

VOLATILES FROM *Ficus hispida* AND THEIR ATTRACTIVENESS TO FIG WASPS

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Abstract—Volatile compositions of receptive (ready to be pollinated), postpollinated, and postparasitized figs, and leaves of *Ficus hispida* were analyzed. Differences among them were examined, and the specificity of fig wasp attractiveness was investigated. Linalool was the major constituent of steam-distilled oil of either male or female receptive figs, while dibutyl phthalate was the major compound of the oils of postparasitized and postpollinated figs. In petroleum ether extracts, palmitic oil, and 9,12-octadecadienoic acid were the main constituents of male and female receptive figs, while hexadecanoic acid ethyl ester was the major compound of postparasitized and postpollinated figs. In dichloromethane extracts, linalool was the major constituent of male and female receptive figs, 1-hydroxylinalool was the major component of male postparasitized figs, and 1-hydroxylinalool and benzyl alcohol were the major constituents of female postpollinated figs. Bioassays with sticky traps showed that *Ceratosolen solmsimarchal* was attracted to dichloromethane extracts of male and female receptive figs and to petroleum ether extracts of female receptive figs, but was not attracted to dichloromethane and petroleum ether extracts of male postparasitized and female postpollinated figs. Figs were attractive to pollinating wasps only at the receptive stage. The volatile constituents of receptive figs were different from those of postpollinated or postparasitized figs. From a receptive to a postpollinated state, figs changed in their volatile composition. Some compounds disappeared or decreased in amount. These include linalool, linalool oxide, α -terpeneol, and 2,6-dimethyl-1,7-octadiene-3,6-diol, which may act as the attractants of the wasps. Others increased in amount, or several additional chemicals appeared. These include dibutyl phthalate, 1-hydroxylinalool, and

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benzyl alcohol, which may be repellents of the wasps. That dichloromethane extracts of male and female receptive figs showed similar activities in attracting fig wasps indicates that receptive figs of both sexes are similarly attractive to fig wasps, which is further supported by their similar volatile composition. Leaf extract was not attractive to the wasps.

Key Words—*Ficus hispida*, *Ceratosolen solmsimarchali*, fig volatile, chemical attraction.

INTRODUCTION

Specialist insects often choose their host plants carefully. They may feed on one or only a few closely related plant species. Although visual factors may be important (Rausher, 1978; Owens and Prokopy, 1986), the search for and identification of preferred host plants are usually olfactory (Miller and Strickler, 1984). The nondirected dispersal flight of the specialist insect is converted into active search behavior once specific host volatile information is perceived (Cardé, 1984).

Mutualism between fig (*Ficus*) and fig wasp (*Agaonidae*) is species-specific. Each species of *Ficus* is generally pollinated by one specific species of fig wasp (Ramirez, 1974; Wiebes, 1979). Fig trees are totally dependent on wasps for pollination, and as a reward, figs provide food for wasp larval development inside the fruit (Janzen, 1979). The high degree of specificity that pollinating wasps show to their particular fig species led to the assumption that the trees attract their specific pollinators through the release of volatile chemicals when the figs are ready to be pollinated (Ramirez, 1970; Hills et al., 1972; Galil, 1977; Janzen, 1979). Olfactory attraction is considered a reasonable way by which fig trees attract huge number of these tiny specific pollinators from far away. Although chemical attraction was suggested more than 50 years ago (Condit, 1947), studies on it are still preliminary (Barker, 1985; Baijnath et al., 1986; Bronstein, 1987, 1992; van Noort et al., 1989; Ware et al., 1993; Ware and Compton, 1994; Gibernau et al., 1997; Grison et al., 1999). An investigation using fig-bearing trees and arrays of sticky traps baited with figs suggested that the wasps are attracted to the trees by volatiles emanating from the figs and that wasps are specifically attracted to figs of their host species only at the time when figs are ready to be pollinated (Ware and Compton, 1994).

In order to elucidate the chemical mechanisms underlying the specific attractiveness of fig trees to their pollinating wasps, we analyzed the chemical constituents of volatiles from receptive (ready to be pollinated), postpollinated, and postparasitized figs of *Ficus hispida* L. and the difference in composition of volatiles among these states of figs. Using arrays of sticky traps baited with fig volatiles, we investigated the specificity of fig wasp attraction to these volatiles.

