A PHOTOPERIODIC MATING RHYTHM FOR THE ANGOUMOIS GRAIN MOTH,
SITOTROGA CEREALELLA (LEPIDOPTERA: GELECHIIDAE), AND THE ROLE OF
MALE RESPONSE TO THE FEMALE SEX PHEROMONE IN ITS DETERMINATION

by 6408

JOHN C. GUERBER

B. S., Albright College, 1969

———————

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1971

Approved by:

Major Professor

TABLE OF CONTENTS

INTRODUCTION

The ubiquity of behavioral rhythms and the importance of sex
pheromones for successful reproduction of insects have enhanced interest
in such phenomena. Undesirable effects created by the continued use of
chemical insecticides have led to a renaissance of interest in the
behavior and ecology of insects, particularly of the more economically
important species.

Attempts in this laboratory to lure male Angoumois grain moths,
Sitotroga cerealella (Olivier), from females using an air stream carrying
the female sex pheromone proved unsuccessful. In those studies no attempt
was made to control the photoperiod under which the insects were reared
or tested. The intriguing possibility of a photoperiodic mating rhythm,
suggested by field observations (Duhamel du Monceau and Tillet 1762,
Simmons and Ellington 1933), and a concomittant rhythm of sex pheromone
communication led to the present study.

Experiments were conducted to determine the existence of a
photoperiodic mating rhythm and whether it is affected by a female rhythm
of pheromone release, a male rhythm of pheromone response, or both.

LITERATURE REVIEW

The Sex Pheromone

The premating behavior of male Angoumois grain moths, Sitotroga cerealella, was observed and recorded by Duhamel du Monceau and Tillet (1762) and Simmons and Ellington (1933) to consist of rapid wing fluttering accompanied by darting about and copulatory attempts. Simmons and Ellington observed this dance behavior in males separated from females by gauze and suggested that it was a response to the odor of the females. Keys and Mills (1968) demonstrated that this behavior was elicited by a substance that could be extracted from females with benzene, ethyl ether, methylene chloride, or acetone, listed in decreasing order of their effectiveness. Using a y-tube olfactometer as well as sticky traps baited with females or their extracts, Keys and Mills found that males were attracted by females over short distances, but the attractive nature of the pheromone was not thoroughly investigated. Neither sex responded to males or elicited a response in females. When males were exposed to air which had passed over virgin females of various ages, the length of the premating dance and the length of the rest period between dances was correlated with the age of the females. In this way, Keys and Mills found that adult female Angoumois grain moths apparently do not release a sex pheromone until they are at least 20 min old and that the pheromone level is high enough when females are 35 min old to elicit male copulatory attempts. The response of males increased gradually to the maximum when females were 2-3 days old. Virgin females were generally more capable of eliciting male response than mated females. Brady (1969) bioassayed the sex pheromone by exposing 2-3 day

old males, which had been isolated from females for 2 days, to serial dilutions of ethanol extracts of female abdominal tips. He found that $2.7-3.5 \times 10^{-4}$ female equivalents placed on the tip of a glass rod and held within 1 cm of the antennae of each male produced the typical wing fluttering response in 50% of the males. This concentration approximates that eliciting a 50% response in males of the cabbage looper, Trichoplusia ni (Hübner) (Shorey and Gaston 1965) and of the Indian-meal moth, Plodia interpunctella (Hübner) (Brady and Smithwick 1968). Brady (1969) also found that the number of males responding increased with the concentration of pheromone to which they were exposed, for concentrations between 10^{-7} and 10^{-1} FE.

Götz (1951) found evidence for the production of sex pheromone by female Lepidoptera only in the posterior portion of the abdomen. Brady (1969) found that male S. cerealella were not responsive to extracts of females from which the abdominal tips had been removed and concluded that the sex pheromone is apparently produced in the terminal 2-3 abdominal segments.

Jones and Jacobson (1968) isolated n, n-diethyl-m-toluamide (deet) from the female pink bollworm, Pectiniphora gossypiella (Saund.), which belongs to the Gelechiidae, the family including the Angoumois grain moth. In testing the possibility of cross attraction between the two species, Brady (1969) found that male Angoumois grain moths were not responsive to deet and that no deet was detectable in extracts of female Angoumois grain moths. Although Jacobson (1965), in a survey of sex pheromone phenomena, reported many cases of non-specificity in sex pheromone response, such responses have not been shown for S. cerealella or any species to which it

is closely related. Roelofs and Comeau (1969) determined reproductive isolation of 2 gelechiids, previously considered the same species, solely on the basis of their sex attractants.

