



History Matters: Oviposition Resource Acceptance in an Exploiter of a Nursery Pollination Mutualism

Pratibha Yadav¹ · Sathish Desireddy¹ · Srinivasan Kasinathan^{1,2} · Jean-Marie Bessière³ · Renee M. Borges¹

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Abstract

In the fig–fig wasp nursery pollination system, parasitic wasps, such as gallers and parasitoids that oviposit from the exterior into the fig syconium (globular, enclosed inflorescence) are expected to use a variety of chemical cues for successful location of their hidden hosts. Behavioral assays were performed with freshly eclosed naive galler wasps. Syconia with different oviposition histories, i.e. with or without prior oviposition, were presented to wasps in no-choice assays and the time taken to the first oviposition attempt was recorded. The wasps exhibited a preference for syconia previously exposed to conspecifics for oviposition over unexposed syconia. Additionally, syconia exposed to oviposition by heterospecific wasps were also preferred for oviposition over unexposed syconia indicating that wasps recognise and respond to interspecific cues. Wasps also aggregated for oviposition on syconia previously exposed to oviposition by conspecifics. We investigated chemical cues that wasps may employ in accepting an oviposition resource by analyzing syconial volatile profiles, chemical footprints left by wasps on syconia, and syconial surface hydrocarbons. The volatile profile of a syconium is influenced by the identity of wasps developing within and may be used to identify suitable host syconia at long range whereas close range preference seems to exploit wasp footprints that alter syconium surface hydrocarbon profiles. These cues act as indicators of the oviposition history of the syconium, thereby helping wasps in their oviposition decisions.

Keywords Aggregation · Fig wasps · Footprints · Host acceptance · Pollinator · Hydrocarbons · Volatile organic compounds · Herbivore-induced plant volatiles (HIPVs) · Floral volatiles · (*E*)- β -ocimene · Methyl salicylate

Introduction

A nursery pollination mutualism where pollinators breed inside the inflorescence that they pollinate (Sakai 2002) is often subjected to exploitation by non-pollinators that utilize host plants for their own breeding (Borges 2015a). Attraction of pollinators in such systems is facilitated by chemical signals released from the host plant in the form of volatile organic compounds (VOCs) (Svensson et al. 2006; Chen et al. 2009; Proffitt et al. 2009; Borges 2015b, 2016); however, host location by exploiters of such mutualisms has rarely been investigated (Hossaert-McKey et al. 2010). An insect's search for

oviposition resources is often a highly complex process (Carrasco et al. 2015) that is executed efficiently using several cues and signals (Bernays 2001; Davis and Stamps 2004; Bruce et al. 2005; Riffell et al. 2013) and can be assumed to involve four hierarchical steps, viz. host habitat location, host location, host assessment and host acceptance. Insects use volatiles at longer distances to locate the habitat and the host itself (Proffitt et al. 2007; Hansson and Stensmyr 2011) whereas compounds of low volatility, surface hydrocarbons, and chemical footprints are exploited for close range host location and host assessment (Ozaki et al. 2005; Rostás et al. 2008; Peri et al. 2013). The final behavioural outcome in an individual insect after processing these various inputs can be additionally affected by extrinsic factors such as host availability and predation risks (Rieger et al. 2004; Hwang et al. 2008).

Once located, an insect's decision to use the available oviposition resource can be affected by the presence of other individuals, whether conspecific or heterospecific. Phytophagous insects such as gallers sometimes tend to join conspecifics for the benefits of feeding when feeding efficiency increases with the number of galler larvae resulting in a component Allee

✉ Renee M. Borges
renee@iisc.ac.in

¹ Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India

² NCF Rainforest Research Station, Coimbatore 642127, India

³ Ecole Nationale Supérieure de Chimie, Montpellier, France

effect (Stephens et al. 1999; Kramer et al. 2009). On the other hand, gravid females may refrain from joining conspecifics at an oviposition site to reduce larval competition (Nufio and Papaj 2001; Prokopy and Roitberg 2001). This joining or avoidance of hosts that are occupied or have been previously exploited by conspecifics is often mediated by cues or signals associated either with eggs or the act of oviposition such as oviposition marking pheromones or chemical footprints (Hemptinne et al. 2001; Nufio and Papaj 2001, 2004; Saleh et al. 2007). Heterospecifics that share similar habitat requirements, however, may also yield important information. Use of resource suitability information based on the presence of heterospecifics, especially if they are not competitors for oviposition resources, may yield benefits with fewer costs (Miller et al. 2013). Unlike conspecifics that provide social cues, heterospecifics may provide non-social cues. To our knowledge, there are very few studies that have investigated the role of heterospecific cues in decision-making during host acceptance behavior (Shiojiri et al. 2002; Miller et al. 2013).

Host location becomes even more complex for phytophagous insects whose oviposition sites are concealed such as for non-pollinating fig galls. The fig–fig wasp system, with its species-specific non-pollinating wasps, provides an excellent system to understand host location behavior and mechanism in specialist parasitic herbivores of a nursery pollination mutualism. The non-pollinating fig wasps (NPFWs) that oviposit from outside the enclosed globular inflorescence (syconium) with the help of their long ovipositors can be gallers (induce gall formation in the flowers in which they lay their eggs), inquilines (feed on the nutritive tissue while the host galler is starved to death), or parasitoids (kill the galler host to complete their own development) (Jousselin et al. 2008; Borges 2015a). Since NPFWs oviposit from the outside, their hosts (flowers or galls containing galler larvae) are hidden inside the syconium and are accessed with the help of their ovipositor by drilling through syconium tissues; the ovipositor, therefore, becomes the major sensory organ involved in locating the suitable host flower (Yadav and Borges 2017a). However, drilling is an expensive process and being time-consuming can also subject wasps to predation risks from ants (Ranganathan et al. 2010; Ghara et al. 2011); therefore, an NPFW has to find the most acceptable syconium before it starts penetrating through the syconium wall.