METHODS AND MATERIALS

Materials. *Ficus hispida* L. is a pioneer tree growing in abundance in the wastelands of the tropics. It is gynodioecious and bears fruit year round. Our previous investigation showed that two or three pollinating wasps were found inside each receptive fig and that all of them were *Ceratosolen solmsimarchali* Mayr (Yang et al., 1997).

Fresh male and female receptive figs, female postpollinated, and male post-parasitized figs and leaves were collected from fruit-bearing healthy *F. hispida* trees inside Xishuangbanna Tropical Botanical Garden, Yunnan, China. Receptive figs are flowering inflorescences that receive pollinating wasps. Figs at different developing stages were determined by fig dissection. Fresh leaves were taken from female and male trees and mixed at a 1 : 1 ratio.

Collection of Volatiles. For steam distillations, immediately following collection, all materials were ground and subjected to vacuum steam distillation for 6 hr at 100°C. Distillates were extracted 3 times with diether ether and dried over sodium sulfate. The oils obtained were then frozen. For solvent extraction, fresh ground plant materials were soaked twice in petroleum ether (30–60°C) or dichloromethane for 24 hr at room temperature. Extracts from each soaking were combined and reduced in volume at 30°C by rotary evaporator. Extracts were dried over sodium sulfate and frozen.

Analysis of Volatiles. Frozen samples were subjected to gas chromatography–mass spectrometry by using a Finnigan 4510 GC/MS/DC. An HP-5 column (30 m long, 0.25 mm ID) was used under the following conditions: 10 psi head pressure, split flow ratio 30 : 1, and oven temperature program of 80–250°C at 5°C/ min. The carrier gas was helium, the injector was maintained at 230°C, and injection volume was 0.2 μ l. Mass spectra conditions: ion source, EI; temperature, 175°C; electron energy, 70 eV; signal-enhancing voltage, 1300 V; bulb current, 0.25 mA; IS scan. Data analyses were carried out by using the EPA/NIH/MASS database (NBS Library database). Retention times and mass spectra of compounds detected in the samples were compared with those of authentic compounds.

Bioassay of Volatiles. A 20 cm⁻² colorless plastic plate sprayed with odorless peach gum was used as a sticky trap. A cotton ball soaked with 0.2 ml dichloromethane solution of the sample (concentration 10% v/v) was placed at the center of the sticky plate to attract fig wasps. Sticky traps were placed on poles. Two arrays of three sticky traps for each treatment were placed 3 m from the fig tree canopy. The plates were positioned 1 m away from each other and 1.5 m above ground. Cotton balls with 0.2 ml dichloromethane were used as solvent controls. The sticky traps were placed at 6:30 AM in May 2000. Insects trapped were recorded and collected at 1200 hr and 1800 hr for two days. The number of the wasps used

is the total of wasps trapped in two days. ANOVA of data were done with Winks software.

RESULTS

Volatiles of figs from steam distillation were present in small quantities, about 20 $\mu\text{l}/\text{kg}$ fresh wt of fig material. Compounds may have been altered by heat. Headspace extraction is nondestructive, but yield of volatiles is even less. Therefore, lower polarity solvents (petroleum ether and dichloromethane) were used to extract enough volatiles from the figs for bioassay. Solvent extraction may also yield some nonvolatile compounds. The following are the comparative analyses of volatiles from figs and leaves of *F. hispida*.

Constituents of Steam Distilled Oils. Large numbers of volatile compounds were obtained through steam distillation (Table 1). Fifty-five compounds from steam distilled oil of male receptive figs were isolated, and 44 of them were identified by GC-MS. Among them, palmitic oil (30.74%), linalool (18.77%), and 9,12-octadecadienoic acid (5.43%) were the major constituents. *cis*-Linalool oxide, *trans*-linalool oxide, 2,6-dimethyl-3,7-octadiene-2,6-diol, and some other compounds were found as well. In the oil of postparasitized male figs, 21 compounds were isolated and 19 were identified. Dibutyl phthalate (41.07%), palmitic oil (27.80%), 9,12 -octadecadienoic acid (9.31%), and heptadecane (3.24%) were the major compounds found. From the oil of female receptive figs, 31 compounds were isolated and 21 were identified. Linalool (19.83%), β -pinene (11.65%), α -terpeneol (9.90%), 3-phenyl-2-propenal (7.83%), sabinene (7.76%), α -pinene (6.85%), terpene-4-ol (6.77%), geraniol (6.00%), 2,6-dimethyl-3,7-octadiene-2,6-diol (4.90%), and γ -terpene (4.80%) were the major compounds. 1-Hydroxy-linalool, 6-methyl-(*E*)-3,5-heptadien-2-one, and 2,6-dimethyl-3,7-octadiene-2,6-diol were not found in oils of other fig materials. From the oil of postpollinated female figs, 13 compounds were isolated, and two were identified. The major compound was dibutyl phthalate (72.71%).

