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Detection Of A Chemical Marker From Ovipositing Females In *Rhynchophorus Ferrugineus* (Coleoptera: Curculiondae)

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Mohamed S. Salem, Mohamed H. Belal, Mahmoud E. Nour, Emad A. Sayed: Detection Of A Chemical Marker From Ovipositing Females In *Rhynchophorus Ferrugineus* (Coleoptera: Curculiondae)

ABSTRACT

The red palm weevil, *Rhynchophorus ferrugineus* **(**Coleoptera: Curculiondae) a relatively new pest in Egypt, is spreading rapidly in the Middle East and causing widespread loss of palms, including date palms. As it spends most of its life deep inside the palm, it has been very difficult to control with currently available methods. Previous observations suggested the existence of a semiochemical which discouraged females from laying in a spot already containing eggs. This study aimed to confirm the existence of a semiochemical and identify it. Firstly, the external and internal morphology of the female ovipositor and related sensilla were carefully examined, photographed and described. Secondly, compounds adhering to the egg surface were extracted, their egg-laying deterrence tested and their constituents analyzed. Twenty-four hours later the sites treated with the extracts contained fewer eggs. Analysis of the extracts by GC-MS revealed several possible signaling compounds, the most abundant of which was tentatively identified as oleic acid.

Key words: red palm weevil, oviposition, semiochemical, deterrent, gland, chemical marker, *Rhynchophorus ferrugineus*

Introduction

The red palm weevil (RPW), *Rhynchophorus ferrugineus (Olivier)*, is an economically important tissue-boring pest of date palm in many parts of the world [8]. In the mid-1980s, the RPW was discovered attacking palms in the Arabian Peninsula [9,3]. and is now causing severe damage to date palms in plantations all over Egypt.

The concealed feeding behavior of the RPW makes it very difficult to detect infestation at early stages. Often rotting of the internal tissue leads to the death of the date palm tree [12]. Current tactics to manage the weevil are largely based on insecticide application, although deepening concerns about environmental pollution have lead to a strong emphasis on the development of integrated pest management (IPM) based on pheromone traps and biological control rather than insecticides. Besides synthetic insecticides [15,1,6,20,4], researchers have investigated the use of plant extracts [18,13], and pheromone trapping of *R. ferrugineus* and *R. palmarum* [10,2,11,16].

R. palmarum, as other coleoptera, depends on chemical communication to coordinate its behavioral activities [17]. Many insects, when depositing their eggs, leave behind oviposition markers, which discourage other females of the species from choosing that site for their own eggs. Oviposition markers have been confirmed in the granary weevil (Sitophilus granarius L.) [14] and *Callosobruchus subinnotatus* (Pic), George [7].

The observation that female RPW tend not to lay their eggs where eggs have already been laid, directed our research to investigate the cause of this behavior. Firstly, the external and internal morphology of the female ovipositor and related sensilla were carefully examined, photographed and described. Secondly, compounds adhering to the egg surface were extracted, their egg-laying deterrence tested and their constituents analyzed.

Materials And Methods

Insects:

Adult weevils were collected from infested palm trees. The insects were maintained in the laboratory in plastic containers at 23-27 ℃ and 70-90% RH, with a 12h light/dark cycle and were fed twice a week with pieces of sugar cane [17].

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Both male and female reproductive segments were dissected, especially the female ovipositor and male copulatory organs. Samples were closely examined and photos were made with a research light microscope (stereoscope).

Extraction techniques:

Extracts with ethanol, methanol, acetone, hexane and dichloromethane were obtained from eggs laid and from the end parts of 3 female abdomens. Groups of 10, 20, 30, 40 and 50 eggs were immersed in one of the solvents used for extraction for 24h.

Semiochemical assay:

Each extract from female genitalia parts and eggs was tested by spraying on 3 separate small pieces of sugar cane which were then provided as oviposition sites for 24h to egg-laying females. For control, the same procedure was followed using pure solvent. At the end of 24h the sugar cane pieces were carefully cut open and the eggs gently collected with a fine camel's hair brush. The results were tabulated and the means recorded.

Chemical analyses:

More than 100 eggs were collected and extracted with acetone. The extract was injected into a Varian 450 GC/Varian 240 MS, with a capillary column GC:VF-5ms 30 m x 0.25 mm, IDDF=0.25µm. Column flow: 1.0 ml/min. Injector: 250ºC. Column oven: 80ºC (2 min.) →290ºC (8 ºC/min.) 290 ºC (2 ºC/min.).

Statistical analysis:

The collected data were subjected to normal statistical analysis as set out by [19]. Treatment mean comparisons were made using least significant difference (LSD) at the 5% level of probability.