The specificity of fig wasps for oviposition into syconia has been investigated up to the level of the developmental stages of syconia (Proffit et al. 2007; Ranganathan et al. 2010). However, there could be variation in syconia of the same developmental stage due to differences in their oviposition history, i.e. previous oviposition into the syconium. Whether the oviposition history of a syconium influences syconium acceptance, and the mechanism for identifying such suitable syconia or rejecting unsuitable ones, have not been previously

investigated. In order to understand oviposition resource acceptance behavior in non-pollinating galls of *Ficus racemosa*, we investigated (a) if these galls discriminate between fig syconia that differ qualitatively (presence of ovipositing conspecific or heterospecific wasps) and quantitatively (varied numbers of previously ovipositing wasps) in their oviposition history, (b) if the acceptability of syconia for oviposition is confined to regions of a single syconium (considering a syconium to be a resource patch), or to the whole syconium, and (c) the possible role of volatiles, syconium surface hydrocarbons and wasp chemical footprints in identification of suitable syconia.

Methods and Materials

Study System Experiments were conducted using *Ficus racemosa* trees within the campus of the Indian Institute of Science, Bangalore, India. The phenology of *F. racemosa* can be divided into five stages (Ranganathan et al. 2010; adapted from Galil and Eisikowitch 1968): A—pre-pollination phase; B—pollen receptive phase; C—interfloral phase; D—wasp dispersal phase; and E—seed dispersal phase. There is one specific pollinator species, *Ceratosolen fusciceps*, whose progeny develops in some of the flowers at the expense of seeds. This fig species hosts six specific NPFWs (*Sycophaga stratheni*, *Sycophaga testacea*, *Sycophaga fusca*, *Sycophaga agransensis*, *Apocrypta* sp. 2, and *Apocrypta westwoodi*) that attack syconia at different stages of its development and differ in their oviposition windows (Ranganathan et al. 2010; Fig. 1). Although NPFWs arrive for oviposition at different stages of syconial development (Wang and Zheng 2008; Ranganathan et al. 2010), they all need to complete their development at the same time in order to exit synchronously with the pollinators since usually only pollinator males cut an exit in the otherwise sealed syconium (Cook and Rasplus 2003; Herre et al. 2008; Suleman et al. 2012). For this reason and also because individual larvae are immobile, confined within single galls, and cannot exploit resources indiscriminately within syconia that may contain hundreds to thousands of galls and seeds, NPFWs have to make very precise oviposition decisions. *Sycophaga stratheni*, *S. testacea*, and *S. fusca* are non-pollinating galls whereas *Apocrypta* sp. 2, and *A. westwoodi* are parasitoids of galls with varying host specificity (Yadav and Borges 2017b). In this manuscript, we report only on experiments conducted with the most abundant non-pollinating galler *S. fusca* whose arrival time overlaps with that of the pollinator and has a slightly longer oviposition window (Ranganathan et al. 2010; Fig. 1). The heterospecific wasp in this set of experiments was the pollinator *C. fusciceps*. Syconia that do not receive pollination services and thereby are not also subjected to oviposition by the pollinator are usually aborted. Therefore, the presence or absence of the pollinator is crucial for these experiments. Syconia in suitable

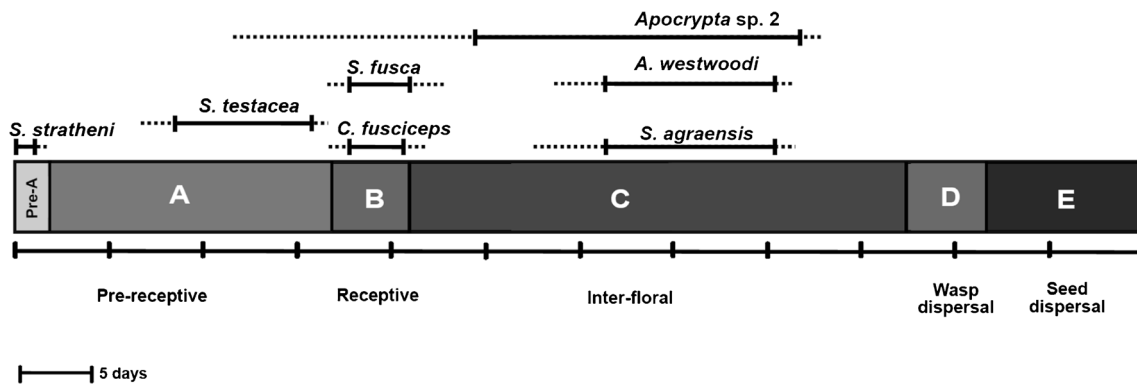


Fig. 1 Wasp arrival sequence for oviposition across syconium development phases (A–E) in *Ficus racemosa* (adapted from Ranganathan et al. 2010). Solid lines represent top 50% of wasp arrival while dotted lines represent the rest

developmental stages and freshly emerging wasps in D-phase for all experiments were collected between 10 am and 12 pm.

