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Field Attraction of Carob Moth to Host Plants and Conspecific Females

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Abstract

The carob moth, *Ectomyelois ceratoniae* (Zeller; Lepidoptera: Pyralidae), is a devastating pest in high-value crops around the world. An efficient sex pheromone attractant is still missing for the management of this pest, because the major pheromone component is unstable. Host plant volatiles attract herbivore insects and have shown to have good potential to be exploited as alternatives or supplements to sex pheromones. To explore this possibility in carob moth, we assessed the attraction of moths to the volatiles of mature pistachio and different fruit stages of pomegranate, alone and in combination with virgin females, using sticky delta traps in pomegranate orchards of Iran. Traps baited with mature pomegranates, whether uncracked or cracked, infested or uninfested, caught significantly larger numbers of male and both mated and virgin female carob moths than unbaited traps. Traps baited with headspace extract of cracked pomegranate only caught mated females, while mature pistachio only attracted males. Pomegranate flowers, unripe pomegranate, and headspace extract of pistachio did not attract moths. Traps baited with cracked fruit caught more mated females than traps baited with uncracked fruit. Males were attracted similarly to traps baited with cracked-infested pomegranate as to traps baited with virgin females alone. Interestingly, the combination of cracked pomegranate and virgin female enhanced the attraction of virgin females. Together, our results show that volatiles from cracked pomegranates alone or in combination with female sex pheromone have great potential for application in pest management programs of carob moth.

Key words: Attractants, fruit bait, plant volatile–pheromone interaction, monitoring, moth

Over the past decades, and mostly driven by the strict regulations imposed on the use of chemical pesticides worldwide (e.g., EU 2009), semiochemical-based behavioral manipulation methods have been increasingly implemented in Integrated Pest Management (IPM) as an efficient environmentally friendly strategy. For moths, many of which are agricultural pests, long-range female sex pheromones are now widely used to monitor and suppress insect populations through mass trapping and mating disruption (reviewed by Witzgall et al. 2010). However, female sex pheromones only target adult males, while females play a prominent role in population dynamics of insects (Caswell 2001). Therefore, behavioral manipulation methods such as monitoring, attract-and-kill, and host finding disruption will be more efficient if they target not only males, but also females. Semiochemicals that would affect both sexes may be good alternatives or supplements to long-range sex pheromones, especially when the sex pheromones are difficult to produce, unstable, and/or inefficient.

In moths, host plant volatiles are involved in host seeking and oviposition behavior of females (Gothilf et al. 1975, Cossé et al. 1994, Landolt and Guédot 2008). Both males and females may also use plant volatiles to find a mate (Coracini et al. 2004, Landolt and Guédot 2008) and adult feeding sites (Lin and Phelan 1991, Tingle and Mitchell 1992, Landolt and Guédot 2008). Moreover, host plant volatiles have been shown to interact with sex pheromones by inducing sex pheromone release of female moths and affecting olfactory perception of the males (McNeil and Delisle 1989, Landolt and Phillips 1997, Groot and Visser 2001, Pregitzer et al. 2012). There are many examples of host plant volatiles that synergize responses of male moths to female sex pheromone and thus enhance their efficiency in pest management (Ochieng et al. 2002, Yang et al. 2004, Schmidt-Buesser et al. 2009, von Arx et al. 2012). Importantly, plant volatiles have great potential for use in IPM compared with pheromones because plant volatiles are often simple compounds, commercially available and cheap.

A drawback of host plant volatiles, however, is that they may be less attractive than pheromones, as they have to compete with volatiles of the host plants in the field. This drawback may be overcome by using the stimuli at specific times, that is, when or where the host plant is absent, for example, before fruiting time in fruit pest, or to attract the pest away from the agricultural area. Another drawback is that individual plant volatiles are generally not specific, and many are shared among a large group of plant species. As behavioral responses of pest insects to host plants are likely encoded by a volatile blend, it is essential to determine which host plants or plant parts are the most attractive sources for the pest, and which volatile blends most attract the insects to their hosts.

The carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), is a polyphagous destructive pest worldwide, attacking different fruits before and after harvest. This insect is recognized as the most economically important pest of date (*Phoenix dactylifera* L.; Arecales: Arecaceae) in the United States (Nay et al. 2006, Vetter et al. 2006) and a key pest of almond (*Prunus dulcis* (Mill) D. A. Webb; Rosales: Rosaceae) in Australia (Madge 2014), and frequently infests stored fruits and nuts in Europe (Carter 1984). In the Middle East, *E. ceratoniae* is the most damaging pest of pomegranate, *Punica granatum* L. (Myrtales: Lythraceae), in almost all pomegranate production areas, causing 30–80% yield loss (Kashkuli and Eghtedar 1975, Al-Izzi et al. 1987, Shakeri 2004, Sobhani et al. 2015). Pistachio (*Pistacia vera* L.; Sapindales: Anacardiaceae) is another host of this insect in the Middle East (Dhouibi 1982, Gothilf 1984, Mehrnejad 1992), which co-occurs with pomegranate in many areas. However, the carob moth has not been reported as an economic pest of pistachio so far, even though it infests the plant.

In pomegranate, larvae feed on internal parts of the fruit, resulting in contamination with saprophytic fungi, which makes the fruit unfit for human consumption and for the food processing industries, and thus unmarketable (Shakeri 2004). Since egg-laying and larval development occurs within the fruits, commercial insecticides are not efficient and thus are not used against this pest. An efficient sex pheromone attractant is still missing for carob moth management. The major female sex pheromone component [(Z,E)-9,11,13-tetradecatrienyl] is unstable (Baker et al. 1991), so that an alternative stable mimic of this component, which is, (Z,E)-7,9,11-dodecatrienyl formate, is currently used in commercial sex pheromone lures. However, these lures are not very attractive in pomegranate orchards (Avand-Faghih et al. 2012, Dhouibi et al. 2016). This situation prompted us to explore the potential of host plant volatiles for IPM against the carob moth.

Pomegranate continuously flowers during the growing season, so that different phenological stages are present to different extents at the same time. Signs of carob moth infestation become visible from the mid-growing season of the pomegranate, that is, when most fruits are at a mature size (Hoseini et al. 2014, Sobhani et al. 2015). In this period, peels of many pomegranate fruits are cracking (Shakeri 2004, Galindo et al. 2014, Saei et al. 2014, Hoseini et al. 2014), a phenomenon that is common throughout the world. Pistachio is infested late in the growing season when the fruits are at mature size and the nut is formed (Mehrnejad 1992).

As a first step in the exploitation of host plant volatiles in the management of carob moth, we determined the attraction of male, mated female, and virgin female moths to different phenological stages of the host fruit and their headspace extracts using sticky delta traps in the field. Subsequently, we tested the attraction of carob moths to pomegranate flowers, unripe pomegranates, mature uncracked pomegranates, mature cracked pomegranates, and mature pistachio. We further investigated whether the combination of pomegranate with virgin females enhances the attraction of male and female moths.

Materials and Methods

Insects

Carob moths were reared from larvae that were collected throughout the growing seasons of 2014 from a commercial pomegranate orchard located in Chandab, Iran (35°25'13"N, 51°56'04"E, and 1,141 m elevation). Insect larvae were maintained on a wheat bran diet [wheat bran (300 g), sugar (50 g), water (120 ml), glycerol (130 ml), and yeast (9 g)], and adults were fed with a 10% honey–water solution. The rearing room was kept at 25 ± 1°C, 60% RH, and a light–dark cycle of 16:8 (L:D) h.

Plants

Pomegranate samples were picked from the cultivar Galu-Barik from the same orchard as the insects, whereas the pistachio cultivar Akbari was obtained from a commercial pistachio orchard in Qazvin, Iran (35°54'07"N, 50°03'06"E, and 1,201 m elevation). Pomegranate materials used in the insect traps were picked early on the same day of the experiment. Pistachio samples were picked from the orchard 1–2 d prior to the experiment, as this orchard was located ~250 km from the experimental orchard, and kept in a refrigerator at 6°C and 90% RH until used in the experiments.

