The chemical ecology of seed dispersal in monoecious and dioecious figs

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Summary

1. In the nursery pollination system of figs (*Ficus*, Moraceae), flower-bearing receptacles called syconia breed pollinating wasps and are units of both pollination and seed dispersal. Pollinators and mammalian seed dispersers are attracted to syconia by volatile organic compounds (VOCs). In monoecious figs, syconia produce both wasps and seeds, while in (gyno)dioecious figs, male (gall) fig trees produce wasps and female (seed) fig trees produce seeds.

2. VOCs were collected using dynamic headspace adsorption methods on freshly collected figs from different trees using Super Q® collection traps. VOC profiles were determined using gas chromatography–mass spectrometry (GC–MS).

3. The VOC profile of receptive and dispersal phase figs were clearly different only in the dioecious mammal-dispersed *Ficus hispida* but not in dioecious bird-dispersed *F. exasperata* and monoecious bird-dispersed *F. tsjahela*.

4. The VOC profile of dispersal phase female figs was clearly different from that of male figs only in *F. hispida* but not in *F. exasperata*, as predicted from the phenology of syconium production which only in *F. hispida* overlaps between male and female trees. Greater difference in VOC profile in *F. hispida* might ensure preferential removal of seed figs by dispersal agents when gall figs are simultaneously available.

5. The VOC profile of only mammal-dispersed female figs of *F. hispida* had high levels of fatty acid derivatives such as amyl-acetates and 2-heptanone, while monoterpenes, sesquiterpenes and shikimic acid derivatives were predominant in the other syconial types. A bird- and mammal-repellent compound methyl anthranilate occurred only in gall figs of both dioecious species, as expected, since gall figs containing wasp pollinators should not be consumed by dispersal agents.

Key-words: bird-dispersed figs, chemical ecology, floral volatiles, frugivory, fruit volatiles, mammal-dispersed figs

Introduction

Considerable research has been done on volatile organic compounds (VOCs) in floral scents (Knudsen, Tollsten & Bergström 1993; Grison-Pigé *et al*. 2001; Knudsen *et al*. 2006), and specific compounds within these bouquets have also been implicated in pollinator attraction (Ayasse *et al*. 2003; Ashman *et al*. 2005). However, similar research on volatiles responsible for attracting dispersers to ripened fruit has been scarce (Dudareva *et al*. 2006) with some research on bats (Mikich *et al*. 2003; Sánchez *et al*. 2006) and primates (Laska *et al*. 2006). Furthermore, the difference in volatile production between flowers and fruit of the same species in relation to the attraction of pollinators or dispersers has not been investigated, largely due to the research priorities of investigators who are either pollination or seed dispersal specialists. In the *Ficus* (Moraceae) system, flowers are borne inside the syconium, which will ripen to form a fig. This system is unique because the syconium is the unit of attraction for both pollinators and seed dispersers. The highly species-specific pollinating fig wasps use VOCs produced by figs to find pollen-receptive figs (Hossaert-McKey, Gibernau & Frey 1994; Grison-Pigé, Bessière & Hossaert-McKey 2002a; Grison-Pigé *et al*. 2002b). Because the fig system is a nursery pollination system (Anstett, Hossaert-McKey & Kjellberg 1997), VOC signatures must vary with the fig developmental cycle, so that pollinators and seed dispersers are attracted to the fig during appropriate time windows. In dioecious fig species, moreover,

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*Correspondence author. E-mail: renee@ces.iisc.ernet.in in which pollinators can only breed within gall figs produced by male trees, and seeds are only produced within seed figs on female trees (Verkerke 1989), there should be selection on gall and seed figs to be indistinguishable from each other at the pollination stage (Grafen & Godfray 1991; Patel *et al.* 1995), and for dispersal agents to be attracted to seed figs rather than gall figs in the dispersal stage (Corner 1978; Lambert 1992; Patel & McKey 1998; Dumont, Weiblen & Winkelmann 2004). Although the pollinators of fig species are closely related to each other, fig seed dispersers are phyletically diverse, including a huge range of birds varying in size and many other traits (Compton, Craig & Waters 1996; Korine, Kalko & Herre 2000; Shanahan *et al.* 2001), as well as mammals including bats, rodents such as sciurids and primates (Shanahan *et al.* 2001).

These features of the fig pollination and seed dispersal system present unique constraints on the chemical ecology of these mutualisms. In this paper, we present the first data comparing differences between the VOC signatures of the receptive (pre-pollination) and dispersal phase of three fig species (one monoecious and two dioecious) and examine the dispersal phase VOCs in greater detail. Based on the ecology of figs and their dispersal agents, we made the following predictions:

1. There should be a distinct shift in volatile signatures of syconia between the receptive (pre-pollination) stage and the post-wasp dispersal (seed dispersal) stage, so that overlap in volatile signatures is minimal.
2. Within a dioecious species, the volatile signatures of ripe seed figs should be distinguishable from that of gall figs.
3. Mammal-dispersed figs should have a volatile signature distinguishable from that of bird-dispersed figs, since mammals and birds have different olfactory sensitivities. Seed figs that are mammal-dispersed should produce volatile signatures of higher scent intensity (greater volatile quantities) compared to gall figs of the same species, or to seed figs of bird-dispersed species.

Materials and methods

STUDY SITES AND SPECIES

The study was conducted at Agumbe, Shimoga District (13°30′N, 75°5′E), and at Kudremukh, Chikmagalur District (13°15′N, 75°7′E) of Karnataka State in the Western Ghats of India. The sites are separated by 75 km. Volatiles were harvested from three sympatric species: two (gyno)dioecious (*Ficus hispida* L.: section *Sycocarpus*, and *Ficus exasperata* Vahl: section *Sycidium*) and one monoecious species (*Ficus tsjahela* Burman: section *Urostigma*). *Ficus hispida*, a short free-standing fig usually found along streams, bears cauli-carpous syconia that are yellowish-green when ripe [fig diameter; female: mean = 23·9 mm (SE = 0·3, *n* = 40 figs); male: 25·3 (0·7, *n* = 40)]. Geocarpic syconia are also present on stolons that emerge from the base of the trunk and are recumbent on the ground. *Ficus exasperata*, a medium-sized free-standing fig bears axillary syconia that are red when ripe [fig diameter; female: mean = 19·7 mm (SE = 0·4, *n* = 50 figs); male: 22·4 (0·4, *n* = 50)]. *Ficus tsjahela*, a tall free-standing fig bears ramicarpous syconia that are creamy-beige when ripe [fig diameter; mean = 5·0 mm (SE = 0·1, *n* = 20 figs)]. The sampled fig trees occurred within a small area (*c.* 5–10 km^2) at each site and belonged to the same population at each site.

