

Signalling receptivity: Comparison of the emission of volatile compounds by figs of *Ficus hispida* before, during and after the phase of receptivity to pollinators

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(Received February 20, 2007; Accepted May 31, 2007)

Abstract

Figs and pollinating fig wasps are involved in highly specific mutualisms. Because associations between figs and their pollinating wasps are horizontally transmitted, partner encounter is a crucial step, and is mediated by the emission by receptive figs of the volatile compounds that are detected by the pollinator. However, pollinator attraction is probably not the only function of the volatile compounds produced by figs. Other likely functions include signalling to wasps that a fig has already been pollinated, and deterring or defending against visitors with negative effects on developing figs or the pollinators they contain. The functions of volatile compounds will also change over the course of fig development, and the composition of the odour bouquet is thus also likely to vary. However, this variation and its likely functional importance have rarely been studied. To address these questions, we investigated changes in the composition of the bouquet of volatile compounds, and in the rate of emission of odour components, before, during and after the phase of receptivity to pollinators in *Ficus hispida*, in Yunnan (China). This first study of the dynamics of variation in signal composition throughout the cycle of fig development, provides support for the hypothesis of chemical mimicry in dioecious figs.

Keywords: Fig-fig wasp mutualism, chemical mediation, volatile organic compounds, temporal variation

1. Introduction

In horizontally transmitted highly specialised mutualisms, partner encounter is a crucial phase in the functioning of the interaction. These include symbiotic protection mutualisms between myrmecophytic plants and their resident plant-ants (Heil and McKey, 2003) and obligate nursery pollination mutualisms. The best-studied examples are the interactions between *Yucca* plants and yucca moths (Dufaÿ and Anstett, 2003) and those between figs and their wasp pollinators (Cook and Rasplus, 2003). In contrast to interactions between plants and herbivores, where plants may escape by their lack of apparency (Feeny,

1976), plants have an interest in signalling their presence to mutualist associates. Signalling by the emission of chemicals, located via olfaction or by contact chemoreception, has been shown to be important in advertising host location and the presence of rewards for the partner in both ant-plant protection mutualisms (Brouat et al., 2000) and nursery pollination mutualisms (Dufaÿ et al., 2003). However, the plant's interest in advertising its location may be restricted to certain times. For example, in pollination mutualisms, attracting the pollinator is only necessary prior to pollination. Following pollination, it may be beneficial to reduce attractiveness of the flower to pollinators, or to other potential visitors, such as herbivores or parasites of developing pollinators or seed predators. Another way to prevent visits of non-mutualists is to emit repellent or camouflage compounds that deter these

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enemies (Schiestl and Ayasse, 2001; Kessler and Baldwin, 2007). However, information on post-pollination changes in the composition and total amounts of volatile compounds is available for only few insect-pollinated species (Arditti et al., 1973; Tollsten and Bergström, 1989; Tollsten, 1993; Schiestl and Ayasse, 2001; Theis and Raguso, 2005; Muhlemann et al., 2006). In these studies, a common trend seems to be a strong quantitative decline of the whole bouquet after pollination and a change in the ratios of compounds in flower scents.

In the interactions between figs and their pollinating wasps, chemical mediation of partner encounter is already known (van Noort et al., 1989; Hossaert-McKey et al., 1994; Gibernau et al., 1998; Song et al., 2001; Grison-Pigé et al., 2002). All of the ~750 *Ficus* species are associated with at least one specific species of pollinating agaonid wasp, which also strictly depends on its host *Ficus* for reproduction. When the fig is receptive, female pollinating wasps, attracted by specific volatile signals emitted by the host fig (Grison-Pigé et al., 2002), penetrate into the fig cavity through the ostiolar bracts (entrance of the fig). Once inside the fig, female wasps pollinate the female flowers and lay eggs in the ovaries of some of them. After several weeks, their offspring emerge in the fig cavity and the new generation of female wasps leaves the fig.

It has been shown that chemical signals emitted by receptive figs appear to enforce specificity of the interaction (Grison-Pigé et al., 2002). As in other specific pollination systems, such as *Silene latifolia* (Muhlemann et al., 2006) or *Ophrys sphegodes* (Schiestl and Ayasse, 2001), we should also expect variation in both the total quantity of odour components emitted and the relative concentrations of the different compounds, once the fig has been pollinated.

This variation is expected for several reasons. First, figs have long-lived inflorescences. The long lifespan of flowers has been interpreted as an adaptation to highly specialized pollination mechanisms (Schiestl and Ayasse, 2000) in which there is often huge temporal variation in pollinator availability (Khadari et al., 1995). The duration of receptivity of figs to pollinators in the absence of wasp visitation is longer than one week for several *Ficus* species (Khadari et al., 1995; Patel and McKey, 1998). After a sufficient number of foundress wasps enter the fig, the attractiveness of the figs to pollinators stops (Khadari et al., 1995; Patel and McKey, 1998). Post-pollination changes in fig traits detected by pollinators (e.g., scent) may signal the closure of the ostiole and the cessation of rewards for the pollinators. In contrast to figs, pollinating fig wasps often live only a few hours. We expect that figs attract pollinators only during the precise period when they can be entered and their ovules fertilized. Also, at that time in their development, figs should emit a very strong signal. Receptivity should thus be marked by sharp peaks in the production of volatile compounds. The production of

volatile compounds by receptive figs has been shown to have low energetic cost (Grison-Pigé, 2001). Thus, in this system an induced change in quantities of volatile compounds produced and in their chemical composition, may be sufficient to advertise the end of the receptive period; complete cessation of the production of volatiles may not be necessary.

