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Effect of Time of Day and Temperature on Sex Pheromone Production and Perception by the Rust-red Flour Beetle, *Tribolium castaneum* (Herbst)

Maissa M. Abdel-Kader [1], R.M. Abdu and M.A. Hussien [1]

Department of Zoology, Faculty of Science, University of Qatar, Doha

ABSTRACT. Sex pheromone production by virgin females of *T. castaneum* (Herbst) was greatly affected by the time of the day. It started at low concentration in the early morning and then increased until it reached its maximum at 1500 hr. It decreased again in the late afternoon and reached its lowest titer at night. Male responsiveness to sex pheromone extracts followed almost a similar trend, with a maximum response between 1300 and 1500 hr.

Sex pheromone production also varied at the different temperature at which the beetles were kept. Temperatures between 30 and 40°C appeared optimum for sex pheromone production. None of the temperatures tested (15-40°C) inhibited pheromone production.

Females of many insect species produce and release sex pheromones that cause a series of mating responses by adult males (Rogoff *et al.* 1964, Burkholder and Dicke 1966, Kuwahara *et al.* 1968, Barrott 1974, and Abdel-Kader and Barak 1979).

The production and the amount of pheromone seem to be closely related to the microclimatic factors of the environment. Little information is available concerning the effect of time of day on sex pheromone production and perception in certain insects (Nagata *et al.* 1972, Vick *et al.* 1973, Chandhury and Ball 1974, Barak and Burkholder 1977, Abdel-Kader and Barak 1979, and Sharma and Deora 1980). No information, on the other hand, is available on the effect of temperature on sex pheromone production by insects.

1 Present address: Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt.

In the previous paper (Abdu *et al.* 1985), we have shown that the production of sex pheromone by female beetles of *Tribolium castaneum* (Herbst) and its perception by males were highly affected by exposure of either pupae or adults to different doses of gamma radiation.

The aim of the present study was to investigate the effect of the time of day and temperature on the production and perception of sex pheromone by the rust-red flour beetle, *T. castaneum*.

Material and Methods

Insect Culture

T. castaneum beetles used in the present study were obtained from a stock culture raised at 30 ± 1°C and 70 ± 10% R.H. The insects were removed from the culture at pupal stage, segregated according to their sex and held separately in petri dishes. The pupae of each sex were kept in a separate incubator adjusted to the same conditions mentioned above until emergence.

Pheromone Extraction and Bioassay

Sexual pheromone was obtained by extracting from the female beetles using diethyl ether, according to the method described by Abdel-Kader and Hussien (1984). The olfactometer and bioassay tests used by Burkholder (1970) and modified by Abdel-Kader and Hussien (1984) were used for testing the responsiveness of males to the female sex pheromone extracts.

Effect of Time of Day

Virgin females (2-5 days old) were extracted with diethyl ether at 2-hour intervals for a 24-hour period beginning at 0500 hr. The female extracts were tested against 2-5 day old unmated males. One hundred males -in 10 replicates- were tested for each female extract [0.3 female equivalent (F.E.) per 10] at each interval. The response of males at each 2-hour interval throughout the photophase was also tested against virgin female extract. The bioassays were conducted at 29-30°C and 30-40% R.H.

Effect of Temperature

Virgin females [0-2 days old] raised at 30°C were incubated at different temperatures (15, 25, 30 and 40°C). When 4-6 days old, their pheromone was extracted. The extracts [0.3 F.E. per 10] were bioassayed with 4-6 day old males raised at 30°C as stated above.

Tests for male response to pheromone extracts were carried out at 1500 hr at 30-31°C and 36-42% R.H.

Results and Discussion

Effect of Time of Day

a. On Sex Pheromone Production

Taking the percentage of male response to sex pheromone as a criterion for assessing sex pheromone production by virgin females, extracted at 2-hour intervals during the photophase and scotophase (Fig. 1), it was noticed that:

(i) When the females were extracted early in the morning (0500 hr) the extract stimulated only about 50% of the males, indicating a somewhat low level of pheromone production by the female.