According to the data, a large change occurred in the volatile composition of figs from the receptive stage to the postpollinated or postparasitized stage. Forty of the 44 identified compounds from male receptive figs were not detected from the oil of postparasitized figs, while 15 compounds from postparasitized figs were not found in the oil of receptive figs. Similarly, 20 compounds from female receptive figs were not found in the oil of female postpollinated figs, which, in turn, have their own special compounds. However, female and male receptive figs share some volatile compounds together. After pollination or parasitization, some volatile compounds in the figs disappeared or were present in reduced amounts (such as linalool and α -terpeneol), while other compounds occurred newly or in increased amount (such as dibutyl phthalate, which is a known insect repellent).

TABLE 1. CONSTITUENTS OF STEAM-DISTILLED OILS FROM DIFFERENT FIG TYPES OF *Ficus hispida*^a

No.	Compound	Male receptive figs	Male postparasitized figs	Female receptive figs	Female postpollinated figs
1	hexanal	0.83			
2	3-hexen-1-ol	0.89			
3	1-hexanol	3.95			
4	2,4,6-trimethyl-1-nonene	0.30			
5	benzaldehyde	0.29			
6	<i>cis</i> -linalool oxide	0.87			
7	<i>trans</i> -linalool oxide	0.76			
8	linalool	18.77		19.83	
9	2,6-dimethyl-3,7-octadiene-2, 6-diol	0.42			
10	(<i>Z</i>)-2-decenal	0.24			
11	1-dodecylne	0.31			
12	borneol	0.19			
13	α -terpeneol	3.90		9.90	
14	2,7-dimethyl-2,6-octadien-1-ol	1.18			
15	geraniol	1.83		6.00	
16	(<i>E</i>)-2-tridecen-1-ol	0.19			
17	3-phenyl-2-propenal	0.40			
18	5-(2-propenyl)-1,3-benzodioxole	0.15			
19	2,4-decadienal	0.23			
20	1-(2,6,6-trimethyl-1,3 -cyclohexadienyl)-2-buten-1-one	0.24			
21	β -elemene	0.60			
22	zingiberene	0.54			
23	12-methyl-(<i>E,E</i>)-1,5,9, 11-tridecatetraene	0.17			
24	β -caryophyllene	0.36			
25	3-phenol-2-propen-1-ol, acetate	0.70			
26	β -farnesene	0.56			
27	curcumene	0.44			
28	β -bisabolene	0.94			
29	elemol	1.38			
30	nerolidol	1.55			
31	caryophyllene oxide	1.00			
32	guaial	1.02			
33	farnesol	5.83			
34	11-octadecenal	0.60			
35	octyl-oxirane	0.62			
36	cyclopentadecanone	0.42			
37	6-octadecenoic acid methyl ester	0.53			
38	dibutyl phthalate	4.59	41.07		72.71