Trapping Experiments

Trapping experiments were conducted in the pomegranate orchard mentioned earlier, during the pest activity season in the area, that is, from mid-July to mid-October (Table 1). For all experiments, green delta traps (280 × 200 × 120 mm [l by b by h] with sticky bottom; Russell IPM, Deeside, United Kingdom) were used. Baits were placed in the middle of the sticky bottom of the traps. Traps were placed between pomegranate trees (outside of the plant canopy) on a rope at a height of 160–220 cm, with at least 10 m between adjacent traps. Treatments (baits) were applied in a randomized complete block design (discussed subsequently). Different treatments were tested at different times, depending on the availability of the plant materials as well as on the results of previous experiments (see Table 1). Traps were checked weekly, at which time the sticky bottoms and the baits were replaced, and treatments were re-randomized within each block. Sticky bottoms that had caught moths were taken to the lab, and kept in the rearing room for 2 d. After 2 d, trapped moths were sexed and counted, and the mating status of the females was determined by checking the color of the eggs that trapped females inadvertently laid on sticky bottoms, which is possible because oviposited fertilized eggs become pinkish red within 48 h (Alrubeai 1987).

Experiment 1. Attraction to host plant materials

To determine the most attractive plant parts to the carob moth, we tested eight different plant materials in five subsequent experiments [experiments (Experiment) 1a–e; Table 1]. The plant materials tested were: 1) fruitful flowers (i.e., flowers that turn to fruits) of pomegranate, referred to as pomegranate flowers; 2) healthy (i.e., uncracked and uninfested by carob moth) immature pomegranate fruits, referred to as immature pomegranate; 3) healthy mature pomegranate, referred to as uncracked pomegranate; 4) naturally cracked, mature pomegranate, referred to as cracked pomegranate; 5) naturally cracked, mature pomegranate infested by carob moth, referred to as cracked-infested pomegranate; 6) mechanically cut (see next paragraph) mature pomegranate, referred to as cut pomegranate; 7) mechanically cut mature pomegranate infested by carob moth, referred to as cut-infested pomegranate; and 8) mature fresh pistachio, referred to as pistachio. For each trap, we used either two

Table 1. Overview of experiments, with different baits (pomegranate and pistachio plant materials and volatiles, and female sex pheromone) tested in sticky delta traps, to evaluate carob moth attraction in the field

		Treatment		Setup date	No. replicates
Experiment 1	Plant materials Healthy, damaged, or infested pomegranate	a	1) Flowers, 2) immature fruit	21 July 2014	14
		b	1) Cracked fruit and 2) cut fruit	13 Aug. 2014	20
		c	1) Uncracked fruit, 2) cut fruit, and 3) cut infested fruit	20 Aug. 2014	12
		d	1) Uncracked fruit and 2) cracked fruit	09 Oct. 2014	20
	Pistachio	e	1) Mature fruit	13 Aug. 2014	14
Experiment 2	Headspace volatiles	a	1) Cracked pomegranate and 2) pistachio	19 Sept. 2013	12
		b	1) Cracked pomegranate	09 Oct. 2014	20
Experiment 3	Damaged or infested pomegranate and virgin female	a	1) Cracked-infested fruit, 2) virgin female, and 3) cracked-infested fruit + virgin female	10 Sept. 2014	12
		b	1) Cracked fruit, 2) virgin female, and 3) cracked fruit + virgin female	25 Sept. 2014	20

flowers of pomegranate, two immature pomegranates of 3–4 cm diameter, one mature pomegranate (uncracked, cracked, cut, infested, or uninfested) of 7–9 cm diameter, or three pistachios, which were 20–25 mm in length. Cracked-infested fruits were naturally cracked fruits on the pomegranate trees with early signs of infestation by carob moths but without visible mold infection, and the cracks of the cracked fruits tested were not wider than 2.5 cm.

