Use of pheromones and other semiochemicals in integrated production
IOBC wprs Bulletin Vol. 25(•) 2002
pp. •-•

Sexual behavior of the *Maladera matrida* male beetle as affected by female cuticular components

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Abstract: The Maladera matrida beetle is a noxious polyphagous pest. Both larvae and adults destroy crops, the former feeding on underground crops such as peanut, sweet potato, potato, carrot, and the latter on flowers and foliage of many plants. In studies conducted in peanut fields it was found that the beetles emerge at sunset from the ground, where they stay all day and most of the night, for a brief (45-90 min) feeding and mating episodes, both taking place at the same time. The males come out first for early feeding, while the females emerge a little later and form a 1:1 (M:F) ratio in aggregations after 15-25 min and most of the active time in the field. In laboratory studies it was demonstrated that females attract males and that the chemicals involved are probably located on the cuticle of mature females. In this paper we describe studies of the behavior of males towards live or frozen females and show that the attracting chemicals may be washed from the cuticle by organic solvents. Both apolar or low polar (hexane or dichloromethane) and polar (methanol) solvents are required for removal of the active components from the cuticle. Washings of females frozen in the morning are not as attractive as those obtained from females at dusk. Preliminary studies show that applying cuticular extracts to washed females restores the sexual activity of males toward females.

Key words: *Maladera matrida*, beetle, female sex pheromone, cuticular components, removing and restoring sexual activity of males.

Introduction

The *Maladera matrida* beetle was first discovered in Israel in 1983 and declared a new species to science (Argaman 1986). The larvae develop in the soil and the adults emerge at sunset from the soil to feed on plants and mate. The males emerge first being followed after a short time by the females, which are probably attracted to volatiles released from the damaged leaves. After 15-25 min a ratio of 1:1 between the sexes is being established. (Harari *et al.*, 1994, Yarden and Shani, 1994, Harari *et al*., 2000). The females and males form aggregates on the plants and stay in these groups for 45-90 min to feed and mate. It was also found that traps baited with volatiles collected from females together with peanut leaves (beetles' food) attract flying beetles to the same extent as do live females together with food (Yarden and Shani, 1994). By the use of gas chromatograph-electroantennodetector (GC-EAD), a bioactive volatile released by the female, identified as (*Z,E*)-_-farnesene, triggered the antennae of both males and females (Yarden *et al.*, 1996). Its activity in both sexes indicates that this compound is probably not the sex pheromone. The high ratio of males to females trapped (80M:20F) in black traps baited with females and food (Falach and Shani, 2000) hints at a long-distance sex pheromone released by the females. Thus we notice two types of behavior, namely, attraction of males to females in traps from long distance at a high proportion of males to females and aggregation of females and males on plants at a ratio of 1:1 between the sexes. The latter aggregations may have a role in the sexual attraction, or they are used for the excitation of the males in a short-range distance. In order to learn more about the sex pheromone complex of the *M. matrida* beetle the cuticular components of the female beetles were washed with organic solvents and their effect on the sexual behavior of the males was studied. The preliminary results are reported here.

Materials and Methods

Rearing of beetles. Third-stage grubs of *M. matrida* were collected in the field and reared individually in 5-ml plastic vials containing humid sand. The grubs were fed with wheat roots. The adult beetles, after being sexed, were reared in sand in groups (up to 100 beetles of the same sex in a 1-liter plastic container) and fed with rose flowers. The containers and vials were kept in an incubator at 16 h light:8 h dark regime at 30:26°C and 60% relative humidity.

Wild beetles for laboratory studies. The study of Harari *et al.* (1997) and our own show that females can mate more than once in their life. The first time takes place at the age of 10-11 days and the second time (under laboratory conditions) is about 14 days later. We therefore used females collected in the field – by traps or manually in the evening – and separated them from males for laboratory studies two weeks later.

Sexing of the beetles. Fast sexing was based on viewing of the last segments of the lower abdomen of adults (see later). For verification sexing was performed by the method described by Gerling and Hefez (1990). Adult beetles were inspected after the pigydium had been opened gently. Females are distinguished by the presence of two elastic chitinic plates located on each side of the pigydium aperture across the abdomen tip. Males are distinguished by a sex organ, which widens into a funnel-like asymmetric chitinic appendage, reminiscent of the stinger of a bee, for grasping the female (Argaman 1986, 1990).

