

# Responses of the Pollinating Wasp *Ceratosolen solmsi marchali* to Odor Variation Between Two Floral Stages of *Ficus hispida*

Chun Chen · Qishi Song

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**Abstract** During development of figs on *Ficus hispida*, only the female floral stage is receptive to its pollinator *Ceratosolen solmsi marchali*. After this stage, the quantity of fig odor decreases. The effects of *F. hispida* volatiles from receptive figs (figs at the female floral stage, when they are pollinated) and interfloral figs (between the female floral and male floral stages) on their pollinator were studied, together with responses to compounds present in the odor. Odors emitted by both receptive and interfloral figs were attractive to the pollinator. However, wasps preferred the odor of receptive figs to that of interfloral figs even though the quantity of interfloral volatiles increased. Three monoterpenes that included linalool (major constituent) and two minor compounds limonene and  $\beta$ -pinene from the receptive fig volatiles were used to test the pollinator responses. The levoisomer and racemic mixtures of linalool were attractive to the pollinator at high doses, but the dextroisomer was neutral. ( $\pm$ )-Limonene and ( $-$ )- $\beta$ -pinene at high doses were even less attractive to the pollinator than clean air and were neutral at low doses, while ( $R$ )-(+)-, ( $S$ )-(-)-limonene were neutral at all doses. In blend tests, all four mixtures of ( $\pm$ )-linalool or ( $S$ )-(-)-linalool combined with ( $\pm$ )-limonene or ( $-$ )- $\beta$ -pinene attracted *C. solmsi marchali* when administered at high

doses. ( $R$ )-(+)-linalool and ( $-$ )- $\beta$ -pinene enhanced the attractiveness of ( $S$ )-(-)-linalool to the pollinator, while enantiomers of limonene did not. These results suggest that both quality and quantity of fig volatiles regulate *C. solmsi marchali* response and that quality is the main host-finding and floral stage-distinguishing cue for the pollinator. Synergistic effects of some compounds may play a role in enhancing attractiveness of the active compounds.

**Keywords** Fig-fig wasp mutualism · *Ficus hispida* · *Ceratosolen solmsi marchali* · Post-pollination odor changes · Behavioral tests · Linalool · Limonene ·  $\beta$ -Pinene · Synergistic effect

## Introduction

In general, floral scents are believed to function in attracting animal pollinators to flowers (van der Pijl 1961). Flower visitors associate floral scent with food, mates, or egg-laying sites (Pellmyr and Thien 1986). Floral signal production is a dynamic process. Changes in floral signal are adaptive, conserve floral resources, and maximize pollinator efficacy (Gori 1983; Weiss 1991; Dudareva and Pichersky 2000). An increasing number of studies have concentrated on floral odor changes that are induced by pollination (Theis and Raguso 2005). Such changes are often observed in orchids, particularly in some species with specialized pollinators. For example, in the sexually deceptive orchid *Ophrys sphegodes*, odor serves as the primary signal that attracts specialized pollinators; changes after pollination result in decreased attractiveness of these flowers (Schiestl et al. 1997).

In the species-specific fig-fig wasp mutualism, post-pollination changes also are important for fig wasps to

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C. Chen · Q. Song (✉)  
Xishuangbanna Tropical Botanical Garden,  
Chinese Academy of Sciences,  
Kunming, Yunnan 650223, China  
e-mail: songqishi@gmail.com

C. Chen  
e-mail: chenchun@xtbg.ac.cn

C. Chen  
Graduate School, Chinese Academy of Sciences,  
Beijing 100039, China

locate inflorescences for efficient pollination. Worldwide, there are about 750 fig species (*Ficus*, Moraceae; Berg 1989), and figs generally are pollinated by female fig wasps (Agaonidae: Hymenoptera) that are specific for each *Ficus* species, and they oviposit in the fig syconium (Wiebes 1979; Ware and Compton 1992). Although visual factors may be involved (Ramirez 1974; Janzen 1979; Verkerke 1989), several studies have shown that the pollinating wasps are attracted by olfactory cues (Bronstein 1987; Van Noort 1989; Ware et al. 1993; Hossaert-McKey et al. 1994; Gibernau et al. 1998; Grison et al. 1999). However, in the five stages of fig development [i.e., prefemale floral stage, female floral stage, interfloral stage (time period between female floral stage and male floral stage), male floral stage, and post-floral stage], only the female stage is receptive to pollinators (Galil and Eisikowitch 1968; Bronstein 1987). Ware and Compton (1994) concluded that figs should either cease to produce attractive signals after being pollinated or that new compounds should be produced (or specific components increased) that repel pollinators. This provides an alternate explanation for the pollinators' indifference to post-pollination flowers (Schiestl and Ayasse 2001; Song et al. 2001). Recent studies of *Ficus hispida* L. volatiles did not detect new compounds after pollination, but the quantity of volatile emission decreased quickly after 24 h (Proffit et al. 2008). Some compounds present only in small amounts disappeared when checked by gas chromatography-mass spectrometry, but the abundant compounds  $\beta$ -ocimene, (*E*)- $\beta$ -farnesene, and linalool still existed 2 weeks after pollination (Proffit et al. 2008).