Results And Discussion

Dissection and careful observation of the hind segments of the red palm weevil *Rhynchophorus ferrugineus* revealed the presence of two symmetrical glands in the ovipositor of the females, but absent in males. *R. ferrugineus,* as other coleopteran species, depends on chemical communication to coordinate its behavioral activities. The dissection of both sexes allowed us to determine that at the posterior end of the ovipositor only the females have a symmetrical pair of glands, which lack a reservoir. Multiple hairs in the area spread a secretion on eggs as they are laid. At the bases of the hairs around the glands small granules were found, possibly formed by waxes secreted from the pairs of glands. In the corresponding position in males we observed hairs on two spots.

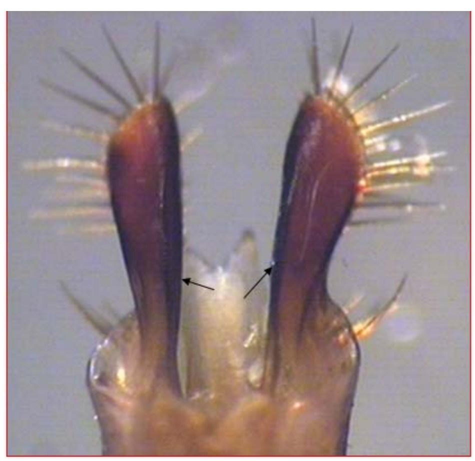

Fig. 1: The external reproductive organs of an adult female, showing the enlargement of two glands in the upper parts (enlarged 360 X)

The morphological examination of the gland suggests that the insect does not possess a reservoir to store the secretion, indicating that this secretion must be synthesized and released in direct response to the stimulus of laying eggs. This gland, due to its location, can be considered as a modified accessory gland which probably releases its secretions onto the eggs as they pass out through the ovipositor. Similar secretion releasing mechanisms could also be expected in insects of the same tribe and even among curculiondae in general.

These two glands were rectangular in shape and could easily be separated in females. On other hand, when observing the posterior end of the copulatory segments or the end of the abdomen in males, the shape of the gland was completely different, like dark spots, which could not be easily separated.

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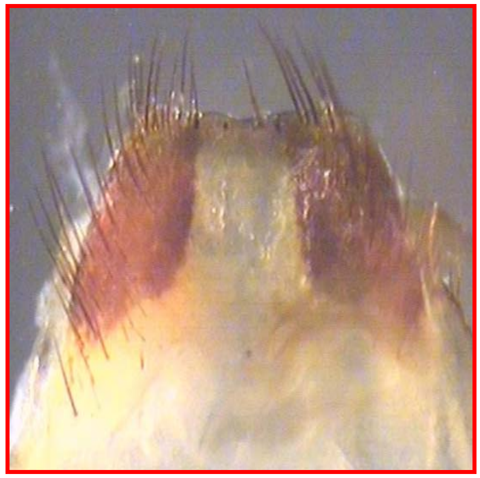

Fig. 2: The external reproductive organs of an adult male, showing the spots like accessory glands in the hind parts of the copulatory organs (enlarged 360 X)

Effect of the egg extract on the number of eggs laid:

The highest numbers of eggs per female were laid where the treatment was weakest, when fewer than 10 eggs had been extracted. Fewer eggs per female were laid where the egg extract was more concentrated (Table 1).

Table 1. The effect of number of eggs extracted by different solvents on the number of eggs laid by females.

Chemical analyses of egg extract:

The GC analysis of an egg extract is presented in Fig. 3. There are a few peaks, but two peaks are the most obvious in this extract. The chemical structure of the curve number one (18.886 min, Scan: 962, 50: 1000, lon: 6248 us, RIC: 183450), shown in the Figs. 4 and 5, may be Z,Z-10,12-Hexadecadienal – $C_{16}H_{28}O$, MW 236 or Oleic Acid – CAS No. 112-80-1, $C_{18}H_{34}O_2$, MW 282.

Table 1: The effect of number of eggs extracted by different solvents on the number of eggs laid by females.