Male behavior in laboratory studies. Males were confronted with frozen-and-thawed females in order to eliminate the effects of non-receptivity and selectivity of females, which drastically reduce the number of incidents of certain modes of sexual behavior. Ten living adult males (marked with white correction fluid) were placed on sand bedding in a glass container (30x30x30 cm) in a dark hood (red light, 3-5 lux) at least five hours before the onset of the experiment. Five females that had been frozen (at -13˚C for 10-15 min or for 1-2 days) and thawed were inserted into the glass container 5 min prior the onset of the experiment. Thawing was done a few minutes before the experiment for females that were frozen in the same evening of the experiment and three hours before the experiment for those frozen one or two days before the experiment. Each experiment was performed in five replications and lasted for 60 min.

Cuticular washings. Adult females were washed with three different solvents of increasing polarities consecutively: n-hexane (non-polar), dichloromethane (low polarity) and methanol (polar). Twelve beetles were immersed twice in 7-8 ml of each solvent for a period of 10 min each time. The washing solvents were combined and evaporated by gentle blowing of N_2 to reduce their volume to 0.07 ml. The beetles were dried in air for 40 min before they were frozen.

Statistical analysis. All results were analyzed by χ^2 test.

Chemicals. n-Hexane, dichloromethane (CH_2Cl_2) and methanol AR grade (Frutarom, Haifa, Israel) were used after checking that no residuals were left after evaporation.

Results and Discussion

External features of the beetle. Emerging beetles are white and within a day change to light brown in color, which turns brown-dark brown within a few days.

Adult female and male beetles can be distinguished by the appearance of the last segments of the lower abdomen (Fig. 1). The last abdominal sternite in the male is round, whereas in the female it is straight. In an earlier study by Ben-Yakir *et al*. (1996) it was found that there are differences between the sexes in the pupae stage, which is sensitive to handling.

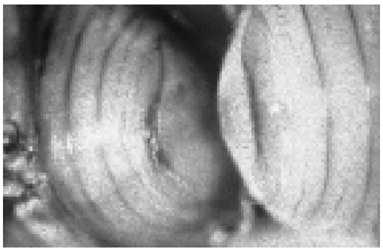

Figure 1

Sexual behavior patterns. Five behavioral patterns of the males were identified: mating, mating attempt, mating disturbance, touching a female, wing fanning. The first three patterns seem to have a direct relation to the sexual behavior of the males toward the females, while the last two patterns are not necessarily related to the sexual ritual and hence were not included in the following report. Some of the behavior patterns are illustrated in a series of photographs (Fig. 2).

Figure 2

The mating posture (mounting of the male on the back of the female) and the copulation with live females lasts for 30-40 min. This behavior was designated “mating”. For mating both excited males and receptive females are required. The case of female refusal to the mounting male was designated “mating attempt”. There is no indication whether this behavior is due to the discrimination of the female against the attempting male or whether it is because the female is not yet receptive. In few cases with live mating females, an attempt of another male to push the mating male from the female was observed. This behavior was designated “mating disturbance”.

Experiments with live pairs of beetles (5 females and 5 males in a glass container) gave low numbers of mating and other sexual activities. Increasing the number of beetles in order to increase the number of mating would make it too complicated to watch and observe the behavior patterns of each male and to record accurately the behavior of all beetles involved. We therefore used another procedure, in which live males were confronted with frozen females. Cooling the female beetles for 10 min at -13^{o}C is enough to kill them without modifying their shape or harming the chemicals on their cuticle. To verify that this procedure does not interfere with the normal behavior patterns, the behavior of males toward live and frozen females was compared (Table 1). The data in Table 1 indicate that the sexual activity of the males is preserved and that the number of incidents in each category of behavior is increased.

Table 1. Sexual behavior of males toward frozen and live females. Values are means ± SE of incidents of each type of behavior per a group of 5 females

Sexual behavior	Frozen females	Live females	χ^2
Mating	7.6±1.1	2.2±1.1	14.9 [a]
Mating attempt	7.2±1.5	1.2±1.1	21.4 [a]
Mating disturbance	5.0±3.0	0.0±0.0	25.0 [a]

[a] Values are significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Mating. The number of mating events per female is much larger with dead females than with live ones, because the former do not refuse, do not have preferences and are inactive. But the chemical cues on the females are still active and attract the males. Moreover, since the length of the copulation is shorter with the dead females (10-15 min) than with live ones, more males mate with the same female, thus the average number of mating per female is increased.

Mating attempt. As for mating, the number of attempts to mate per female is increased due to the inability of the dead females to refuse the males, thus more males have the chance to try to copulate.

Mating disturbance. It is clear that this type of behavior is relatively rare with live females, but is very frequent with dead females. The reason for this difference might be the small chance of a male to push away a mating male; such a behavior may be considered a waste of energy and time for the disturbing male. But with dead females, which do not react, the trials of many males to replace the mating male are more frequent and a large number of events are recorded.