The Mating rhythm

Duhamel du Monceau and Tillet (1762) recorded a nocturnal mating rhythm for S. cerealella; the moths flew from infested granaries at dusk and mated and oviposited on the heads of wheat in the fields nearby. Apparently no subsequent studies have determined the exact temporal relationship between mating and photoperiod and the role of the sex pheromone in determining the mating period.

Many species of moths have been shown to display a photoperiodic mating rhythm and a survey of these phenomena is given by Beck (1968). Photoperiodic rhythms in the responses of males to female sex pheromone have been shown to influence the mating time of the cabbage looper, T. ni, alfalfa looper, Autographa californica (Speyer), tobacco budworm, Heliothis virescens (F.), beet armyworm, Spodoptera exigua (Hubner) (Shorey and Gaston 1965), red-backed cutworm, Euxoa ochrogaster (Guenée) (Struble and Jacobson 1970), smaller tea tortrix, Adoxophyes orana Fisher von Roslerstamm (Tamaki et al. 1969), forest tent caterpillar, Malacossoma disstria Hubner (Struble 1970), and the Mediterranean flour moth, Anagasta kuhniella (Zell.) (Traynier 1970). In several species a daily mating rhythm has been correlated with the time of female "calling" (extrusion of scent-producing glands in the posterior abdomen). Among these are the cabbage looper, T. ni (Shorey and Gaston 1964), and the Mediterranean flour moth, A. kuhniella (Traynier 1970).

In several other species, the production or release of sex pheromone by female moths does not appear to be correlated with the time of day (Jacobson 1965). Among them are the Indian-meal moth, P. interpunctella (Brady and Smithwick 1968), lesser wax moth, Achroea grisella (Fabricius), and greater wax moth, Galleria mellonella (L.) (Barth 1937). Those 3 species and Sitotroga cerealella have adapted their biology and behavior to relatively enclosed environments.

Studies of the general role of sex pheromones in determining mating time are surveyed by Jacobson (1965), although in most species including S. cerealella field observations could not reveal whether mating periods were determined by periodicity in male response to pheromone, female release of pheromone, or both.

TEST 1: THE MATING RHYTHM

Materials and Methods

Moths used were from stock cultures in the Department of Entomology,
Kansas State University, reared from Sitotroga cerealella originally
collected in Anderson County, Kansas in August, 1960. The culture medium
was hard red winter wheat with a moisture content of approximately 13%,
and the rearing containers, which differed for the various studies, will
be described below. Unless otherwise stated, moths were reared, observed,
and tested at $26 \pm 2^{\circ}C$ and $65 \pm 5\%$ relative humidity. For simplicity, a 12L:12D
photoperiod was used with the lights on at 6 AM and off at 6 PM. During
the photophase light was provided by overhead fluorescent tubes of the
"daylight" type at an intensity of 8 ± 1 lux (25 ± 2 ft-c), measured at the
average level of the moths with a Weston illumination meter, model 756,
with ultraviolet sensitive probe.

To simulate a natural stored grain infestation, an estimated
population of 200,000 to 400,000 adults was established in a cage measuring
1.83 m on each side constructed by supporting nylon screening with a wooden
frame. The base of the cage was made from 6.3-mm Masonite sheet and was
covered with wheat to a depth of about 1 cm. An original population was
cultured in a galvanized metal tray measuring 45 x 122 x 25 cm high covered
with a piece of hardware cloth to retain moths but admit light, and contain-
ing wheat to a depth of 5 cm. After 1 month the tray was transferred to
the large cage and its screen top removed. Moths continued to emerge and
reinfest the grain in the tray and on the floor of the cage, maintaining a
relatively stable population while observations of mating and other behavior

were made. All moths used for these observations had been reared under
the described photoperiod for at least 7 generations.

