

MECHANISM OF AGGREGATION BEHAVIOR IN *Maladera matrida* ARGAMAN (COLEOPTERA: SCARABAEIDAE)

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(Received May 24, 1993; accepted September 28, 1993)

Abstract—Adult *Maladera matrida* Argaman (Coleoptera: Scarabaeidae: Melolonthinae) males emerge from soil for an active period at dusk, a few minutes before the females. Adults are found during most of the active hours on the foliage in aggregations composed of an equal sex ratio. The mechanism of aggregation behavior of *M. matrida* beetles was studied in a Y-shaped olfactometer. No evidence was found for the existence of an aggregation pheromone released either by males or by females, but behavior tests indicate that adult *M. matrida* beetles, males as well as females, are attracted to volatiles of an injured host plant. The following scenario is suggested: Males emerge daily from soil at dusk, a few minutes before the females, and immediately start feeding. Additional males are attracted to the injured host's volatiles and form aggregations. When females emerge from soil, the attractant volatiles are concentrated in spots, and the females join the aggregations, forming an equal sex ratio.

Key Words—Coleoptera, Scarabaeidae, *Maladera matrida*, aggregation, plant volatiles, olfactometer, attractant, behavior

INTRODUCTION

Maladera matrida Argaman (Coleoptera: Scarabaeidae, Melolonthinae), a polyphagous pest, was first detected in Israel in 1983 (Klein and Chen, 1983), was described as a new species by Argaman (1986, 1990) and has lately been reported

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also from Saudi Arabia (Anonymous, 1991). Adult beetles are found feeding on foliage in irrigated fields, orchards, ornamental home gardens, and parks; the grubs develop in soil around root systems, causing damage mainly to root crops such as potatoes, sweet potatoes, and peanuts. Adult *M. matrida* beetles emerge from soil at dusk to feed and mate (Gol'berg et al., 1989).

Our preliminary field observations have indicated that emergence of males from soil for the active period at dusk always precedes that of females by a few minutes. Among scarabaeids, seasonal protandry was reported in various June beetles (Kard and Haim, 1990), whereas daily preemergence of males was seen in two species of *Cyclocephala* (Potter, 1980).

Preliminary observations also indicate that *M. matrida* adults aggregate on foliage in an equal sex ratio. In other scarabaeids, aggregations have been described in the Japanese beetle, *Popillia japonica* Newman (Ladd, 1970), and in two species of *Cyclocephala* (Potter, 1980), in which one female, or a mating couple, were surrounded by several males. Aggregations of equal sex ratio were reported in the green June beetle, *Cotinis nitida* (L.) (Domek and Johnson, 1988, 1990) and in other Coleoptera, e.g., the nitidulids *Carpophilus hemipterus* (L.) (Bartelt et al., 1990a), *C. lugubris* Murray (Lin et al., 1992), and *C. freemani* Dobson (Bartelt et al., 1990b); the chrysomelid, *Phyllotreta cruciferae* (Goeze) (Peng and Weiss, 1992); the scolytid, *Dendroctonus pseudotsugae* Hopkins (Knope and Pitman, 1972), and others.

Protandry may evolve under natural selection by conferring an advantage upon males that emerge early and maximize the number of females located during the time of activity (Potter, 1980), whereas the advantages of aggregation may lie in providing protection and an opportunity to locate food or mates (Bailey, 1991). A combination of protandry and aggregation of equal sex ratio may provide several of these advantages.

Several possibilities have been described for pheromone-mediated aggregation behavior in insects: (1) aggregation pheromone produced and released by insects (Phillips and Burkholder, 1981); (2) synergism between host plant volatiles and aggregation pheromone produced by insects (Bartelt et al., 1990a, b; Dowd and Bartelt, 1991; Lin et al., 1992); (3) contact with host plant required for production of aggregation pheromone by insects (Peng and Weiss, 1992); (4) aggregation pheromone released by insects while feeding (Domek and Johnson, 1988); (5) pheromone produced by insects as a derivative of consumed host plant materials (Eisner and Meinwald, 1987); (6) attractant produced by an interaction between microorganisms and feeding insects (Domek and Johnson, 1990); and (7) attractant produced by microorganisms developing upon damaged host plant (Dolinski and Loschiavo, 1973).

The goal of the present study was to elucidate the mechanism of aggregation behavior in *Maladera matrida* beetles.