In addition, many non-pollinating wasp species, also highly specific, exploit fig pollination mutualisms and also use chemical cues to locate their hosts (Proffitt et al., 2007). These wasps, like pollinators, oviposit on figs at very precise stages of fig development (Kerdelhué and Rasplus, 1996; Peng et al., 2005). These non-pollinating wasps can discriminate among volatile chemicals emitted by figs at different developmental stages (Proffitt et al., 2007). Qualitative composition of the bouquet may thus be expected to change over time, according to its function (e.g., attraction of pollinators, defence against herbivorous insects; attraction of seed dispersal vectors). However, few studies have investigated changes in chemical mediation before, during and after the phase of receptivity, or shown how these changes influence behaviour of insects associated with figs (Song et al., 2001; Proffitt et al., 2007).

The objective of the present study was to characterize variation in quantity and composition (relative abundance, as well as qualitative differences) of volatile compounds emitted before, during and after receptivity (which corresponded here with pollination) in figs of *Ficus hispida*. We also wished to compare the bouquets of male and female figs in this dioecious fig species.

2. Materials and Methods

Study site and life history of the studied species

The study was carried out at Xishuangbanna Tropical Botanical Garden (101°15'E, 21°55'N), Yunnan province, China, between November and December 2005. *Ficus hispida* (section *Sycocarpus*) is a functionally dioecious species. In dioecious fig species, female trees, whose figs contain only female flowers, produce seeds while functionally male trees produce pollen (male flowers) and pollinators (in ovaries of gall flowers) (Janzen, 1979; Kjellberg et al., 1987). The pollinator, *Ceratosolen solmsi marchali* Mayr (Chalcidoidea, Agaonidae), like other pollinating fig wasps, is attracted specifically to trees (both sexes) of its host species by volatile signals emitted by receptive figs (Chen and Song, unpublished data). In the functionally male figs, the pollinator lays eggs in some ovaries of the gall flowers and pollinates only these flowers, so that no seeds are produced. In female figs, pollinators are unable to deposit eggs, and ovules of pollinated flowers develop into seeds. Three species of non-pollinating fig wasps (NPFW) are also specifically

associated with *F. hispida* at Xishuangbanna (*Philotrypesis pilosa* Mayr, *Philotrypesis* sp., and *Apocrypta bakeri* Joseph, identified by J.-Y. Rasplus [pers. comm.]). These wasps oviposit from outside male figs into ovaries containing pollinator eggs (Abdurahiman, 1986; Peng et al., 2005; Proffitt et al., submitted). Like the pollinators, females of NPFW are attracted to male trees of their host species by species-specific volatile signals emitted by receptive figs (Proffitt et al., 2007; Proffitt et al., submitted). After completing development, pollinators and NPFW mate inside the fig, then leave their natal fig in search of a receptive fig. Receptivity is thus a key period in fig development for the persistence of the fig-fig wasp mutualism. Mean duration of receptivity in *F. hispida* is the same in both sexes, 2.4 ± 0.2 weeks, if figs stay unpollinated (Patel and McKey, 1998).

Volatile collections

Collecting sufficient amounts of volatile for analysis can be a limiting factor in odour extraction, especially when individual figs are small and when figs in the appropriate stage are present in limited numbers on each plant. We conducted preliminary extractions to standardize our sampling protocol. In most of trees, for the different stages of development we studied, the number of figs per branch was too low to collect enough amount of volatile for each analysis. Thus because of the necessity to obtain concentrated extracts, odour collections were performed *ex situ*. In previously performed tests on several *Ficus* species, no significant differences were found in the blends of volatiles produced by figs *in situ* and *ex situ* (Grison-Pigé, 2001; M. Proffitt, unpublished data). To allow a precise comparison of changes in the composition of odour bouquets over time, and to compare male and female figs in this respect, we thus combined samples for a given developmental phase from several individuals of each sex, taking into account the inter-individual variation between trees (Borges et al., submitted). For each developmental phase, we pooled samples from an equivalent number of figs originating from four individual trees of each sex. Figs in pre-receptive phase were placed in nylon bags to prevent oviposition by pollinators and parasites.

When figs reached receptivity (change in the physical aspect of the figs, opening of the ostiole, and fig wasps becoming attracted to the bagged figs), we experimentally introduced at least three pollinators into each fig tested, using the technique described by Hossaert-McKey and Bronstein (2000), and then marked the experimentally pollinated figs. Experimental introduction permits knowing the exact date figs were pollinated. In our study, receptivity was thus always followed, after a constant time interval, by pollination. For figs at receptivity, at pre-receptive phase, as well as all later developmental stages, we prepared one pooled sample of male figs and one of female figs for odour extraction for each developmental phase (Table 1). To remove environmental or technical effects, volatile chemicals of each sample were extracted using precisely the same method. For each development stage of the figs, female and male figs were extracted the same day.

