RUNNING HEAD: THE EFFECT OF TEMPERATURE ON THE VOLATILES EMISSION FROM DIOECIOUS FIGS

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Influence of temperature on emission of volatiles from some dioecious figs (*Ficus sps.*)

Abstract

We studied the effects of temperature in the quantity and composition of the volatiles emitted by receptive female and male figs of *Ficus aurata* and *F. condensa* two dioecious fig species of secondary forest that occur in Brunei and of *F. carica* a mediterranean dioecious species. Head-space extraction of volatiles emitted by receptive figs were performed in the field and in the laboratory under temperature controled conditions. We found that temperature has an influence on the intensity of the emission as well as on the relative abundances of the different compounds in the floral fragrance. The significance of our results will be discussed in relation to the ecology and reproductive biology of the tropical dioecious figs *F. aurata* and *F. condensa* in comparison with the mediterranean species *F. carica*…or bla, bla, bla..

Introduction

One of the most common ways that entomophilous flowers use to attract pollinators is the production of specific odours in order to signal their presence (Faegri and Van der Pijl 1966). Volatile compounds have been documented as pollinator-attractants in a diversity of pollination syndromes (Pellmyr and Thien 1986): pollination by bats (Knudsen and Tollsten 1995), beetles (Thien et al 1975), bees (Williams 1983 and Bergstrom 1978), and flies (Meeuse 1966). The pantropical genus *Ficus* in the family *Moraceae* includes more than 800 species of trees and scrubs (Willis 1973), they form a distinctive component of tropical floras world wide and, together with their species-specific pollinator wasps (*Agaonidae*) form one of the most extreme cases of mutualism known (Herre 1989).

It has been demonstrated that pollinator attraction to figs is due to the emission of a complex mixture of aromatic compounds emitted from receptive figs (Dobson??, Hossaert-Mckey et al 1994, Barker 1985, Gibernau 1997, Grison??). The volatile compounds emitted by receptive figs function as long and short distance attractants for their mutualistic pollinators, thereby olfactory cues are particularly important to the reproductive success of both fig and wasp due to various constraints affecting encounter of the mutualists: (1) low density of fig trees (Foster and Hubbell 1990), (2) small proportion of trees at the appropriate reproductive phase stage (Anstett et al 1997), (3) presence of several other fig species within the same site (Michaloud 1988) and (4) the short life span of the adult wasp (Kjellberg et al 1988).

Rationale

1) Volatiles compounds from *Figs* are quite common in flower fragrances (??XX Givernau 1997, Grison et al XX). This suggest that only the qualitative and quantitative combination of the compounds may ensure the specific attraction of fig pollinators

2) Studies on *Ribes nigrum* (Hanstel et al 1994) and *Trifolium repens* (Jakobsen and Olsen 1994), more studies?? (xx) demonstrated that temperature has strong effect on the qualitative emission of volatiles. 3) In monoecious fig species in Panama it was demonstrated that figs avoid internal high temperature by evapotranspiration (Patiño, Herre and Tyree 1994).

Preliminary results showed ....

Do figs keep the "optimum" temperature only to keep the pollinators alive? Or additionally to keep running other physiological and reproductive processes?

From (1), (2) and, (3) it seems that figs present very precise combination of volatiles and they avoid internal high temperature by evapotranspiration.

In this study, we aim to test the hypothesis that figs need an optimum temperature to release the right combination of volatiles that attract the pollinator wasps.

In order to address the hypothesis we select two dioecious species *F aurata* and *F. condensa* from Brunei and *F. carica*, a well known mediterranean species. The advantage of studying these species is that the composition and the quantity of the volatiles involved in pollination have been identify (REFS), so we would be able to quantify the effect of temperature in the emission of the volatiles in these species and discuss the results in a more broad evolutionary perspective.

Methods

Plant material. Experiments were conducted on two dioecious *Ficus* species (*F. aurata* Miq., *and F. condensa*) from Brunei, Borneo and *F. carica* a seasonal Mediterranean dioecious species. *F. aurata, and F. condensa* grow in forest understory, secondary forests and, specially along river banks in all Borneo, Annam (Central Vietnem), Malaya, Sumatra, Riouw archipielago, Bangka (Malay Archipielago). Headspace collections under different controlled temperatures and *in situ* were made on female and male figs looking morphologically receptive (ostiole opened, flowers within the fig with turgescent stigma). Additional temperature experiments on transpiring and non-transpiring figs were performed in receptive male and female as well as visited (pollinated) figs of both sexes.