VOC COLLECTION AND IDENTIFICATION

VOCs from fig syconia were collected by dynamic headspace adsorption methods as described in Grison, Edwards & Hossaert-McKey (1999). Freshly harvested receptive or dispersal phase figs from each single tree were enclosed in a polyethylene terepthalate (Nalophan®) bag (Kalle Nalo GmbH, Wursthüllen, Germany) through which a constant airflow was maintained by two micropumps (incoming flow rate: 400 mL min^{-1} and outgoing flow rate: 300 mL min^{-1}) over an Alltech Super Q® volatile collection trap (VCT; ARS Inc., Gainesville, FL) for 3 h per sample. Incoming air was cleaned using activated charcoal filters. For *F. tsjahela*, since the branches of this species are also strongly aromatic (unlike those of the other two species), we also extracted VOCs from branches at receptive and dispersal stages. Controls (empty bags) were run at the same time to determine any ambient airborne contamination. VCTs stored at −20 °C until analysis were later eluted with 150 µL dichloromethane to which 20 µL of a 200-ng µL^{-1} solution of nonane and dodecane were added as internal standards. The eluates were analysed in a CP-3800 (Varian Inc., Palo Alto, CA) gas chromatograph with an FID detector coupled with a Saturn 2000 mass spectrometer (Varian Inc.) in the split mode at 1 : 4 split ratio. For both gas chromatography (GC) and mass spectrometry (MS), a CP-SIL low bleed Varian column (30 m, ID 0·25 mm, film thickness 0·25 µm), and helium carrier gas (at 1 mL min^{-1}) were used. The temperature programme for the analysis was: 50 °C for 3 min, ramped 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. Compound identification was based on comparison of mass spectra with the NIST 98 MS library and on retention indices reported in literature.

Each sample reported in this paper was collected from a different tree. Each tree was sampled only for a particular phenophase. The numbers of figs from which volatiles were extracted varied for each sample depending on availability of the phenophase on the tree. The numbers of figs from which volatiles were extracted for each sample were as following: Receptive phase: *F. hispida*: mean = 117·3 figs (SE = 10·1, *n* = 30 trees), *F. exasperata*: 230·5 (18·5, *n* = 33), *F. tsjahela*: 604·3 (200·4, *n* = 6); Dispersal phase: *F. hispida*: mean = 37·1 figs (SE = 3·7, *n* = 16 trees), *F. exasperata*: 96·5 (9·5, *n* = 24), *F. tsjahela*: 618·6 (144·0, *n* = 12). Therefore, the VOC signature is represented as percentages of each volatile in the sample. Absolute quantities of VOCs per sample were calculated on a per fig and per hour basis (ng fig^{-1} 3 h^{-1}), for comparison across species and phenophases.

DATA ANALYSIS

Principal component analysis (PCA, covariance matrix, STATBOX v6·3) was used to compare patterns of scent composition between the different stages and sexes for each species, or among species for the dispersal stage. For these multivariate analyses, we used the relative amounts (percentages) of the compounds. We also estimated the total quantities of volatiles produced by figs using calibrated quantities of the two internal standards. We then tested the effect of sex and stage on the intensity (i.e. quantity) of VOC emission for each species using ANOVAS (on log-transformed data when required) (PROC GLM, type 3, SAS v9) followed by a multiple comparison of means (LSMEANS with Tukey–Kramer multiple comparison tests)

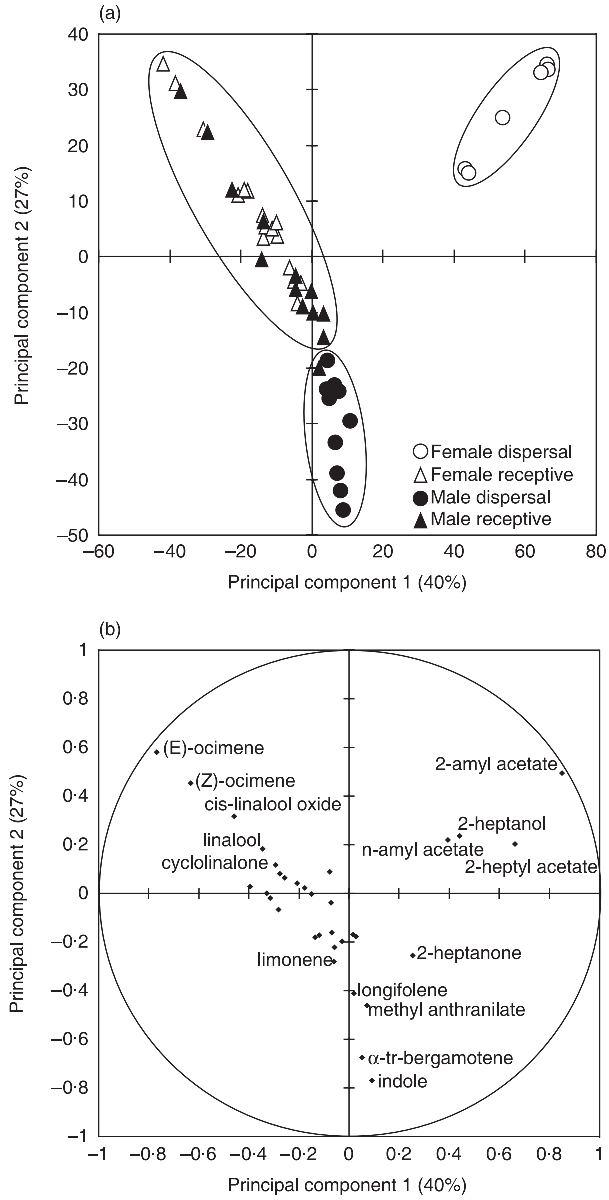

Fig. 1. Volatile profiles of *Ficus hispida*. (a) PCA analysis of VOCs produced by male and female figs in receptive and dispersal phases. (b) Factor loadings of VOCs in the above PCA analysis.

when appropriate. Finally, for each species, we investigated stage and sex effects on the overall abundance of the compounds using a multivariate analysis of variance (MANOVA, PROC GLM, SAS v9), followed by univariate (i.e. sequential analyses) on each dependent variable (quantity of each VOC) to test which compounds contribute to the overall significance in this analysis (Stevens 1992).