Time of sexual attractiveness. Since the beetles emerge at sunset to feed and mate, it was assumed that this time is the only period of sexual activity of the adults of the day. It was therefore important to verify that the males would not react to females at other times (Table 2). The small number of sexual events toward the females frozen in the morning as compared with those frozen in the evening indicates that no chemical cues were present on their cuticle to excite the males. To test this possibility, we compared the organic-solvents washes of the cuticle of females either frozen in the evening or in the morning (to be published). Preliminary results revealed several differences between the chemicals present on the cuticle at the different times of the day, but no biological studies have yet been conducted with the unique chemicals found in the evening.

Table 2. Sexual behavior of males toward females that were frozen either in the morning or in the evening hours and live females as the control. Values are means ± SE of incidents of each type of behavior per a group of 5 females

Sexual behavior	Frozen females *Morning*	Frozen females Evening	Live females (control)	χ^2 Morning/ control	χ^2 Evening/ control	χ^2 Morning/ evening
Mating	2.6 ± 1.7	9.2 ± 2.6	2.6 ±1.7	0.0	18.5 [a]	18.5 [a]
Mating attempt	0.0 ± 0.0	1.8 ± 1.9	1.4 ±1.1	7.0 [a]	0.3	9.0 [a]
Mating disturbance	0.2 ± 0.4	4.8 ± 3.3	1.6 ±1.5	5.4 [a]	8.0 [a]	21.2 [a]

[a] Values are significantly different according to $\_^2$ tests (df = 1, $P < 0.05$)

Effectiveness of the frozen beetles. The findings presented in Table 2 and the pattern of behavior of the beetles in the field suggested that both the behavioral studies and the washing should be limited to a short period of 30-60 min at sunset. The consequence was that we could not conduct both washing and behavioral observation at the same evening. This meant that we had to keep the frozen beetles for at least 24 hours before conducting the experiment. The results in Table 3 demonstrate that freezing for as long as 48 h did not impair the attractiveness of the females to males.

Table 3. Effect of freezing duration before the experiment on male sexual behavior. Values are means ± SE of incidents of each type of behavior per a group of 5 females

Sexual behavior	Duration of freezing					
	24 h	0 h (control)	χ^2	48 h.	0 h (control)	χ^2
Mating	7.2±0.8	7.6±0.9	0.1 [a]	8.4±1.1	7.4±1.5	0.3 [a]
Mating attempt	2.8±1.9	3.6±1.9	0.5 [a]	3.8±2.6	2.6±3.7	1.1 [a]
Mating disturbance	8.4±6.3	8.4±1.3	0.0 [a]	5.8±1.8	6.4±2.4	0.1 [a]

[a] Values are not significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Repellence by frozen males. To assure that males do not attract males by an aggregation pheromone, we checked the behavior of males toward frozen males. The deterrence of males from frozen males, shown in Table 4, verifies previous observations (Yarden and Shani, 1994) and living males emerging from the soil were repelled by frozen males and immediately returned to their holes in the soil. This behavior may have resulted from the occurrence of repellents on the cuticle of the males or from the absence of female chemical attractants.

Table 4. Sexual behavior of males toward frozen males as compared with that toward frozen females. Values are means ± SE of incidents of each type of behavior per a group of 5 beetles of each sex

Sexual behavior	Frozen males	Frozen females	χ^2
Mating	0.0±0.0	7.4±1.3	37.0 [a]
Mating attempt	0.0±0.0	9.8±4.7	49.0 [a]
Mating disturbance	0.0±0.0	6.0±4.4	36.0 [a]

[a] Values are significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Effect of solvent trickled in frozen females. As mentioned above, the difference in male behavior in the evening and morning hours could stem from the presence of attracting or exciting chemicals on the female cuticle in the evening but not in the morning. Washing of the cuticle by three solvents of increasing polarities (hexane, dichloromethane and methanol) is expected to release most of chemicals adhered to the cuticle. The solvents themselves were found to have no ill effect on the male behavior, as no reduction in the normal sexual behavior of the males was found after the evaporation of 10 _L of each of the three solvents trickled on frozen females (Table 5).