The simple method of Bünning (1964) was used to determine the
controlling influence of photoperiod on mating by observing mating behavior
before and after reversing the light and dark phases. A grid covering
6000 cm^2 was drawn on one side of the cage and counts were made of the
number of mating pairs within the grid at 2, 6, and 10 hr after the onset
of both the light and dark phases (8 AM, 12 N, 4 PM, 8 PM, 12 M, and 4 AM).
A red 25-watt incandescent bulb suspended in the center of the cage and
constantly illuminated made it possible to see the silhouettes of the moths
during the dark phase. After counts of mating pairs had been made for a
3-day period under the 12L:12D photoperiod, the dark phase of day 3 was
extended 12 hr to reverse the photoperiod (12D:12L). After 3 days, another
3-day observation period was initiated to determine if the mating rhythm
phase-adjusted to the reversed photoperiod.

Results

A pronounced daily rhythm in mating occurrence was displayed by
Angoumois grain moths held in a 12L:12D photoperiod. Three days after
photoperiod reversal the mating rhythm was completely re-entrained,
constituting a phase shift of 12 hr (Table 1). No data were collected
during the first 3 days after reversing the photoperiod; the time required
for re-entrainment and the nature of transient cycles in the endogenous
mating rhythm remain undetermined, except that complete re-synchronization
with the new photoperiodic regime occurred within 3 days.

By pooling the data collected before and after reversal of the

photoperiod, a slightly more accurate description of the mating periodicity is possible than by using data from one 3-day observation period alone (Table 2).

Table 1. Number of mating pairs of S. cerealella observed on a 6000-cm^2 cage surface at each of 6 daily times before and after reversal of a 12L:12D photoperiod. Dawn and dusk occurred at 6 AM and 6 PM CST on days 1-3 and 6 PM and 6 AM CST on days 4-9.

Phase and time in photoperiod		CST	Day			Total	% mating
			Before reversal of photoperiod				
			1	2	3		
light	8 AM	8 AM	7	12	14	33	2.5
light	12 N	12 N	0	2	1	3	0.2
light	4 PM	4 PM	2	8	8	18	1.4
dark	8 PM	8 PM	21	20	56	97	7.4
dark	12 M	12 M	163	211	195	569	43.6
dark	4 AM	4 AM	178	200	208	586	44.9
						1306	100.0
			After reversal of photoperiod				
			7	8	9		
light	8 AM	8 PM	15	19	24	58	6.8
light	12 N	12 M	2	4	0	6	0.7
light	4 PM	4 AM	0	3	5	8	0.9
dark	8 PM	8 AM	29	20	38	87	10.2
dark	12 M	12 N	98	120	123	341	40.0
dark	4 AM	4 PM	121	113	119	353	41.1
						853	100.0

Table 2. Total number of mating pairs of S. cerealella observed on a
          6000-cm^2 cage surface for 6 days at each of 6 times in a 12L:12D
          photoperiod with dawn and dusk corresponding to 6 AM and 6 PM
          respectively.

Phase and time in photoperiod	6-day total of mating pairs	% of 6-day total of mating pairs
light 8 AM	91	4.2
light 12 N	9	0.4
light 4 PM	26	1.2
dark 8 PM	184	8.5
dark 12 M	910	42.2
dark 4 AM	939	43.5
	2159	100.0

During the light phase, mating was infrequent, with the lowest
incidence observed at 12 N and 4 PM. Mating greatly increased during the
first half of the scotophase, reflected by an increase from 8.5% of the
total observed mating pairs at 8 PM to 42.2% at 12 M. At 4 AM there was
only a slight increase in mating from that observed at 12 M. A total of
94.2% of the pairs in copulo were observed during the scotophase with 85.7%
at 12 M and 4 AM. The percents of total pairs in copulo observed at each
time for the 6 days are used to eye-plot a smooth curve representing one
cycle of the daily mating rhythm (Fig. 3, A; p. 27).

TEST 2: PHEROMONE RELEASE RHYTHM

Materials and Methods

Experiments to test the hypothesis that the mating rhythm observed was due, at least in part, to a daily fluctuating pheromone level in the environment required bioassays of the air in the rearing room at various times. Air was forced through a 2-m length of 1.2-cm-ID polyethylene tubing by a "squirrel cage" fan, from the room holding the large population cage into a smaller test population cage held outside the room in constant illumination (4.6-6.1 lux, 15-20 ft-c). The pheromone concentration was tested by counting the number of mating pairs and the number of males displaying the pheromone response dance at different times in the test population (24L:0D) and comparing these with similar data, collected at the same times, for the photoperiodically entrained population (12L:12D). Counts were made at the same times as those used earlier for mating observations. A daily fluctuation in pheromone level would presumably be reflected in periodicity of mating or male dancing in the test population.