Study sites. Laboratory experiments were carried out at the Universiti Brunei Darussalam, Brunei and in the CNRS, Montpellier, France. *In situ* experiments where performed either in the Brunei Museum, Bandar Seri Begawan or Forestry Department, Sungai Liang in Brunei and in the CNRS in Montpellier. Experiments were carried out in June, November, December 1998 and January, February 1999 in Brunei and June and July 1999 in Montpellier.

Collection of volatiles under different temperature treatments. For *F. condensa* and *F. aurata* in Brunei, branches bearing figs were collected in the field and returned to the laboratory within one hour. Twenty five to ninety four figs detached from the branches were placed in glass petry dishes containing 3 mml of destilled water (to avoid fig dehydration). Two dishes were enclosed in each polyethylene terephtalate (Nalophan®) bag (two to four bags per experiment). One thermocouple per fig was inserted into the centre of three to four figs per bag. An additional thermocouple was placed inside the bag to monitor the air temperature in the bag. The figs, initially at room temperature (20 to 24 °C), were placed in a temperature – light controlled chamber (model XX, XX, XX) pre-set at the test temperature, which equalled 25, 40 or 50 ± 1.0. The test for 16 ± 1.0 °C was performed in an air conditioned laboratory set at 16 °C. The test for 30 ± 1.0 was made outside the laboratory in a shade balcony. Fig temperatures were monitored at 5-min intervals and all the figs reached the desired temperature in about 30-45 min for *F. aurata* and *F. condensa*, respectively.

For *F. carica,* receptive figs (either female and male) were detached from trees growing in the CNRS and placed inside bags (10 – 20 figs per bag and 2 – 4 bags per temperature). Cool temperature experiments were done by placing the bags inside a stereoform box containing ice, warm temperature experiments were performed using an oven (model XXX), ambient temperature experiments were done inside and outside the laboratory. The temperature measurements follows the procedure described above. For the three species, volatiles released by figs were collected at the same time using an adsorption–desorption (headspace) technique [25, (Grison in press)]. Briefly, air was purified by charcoal filters and drawn by a Millipore® pump into the bag (entrance flux: 400 ml/min), through Teflon tubing. It was then drawn out of the bag (exit flux: 300 ml/min) over a Porapak®Q (25ng, 80-100 mesh) collection trap, and pulled through Tygon tubing. Each headspace collection lasted 5 hours. The Porapak®Q collection trap was then eluted with 150 µl of dichloromethane.. *In situ* collections of volatiles were made by enclosing branches bearing figs in the polyethylene terephtalate (Nalophan®) bags following the procedure described elsewhere (Grison et al. Her first paper!!!). Fig temperatures were measured and recorded as described above. Photosyntetic active radiation (PAR) and air temperature were measure to characterise the field site. Tables 1 (a,b) present a summary of different experiments/treatments and figs characteristics.

Chemical and data analysis. GC-MS analysis were carried out using an HP -5 (5% phenyl methyl siloxane) column (length 50m, internal diameter 0.22mm, film thickness 0.33µm), in an HP 5890 Series II GC with mass-selective detector 5971A MSD under the following conditions : carrier gas, helium, 5 psi head pressure, split flow ratio 20:1; column flow ≤ 2ml/min ; injector 280°C, detector 300°C; oven temperature program : 50°C for 1 min then 5°C/min to 200 held for a total run time of 46 minutes. Injection volumes were 1-3µl. Data analysis were carried out using HP G1030A MS Chemstation software and a Wiley 138K Spectral Database.

Fig physiology. To relate volatile emissions to fig physiology, temperatures were monitored in transpiring and nontranspiring figs to see if test temperatures are reached by figs under experimental or field conditions and to determine if evapotranspiration cooling is present in these dioecious species.

Additional measurements

Stomata

Biological tests. We tested the volatiles extracted during the experiments under different temperature regimes….?