Results

FICUS HISPIDA

The volatile signature of the receptive phase figs had, on average, 36·0 compounds (SE = 1·4, n = 30 trees) while that of the dispersal phase had 25·7 compounds (1·4, n = 15 trees). A PCA performed using only VOCs that occurred at least once in a sample at > 5% levels (33 compounds) showed clear separation of receptive and dispersal phase figs (Fig. 1a). Moreover, seed (female) and gall (male) figs were also clearly separated in the dispersal phase (Fig. 1a). The first two components explained 67% of the variance. Similar results were obtained with VOCs at > 1% levels. Several VOCs were responsible for this separation (Fig. 1b). The VOCs that were largely responsible for the separation of female dispersal figs in this PCA analysis were 2-amyl acetate, 2-heptanol, *n*-amyl acetate and 2-heptyl acetate. Dispersal phase male figs were characterized by indole, α-*trans*-bergamotene and methyl anthranilate. For these dispersal figs, we had sufficient samples from two calendar years to examine a year effect, and we found none (analysis not shown), indicating that the VOC difference between sexes was stable across time.

Table 1. Results of analysis of variance on number of compounds produced by male and female figs in receptive and dispersal stages

Species	Effect	F	df	P
Ficus hispida	Sex	7·33	1, 44	0·010
	Stage	20·81	1, 44	< 0·0001
	Sex × stage	0·06	1, 44	NS
Ficus exasperata	Sex	2·93	1, 52	0·093
	Stage	34·56	1, 52	< 0·0001
	Sex × stage	16·56	1, 52	0·0002
Ficus tsjahela	Stage	1·49	1, 17	NS
	Year	1·67	2, 17	NS
All three species	Species	2·36	2, 115	< 0·0001
	Stage	0·21	1, 115	< 0·0001
	Species × stage	12·61	2, 115	NS

We found a significant effect of sex and stage on the total number of compounds emitted by figs of both sexes and between figs at receptive and dispersal stages while the interaction effect was non-significant (Table 1). Figs of both sexes produced more compounds at receptive stage than at dispersal stage [females: mean = 38·8 compounds (SE = 1·8, n = 16 trees), and 28·8 (2·1, n = 6); males: mean = 32·7 compounds (SE = 1·9, n = 14 trees), and 23·7 (1·7, n = 9); LSMEANS $P < 0{\cdot}01$]. Using the total quantity of VOCs emitted by the sexes in receptive and dispersal stages (log-transformation employed to meet ANOVA requirements for normality), we also found significant effects of sex and of stage, as well as a significant interaction effect between sex and stage in scent quantity (Table 2). These effects were due to the large quantities of VOCs emitted by female figs in the dispersal stage [mean = 58·1 ng fig^{-1} 3 h^{-1} (SE = 7·3, n = 6 trees)] which were significantly greater ($P < 0{\cdot}0001$ in all cases) than those emitted by female figs in the receptive stage [mean = 2·5 ng fig^{-1} 3 h^{-1} (0·7, n = 16)], males in the receptive stage [mean = 2·9 ng fig^{-1} 3 h^{-1} (0·6, n = 14)] and males in the dispersal stage [mean = 2·7 ng fig^{-1} 3 h^{-1} (0·6, n = 9)].

To investigate the contribution of some specific VOCs to these results, we selected those compounds with mean quantities > 0·04 ng fig^{-1} 3 h^{-1} for receptive figs and > 0·14 ng fig^{-1} 3 h^{-1} for dispersal figs, and performed a MANOVA which showed a significant effect of stage (Wilks' λ = 0·0044, $F_{26,16}$ = 140·41,

Table 2. Results of analysis of variance on total quantity (ng fig^{-1} 3 h^{-1}) of different compounds produced by male and female figs in receptive and dispersal stages (log quantities used in analysis)

Species	Effect	*F*	df	*P*
Ficus hispida	Sex	16·89	1, 44	0·0002
	Stage	34·48	1, 44	< 0·0001
	Sex × stage	28·47	1, 44	< 0·0001
Ficus exasperata	Sex	0·73	1, 52	NS
	Stage	5·72	1, 52	0·02
	Sex × stage	0·57	1, 52	NS
Ficus tsjahela	Stage	0·01	1, 17	NS
	Year	1·30	2, 17	NS
All three species	Species	2·36	2, 115	0·099
	Stage	0·21	1, 115	NS
	Species × stage	12·61	2, 115	< 0·0001

$P < 0{\cdot}0001$) and of sex (Wilks' $\lambda = 0{\cdot}0047$, $F_{26,16} = 129{\cdot}05$, $P < 0{\cdot}0001$) as well as an interaction effect of stage and sex (Wilks' $\lambda = 0{\cdot}0045$, $F_{26,16} = 135{\cdot}88$, $P < 0{\cdot}0001$). From the univariate sequential ANOVA analysis on these VOCs included in the MANOVA procedure, we found that several fatty acid derivatives such as 2-amyl acetate, *n*-amyl acetate, 2-heptanol, 2-heptanone, 2-heptyl acetate and 2-nonyl acetate were significantly higher in the female dispersal phase than in other combinations of sex and phase (Table 3), as were compounds such as β-elemene, β-caryophyllene and α-humulene, while indole was higher in male dispersal figs (Table 3). Methyl anthranilate was absent in the female dispersal phase (Table 3, see Appendix S1 in Supplementary material).