METHODS AND MATERIALS

Beetles. *M. matrida* was reared in a climatic room at 27°C, 50–70% relative humidity, and 16:8 hr light–dark. The beetles were sexed at the pupal stage, because it is difficult to distinguish adult males from females. Fifteen to 20 beetles of one sex were placed in plastic boxes, 10 × 10 × 25 cm, containing humid sand and *Duranta repens* L. leaves as a food substrate. Beetles used in this study, either as attractant sources or as subjects attracted to a volatile source, were 10–35 days old and were starved for 48 hr prior to the experiments.

Bioassay Setup. All experiments were held in an olfactometer (Figure 1), composed of a vacuum pump connected, through a flowmeter, to a Y-shaped glass tube (stem 2 × 14 cm, each arm 2 × 10 cm). Each of the two arms led to a spherical glass trap, followed by a glass bulb containing a volatile source. Another flowmeter was connected by Tygon hoses to the glass bulb and to a bottle of activated charcoal. The assay beetles were released individually at the starting point and would walk or fly against the air current toward the attractant source, falling into the spherical trap, from which they would be removed to avoid interruption of the system. The airflow along the stem tube and in both arms was 244 ml/min. A smoke test demonstrated a laminar airflow in the stem tube and in both arms.

Because the beetles are active from sunset on for 2.5 hr (Gol'berg et al., 1989), the experiments began when the lights were switched off and lasted for 2.5 hr under red light (located above the olfactometer), which in preliminary observations appeared to have no effect on the behavior of the beetles.

Because of the short daily activity period of the beetles, only one set of experiments was held in a given day, and the behavior of only one sex, either males or females, of *M. matrida* was tested toward a given attractant. Every set of experiments was repeated five times on different days, with 20–55 subjects tested in a given day. At the end of an experiment set, the olfactometer was rinsed with analytical grade acetone.

Treatments. The following sets of experiments were conducted in order to determine whether *M. matrida* beetles are attracted to one or both sexes of their own species, to an interaction between their own species and a host plant, or

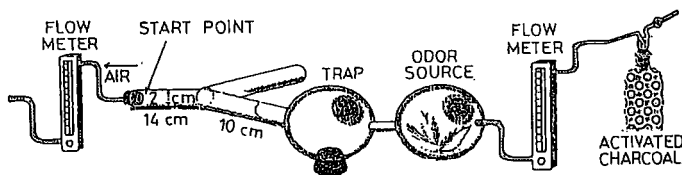


FIG. 1. The olfactometer.

to any insect-host interaction. In any given experiment, different sources of attractant were placed in the bulb of each arm of the olfactometer.

1. Seven *M. matrida* males vs. seven *M. matrida* females, no food; a total of 121 males and 158 females were individually tested in this experiment against these attractant sources.

2a. Blank (no volatiles) vs. seven *M. matrida* males feeding on five leaves of *D. repens*; 118 males and 136 females of *M. matrida* were tested.

2b. Blank (no volatiles) vs. seven *M. matrida* females feeding on five leaves of *D. repens*; 129 males and 113 females were tested.

3a. Seven nonfeeding *M. matrida* males vs. seven *M. matrida* males feeding on five leaves of *D. repens*; 130 males and 107 females were tested.

3b. Seven nonfeeding *M. matrida* females vs. seven *M. matrida* females feeding on five leaves of *D. repens*; 131 males and 122 females were tested.

4. Seven *M. matrida* females feeding on five leaves of *D. repens* vs. seven *M. matrida* males feeding on five leaves of *D. repens*; 140 males and 132 females were tested.

5. Five leaves of *D. repens* vs. seven *M. matrida* males feeding on five leaves of *D. repens*; 111 males and 126 females were tested.

6. Four fifth-instar nymphs of the desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae), feeding on five leaves of *D. repens* vs. seven *M. matrida* males feeding on five leaves of *D. repens*; 148 males and 171 females were tested.

7. Four nonfeeding fifth-instar nymphs of *S. gregaria* vs. four *S. gregaria* nymphs feeding on five leaves of *D. repens*; 119 males and 137 females of *M. matrida* beetles were tested.

Only active beetles (found above soil) were used as an attractant source and as subjects in the behavior tests. Beetles that chose a direction and fell into the trap (Figure 1) within 5 min were recorded as a "response," while those that did not choose a certain arm within 5 min or moved in the opposite direction (i.e., with the air current) were scored as "no response." Every five runs, the olfactometer was turned 180° in order to change the direction of the volatile sources, to avoid bias from uncontrolled directional factors.