To check whether mechanical damage or mechanical damage plus an artificial infestation in pomegranate fruits makes these fruits as attractive as naturally cracked or cracked-infested fruits, cut and cut-infested fruits were also tested. To make a mechanically cut fruit, an incision was made on the peel of a healthy mature fruit from crown to base on both sides, after which the fruit was split in half along the incision, such that the two halves were still attached at the base while creating a maximum 2-cm-wide gap at the crown of the fruit. To make a mechanically cracked-infested fruit, five third-instar larvae were placed in the mechanical gaps early in the day and larval settlement in the fruit was checked before using them in the traps. In all experiments with plant materials, unbaited traps were used as control.

Experiment 2. Attraction to the headspace extracts of host fruits

To determine whether the volatiles from cracked pomegranate and pistachio were attractive for carob moths, headspace extracts were tested in sticky traps (Experiment 2a and b, Table 1). Volatiles emanating from 2 kg of cracked pomegranate (i.e., naturally cracked mature pomegranate) and 1 kg of pistachio (i.e., mature fresh pistachio), placed in a 4-liter glass jar, were collected separately as follows. A charcoal-filtered air stream was pulled over the enclosed fruits, and headspace volatiles were collected on an adsorbent trap, containing 50 mg of Tenax-GR 60/80 (Restek, Bellefonte, PA, United States) in a 60-mm-long × 6-mm-diameter glass tube. For collection of control samples, a charcoal-filtered air stream was pulled through the empty glass jar. Tenax traps were thermally conditioned at 200°C for 30 min under a stream of nitrogen before use. The air flow in the headspace collection system was 0.5 liter/min, and each collection session lasted for 22 h. The Tenax traps were extracted with 2 ml of *n*-hexane (Merck, Darmstadt, Germany). Samples were sealed and stored at –20°C until use in the traps. One milliliter of each headspace extract was pipetted in a gray rubber septum (Econex, Murcia, Spain) and left to dry in the lab. The septa were then sealed in glass vials 3–4 h before use in the field. Only one dispenser was used per trap.

Experiment 3. Attraction to a combination of virgin females and host fruits

To determine whether host fruits interact with the female sex pheromone in the attraction of carob moth, we baited traps with combinations of pomegranate fruits and living virgin females as pheromone source (Experiment 3a and b; Table 1). The experiment consisted of four treatments: 1) one 2–3 d-old virgin female, with a small piece of cotton wool soaked in tap water for feeding, in a cylindrical cage (2 cm height × 2.7 cm diameter) covered by gauze, referred to as virgin female; 2) one virgin female plus one cracked pomegranate; 3) one cracked pomegranate; and 4) a cage with cotton-water but without insect and fruit (control).

Statistical Analysis

The different trapping experiments (Table 2) were analyzed separately. A generalized linear model (GLM) with a negative binomial error distribution using the Simultaneous Inference Procedure described by Hothorn et al. (2008) was used to compare treatments in each experiment. Treatments were compared with each other using a Tukey's test and treatments were compared with the control using Dunnett's test. To analyze single and interaction effects of cracked pomegranate and virgin female carob moth in total (male + female) moth catch in Experiment 3b, a two-by-two factorial analysis was performed using GLM. Correlation analysis was performed between the numbers of trapped males and trapped mated and virgin females in all experiments. All analyses were conducted in R version 3.2.3 (R Core Team 2015).

Results

Effects of different treatments (baits) in different experiments on the catch of male, mated female, and virgin female carob moths are shown in Table 2.

