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Variation in Volatiles from Fruits of Mango and Marula Attractive to the Mango Fruit Fly, *Ceratitis cosyra* (Walker)

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Wild mango fruit fly *Ceratitis cosyra*, was attracted to and oviposited preferentially on immature and mature green than ripe yellow mango fruits in the field. Volatile compounds from fruits of mango and marula, at different ripeness stages, were trapped on octadecyl reversed-phase silica. The volatile compounds were identified using gas chromatography, gas chromatography-mass spectrometry and by chromatographic comparisons with authentic samples. Immature and mature green mango fruits on trees emitted similar compounds, comprising of monoterpenoids and sesquiterpenoids. A detached mature green mango fruit emitted a few esters in addition to monoterpenes and sesquiterpenes. The ripe yellow mango fruit emitted large quantities of esters and smaller proportions of terpenoids. Several esters, similar to ripe yellow mangoes, were identified in volatiles of ripe yellow marula fruits. A total of 17 terpenoids and 19 esters were identified. Some of the identified compounds in green mangoes, particularly the terpenoids, constitute candidate kairomones for *C. cosyra*.

Key words: *Ceratitis cosyra*, mango, marula, fruits, volatiles, kairomones, oviposition.

INTRODUCTION

Mango *Mangifera indica* (Anacardiaceae) fruits are attacked by three types of fruit flies: the Natal fruit fly *Ceratitis rosa*, the Mediterranean fruit fly *C. capitata* and the Mango fruit fly *C. cosyra* [1]. *Ceratitis cosyra* is the major pest of, mangoes in eastern and southern parts of Africa. The wild host of *C. cosyra* is marula *Sclerocarya birrea* (Anacardiaceae), a tree that grows from Kenya to South Africa. Mangoes are severely damaged by the females laying eggs in the fruit and by the maggots developing in the flesh of the fruit. Infestation levels of up to 90% in mangoes have been recorded in Kenya [2].

Fruit flies are attracted to a potential host plant through olfactory cues and recognize fruits via both olfactory and visual cues such as color, shape and size [3-9]. Olfactory attractants (kairomones) are considered the most important in the recognition of a suitable fruit for oviposition and have been used to develop effective, nontoxic traps for detection, monitoring and control of fruit fly pests [10,11]. In general, kairomones are semiochemicals (chemicals that carry messages from one organism to another) perceived by an organism to the detriment of the emitter.

Although the population density of fruit flies affects infestation on fruits, ripeness and associated volatiles are important in determining the level of infestation. For example, infestation rate of papayas by the oriental fruit fly, *Dacus dorsalis* and melon fly, *D. cucurbitae* was found to increase with increase in degree of ripeness [12,13]. However the flies were attracted to volatiles from all stages of ripeness [10]. When investigating volatiles attractive to the apple maggot fly *Rhagoletis pomonella*, Fein and others [14] identified seven esters in the active fraction. The variation in the apple fruit volatiles with ripeness revealed 52 esters with the quantity of these compounds increasing with ripeness [15]. Volatile fruit odors have also been investigated as potential attractants for *C. capitata* [16-18], Caribbean fruit fly, *Anastrepha suspensa* [19], oriental fruit fly *Bactrocera dorsalis* [20] and the Queensland fruit fly *Dacus tyroni* [21].

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No systematic studies have been carried out on the behavioral responses of *C. cosyra* to mango fruits or to elucidate the mediating semiochemicals. This has posed a significant drawback in the development of an effective technique for its detection, monitoring and control. In this study, we investigated the attractiveness of mangoes at different ripeness stages to wild *C. cosyra* and then trapped and analyzed the volatiles emanating from these fruits while undisturbed, on tree, and when detached. We also trapped and analyzed volatiles from marula fruits, the native host of *C. cosyra*.