Statistical Analysis. The results were analyzed as two-choice data. Choices of any arm were pooled as a response category, versus the no-response category. The nonresponsive individuals (less than 10% in any experiment) were omitted from further calculations, and the choice of one arm was compared to the choice of the other. G statistics (Sokal and Rohlf, 1969) were used for replicated goodness-of-fit tests. The heterogeneity of the replicates for each experiment was tested first and was found to be nonsignificant ($P \gg 0.1$), indicating that replicates of the same experiment did not differ significantly between different days. A second goodness-of-fit test was performed for the pooled data. Both

tests were used to compute the significance of deviation from expectation (1:1) for each experiment ($\alpha = 5\%$).

RESULTS AND DISCUSSION

In preliminary experiments, seven *M. matrida* males without food were placed in one arm of the olfactometer vs. no volatile source in the other. Male and female subjects ($N = 78$ and 54 , respectively) did not differ significantly ($P < 0.05$) in their choice of arm. When seven *M. matrida* females without food were placed in one arm of the olfactometer vs. no volatiles in the other, females ($N = 66$) and males ($N = 76$) exhibited similar nonpreference toward either arm. These preliminary results indicated that *M. matrida* beetles, males as well as females, are not attracted to volatiles emitted by conspecific beetles per se. Nevertheless, the first set of experiments was carried out to test the possibility of differences in attraction to the different sexes.

1. *Nonfeeding M. matrida females vs. nonfeeding M. matrida males.* Male as well as female subjects did not differ in their response toward either males or females ($P \gg 0.05$) (Figure 2A and B). Therefore, the possibility of an aggregation pheromone, produced and released by either one of the sexes in the absence of food (Phillips and Burkholder, 1981), could be excluded. Inasmuch as beetles serving as an attractant source were fed until 48 hr prior to testing, the possibility of a pheromone produced by the insects as a derivative of consumed host plant materials (Eisner and Meinwald, 1987) was excluded as well.

2a. *Blank vs. M. matrida males feeding on D. repens leaves.* Male and female subjects did not differ significantly in their choice of arm. Both males and females were significantly more attracted to the feeding males ($73.3 \pm 2.1\%$ males and $69.7 \pm 1.9\%$ females of response category beetles, $P \ll 0.05$) than to the blank (Figure 3A).

2b. *Blank vs. M. matrida females feeding on D. repens leaves.* There was

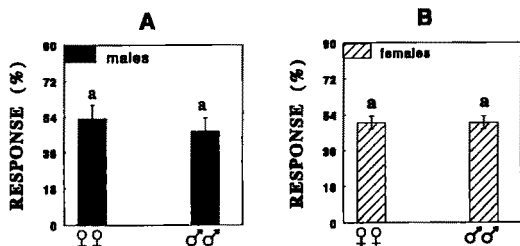


FIG. 2. Nonfeeding *Maladera matrida* females vs. nonfeeding *M. matrida* males. (A) Testing the behavior of 121 *M. matrida* males ($P \gg 0.05$). (B) Testing the behavior of 158 *M. matrida* females ($P \gg 0.05$).

no significant difference between the choice of arm by either male or female subjects. Most of the responding beetles were attracted to the feeding females ($70.6 \pm 5.0\%$ and $68.2 \pm 4.2\%$, respectively) (Figure 3B).

3a. *Nonfeeding M. matrida males vs. M. matrida males feeding on D. repens leaves.* Male as well as female subjects were significantly more attracted to the feeding males ($67.0 \pm 3.1\%$ and $69.4 \pm 6.5\%$, respectively) (Figure 4A).

3b. *Nonfeeding M. matrida females vs. M. matrida females feeding on D. repens leaves.* Male as well as female subjects were significantly more attracted to the feeding females ($66.6 \pm 5.1\%$ and $60.3 \pm 3.2\%$, respectively) (Figure 4B).

These results suggested that *M. matrida* beetles are attracted to feeding beetles of both sexes, rather than to beetles alone. Therefore, the following set of experiments was conducted in an attempt to distinguish between the attraction of feeding males and feeding females.