Table 1a *F. aurata*

Sex	Bag	N	D (SD) mm	V (SD) Mm³	T (SD) °C
Female	1	92	13.7 (1.0)	1301.8 (231.9)	16.7 (0.2)
	2	94	13.7 (0.8)	1373.8 (218.9)	16.5 (0.2)
	3	60	11.6 (1.1)	815.6 (152.8)	16.8 (0.2)
	4	60	11.6 (1.1)	834.2 (203.3)	48.7 (0.2)
	5	46	12.0 (1.4)	943.2 (260.2)	47.6 (0.1)
	6	34	10.1 (0.9)	538.7 (111.3)	51.0 (0.2)
	7	34	13.4 (0.7)	12.5 (0.8)	37.1 (1.1)
	8	44	13.8 (1.2)	1413.3 (257.8)	32.4 (0.6)
	9	39	12.5 (0.8)	1034.3 (191.6)	33.9 (0.5)
	10*	26?	12.3 (0.7)	984.1 (160.0)	29.6 (0.7)
	11*	26?	11.7 (0.9)	859.5 (202.3)	29.6 (0.8)
	12	40	13.4 (0.9)	1275.9 (259.9)	31.5 (2.3)
	13	37	13.9 (0.7)	1407.9 198.2	31.0 (2.0)
	14	31	11.8 (0.9)	878.3 (205.8)	32.5 (3.4)
	15	50	13.2 (0.6)	1218.9 (171.3)	40.7 (0.7)
	16	57	13.2 (1.0)	1224.4 (260.3)	39.6 (0.7)
	17	37	12.6 (1.0)	1074.9 (237.1)	41.1 (1.1)
	18	64	13.1 (1.0)	1196.0 (250.2)	23.2 (1.5)
	19	56	13.7 (0.6)	1361.6 (176.2)	23.2 (1.3)
Male	1				29.3 (0.3)
	2				28.9 (0.2)
	3				39.2 (0.2)
	4				37.4 (0.2)
	5	40	13.1 (1.0)	1162.6 (321.2)	45.7 (0.4)
	6	40	13.1 (1.0)	1194.5 (262.1)	47.5 (0.4)
	7	43	13.8 (1.0)	1275.4 (291.9)	24.8 (0.3)
	8	45	13.0 (1.0)	1168.6 (252.7)	24.6 (0.1)
	9*	48	13.7 (1.2)	1370.9 (344.7)	26.9 (1.8)
	10*	25	~13.9 (1.1)	~1441.4 (325.2)	27.1 (2.0)
	11*	27	13.9 (1.1)	1433.9 (299.9)	26.9 (1.8)
	12*	24	14.4 (0.9)	1593.9 (265.7)	27.2 (2.5)

Table 1b *F. condensa*

Sex	Bag	N	D (SD) mm	V (SD) Mm³	T (SD) °C
Female	1				25.1 (0.2)
	2				25.0 (0.2)
	3				28.8 (0.3)
	4				28.7 (0.5)
	5				37.5 (0.1)
	6				39.0 (0.6)
	7				47.2 (0.1)
	8				47.1 (0.1)
	9*	-	-	-	30.3 (1.5)
	10*	30	19.0 (0.9)	3463.2 (817.7)	30.7 (1.3)
	11*	-			
	12*	30			
Male	1	40 ± 2	19.1 (1.9)	3756.9 (1155.5)	28.6 (0.2)
	2	40 ± 2	17.3 (2.8)	2916.0 (1265.2)	29.0 (0.2)
	3	40 ± 2	17.6 (1.4)	2883.6 (663.0)	25.0 (0.2)
	4	40 ± 2	19.4 (2.1)	3961.7 (1150.3)	24.9 (0.1)
	5	40 ± 2	19.5 (1.7)	3933.7 (968.3)	39.3 (2.8)
	6	40 ± 2	18.6 (2.7)	3576.7 (1306.9)	38.9 (2.8)
	7	40 ± 2	19.8 (1.7)	4152.1 (1049.0)	47.8 (0.2)
	8	40 ± 2	20.0 (1.7)	4295.9 (1038.3)	47.6 (0.3)
	9*	26	17.8 (2.7)	3142.5 (1245.8)	26.9 (2.0)
	10*	26	18.6 (1.7)	3425.9 (913.5)	26.8 (1.9)
	11*	23	20.6 (1.6)	20.6 (1.6)	26.8 (1.9)