TABLE 1. CONTINUED

No.	Compound	Male receptive figs	Male postparasitized figs	Female receptive figs	Female postpollinated figs
39	hexadecanoic acid	30.74	27.8		
40	12,15-octadecadienoic acid, methyl ester	0.09			
41	9,12,15-octadecatrienoic acid, methyl ester (<i>Z,Z,Z</i>)	0.34			
42	1,7,11-trimethyl-4- <i>l</i> (1-methylethyl)-cyclotetradecanol	0.94			
43	9,12-octadecadienoic acid	5.43	9.31		
44	octadecanoic acid	0.40	0.82		
45	6-ethyltetrahydro-2,2, 6-trimethyl-2H-pyran-3-ol		0.34		
46	dodecanoic acid		0.81		
47	1-chloro-octadecane		0.67		
48	heptadecane		3.24		
49	octadecanal		2.32		
50	8,11-octadecadienoic acid, methyl ester		1.58		
51	octadecanol		1.16		
52	2,6-dimethyl-heptadecane		1.25		
53	2,3-dimethyl-heptadecane		1.64		
54	nonadecane		0.86		
55	11-dodecen-2-one		0.86		
56	17-octadecenoic acid, methyl ester		1.75		
57	(<i>Z</i>)-9-octadecenoic acid, methyl ester		0.59		
58	9-octadecenal		1.10		
59	(<i>Z</i>)-9-tricosene		0.83		
60	α -pinene			6.85	
61	1-tetradecen-3-yne			3.70	
62	sabinene			7.76	
63	β -pinene			11.65	
64	α -terpene			2.72	
65	1-methyl-4-(1-methylethyl)-benzene			2.74	
66	limonene			3.88	
67	eucalyptol			2.78	
68	γ -terpene			4.80	
69	6-methyl-(<i>E</i>)-3, 5-heptadien-2-one			2.89	
70	terpene-4-ol			6.77	
71	2,6-dimethyl-3,7-octadiene-2,6-diol			4.90	
72	2-ethenyl-2,5-dimethyl-4-hexen-1-ol			2.86	
73	3-phenyl-2-propenal			7.83	
74	1-hydroxylinalool			2.88	
75	<i>trans</i> -2-tridecenal			2.97	

TABLE 1. CONTINUED

No.	Compound	Male receptive figs	Male postparasitized figs	Female receptive figs	Female postpollinated figs
76	pentadecane			1.76	
77	camphene			2.88	
78	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester				5.24
	unidentified compounds	11 ^b , 4.30 ^c	2 ^b , 1.99 ^c	10 ^b , 11.38 ^c	11 ^b , 21.05 ^c

^aValues are the percentage of each compound in whole steamed oil.

^bNumber of unidentified compounds.

^cCombined percentage of unidentified compounds.

Constituents of Solvent Extracts. Sixteen compounds were isolated from petroleum ether extracts of male receptive figs (Table 2). Eight were identified. Palmitic oil (51.90%) and 9,12-octadecadienoic acid (39.39%) were the major constituents. Fifteen compounds were isolated from petroleum ether extracts of male postparasitized figs, and 10 were identified. Hexadecanoic acid ethyl ester (29.69%) and hexadecanoic methyl ester (22.26%) were the major constituents. Thirteen compounds were isolated from petroleum ether extracts of female receptive figs, and four were identified. Palmitic oil (68.73%) and 9,12-octadecadienoic acid (25.99%) were the major constituents. Eleven compounds were isolated from female postpollinated figs, and five were identified. Major compounds were hexadecanoic acid ethyl ester (34.32%), hexadecanoic methyl ester (24.01%), and palmitic acid (22.35%). Sixteen compounds were isolated from petroleum ether extracts of leaves, and seven were identified. Major constituents were 9,12-octadecadienoic acid (43.39%) and palmitic acid (28.82%). In comparison with steam distillation, petroleum ether extraction yielded fewer volatile compounds. That female and male receptive figs were different in volatile composition from female postpollinated or male postparasitized figs was confirmed by petroleum extraction. Female and male receptive figs have their own special volatiles, characterized by a greater proportion of 9,12-octadecadienoic acid and palmitic acid. The main constituents of leaf extracts were similar to those of female and male receptive figs.

In dichloromethane extracts of male receptive figs (Table 3), 17 compounds were detected, and eight were identified. The major constituents were linalool (51.20%), 1-hydroxylinalool (11.45%), bicyclo[2,2,1]hepta-2,5-dien-7-ol (11.46%), and 2,6-dimethyl-1,7-octadiene-3,6-diol (5.22%). Fifteen compounds were isolated from dichloromethane extract of male postparasitized figs, and 11 were identified, with 1-hydroxylinalool (33.00%), linalool (22.32%), and benzyl alcohol (20.56%) being the main constituents. In dichloromethane extracts of female receptive figs, 20 compounds were detected, and 13 were identified. The