It is clear that pollinators can distinguish between receptive and non-receptive figs (Van Noort et al. 1989; Ware and Compton 1994; Song et al. 2001). However, few bioassays have been conducted to test the pollinators' responses to figs at different developmental stages (Grison-Pigé et al. 2002a). Because floral odors are complex mixtures of volatiles (Knudsen et al. 1993, 2006), it is difficult to determine how alterations in the composition, proportion, and concentration of floral odor components influence pollinator behavior (Patt et al. 1995). Nonetheless, tests of individual volatile compounds and combinations may shed light on the chemical mechanism involved in post-pollination volatile changes.

In this study, we used a Y-tube olfactometer to test fig wasp, *Ceratosolen solmsi marchali* Mayr, behavior to volatile compounds from *F. hispida* and preferences of pollinators to receptive and interfloral figs from their dioecious host. Based on previous studies (Song et al. 2001; Proffit et al. 2008), we also chose three monoterpene compounds from receptive figs, one abundant compound (linalool) and two minor ones (limonene and  $\beta$ -pinene), to test the response of the pollinator. All of these volatile compounds are found in the odor of receptive figs of *F.*

*hispida*, and their quantities have decreased by the interfloral fig stage. The specific purpose of our study was to (1) determine experimentally whether the pollinator was sensitive to floral scent changes between receptive and interfloral figs of *F. hispida*; (2) investigate whether quantity or quality of fig volatiles play an important role in attracting fig pollinators; (3) examine whether linalool, the main constituent of the receptive fig odor of *F. hispida*, plays an important role in attracting pollinators; and (4) whether the minor constituents of fig odor enhance attractiveness.

## Methods and Materials

**Plants and Insects** *F. hispida*, growing naturally at Xishuangbanna Tropical Botanical Garden (101°15' E, 21°55' N) in southwest China, was used to obtain both plant and insect materials. *C. solmsi marchali*, the pollinator of *F. hispida*, develops in syconia of this species. Both the fig and its pollinator have been studied and described in earlier publications (Hill 1967; Abdurahiman and Joseph 1976; Patel et al. 1995; Patel 1996; Patel and Hossaert-McKey 2000; Moore et al. 2003; Peng et al. 2005; Proffit et al. 2008). Pre-receptive figs were isolated from pollinators by using mesh bags. Fresh figs with their branches were cut in the morning, placed into a vase containing water, and used for bioassays of volatiles. When a fig is nearly mature, fertilized female wasps will leave via a channel bored by males (Galil and Eisikowitch 1968). Pre-mature male figs without holes were collected at dusk and put into mesh bags. The next morning, wasps came out of those figs and were selected for olfactometer studies. Fig floral stages were estimated by their physical characteristics: loose obstacle, heavy-sweet scent, and pollinators being attracted outside the bag are signals of a receptive fig; interfloral figs have a hard, solid feel and a deeply colored surface, while a mature fig has a soft, white and smooth surface.

**Chemicals** Previous studies indicate that linalool is the most abundant compound in the odor of receptive *F. hispida* (Song et al. 2001; Proffit et al. 2008). For this reason, the response of *C. solmsi marchali* was tested against (*R*)-(+)-linalool (Linke Reagent Co., Ltd., China,  $\geq 93\%$ ), (*S*)-(-)-linalool (Juhua Reagent Co., Ltd., China,  $\geq 98\%$ ) and a 1:1 mixture of these two enantiomers. Limonene and  $\beta$ -pinene are minor volatiles in the odor of receptive *F. hispida* and numerous other receptive *Ficus* species (Grison-Pigé et al. 2002b). Enantiomers of limonene were tested against *C. somisi marchali* by using (*R*)-(+)-limonene (Fluka,  $\geq 98\%$ ), (*S*)-(-)-limonene (Fluka,  $\geq 97\%$ ) and a 1:1 mixture of these two enantiomers. The levoisomer of  $\beta$ -pinene [(*-*)- $\beta$ -pinene (Cole-Parmer,  $\geq 98\%$ )] was also tested against *C. somisi marchali*.

