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RESEARCH NOTE

Volatiles from *Carissa carandas* fruits damaged by conspecifics attract chafer beetles, *Protaetia alboguttata* (Vigors)

P. D. KAMALA JAYANTHI[1*], K. VIVEK [1], M. A. RAVINDRA[1], P. SARAVAN KUMAR[1], B.N.S MURTHY[2] and N. BAKTHAVATSALAM[3]

[1]Division of Entomology and Nematology, [2]Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru 560089, Karnataka, India.

[3]ICAR-National Bureau of Agricultural Insect Resources, Bellary Road, Bengaluru, Karnataka, India

*** E-mail**: jaiinsect@gmail.com

ABSTRACT: The present study highlights the importance of herbivore induced plant volatiles (HIPVs) in attracting the cetoniid beetles, *Protaetia alboguttata* (Vigors) to ripe conspecific damaged *Carissa carandas* L.fruits. Olfactometer assays revealed that volatiles from damaged fruits are significantly attractive to the adult beetles of *P. alboguttata*. Gas chromatography electroantenno-detection studies revealed that presence of α-copaene and carvacryl acetate in the damaged *C. carandas* fruits that might be involved in guiding the adult *P. alboguttata* beetles to their host plants.

Keywords: HIPVs, Karonda, *Carissa carandas*, GC-EAD, Cetoniid beetle

Plants when attacked by phytophagous insects, produce a bouquet of odour plumes often termed as Herbivore Induced Plant Volatiles (HIPVs) which play a crucial role in attracting natural enemies of the insect pests. However these HIPVs were later on established to have multiple ecological functions and services encompassing inter-and intra-plant communication, conspecific- and heterospecific herbivore foraging strategies (Kamala Jayanthi *et al.*, 2020). The cetoniid beetles popularly called fruit and flower chafers are mainly diurnal, and visit flowers for pollen and nectar thereby rendering vital pollination services. However, genus *Protaetia* includes agriculturally important phytophagous pest species like *Protaetia alboguttata* (Vigors) (Coleoptera: Scarabeidae) that is known to attack the crops in swarms causing severe damage (Kamala Jayanthi *et al.,* 2017; Veeresh *et al.,* 1980; Sekhar *et al.*, 2000). Like other phytophagous herbivores, chafer beetles depend on the host volatiles (=kairomones) to locate their hosts as these sites are used not only as their food resources but also as potential mating sites (Ruther, 2004). In case of cockchafers of the genus *Melolontha* (Scarabaeidae: Melolonthinae), leaf alcohols released from host plants upon insect feeding selectively attracted males, thereby enabling mate finding (Reinecke *et al.,* 2002; Ruther *et al.,* 2002a, b). Therefore the term 'sexual kairomone' has been introduced to describe this type of novel function of plant chemicals in the reproductive behaviour of insects (Ruther *et al.,* 2002a, b). Thus, volatile compounds emitting from hosts might serve as attractants to scarab beetles. Even in the case of *P. alboguttata*, congregations of female and male beetles were found making distinctive high-pitched buzzing around the fruiting trees and found to be involved in feeding as well as courtship (Kamala Jayanthi *et al.,* 2017). Earlier studies revealed that the *P. alboguttata* beetles were attracted to ripe karonda, *Carissa carandas* fruits when compared to unripe fruits (Kamala Jayanthi *et al.,* 2017). Further, they noticed that the plants with damaged fruits were more attractive to adult beetles of *P. alboguttata* than the plants with undamaged fruits.

In the present study, we compared the volatiles of fresh ripe fruits of *C. carandas* (undamaged as well as damaged by *P. alboguttata*) for their attractiveness to the conspecifics. The study was conducted in the Fruit Entomology Laboratory of Indian Institute of Horticultural Research (IIHR), Bengaluru, India. The adult beetles of *P. alboguttata* hovering, feeding on ripe *C. carandas* fruits were collected from the experimental fields of IIHR brought to laboratory and kept in cages (1.5 x 1.5 x 1.5 m). Fresh ripe *C. carandas* fruits and water were provided daily *ad libitum*. Prior to bioassays the beetles were starved for 24 h.