Volatile compounds released by figs were collected by adsorption-desorption (dynamic headspace technique) (Turlings et al., 1991; Grison-Pigé et al., 2002; Dufaÿ et al., 2003). For odour collection, figs, between 14 and 60 figs (see Table 1), were enclosed in polyethylene terephthalate (Nalophan®) bags, which have been shown not to release volatiles. Airflow was maintained through the bags by two pumps. Entrance and exit flow rates (controlled by flowmeters) were 400 and 300 ml/min. The difference in flux ensured that the system was continuously purged, to compensate for the inevitable leaks, and that no contaminated outside air would enter the system. At the entrance of the bag, air was purified in a charcoal filter. At the exit, air flowed through a trap containing 30 mg of Alltech Super Q absorbent (ARS Inc., Gainesville, FL). Odour collection lasted 3 hours. Blanks were collected in parallel using an empty bag.

Analysis of volatile compounds

Trapped odour samples were stored at -18°C until analysis. Trapped volatiles were eluted with 150 μl of dichloromethane; two internal standards (IS, nonane and dodecane, $200 \text{ ng } \mu\text{l}^{-1}$) were added to each sample for gas chromatography. Volatile compounds were analysed by injection in a CP-3800 (Varian Inc., Palo Alto, USA) gas

Table 1. Number of figs of *F. hispida* used for dynamic headspace extractions for male and female figs sampled at the seven different stages of fig development recognized (R-1: Pre-receptive stage, Receptivity and R+2, R+4, R+8, R+12 and R+16: two, four, eight, twelve and sixteen days, respectively, after pollination). For each developmental phase, we pooled samples from an equivalent number of figs originating from four individual trees of each sex.

	R-1	Receptivity	R+2	R+4	R+8	R+12	R+16
Female	50	24	31	28	35	32	14
Male	60	24	36	22	35	35	40

chromatograph with FID detector coupled with a Saturn 2000 mass spectrometer (Varian Inc., Palo Alto, USA). For both analyses a Varian column CP-SIL low blend MS (30 m, ID 0.25 mm, film thickness 0.25 μm , carrier with helium (at 1 ml/min) was used. The injector split vent was opened (1/4). Oven temperature was programmed at 50°C during 3 min; then increasing by 3°C min⁻¹ to 100°C, by 2.7°C min⁻¹ to 140°C, by 2.4°C min⁻¹ to 180°C and by 6°C min⁻¹ to 250°C. Component identification was based on computer matching of the mass spectra with NIST 98 MS library and on retention indices reported in the literature (Adams, 1995). By comparing spectra of each sample with the respective blank (empty bag, same day of collection), supposedly contaminant compounds were subtracted.

We estimated the total quantities of volatile compounds by using the IS method, by integrating the peak of every substance and dividing every peak area with the integrated IS peak area and multiplying by the amount of IS. We then compared the intensity of volatile emission for different stages of fig development. The relative proportions of the different compounds emitted were also determined for each sample. Principal component analysis (PCA, covariance matrix, StatBox, 2002) was used to compare patterns of scent composition among samples. We compared the relative amounts of all the compounds. Percentage similarity (PS) (Whittaker and Fairbanks, 1958) of odour composition for one stage among sexes was also estimated. The formula of the percentage of similarity for one stage of fig development is:

$$PS = 1 - 0.5 \sum_{i=1}^X [p_{M,i} - p_{F,i}] = \sum \min(p_{M,i} - p_{F,i})$$

Where $p_{M,i}$ is the proportion of the compound i for male (M), and there are X compounds in the blend of male and female figs at this stage. $p_{F,i}$ is the same but for female (F).

3. Results

Temporal changes in total amount of volatile compounds emitted during fig development

Temporal patterns of the emission of volatile compounds by figs of both sexes showed one major peak (at receptivity), then a decline in odour production which was sometimes followed by another peak of lower intensity (Fig. 1). Maximum volatile production was reached at full receptivity, when the ostiole opens and the bracts loosen to allow entrance of pollinating fig wasps. Once pollinators were experimentally introduced into a fig, the emission of volatile compounds by the fig decreased rapidly, reaching minimum values 12 to 16 days after pollination. This decrease in quantity of volatile production following

pollination was all the more striking in that it occurred two days after pollination. Overall, there was no correlation between diameter of a fig and the quantity of odour components emitted ($r^2 = -0.14$, $P=0.61$). At receptivity, production of volatile compounds was two to four times higher than in other periods.

Changes in chemical composition of the bouquet of F. hispida over fig development

The chemical composition of odour bouquets emitted by figs at the different stages is summarised for each sex in Table 2. The detected compounds are divided into three groups based on biosynthetic origin (Knudsen et al., 2006). Within each group compounds are listed in order of retention time in Table 2.

A total of 67 compounds were identified, with sesquiterpenes comprising the largest number of compounds. Depending on the stage of development, the odour blend of *F. hispida* figs comprised between 31 to 54 compounds, and 19 to 34 compounds with relative abundance greater than 1% (Table 2), these latter compounds accounting for 88 to 100% of the blend. Linalool, (E)- β -farnesene and indole were the most abundant compounds at receptivity (Table 2). For both male and female figs, the number of volatile compounds emitted increased around the period of receptivity, then declined rapidly after 2 days for female and 12 days for male. The maximal number of volatile compounds at receptivity was 53 in female figs, and 48 for male figs. The ratio between the number of volatile compounds present at receptivity and those present sixteen days after receptivity was similar in male and female figs (1.63 and 1.51, respectively). At the end of our collection period (16 days after pollination), the number of compounds was reduced to 33 in female figs and 32 in male figs.