The test population was held in a cage measuring 43 x 29 x 44 cm high the sides and top of which were a continuous curved sheet of clear plastic on which a grid covering an area of 3000 cm^2 was drawn to facilitate counting mating pairs and dancing males. Air from the room holding the 12L:12D population entered one end of the cage at a rate of 130 ml/min via an 8-cm-diam screened port and exhausted through a similar 6.5-cm-diam aperture at the opposite end.

Moths used in the bioassay cage were obtained by placing wheat from stock cultures (which had never been exposed to a regularly cyclic

photoperiod) in the bottom of the cage and allowing the moths to emerge.
The adult population increased during the 3-day period. Because of the
variation in the number of males displaying the dance from one moment to
the next, the average of 3 counts was recorded for each time as well as
the number of mating pairs for both the 12L:12D and 24L:0D population
for 3 days.

Results

Moths held under constant illumination did not display the periodicity
of mating or dancing shown by the photoperiodically exposed moths, although
both populations were exposed to similar atmospheres (Table 3). This
suggested that the pheromone concentration in the 12L:12D population cage
was stable throughout the day or, if fluctuating, did not affect the daily
mating rhythm observed.

The gradually increasing mating and dancing displayed by the 24L:0D
population over the 3-day observation period was due to the increasing
population size, and the minor fluctuations in dance frequency are at the
level of random variations in the 3 counts averaged for each observation
time. It is therefore concluded that a basis other than a female pheromone
release rhythm must be sought for the mating rhythm of S. cerealella held
in a photoperiod.

Comparison of the mating frequencies (Table 3, a) and dance frequencies
(Table 3, b) of the 12L:12D population reveals a close correlation between
the number of males displaying the premating pheromone-elicited dance and
the number of mating pairs, although, as would be expected, the dance
frequency increases slightly earlier in the scotophase. This suggests the

role that male response to pheromone may play in the expression of the

mating rhythm.

Table 3. Number of S. cerealella mating pairs (a) and males dancing (b)
observed on constant cage surfaces at 6 times in 12L:12D and
24L:0D photoperiods for 3 days. Both populations were exposed
to similar atmospheres.

a. Mating pairs

| Time | 12L:12D | | | | | 24L:0D | | | | |
| | Day | | | Total | % | Day | | | Total | % |
	1	2	3			1	2	3		
8 AM	15	19	24	58	6.8	15	30	49	94	13.5
12 N	2	4	0	6	0.7	24	47	53	124	17.8
4 PM	0	3	5	8	0.9	28	31	69	128	18.4
8 PM	29	20	38	87	10.2	28	25	64	117	16.8
12 M	98	120	123	341	40.0	21	38	55	114	16.3
4 AM	121	113	119	353	41.4	21	36	63	120	17.2
				853	100.0				697	100.0

b. Males dancing

| Time | 12L:12D | | | | | 24L:0D | | | | |
| | Day | | | Total | % | Day | | | Total | % |
	1	2	3			1	2	3		
8 AM	13	5	7	25	3.5	19	55	95	169	14.3
12 N	1	1	0	2	0.3	32	58	90	180	15.2
4 PM	0	1	0	1	0.1	37	64	104	205	17.3
8 PM	33	37	44	114	15.9	28	65	98	191	16.1
12 M	77	94	110	281	39.1	41	80	101	222	18.7
4 AM	104	88	103	295	41.1	36	79	103	218	18.4
				718	100.0				1185	100.0

TEST 3: RHYTHM OF MALE RESPONSE TO FEMALE SEX PHEROMONE

Materials and Methods

The hypothesis that the observed mating rhythm was due to daily
changes in male response to the female sex pheromone was tested by exposing
males in a specially-constructed test apparatus to a standard dose of
female extract at various times in a 12L:12D photoperiod and recording
the number of responding moths. Moths were cultured under the previously
described photoperiod and atmospheric conditions in quart mason jars
containing 200 g (4 cm depth) of wheat. The jar lids held screening and
Kelthane-treated filter paper discs to prevent mite infestation. Males
were tested for pheromone response under conditions of temperature, humidity,
photoperiod and light intensity approximating those under which they were
reared. For these tests, the scotophase was absolutely dark.