MATERIALS AND METHODS

Behavioral Responses of Wild *Ceratitis Cosyra* to Mango Fruits in the Field

Field experiments were carried out in Nguruman (South-West Kenya) where small-scale mango orchards are predominantly infested with *C. cosyra*. Three ripeness stages of freshly detached mango fruits (round variety), ripe (yellow), mature (green) and immature (green) were arranged on the ground under a mango tree shade in a completely randomized design with 5 replicates in 3 rows and 5 columns at a spacing of 20 cm within rows and between rows. Observations were made every 15 min beginning at 0730 h up to 1930 h. The number of flies landing or visiting each fruit and their activities were recorded. Fly activities on the fruit included walking, sitting, probing using the ovipositor or the proboscis, and ovipositing. The experiment was carried out for two consecutive days. Harvesting of mangoes on this orchard was not done on the days of experiments.

Adsorbent for Trapping Volatiles

The adsorbent used for trapping volatiles from the fruits was octadecyl bonded silica gel, having a particle size of 40 μm. The adsorbent was first Soxhlet cleaned using dichloromethane for 24 h and dried in an oven. Sachets of dimensions 4 cm x 4 cm, each holding 0.45 g of the adsorbent, were made from filter paper (Whatman No. 1), as described by Gikonyo *et al.* [22].

Trapping of Volatiles from Fruits

Trapping of volatiles was carried out from fruits on trees in the field at Machakos (East of Nairobi) and Nguruman. The trapping was carried out from fruits of mango *Mangifera indica* (cultivated host) and marula *Sclerocarya birrea* (wild host of *C. cosyra*). A basket (made from coconut fibers) having dimensions of 20-22 cm (mouth) and 10 cm depth was hung from the branch bearing the fruit or nearby branches using a wire such that the fruit was inside the basket but about 2-4 cm from the bottom. Care was taken not to injure the fruit, leaves or branches. A clean glass jar was placed inside the basket such that the fruit was inside this jar. The fruit was enclosed by covering the mouth of the jar with freshly cut aluminium foil. This setup was allowed to stabilize for 30 min before introducing adsorbent sachets. Detached fruits were placed in a jar, covered using aluminium foil and allowed to stabilize for 30 min before introduction of adsorbent sachets. Trapping was carried out under the shade of a mango or marula tree. For the control, adsorbent sachets were placed in an empty clean jar, covered using aluminium foil and kept under the same mango or marula tree. The trapping was carried out for 24 h. At the end of trapping, the sachets were removed using forceps, wrapped in clean aluminium foil, placed in a clean glass jar and kept in a cool box. This was then transferred into a freezer (-20 ^{0}C) awaiting extraction of adsorbed volatiles.

Extraction of Volatiles from Adsorbents

The adsorbent material in sachets was carefully transferred into elution tubes (either Pasteur pipettes or funnel shaped tubes made from the female part of a ground joint glass tube). Redistilled dichloromethane was passed through the adsorbent and collected in a clean 4 ml vial. The sample was concentrated under ice to about 100 μl using a gentle stream of high purity nitrogen and stored in a freezer at -20 ^{0}C until analyzed.

Gas Chromatography

Gas chromatographic (GC) analysis were conducted on a Hewlett Packard (HP) (Avondale, PA, USA) model 5890 Series II gas chromatograph equipped with a splitless capillary injector system, a flame ionization detector (FID) and an HP 3396 Series II integrator. A fused silica capillary column, 50 m x 0.2 mm internal diameter (i.d.) coated with cross-linked methyl silicone gum (0.11 μm film

thickness) HP was used with nitrogen as carrier gas. The oven temperature was kept isothermal at 40 ^{0}C for 10 min following injection of a sample and then programmed at 5 ^{0}C/min to 280 ^{0}C where it was held for 10 min. In all cases, 1-2 µl of the sample was injected in the splitless mode with a 45 sec delay before injection purging. The injector and detector temperatures were held at 250 ^{0}C.

Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on a VG Platform II (Fisons instruments) mass spectrometer coupled to Series 8000 gas chromatograph, model 8060 MS II (Fisons instruments, Cheshire, UK). The mass spectrometer was operated in the electron ionization mode having an electron energy of 70 eV and an emission current of 200 mA. The GC column and oven temperature programming were the same as those described for the GC analysis.

Compounds in the trapped volatiles were identified from their mass spectra and by comparing with a computerized library of mass spectra. The identity of the compounds was further confirmed by GC co-injection of authentic compounds and trapped volatiles.

RESULTS AND DISCUSSION

Responses by wild *C. cosyra* to mango fruits at various stages of ripeness, was investigated in a mango orchard at Nguruman. Table 1 shows that *C. cosyra* was attracted to the mango fruits of all ripeness stage. Although the flies showed a preference for green fruits, immature fruits attracted more flies than mature and ripe ones. Once the flies landed, similar proportions of flies walked, sat, or probed using proboscis on all stages of fruits (Table 1). Although *C. cosyra* could lay eggs on any stage of fruit, there was a preference for ovipositing on immature mango fruits. Ovipositing on an immature fruit will provide nutrients for development of larvae over a long time. On the other hand, it may be a survival strategy aimed at ensuring that larvae are fully developed and have popped out of the fruit before onset of ripeness when fruits would be harvested. Whereas ovipositing on green mango fruits may be beneficial to the fly, this may pose the serious risk of translocating the fly to other regions, especially during mango export.

Table 1: Responses of wild mango fruit flies, *Ceratitis cosyra* to detached mango fruits at different ripeness stages in the field

	Mango fruit		
	Immature green	Mature green	Ripe yellow
Number of flies attracted	338	292	95
Walking (%)	39.6	42.1	43.2
Sitting (%)	24.6	30.8	37.9
Probing with proboscis (%)	7.1	4.1	5.3
Probing with ovipositor (%)	8.9	9.6	2.1
Ovipositing (%)	19.8	13.4	11.6

Compounds volatilizing from immature green and mature green mango fruits while still up on the tree were qualitatively and quantitatively similar. The identified compounds from these green fruits were monoterpenoids and sesquiterpenoids. Monoterpenes included ocimene , β-myrcene, α-phellandrene, 3-carene, limonene, terpinolene and 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one, while sesquiterpenes were α-copaene and germacrene-B (Table 2). Detaching a mature but green mango fruit off the tree resulted in the emission of esters including ethyl methacrylate, ethyl octanoate, methyl-*z*-butenoate, ethyl butyrate and ethyl crotonoate. Additional monoterpenoids, camphene and γ-terpinene as well as the sesquiterpenes, α-cubebene, β-cubebene and α-caryophyllene were emitted when a mature green mango was detached (Table 2). Detaching the green mature mango fruit also resulted in a quantitative increase in the emission of ocimene and β-myrcene and a decrease in 3-carene. The detached ripe yellow mango fruit emitted large quantities of compounds, especially the esters. Esters such as ethyl hexanoate, ethyl-*E*-4-decenoate, ethyl propanoate, butyl octanoate and ethyl-*E*-2-octenoate were detected in addition to those from the detached mature green fruit. Trace amount of odor was trapped from the immature/mature green marula fruits that were on tree. The ripe yellow detached marula fruits emitted a large number of compounds constituting mainly of esters, some of which were also found in ripe yellow detached mangoes.

Table 2: Compounds and their percentages, detected in volatiles of mango and marula fruits at different ripeness stages