Mature Pomegranates Are Attractive to Males as well as Mated and Virgin Females

Numbers of male, virgin female, and mated female carob moths trapped by mature pomegranates, whether uncracked (Experiment 1d; Dunnett's post-hoc test, male: $P < 0.001$, mated female: $P < 0.001$, virgin female: $P = 0.01$), cracked infested (Experiment 3a; Dunnett's post-hoc test, male: $P < 0.001$, mated female: $P = 0.005$, virgin female: $P = 0.04$), or cracked uninfested (Experiment 1d;

Table 2. Statistical significance as indicated by GLM in each of the trapping experiments

Experiment		Treatment(s)	Male			Mated female			Virgin female		
			χ^2	df	P	χ^2	df	P	χ^2	df	P
Exp.1	1a	Fl; ImP	2.19	2	0.333	2.19	2	0.333	2.19	2	0.333
	1b	CkP; CutP	0.46	1	0.493	0.32	1	0.566	0.02	1	0.865
	1c	UckP; CutP; CutIP	19.31	3	<0.001**	30.78	3	<0.001**	5.38	3	0.145
	1d	UckP; CkP	47.95	2	<0.001**	84.56	2	<0.001**	19.3	2	<0.001**
	1e	Ps	3.92	1	0.047*	1.04	1	0.306	0.33	1	0.56
Exp. 2	2a	CkPVol; PsVol	2	2	0.367	7.98	2	0.018*	0.47	2	0.79
	2b	CkPVol	1.84	1	0.174	9.03	1	<0.001**	0.33	1	0.56
Exp. 3	3a	CkIP; VF; CkIP + VF	63	3	<0.001**	35.9	3	<0.001**	17.6	3	<0.001**
	3b	CkP; VF; CkP + VF	77.45	3	<0.001**	101.9	3	<0.001**	35.52	3	<0.001**

Each experiment contains a set of unbaited traps as control.

Fl (pomegranate flowers); ImP (immature pomegranate); UckP (uncracked pomegranate); CkP (cracked pomegranate); CkIP (cracked-infested pomegranate); CutP (cut pomegranate); CutIP (cut infested pomegranate); CkPVol (headspace extract of pomegranate); Ps (pistachio); PsVol (headspace extract of pistachio); VF (virgin female carob moth).

Dunnnett's post-hoc test, male: $P < 0.001$, mated female: $P < 0.001$, virgin female: $P = 0.008$) were significantly higher than in unbaited control traps. Traps baited with pomegranate flowers (Experiment 1a; Dunnnett's post-hoc test, male: $P = 0.33$, mated female: $P = 0.33$, virgin female: $P = 0.33$), unripe pomegranate (Experiment 1a; Dunnnett's post-hoc test, male: $P = 0.33$, mated female: $P = 0.33$, virgin female: $P = 0.33$), and headspace extract of pistachio (Experiment 2a; Dunnnett's post-hoc test, male: $P = 0.99$, mated female: $P = 0.99$, virgin female: $P = 0.78$) did not catch numbers of moths that were different from the control traps (Fig. 1).

Mature Pistachio Only Attracts Males, While Headspace Extract of Pomegranate Only Attracts Mated Females

When traps were baited with mature pistachio (Experiment 1e), only the number of trapped males was higher than in control traps, whereas the number of trapped mated and virgin females did not differ from control (Table 2; Fig. 1). In both Experiment 2a (Dunnnett's post-hoc test, $P = 0.05$) and Experiment 2b (Table 2), only mated females were significantly more trapped in the traps baited with the headspace extract of cracked pomegranate than in controls. Traps baited with the headspace extract of cracked pomegranate caught similar numbers of males and virgin females as control traps in both Experiment 2a (Dunnnett's post-hoc test, males: $P = 0.32$, virgin females: $P = 0.78$) and Experiment 2b (Table 2; Fig. 1).

Mechanical Damage on the Peel Increases Attraction of Mated Females to Mature Fruit

Compared with uncracked mature pomegranates, both cut (Experiment 1c; Tukey's post-hoc test, $P = 0.02$) and cracked (Experiment 1d; Tukey's post-hoc test, $P < 0.001$) pomegranates trapped significantly more mated females. Cracked and cut pomegranates trapped similar numbers of mated females, as well as males and virgin females (Experiment 1b; Table 2).

