a lack of field data to confirm this hypothesis: the number of fruits *F. arisanus* locates in a given environment, and the fraction of these fruits that are not host food plants, is not known. This hypothesis converges with a similar one offered by Krainacker *et al.* (1987) to explain the polyphagous behaviour of *C. capitata*, which sometimes oviposits in nonhost food fruits in the field.

Infested fruits induce a stronger response by the females compared with healthy fruit. The greater attraction observed could be due to kairomones left by the host and the host population on the fruit, or to synomones induced by the infestation (Turlings *et al.*, 1995). Various studies show the greater attractiveness for opiine parasitoids of plants infested by their hosts compared with uninfested ones. For example, Eben *et al.* (2000) observe that *D. longicaudata* prefers fruits infested by its hosts, and Messing *et al.* (1996) arrive at a similar conclusion for *P. fletcheri*.

Infested fruits are still more attractive than the uninfested ones 1 week after infestation (i.e. when they were decaying and contained only larvae that *F. arisanus* could not parasitize). Such a strategy might be profitable for a parasitoid in the field when the decaying plant becomes more and more attractive for ovipositing hosts. This is the case in some Anthomyiidae (Dindonis & Miller, 1980; Baur *et al.*, 1996) but such a behaviour is uncommon in Tephritidae. Fitt (1984) show that *Bactrocera jarvisi* and *Bactrocera tryoni* oviposited preferentially in fruits without any larva inside. It is thus surprising that *F. arisanus* is attracted by fruits in which it has few opportunities to find eggs, although the presence of decayed fruits may indicate the vicinity of fresher ones.

Females of *F. arisanus* are attracted also by volatiles left by tephritid adults. According to Noldus *et al.* (1988), insect eggs emit few or no volatiles and egg parasitoids usually rely on cues from the host community to detect the microhabitat. This attractiveness depends neither on sex, nor on sexual maturity of the fly. Some tephritid species are known to deposit host marking pheromones (HMP) near their oviposition site (Averill & Prokopy, 1989). In some examples, this kind of pheromone is known to act as kairomone for opiine parasitoids (Prokopy & Webster, 1978; Roitberg & Lalonde, 1991). Nevertheless, these pheromones are deposited by ovipositing tephritid females only. Moreover, some tephritid species do not deposit such kairomones: this is the case with *B. zonata*, which does not mark its oviposition sites with HMP, although it exhibits some remnant of marking behaviour (S. Quilici and P. F. Duyck, unpublished data). The hypothesis of detection of HMP by *F. arisanus* is therefore hardly supported by the present data. It is assumed that *F. arisanus* females respond to the odour of faeces. Such a strategy has already been shown in egg parasitoids (Meiners *et al.*, 1997) as well as in Braconidae (Parra *et al.*, 1996).

Females of *F. arisanus* also respond to a volatile and hydro-soluble kairomone (or a mixture of such kairomones) emanating from the egg masses of Tephritidae. Such a volatile is present in the egg masses of the four tested tephritid species but absent in the egg-masses of *S. calcitrans*. It could therefore be more a family specific than a species-specific kairomone. The chemical nature and physiological role of this compound is unknown and requires further investigation. It is perceived at shorter range than volatiles from fruit and faeces and may therefore be only moderately volatile. Indeed, this kairomone appears to act less as an attractant than as an arrestant molecule, increasing the search activity in a small area (Tumlinson *et al.*, 1992). As with fruits, the preferences shown by the female parasitoid might indicate a difference in concentration or nature of this kairomone among tephritid species.

Few studies mention volatiles emitted by egg masses. Usually, the kairomones deposited with eggs during oviposition elicit the recognition and/or acceptance by egg parasitoids (Strand & Vinson, 1982, 1983a,b; Kainoh & Tatsuki, 1988; Bin *et al.*, 1993; Borges *et al.*, 1999). In other words, these kairomones usually belong to the group 3 of infochemicals as defined by Vinson (1991) (i.e. nonvolatile cues perceived by contact chemoreceptors). Similar cases have been mentioned that deal with oophagous parasitoids (Conti *et al.*, 2003; Sales, 1979; Wysoki & De Jong, 1989; Hofstetter & Raffa, 1998). These olfactometry tests show that host egg masses are attractive at a maximal distance of approximately 10 cm.