Table 5. Male sexual behavior as affected by solvents trickled on frozen females. Frozen females were trickled with 10 µl of solvent and dried for 3 h before the experiment. Values are means ± SE of incidents of each type of behavior per a group of 5 females

Solvent	Mating			Mating attempt			Mating disturbance		
	Trickled	Not trickled	χ^2	Trickled	Not trickled	χ^2	Trickled	Not trickled	χ^2
n-Hexane	4.2±2.3	5.2±2.6	0.5	0.8±0.8	2.0±1.0	2.5	3.6±4.1	2.0±2.0	2.3
CH_2Cl_2	2.8±1.1	3.2±2.1	0.1	1.0±1.2	0.2±0.4	2.7	1.0±1.2	3.6±3.5	7.3 [a]
Methanol	4.4±2.1	4.2±1.8	0.0	2.4±3.4	2.4±1.8	0.0	3.0±2.0	4.0±1.2	0.7

[a] Values are significantly different according to χ^2 tests (df = 1, $P < 0.05$)

Cuticle washing with organic solvents. The sexual activity of males toward females was totally eliminated by washing the females with three solvents of increased polarities: hexane, dichloromethane and methanol (Table 6). Two solvents only were also effective if one of them was methanol. The nonpolar hexane plus the low polar dichloromethane solvents were incapable of complete removal of the sexual activity. These results indicate that a mixture of both polar and less polar components constitute the short-range sex pheromone of the female *M. matrida* beetle.

Table 6. Sexual activity of males toward frozen females washed with different solvents. Values are means ± SE of incidents of each behavior per a group of 5 females

Washing solvent				Mating		Mating attempt		Mating disturbance	
Hexane	CH_2Cl_2	Methanol		Washed females	Unwashed females	Washed females	Unwashed females	Washed females	Unwashed females
+	-	-		6.6±2.9	5.0±0.7	4.4±1.8	2.8±1.6	3.4±3.2	6.2±3.8
-	+	-		5.4±1.7	7.8±2.0	1.0±1.2	1.8±0.4	3.8±2.6	6.4±1.5
-	-	+	*	3.2±1.3	6.8±3.8	1.2±0.8	2.8±0.4	2.0±2.0	5.6±5.5
+	+	-	*	2.4±1.1	6.0±1.0	1.4±1.3	1.8±1.1	0.8±1.3	3.0±1.9
+	-	+	*	0.4±0.5	6.8±1.1	0.0±0.0	1.0±1.0	0.4±0.9	7.2±0.4
-	+	+	*	0.4±0.5	6.8±0.4	0.0±0.0	0.6±0.5	0.0±0.0	6.8±1.6
+	+	+	*	0.0±0.0	7.8±1.3	0.6±1.3	9.8±5.5	0.0±0.0	8.4±3.5

[a] Values are significantly different according to χ^2 tests for all types of behavior (P = 0.05).

Acknowledgements

We thank Mr. Ido Yosha for taking the photographs of the beetles and Ms. Dorot Imber for editing the manuscript.

References

Argaman, Q. 1986: *Maladera matrida,* a new Scarabaeidae in Israel. Shapirit 4:40-46 (In Hebrew), 67-68 (In English).

Argaman, Q. 1990: Redescription of *Maladera matrida* (Coleoptera, Scarabaeidae, Melolonthinae). Isr. J. Entomol. 24: 21-27.

Ben-Yakir, D. Koren, L. & Harari, A. 1996: Biometric discrimination of the larval instars and sexes of *Maladera matrida* (Coleoptera, Scarabaeidae, Melolonthinae). Isr. J. Entomol. 30: 47-52.

Falach, L. & Shani, A. 2000: Trapping efficiency and sex ratio of *Maladera matrida* beetles in yellow and black traps. J. Chem. Ecol. 26: 2619-2624.

Gerling, D. & Hefez, A. 1990: The ecology of the *Maladera* beetle. *In* R&D for Pest Management of *Maladera*. Israel Ministry of Agriculture (in Hebrew).

Harari, A.R., Ben-Yakir, D. & Rosen, D. 1994: Mechanism of aggregation behavior in *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). J. Chem. Ecol. 20: 361-371.

Harari, A. R., Ben-Yakir, D., Chen, M. & Rosen, D. 1997: Life- and fertility- tables of *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). Environ. Entomol. 26: 1073-1078.

Harari, A. R., Ben-Yakir, D. & Rosen, D. 2000: Male pioneering as a mating strategy: the case of the beetle *Maladera matrida*. Ecol. Entomol. 25:1-8.

Yarden, G. & Shani, A. 1994: Evidence for volatile chemical attractants in the beetle *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). J. Chem. Ecol. 20: 2673-2685.

Yarden, G., Shani, A. & Leal, W.S. 1996: (*Z*,*E*)-_-Farnesene _ an electroantennogram-active component of *Maladera matrida* volatiles. Bioorg. Med. Chem. 4: 283-287.