Behavioural Assays. Acceptability of Syconia Differing Qualitatively in Oviposition History Three experimental set-ups were used: (a) receptive-phase figs unexposed to oviposition by wasps (ensured by bagging of syconial bunches in primordia stage to ensure that no oviposition occurred; this set-up was used as a control), (b) pollen receptive-phase figs exposed to oviposition by *C. fusciceps* (pollinator) only, and (c) pollen receptive-phase figs exposed to oviposition by *S. fusca* only. Naive *S. fusca* wasps (with no prior oviposition experience) freshly emerging out of fig syconia were used for the oviposition experiment. Receptive-phase figs (B-phase) were collected from the experimental bunch on the fig tree not more than 30 min before starting the experiment. The latex exuding from the cut pedicel of the syconium was wiped off with tissue paper and the cut end was sealed with parafilm at the time of collection. The experiment was conducted under red light to prevent any visual cues from interfering with the experiment. The experimental set-up had three fig syconia (from the same bunch) placed in a glass chamber. Four female wasps were then released into the set-up and observed for 30 min; 4–5 wasps was the maximum number that an observer could individually track during the behavioral experiments because these wasps are too tiny to be marked. The time taken to the first oviposition attempt was recorded. The same procedure was repeated for the other two set-ups. The experiment was repeated six times.

Behavioural Assays. Acceptability of Syconia Differing Quantitatively in Oviposition History A varied number of *S. fusca* wasps (200–700) were released per bunch of receptive phase fig syconia into bagged bunches with 10–20 figs to achieve a desired average number of wasps (10/15/20 ovipositing wasps) per syconium (Table 1). Wasps were allowed to oviposit for 24 h. These syconia were then

collected, wasps removed, and a new batch of five freshly enclosed naive wasps was released into the experimental set-up as described for the first experiment. The time taken to the first oviposition attempt and syconium preference, if any, were noted. This experiment could be performed only once per treatment owing to the unavailability of the large number of wasps required for each treatment.

Behavioural Assays. Acceptability of Regions Differing in Oviposition History Within a Syconium We consider a syconium to be a resource patch filled with hundreds to thousands of uniovulate flowers each of which can be individually galled, and therefore such a patch is divisible into regions. To investigate whether regions of an individual syconium can become unacceptable based on prior oviposition within that region, the following experiment was conducted. Receptive phase figs were collected from a bunch of fig syconia previously exposed to pollinators (control). In a group of five syconia, each syconium was entirely covered with aluminum foil except for a circular region 0.75 cm in diameter that was left exposed to allow oviposition access (Fig. 2). Ten *S. fusca* wasps were allowed to exploit the exposed region for oviposition for 30 min; this number was a compromise between enhancing the probability of oviposition within the exposed patch and reducing competition between ovipositing wasps. The wasps were then removed and another circular region of the same diameter was made available on each syconium diametrically opposite to the original one, with the original exposed area still left exposed. Four freshly enclosed naive *S. fusca* wasps were then released on these fig syconia (Fig. 2). The time taken to the first oviposition attempt and the number of wasps on each region were noted. This experiment was repeated thrice.

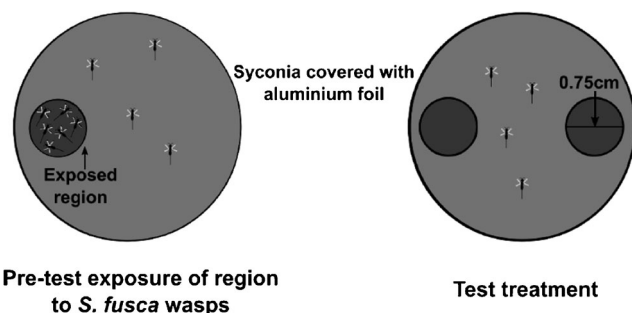
Volatile Organic Compounds (VOCs) of Syconia We collected and analyzed VOCs released by receptive-phase syconia subjected to three treatments: (a) unexposed syconia, (b) syconia exposed only to pollinators, and (c) syconia exposed only to *S. fusca* ($N = 3$ trials for each treatment; exposure for 24 h). A

Table 1 Number of *S. fusca* wasps released per bunch of receptive phase fig syconia to achieve the following desired number of wasps per syconium

Bunch	Number of figs	Number of wasps released	Average number of wasps/fig
1	15	150	10
2	20	300	15
3	35	700	20
4	18	360	20

single syconium in each treatment was enclosed in a polyethylene terephthalate bag (Nalophan® [KalleNalo GmbH, Würsthüllen, Germany]) *in situ* on the tree. A 5 mm long PDMS tube (1 mm i.d.*1.8 mm o.d., Carl Roth) was placed inside and left for 2 h for passive volatile collection. Samples were analyzed by thermal desorption gas chromatography-mass spectrometry (TD-GC-MS) using a GC-MS instrument (Agilent-HP GC model 6890 N, MS model 5973 N). The gas chromatograph was equipped with an HP 5-MS column (60 m × 0.25 mm × 0.25 μm). Trapping tubes were placed in the injector equipped with a Markes thermal desorption unit (Unity²). The instrumentation and temperature programs were as follows. Electronic flow control was used to maintain a constant helium carrier gas flow of 1.0 ml/min. The GC oven temperature was held at 40 °C for 5 min, then increased by 5 °C/min to 180 °C followed by an increase by 30 °C/min to 280 °C. The GC-MS data were processed using the Agilent Chemstation software package. Chemical identification was carried out by matching the mass spectra with the NIST 2.2 spectra library and compound retention time. For each sample run, we estimated the total quantity of VOCs identified and calculated proportional abundance of each compound in the total volatile content.