FICUS EXASPERATA

The volatile signature of the receptive phase figs had, on average, 37·9 compounds (SE = 1·1, $n = 33$ trees) while that of the dispersal phase had 29·4 compounds (1·8, $n = 24$). A PCA performed using only VOCs that occurred at least once in a sample at > 5% levels showed no clear separation of receptive and dispersal phase figs (Fig. 2). A PCA using only compounds > 1% gave similar results. There was also no striking separation of male (gall) and female (seed) figs like that found for *F. hispida*. Here also, as in *F. hispida*, methyl anthranilate was present in gall figs but absent in seed figs (Supplementary Appendix S1).

Unlike for *F. hispida*, we found no effect of sex in the total number of compounds produced. However, the effect of stage and the interaction effect between sex and stage were significant

Table 3. MANOVA on quantities of compounds (ng fig^{-1} 3 h^{-1}) for the three *Ficus* species, followed by univariate sequential ANOVA analysis on some relevant compounds

	MANOVA effect			
Compound	Sex	Stage	Sex × stage	Remarks (results of Tukey–Kramer tests)
Ficus hispida				
Overall MANOVA	***	***	***	
2-amyl acetate	***	***		Higher in female dispersal
2-heptanol	**	**		”
2-heptanone	***	***		”
n-amyl acetate	*	*		”
2-heptyl acetate	***	***		”
2-nonyl acetate	***	***		”
β-elemene	**	**		”
β-caryophyllene	***	**		”
α-humulene	**	**		”
Methyl anthranilate	**	*		Absent in female dispersal
Indole	*	*		Higher in male dispersal
cis-linalool oxide	*	NS		
Cyclolinalone	*	NS		
α-copaene	*	NS		
α-*trans*-bergamotene	**	NS		
Germacrene D	*	NS		
δ-cadinene	*	NS		
Ficus exasperata				
Overall MANOVA	*	*	NS	
β-caryophyllene	*	NS	NS	
α-copaene	*	NS	NS	
Germacrene D	*	NS	NS	
α-humulene	*	NS	NS	
(*Z*)-ocimene	**	*	NS	
Myrcene	NS	*	**	
p-cymene	NS	NS	*	
γ-terpinene	NS	NS	**	
Ficus tsjahela				
Overall MANOVA		NS		

*$P < 0{\cdot}05$, **$P < 0{\cdot}001$, ***$P < 0.0001$.

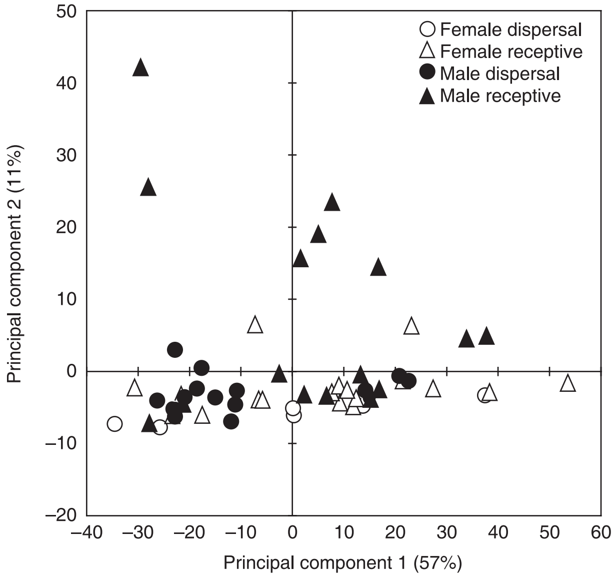

Fig. 2. Volatile profiles of *Ficus exasperata*. PCA analysis of VOCs produced by male and female figs in receptive and dispersal phases.

(Table 1). Males produced the same number of compounds at receptive and dispersal phase [mean = 35·2 compounds (SE = 1·4, n = 16 trees); and 31·8 (1·7, n = 17)], while females produced more compounds in receptive [mean = 39·6 compounds (1·5, n = 14 trees)] compared to dispersal phase [mean = 20·8 (3·0, n = 6); LSMEANS $P < 0{\cdot}01$]. We found no effect of sex in the total quantity (log-transformed) of VOCs (data only from Agumbe), but a significant effect of stage, and a non-significant interaction effect between sex and stage in scent intensity (Table 2). The effect of stage was due to the larger quantities of VOCs produced by male figs in the receptive stage [mean = 0·6 ng fig^{-1} 3 h^{-1} (SE = 0·1, n = 16 trees)] which were significantly different ($P < 0{\cdot}05$) from that of males in the dispersal stage [mean = 0·2 ng fig^{-1} 3 h^{-1} (0·02, n = 17)] but not significantly different from that of females in the receptive stage [mean = 0·3 ng fig^{-1} 3 h^{-1} (0·1, n = 14)], and females in the dispersal stage [mean = 0·2 ng fig^{-1} 3 h^{-1} (0·1, n = 6)].

To investigate the contribution of some specific VOCs to these results, we selected VOCs with mean quantities > 0·05 ng fig^{-1} 3 h^{-1} for both receptive and dispersal figs and using a MANOVA we found a significant effect of stage (Wilks' $\lambda = 0{\cdot}38$, $F_{20,30} = 2{\cdot}48$, $P = 0{\cdot}012$) and of sex (Wilks' $\lambda = 0{\cdot}43$, $F_{20,30} = 1{\cdot}98$, $P = 0{\cdot}04$), but a non-significant interaction between sex and stage. From the univariate sequential ANOVA analysis on these VOCs included in the MANOVA procedure, we found that only myrcene was emitted in greater concentration in the male dispersal phase, while (*Z*)-ocimene was significantly greater in the receptive phase (Table 3). Myrcene, *p*-cymene, and γ-terpinene showed significant interactions between sex and stage (Table 3).