(ii) Females extracted two hr later (0700 hr) produced a higher amount of sex pheromone. The percentage of male responsiveness significantly increased ($Z = 2.29$).

(iii) Pheromone production reached a peak at 0900 hr and remained at the same level until 1300 hr.

(iv) The peak of male responsiveness was reached at 1500 hr, indicating the highest sex pheromone titer produced at that time of the day.

(v) Male response to female extracts taken late in the afternoon (1700 hr) decreased significantly ($Z = 3.25$). It continued to decrease towards evening (1900 hr), indicating a progressive reduction in sex pheromone production.

(vi) Females extracted during the scotophase (2100-0300 hr) produced lower but constant amounts of sex pheromone. The percentage of male response was less than 50%.

b. Effect of the time of day on Male Responsiveness to Sex Pheromone

Results on responsiveness of 2-5 day old males to sex pheromone extract of virgin females, at 2-hour intervals during the photophase (Fig. 2) indicated that:

(i) The male response was at a low level in the early morning (0500 hr). It then started to increase progressively towards noon.

(ii) The maximum response of males to sex pheromone was reached at 1300 hr and continued until 1500 hr.

(iii) The male response decreased significantly ($Z = 1.96$) towards the evening and reached its lowest level at 1900 hr.

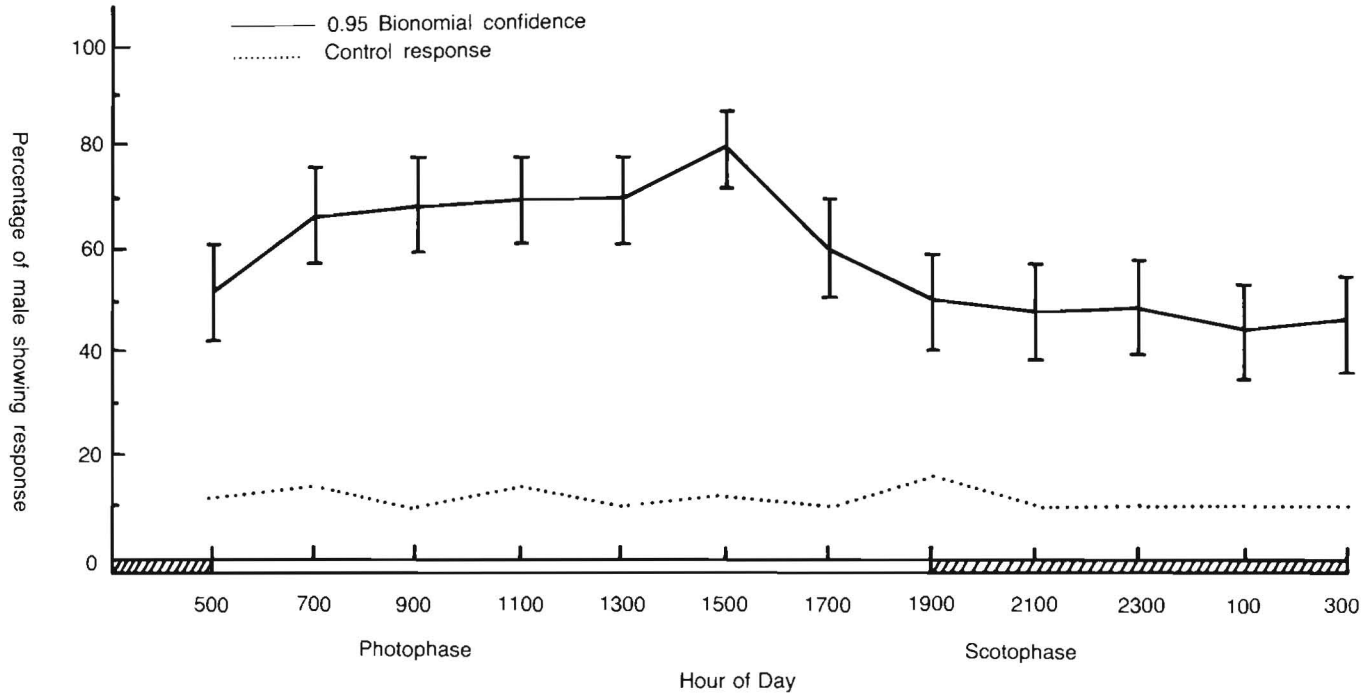

Fig. 1. Response to *T. castaneum* males to sex pheromone extracts of females made at 2 hr intervals during the photophase and scotophase