Wasp Footprint Analysis We collected and analyzed chemical footprints of the aggregating galler *S. fusca*. A total of 60, 1-day old, naive wasps was released and left for 4 h in a transparent plastic chamber whose sides were covered with black paper with a glass lid on the top. Light was provided from above to ensure

**Fig. 2** Experimental set-up to investigate the acceptability of regions within a syconium for oviposition

movement of wasps on the lid owing to their known phototactic response. This was done to ensure that wasps left footprints on the glass. The glass lid was then removed and washed with *n*-pentane for 1 min ($N=8$). Samples were analyzed by the same GC-MS equipment as for the volatile analysis. The instrumentation and temperature programs were as follows. Electronic flow control was used to maintain a constant helium carrier gas flow of 1.0 ml/min, the GC oven temperature was held at 150 °C for 3 min, then increased by 2 °C/min, 1.9 °C/min, 1.8 °C/min, 1.7 °C/min, and 1.6 °C/min to 170 °C, 190 °C, 210 °C, 230 °C, and 250 °C respectively and was maintained at the final temperature for 30 min. The GC-MS data processing, chemical identification and volatile abundance calculation were done as for the volatile analysis mentioned above.

Syconium Surface Hydrocarbons We collected and analyzed surface hydrocarbons of receptive phase syconia exposed to *S. fusca* for 24 h (10 wasps/syconium on average) and compared them with surface hydrocarbons of unexposed syconia, which were in pollen-receptive phase. For each treatment, five syconia were collected and washed with *n*-pentane for 1 min ($N=3$). Samples were analyzed as described for footprints. The instrumentation, temperature programs, and data processing were the same as those for wasp footprints.

Statistical Analysis Data were examined for normality using the Shapiro-Wilk test. Student's *t*-tests were performed on normally distributed data and Kruskal-Wallis one-way ANOVAs were used to determine the significance of difference between treatments for non-normally distributed data. Mann-Whitney *U* tests were used for pairwise comparisons. Bonferroni corrections were applied. All tests were carried out using RStudio Version 0.99.902 for Linux.

Using the 'vegan' package (Oksanen et al. 2013), the volatile profile (relative proportions of all VOCs) of syconia with different oviposition histories were compared using non-metric multi-dimensional scaling (NMDS) based on the Bray-Curtis distance index (Bray and Curtis 1957) to calculate the data matrix of pairwise comparisons among samples. Prior to this, data were standardized using a Wisconsin double standardization. The null hypothesis of no difference in patterns of volatile profiles between groups of syconia was tested for significance using permutational multivariate analysis of variance (PERMANOVA) by employing the function 'adonis' in the 'vegan' package with the Bray-Curtis similarity measurement and 999 permutations.

Results

Behavioural Assays. Acceptability of Syconia Differing Qualitatively in Oviposition History The time taken to the first oviposition attempt on syconia exposed to *S. fusca* was significantly lower (~5 min) as compared to those exposed to

pollinators (~12 min) or to completely unexposed figs (>30 min) (*Kruskal-Wallis test*, $\chi^2 = 59.67$, $N = 24$, $P < 0.0001$, Fig. 3a). This indicates that the wasps preferred figs exposed to conspecifics over heterospecifics or to completely unexposed figs (*Mann-Whitney tests*: Unexposed versus *S. fusca*-exposed: $W = 33.5$, $N = 24$, $P < 0.01$; Unexposed versus Pollinator-exposed: $W = 0$, $N = 24$, $P < 0.01$; Pollinator-exposed versus *S. fusca*-exposed: $W = 0$, $N = 24$, $P < 0.01$). Wasps also showed aggregation behavior during the experiment (Fig. 3b). An oviposition attempt by the first wasp attracted other wasps on to the same figs.

Behavioural Assays. Acceptability of Syconia Differing Quantitatively in Oviposition History *Sycophaga fusca* wasps did not attempt oviposition in syconia that were previously exposed to >20 wasps ($N = 15$). The time taken to the first oviposition attempt by naive wasps on syconia that had ≤ 15 wasps per syconia was similar to that for exposed syconia in the first experiment (5.1 ± 1.2 min, mean \pm sd, $N = 18$). Wasps did not show any gradual change in acceptance time over the range of wasp numbers that we tested. They either accepted

the offered syconium readily (exposure to ≤ 15 wasps) or not (exposure to >20 wasps) for the observed duration of experiment (maximum 45 min after which the experiment was concluded owing to possible changes in the volatile profile of collected syconia).

Behavioural Assays. Acceptability of Regions Differing in Oviposition History Within a Syconium *Sycophaga fusca* wasps did not show any preference or avoidance for the region within a syconium already exploited for oviposition (*Mann-Whitney test*: $W = 50$, $N = 10$, $P = 0.49$, Fig. 3c).