Combination of Pomegranates and Virgin Females Increases Attraction of Virgin Females and Total Moth Catch

The combination of cracked pomegranate and virgin female significantly increased virgin female trap catch compared with cracked pomegranate only (Experiment 3b; Tukey's post-hoc test, $P = 0.04$), whereas traps baited with virgin females did not catch numbers

of conspecific virgin females that were more than control traps (Experiment 3b; Dunnnett's post-hoc test, $P = 0.27$). A significant interaction was observed between cracked pomegranate and virgin females ($\chi^2 = 14.2$, $df = 1$, $P \leq 0.0001$) in total moth catch, so that total numbers of moths trapped in the traps baited with the combination of these baits was significantly higher than that of the traps baited with each one alone [i.e., virgin female only (Experiment 3b; Tukey's post-hoc test, $P = 0.03$), and cracked pomegranate only (Experiment 3b; Tukey's post-hoc test, $P = 0.002$)] (Fig. 2; Experiment 3b). Traps baited with a cracked or cracked-infested pomegranate plus a virgin female (Experiment 3a and b) did not increase male catch compared with the traps baited with a virgin female only (Tukey's post-hoc test, Experiment 3a: $P = 0.99$, Experiment 3b: $P = 0.62$). Cracked-infested pomegranate attracted the same number of males as virgin female only (Experiment 3a, Tukey's post-hoc test, $P = 0.06$). Traps baited with a virgin female only did not catch mated females (Dunnnett's post-hoc test, Experiment 3a: $P = 0.99$, Experiment 3b: $P = 0.78$). Combination of cracked or cracked-infested pomegranates with a virgin female did not increase mated female catch compared with cracked or cracked-infested only (Tukey's post-hoc test, Experiment 3a: $P = 0.99$, Experiment 3b: $P = 0.16$) (Fig. 1).

Significant correlations were observed between the numbers of males and virgin females ($n = 422$; $r = 0.655$, $P < 0.0001$) and males with mated females ($n = 422$; $r = 0.457$, $P < 0.0001$) trapped in all experiments (Fig. 3).

Discussion

Our study provides the first experimental evidence of attraction of adult carob moths to host fruit, as well as to volatile extracts of pomegranate in the field. Previous research had shown behavioral responses of female carob moths to volatiles of carob (*Ceratonia siliqua* L.; Fabales: Caesalpinaceae) (Gothilf et al. 1975) and date (Cossé et al. 1994) under laboratory conditions. We also demonstrated that male and female carob moths discriminate between different phenological stages of pomegranate.

Before discussing our results in more detail, we find it important to point out that trapped moths may have affected subsequent catches, because in delta traps each moth caught could be a new odor source that may attract other moths, for example, trapped females could become sex pheromone sources for males and vice versa, as in moths attraction of females to male pheromone has been observed as well (Landolt and Heath 1989). We indeed found strong correlations

Fig. 1. Number of male, mated female, and virgin female carob moths trapped per week in Experiments 1a to 3b (see Table 2). Within each panel, trap catch results with different letters (a, b, and c for males; A, B, and C for virgin females; α , β , and γ for mated females) are significantly different across the treatments at $\alpha = 0.05$. The central line shows the median, and the whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. C (control); FI (pomegranate flowers); ImP (immature pomegranate); UckP (uncracked pomegranate); CkP (cracked pomegranate); CkIP (cracked infested pomegranate); CutP (cut pomegranate); CutIP (cut infested pomegranate); CkPVol (headspace extract of pomegranate); Ps (mature pistachio); PsVol (headspace extract of mature pistachio); VF (virgin female carob moth).

between the number of males and both the number of virgin and mated females trapped in the various experiments. However, as we do not know which sex was trapped first, we find it more accurate to not take this interaction into account. Below, we discuss different hypotheses of why different treatments were attractive or unattractive for males and/or females.

1. Cracked and infested fruits serve as mating site

Similar numbers of males were caught in the traps baited with a virgin female and the traps baited with carob moth-infested fruits. Carob moth larvae develop entirely in one and the same fruit, in which they also pupate (Al-Izzi et al. 1985, Shakeri 2004) and from which the adult emerges. Carob moth females can mate in the night of emergence, so the high attraction of males to the infested fruits is likely a response to female emergence sites. Interestingly, we found that the presence of a virgin female next to the fruit enhances the attraction of conspecific virgin females, which are likely searching

for a calling or mating site. Females can probably reduce the cost of calling or finding suitable calling sites, or both, by being close to other females (Harari et al. 2011).