Peak number	Compound	Mango fruit				Marula fruit	
		IGT	MGT	MGD	RYD	IMGT	RYD
	ESTERS						
1	Ethyl propanoate	-	-	-	0.40		2.38
2	Methyl butanoate	-	-	-	0.17		-
3	Methyl-Z-butenoate	-	-	0.21	-		-
4	Methyl crotonoate	-	-	-	0.20		-
5	Ethyl isobutyrate	-	-	-	-	-	1.19
6	Methyl isovalerate	-	-	-	-		0.59
7	Ethyl butyrate	-	-	0.13	11.25		0.79
8	Ethyl methacrylate	-	-	2.64	7.49		-
9	Ethyl crotonoate	-	-	0.07	2.16	-	-
10	Ethyl-2-methyl butyrate	-	-	-	-	2.82	2.46
11	Ethyl isovalerate	-	-	-	0.12	-	31.93
12	Ethyl tiglate	-	-	-	0.12		-
15	Ethyl hexanoate	-	-	-	4.10		-
22	Methyl octanoate	-	-	-	0.37		-
24	Ethyl octanoate	-	-	2.38	22.23	-	-
25	Ethyl-E-2-octenoate	-	-	-	0.41		-
26	(E)-7-Decenylacetate	-	-	-	-		15.50
27	Ethyl-E-4-decenoate	-	-	-	4.37		3.16
28	Butyl octanoate	-	-	-	0.50	-	1.08
	TERPENES						
13	Ocimene	0.95	1.25	18.04	0.92	-	-
14	Camphene	-	-	2.44	-	-	
16	β-Myrcene	2.81	2.78	46.93	-	-	-
17	α-Phellandrene	0.96	0.97	1.04	-		-
18	3-Carene	66.14	71.87	5.31	29.19		-
19	Limonene	2.34	2.58	1.93	1.18		
20	γ-Terpinene	-	-	0.33	-		
21	Terpinolene	4.32	3.65	0.44	1.40		
23	TBH	1.09	0.74	-	-	-	-
29	α-Copaene	1.94	1.19	1.03	0.12	2.50	
30	Isocryophyllene	-	-	-	-	9.58	
31	α-Cubebene	-	-	1.20	0.27	-	
32	Longipinene	-	-	-	-		6.09
33	β-Cubebene	-	-	0.77	0.16		-
34	α-Caryophyllene	-	-	0.67	-		-
35	Germacrene-D	-	-	-	2.80		-
36	Germacrene-B	1.41	0.86	0.38	-		-

Peak number indicates the order of elution from GC column. IGT: immature green on tree, MGT: mature green on tree, IMGT: immature/mature green on tree, MGD: mature green detached from tree, RYD: ripe yellow detached from tree. TBH: 1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one. -: undetected.

The major compounds in volatile of green mango fruits on tree were 3-carene at about 70% followed by terpinolene at about 4% and β-myrcene at 3% (Table 2). However, β-myrcene was present at about 47% while ocimene was 18% and 3-carene about 5% in the volatiles of the detached mature green mango. Volatiles of the detached ripe yellow mango constituted about 30% 3-carene while the other major compounds were the esters, ethyl octanoate at 22% and ethyl butyrate at 11%.

The emission of esters from ripe fruits has been reported widely and these have been used to formulate attractants for fruit flies [11, 14, 23]. Fein and others [14] reported seven esters from an attractive fraction of volatiles of ripe apple fruits. Carle and others [15] identified a total of

52 esters from apples of different ripeness stages. However, they detected no other groups of compounds and no variations in composition of volatiles between detached and fruits on tree. In addition to esters, some of the terpenoids that elicit behavioral responses to fruit flies include camphene, α-pinene, eugenol, geraniol, asarone, limonene, β-caryophylene, 3-carene and myrcene [24]. The sesquiterpenes α-copaene and α-ylangene are potent attractants to male *C. capitata* [25].

Results of this study indicate that attractiveness of mangoes to *C. cosyra* is related to ripeness, with green fruits being more attractive than ripe yellow ones. The change in the emitted volatiles with ripeness of fruits is suspected to influence the observed gradation of the mangoes as oviposition and/or feeding sites by *C. cosyra*. Presence of some known attractive esters and terpenoids in the volatiles of fruits of both mango and marula, leads us to suggest that they play a key kairomonal role for *C. cosyra*. Identification of these kairomones is important for development of baits for monitoring and controlling *C. cosyra*.

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