S3.1 Windtunnel experiments (orientation behaviour)

Experimental setup

Wind tunnel experiments were conducted in 2013 and 2014 at the University of Tehran, Karaj, Iran with the moths that had been reared for at most two generations at a light–dark cycle of 16 L: 8 D.

Responses of mated female, and virgin female and male carob moths to different odour sources were explored in a Plexiglas wind tunnel with a flight section of 120 × 50 × 50 cm^3 (l × w × h), placed in a walk-in climate chamber, at 23 ± 1 ºC and 50–60% RH. Air was blown by a fan at 50 cm/sec through a charcoal filter into the tunnel. The flight section was lit diffusely from the sides, yielding a light intensity of < 1 lux along the wind tunnel floor.

Test insects were placed individually in cylindrical, transparent plastic cups with 8 × 7 × 5 cm (height × top × base). We used 2- to 3-day-old virgin females and males, and 4- to 5-day-old mated females. The containers with moths were placed in the wind tunnel at least 1 h before testing to let them adjust to wind tunnel conditions. Each insect was tested for 15 min. All experiments were conducted 1-4 h into the scotophase, which is the optimal oviposition period for this moth (Cossé et al. 1994 [1]). During this period, behavioural responses of 4-10 moths to one odour stimulus were tested.

The container with a test moth was positioned horizontally on a glass platform at 20 cm high and 100 cm downwind from the odour source. The upwind end of the container was open, whereas at the downwind side pores (smaller than the insect) allowed air to pass through the container. Odour stimuli were placed on a similar glass platform at 20 cm height and 20 cm from the tunnel's upwind end. As test stimuli either two pomegranate flowers, two immature pomegranates of 3-4 cm diameter, one mature pomegranate (either cracked or uncracked) of 7-9 cm diameter, or three pistachios of 20-25 mm in length were used. The cracks in the cracked fruits were not wider than 2 cm.

Headspace extracts were tested on a gray rubber septum (Econex, Murcia, Spain). Half a ml of headspace extract was pipetted on the septum and left to dry in the lab for 30 min. The dispensers were charged immediately before the start of the experiment in the wind tunnel and sealed in 14-ml vials until use. One dispenser was used per tested insect.

Two types of controls were used, a blank platform (blank) and dispensers filled with 0.5 ml of the control samples (control). The following sequence of behaviours was recorded for each test insect: take off, upwind flight to 50 cm of the odour source, upwind flight to 7 cm of the odour source, and landing. Moths that landed on a wall for > 10 s and moths that did not take off within 10 min from the start of the experiment were discarded.

Statistical Analysis.

All analyses were performed in R, version 3.3.1 (R Core Team 2016). Overall behavioural performance of the moths in the wind tunnel assays was analysed by Kaplan-Meier survival analysis (Package survival; Therneau and Grambsch, 2000 [2]). Differences between the plant volatiles and controls in terms of the number of moths that reached different behavioural steps (take off, flight to 50 cm upwind, flight to 7 cm upwind, and landing) were determined using a logistic regression model followed by the Dunnett's post hoc test. In this model, the odour source (plant materials and headspace extracts) and insect response (0 = did not reach the behavioural step; 1 = reached the behavioural step) were analysed as the explanatory and response variables, respectively. A general linear model with the Tukey's HSD test was used to determine the differences between the plant materials in terms of the amount of β-caryophyllene in their headspace extracts. Impact of different

A general linear model with the Tukey's HSD test was used to determine the differences between the plant materials in terms of the amount of β-caryophyllene in their headspace extracts. Impact of different concentrations of chemicals on the oviposition of the moths was analysed using a generalized linear model with a Poisson error distribution (the best fit) followed by Tukey's HSD test.

Windtunnel results and statistics

In the wind tunnel, mated females were attracted to pistachio and both cracked and uncracked pomegranates, but not to pomegranate flowers or unripe pomegranates. Males were only attracted to pistachio and cracked pomegranates, and virgin females only responded to cracked pomegranates. When testing the headspace extracts, only mated females responded to the pomegranate and pistachio headspace extracts (Table S3.3.1).

Table S3.1.1 Attraction of carob moths to different host plant materials and their headspace extracts in comparison with controls in wind tunnel bioassays, analysed using Kaplan-Meier survival curves. Mated females were attracted to pistachio and both cracked and uncracked pomegranates, but not to pomegranate flowers or unripe pomegranates. Virgin females only responded to cracked pomegranate. NT = not tested.

	Male		Mated female		Virgin female	
Odour source	N	P	N	P	N	P
Pistachio	44	0.021	44	<0.001	0	NT
Cracked pomegranate	60	0.019	90	<0.001	30	0.0085
Uncracked pomegranate	44	0.12	44	0.0039	30	0.18
Unripe pomegranate	30	0.65	30	0.34	30	0.14
Flowering pomegranate	30	0.89	36	0.43	30	0.18
Pistachio headspace extract (HE)	15	0.14	18	0.059	15	0.29
Cracked pomegranate HE	24	0.25	28	0.007	16	0.23

Significant P-values are highlighted in grey.

