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Attraction behavior of the Melon Weevil, *Acythopeus curvirostris persicus*, to conspecific insects and host plants

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ABSTRACT

Melon weevil, *Acytopeus curvirostris persicus* Thompson (Col.: Curculionidae), is an important pest of cucurbits. Understanding the chemical ecology of this species could provide information for the development of nonchemical control measures. Field trapping experiments indicated adult insects respond to conspecific (a member of the same species) insects and host fruit. To determine attractiveness rate, two-way static experiments were carried out under laboratory conditions using conspecific insects, and to evaluate the role of the host plants melon (*Cucumis melo* L.), watermelon [*Citrullus lanatus* (Thunb)], cucumber (*C. sativus* L.), and colocynth [*C. colocynthis* (L.)]. Cucumber fruit had the most, and colocynth fruit, the least attraction. Adult insects were more attracted to a mated, or unmated, conspecific insect indicating existence of an aggregation pheromone. Results of the study provided the necessary background for the identification of host plant kairomone and aggregation pheromone of melon weevil.

KEYWORDS

Aggregation pheromone; cucurbitaceae; melon weevil; two-way static olfactometer

Melon weevil, *Acythopeus curvirostris persicus* Thompson (Col.: Curculionidae), attacks members of the Cucurbitaceae (Mohammadpour et al., 2015). A wild host of melon weevil is the colocynth, *Citrullus colocynthis* (L.) Schrad. Due to intensive agriculture, colocynth populations have been reduced, or destroyed, and melon weevil has adapted to new hosts and become a major pest. The fruit is destroyed by feeding by larvae and can become infected with bacteria to become unmarketable.

This weevil selects cucumber (*Cucumis sativus* L.) fruit, 5–8 cm in dia, which have a thin, succulent, skin for egg-laying, and after 1 to 2 days of tunneling in fruit and feeding, it begins to lay eggs. The *A. curvirostris persicus* subspecies usually lays a single egg in each cavity, the duration of egg-laying is about 3–4 min. Oviposition location AA foam that are countable and a maximum of 38 eggs occurs in each oviposition hole. Holes used for feeding are not countable due to high numbers (Mohammadpour et al., 2015).

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Host-selection behavior of herbivorous arthropods provided evidence for the role of secondary plant chemicals as a source of information in behavioral decisions of herbivores (Dicke, 2000) and searching behavior of their natural enemies (Fürstenberg-Hägg et al., 2013). Use of pheromone and kairomone are used as bait traps as a monitoring device to determine when to apply insecticides for controlling insect pests (Rechcigl and Rechcigl, 2010). The level of insect attraction of several related, and unrelated insects (Farazmand et al., 2001; Izadi et al., 2011; Moayeri et al., 2008; Mohammadpour, 2005; Mohammadpour and Avand Faghih, 2008), has determined that attractiveness rate is related to plant volatile materials and sexual pheromones (Mohammadpour et al., 2013; Zhang and Sun, 2006).

Due to the multi-generational nature of the pest, farmers use large amounts of insecticides during the growing season for control (Mohammadpour et al., 2013). Excessive use of synthetic pesticides results in accumulation of residues in the environment and on, or in, cucurbits. Little is known about daily and seasonal activities, including movement patterns and reproductive processes of the melon weevil (Mohammadpour et al., 2013). The combination of biological, behavioral, and chemical studies for use of semiochemicals, particularly pheromones, has been used against agricultural pests, representing a safe and lasting alternative in control against insects by employing monitoring, mass trapping, or disruption, to detect pests or prohibit entry of nonnative species in cultivated areas. Understanding the chemical ecology of melon weevil could provide information for control. Field trapping has shown that adult insects respond to conspecific (a member of the same species) insects and host fruit. This experiment was carried out to determine the attractiveness rate that may have potential application in monitoring and control of Melon weevil.

Materials and methods

Melon weevil adults were collected from melon and watermelon fruit infested with larvae, or adults, from the Agricultural Research Station of Birjand, South Khorasan province, Iran, and placed in a growth chamber at 26 ± 1°C, 50–60% RH, and 12:12 h (L:D) photoperiod. The colony was periodically supplemented with weevils collected from the same field locations. As soon as insects emerged from pupae, females, and males were separated (Khanjani, 2005) according to morphological characteristics. Male and female insects were placed into plastic containers, 10 × 10 × 20 cm, each side of which had a test chamber. The insects were fed pieces of cucumber and watermelon fruit. One day before tests, insects were isolated in small boxes without food.

Experiments were carried out using a two-way static olfactometer (Avand-Faghih, 2004). The surface of the olfactometer's transparent plastic sheet was circular, 30 cm dia, and 10 cm tall, with 2 holes in the bottom of the plate with 2 cm dia holes spaced 15 cm from each other. Beneath each hole was placed a 2 cm high Petri dish. After each test with each replication, the olfactometer was rotated 90° clockwise to eliminate error due to conditions of the test room.