Female extracts. An extract of female moths was prepared using a
modification of the technique of Brady (1969) to control accurately the
pheromone dosage to which the test males were exposed. Less than 1 hr
after emergence 360 females were isolated from males. When 56-64 hr old
(average, 60 hr) they were homogenized in 36 ml of cold 95% ethanol in an
ice bath using a Sorval Omnimixer. The homogenate was centrifuged at
20,000 rpm and $-18^{\circ}C$. The supernatent, 32 ml of crude female extract at a
concentration of 10 female equivalents/ml, was stored in an air-tight glass
vial in the dark at $-12^{\circ}C$. Ethanol was stored similarly to be used in
controls (described below).

Attraction test apparatus. Ten test chambers were constructed to
measure either vigorous locomotion or attraction when exposed to an air
stream carrying vapors of female extract. Each test chamber was essentially
a hollow cylinder divided by a funnel which permitted moths to move easily
in one direction but inhibited movement in the opposite direction (Figs. 1
and 2). During testing, males placed in the distal end, called the "release
chamber" (Fig. 1, a) were exposed for 1 hr to a slow air stream carrying
vapors of female extract. A "response" was defined as the movement of a
moth through the funnel, toward the pheromone source, into the "response
chamber" (b). Due to the shape of the release chamber, most of the moths
stimulated by pheromone to display the agitated movements and locomotion
characteristic of pheromone response would move into the release chamber
due to increased, even if nondirected, movements.

To prevent the interruption or alteration of the photoperiod,
temperature, and particularly the light intensities to which the test
insects were exposed, the test chambers were used in the rearing room
and testing was independent of direct observations (discussed below).
To minimize possible effects of directional light, the test chambers were
mounted in a cabinet painted flat white inside and illuminated during the
photophase by light (diffused by groung Plexiglas) provided by fluorescent
tubes mounted above the chambers. The photoperiod and light intensity
inside the cabinet were the same as those of the rearing room. Test
chambers were transparent throughout, minimizing shadows.

A 2 ml/min air stream was provided in each test chamber by a "squirrel
cage" fan (Fig. 1, c) which drew fresh air from outside the building via a
3.3-cm-ID plastic tube. To equilibrate the temperature of the air with that

Explanation of Figure 1. Diagram of Attraction Test Apparatus
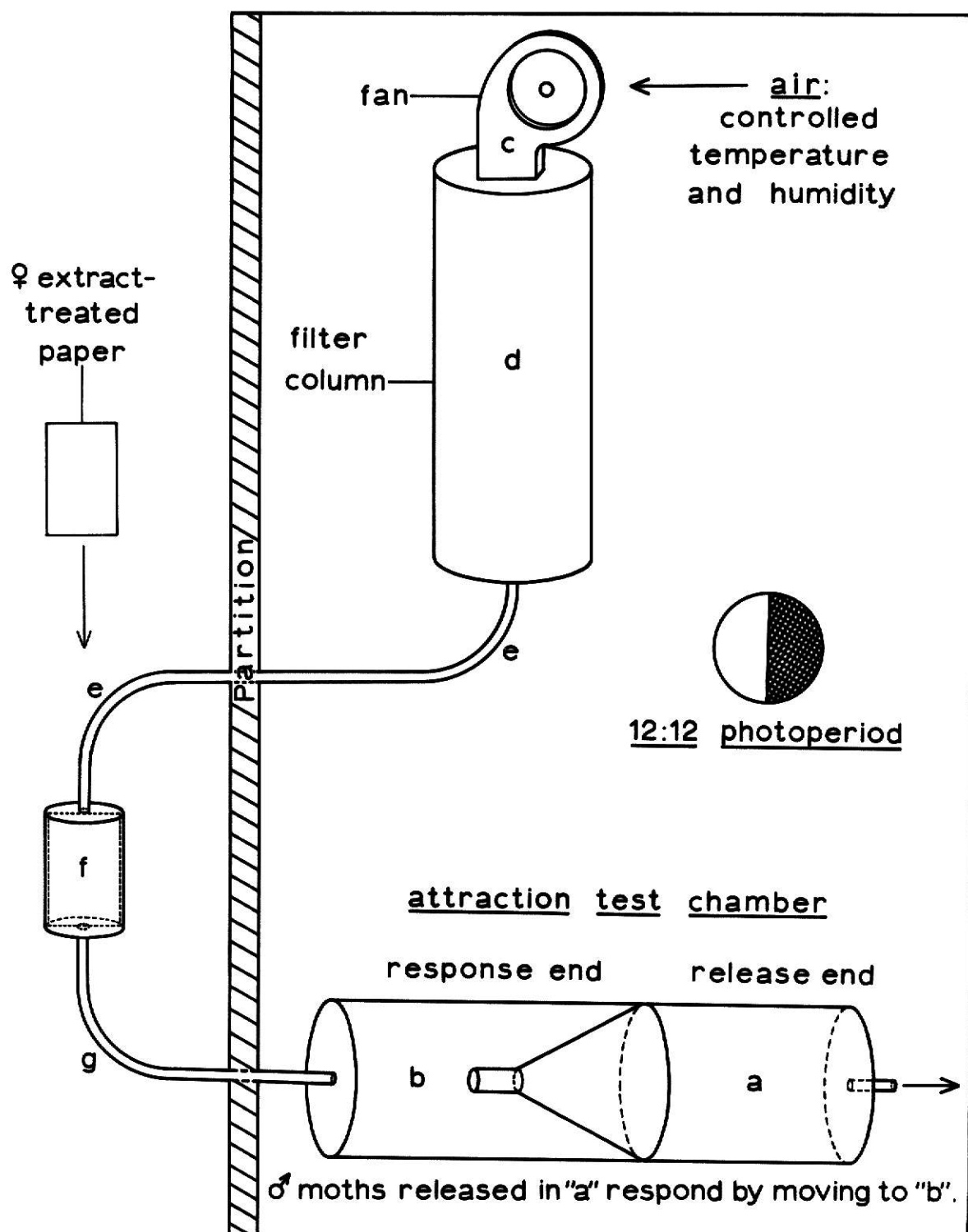
(not drawn to scale)