**Olfactometer Methodology** All experiments were conducted in a Y-tube olfactometer (stem, 8 cm; arms, 9 cm; at 55° angle; ID 1.5 cm) with each of the two arms connected to a glass container or a polyethylene terephthalate (Nalophan) bag (Kalle Nalo GmbH, Würsthüllen, Germany) that contained an odor source. This small dimension olfactometer was adopted to test small wasps that respond to attractants by walking. It is similar to the one described by Tooker et al. (2005). Air is drawn through Teflon tubing by an air pump and passed through a charcoal filter and distilled water. The cleaned and humidified air stream, regulated to a flow rate of 0.2 L/min with two flowmeters, was split via a “Y” hose junction to create two equal air streams. To limit visual distractions for the insects, the olfactometer was placed in the center of a flat, white table illuminated with three 40-W cool white fluorescent tubes placed above the arms of the Y tube. The windows of the bioassay room were covered by black fabric curtains, and the air temperature was monitored and maintained at ~26°C.

Each wasp was allowed 5 min to respond to odors, and a choice of the left or right arm of the olfactometer was noted when the insect went 1 cm (decision line) past the Y junction and stayed for at least 1 min. Wasps not reaching the decision line within 5 min were removed and recorded as “no choice”. Every four to five bioassays, the treatment arm was switched between the two arms of Y-tube to avoid any influence of unforeseen asymmetries in the setup. The olfactometer was rinsed with absolute alcohol and then dried by an air blower after every bioassay.

**Bioassays of Odors of Receptive and Interfloral Fig Sources (Experiment A)** In order to compare responses of *C. solmsi marchali* to receptive and non-receptive floral scents, experiments (Table 1, Exp. A1, A2, A3, and A4) were conducted with four different types of *F. hispida* scents vs. clean air.

**Table 1** Response of the pollinator wasp *Ceratosolen solmsi marchali* to odors of receptive and interfloral fig(s) of *Ficus hispida* (Experiment A)

Experiment	Type and (number of figs) in the arms of the Y tube	
	Arm 1	Arm 2
A1	Male receptive fig (1)	Clean air
A2	Male interfloral fig (1)	Clean air
A3	Female receptive fig (1)	Clean air
A4	Female interfloral fig (1)	Clean air
A5	Male receptive fig (1)	Male interfloral fig (1)
A6	Male receptive fig (1)	Male interfloral fig (9)
A7	Male receptive fig (1)	Male interfloral fig (25)
A8	Female receptive fig (1)	Female interfloral fig (1)
A9	Female receptive fig (1)	Female interfloral fig (9)
A10	Female receptive fig (1)	Female interfloral fig (25)

Grison-pigé et al. (2001) reported that figs have a higher quantity of volatile emission in their receptive stage. The ratios of scent quantity emitted by one fig between receptive fig vs. interfloral fig were about 13:1 for male *F. hispida* and 17:1 for female *F. hispida* (Proffitt et al. 2008). In six subsequent experiments (Table 1, Exp. A5, A6, A7, A8, A9, and A10), we set 1:1, 1:9, and 1:25 ratios for the number of receptive figs vs. interfloral figs to balance the higher quantity of odor emission from receptive figs.

Fresh figs were packed into a polyethylene terephthalate bag and used as a sample odor source. A similar sized bag was used as a control for supplying clean air in the first four experiments. Fig sources were changed every 1–2 h. Trials were replicated until there were at least 24 wasps responding to one of the odor sources in the Y-tube. In each experiment, all replicates were tested over 3 days. All bioassays of fresh fig sources were conducted between 08:30 to 11:30 during November and December 2005.

**Bioassay of Fig Volatiles (Experiment B)** The olfactory responses of *C. solmsi marchali* to three fig volatiles emitted from receptive *F. hispida* (e.g., linalool, limonene,  $\beta$ -pinene, and blends of linalool and limonene and  $\beta$ -pinene). This experiment examined whether a major or a minor constituent of volatiles attracts the pollinator at different quantities and determined whether these compounds could enhance the attraction of pollinators.

The concentrations of volatiles in the blends were set according to the amount emitted by the receptive figs. In the case of *F. hispida*, linalool, limonene, and  $\beta$ -pinene comprise 13.57%, 1.23%, and 0.24% of the total volatiles emitted by the receptive figs of male trees, and 15.74%, 2.90%, and 1.21% of the volatiles emitted by the receptive figs of female trees, respectively (Proffitt et al. 2008). The compounds were diluted separately in dichloromethane ( $10^{-6}$   $\mu$ l ~ 1  $\mu$ l/100  $\mu$ l). Cellulose sponge pieces (1×1×0.2 cm) were impregnated with 100  $\mu$ l of diluted compounds. In the same way, a piece of cellulose sponge with pure solvent (100  $\mu$ l of dichloromethane) was prepared as the control. After 20 min of solvent evaporation (to get rid of dichloromethane completely), each piece of sponge was stored in a pipette tube, the tube was put into a 30 ml glass container, and the container was connected with one arm of the Y-tube. The compound put into the pipette tube was used as a sample odor source and tested individually on one wasp. Each pipette tube was made from a 7 cm long, 1 ml polypropylene pipette tip, from which 3 cm of the bottom and 1.5 cm of the tip were removed. Trials were replicated until there were at least 40 wasps responding to either of the odor sources in the Y-tube.