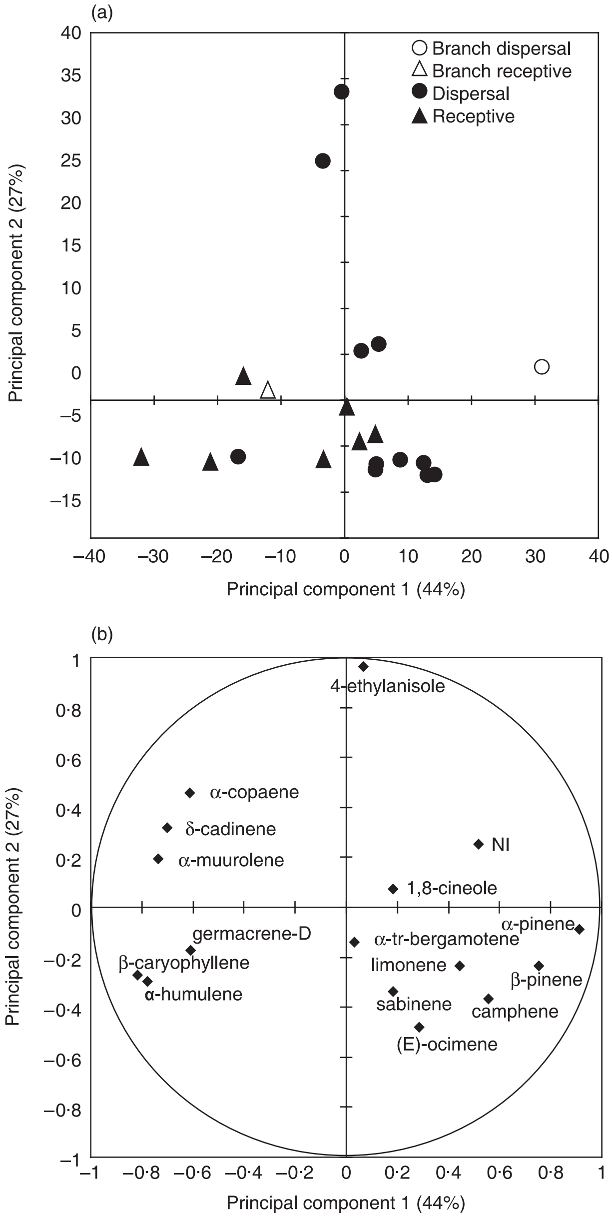

Fig. 3. Volatile profiles of *Ficus tsjahela*. (a) PCA analysis of VOCs produced by male and female figs in receptive and dispersal phases, as well as branches collected during receptive and dispersal phases. (b) Factor loadings of VOCs in the above PCA analysis. NI, not identified compound.

FICUS TSJAHELA

The volatile signature of the receptive phase had, on average, 54·3 compounds (SE = 2·7, n = 6 trees) while that of the dispersal phase had 49·2 compounds (2·7, n = 12). A PCA performed using only VOCs that occurred at least once in a sample at > 3% levels showed no clear separation of receptive and dispersal phase figs (Fig. 3a); some VOCs such as β-caryophyllene and α-humulene seemed to be more associated with receptive figs (Fig. 3b). In the PCA analysis, we found no effect on the results if we included or removed the data of the aromatic branches. This indicates that the branches cannot be separated from the aroma of the figs at the corresponding stages (Fig. 3a) and also that figs in receptive or dispersal stages are themselves indistinguishable, at least by this analysis.

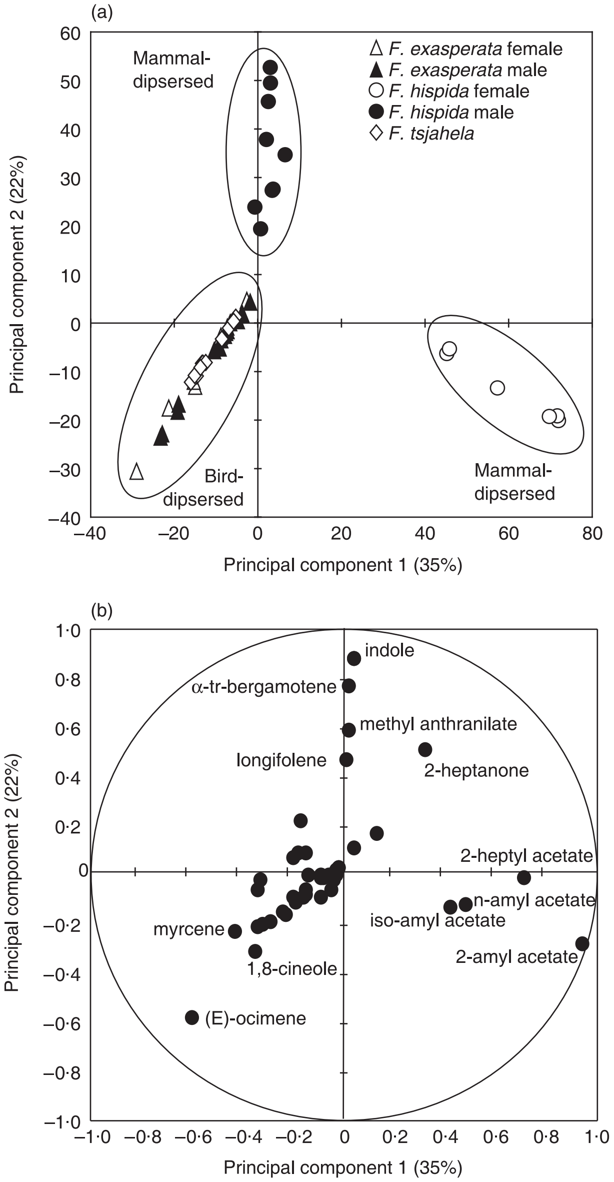

Fig. 4. Volatile profiles of all *Ficus* species examined together. (a) PCA analysis of dispersal phase VOCs of male and female figs of *Ficus hispida* and *F. exasperata*, and of monoecious *F. tsjahela.* (b) Factor loadings of VOCs in the above PCA analysis.

There was no difference between receptive and dispersal stages in the number of VOCs emitted by figs [Table 1; receptive phase: mean = 54·3 VOCs (SE = 2·7, n = 6); dispersal phase: mean = 49·2 VOCs (2·7, n = 12)]. There was also no significant difference in the total quantity of VOCs produced between receptive and dispersal stages (Table 2), and no effect of the year of collection [Tables 1 and 2; receptive figs: mean = 5·6 ng fig^{-1} 3 h^{-1} (SE = 2·6, n = 6); dispersal figs: mean = 3·1 ng fig^{-1} 3 h^{-1} (1·0, n = 12)]. Seven VOCs were most important in the signature: α-pinene, (*E*)-ocimene and β-caryophyllene were present in > 10% quantity on average, while β-pinene, 4-ethyl anisole, α-copaene and germacrene D were present in > 3% quantity on average (Fig. 3b, Supplementary Appendix S1). However, there was no effect of stage on VOC quantities (MANOVA: Wilks' λ = 0·54, $F_{6,11}$ = 1·6, P = 0·24).