A 2 ml/min stream of conditioned air (see text) was provided in each test chamber by a "squirrel cage" fan (c). The fan forced the air through an 8-cm-diam x 12-cm column of activated charcoal granules (d). From the filter column, ten 6-mm-ID polyethylene tubes (e) passed through a partition to outside of the rearing-testing room where each was fitted with a 19-ml polyethylene vial to provide for the insertion of a 2.5 x 3.3-cm filter paper treated with female extract or ethanol. Air tubes (g) returned through the partition to the testing cabinet where each communicated with a test chamber.

During testing, males placed in the distal or "release" end of the chamber (a), were exposed for 1 hr to the air stream carrying vapors of the female extract or blank controls. A "response" was defined as the movement of a moth through the funnel dividing the chamber, toward the air source into the response end of the chamber (b).

16

Figure 1. Diagram of Attraction Test Apparatus

fan

air: controlled temperature and humidity

o

c

♀ extract-treated paper

filter column

d

e

12:12 photoperiod

e

Partition

f

attraction test chamber

response end   release end

g

b   a

♂ moths released in "a" respond by moving to "b".

of the rearing-testing room, the air was drawn through several layers of
moist cheesecloth in an air-tight 74-1 plastic tub and monitored with a
thermometer. This also increased the humidity of the air to near saturation
although humidity was not monitored. The fan forced this fresh air through
an 8-cm-diam x 12-cm column of activated charcoal granules (2.4-5.5 mesh/cm)
