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Response of the red palm weevil *Rhynchophorus ferrugineus* to its aggregation pheromone under laboratory conditions

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Abstract

Behavioural responses of insects to semiochemicals may be affected by physiological status. The effect of different dosages of aggregation pheromone at 1, 10, 100 and 1000 ng/1 µl hexane on the diurnal responses of virgin and mated male and female *Rhynchophorus ferrugineus* (Olivier) (Coleoptera Dryophthoridae) was studied under laboratory conditions, using a two-choice pitfall static olfactometer. Pheromone concentration, as well as sex and mating status, was found to affect the behavioural responses of the weevils. In both sexes the response to ferrugineol increases with mating. Based on the results, due to searching behaviour of mated females to find the new oviposition sites, they showed stronger response to aggregation pheromone than the unmated ones.

Key words: aggregation pheromone, ferrugineol, olfactometer, laboratory bioassay, palm pest, red palm weevil, *Rhynchophorus ferrugineus*.

Introduction

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera Dryophthoridae) is the major pest of various palms in the Middle East, South and South-East Asia, North Africa and South Europe (Murphy and Briscoe, 1999). Females lay eggs in damaged or wounded palms; upon emergence the larvae burrow into the fresh tissue, and migrate to the bud region and the heart of the crown where they feed for two to four months and eventually kill the host plant (Rajapaksha and Kanagratnam, 1988).

RPW males produce an aggregation pheromone comprising: 4-methyl-5-nonanol (ferrugineol), 4-methyl-5-nonanone (ferrugineone, the less abundant compound) (Hallet *et al*., 1993a) and 3-methyl-4-octanol (phoenicol, the minor compound) (Rochat *et al*., 1993). The most abundant compound, ferrugineol attracts both sexes (Hallet *et al*., 1993b). As with other weevil species (Rochat *et al*., 1991b, Tinzaara *et al*., 2007), the RPW aggregation pheromone is more attractive when it is emitted in the presence of host plant volatiles, and mass trapping with lures combining the two odour sources is used in management programs of this pest (Giblin-Davis *et al*., 1996; Hallet *et al*., 1999). Both virgin and mated individuals of both sexes are captured using this method, but in all field tests the majority of adults caught were mated females (Avand-Faghih, 1996; Avand-Faghih *et al*., 2005; Faleiro *et al*., 2000; 2003; El-Sabay, 2003).

There is a paucity of detailed information on the response of RPW to the aggregation pheromone alone, as it has always been used in combination with host plant odours under field conditions. It is not known whether more mated females are captured because they have a higher sensitivity or whether is the result of them being more active than virgin females and all males. The purpose of this research was determination the response of virgin and mated male and female weevils to different concentrations of the aggregation pheromone under constant laboratory conditions. The hypothesis was that the sex, and mating status change the behavioural response of the weevils, and mating cause them more sensitive.

Materials and methods

Insects

Pupae of *R. ferrugineus* were obtained from date plantation in the Saravan region, Sistan and Baluchistan provinces, Iran. The adults were kept at 29 ± 1 ºC, 60-70 % RH 12:12 L:D photoperiod in individual covered plastic boxes (10 cm Ø × 7 cm high) and were fed with sugarcane stem. To obtain mated individuals for the bioassays, a male and a female were housed together for 72 h (at least one mating was observed) and 48 h later used to testing. Mated individuals were exactly at the same age as the virgins. The individuals in all tests, on average, were 17 days old. Insects were tested once only and then discarded.

Insect bioassays

Weevils were transferred into the testing room (29 ± 1 °C) an hour prior to each bioassay at the beginning of the photophase when the weevils were most active (Avand-Faghih, 2004). To determine the sensitivity of different status of the individuals virgin/mated and male/female the responses of individual were tested at concentrations of 1, 10, 100 and 1000 ng of ferrugineol (>99% purity, E.G.N.O. Chimie, France) in 1 µl of hexane (Merck®, 98% purity) applied to 1 cm^2 pieces of filter paper. The dosages were selected from the results of previous work (Avand-Faghih, 2004; Mura, 2005). Hexane on filter paper was considered as control. The assays were carried out using a two-choice pitfall static

olfactometer (Rochat *et al*., 1991a) composing a circular arena (50 cm Ø × 15 cm high) connected to two glass jars. The Plexiglas floor of the arena was covered with white paper to allow the weevils to walk easily, while the top was covered with a glass plate (60 × 60 cm). A single weevil was released at the centre of the arena, with a filter paper of pheromone in one jar and the solvent control in the other. If a weevil remained motionless for 15 min it was classified as a non-mover and the assay was terminated. Also the assay was terminated when the individual made a choice (entered the jar for >1 min) or after 30 min if it did not choice. There were nine replicates, and in each one a minimum of 4 weevils of each sex and mating status (virgin/mated individuals) were tested for each dosage of pheromone (randomized complete block). Thirty six to 43 of both virgin and mated females, and the same number of both virgin and mated male weevils were bioassayed in each of the different doses. The responses of RPWs also were recorded in a control treatment with two empty jars (clean air). To show the effect of hexane, 1µl of hexane was applied to a filter paper (one jar) and the other, empty jar as control was assayed with 36-43 mated female. After each assay the chamber was washed and cleaned by acetone, rotated by 180° and the test filter papers were replaced.