TABLE 2. COMPOUNDS FROM PETROLEUM ETHER EXTRACTS OF FIGS AND LEAVES OF *Ficus hispida*^a

No.	Compound	Male receptive fig	Male postparasitized fig	Female receptive fig	Female postpollinated fig	Leaves
1	α -methylstyrene	0.64				
2	1-ethyl-2- methylbenzene	0.22				
3	2,6-dimethyl-2,7- octadiene-1,6-diol	0.17				
4	butanone		0.34			
5	dibutyl phthalate	4.33		1.78		4.88
6	hexadecanoic acid methyl ester	0.35	22.26		24.01	1.5
7	hexadecanoic acid ethyl ester		29.69	1.61	34.32	
8	palmitic acid	51.90	15.91	68.73	22.35	28.82
9	9,12-octadecadienoic acid	39.39	4.84	25.99		43.39
10	octadecanoic acid	1.16	1.3			2.19
11	9-octadecenoic acid methyl ester		6.53			
12	octadecanoic acid methyl ester		2.46			
13	octadecanoic acid ethyl ester		7.53			
14	9,15-octadecadienoic acid methyl ester				4.12	
15	9,12,15-octadecatrienoic acid methyl ester					9.82
16	9,12,15-octadecatrienoic acid ethyl ester					6.43
17	oleic acid ethyl ester		7.03			
18	linoleic acid ethyl ester				8.82	
	unidentified compounds	8 ^b , 1.84 ^c	5 ^b , 2.09 ^c	9 ^b , 1.89 ^c	6 ^b , 6.37 ^c	9 ^a , 2.96 ^c

^a Values are the percentage of each compound in whole extract.

^b Number of unidentified compounds.

^c Combined percentage of unidentified compounds.

main constituents were linalool (33.64%), 1-hydroxylinalool (12.15%), benzyl alcohol (11.20%), 4-methyl-2-pentadecyl-1,3-dioxolane (10.43%), and α -farnesene (6.98%). In dichloromethane extracts of female postpollinated figs, 20 compounds were detected, and 16 were identified. The major compounds were 1-hydroxylinalool (23.77%), benzyl alcohol (19.55%), linalool (16.55%), and methyl-(3-methoxy- 4-hydroxy-benzyl)-ether (8.90%). The number of compounds recovered from dichloromethane extraction was less than that from steam distillation,

TABLE 3. COMPOUNDS FROM DICHLOROMETHANE EXTRACTS OF *Ficus hispida*^a

No.	Compound	Male receptive fig	Male postparasitized fig	Female receptive fig	Female postpollinated fig
1	benzyl alcohol		20.56	11.20	19.55
2	benzeneacetaldehyde		3.80	1.05	3.86
3	linalool	51.20	22.32	33.64	16.55
4	4-methyl-2-pentadecyl- 1,3-dioxolane			10.43	
5	(Z)butanoic acid 2-hexenyl ester			4.89	
6	1-(2,2-dimethylcyclopentyl) -ethanone			1.00	
7	1-(dichloromethyl)-3-methyl- benzene			0.71	
8	trans,trans-2,6-dimethyl-2, 6-octadiene-1,8-diol			0.84	
9	dodecyl-oxirane			1.08	
10	1-hydroxylinalool	11.45	33.00	12.15	23.77
11	β -farnesene	4.00	1.78	2.90	
12	α -farnesene	2.67	1.88	6.98	0.87
13	methyl-(3-methoxy-4- hydroxy-benzyl)-ether			1.80	8.90
14	1-(1-cyclohexen-1 -yl)ethanone		1.05		2.95
15	1-undecyne		2.12		0.97
16	tetradecanal				1.73
17	vanillin				0.79
18	caryophyllene				1.55
19	4-ethyl-1,4-dimethyl -2-cyclohexen-1-ol				1.00
20	nerolidol				0.88
21	4-hydroxy- β -ionone				3.67
22	allyl undecylenate				2.00
23	bicyclo[2,2,1]hepta- 2,5-dien-7-ol	11.46			
24	2-amino-4-nitro-phenol	4.50			
25	2,6-dimethyl-1, 7-octadiene-3,6-diol	5.22			
26	decyl-oxirane	2.60			
27	phenylethyl alcohol		1.08		0.90
28	2,5-dimethyl-1, 5-hexadien-3-ol		1.15		
29	dodecanal		2.83		
	unidentified compounds	9 ^b , 6.90 ^c	4 ^b , 8.43 ^c	7 ^b , 11.33 ^c	4 ^b , 10.06 ^c

^aValues: the percentage of each compound in whole extract.^bNumber of unidentified compounds.^cCombined percentage of unidentified compounds.

but more than that from petroleum ether extraction. The chemical constitution of dichloromethane extracts was similar to that of distilled oil; however, it was rather different from that of petroleum ether extracts. For dichloromethane extracts, female and male receptive figs were similar, while both were different from female postpollinated figs and male postparasitized figs. Female and male receptive figs have their own special volatile compounds. The content of linalool was higher than that for female postpollinated figs and male postparasitized figs. On the other hand, the content of 1-hydroxylinalool and benzyl alcohol of female postpollinated figs and male postparasitized figs was higher than that for receptive figs.