2. Cracked pomegranate serves as the best oviposition site for mated females

Mated females preferred both cracked and cut pomegranates over uncracked pomegranates. Headspace extract of cracked pomegranate also significantly attracted mated female moths but not virgin females and males. This is likely due to the fact that gravid females use cracked fruits for oviposition, because cracks on fruit peel can facilitate larval penetration of the fruit. Female carob moths oviposit inside the pomegranate crown (calyx) and in cracks on the fruit (Shakeri 2004, Talaie et al. 2010). In our field observations, we found that first and many second instars cannot enter the fruit, while in cracked fruits first instars are, almost without exception, found deep inside the fruits. Ovipositing eggs in the cracks may also

decrease the risk of predation, as common predators such as spiders and lady beetles are generally present in the fruit crown (Shakeri 2004, Mehrazin et al. 2016). Female carob moths may thus likely increase their fitness by choosing cracked fruits for egg laying. This idea is supported by the findings of Talaie et al. (2010), who found more carob moth eggs in cracked fruits than on uncracked fruits, as well as by our previous study, in which we showed significantly higher larval population size and infestation rates in cracked than in uncracked pomegranates (Hoseini et al. 2014).

3. Microbial volatiles attract carob moths

Previous research has shown that mated carob moth females prefer injured and mold-infected carob (Gothilf et al. 1975) and date fruit (Cossé et al. 1994) for oviposition. Gothilf et al. (1975) further demonstrated that fungal growth on injured carobs leads to the production of some alcohols that stimulate oviposition by female carob moths. Since cracked pomegranates are usually infected with

fungi (Shakeri 2004), a similar mechanism may have been involved in the attraction of mated females to cracked and cut pomegranate in our study. Also, mechanically damaged fruit is likely to be quickly infected with fungi, which could explain the observation that mated females were similarly attracted to naturally cracked or cracked-infested and mechanically cut or cut-infested fruits.

4. Mature pomegranates serve as adult feeding site for male and female carob moths

Mature pomegranates, whether cracked or cut or uncracked and infested or uninfested, were attractive to males, mated females, and also virgin females. This suggests that adult moths use the volatile blend that emanates from mature pomegranates to locate adult feeding sites. Carob moths feed on sugar and are attracted to ethyl alcohol (Gothilf et al. 1975, Cossé et al. 1994), which is a volatile product of sugar fermentation by bacteria and other microbes and is found in sweet baits (Utrio and Eriksson 1977). Both males and females of other polyphagous pyralid moths are attracted to water (Ryne et al. 2002), as well as chocolate odor (Olsson et al. 2005), and these components are now used in the management of some of these moths (Anderbrant et al. 2009). Therefore, we hypothesize that ripe fruit may provide a source of water and sugar for carob moth adults, and responses to the volatiles of these fruits may be a strategy to locate adult food. These locations can also serve as rendezvous sites for mating. Males can find females in the adult feeding sites, and females mate males that can find such sites.

5. Pomegranate is a better oviposition substrate than pistachio

We did not find an attraction of female carob moths to pistachio or its headspace extract, which suggests that female carob moths prefer pomegranate to pistachio for oviposition, as we conducted our experiments in a pomegranate orchard. Such a preference also appears from the observation that both pistachio and pomegranate are widely grown in the Middle East, but the carob moth has not been reported as an economic pest of pistachio, while it causes heavy losses in pomegranate orchards (Kashkuli and Eghtedar 1975, Shakeri 2004, Sobhani et al. 2015).