(Fig. 1, d) to minimize any odors which passed through the cheesecloth.
From the filter column, ten 6-mm-ID polyethylene tubes passed through a
partition to outside of the rearing-testing room (e). At this point, each
tube was fitted with a 19-ml polyethylene vial (Fig. 1, f; Fig. 2, a) to
provide for the insertion of a 2.5 x 3.3-cm filter paper (Fisher No. 9-795)
treated with female extract or ethanol. Air tubes (Fig. 1, g) returned
through the partition to the testing cabinet where each communicated with
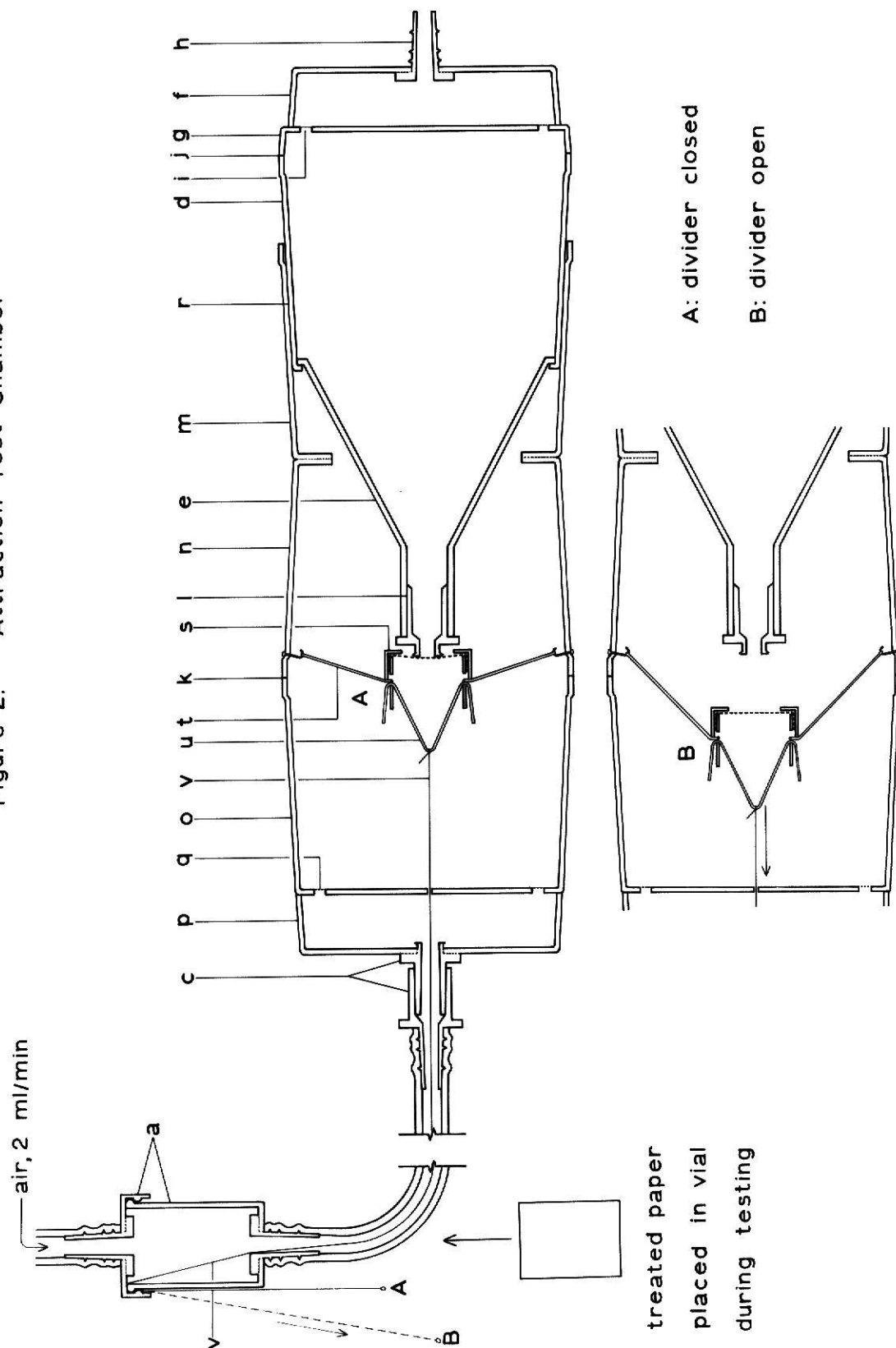a test chamber via a polypropylene connecting tube (Fig. 2, c).

Test chambers (Fig. 2) were fabricated from inexpensive and easily
obtained materials. Each release chamber was made from a transparent plastic
dish 6.8 cm deep with a diameter tapering from 9.5 to 8.7 cm (Fig. 2, d),
with its base removed to attach an 8-cm plastic powder funnel (e). The
distal end of the release chamber was the base and top of a plastic petri
dish, 9.5 x 2 cm, joined together with top upside down (f, g). The base
of the dish (f) was provided with a connecting tube (h) and in the top (g)
were drilled 12 3.5-mm holes (i) around its periphery for air escape.
These holes were covered with fine brass strainer cloth to prevent the
escape of moths. To facilitate dismantling and cleaning, this unit was
attached to the body of the release chamber at point j using masking tape;
a similar joint was made at point k. Unless otherwise described, pieces
were bonded with styrene plastic cement; joints involving polyethylene or