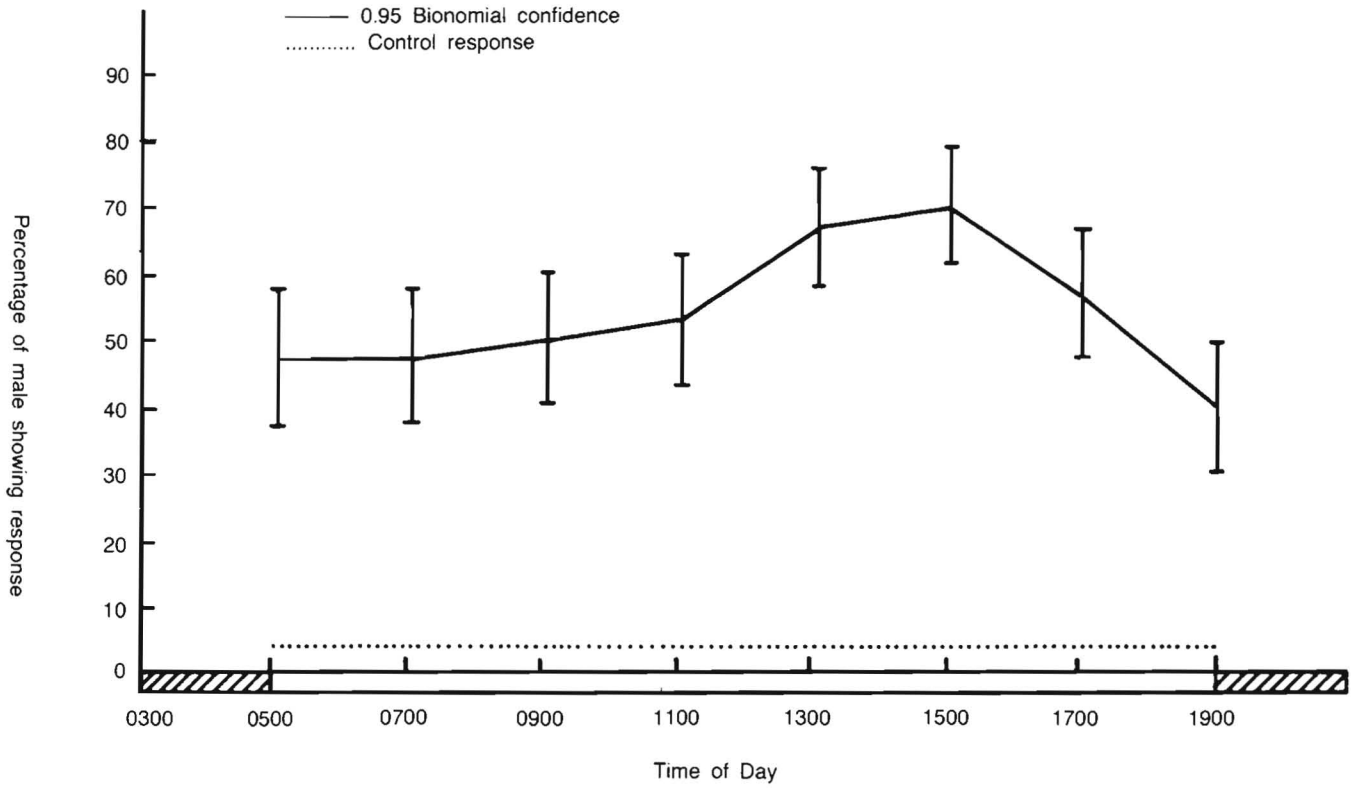

Fig. 2. Response of *T. castaneum* (2-5 days old) males at 2 hour interval over the photophase to a single sex pheromone extract of virgin females

It is evident that sex pheromone production by the rust-red flour beetle, *T. castaneum*, follows a diurnal rhythm with a peak at approximately 1500 hr. This trend is also evident for male responsiveness to sex pheromone extract of virgin females, with a peak response between 1300-1500 hr. Such rhythms in sex pheromone production and responsiveness appear to be common among other insects (Shorey 1974).