Number of eggs extracted	Extractors					Mean
	Ethanol	Methanol	Acetone	Hexane	Dichloromethane	
0	17.33	14.33	14.67	16	18.67	16.20 A
10	5.33	3.67	4.67	5.67	4.33	4.73 B
20	3.67	3.67	3.00	4.00	3.00	3.47 C
30	2.33	3.00	1.67	2.67	1.67	2.27 CD
40	1.33	1.67	0.67	1.67	1.00	1.27 DE
50	0.67	0.33	0.00	1.33	0.33	0.33 E
	5.11 A	4.44 A	4.11 A	5.22 A	4.83 A	

-Means within column and row followed by the same letter are not significantly different at 5% probability.
-Eggs used (A): L.S.D $_{0.05}$ =1.26, Extractors (B): N.S, A*B: N.S

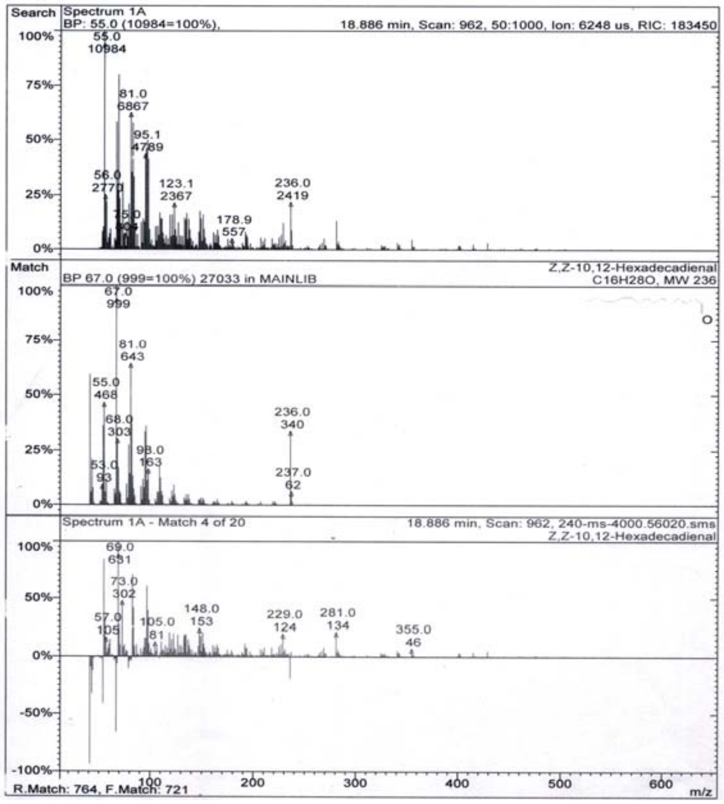

Fig. 3: GC_FID chromatogram of egg extract. Z,Z-10,12-Hexadecadienal, C16H280, MW 236.

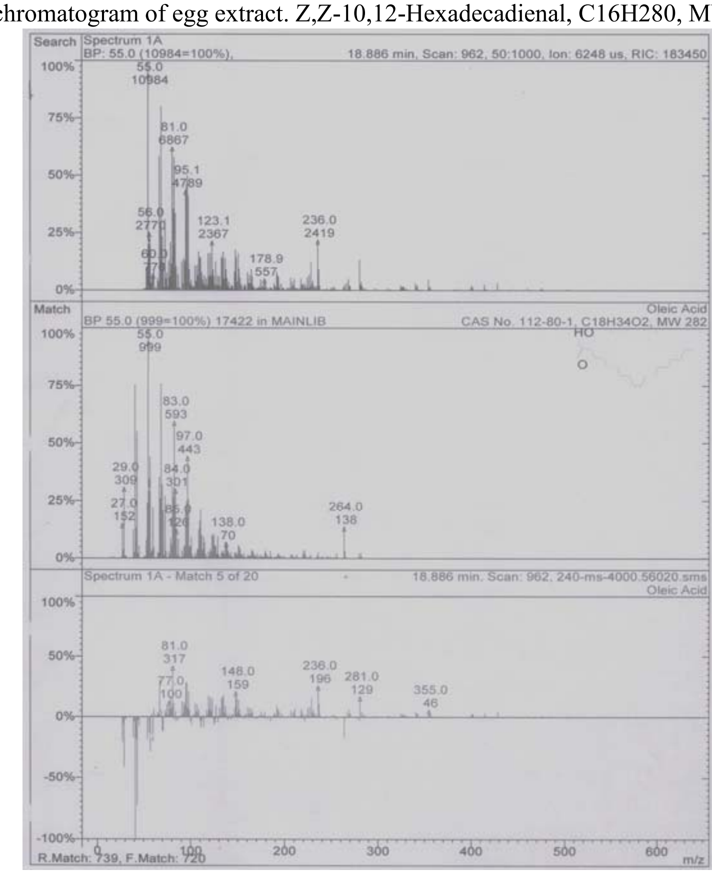

Fig. 4: GC_FID chromatogram of egg extract. Oleic Acid – CAS No. 112-80-1, C18H34O2, MW 282.

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