	12*	35	19.5 (1.6)	3980.1 (908.0)	27.0 (2.1)

Table 1c *F. carica*

Sex	Bag	N	D (SD) mm	V (SD) Mm³	T (SD) °C
	18	16	14.0 (2.4)	1.9 (1.0)	0.2 (0.7)
	17	15	13.8 (2.5)	1.8 (0.9)	0.4 (0.6)
	14	18	12.3 (2.9)	1.3 (1.0)	0.9 (0.6)
	13	18	13.2 (2.3)	1.5 (1.0)	2.1 (0.7)
	8	19	13.4 (1.5)	1.6 (0.5)	9.8 (3.9)
	6	11	13.5 (2.4)	1.7 (1.0)	15.0 (6.3)
	4	11	16.4 (2.1)	2.8 (1.0)	14.7 (5.6)
	5	11	13.8 (2.5)	1.8 (0.9)	15.8 (5.5)
	7	18	13.1 (1.9)	1.5 (0.6)	12.6 (1.9)
	3	11	15.2 (2.0)	2.3 (0.9)	18.8 (4.1)
Female	1	10	14.5 (2.1)	2.0 (0.8)	22.1 (1.2)
	2	10	13.8 (1.5)	1.7 (0.6)	22.5 (1.4)
	9	19	13.4 (1.9)	1.6 (0.6)	22.9 (0.9)
	20	26	12.9 (1.7)	1.4 (0.6)	23.5 (0.9)
	19	31	12.1 (1.5)	1.1 (0.4)	23.8 (1.1)
	10	18	12.8 (1.6)	1.4 (0.5)	25.9 (2.2)
	11	18	14.7 (2.9)	2.2 (1.2)	47.4 (1.7)
	12	18	13.2 (2.4)	1.6 (0.9)	48.3 (1.4)
	15	16	13.6 (1.8)	1.7 (0.7)	48.3 (0.5)
	16	16	13.4 (1.8)	1.6 (0.6)	48.7 (0.3)

Table 1 (a, b, c) Species of *Ficus* used in this study with summary of fig characteristics and temperature treatment. N (number of figs per bag), D (diameter, mm), V (volumen, mm), T (fig temperature). D and V are mean values of the number of figs per bag. D, V and T values are reported as a mean and SD based on 4-5 hours of recordings (80 – 100 values of temperauture), (*) *in situ* experiments.

Results

aurata

Sex	Temperature (°C)	Total volume of figs (mm3)	Total quantity of volatile compounds (10^{-2}ng/mm3 hr)	Percentage of b ocimene
Male				
Female				

Table 2. Effect of temperature on concentration of volatile emission from *F. aurata* figs.

NB : Chemical analysis …..
We expressed the concentration by mm3 of figs to control 1) for the mean diameter of the figs in the bag –who is not the same in all the bags (the effect is significant) and should be use as a covariate in further analysis, and 2) for the total number of figs within bags

condensa

Sex	Temperature (°C)	Total volume of figs (mm3)	Total quantity of volatile compounds (10^{-2}ng/mm3 hr)	Percentage of
Male				
Female				

Table 2. Effect of temperature on concentration of volatile emission from *F. aurata* figs.

NB : Chemical analysis ....
We expressed the concentration by mm3 of figs to control 1) for the mean diameter of the figs in the bag –who is not the same in all the bags (the effect is significant) and should be use as a covariate in further analysis, and 2) for the total number of figs within bags

carica

Sex	Temperature (°C)	Total volume of figs (mm3)	Total quantity of volatile compounds (10^{-2}ng/mm3 hr)	Percentage of Linalool
Female				

Table 2a, b, c. Effect of temperature on concentration of volatile emission from *F. aurata* figs.