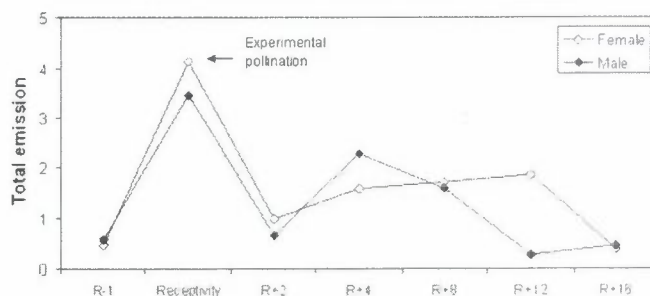


Figure 1. Temporal changes in the total amount of volatile compounds emitted by figs of *F. hispida* before, in, during and after pollination (R-1: Pre-receptive stage, Receptivity and R+2, R+4, R+8, R+12 and R+16: two, four, eight, twelve and sixteen days, respectively, after pollination).

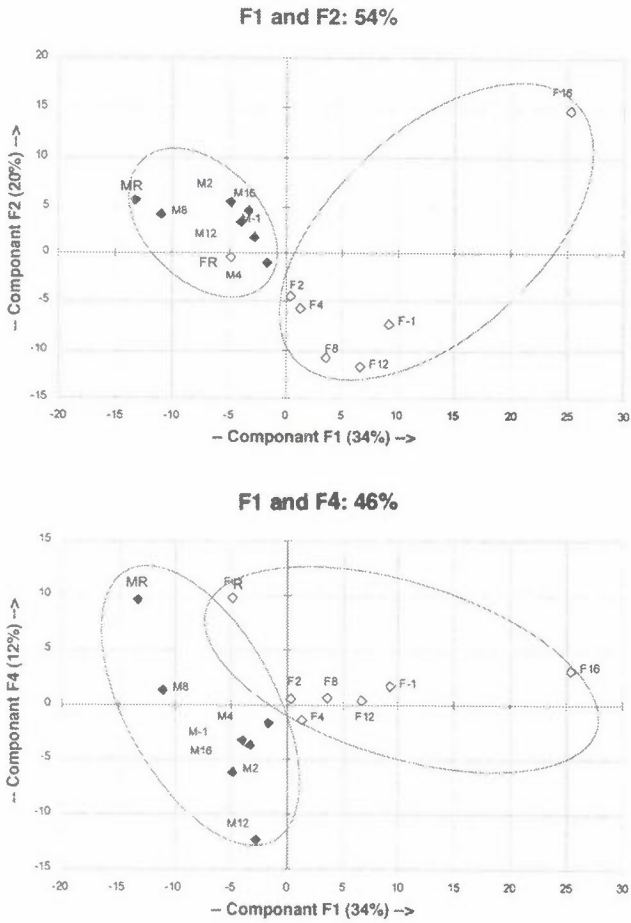


Figure 2. Plot of the principal component analysis (F1 x F2, and F1 x F4) based on the relative proportions of the different volatile compounds in blends of male (M) and female (F) figs of *F. hispida* at the seven different stages of fig development recognized (-1: pre-receptivity stage, R: receptivity and 2, 4, 8, 12 and 16: two, four, eight, twelve and sixteen days, after pollination, respectively).

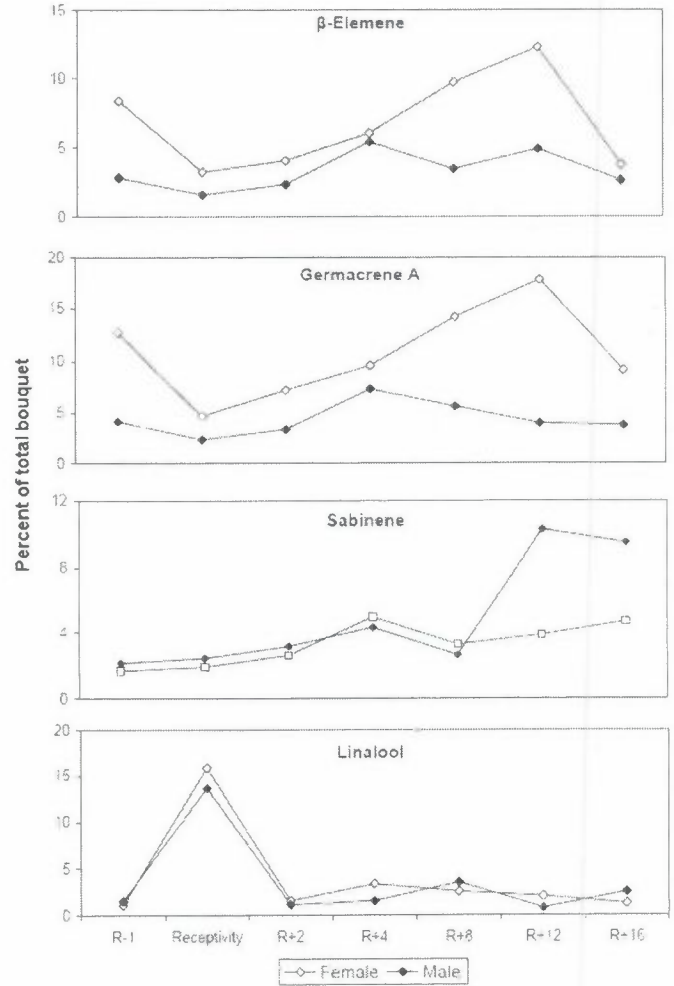


Figure 4. Temporal change in the relative proportions (% of total bouquet) of four major compounds, plotted separately for male and female figs of *F. hispida*.