Comparison of dispersal phase volatiles of the three species

We attempted to determine whether the volatile signatures of the dispersal stages of the three species could be separated using a PCA analysis. For this, we employed only VOCs that occurred at least once in a sample at > 5% levels (43 compounds). In this combined analysis, we found three distinct clusters: gall figs of *F. hispida*, seed figs of *F. hispida*, and a combined cluster of dispersal figs of *F. exasperata* and *F. tsjahela* (Fig. 4a). Furthermore, an ANOVA on the scores of these individuals on the three main principal components showed that Principal Component 1 (35% of variance) clearly separated gall and seed figs of *F. hispida* from the others ($F_{4,44}$ = 134·73, P < 0·0001) (Fig. 4a,b), while Principal Component 2 (22% of variance) separated male *F. hispida* from the rest ($F_{4,44}$ = 43·44, P < 0·0001) and Principal Component 3 (8% of variance) separated *F. tsjahela* from the rest ($F_{4,44}$ = 24·37, P < 0·0001).

There was a significant effect of species and of stage on the total number of VOCs produced, but a non-significant interaction between species and stage (Table 1). However, for the total quantity of volatiles produced, there was a non-significant effect of species and stage but a significant interaction effect between species and stage (Table 2). This was due mainly to the large amounts of compounds produced by female seed figs of *F. hispida* (Fig. 5a,b) compared to those produced by other figs. An analysis of quantities of compounds produced by each dioecious species, conducted to understand the interaction effect of sex, stage and species, showed that only seed figs of *F. hispida* were significantly different from the other categories (LSMEANS, Tukey–Kramer multiple comparisons, P < 0·0001). Fatty acid derivatives were higher both in percent as well as in absolute concentration only in seed figs of *F. hispida* relative to all other figs while shikimic acid derivatives, monoterpenes and sesquiterpenes characterized the other syconial stages (Fig. 5a,b).

Discussion

We have shown that, within species, volatile signatures of figs vary with stage of development of the fig as well as with sex. Volatile signatures are also species-specific. Moreover, these differences and similarities can be put into the context of the particular biology of the fig species, especially whether the species is dispersed by birds, mammals or both, and whether the species, if dioecious, has its male and female syconia available simultaneously or at different times.

VOLATILE SIGNATURES OF RECEPTIVE VS. DISPERSAL PHASES

The fig is a climacteric fruit wherein sharp rises in respiration and ethylene production within the syconium precede the

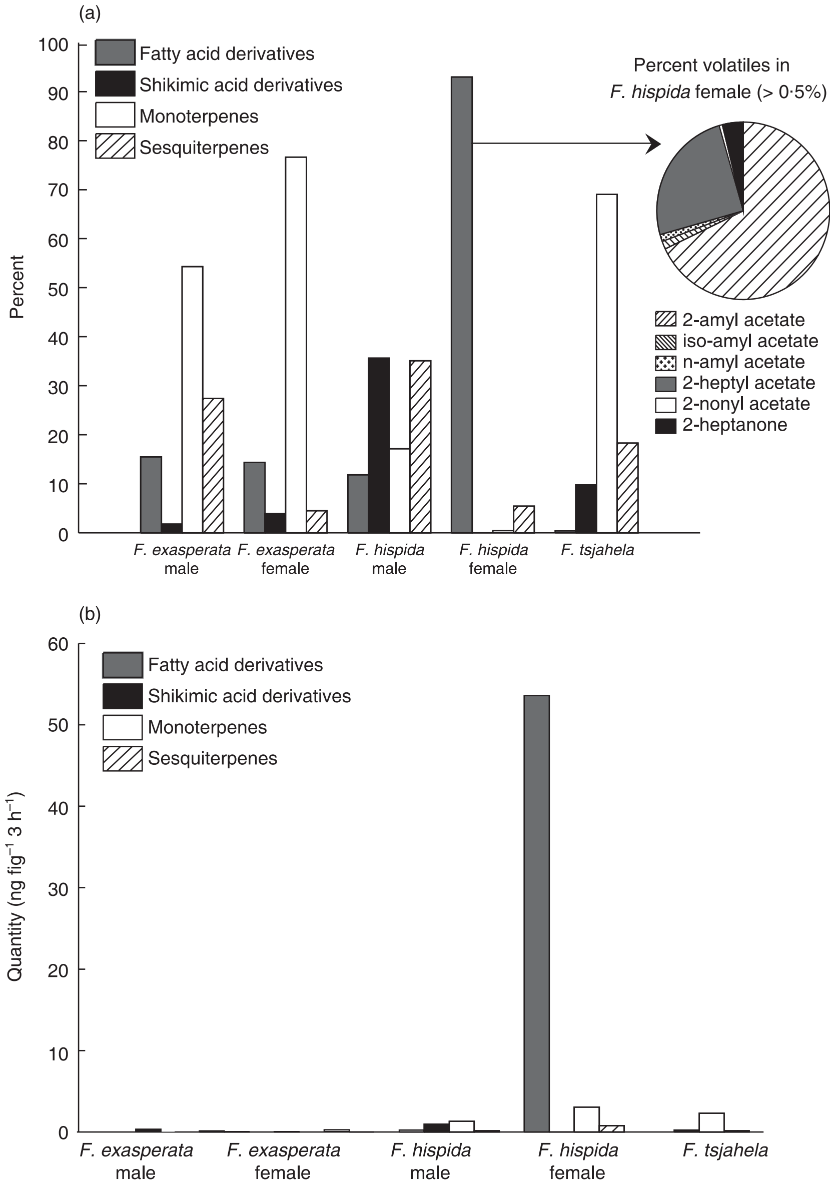

Fig. 5. (a) Mean percent VOCs in different classes in the different *Ficus* species and sexes. The relative composition of fatty acid derivatives is detailed (pie chart) for female dispersal figs of *F. hispida*. (b) Mean quantity of VOCs (ng fig^{-1} 3 h^{-1}) in different classes in the different *Ficus* species and sexes.