Seven experiments (Table 2, Exp. B1-1, B1-2, B1-3, B2-1, B2-2, B2-3, and B3) were conducted to test single compounds. Based on the results of single compound tests,

the attractive enantiomers of linalool were mixed with the other two compounds (Table 2, Exp. B4 and B5). Each blend was tested respectively in four doses.

All the bioassays on compounds were conducted between 07:30 to 11:30 from February to May 2006 and from May to July 2007.

**Statistical Analyses** The results from each bioassay experiment were subjected to a chi-square ( $\chi^2$ ) test. The null hypothesis was that wasps had a 50:50 distribution between the two odor sources in two arms, respectively. Given the sample sizes in the experiments, statistical analyses were not needed when all the responding wasps entered one of the arms.

## Results

Before testing all odor sources, a series of control tests were performed with arms of the olfactometer permeated with clean air. There was no observed difference in the responses of wasps to the two arms of the Y-tube: 20 directed to the right and 20 to the left when the arms were switched by turning the Y-tube over every five tests ( $\chi^2=0$ ,  $df=1$ ,  $P=1.000$ ).

**Bioassays of Odors from Receptive and Interfloral Fig Sources (Experiment A)** In the treatment of fig odor vs. clean air, wasps chose the arm of the olfactometer with fig odor over the control, even when odors were emitted from interfloral figs (Fig. 1a). Subsequent experiments investi-

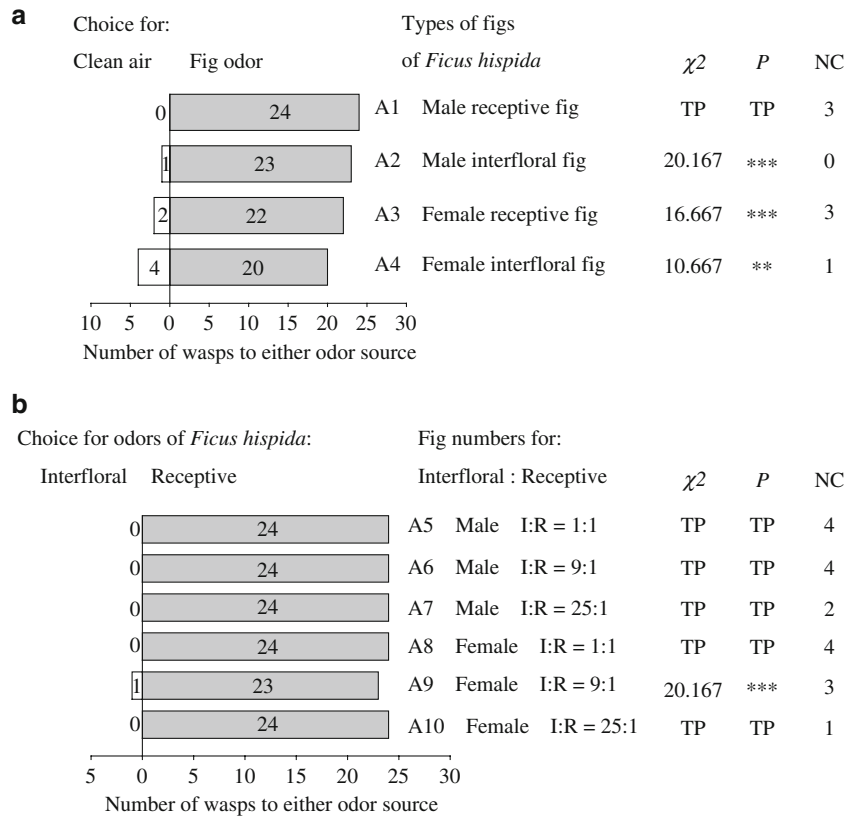
gated the choices of *C. solmsi marchali* made between receptive and interfloral fig odors. Pollinators always showed a strong preference to the receptive fig odor, regardless of increases in interfloral fig numbers (Fig. 1b).