When comparing the behavioural elements of attraction in the wind tunnel separately, i.e., take off, upwind flight towards odour source to within 50 cm and to within 7 cm, and landing, mated females only took off and flew upwind to within 50 cm and 7 cm of the odour sources when the odour source was cracked pomegranates, uncracked pomegranates or pistachio Virgin females did not start flying towards any of the odour sources. Virgin males flew upwind only to cracked pomegranate). However, only mated females actually landed on the cracked pomegranates (Fig. S3.1 ; Figure 2 in S0 and main paper]).

References

1. Cossé, A.A.; Endris, J.J.; Millar, J.G.; Baker, T.C. Identification of volatile compounds from fungus-infected date fruit that stimulate upwind flight in female *Eectomyelois ceratoniae*. *Entomol. Exp. Appl.* **1994**, *72*, 233–238.
2. Therneau, T.M.; Grambsch, P.M. Modeling Survival Data: Extending the Cox Model. In Proceedings of the First Seattle Symposium in Biostatistics, Seattle, WA, USA, 20–21 November 2000; https://doi.org/10.1007/978-1-4757-3294-8.

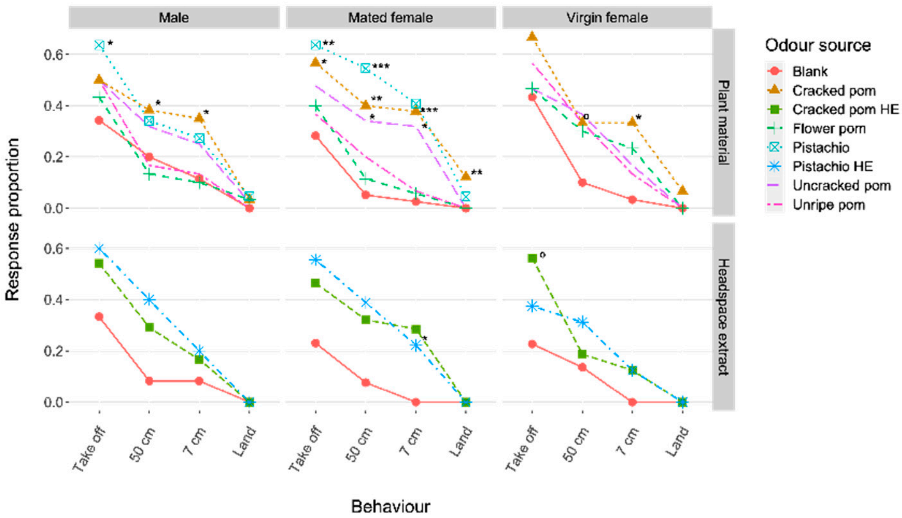

Fig. S3.1 (low resolution) Behavioural responses of mated female and virgin female and male carob moths to a series of plant materials (pomegranate and pistachio) and their headspace extracts (HE) in wind tunnel bioassays. Differences between the odour sources and controls at each behavioural step are depicted using the following codes: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ° $P \leq 0.1$.

Table S3.1.2 Logistic regression analysis of the behavioural responses of carob moth to different stages of pomegranate fruit and mature pistachio as well as their headspace extracts, tested in the wind tunnel. The behaviours recorded include take off, upwind flight sustained to 50 cm of odour source, upwind flight to 7 cm of odour source, and landing. Mated females responded to the best.
The script producing these statistics can be found in the file Caryophyllene_Statistic.R.txt under S0/Data and Scripts/

	Plant material									Volatile extract								
	Male			Mated female			Virgin female			Male			Mated female			Virgin female		
Behavioural response	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
Take off	7.33	5	0.20	15.89	5	0.01	4.49	4	0.34	2.11	2	0.35	3.51	2	0.17	4.51	2	0.11
50 cm	11.09	5	0.05	39.58	5	< 0.001	7.77	4	0.10	3.90	2	0.14	4.58	2	0.10	1.75	2	0.42
7 cm	13.23	5	0.02	45.01	5	< 0.001	11.27	4	0.02	0.79	2	0.67	7.03	2	0.03	4.40	2	0.11
Land	4.14	5	0.53	22.28	5	< 0.001	6.55	4	0.16	0.00	2	1.00	0.00	2	1.00	0.00	2	1.00

S3.2 Oviposition Experiment

To test the oviposition response of carob moth to β-caryophyllene, gravid females were exposed to different amounts of β-caryophyllene (10 - 400 ng) diluted in 10 µl hexane, which was sprayed on ~ 1.5 m^2 clean paper towel pieces using a 10 µl Hamilton syringe (Reno, Nevada). The treated papers were placed individually in small transparent cups (37 ml; Solo, Lake Forest, Illinois) along with one 4-5 day-old mated female moth 10 – 80 min before the start of the scotophase. The number of eggs laid in the cup was recorded directly after the 10-h dark period. Papers with 10 µl hexane were used as a control. To ensure that our result could be attributed to β-caryophyllene and not to any random plant odour, we also tested the impact of the green leaf odour cis-3-hexene-1-ol on the insect's oviposition over the same concentration range and in the same way as described above.