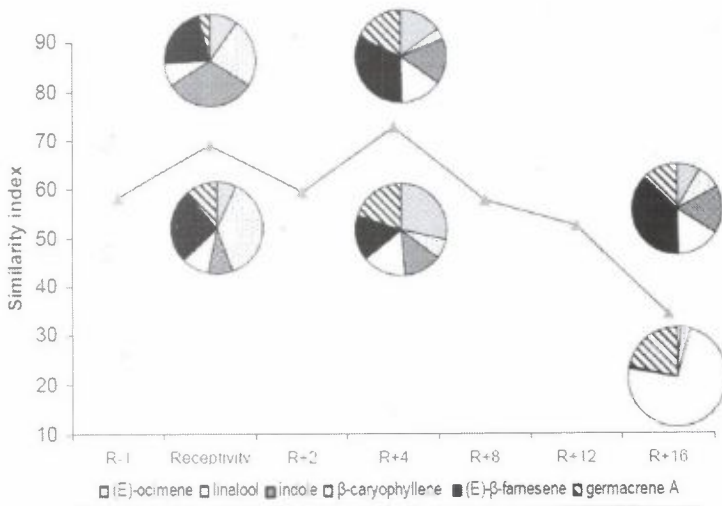


Figure 3. Temporal change in percentage of similarity between male and female figs of *F. hispida* over development. The pie charts present temporal change in the relative proportions of the major compounds ($\geq 4.5\%$ of the total blend) for male (top) and female (bottom) figs at three of the seven developmental stages (see Fig. 1 for legend).

Table 2. Relative proportions of volatile compounds in male and female figs of *Ficus hispida*. The compounds detected in each species are divided into three groups based on general biosynthetic origin (Knudsen et al., 2006) and within all the groups in four classes. Within each class they are listed in order of their Kovat's retention index (RI, see Fig. 1 for legend).