**Bioassay of Fig Volatile Compounds (Experiment B)** In the assays with single volatile compounds, all were tested with less than 1  $\mu\text{l}$ . Among them, the highest doses of enantiomers of linalool were presented as 0.1  $\mu\text{l}$  because the pollinators reacted in a confused, disordered way to it at a dose of 0.25  $\mu\text{l}$ . At all tested doses, (*R*)-(+)-linalool, (*R*)-(+)- and (*S*)-(-)-limonene were never active (Fig. 2a,d,e). However, the other four compounds all evoked dose-dependent responses from *C. solmsi marchali*, although the threshold doses at which each first induced a significant response were markedly different. The wasp pollinators showed a strong preference for (*S*)-(-)-linalool when offered at amounts of 0.01 or 0.1  $\mu\text{l}$  (Fig. 2b). The mixture of (*R*)-(+)- and (*S*)-(-)-linalool was attractive to wasps at doses of 0.001, 0.01, and 0.1  $\mu\text{l}$  (Fig. 2c). No response was observed at lower doses for (*S*)-(-)-linalool or ( $\pm$ )-linalool. At the higher doses, ( $\pm$ )-limonene and (-)- $\beta$ -pinene both evoked a significant wasp avoidance response, while they were neutral at the lower doses (Fig. 2f,g). Compared with either enantiomer of linalool and limonene, the mixture of (*R*)-(+)- and (*S*)-(-)-linalool enhanced pollinator attraction, while (*R*)-(+)-linalool and (*S*)-(-)-limonene enhanced wasp avoidance.

Subsequent experiments with volatile blends utilized a 10:1 ratio of (*S*)-(-)-linalool or ( $\pm$ )-linalool vs. ( $\pm$ )-limonene, or a 20:1 ratio of (*S*)-(-)-linalool or ( $\pm$ )-linalool vs. (-)- $\beta$ -pinene. The ( $\pm$ )-limonene and (*S*)-(-)-linalool

**Table 2** Response of the pollinator wasp *Ceratosolen solmsi marchali* to fig volatile compounds from *Ficus hispida* compared with control (Experiment B)

Experiment	Compound	Dose ( $\mu\text{l}$ )				
		1	2	3	4	5
Single compound						
B1-1	( <i>R</i> )-(+)-linalool	0.00001	0.0001	0.001	0.01	0.1
B1-2	( <i>S</i> )-(-)-linalool	0.00001	0.0001	0.001	0.01	0.1
B1-3	( $\pm$ )-linalool	0.00001	0.0001	0.001	0.01	0.1
B2-1	( <i>R</i> )-(+)-limonene	0.0001	0.001	0.01	0.1	1
B2-2	( <i>S</i> )-(-)-limonene	0.001	0.01	0.1	0.25	1
B2-3	( $\pm$ )-limonene	0.0001	0.001	0.01	0.1	1
B3	(-)- $\beta$ -pinene	0.001	0.01	0.1	0.25	1
Blends						
B4	Linalool + ( <i>R</i> )-(+)-limonene + ( <i>S</i> )-(-)-limonene=10:0.5:0.5					
B4-1	( <i>R</i> )-(+)-linalool + ( $\pm$ )limonene	0.0001	0.001	0.01	0.1	
B4-2	( <i>S</i> )-(-)-linalool + ( $\pm$ )limonene	0.0001	0.001	0.01	0.1	
B4-3	( $\pm$ )-linalool + ( $\pm$ )limonene	0.0001	0.001	0.01	0.1	
B5	linalool + (-)- $\beta$ -pinene=20:1					
B5-1	( <i>R</i> )-(+)-linalool + (-)- $\beta$ -pinene	0.0001	0.001	0.01	0.1	
B5-2	( <i>S</i> )-(-)-linalool + (-)- $\beta$ -pinene	0.0001	0.001	0.01	0.1	
B5-3	( $\pm$ )-linalool + (-)- $\beta$ -pinene	0.0001	0.001	0.01	0.1	



**Fig. 1** Responses of females of *Ceratosolen solmsi marchali* to (a) volatiles of male or female figs of *Ficus hispida* at receptive or interfloral stages compared with clean air. The volatiles tested were emitted by one fig; (b) volatiles of male or female figs of *F. hispida* compared between receptive and interfloral stages. The ratios of number of interfloral vs. receptive figs (I:R) were set as 1:1, 9:1 and 25:1. Numbers in the bars are the number of wasps responded to the

arms of the Y-tube.  $\chi^2$  test was used to evaluate whether the number of responding wasps differed from a 50:50 distribution between the two olfactometer arms (TP: there was no  $\chi^2$  test performed because one of the odor sources in either arm had a total preference (TP) of pollinators; \*\*\* $P$ <0.001; \*\* $P$ <0.01). Wasps that did not respond within 5 min [no choice (NC)] were excluded from statistical analysis

mixture was attractive to pollinators at the two higher doses (Fig. 2h). ( $\pm$ )-Limonene plus ( $\pm$ )-linalool was attractive at the three higher doses (Fig. 2i); however, at lower doses, pollinators did not respond to either of the two blends (Fig. 2h,i). The 20:1 ratio mixtures of both (*S*)-(-)-linalool and ( $\pm$ )-linalool to (-)- $\beta$ -pinene were attractive to pollinators at the three higher doses but were neutral at the lower doses (Fig. 2j,k).