Headspace samples of volatiles from fresh ripe fruits of *C. carandas* (cv. sweet type) were collected by air entrainment (Kamala Jayanthi *et al.,* 2012). The

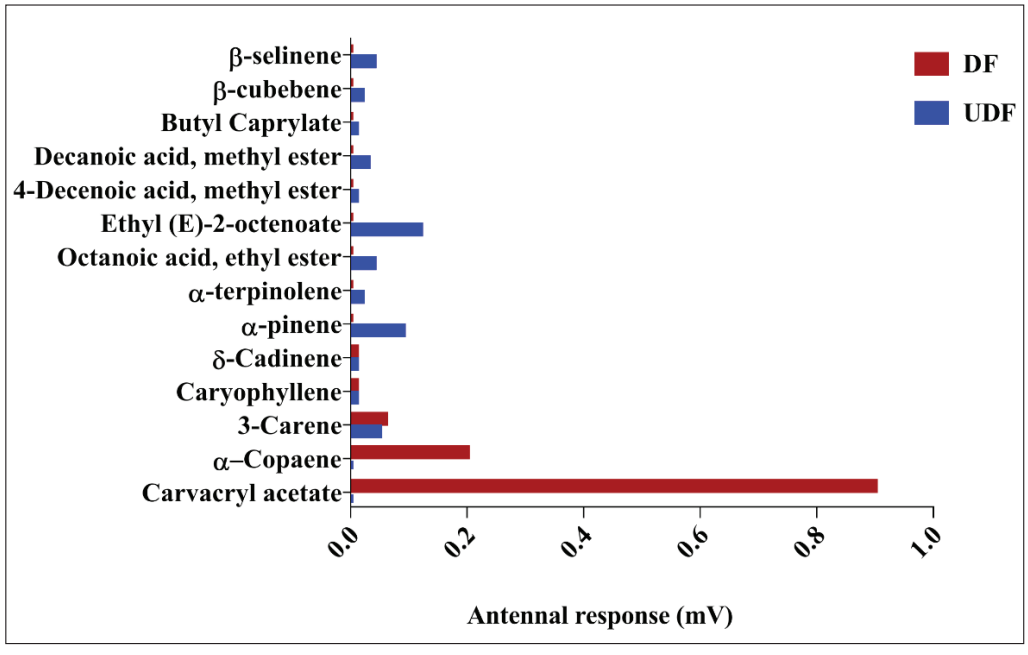

Fig.1. Antennal response of adult chafer beetles, *P. alboguttata* to damaged (DF) and undamaged (UDF) fruit volatiles

fruits [both undamaged (UDF) and damaged (DF) by *P. alboguttata*] were collected from the field and brought to the laboratory to collect the volatiles. The volatiles from fruits were entrained for 12 h and the Porapak Q filters were eluted with 750 µl of redistilled diethyl ether, providing a solution that contained the isolated volatile compounds served as test sample. Samples were stored in a freezer (-20°C) until use.

The behavioral responses of the adult *P. alboguttata* beetles to headspace samples of fruit volatiles were tested in single/dual-choice Y-tube olfactometer bioassays. Olfactometer assays were carried out as described in Piñero *et al.,* (2008). Briefly, the Y-tube olfactometer consisted of a Y-shaped glass tube (6.5 cm diam., 13 cm arm length and 21 cm common arm length) connected to two tubular glass chambers (5.5 cm long and 2.5 cm in diameter), where the odor sources were placed (one in each arm). Charcoal-filtered and moistened air was drawn into each of the two glass chambers and Y-tube arms at a rate of 750±10 mL min^{-1} at the entrance. Prior to each experiment, all glassware was washed with teepol, rinsed with acetone and distilled water, and baked in an oven overnight at 160°C. Experiments were conducted in a isolated room (25±2°C, 60% RH) to avoid contaminant odours. Mixed populations of both female and male beetles were brought into the experimental room 30 min before the start of the experiments to allow acclimatization to the room conditions. A single beetle was released at the entrance of the common arm of the Y-tube and exposed to a test sample (10 µl) and the same quantity of solvent, diethyl ether served as control in single choice assays. In dual-choice assays, comparison was made between the test samples namely UDF and DF. The filter paper strips containing the test sample and solvent (as the case may be) were placed individually inside the two chambers that connected to the two arms of the Y-tube olfactometer. Once inside the Y-tube, the behavior of each beetle was observed for 5 min. A beetle was considered to have made a choice if it entered either arm, and a beetle was considered not having made a choice if it remained in the common arm of the Y-tube by the end of the observation period. A fresh filter paper strip with odour was used for each individual beetle tested, and the position of the chambers containing the odour source, as well as the position of the two arms of the olfactometer, was systematically changed for each set in order to avoid positional bias. For each set the sample size consisted of 30 beetles of mixed sexes and a total of four sets were carried out for each assay. Individuals that did not make a choice were excluded from the statistical analysis. A new 'Y' tube olfactometer was used for each replication.