Compounds	Female								Male							
	RI	R-1	Recep	R+2	R+4	R+8	R+12	R+16	R-1	Recep	R+2	R+4	R+8	R+12	R+16	
Fatty acid derivatives																
Ethyl 2-methyl-																
butanoate	827	0.53	1.28	1.66	0.23	0.51			0.42	1.76	3.28				1.62	
(Z)-3-hexenol	857	1.86	0.52	0.59				0.90	1.24	0.60	1.57	0.49			3.24	
2-heptanone	892	0.30	0.47	0.94						0.67	1.72				2.53	
2-hexyl acetate	937	0.39	0.54	0.86						0.69	1.82					
(Z)-3-hexenyl																
Acetate	1006	5.94	3.06	4.02	1.94	2.42	1.55		3.53	3.22	7.92	4.50	1.28	0.90	4.22	
Hexyl acetate	1009	0.61	0.41	0.45		0.65			0.39	0.60	1.38	0.68	0.34		1.42	
(E)-2-hexenyl																
Acetate	1010	0.91				0.42			0.39		0.58	0.33	0.17			
Butyl 2-methyl-																
butanoate	1038		0.08	0.29						0.14	0.38					
Decane	1000		1.09							1.07						
Undecane	1100										0.56			1.40		
Tetradecane	1400	2.09	0.27	0.34	0.12		0.32	5.43	1.13		1.38	1.17	0.79	3.10		
Pentadecane	1500	1.56	1.56	1.57	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	
Total percent		14.19	7.72	9.15	2.29	4.00	1.87	6.33	7.10	8.75	20.59	7.17	2.58	5.40	13.03	
Terpenes																
Monoterpenes																
α -pinene	933	0.63	1.56	0.68	0.99	0.73	1.02	1.36	0.77	1.33	1.30	1.45	0.70	2.39	3.21	
Sabinene	965	1.67	1.91	2.52	4.88	3.30	3.73	4.72	2.14	2.43	3.06	4.34	2.65	10.10	9.32	
β -pinene	979	0.76	1.21	1.59	0.61	0.50	0.75	1.44	0.70	0.24	1.15	1.00	1.10	2.50	7.87	
Myrcene	990	0.50	0.09	0.16	0.49	0.23	0.39	0.69	1.36		0.34	1.06	0.30	0.90	1.80	
p-cymene	1023		0.87					0.25		0.26						
Limonene	1029	1.49	2.90	1.35	0.57	0.50	0.39	9.37	1.15	1.23	3.33	1.70	0.39	2.24	3.10	
1,8-cineole	1034	1.64	2.46	1.96	4.73	3.49	4.83	0.46	1.86	2.89	2.86	4.00	4.58	8.80	2.06	
(Z)- β -ocimene	1037	2.17			2.00	0.19	0.16	5.53	1.17			0.81		0.40	2.96	
(E)- β -ocimene	1047	3.59	2.90	2.73	13.62	7.09	5.10	0.57	4.92	5.51	3.25	6.22	5.90	15.08	2.43	
Linalool-oxide	1090	0.62	0.52	0.42	0.79	0.55	0.42	0.12	0.39	0.04		0.55	0.45			
Linalool	1102	1.08	15.74	1.58	3.28	2.56	2.07	1.29	1.53	13.57	1.16	1.56	3.55	0.81	2.54	
3-oxa-2,2,4-tri-																
methyl-4vinyl-																
Cyclohexanone	1108	1.28	0.28	0.88	0.96	0.68	1.24	1.74	0.68			0.59	0.39	0.33	1.70	
Perillene	1115	0.79	0.82	0.44	0.40	0.21	0.49		1.55	1.11	1.03	0.50	0.43	0.09	1.69	
Total percent		16.22	31.26	14.31	33.32	20.03	20.59	27.54	18.22	28.61	17.48	23.78	20.44	43.64	38.68	
Sesquiterpenes																
Unknown 1	1335	1.86	1.07	1.24	1.34	1.26	1.03	2.15	3.56	1.47	1.65	3.50	1.40	0.40	1.79	
Clovene	1351							0.35	0.58	0.44	0.34	0.62	0.28			
α -longipinene	1360	1.08	0.68	0.70	0.82	0.75	0.55	1.67	1.78	0.87	1.33	1.83	0.75	1.61		
Unknown 2	1373			0.26	0.26	0.23	0.28			0.17						
Isodene	1376		1.18	1.10	0.41	0.32	0.58			0.24	0.47	0.27	0.22			
α -copaene	1379	0.95	0.45	0.12	0.47	0.47	0.79	1.70	2.32	0.82	1.46	0.80	0.83	0.93		
Daucene	1381	5.06	2.26	2.71	1.84	2.28	1.13			0.79	1.13	1.16	0.89		2.41	
Bicycloopposit-																
4-ene	1383	5.06	3.50	6.41	6.32	6.68	4.78	2.34	3.50	2.40	1.26	4.19	1.44	2.78		
α -isocomene	1392		1.80	0.31	1.61	1.66	2.41	1.47	3.60	0.53	1.26	2.86	1.13	2.34		
β -elemene	1392	8.39	3.15	3.98	5.95	9.66	12.14	3.79	2.85	1.61	2.38	5.31	3.48	4.80	2.66	
β -longipinene	1403		0.52	0.49	0.50	0.42	0.35					1.10	0.79	0.31	1.42	
7-epi-a-cedrene	1403		0.24	1.37	0.17						0.16	0.38	1.57	0.48		
Unknown 4	1404			1.20					0.23					0.76		
Isocaryophyllene	1409		0.27		0.17			0.33		0.89	0.23	0.20	1.03			
cis- α -bergamotene	1415		0.95	2.32	0.24	0.20				0.09		0.16	1.29			
β -caryophyllene	1423	9.64	3.91	5.94	7.45	4.90	6.56	30.91	7.92	4.18	5.08	6.00	3.86	4.76	4.40	

Table 2. Continued.

Compounds	RI	Female							Male						
		R-1	Recep	R+2	R+4	R+8	R+12	R+16	R-1	Recep	R+2	R+4	R+8	R+12	R+16
Oppositadiene	1423							0.58			0.38	0.71	0.51	0.89	
Unknow7	1430								0.71				0.80		
Trans- α -bergamotene	1435		0.82	3.35	0.75	0.40	0.95	1.52	1.05		0.30	1.15	1.48	0.44	
Aromadendrene	1443		0.30	0.91			0.49		0.57			0.63	0.43		
(E)- β -farnesene	1454	5.63	10.95	15.44	7.69	6.58	7.80	0.35	20.40	12.36	13.03	13.09	8.61	6.17	10.90
α -humulene	1458	1.27	0.43	0.16	0.88	0.51	1.35	3.03	1.22	1.36	1.05	1.41	0.70	2.67	1.62
α -neoclovene	1460	2.50	1.69	2.55	1.77	2.05	1.35	3.01	1.22	1.44	1.13	2.83	1.72	2.67	
Alloaromadendrene	1464	1.98	0.57			5.33			2.24		0.23	1.15	1.05		2.02
Selina-7,11-diene	1471	8.99	4.00	6.22	5.50	6.26	6.67		0.70	0.80					
Amorpha-4,7-diene	1476		2.19	2.17	0.36	1.68	1.64			0.47	2.59	0.23			1.39
γ -muurolene	1477					0.45		0.46		1.14	0.86	0.48	0.84	0.71	
α -curcumene	1484	1.00	0.34			0.39			0.57		0.35				
Germacrene D	1484	0.87				0.34	0.58		1.56						
β -selinene	1492		0.63					0.39	5.38	3.41	11.24		8.48	8.38	3.17
γ -amorphene	1497			0.68					0.61	0.75	1.58	0.78			
α -selinene	1497		0.54		0.74	0.75	1.40	1.56	0.72	1.03	2.03		2.06		
(E,E)- α -farnesene	1506			0.42											2.91
Germacrene A	1511	12.84	4.55	7.13	9.51	14.15	17.68	8.96	4.90	2.24	3.23	7.24	5.64	3.83	3.57
δ -cadinene	1521	1.07				0.34	0.27				0.62	0.59	0.59	0.64	
β -sesquiphellandrene	1526		2.59	4.56	1.74	1.40	1.89			0.99	2.12	1.70	2.20	0.22	2.10
Unknown 5	1529		2.80	1.10		0.33	0.29		1.41	0.78	0.28	0.41	0.38		
α -alaskene	1532			1.21		0.34	0.21			0.57	0.23	0.31	0.21		
Unknown 6	1613		2.60				0.06								1.58
Total percent		68.19	54.98	74.05	56.49	70.13	73.23	64.57	69.60	41.84	58.00	61.09	55.42	45.03	41.94
Shikimic acid derivatives															
Benzyl alcohol	1030		0.47							0.48					
Methyl benzoate	1091		0.38							0.50					
Indole	1298	1.40	3.63	0.92	6.34	4.28	2.75		3.52	18.26	2.37	6.40	20.00	4.37	4.79
Total percent		1.40	4.48	0.92	6.34	4.28	2.75	0.00	3.52	19.24	2.37	6.40	20.00	4.37	4.79
Total number of compounds		38	53	48	40	46	42	33	44	48	50	48	48	35	32