At higher doses, (*S*)-(-)-linalool and ( $\pm$ )-linalool actively attracted *C. solmsi marchali*, but a blend of (-)- $\beta$ -pinene and ( $\pm$ )-limonene was repellent. (*R*)-(+)-Linalool, (*R*)-(+)-limonene, and (*S*)-(-)-limonene did not induce any response from the wasps. The non-active compounds (*R*)-(+)-linalool and (-)- $\beta$ -pinene enhanced the attractiveness of (*S*)-(-)-linalool, while enantiomers of limonene did not.

**Discussion**

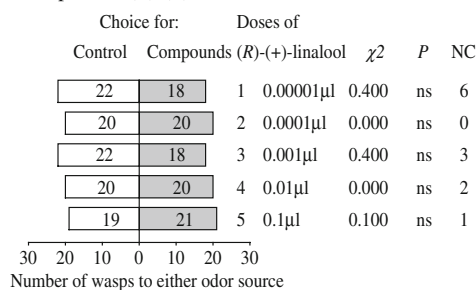
Post-pollination changes in floral characteristics, which include shape and color changes, cessation of nectar

production, and scent alteration, can be detected by pollinators (Arditti 1979). The structure of inflorescences and specificity of the association make pollinator attraction by *Ficus* species unique. Both tactile and olfactory cues have been suggested to be involved in fig wasp's localization (Ramírez 1974; Janzen 1979; Ware and Compton 1992). Long-distance olfactory cues are used by wasps to find host trees that bear receptive figs. Tactile cues are used once the insect has landed on the fig surface to determine whether it is suitable (Gibernau et al. 1998). However, in this study, without any visual or tactile cues, fig wasps were attracted to host fig volatiles in the Y-tube. Thus, at short distances, tactile cues may not be indispensable for the pollinator to locate a receptive fig.

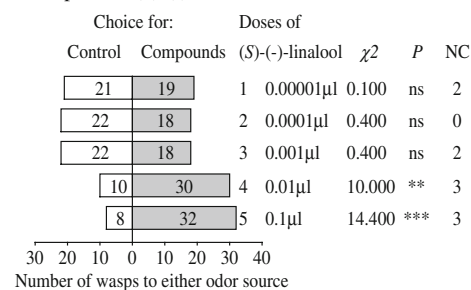
Few studies have tested directly pollinator responses to pollination-induced floral changes. (Gori 1983; Primack 1985; van Doorn 1997). The behavior of pollinating wasps at the receptive stage compared with that at the interfloral stage has not been studied, probably because the wasps are rarely observed on figs at the interfloral stage (van Noort et al. 1989; Ware and Compton 1994). Our findings showed

**Fig. 2** Responses of females of *Ceratosolen solmsi marchali* to: (a–g) single volatile component mainly found in receptive figs of *F. hispida*, (h–k) blends mixed by a 10:1 ratio of (*S*)-(-)-linalool or (±)-linalool to (±)-limonene, and a 20:1 ratio of (*S*)-(-)-linalool or (±)-linalool to (-)-β-pinene. Each blend was tested respectively in four doses. The doses labeled in the figure belong to (*S*)-(-)-linalool or (±)-linalool in blends. Numbers in the bars are the number of wasps responded to the arms of the Y-tube.  $\chi^2$ -test was used to evaluate whether the results differed from a 50:50 distribution between the two olfactometer arms (\*\* $P < 0.001$ ; \* $P < 0.01$ ; \* $P < 0.05$ ; ns:  $P \geq 0.05$ ). Wasps that did not respond within 5 min [no choice (NC)] were excluded from the statistic analysis