Table1. The ANOVA testing for the effect of dose, sex and mating status on the number of RPW responses to the aggregation pheromone in two-choice pitfall olfactometer.

Source	Df	F
Dose	3	2.88*
Sex	1	17.52***
Status	1	14.67***
Sex × Status	1	0.01
Dose × Sex	3	0.52
Dose × Status	3	0.33
Dose × Sex × Status	3	0.35
Repetition	8	2.20

Levels of significance: $^{*}P < 0.05$, $^{***}P < 0.001$.

Data Analysis

Normality of data was tested. The differences between the responses of the sex, mating status and doses were analysis of variance (Multy-way ANOVA) and ranked by Tukey Test. A χ^2 analysis was used to compare between number of weevils choosing the treatment and control jars, with the null hypothesis that there would be no differences between stimulus and control.

Results

Very few individuals responded in the absence of pheromone, whether it was a choice between two empty jars or one containing the hexane solvent (df = 1, χ^2 = 0.14; df =1, χ^2 = 0.67, respectively). Pheromone concentration, sex and mating status had significant effect on the level of responses (table 1). However, in all treatments, a high number of individual were active in the arena, but did not make a specific choice (table 2). In the case of aggregation pheromone at 1000 ng dose elicited a significantly greater response that of 1 ng (df = 3, p = 0.039), while the remaining doses gave intermediate responses. Based on Tukey test, the doses were classified in three groups: 1000 ng in group (a), 1 ng in group (b), 10 ng, 100 ng in group (ab). Female weevils responded to the pheromone more than males, and mated individuals more than their virgin counterparts (table 2). The number of tested individuals that explored the test arena was higher for mated than virgin adults but did not differ between sexes (df = 1, p = 0.295).

Table 2. The number of RPW responses to aggregation pheromone in Two-Choice Pitfall Olfactometer after 30 minutes.

Dose	Sex	Mating status	Number of non mover RPWs	Number of mover RPWs		
				No choice	Choice	
					Treatment	Control
1 ng	Male	Virgin	6	29	3 ns	4
		Mated	-	25	8 ns	3
	Female	Virgin	3	28	5 ns	3
		Mated	4	20	13*	3
10 ng	Male	Virgin	4	30	4 ns	2
		Mated	-	26	9*	1
	Female	Virgin	6	20	12*	4
		Mated	3	20	15***	1
100 ng	Male	Virgin	4	29	5 ns	2
		Mated	-	25	11***	-
	Female	Virgin	5	19	15***	2
		Mated	2	17	17***	2
1000 ng	Male	Virgin	7	27	7 ns	2
		Mated	1	23	13***	-
	Female	Virgin	4	23	11*	2
		Mated	4	17	19***	-

Difference from the control (χ^2 test) indicated by: ns = not significant, * $P \leq 0.05$, *** $P \leq 0.001$. Under the null hypothesis to respond to the stimulus and to the control with an equal probability of ½.

Discussion

Unlike the situation described for the weevil *Rhynchophorus palmarum* L., where neither sex nor mating status affected the response to aggregation pheromone (Rochat *et al*., 1991a), RPW females, especially mated ones, showed a significantly more response than males. A detailed comparison of the ecology and reproductive biology of the two species could elucidate possible reasons for such interspecific differences. Differential responses to aggregation pheromones by sex have been reported in a number of other beetles (Dickens, 1986; Graaf *et al*., 2005; White and Chambers, 1989) but differences resulting to mating status was not reported (e.g. Oceallachin and Pyan, 1977; Phillips and Burkholder, 1981; Rochat *et al*., 1991a).

Avand-Faghih (1998) suggested that the female sex bias in field trap catches was due to their higher activity levels. However, in current experiments, sexes did not differ in their level of movement within the arena. So, these results suggest that females may have a higher sensitivity to the aggregation pheromone than males. This possibility is supported by the fact that RPW females have more basioconic sensillae on antenna than males (Avand-Faghih, 2004). The basioconic sensillae in the weevil *R. palmarum*, are known to be sensitive to the aggregation pheromone (Said *et al*., 2003).

RPW females mate on the host where they completed larval development (Avand-Faghih, 1998). Due to larval feeding, the hosts become a poor quality site. Thus, to leave low quality hosts, high sensitivity to the aggregation pheromone may ensure that females find more suitable oviposition sites and potential mates. Clearly, additional research is required to reveal oviposition behaviour and the incidence of polyandry, for better understand the observed differences in male and female responses. Furthermore, such information may help to interpret the field results of the use of the aggregation pheromone/plant volatile combinations for mass trapping programmes, as the current lures are estimated to capture only about 30% of the population under field conditions (Abbas *et al*., 2006). An accurate understanding of these factors and the effects on the behavioural response of the RPW to the aggregation pheromone alone or in combination with host plant volatiles, will contribute to the effective use of traps in the field. Abbas *et al*., (2006) showed the trap baited with pheromone-host plant volatiles caught one third of the population under field conditions. Thus, better understanding of these captured individuals could enhance the efficiency of mass trapping method in management of the RPW.

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