Components Analysis (Fig. 2) illustrates the variation in the chemical signature of the odour bouquet over the cycle of fig development for each sex. Although volatile composition varied substantially in figs of each sex over the course of development, principal components analysis showed that odours of male figs and those of female figs were quite well separated (Fig. 2). The first four principal components together explained 80% of the variance. The chemical signature of male figs and those of female figs remained quite distinct, except at receptive phase, when the signature of female figs fell within, or very close to, the space delimited by male figs. Only at receptivity and immediately afterwards were odours produced by the two sexes so similar. The odour of female figs at sixteen days after pollination was always in an extreme position within the space occupied by female figs. This pattern was not found in male figs, for which odour of male figs at sixteen days after receptivity remained within the space defined by

the chemical bouquet of male figs at other stages of development.

Similarity of the chemical signature among sexes

Percentage similarity of volatile bouquets between male and female figs at the different stages showed a first peak (69.1%) at receptivity and a second, slightly higher peak (72.9%) four days after pollination (Fig. 3). However, different compounds were responsible for the similarity of the chemical signature at these two stages (Fig. 3), with germacrene A and indole replacing linalool after pollination. Percentage similarity declined rapidly after this post-pollination stage (4 days after) to reach the minimal value (34.4%) sixteen days after receptivity. Volatile bouquets were thus most divergent between male and female figs at the end of our sampling period 16 days after pollination.

Temporal dynamics of emission of some major compounds

Different odour components showed different patterns of variation over fig development. Fig. 4 presents some examples, drawn from among compounds that contributed strongly to the variation. Some compounds were abundant before receptivity, as for example β -elemene and germacrene A. In figs of both sexes, the receptive phase was marked by the emission of large quantities of linalool (Fig. 4), which was the predominant component of the bouquet at that phase. In both sexes, the stages soon after pollination are marked by an increase in concentrations of two compounds, β -elemene and germacrene A (Fig. 4), which continued to increase with fig development. While also presenting a post-pollination increase in production, indole presented a different pattern, with male figs always producing higher concentrations of this compound than female figs, and with an apparent temporary decrease in odour production in the middle of the interfloral stage (Table 2). Finally, sixteen days after pollination male and female figs had very different chemical signatures, with female figs emitting higher concentrations of sabinene (Fig. 4).

4. Discussion

In this study, we have examined the dynamics of variation in the composition and emission rate of volatile compounds throughout fig development. As pointed out by Dufaÿ et al. (2003), the likely diversity of biological functions of odour components in nursery pollination mutualisms render inadequate a simple comparison of odours at two points in time, pollination and post-pollination. Other studies have produced evidence that pollinators are attracted only to receptive-phase figs (Chen and Song, unpublished data), and that post-pollination variations in odour emitted by figs are used by non-pollinating fig wasps to locate oviposition sites at a particular moment during the development of figs and pollinator larvae (Proffit et al., 2007). We show here quantitative and qualitative differences in composition of volatile compounds that can account for such stage-specific attraction. However, our results also show other changes in fig odours over time, suggesting additional potentially adaptive patterns of variation.

The composition of odour extracts of receptive figs of the species we studied, *F. hispida*, has already been reported by Song et al. (2001). Our results differ from those presented in that study, which used solvent extraction, a passive technique that recovers not only volatile compounds emitted by figs, but in addition chemicals present in the superficial cell layers of the plant. Our study, based on dynamic headspace extraction, reported only volatile compounds, which are potentially detectable by