Each experiment had 30 replicates and replications were performed separately for males and females. Melon weevils were individually placed in the middle of the olfactometer apparatus and their behavior recorded after 5 min (Alizadeh et al., 2009; Saïd et al., 2006). The olfactometer apparatus was washed with water and detergent after each test with each treatment. After drying, it was again completely washed with alcohol and reused.

Behavior of mated and unmated male and female melon weevil was tested with: 1) both empty holes (control); 2) melon fruit vs. control; 3) watermelon fruit vs. control; 4) cucumber fruit vs. control; and 5) colocynth fruit vs. control. The fruit were collected daily from the field and 1 × 1 × 2 cm pieces of fruit with peel were used. Fruit pieces were replaced every five replicates. Due to the sensitivity and accuracy of olfactometry assays, disposable gloves were used to replace fruit pieces so as not to affect the insect selection process. In the olfactory assay with conspecific insects, treatments were: A) mated male insect vs. empty hole (control); B) unmated male insect vs. control; C) mated female insects vs. control; and D) unmated female insect vs control.

To compare the selection of insects, a binomial test was used (Excel 2013, Microsoft,

Redmond, WA). Analysis of olfactometer tests was in a factorial experiment based on a completely randomized design using SPSS (ver. 24.0, IBM, Armonk, NY). If interactions were significant, they were used to explain results. If interactions were not significant means were separated with Tukey's Honest Significant Difference test.

Results and discussion

Behavioral responses of *A. curvirostris persicus* males and females were affected by host fruit, and whether conspecific insects were mated (Table 1). Response to cucumber fruit was highest; the least amount of attraction was for colocynth fruit (Table 2). Mated insects were more attracted to host plants (42.08 ± 4.23) than unmated insects (32.08 ± 4.10) according to the analysis of variance. Attraction of adult weevil was affected only by the presence of conspecific insects (Table 3). Presence of conspecific insects had a greater attraction for melon weevil (avg. 38.5%) than the absence of conspecific insects (avg. 18%), according to ANOVA.

Melon weevil was attracted to traps baited with watermelon fruit and male insects (Mohammadpour et al., 2013) which appears to be different to results for watermelon reported here. This may be a result of differences in study conditions where results under controlled conditions need to be tested under field conditions to prove their applicability. For melon weevil, the reason for

Table 1. Analysis of variance factors affecting attraction of adult melon weevil, *Acythopeus curvirostris persicus*.

P	F Statistic	Mean squares	df	Variable
0.04*	2.927	1250	3	Fruit type (F)
0.70	0.156ns	66.66	1	Sex (S)
0.02*	5.614	2400	1	Mated (M)
0.65	0.671	286.66	5	Replicate
0.99	0.26ns	11.11	3	F × S
0.62	0.598ns	255.55	3	F × M
0.84	0.039ns	16.66	1	S × M
0.47	0.845ns	361.11	3	F × S × M
		427.11	75	Error

ns, * not significant or significant at 5%, ANOVA.

Table 2. Attraction of adult melon weevil, *Acythopeus curvirostris persicus* to host fruit.

Mean (percent)	Crop
43.33a[a]	Cucumber
39.16ab	Watermelon
39.16ab	Melon
36.66b	Colocynth

[a]values in the column followed by the same letter are not significantly different, $P \le 0.05$, Tukey's HSD test.

Table 3. ANOVA results for factors affecting attraction of melon weevil, *Acythopeus curvirostris persicus* to adult conspecific insects.

P	F Statistic	Mean squares	df	Variable
0.001*	185.6	1804.17	3	Insect (I)
0.721	129.0ns	37.5	1	Sex (S)
0.077	3.214ns	937.5	1	Mated (M)
0.953	3.214	937.5	5	Replicate
0.413	0.966ns	281.94	3	I × S
0.431	0.928ns	270.83	3	I × M
0.286	1.157ns	337.5	1	S × M
0.332	1.151ns	337.5	3	I × S × M
		611.322	75	Error

ns, * not significant or significant at 5%, ANOVA.

differences in attraction may be attributed to the insect's detection of presence, or amount, of volatile chemicals in these fruit (Reddy and Guerrero, 2000).

Melon weevil was attracted to conspecific insects. The adults may use volatiles induced by conspecific insects as olfactory cues when foraging for hosts (Mohammadpour et al., 2015, 2013), and may, in part, explain aggregation behavior of these herbivores in the field. Herbivore-induced host plant volatiles, and aggregation pheromone, may have potential application in monitoring and control of Melon weevil. The chemical composition of *A. curvirostris persicus* aggregation male pheromone has not been identified.

Additional experiments in controlled conditions are needed to identify this pheromone and evaluate the combined effectiveness of aggregation pheromone and fruit kairomone for potential trapping. These semiochemicals can be used in the development of tools for enhancement of bio-rationale alternatives to synthetic pesticides.

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