*Statistical Analysis***.** A general linear model with the Tukey's HSD test was used to determine the differences between the plant materials in terms of the amount of β-caryophyllene in their headspace extracts. Impact of different concentrations of chemicals on the oviposition of the moths was analysed using a generalized linear model with a Poisson error distribution(providing the best fit), followed by Tukey's HSD test. See S0 for the R script and raw data.

*Oviposition Experiment***.** Carob moths laid different numbers of eggs on the papers perfumed with different amount of both β-caryophyllene (χ^2 = 1283.7, d.f. = 7, P < 0.0001) and the green leaf odour cis-3-hexene-1-ol (χ^2 = 55.85, d.f. = 7, P < 0.0001) (Fig. 4). β-Caryophyllene with the amounts of 40 ng, 80 ng, and 100 ng and cis-3-hexen-1-ol with 80 ng stimulated oviposition (Fig. 4). High amounts of β-caryophyllene (200 and 400 ng) inhibited oviposition, whereas cis-3-hexen-1-ol did not affect oviposition at these high doses (Fig. S3.2).

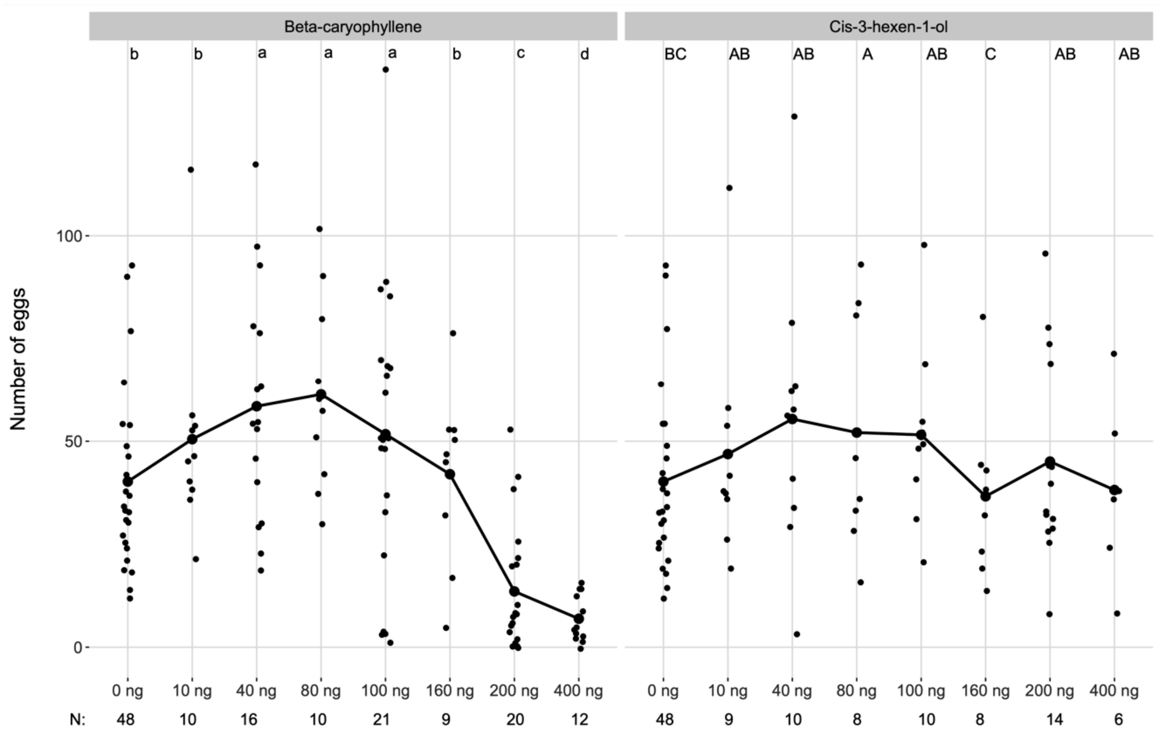

Fig. S3.2 Number of eggs laid by mated carob moth females on filter papers perfumed with 10 µl hexane containing different amounts (0 – 400 ng) of β-caryophyllene or cis-3-hexen-1-ol. Means with different letters are significantly different. N = number of moths tested