FIG. 3. (A) Blank vs. males of *M. matrida* feeding on *Duranta repens* leaves. Testing the behavior of 118 males and 136 females of *M. matrida* ($P \ll 0.05$). (B) Blank vs. females of *M. matrida* feeding on *D. repens* leaves. Testing the behavior of 129 males and 113 females of *M. matrida*. ($P \ll 0.05$).



FIG. 4. (A) Non-feeding *M. matrida* males vs. *M. matrida* males feeding on *D. repens* leaves. Testing the behavior of 130 males and 107 females of *M. matrida* ($P \ll 0.05$). (B) Non-feeding *M. matrida* females vs. *M. matrida* females feeding on *D. repens* leaves. Testing the behavior of 131 males and 122 females of *M. matrida* ($P \ll 0.05$).

4. *M. matrida* females feeding on *D. repens* leaves vs. *M. matrida* males feeding on *D. repens* leaves. Female subjects did not exhibit significant preference to either feeding females or feeding males ($P \gg 0.05$); however, male subjects were significantly more attracted to feeding females ($63.3 \pm 2.4\%$) (Figure 5). The latter finding led us to the assumption that a sex pheromone released by the females might be involved in male attraction. However, the fact that males did not exhibit significant attraction toward nonfeeding females may suggest that a sex pheromone is released by the females while feeding on the host plant. This assumption is supported by field observations (G. Yarden, personal communication). Since we were looking for an aggregation factor, which should be attractive to both males and females (Alcock, 1982), females were excluded as an attractant source from subsequent tests, to avoid influence of a female sex pheromone on male behavior.

The next test was performed in order to distinguish the influence of volatiles emitted by the host plant from those of the feeding males.

5. *D. repens* leaves alone vs. *M. matrida* males feeding on *D. repens* leaves. Male as well as female subjects were significantly more attracted to the feeding males ($69.2 \pm 1.6\%$ and $64.9 \pm 5.0\%$, respectively) (Figure 6).

The results obtained so far could be explained by the presence of an attractive factor resulting from the feeding action of adult *M. matrida* beetles of both sexes, a mechanism suggested for aggregation-inducing behavior by Domek and Johnson (1988). In order to test this hypothesis and to distinguish volatiles emitted by the feeding beetles from those emitted by a damaged host plant, the feeding action of the beetles was mimicked by placing fifth-instar nymphs of *Schistocerca gregaria* (Forskål) on the host plant.

6. *S. gregaria* nymphs feeding on *D. repens* leaves vs. *M. matrida* males feeding on *D. repens* leaves. Male as well as female subjects did not favor feeding beetles over feeding locusts (Figure 7). The possibility that an aggregation pheromone is produced upon contact between host plant and beetle (Peng

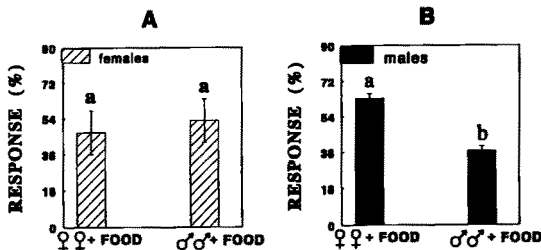


FIG. 5. *M. matrida* females feeding on *D. repens* leaves vs. *M. matrida* males feeding on *D. repens* leaves. (A) Testing the behavior of 132 females of *M. matrida* ($P \gg 0.05$). (B) Testing the behavior of 140 males of *M. matrida* ($P \ll 0.05$).

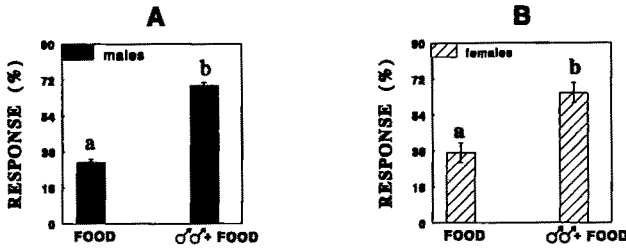


FIG. 6. Intact leaves of *D. repens* vs. *M. matrida* males feeding on *D. repens* leaves. (A) Testing the behavior of 111 *M. matrida* males ($P \ll 0.05$). (B) Testing the behavior of 126 *M. matrida* females ($P \ll 0.05$).