Fopius arisanus is known to recognize and parasitize about 40 tephritid species. This list contains 18 species tested in the laboratory (Calvitti *et al.*, 2002; Harris & Bautista, 1996; Lawrence *et al.*, 2000; Quimio & Walter, 2001; Zenil *et al.*, 2004; Rousse *et al.*, 2006), whereas 33 species have been recorded from field collections (Carmichael *et al.*, 2005; Snowball & Lukins, 1964; Wharton & Gilstrap, 1983; Chinajariyawong *et al.*, 2000). In essence, *F. arisanus* locates and accepts all tephritid species presented experimentally on juicy fruits, including nonhost species and species that it never encountered in its evolutionary history (Rousse *et al.*, 2006). During the host selection process, *F. arisanus* females probably do not recognize each host species separately, but rather react globally to members of the family Tephritidae. As discussed in relation to the response to fruit species, the occurrence of nonhost Tephritid species is low, and the risk of failure for naive females is weak.

The precise host range of *F. arisanus* is still to be defined. The stimuli involved in host location and host recognition might be present in many Tephritidae feeding on juicy fruits. Because this family contains currently 4000 species of which approximately 50% feed on juicy fruits (White & Elson-Harris, 1992), this might eventually suggest a large potential spectrum of hosts for *F. arisanus*.

But what about its actual host range in nature? Although the possibility for *F. arisanus* to develop on such numerous host species is proven, its development is highly variable depending on the host species (Harris & Bautista, 1996; Quimio & Walter, 2001; Zenil *et al.*, 2004; Rousse *et al.*, 2006). Furthermore, choice experiments show clear preferences during olfactory location of host and of its microhabitat (present study) and host acceptation (P. Rousse and S. Quilici, unpublished data). Heterogeneous distribution of the parasitoid may therefore be expected in the field among hosts and host food plants. This parasitoid would mainly respond to the more attractive fruits and develop on the more favourable tephritid species in the field. Rather than a polyphagous generalist, *F. arisanus* is more probably an oligophagous and opportunist parasitoid specialized on Tephritidae.

Nevertheless, the field data published to date hardly support this expectation. In Hawaii, Costa Rica, Fiji, India, Malaysia, Thailand and Australia, *F. arisanus* has been collected from 33 Tephritidae (Carmichael *et al.*, 2005; Snowball & Lukins, 1964; Wharton & Gilstrap, 1983; Chinajariyawong *et al.*, 2000) and 77 fruit species, from 35 families, in the world (Chinajariyawong *et al.*, 2000; Snowball *et al.*, 1962; Snowball & Lukins, 1964; Vargas *et al.*, 1993; Purcell, 1998). Most of the available numeric data come from Hawaii where the parasitoid is widespread. Although *B. dorsalis* appears to be a far better host than *C. capitata* for the development of *F. arisanus* (Harris & Bautista, 1996), no clear difference can be identified in parasitism rates between both in the field (Haramoto & Bess, 1970; Vargas *et al.*, 2001). Similarly, *F. arisanus* is the predominant opiine parasitoid of Tephritidae on all collected fruit species (Vargas *et al.*, 1993).

This discrepancy between expectations and field observations may be explained in terms of information known about its behaviour. The present study shows that newly emerged parasitoids present a large set of innate responses, and Dukas & Duan (2000) show that the innate response of *F. arisanus* females to fruit odour can be modified by rewarding experiences. *Fopius arisanus* has evolved in a tropical environment, with numerous different suitable resources (host and host plants) having different phenologies. According to the resources available at the time of their emergence, some of these innate responses may so be reinforced by associative learning (Vet & Dicke, 1992; Vet *et al.*, 1995). The generalist foraging behaviour shown by naive wasps in our experiments could therefore evolve with experience towards a more specialized one. In a heterogeneous environment, it is assumed that distinctly specialized populations of *F. arisanus* coexist, temporally structured by their dispersion abilities and the spatial distribution of resources (Horner *et al.*, 1999; Steidle & Van Loon, 2003). Snowball & Lukins (1964) and Snowball (1966) report clear preferences of *F. arisanus* for some fruits in the field in Australia, but note that these preferences were variable in time and space. From this point of view, the natural populations of *F. arisanus* might be a complex of different oligophagous subpopulations giving the false impression of a large polyphagy in the species. The confirmation of such a hypothesis requires future studies on the specificity and individual preferences of parasitoids collected in the wild.

Acknowledgements

We thank F. Gourdon and C. Simiand for maintaining the parasitoid colony, as well as J. Payet and S. Glénac for rearing the flies. Many thanks to R. Ceniceros and T. Hue (GRDSR) for providing the *S. calcitrans* eggs. We are grateful to S. Nibouche, and L. Kaiser for their advice and suggestions. This work was supported technically by F. Sauveur (MS400) and funded by the Conseil Régional de La Réunion and the European Union.

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Accepted 7 March 2007