6. Pomegranate flowers and immature fruits are not oviposition sites for females

As we found that pomegranate flowers and immature fruits were not attractive to carob moths, while the insects were actively attacking the mature fruits of the orchard at the same time, flowers and immature fruits do not seem to be suitable oviposition sites for female

Fig. 2. Total (male + female) number of carob moths trapped per trap per week in the traps baited with different baits in Experiment 3b. Treatments with different letters are significantly different at $\alpha = 0.05$. The central line shows the median, and the whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. C (control); CkP (cracked pomegranate); VF (virgin female carob moth).

Fig. 3. Correlation between the numbers of virgin and mated female carob moths and the number of male carob moths trapped per week in the experiments. ** $P < 0.001$.

carob moths. This hypothesis is supported by the recent findings that 1) carob moths attack pomegranate starting at midseason when most fruits are mature (Sobhani 2011, Hoseini et al. 2014, Sobhani et al. 2015) and 2) no carob moth eggs or larvae was found on pomegranate flowers and immature fruits earlier in the season (Talaie et al. 2010, Sobhani 2011).

Possible Use of Pomegranate Volatiles in IPM Against Carob Moth

As we found that cracked pomegranate are very attractive to mated females, alone or in combination with female sex pheromone lures, volatiles from this source may be exploited in behavioral manipulation methods as part of integrated pest management programs of the carob moth. Field evidence of attraction of carob moth to host plant materials and their headspace extracts warrants efforts to identify host plant volatiles or volatile blends for trapping both male and female moths. Behavioral manipulation methods to be exploited include monitoring, mass trapping, and push-pull and attract-and-kill.

Monitoring

Sex pheromone lures of the carob moth are relatively expensive to make, and as attractant they are not very efficient in pomegranate orchards (Avand-Faghih et al. 2012, Dhouibi et al. 2016), attracting an average of nine males per week at most (Dhouibi et al. 2016). In traps baited with cracked pomegranate only, we trapped similar numbers of moths per week, but mostly females. We thus show that traps baited with pomegranate fruit or their volatile extracts can be used at least to monitor female populations of the carob moth. This method has been successfully developed in management of a closely related moth, the navel orangeworm, *Amyelois transitella* (Walker; Lepidoptera: Pyralidae) (Beck et al., 2012), for which sex-pheromone attractants are similarly expensive and difficult to make as for the carob moth. Monitoring populations of carob moth females can be especially useful to time the augmentative release of, for example, trichogrammatid egg parasitoids (Moezipour 2006, Poorjavad et al. 2011) and the larval parasitoid *Bracon hebetor* (Say; Hymenoptera: Braconidae) (Saadat et al. 2016) in biological control programs.

Mass Trapping

As we found that the combination of volatiles of cracked pomegranates and virgin females enhances attraction of female carob moths and increases total (i.e., male and female) moth catch, mass trapping female carob moths might be achieved by using the combination of volatiles of cracked pomegranate with female sex pheromone lures when pomegranates of the orchards are not yet mature and cracked, that is, earlier in the season during flowering/early fruiting stages of the plants, when the fruits do not crack yet (Hoseini et al. 2014).

Push-Pull and Attract-and-Kill

The most effective behavioral manipulation in IPM of the carob moth may be achieved by the so-called push-pull strategy, which involves the behavioral manipulation of insects via the integration of stimuli that make one source (the crop) unattractive or unsuitable (push) for the pest insect, while luring them toward an attractive source (pull), from where the pest insects are subsequently removed (Cook et al. 2007). A carob moth repellent, such as the essential oils of *Ferula assa-foetida* L. (Apiaceae) (Peyrovi et al. 2011), could be used on pomegranate to push the moths away, while traps baited with cracked-mature pomegranate

volatiles, or better, with a combination of pomegranate volatiles and female sex pheromone lures, could be used to pull the female moths to a removable source.

In conclusion, we have identified sources of attractive volatiles to which both carob moth males and females are attracted. The next step will be to identify which of these volatiles can be used for the development of attractive lures. Meanwhile, delta traps with live virgin females and cracked pomegranate may be used as attractive lures, which are likely to be sufficiently effective to monitor moth populations and possibly even to be used in push-pull and/or attract-and-kill strategies.

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