ripening process (Marei & Crane 1971) and influence release of pollinating wasps from the syconium (Galil, Zeroni & Bar Shalom 1973). Thus, differences between the VOCs of receptive and dispersal stages of figs are expected. *Ficus hispida*, which is a mammal-dispersed fig (Tang *et al*. 2007) showed clear separation between the volatile signatures of receptive and dispersal stages. However, in the largely bird-dispersed *F. exasperata* (Whitney *et al*. 1998) and *F. tsjahela* (R.M. Borges, personal observation) there was no such clear separation. The reasons for such a difference between the species are not yet clear. Furthermore, since these are the first data on comparisons between volatile signatures of figs in the receptive and dispersal stages, we are unable to determine whether this is a general difference between mammal- and bird-dispersed figs in receptive and dispersal syconia. That figs do differ in VOC profile even between pre-pollination and post-pollination stages is, however, known for one of our studied species, *F. hispida* (Proffit *et al*. in press).

VOLATILE SIGNATURES OF THE DISPERSAL PHASE OF SEED FIGS VS. GALL FIGS

The VOC signature of the dispersal phase of the seed figs of *F. hispida* was significantly different from that of the gall figs, as predicted (Figs. 1 and 5). However, this prediction was not borne out in *F. exasperata*, in which the signatures of seed figs and gall figs were not as clearly separated (Figs 2 and 5). How might these differences between the two dioecious species be explained? The phenology of syconium production in *F. hispida* differs from that of *F. exasperata* (Patel 1996). In *F. hispida*, receptive or dispersal stage figs are available simultaneously on trees of both sexes in the population (synchrony between sexes) while in *F. exasperata*, there is low overlap between syconia production in the sexes (asynchrony between sexes) (Patel 1996). There should be selection on seed figs to be different from gall figs such that only seed figs are consumed (Corner 1978; Lambert 1992; Patel & McKey 1998; Dumont *et al.* 2004). However, selection for this difference is expected to be greater in dioecious species that produce seed and gall figs simultaneously in the same season (Lambert 1992). Thus, there should be less selection pressure on *F. exasperata* than on *F. hispida* in the dispersal phase to make the gall fig volatile signature different from that of seed figs. From the perspective of attraction using volatiles, we suggest that one likely response to such selection could be the evolution of a volatile signature in seed figs of *F. hispida* that is more attractive to mammalian dispersers, since frugivorous birds are known to be more responsive to visual cues (Sallabanks 1993). The ripe figs of *F. exasperata* are bright red in colour in contrast with the dull colour of the ripe figs of *F. hispida*. Sex differences in VOC signature could also result from other differences in chemical composition reflecting the higher nutrient quality of seed compared to gall figs, as was found for *F. pungens*, in which seed figs had higher levels of soluble carbohydrates and fat and lower fibre contents compared to gall figs (Dumont *et al.* 2004). Such differences could lead to the higher levels of fatty acid derivatives found in the VOC signature of the seed figs of *F. hispida* (Fig. 5a,b). Since mammals, including bats, are sensitive to such compounds which include aliphatic esters and alcohols (Laska 1990; Laska & Seibt 2002a, b), incorporating these compounds in VOC signatures could be a strategy to target mammals as preferred dispersers for such figs. Furthermore, *F. hispida* is cauliflorous and geocarpic, and therefore, more likely to be preferentially consumed by bats and other mammals such as rodents (Lambert & Marshall 1991).

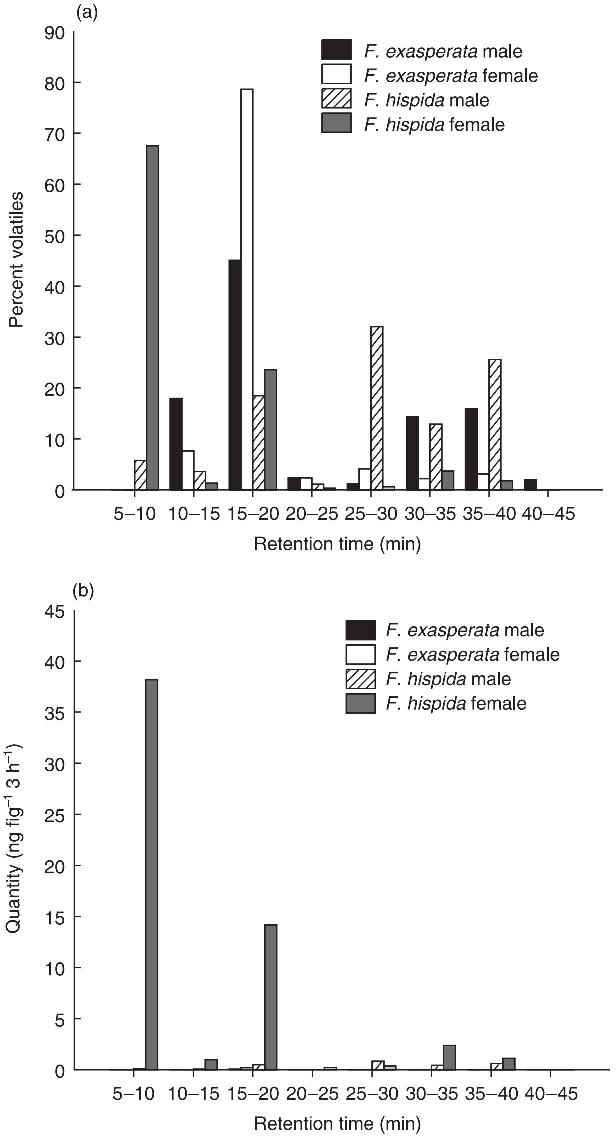

Fig. 6. (a) Mean percent volatiles in 5-min intervals of retention time in the GC analysis in the different *Ficus* species and sexes. (b) Mean quantity (ng fig^{-1} 3 h^{-1}) in 5-min intervals of retention time in the GC analysis in the different *Ficus* species and sexes.