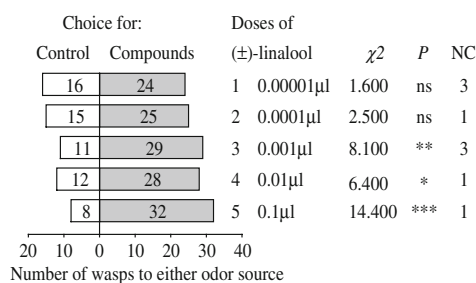
**a** Exp. B1-1 (*R*)-(+)-linalool



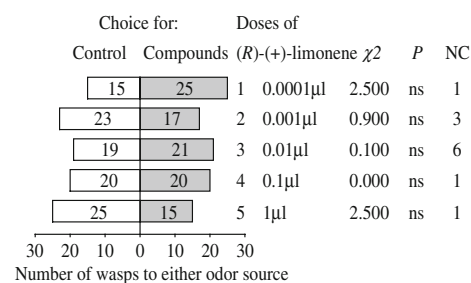
**b** Exp. B1-2 (*S*)-(-)-linalool



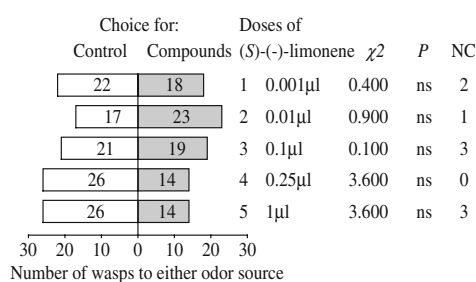
**c** Exp. B1-3 (±)-linalool



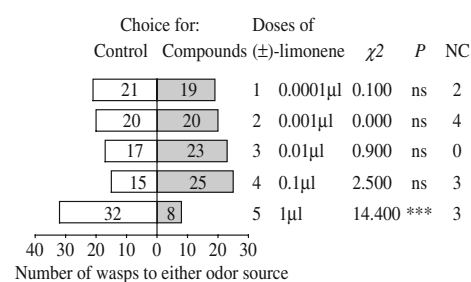
**d** Exp. B2-1(*R*)-(+)-limonene



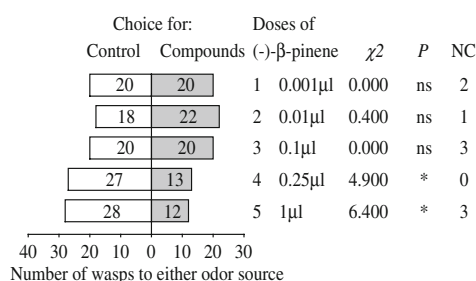
**e** Exp. B2-2(*S*)-(-)-limonene



**f** Exp. B2-3 (±)-limonene



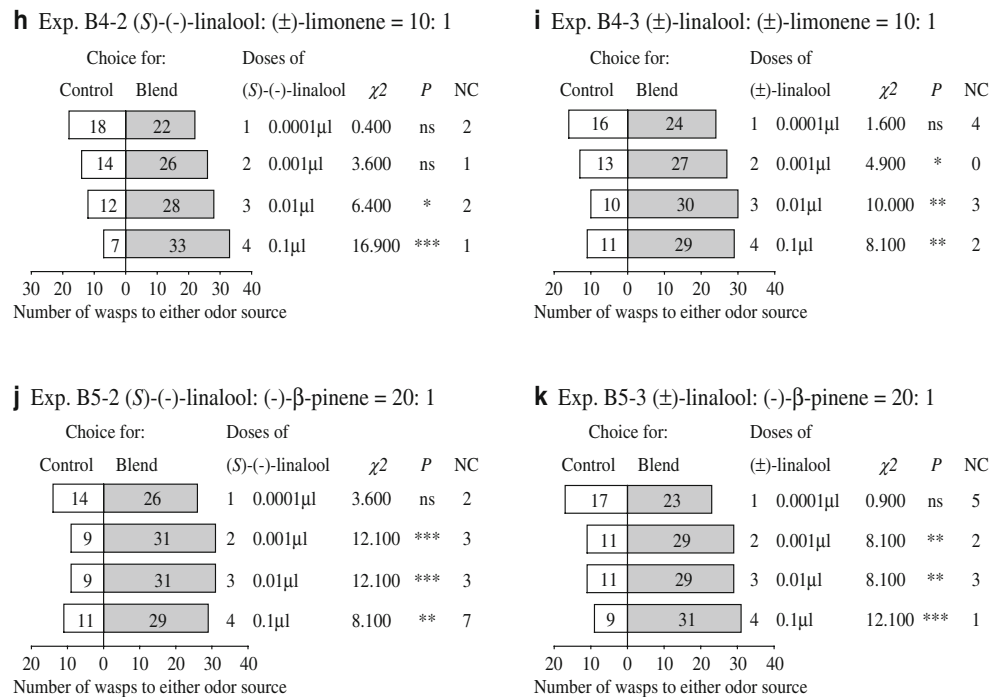
**g** Exp. B3 (-)-β-pinene



that two odor sources emitted from receptive figs and interfloral figs of *F. hispida* caused different behavioral responses of *C. solmsi marchali*. These results parallel previous studies in which pollinators were found to be attracted only to receptive figs (Ware and Compton 1994; Gibernau et al. 1998; Song et al. 2001). All previous studies were carried out under conditions when non-receptive figs coexisted with receptive figs. In the present study, we found unexpectedly that pollinators were attracted also to interfloral figs in the absence of receptive figs.