Volatile Organic Compounds (VOCs) of Syconia The volatile profiles of syconia with different oviposition histories showed quantitative and qualitative differences (*PERMANOVA*, $F_{2,8} = 4.9$, $P = 0.005$; Table 2) with some overlaps. A total of 17 compounds comprising of monoterpenes, sesquiterpenes, aliphatics, fatty acid derivatives and shikimic acid derivatives were identified. Unexposed syconia showed high proportions of benzyl alcohol whose levels declined after oviposition. (*E*)- β -ocimene, on the other hand, peaked after oviposition by either of the concurrently

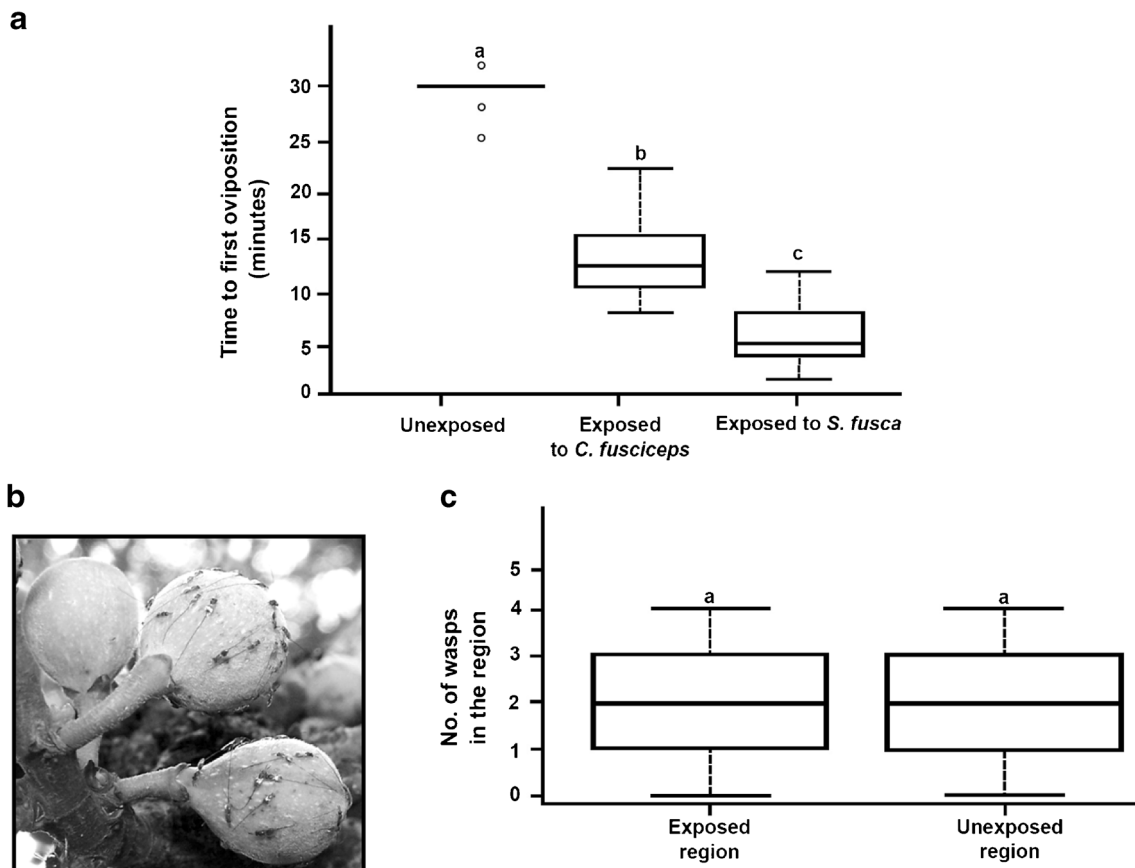


Fig. 3 Acceptance of syconia with different oviposition histories by freshly eclosed naive *S. fusca* wasps. **a** Time of acceptance of receptive phase syconia with different oviposition histories offered to freshly eclosed *S. fusca*. **b** *S. fusca* wasps aggregating during oviposition. **c**

Number of freshly eclosed naive *S. fusca* wasps on the exposed and unexposed region of the same receptive phase syconium. Different alphabets denote statistical significance at $\alpha = 0.05$

Table 2 Proportional abundance of individual compounds from volatile profiles of differentially exposed syconia

Compounds	RI ^a	Unexposed % abundance (mean ± sd)		<i>C. fusciceps</i> -exposed % abundance (mean ± sd)		<i>S. fusca</i> -exposed % abundance (mean ± sd)	
Aliphatics							
(<i>Z</i>)-3-hexenol	836	4.71	4.7	11.69	19.93	0.57	0.75
Nonanal	1104	0.92	0.63	1.22	1.31	1.94	2.48
Decanal	1205	0.54	0.48	5.25	6.51	2.63	2.39
Total percent		6.17		18.16		5.14	
Fatty acid derivatives							
(<i>Z</i>)-3-hexenyl acetate	1009	51.97	2.16	20.17	10.28	34.41	20.46
Total percent		51.97		20.17		34.41	
Monoterpenes							
β-pinene	900	0.08	0.13	1.09	0.94	0.23	0.39
α-pinene	928	0.40	0.36	3.92	6.28	1.05	1.81
3-carene	1005	1.58	2.39	3.24	2.81	0.03	0.049
Limonene	1025	0.25	0.12	2.57	2.25	1.34	0.18
(<i>E</i>)-β-ocimene	1048	11.61	12.46	28.38	19.09	32.03	15.42
Linalool	1100	0	0	0	0	3.15	4.84
Total percent		13.92		39.20		37.83	
Sesquiterpenes							
Copaene	1370	0	0	2.54	3.77	3.70	6.35
β-caryophyllene	1424	1.54	1.4	2.02	3.09	3.64	6.3
Total percent		1.54		4.56		7.34	
Shikimic acid derivatives							
Anisole	914	1.84	1.19	2.64	3.72	3.20	4.81
Benzaldehyde	993	1.19	1.11	1.02	1.04	0.59	0.56
Benzyl alcohol	1043	15.54	8.56	1.38	2.18	0.49	0.45
Methyl salicylate	1189	1.36	0.27	0.85	0.71	9.81	10.52
Total percent		19.93		5.87		14.09	
Miscellaneous							
6-methyl-5-hepten-2-ol	955	6.47	4.87	12.07	17.67	1.19	1.43
Total percent		6.47		12.07		1.19	

^a RI: Kovats Retention Index

ovipositing species, *C. fusciceps* or *S. fusca*. While syconia exposed to only the pollinator *C. fusciceps* showed high abundance of (*Z*)-3-hexenol, only *S. fusca*-exposed syconia showed a higher proportional abundance of methyl salicylate.