Attractiveness of Volatiles of F. hispida to C. solmsimarchali. Bioassays using sticky traps showed that there were significantly more *C. solmsimarchali* trapped by dichloromethane extracts of female and male receptive figs and by petroleum ether extract of female receptive figs ($P < 0.05$) and that there were no significantly more fig wasps trapped by petroleum ether extract of leaves than by solvent controls (Table 4). Moreover, there were more wasps trapped by petroleum ether extracts of female receptive figs than by those of postpollinated figs ($P < 0.05$). As for dichloromethane extracts, those of receptive figs attracted significantly more wasps than those of postpollinated or postparasitized figs, either female or male ($P < 0.05$). However, there were no differences in fig wasp numbers trapped by either dichloromethane or petroleum ether extracts of female postpollinated and male postparasitized figs and solvent control ($P > 0.05$). Wasps trapped by dichloromethane extracts of female and male receptive figs were not different either ($P > 0.05$). The sticky plate trapped many other insects but no other fig wasp species.

DISCUSSION

Results of bioassays indicate that receptive figs are attractive but postpollinated and postparasitized figs are not attractive to the pollinating wasp in the case of *F. hispida*. Different responses of wasps to receptive figs, postpollinated figs, postparasitized figs, and leaves of the *Ficus* tree were caused by the varying volatile constitutions of these plant materials. The unique volatile constituents and special volatile blend-up are the chemical basis of the receptive figs of *F. hispida* to attract *C. solmsimarchali*.

Field bioassay results of fig volatile extracts of *F. hispida* confirmed conclusions drawn from previous studies on other fig species (Ware and Compton, 1994). Figs are attractive to their wasp pollinator only at the receptive stage.

To identify chemical cues for fig wasps, we adopted a comparative approach. Figs changed in their volatile constituents from receptive to postpollinated or postparasitized states. Some compounds decreased in concentration or disappeared,

TABLE 4. ATTRACTION OF VOLATILE EXTRACTS OF *Ficus hispida* TO *Ceratosolen solmsimarchali*

Treatment	Traps (N)	Mean Fig wasps trapped (±SE)	Newman-Keuls multiple comparison (F values)			
			Against control	Against A	Against E	Against G
A. Petroleum ether extract of female receptive figs	6	14.3 ± 4.2	4.2*		2.9 ^{NS}	3.4*
B. Petroleum ether extract of female postpollinated figs	6	0.5 ± 0.8	NS	4.5*	4.4*	4.2*
C. Petroleum ether extract of male receptive figs	6	1.5 ± 1.4	NS	4.0*	3.8*	3.4*
D. Petroleum ether extract of male postparasitized figs	6	0.3 ± 0.5	NS	4.6*	4.5*	4.4*
E. Dichloromethane extract of female receptive figs	6	12.5 ± 1.4	4.0*	2.9 ^{NS}		2.9 ^{NS}
F. Dichloromethane extract of female postpollinated figs	6	0.5 ± 0.5	NS	4.4*	4.2*	4.0*
G. Dichloromethane extract of male receptive figs	6	11.0 ± 2.6	3.8*	3.4*	2.9 ^{NS}	
H. Dichloromethane extract of male postparasitized figs	6	0.2 ± 0.4	NS	4.7*	4.6*	4.5*
I. Petroleum ether extract of leaves	6	2.7 ± 0.8	NS	3.8*	3.4*	2.9*
J. Control	6	0.7 ± 0.8		4.2*	4.0*	3.8*

*P < 0.05, significant difference; NS: no significant difference at P > 0.05.

while others increased in concentration or several additional chemicals appeared. The compounds that disappeared or decreased in amount after pollination or parasitization were linalool, linalool oxide, α-terpeneol, and 2,6-dimethyl-1,7-octadiene-3,6-diol, which may act as attractants for the wasps. Compounds that increased in amount or newly occurred were dibutyl phthalate, 1-hydroxylinalool, and benzyl alcohol, which could be deterrents to the wasps.