NB : Chemical analysis still in process for female figs, so we were not able to complete this table , just say preliminary results
We expressed the concentration by mm3 of figs to control 1) for the mean diameter of the figs in the bag –who is not the same in all the bags (the effect is significant) and should be use as a covariate in further analysis, and 2) for the total number of figs within bags

Species	Sex	Stage	Treatment	N r	T_f (SD)	$T_f - T_a$
F. aurata	Female	R	C	283	32.3 (1.3)	0.1 (0.9)
			G	283	34.6 (2.1)	2.5 (1.2)
		V	C	92	31.6 (1.0)	1.6 (0.9)
			G	92	32.7 (2.3)	2.7 (2.1)
	Male	R	C	283	33.7 (0.6)	0.1 (0.9)
			G	283	34.9 (0.5)	1.3 (0.8)
		V	C	92	30.5 (0.9)	0.6 (1.0)
			G	92	32.1 (1.2)	2.1 (1.1)
F. condensa	Female	R	C	231	32.3 (1.3)	0.7 (1.3)
			G	231	33.4 (1.5)	1.8 (1.4)
		V	C	131	32.1 (2.0)	1.6 (0.9)
			G	75	34.5 (1.3)	2.5 (0.6)
	Male	R	C	231	33.1 (1.7)	1.4 (0.8)
			G	131	33.4 (2.1)	2.9 (0.9)
		V	C	231	33.7 (2.2)	2.1 (0.8)
			G	231	35.2 (1.0)	2.6 (0.3)
F. carica	Female	R	C	122	28.1 (1.7)	1.6 (0.7)
			G	122	28.8 (2.1)	2.3 (0.9)
		V	C	201	29.3 (1.6)	1.6 (0.5)
			G	201	30.0 (1.4)	2.4 (0.6)
	Male	R	C	122	29.8 (2.4)	3.3 (1.5)
			G	122	30.8 (2.1)	4.3 (1.0)
		V	C	201	29.1 (1.1)	1.5 (1.6)
			G	201	30.8 (1.1)	3.2 (1.5)

Mean								
Std.Dev								
Std.Err								

Discussion

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Table 2 Summary of temperature measurements of transpiring and nontranspiring figs in a sunny grass area outside the laboratory . Nr. (number of readings), SD (standard deviation), R (receptive fig), V (pollinated fig), C (control), G (greased), Tf (internal fig temperature), Ta (air temperature). The temperature shown when PAR > 800 µmol m^{-2} s^{-1}

Stomata

Species	**Sex**			
	F		**M**	
	R	**V**	**R**	**V**
F. cond	**0.25**	**0.7**	**0.45**	**4.7**
F aurata	**18.75**	**19.35**	**22.35**	**18.4**
F. carica				

2 figs 10 countings per fig

Biological test F. condensa male

	In situ		**25 °C**		**40 °C**		**50 °C**	
	C (s)	**E (s)**	**C (s)**	**E (s)**	**C**	**E**	**C**	**E**
	3	8	2	19	3	135	3	15
	2	17	12	5	8	12	12	6
	40	25	4	5	225	13	6	3
	19	190	3	50	2	275	75	20
	2	350	2	44	95	35	1	4
	2	5	25	45	17	70	8	225
	1	3	21	4	9	5	15	25
	2	12	7	3	14	3	7	20
	1	105	32	70	2	25	60	6
	5	60	15	9	30	7	2	7
	3	85	35	6	3	35	5	3
	6	4	105	4	5	37	2	2
	2	3	12	20	2	20	220	90
	3	230	1	5	4	3	3	2
	7	8	5	5	70	7	8	5
	3	70	7	3	2	40	8	2
	1	2	2	5	15	7	27	4
	5	3	5	2	14	25	17	7
	12	4	1	1	7	7	11	6
	2	4	2	50	3	150	3	3
Mean	6.05	59.4	14.9	17.75	26.5	45.55	24.65	22.75
Std.Dev	9.092941	94.30432	23.62403	21.29708	52.61029	67.81358	49.90441	51.46575
Std.Err	2.033243	21.08709	5.282493	4.762173	11.76402	15.16358	11.15897	11.50809

Biological test F. carica female

	In situ		**15 °C**		**30 °C**		**50 °C**	
	C (s)	**E (s)**	**C (s)**	**E (s)**	**C**	**E**	**C**	**E**