Wasp Footprint Analysis The extracts of *S. fusca* footprints consisted largely (>90%) of a homologous series of saturated alkanes ranging from pentacosane (*n*-C₂₅) to dotriacontane (*n*-C₃₂) with the monomethyl branched alkane 5-methyl nonacosane (5MeC₂₉) being the most abundant (Fig. 4b). Some alkenes, alcohols and four unidentified compounds were also present in minor quantities among a total of 15 distinctively identifiable peaks (Fig. 4a).

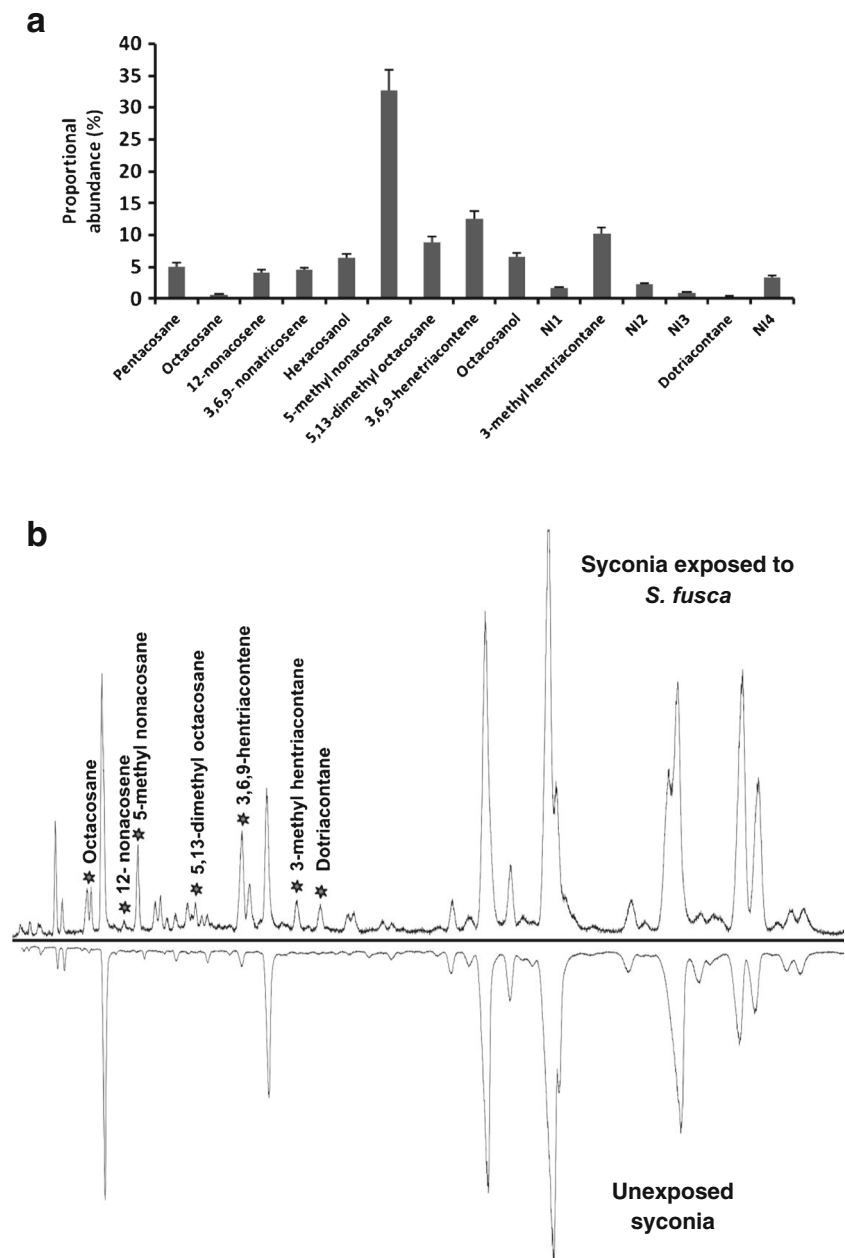
Syconium Surface Hydrocarbons The hydrocarbon profile of unexposed syconia and those exposed to oviposition by *S. fusca*

differed in that *S. fusca* footprint compounds were additionally present in the profile of exposed syconia (Fig. 4b).

Discussion

The externally ovipositing non-pollinating galler *S. fusca* reacted to syconia with different oviposition histories in making decisions to oviposit and joined conspecific aggregations on syconia. Syconia with varied oviposition histories differed in their volatile profiles that may be used by wasps for differentiating between syconia before landing on them for oviposition. Syconial surface hydrocarbons as host plant cues and chemical footprints as conspecific cues may also be used in order to assess and utilize the most acceptable host syconium after alighting on it.