The composition of volatiles of receptive figs produced by steam distillation and dichloromethane extraction was similar to that from the headspace collection (Grison et al., 1999) and pentane extraction (Gibernau et al., 1997) used in investigations of other fig species. The essential chemicals suggested to attract fig

wasps, such as linalool, linalool oxide, and benzyl alcohol, were detected in this investigation also. Linalool and linalool oxide were found in the distilled oil, and linalool, benzyl alcohol, and 1-hydroxylinalool (rather than linalool oxide) were found in dichloromethane extracts. However, benzyl alcohol did not appear to act as a fig wasp attractant but rather as a repellent, because its content in figs increased after pollination or parasitization. Characteristic or major volatile compounds and special blends of volatiles may be important in attractiveness of receptive figs to wasps. Some volatile compounds found in trace amounts may also play a role in this attraction. A much greater amount and number of volatile compounds were produced from steam distillation and dichloromethane extraction compared with previous methods (Gibernau et al., 1997; Grison et al., 1999). It is interesting that dibutyl phthalate, a known insect repellent, was found in large quantity in postpollinated and postparasitized figs. This indicates that figs may, in fact, be repellent to fig wasps after losing attractiveness following pollination or parasitization. Compounds yielded by petroleum ether extraction were largely fatty acid derivatives. Only the extract from female receptive figs showed activity in fig wasp attraction. Extraction with petroleum ether is not ideal for obtaining fig volatiles in large quantity.

Although volatile compositions of receptive figs, male or female, proved much different from those of postpollinated or postparasitized figs, compositions of male and female receptive figs proved similar. They differed only in the quantity of some major compounds; this corresponds to findings on other fig species (Grison et al., 1999). That dichloromethane extracts of male and female receptive figs showed similar activities in attracting fig wasps indicates that receptive figs of both sexes are similarly attractive to fig wasps. This is further supported by their similar volatile composition. Pollinating fig wasps should favor male receptive figs and avoid female figs because they can lay eggs only in male figs, not in female ones, where they pollinate and die. On the other hand, in order to attract wasps for pollination, female receptive figs could imitate the odor of male receptive figs. There could be an intersexual mimicry of odor in the case of *F. hispida*.

Leaf extracts did not show significant activity in the wasp attraction. This suggests that the canopy of fig trees may not play a role in long distance odor attraction of fig wasp. Compounds in leaf extracts were mainly fatty acid derivatives; this is similar to previous findings on other fig species (Buttery et al., 1986).

The species-specific mutualism between a fig tree and its pollinating wasps is based on some unique volatile compounds and special volatile blends of receptive figs. Our bioassays showed that volatile extracts of receptive figs of *F. hispida* were attractive only to one species of fig wasp, its pollinator *C. solmsimarchali*. They were not attractive to other fig wasps, although the sticky plates baited with the extracts trapped some other insects. Furthermore, only *C. solmsimarchali* were found in more than 10,000 dissected receptive figs.

About 900 species of *Ficus* (Janzen, 1979) are widespread in tropical ecosystems. They not only provide food to many diverse animals and microorganisms, but also act as habitats for epiphytes, saprophytes, parasites, and shade-requiring plants. They are keystone species in tropical ecosystems (Wiebes, 1979; Xu, 1994; Yang et al., 1997, 1999). In Xishuangbanna, the tropical area of China, *Ficus* species (such as *F. hispida*, *F. semicordata*, and *F. tinctoria* subsp. *gibbosa*) grow quickly as pioneer woody plants after deforestation. Inside or under the canopy of these plants, a community with rich animal, plant, and microorganism diversity is likely to build and expand gradually. Thus, *Ficus* trees are important in the restoration of tropical ecosystems. The species-specific mutualism between *Ficus* trees and their pollinating wasps makes their reproduction extremely efficient. However, this special relationship is vulnerable. The decrease or extinction of any partner in the mutualism could lead to a decrease or extinction of the corresponding other partner. The study of chemically based mechanisms of species-specific pollination of *Ficus* trees might be significant in the conservation of these keystone species and the whole tropical forest ecosystem. The collection, isolation, identification, and bioassay of the fig wasp attractants from *Ficus* trees deserve further attention.