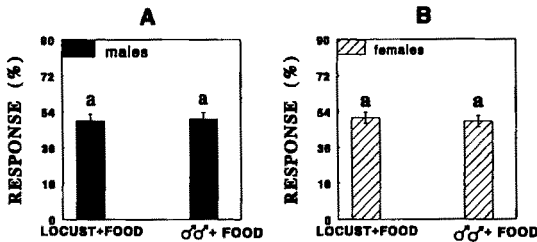


FIG. 7. *S. gregaria* nymphs feeding on *D. repens* leaves vs. *M. matrida* males feeding on *D. repens* leaves. (A) Testing the behavior of 148 *M. matrida* males ($P \gg 0.05$). (B) Testing the behavior of 171 *M. matrida* females ($P \gg 0.05$).

and Weiss, 1992) or that of synergism between food volatiles and an aggregation pheromone (Bartelt et al., 1990a,b; Lin et al., 1992) were therefore discounted.

The following experiment was carried out to ascertain that *M. matrida* was not attracted to the locusts themselves.

7. *Nonfeeding S. gregaria nymphs vs. S. gregaria nymphs feeding on D. repens leaves.* Males and females of *M. matrida* were significantly more attracted to feeding *S. gregaria* nymphs ($65.4 \pm 3.1\%$ and $63.7 \pm 5.9\%$, respectively) (Figure 8). In field observations, aggregations of *M. Matrida* adults were seen close to feeding larvae of *Spodoptera littoralis* (Buisduval) (Lepidoptera: Noctuidae). Inasmuch as the beetles exhibited a similar degree of attraction to feeding insects belonging to three different orders, it may be assumed that they are not attracted to volatiles emitted by the insects while feeding, but rather to the volatiles of the injured host plant itself. Attraction to injured (cracked) host plant was reported by Trematerra and Giragenti (1989) and by Walgenbach et al. (1987). The possibility that microorganisms in the food were involved in inducing aggregation behavior (Dolinski and Loschiavo, 1973) in *M. matrida* was not tested.

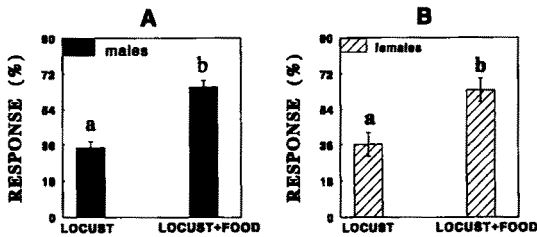


FIG. 8. Nonfeeding *S. gregaria* nymphs vs. *S. gregaria* nymphs feeding on *D. repens* leaves. (A) Testing the behavior of 119 *M. matrida* males ($P \ll 0.05$). (B) Testing the behavior of 137 *M. matrida* females ($P \ll 0.05$).

Our observations and experiments indicate the following scenario: Males emerge daily from soil at dusk, a few minutes before females, and immediately start feeding. Additional males are attracted to the injured host plant volatiles and form aggregations. When females emerge from the soil, the attractant volatiles are concentrated in spots, and the females join the aggregations, forming an equal sex ratio.

M. matrida is a polyphagous pest, and since the same aggregation pattern was seen on numerous host plants (Gol'berg et al., 1989; Harari et al., in preparation), it may be assumed that the volatiles emitted from the injured plant are general odor components such as leaf alcohols, aldehydes, and derivatives. Such green odor components are known to affect the behavior of many insects, e.g., the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae); the cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae); the carrot rust fly, *Psila rosae* (Fabricius) (Diptera: Psilidae), and others (Visser, 1986).

The type of aggregation behavior described herein may either be an adaptive behavior, enabling the beetle to respond to volatiles emitted by an injured host plant to ensure food availability (Shorey, 1973) and mate location (Bailey, 1991) in a patchy environment, or may play a role in sexual selection. Preliminary field observations have indicated that there may be a sexual advantage to aggregation, as most of the copulations in *M. matrida* populations seem to occur within the aggregations, but these observations have to be corroborated.

Acknowledgments—We thank Professors A. Hefetz and D. Gerling, Tel-Aviv University, for providing good suggestions and technical support throughout the study and for helpful comments on an early draft of this manuscript, and Dr. H. Voet, Faculty of Agriculture, The Hebrew University, Rehovot, for advice on statistical analysis. This study was partly supported by the Fund for Encouragement of Research of the General Federation of Labor in Israel, and by the Chief Scientist of the Ministry of Agriculture of Israel.

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