Fig. 4 a Histogram showing proportional abundance of different compounds in footprint extracts of *S. fusca* arranged by retention time. Error bars represent standard error ($N=8$). NI = Not identified. **b** Chromatograms comparing SHC profile of syconium exposed to *S. fusca* (top) with that of unexposed syconium (below). * = compounds within *S. fusca* footprints



Acceptability of Syconia Differing Qualitatively in Oviposition History Up to now, in the highly specific community of fig wasps, the only known preferences for oviposition were for specific phenological phases of syconia (Hossaert-McKey et al. 1994; Grison-Pigé et al. 2002; Proffit et al. 2007; Ranganathan et al. 2010). Many insects prefer hosts exposed to oviposition by conspecifics since this enhances mate availability, resource utilization, defense against predators, and may also help to overcome host plant defenses (Aukema and Raffa 2004; Desurmont and Weston 2011; Woodbury and Gries 2013). The preference exhibited by *S. fusca* for syconia of the appropriate phenological stage that were previously exposed to oviposition by conspecifics, that we demonstrated

in this study, can be attributed to the increased probability of finding mates for offspring since mating takes place only within the syconium (Weiblen 2002). Further, as mentioned earlier, there are very few studies that have investigated the role of heterospecific cues in oviposition choice in insects (Shiojiri et al. 2002; Michaud and Jyoti 2007; Miller et al. 2013). The preference of *S. fusca* wasps for syconia exposed to oviposition by the heterospecific pollinator wasp *C. fusciceps* over unexposed syconia is possibly explained by the fact that non-pollinated syconia carry a high risk of being aborted by the tree since they will not produce any seeds (Jandér et al. 2012). The presence of pollinator eggs within a syconium might provide two types of information to *S. fusca*: it increases the

probability that syconium development will go to completion and at the same time makes these syconia preferable to other conspecifics. Therefore, the benefits of combined oviposition by heterospecific and conspecific wasps may overcome the costs associated with interspecific competition for resources within syconia. However, the relative fitness cost to *S. fusca* of laying eggs in an unpollinated syconium versus a syconium without conspecific eggs are expected to be different. Being the first *S. fusca* individual to lay eggs in an unpollinated syconium may result in complete clutch loss if the syconium is aborted. On the other hand, since these wasps have a haplodiploid breeding system, they could lay unfertilized eggs resulting in male offspring; therefore, oviposition in a pollinated syconium in which conspecifics have not oviposited could result in sons mating with daughters under a scenario of reduced inbreeding depression (Henter 2003; Charlesworth and Willis 2009; Greeff et al. 2009; Boulton et al. 2015). The age and egg load of an individual will obviously dictate these choices. A freshly eclosed female can afford to be choosier and more risk-taking whereas a female towards the end of its lifespan may accept a less-suitable host based on her remaining egg load (Minkenberg et al. 1992; Rosenheim et al. 2008; Yadav and Borges, unpublished data).

Acceptability of Syconia Differing Qualitatively in Oviposition History

The low oviposition response of *S. fusca* to syconia earlier exposed to oviposition by >20 *S. fusca* wasps may be the result of more than one factor acting simultaneously. The increased probability of finding mates for their offspring due to the presence of *S. fusca* eggs increases the suitability of a syconium with prior oviposition; however, occupancy of oviposition sites by a large number of conspecifics might result in crowded syconia and therefore a reluctance to oviposit. A syconium in *F. racemosa*, with ~2500 flowers inside, offers sufficient resources for many individuals to lay their full egg complement (each *S. fusca* individual ecloses with ~120 eggs; Ghara and Borges 2010). However, drilling into the syconium is an expensive process that also entails predation risks (Ranganathan and Borges 2009; Ranganathan et al. 2010; Ghara et al. 2011); spending energy in drilling through one syconium to lay only a few eggs (if oviposition sites are occupied by prior visits by conspecifics or heterospecifics) would entail loss of time and resources when wasps can afford to lay several eggs in a single oviposition attempt in a syconium that bears a sufficient number of empty flowers. In this experiment, it is safe to assume that wasps lay the maximum possible complement of their eggs in the available syconium since *S. fusca* has 100% accessibility to oviposition sites within a syconium at the offered B-phase (Ghara et al. 2014) and these wasps are restricted to syconia offered in the experimental set-up. As a result, naive wasps exhibit a reluctance to oviposit in such syconia that are expected to be over-exploited (those into which >20 wasps have already oviposited).

Acceptability of Regions Within a Syconium Differing in Oviposition History Joining and avoidance of a site/patch previously used by conspecifics have been studied in many insect species (Prokopy and Roitberg 2001; Rohlf and Hoffmeister 2004; Wertheim et al. 2005). In our study, wasps clearly exhibited preferences for conspecific-exposed over unexposed syconia. However, they did not show any preference or avoidance for the already exploited region within a syconium. As mentioned earlier, a single syconium can contain up to ~2500 flowers and therefore provides abundant resources for an ovipositing female. The first visiting female can ideally exploit/use all the flowers within the reach of her ovipositor in a single drilling attempt owing to the flexible nature of the ovipositor that can bend, flex, and exhibit complex trajectories within the syconium lumen (Ghara et al. 2011; Yadav and Borges 2017b). However, it is likely beneficial for the female to distribute her eggs over a number of patches (syconia in this case) when resources are patchily distributed and there is competition with coexisting species (Root and Kareiva 1984; Chesson 2000; Campbell and Runnion 2003; Mitsui et al. 2006). It is, therefore, not surprising that not all flowers within a syconium, or even within the same local area of the syconium, are exploited by ovipositing females. Consequently, several females could still exploit the same local region of the syconium.