Cetoniid beetle, *P. alboguttata* antennal responses to extracts of porapak volatiles from undamaged and damaged karonda fruits were studied by gas chromatography coupled electroantennographic detection (GC-EAD) as per the procedure described earlier (Shivaramu *et al.,* 2017). The electroantennogram preparations were obtained using the indifferent electrode being placed at the base of beetle antenna and

the recording electrode was slipped over the tip of the antennae and the electrodes are filled with 25 mM Nacl (Ringer solution). The Purified fraction eluted from the transfer line were carried out by a flow of humidified air with flow rate of 80 cm/s. The antennal responses eluted against the GC peaks were recorded by Syntech GCEAD software (V.2010). Peaks eluting from the GC column were judged to be active if they elicited EAG activity in two or more of the coupled runs.

Chemical composition of Porapak Q elutes were analyzed by Coupled Gas Chromatography-Mass Spectrometry (GC-MS) using Varian 3800 apparatus equipped with coupled MS/MS (Saturn 4000) as described earlier (Shivaramu *et al.,* 2017). Two micro liter sample was injected in split mode (1:20) with injection temperature at 270°C. Compounds were identified by GC retention time, mass spectrum and KOVATS index using NIST 2007 and Wiley library as reference.

Data of single-choice behavioral bioassays were analyzed for preference (percentage of adults that made a choice between a test odor and the solvent in each set) and a paired-sample *t*-test was carried out to compare the beetle response. The data of dual-choice assay was subjected to one way ANOVA.

The adult beetles of *P. alboguttata* exhibited significant attraction to the volatiles collected from damaged fruits of *C. carandas* (DF) (beetle response, Mean±SE, 69.83± 2.78%; $P = 0.003$; $t = 6.90$) compared to the solvent control (29.67± 3.06%) in single-choice assay. Similarly when the beetles were exposed to the volatiles of undamaged fruits (UDF), no significant difference was found between their response over solvent control (Mean±SE, UDF: 69.83± 2.78%; control: 53.93±2.76%; $P = 0.12$). In the dual-choice assay, when the beeltes were given a choice between UDF and DF volatiles, the beetles showed significant response to UDF (Mean±SE, 73.08± 5.87%; $P = 0.01$; $F = 16.69$) over DF (Mean±SE, 40.42 ± 5.43%).

The GC-EAD and GC-MS analysis of damaged and undamaged *C. carandas* fruit volatiles revealed significant differences in the antennal response of *P. albogutta* to various compounds belonging to monoterpene esters, terpenes, mono- and sesqui-terpenes and fatty acid esters. The volatile compounds namely β-selinene; β-cubebene; butyl caprylate; decanoic acid, methyl ester; 4-decenoic acid, methyl ester; ethyl (*E*)-2-octenoate; octanoic acid, ethyl ester; α-terpinolene and α-pinene in the undamaged fruit volatiles elicited antennal response in *P. alboguttata*. The compounds like α-copaene and carvacryl acetate that were present in damaged fruit volatiles elicited maximum response in the adult *P. alboguttata* compared to other volatile compounds. The compounds like 3-carene, δ cadinene and caryophyllene were present in both the fruit volatile samples (UDF and DF) and elicited antennal response in the adult beetles (Fig.1). The results precisely indicate that the HIPVs like α-copaene and carvacryl acetate emitted from the damaged fruits might be involved in host orientation, guiding the adult *P. alboguttata* beetles to their host plants. Our earlier observations also revealed that relatively more numbers of *P. alboguttata* beetles (both the sexes) were noticed on the *C. caradas* trees having damaged fruits compared to the trees with undamaged fruits (Kamala Jayanthi *et al.,* 2017). Earlier studies established that the utilization of conspecific produced HIPVs by phytophagous insects have the advantage of easy host location (Kessler and Baldwin, 2001; Shivaramu *et al.,* 2017) and easy access to cohorts and mates (Kalberer *et al.*, 2001) as observed in the present study.

Considering the polyphagous nature of chafer beetles, *P. alboguttata* and their seasonal flight habit during monsoon showers, these beetles can pose direct threat to the cultivation of several fruit crops that have May-June fruit bearing period (Kamala Jayanthi *et al.,* 2017) and the utilization of the conspecific feeding induced fruit volatiles like α-copaene and carvacryl acetate as aggregation kairomones may be worth exploring as similar attempts in several other phytophagous chafers have yielded positive results (Ruther, 2004; Ruther and Hilker, 2003; Ruther and Tolasch, 2004; Ruther and Mayer, 2005).

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