Raguso (2001) has summarized the three roles of post-pollination volatile emission in pollinator attraction as follows: distance attraction, futile visits prevention, and promotion of learned avoidance of reward-depleted flowers. It seems that the attractive effect of *F. hispida* interfloral figs cannot be explained by the above. We suggest that the similarity of odor composition in the two stages may be responsible for the wasps' tendency for futile attraction to interfloral inflorescences in the Y-tube olfactometer. Nearly all volatiles found in *F. hispida* interfloral figs occur also in

Fig. 2 (continued)



receptive figs. Linalool, the major volatile, continued to be emitted from the interfloral figs and was a strong attraction to pollinators in our tests. There might be an adaptive advantage to having pollinated figs that still draw wasps to a tree. In situations where pollinators are limiting, any extra attraction to trees could be advantageous—i.e., as long as wasps are drawn to figs that need to be pollinated, in preference to pollinated ones. In particular, this might be valuable for female trees where relatively few figs are attractive at any one time. Although pollinated figs are still attractive, wasps cannot get into them because the figs tightened obstacles after pollination. However, not all interfloral figs of *Ficus* species are attractive to their respective pollinators. In other studies, the interfloral figs of dioecious *Ficus semicordata* were not attractive to their pollinator *C. gravey*, (Chen and Song, unpublished data), probably because of differences in the volatile composition compared with receptive figs). The fig–fig wasp mutualism is a complex and diverse system, so conclusions deduced from one species should be carefully applied to another species.

Numerous studies have focused on whether quantity or quality plays a more important role in plant–insect chemical attraction (e.g., Visser 1986; Hoballah et al. 2002). Since *F. hispida* has lower volatile emission following the receptive stage, another aim of our study was to elucidate whether the quantity of odor in this fig species influenced the cognitive behavior of its pollinator. Our results indicate that higher quantities of interfloral fig odor were less attractive to pollinators than receptive fig odor. Although receptive figs may emit higher levels of volatiles, the stage-dependent

host localization behavior of wasps is regulated by the composition and proportion of the compounds (i.e., quality), not just the quantity of volatiles. Moreover, all of the volatiles studied induced a behavioral response in wasps that was dose-dependent. All compounds tested at the lower concentrations were neutral, even those that were attractive to pollinators at higher concentrations. There is little doubt that compounds termed “attractants” can also act as neutrals or repellents at high concentrations. Single compound tests indicated that some compounds are more important than others in attracting wasps. Attractive compounds that were identified in this study, e.g., linalool, are common in many floral fragrances (Gibernau et al. 1997). In simulating changes in fig odors from the receptive to interfloral stage, we set four concentration degrees in the blend tests. Just as the interfloral figs with smaller volatile quantities were less attractive to pollinators, the attractive compounds were not attractive to the pollinator when their concentrations were comparatively low. At high concentrations, all blends of (*S*)-(-)-linalool or ( $\pm$ )-linalool with one of the minor components attracted pollinator wasps. However, at low concentrations, all blends were neutral. In addition to host quality odor, i.e., volatile composition, the quantity of active volatiles is also important.

Our mixture experiments confirmed synergistic effects of volatiles in plant odors (Visser 1986). In several cases, blends of active and non-active compounds are more attractive to parasites or pollinators than single compounds (Visser and Avé 1978; Dariusz and Stephen 1999). In this study, neither ( $\pm$ )-limonene nor (-)- $\beta$ -pinene were attractive to *C. solmsi marchali* when applied singly; however, blends

of either of those compounds with linalool were more attractive. The attractiveness of (*S*)-(-)-linalool to *C. solmsi marchali* was enhanced by decreasing the active threshold dose from 0.01 to 0.001  $\mu$ l when mixed with non-active (*R*)-(+)-linalool, ( $\pm$ )-limonene, or (-)- $\beta$ -pinene.

The fig–fig wasp mutualism is a complex system, with variation among *Ficus* species and fig wasp species, volatile compounds, and volatile emission rhythms (Wiebes 1979; Berg 1989; Grison et al. 1999; Grison-Pigé 2001, 2002b). Our experiment is an initial exploration of pollinating fig wasp responses to pollination-induced changes. In the future, comprehensive studies should be undertaken to investigate further the chemical interaction in this specialized insect–plant mutualism.

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