Mechanisms Used by Wasps to Identify Acceptable Syconia.

Changes in VOC Profile The VOC profile of *F. racemosa* and other *Ficus* species changes dynamically through the phases of syconial development (Grison-Pigé et al. 2002; Proffit et al. 2007, 2008; Ranganathan and Borges 2009; Hossaert-McKey et al. 2010; Borges et al. 2013). However, change in syconial volatile profile within a development phase due to oviposition by different species has not been investigated in any *Ficus* species. Suppression of emission of certain volatiles and a change in the ratio of constituents of the floral blend may occur post-oviposition in several flowering plants (Bruce et al. 2005; Peñaflor et al. 2011). Syconia exposed to oviposition by *S. fusca* or pollinators emitted a burst of (*E*)- β -ocimene, which is a common herbivore-induced plant volatile (HIPV) (Dicke and Baldwin 2010; Borges et al. 2013). These syconia also emitted an extremely low proportional abundance of benzyl alcohol compared to unexposed syconia indicating that a change in volatile profile may be used to differentiate an exposed syconium from a completely unexposed syconium but does not provide information about the ovipositing species. This could be due to a similar mechanism of oviposition by the two concurrently ovipositing species in this study and is probably used as a first-level cue in the hierarchy of events in finding the most acceptable host. A volatile blend can elicit a more specific and stronger response than individual compounds constituting the blend indicating

that insects are sensitive to combinations of cues (Webster et al. 2010; Riffell et al. 2013). It is possible that the absence of benzyl alcohol along with the presence of (*E*)- β -ocimene in the syconial volatile profile is used as a cue to differentiate exposed syconia from unexposed syconia. The relatively higher abundance of (*Z*)-3-hexenol in the volatile profile of pollinator-exposed syconia and of methyl salicylate in *S. fusca*-exposed syconia may also perhaps be used to distinguish the oviposition history of an exposed syconium. It is possible that increased proportional abundance of HIPVs such as methyl salicylate in *S. fusca*-exposed syconia is due to greater damage caused by *S. fusca* by mechanically drilling through the syconium wall, or by the presence of the egg itself that results in oviposition-induced plant volatiles (OIPVs) (Fatouros et al. 2012). Flight assays with these volatile compounds as stimuli would need to be performed to investigate this possibility.

Mechanisms Used by Wasps to Identify Acceptable Syconia.

Chemical Footprints Chemical trails to facilitate aggregation have been studied in several insects (Prokopy and Duan 1998; Saleh et al. 2007). The footprint extract analysis showed chemical signatures left by *S. fusca* wasps on the surface of figs that they walked upon, with 5-methyl nonacosane (5-MeC₂₉) being the most abundant compound. Hydrocarbons ranging from C₁₅ to C₃₂ are abundantly present in the footprint profile of insects and can last for days without being degraded (Rostás and Wölfling 2009). 5-methyl nonacosane is highly stable (m.p. 442 K and b.p. 885 K), hydrophobic (log O/W coefficient 11), is expected to have a long half life, and may provide a long-lasting chemical cue to visiting wasps with information about previous visits to the syconium by conspecifics.

Understanding Aggregation Behavior in *S. fusca* Chemical footprints and volatiles may be used simultaneously to facilitate the aggregation that we observed in *S. fusca* wasps. There are two possible explanations behind this aggregation behavior: predation dilution (Aukema and Raffa 2004; Wertheim et al. 2005) and the component Allee effect (Stephens et al. 1999). Aggregation could be a strategy to dilute predation since all the externally ovipositing NPFWs face attack by aggressive predators such as *Oecophylla smaragdina* ants (Ranganathan et al. 2010). In addition to predation dilution, aggregation for oviposition may also decrease the per capita risk of parasitism faced by *S. fusca* offspring developing within the same syconium. A component Allee effect might not seem to be acting at first since the developing larvae are individually confined inside each galled flower. However, it is also known that host sanctions occur in figs, i.e. syconia that do not receive sufficient pollen or that are over-exploited by wasps are aborted; these sanctions occur at the level of the syconium (Jandér et al. 2012). Conversely, since syconia act

as a nutrient sinks owing to the growth demands of seeds and wasps (Krishnan and Borges 2014), the phenomenon of aggregation by joining conspecifics in ovipositing into the same syconium may benefit offspring by drawing more nutrition to the syconium. However, excessively active sinks (syconia) as a result of over-exploitation may provide an abortion signal to the fig tree and result in loss of reproductive success. This phenomenon could restrict the exploitation of individual syconia and thus explain the response of wasps to syconia that have been exposed to oviposition by >20 wasps.

This is one of few studies (e.g. Braccini et al. 2015; Mathis and Tsutsui 2016) that has examined behavior and the cues that are likely perceived at different levels in the hierarchical processes of host selection for oviposition by a single insect species. While insects are faced with vast amounts of information, only some cues are likely used to obtain relevant information as suggested by *S. fusca*'s utilization of volatile and surface cues in assessing syconial quality before oviposition. Host selection by phytophagous insects is not merely finding the best quality plant or plant part for reproduction and development; it is also affected by factors outside the host plant–herbivore context especially in a system where multiple species use the same resource as evident from *S. fusca*'s response to host syconia that had been exposed to heterospecifics for oviposition. A multiplicity of factors, as revealed by the oviposition history of the oviposition resource, interact to determine host choice.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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