pollinators and other animals. Previous studies show that chemical compounds emitted by receptive figs of *F. hispida* are the same but varied greatly among plants in relative proportions, within *Ficus* populations (Borges et al., submitted; Proffit et al., submitted). In our study population, the odour bouquet is dominated by terpenes (13 monoterpenes and 39 sesquiterpenes, Table 2). Comparing the scent produced by receptive figs of *F. hispida* with those of other *Ficus* species (Grison-Pigé et al., 2001; 2002; Borges et al., submitted; Proffit et al., submitted), we found a similar blend. In each *Ficus* species, floral scents have fewer than 50 compounds belonging to four different chemical classes: monoterpenes, sesquiterpenes, fatty acid derivatives and shikimic acid derivatives. One to three main compounds always represent 20% to 92% of the total blend (Grison-Pigé et al., 2001; 2002). These main compounds are widespread in floral scents of plants of different families (Knudsen et al., 2006), but they generally differ among fig species (Grison-Pigé et al., 2001, 2002). By controlling the synthesis and emission of volatile compounds to produce a strong and specific signal during receptivity, figs can advertise their availability to pollinators. The post-pollination decline in odour production we found is consistent with results of other studies on *Ficus* (Grison-Pigé et al., 2002), on other nursery-pollination mutualisms (Muhlemann et al., 2006) and other pollination mutualisms (Arditti et al., 1973; Tollsten and Bergström, 1989; Tollsten, 1993; Schiestl and Ayasse, 2001; Theis and Raguso, 2005).

Our results also reveal three other striking phenomena. First, although female and male figs tend to have quite distinctive odours throughout much of their development (Fig. 2), at receptivity their odour bouquets seem similar (Borges et al., submitted). This pattern is consistent with the hypothesis that in dioecious figs, selection results in reciprocal chemical mimicry between male and female figs (Grafen and Godfray, 1991; Patel et al., 1995; Anstett et al., 1997). In our study, odours produced by figs of the two sexes were most similar precisely at the point in the cycle when it is important for the functioning of the system that pollinators be unable to distinguish between figs of the two sexes (Patel et al., 1995; Grison-Pigé et al., 2001). In both female and male receptive figs, the odour bouquet is dominated by the production of large amounts of linalool. Linalool is a frequent component of many floral odours (Knudsen et al., 2006). Gibernau (1997) showed that a mixture of linalool with two monoterpene compounds (linalyl oxides and benzyl alcohol) in certain proportions was necessary and sufficient to attract *Blastophaga psenes*, the specific pollinator of *F. carica*. Alone, linalool is thus unlikely to ensure specific attraction of the pollinator of *F. hispida*. However, in receptive figs of this species, linalool is associated with lower concentrations of other compounds, such as indole and ocimene, and the composition of this bouquet could ensure the specificity of

attraction (Proffitt et al., submitted). Not only the qualitative composition, but also the quantitative strength of the message, showed no apparent difference between male and female figs.

The second striking phenomenon found in our study was the shift in composition of the odour bouquet following pollination. Male and female figs underwent similar changes, so that although they continued to resemble each other, different compounds were responsible for this resemblance. In figs of both sexes, there was an increase in the concentration and relative proportions in the bouquet of germacrene A and indole. Together with changes in the physical structure of the figs likely to be perceptible by pollinators (e.g., closing of the ostiole), the shift in composition of the odour bouquet could constitute a signal that repels pollinating fig wasps. Compounds that are emitted at a higher flux rate following pollination are likely candidates as repellent compounds, that function either to prevent additional pollinator visits or to deter enemies such as seed predators (Raguso, 2003). Immediately after pollination, as at receptivity, the interests of male and female figs, and the function of the odours they emit, are similar. While they are likely candidates for such roles, the deterrent function of germacrene A and indole remains to be demonstrated (Chen and Song, unpublished data).

The third striking result of our study was the increasing divergence between the odour bouquets of male and female figs as they developed. This divergence can be seen from day 8 after pollination onwards, and the separation in chemical signature between the two sexes was maximal 16 days after pollination (Fig. 2). Percentage similarity between odour bouquets of male and female figs also reached its minimum observed value at this stage of development, which corresponded to the time in the cycle when the odour bouquets of female figs included the smallest number of compounds. In *F. hispida*, figs at this stage are nearing the end of the interfloral phase and will soon reach maturity, when they must attract frugivorous vertebrates that disperse their seeds (mainly bats in the case of *F. hispida*). Divergence in the odour bouquets of female figs at this stage might be linked to changes associated with this specific biological function (or in the related function of avoiding seed predators), which is not performed by male figs (Lambert, 1992; Patel and McKey, 1998; Dumont et al., 2004).

This first study of the dynamics of variation in signal composition throughout the cycle of fig development has provided support for the hypothesis of chemical mimicry in dioecious figs, and has demonstrated the change, in both male and female figs, in the composition of the odour bouquet following pollination. Our study also suggests many interesting perspectives that should be pursued in more detailed studies. Male figs must protect the developing pollinators. What defensive roles, if any, are played by odour compounds? Do developing figs produce

compounds that deter parasites of pollinators? Alternatively, is there selection against production of distinctive compounds that might attract these enemies? In this study, sampling of female figs ceased before their maturity and so further studies should sample mature female figs, to test the prediction that they produce distinctive odours specifically attractive to frugivorous vertebrates (R. Borges, pers. comm.).

Acknowledgements

This research was supported by a grant from the CAS-CNRS (DREI, CNRS) to Martine Hossaert-McKey, by a grant from the Agence Nationale de la Recherche (ANR), and by the CNRS's Research Group (GDR) in chemical ecology. We wish to thank Xishuangbanna Tropical Botanical Garden, Yunnan, China, and its director, Chen Jin, for facilitating our stay and field work in China. We also thank Jean-Yves Rasplus for identifying wasps, Bruno Buatois for help in chemical analysis, and Doyle McKey, Rhett Harrison and the anonymous referees for their helpful comments on the manuscript.

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