Mammalian dispersers of figs usually occur at lower densities compared to birds (Compton *et al.* 1996; Shanahan & Compton 2001). This could result in selection for a stronger VOC signal, especially in seed figs, of bat- or rodent-dispersed fig species. Consistent with this prediction is the fact that in our study, the highest concentration of the VOC signal was found in the dispersal-phase seed figs of *F. hispida* (Fig. 5b). Bats are indeed effective seed dispersers of *F. hispida* in south Asia (Tang *et al.* 2007), and the continuously available steady-state fruiting of *F. hispida*, in which only few ripened fruit are available at any one time, attracts solitary bat species (Corlett 2006). Furthermore, there is evidence that the olfactory detection threshold of many mammals including bats, primates and rats, is negatively correlated with carbon chain length of the compounds (Laska, Seibt & Weber 2000), which means that the longer the carbon chain length, the better the detection at lower quantities. This also means that the higher the volatility of the compound (i.e. in approximate terms, the fewer carbon atoms it possesses), the higher the olfactory detection threshold. This suggests that *F. hispida*, which produces a few mature seed figs each night for dispersers, will have a higher probability of attracting low-density mammalian dispersers if it produces compounds of higher volatility in higher amounts and compounds with lower volatility in lower amounts, in order to facilitate detection. Since volatility of a compound can be approximated by its retention time in the GC analysis, we grouped volatiles according to their retention times (in 5-min intervals) and determined their representation in the signals of the various species and sexes (Fig. 6a,b). As predicted, the seed figs of *F. hispida* produced the highest percents and quantities of compounds with the highest volatility, while in the other figs, the opposite pattern was observed (Fig. 6a,b).

VOLATILE SIGNATURES OF MAMMAL-DISPERSED *F. HISPIDA* VS. BIRD-DISPERSED *F. EXASPERATA* AND *F. TSJAHELA*

Of the various mammal dispersers of fig seeds (Shanahan *et al.* 2001), bats are the most important in terms of numbers as well as the distances to which fig seeds are carried (Lambert 1990; Kalko, Herre & Handley 1996; Shilton *et al.* 1999; Korine *et al.* 2000). However, figs appear to be one group in the Asian tropics in which specialist fig feeders have evolved among birds. For example, some fruit pigeons (*Treron*), some barbets (*Megalaima*) and one hornbill are specialist fig consumers that travel large distances in search of fig crops (Lambert & Marshall 1991; Shanahan *et al.* 2001). Furthermore, if plants evolve to utilize the services of specialist fig consumers such as *Treron*, the lipid (fatty acid) content of these fruits should be lower since some birds such as *Treron* lack a gall bladder (Garrod 1874). *Treron phoenicoptera* and *Megalaima* (*M. zeylanica* and *M. rubricapilla*) are important visitors to both *F. exasperata* and *F. tsjahela* at our study sites (R.M. Borges, personal observation).

Since bird-dispersed figs seem to have no obvious odour associated with them (Compton *et al.* 1996; Kalko *et al.* 1996), it appears that birds use other cues such as visual cues to locate ripe figs. However, with the exception of our present study, no investigation of volatiles produced by bird-dispersed figs has been conducted. Our study has shown that even bird-dispersed figs such as those of *F. exasperata* and *F. tsjahela* produce volatiles, but that these volatiles are substantially different in quality and quantity from those produced by mammal-dispersed figs (Fig. 4a). Furthermore, the volatiles produced by gall figs of the continuously fruiting mammal-dispersed *F. hispida* were also indistinguishable from those of bird-dispersed figs. Although birds use olfaction in social behaviour (Hagelin, Jones & Rasmussen 2003), foraging (Bonadonna *et al.* 2006), homing (Wallraff 1990) and self-medication (Petit *et al.* 2002), the role of dispersal-phase VOCs in attracting or repelling birds is completely unexplored. We found methyl anthranilate [a bird- and mammal-repellent compound (Mason, Adams & Clark 1989; Clark & Mason 1993; Nolte, Mason & Clark 1993)] in the gall figs of both *F. hispida* (in very high quantities) and *F. exasperata* but none in the seed figs of either species. Figs are thus possibly using specific compounds to repel dispersal agents from gall figs and to facilitate preferential consumption of seed figs, as earlier predicted.

Research on the salience of cues or signals that can evolve to facilitate communication between plants and vertebrates is growing. However, this research has largely focused on mammals rather than on birds. For example, 2-heptanone which was found in our study to be characteristic of seed figs of *F. hispida*, is associated with oestrus in mammals such as rodents (Schwende, Wiesler & Novotny 1984). A generalist mammal such as the rat, *Rattus norvegicus*, is also highly sensitive to the banana-like odour of esters such as amyl acetate (Alberts & May 1980). 2-heptanone and amyl acetates constituted 68·7% of the VOC profile of seed figs of *F. hispida* (Fig. 5a). Bats have been found to be attracted to fruity odours (esters, alcohols) (Laska 1990), although they avoid higher concentrations of ethanol (Sánchez *et al.* 2006). As in other mammals in which innate or acquired olfactory sensitivity is context-dependent (Laska *et al.* 2000), bats may also differ in their preference for volatile cues based on their food preferences (Mikich *et al.* 2003). There may also be region-specific olfactory adaptations of taxa as found for flower-visiting bats (von Helversen, Winkler & Bestmann 2000; Pettersson, Ervik & Knudsen 2004). However, the frequency of occurrence of compounds in the environment of a species may not necessarily determine olfactory sensitivity in that species to those compounds (Laska *et al.* 2006). It is clear that research on olfactory adaptations is in its infancy (Laska *et al.* 2000), and much more needs to be determined about the chemical environment of species and the salience of potential communication signals. Thus, just as mammal- and bird-dispersed fruit differ in sugar types based on differences in disperser physiology and nutritional ecology (Martínez del Rio & Restrepo 1993), so also volatiles may differ between bird- and mammal-dispersed fruit, as we found for *F. hispida*, *F. exasperata* and *F. tsjahela*. More comparative data performed in the context of phylogenetic contrasts are needed to validate this prediction.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1. Dispersal phase volatile compounds (percentages and quantities) in the various fig species

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2435.2008.01383.x
(This link will take you to the article abstract).