Apparent female circadian rhythmicity of sex pheromone production has also been demonstrated in several species of the genera, *Attagenus*, *Anthrenus* and *Trogoderma* (Burkholder *et al.* 1974, Barak and Burkholder 1977, 1978, and Ma and Burkholder 1978), and in *Dermestis maculatus* (Abdel-Kader and Barak 1979).

If the mating activity coincides with increased sex pheromone production, as in the female moth, *Trichoplusia ni* (Hubner) (Sower *et al.* 1970), it would be expected that mating of *T. castaneum* (Herbst) takes place during the day with a peak of mating activity at 1500 hr. This was confirmed by the activity of male beetles, which followed a similar diurnal rhythm as pheromone production in the females.

The information obtained may help in applying sex pheromone for the control of this stored grain insect pest, using the technique commonly known as "Male Confusion". This is now one of the most promising techniques for the control of insect pests with pheromones (Birch and Haynes 1982).

Effect of Temperature

The temperature at which the insect is kept, actively affected the amount of sex pheromone production by female beetles of *T. castaneum* (Table 1). According to the percentage of male response, the amount of sex pheromone production was

Table 1. Response of male *Tribolium castaneum* beetles to sex pheromone extracts of females subjected to different temperatures. Bioassays were conducted at 30-31°C and 36-42% R.H.

Female Rearing Temp. (°C)	Percentage of males showing response			
	Experiment	Control	Corrected experiment	Binomial limits
15	54	8	50.00	44.23 - 63.77
25	64	12	59.09	54.59 - 73.41
30	80	12	77.27	72.16 - 87.84
40	73	10	70.00	64.30 - 81.70

significantly low at low temperature (15°C). It increased gradually at higher temperatures and reached its maximum titer at 30°C. It started to decrease again with increasing temperature up to 40°C, but the difference was not significant (Z = 1.17). None of the temperatures tested, however, inhibited pheromone production by *T. castaneum*.

The results clearly indicate that the optimum temperature for sex pheromone production by virgin females of *T. castaneum* occurs between 30 and 40°C, below or above which pheromone production decreases. The results also indicate that *T. castaneum* beetles are sexually active throughout a wide range of temperature. This activity, however, differs according to the temperatures at which the beetles are kept, and higher temperatures are more favourable than lower ones.

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تأثير فترات اليوم ودرجات الحرارة على الإنتاج والاستجابة للفيرومون الجنسي في خنفساء الدقيق الصدئية

مائسة محمد عبدالقادر[1] و رضا محمد عبده و محمد عادل حسين[1]

قسم علم الحيوان ـ كلية العلوم ـ جامعة قطر ـ الدوحة ـ قطر

لقد كان لفترات اليوم تأثيراً واضحاً على معدل إنتاجية فيرومون الجنسي لإناث خنفساء الدقيق الصدئية وقد بدأت بإنتاج تركيزات قليلة في الصباح الباكر ثم أزدادت الإنتاجية إلى أن وصلت مداها في الساعة الثالثة بعد الظهر وتناقصت مرة أخرى بعد هذه الفترة من اليوم إلى أن وصلت أدنى إنتاج في المساء.

وتأثرت كذلك استجابة ذكور الخنافس للفيرومونات الجنسية التي تنتجها الإناث بفترات اليوم المختلفة حيث وصل الحد الأقصى من الاستجابة في الفترة ما بين الساعة الواحدة والساعة الثالثة بعد الظهر.

ولقد كان لدرجات الحرارة تأثيراً على إنتاجية هذا الفيرومون حيث كان أكبر إنتاجية للفيرومون عند درجة الحرارة ٣٠ ـ ٤٠°م. ولكن لم يثبط إنتاج هذا الفيرومون عند أي درجة من درجات الحرارة المستخدمة (١٥ ـ ٤٠°م).