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REFERENCES

- BAIJNATH, H., NAICKER, S., and RAMCHARUN, S. 1986. The interaction of figs and fig wasps, pp. 348–349, in B. E. S. Juniper and T. R. E. Southwood (eds.). *Insect and the Plant Surface*. Edward Arnold, London.
- BARKER, N. P. 1985. Evidence of a volatile attractant in *Ficus ingens* (Moraceae). *Bothalia* 15:607–611.
- BRONSTEIN, J. L. 1987. Maintenance of species specificity in a neotropical fig-pollinator wasp mutualism. *Oikos* 48:39–46.
- BRONSTEIN, J. L. 1992. Seed predators as mutualists: ecology and evolution of the fig/pollinator interaction, pp. 1–44, in E. Bernays (ed.). *Insect-Plant Interactions*, Vol. IV. CRC Press, Boca Raton, Florida.
- BUTTERY, R. G., FLATH, R. A., MON, T. R., and LING, L. C. 1986. Identification of germacrene D in walnut and fig leaf volatiles. *J. Agric. Food Chem.* 34:820–822.
- CARDÉ, R. T. 1984. Chemo-orientation in flying insects, pp. 111–124, in W. J. Bell and R. T. Cardé (eds.). *Chemical Ecology of Insects*. Chapman and Hall, London.
- CONDIT, I. J. 1947. *The Fig*. Chronica Botanica. Waltham.
- GALIL, J. 1977. Fig biology. *Endeavour* 1:52–56.
- GIBERNAU, M., BUSER, H. R., FREY, J. E., and HOSSAERT-MCKEY, M. 1997. Volatile compounds from extracts of figs of *Ficus carica*. *Phytochemistry* 46:241–244.
- GRISON, L., EDWARDS, A. A., and HOSSAERT-MCKEY, M. 1999. Interspecies variation in floral fragrances emitted by tropical *Ficus* species. *Phytochemistry* 52:1293–1299.

- HILLS, H. G., WILLIAMS, N. H., and DOBSON, C. H. 1972. Floral fragrances as isolating mechanisms in the genus *Catasetum* (Orchidaceae). *Biotropica* 4:61–76.
- JANZEN, D. H. 1979. How to be a fig. *Annu. Rev. Ecol. Syst.* 10:13–51.
- MILLER, J. R. and STRICKLER, K. L. 1984. Finding and accepting host plants, pp. 127–155, in W. J. Bell and R. T. Cardé (eds.). *Chemical Ecology of Insects*. Chapman and Hall, London.
- OWENS, E. D. and PROKOPY, R. J. 1986. Relationship between reflectance spectra of host plant surfaces and visual detection of host fruit by *Rhagoletis pomonella*. *Physiol. Entomol.* 11:297–307.
- RAUSHER, M. D. 1978. Search image for leaf shape in a butterfly. *Science* 200:1071–1073.
- RAMIREZ, B. W. 1970. Host specificity of fig wasps (Agaonidae). *Evolution* 24:680–691
- RAMIREZ, B. W. 1974. Coevolution of *Ficus* and Agaonidae. *Ann. Mo. Bot. Gard.* 61:770–780.
- VAN NOORT, S., WARE, A. B., and COMPTON, S. G. 1989. Pollinator specific volatile attractants released from the figs of *Ficus burtt-davyi*. *S. Afr. J. Sci.* 85:323–324.
- WARE, A. B. and COMPTON, S. G. 1994. Responses of fig wasps to host plant volatile cues. *J. Chem. Ecol.* 20:785–802.
- WARE, A. B., COMPTON, S. G., KAYE, P. T., and VAN NOORT, S. 1993. Fig volatiles: Their role in attracting pollinators and maintaining pollinator specificity. *Plant Syst. Evol.* 186:147–156.
- WIEBES, J. T. 1979. Co-evolution of figs and their insect pollinators. *Annu. Rev. Ecol. Syst.* 10:1–12.
- XU, Z. 1994. *Ficus* trees: Keystone species of tropical rain forest ecosystem in southern Yunnan, China. *Chin. Biodivers.* 2:21–23 (in Chinese with English abstract).
- YANG, D., LI, C., and YANG, B. 1997. Studies on the diversity and structure of animal community living on the *Ficus* trees in Xishuangbanna tropical rain forest. *Zool. Res.* 18:189–196 (in Chinese with English abstract).
- YANG, D., LI, C., HAN, D., and YAO, R. 1999. The impact of the fragmentation of tropical rain forest on fig wasps and *Ficus* species. *Zool. Res.* 20:126–130 (in Chinese with English abstract).