Explanation of Figure 2. Attraction Test Chamber

Conditioned air (see text) was forced through a 6-mm-ID polyethylene tube at a rate of 2 ml/min into a 19-ml polyethylene vial (a) provided for the insertion in the air stream of a treated filter paper. The tubes were connected to test chambers by polypropylene connecting tubes (c). Release chambers were made from a plastic dish (d), an 8-cm plastic powder funnel (e), and the base and top of a plastic petri dish (f, g). The base of the petri dish (f) was provided with a connecting tube (h), and 12 screened 3.5-mm holes (i) in the top (g) to allow air to exhaust from the chamber. A cut-off connecting tube (1) restricted the funnel aperture. Response chambers were made from 3 plastic dishes (m, n, o), and the base of a plastic petri dish (p) with 6 3.5-mm air inlet holes (q). The passage of moths through the chamber was controlled (A, B) by a divider (s) suspended by a rubber band (t) and operated by a thread (v) tied to the divider by means of a w-shaped wire (u). Joints j and k were made with masking tape; r was a press fit.

Figure 2.   Attraction Test Chamber

air, 2 ml/min

a

A

v

B

treated paper
placed in vial
during testing

A: divider closed

B: divider open

h

f

g

j

i

d

r

m

e

n

l

s

k

t

u

v

o

q

p

c

polypropylene were made with epoxy cement. Through preliminary testing it was found that a cut-off connecting tube (1) pressed into the funnel aperture greatly reduced random movement of moths through the chamber without substantially interfering with responses to pheromone.

Each response chamber was made in similar fashion from 3 plastic dishes (m, n, o) and the bottom of a plastic petri dish (p). Six air inlet holes (q) were made in the end of the chamber, similar to the outlet holes (i). To minimize handling the moths prior to testing, the release chambers were removable at the air-tight joint (r). It was possible to aspirate moths directly into the release chamber through the funnel aperture by attaching a vacuum pump to the connecting tube (h).

The passage between the release and response chamber was controlled by a door operated from outside the testing room (Fig. 2; A, B). A piece of nylon screen covered a 2-cm hole in the top half of a capped 2.3-cm-diam polyethylene vial (s). The half vial was suspended in the center of the response chamber by one rubber band (t) threaded through 4 6-mm holes in the vial wall and attached to 4 wire hooks mounted 90° apart in the test chamber. The elasticity of the rubber band held the screen securely against the end of the funnel (A). By pulling on the thread (v) attached to a w-shaped piece of wire (u) the door could be opened from outside the room and held open during testing by the lid of the extract vial (a).

Collection and handling of test insects. Males were collected 0-24 hr after emergence by aspirating them diectly into 1-gallon glass jars where they were held (isolated from females) for 36 hr. Fresh air was provided during this holding period by a 1.2-cm-ID polyethylene tube between the jars and the lower end of the activated charcoal column. Thus

the moths were held in the same type of air in which they were to be tested and were not exposed to pheromone emanating from cultures in the room. Thirty males were aspirated into each response chamber which was then attached to a release chamber. During the following acclimation period of 6-14 hr, the moths were exposed to the 2 ml/min air current with the chamber doors closed.

Because all manipulations of insects inside the rearing room had to be done during the light phase, it was not possible to test all moths at the same average group age or after the same acclimation period. By staggering the collection of moths and their insertion into the release chambers, however, it was possible to provide the same preparatory manipulations for moths to be tested at 8 AM and 8 PM, 12 N and 12 M, and 4 AM and 4 PM (Table 4). In this way, no bias was created in the treatment of moths to be tested in the photophase or the scotophase.

Table 4. Schedule for handling males to be tested for pheromone response.

Collection		Holding	Acclimation	Testing	
Time	Age (hr)	period (hr)	period (hr)	Time	Age (hr)
6 AM	0-24	36	6	12 M	42-66
6 AM	0-24	36	10	4 AM	46-70
6 AM	0-24	36	14	8 AM	50-74
6 PM	0-24	36	6	12 N	42-66
6 PM	0-24	36	10	4 PM	46-70
6 PM	0-24	36	14	8 PM	50-74

Testing procedure. At the time of testing all manipulations were
done from outside the room containing the test chambers. For controls,
5 filter papers were treated with 0.1 ml of cold 95% ethanol and evaporated
for 50 sec in an air stream provided by a fan. Each paper was then inserted
into a vial selected at random and the chamber doors were opened. The
remaining 5 chambers had filter papers treated with 0.1 ml of cold female
extract (1 FE). After 60 min the chamber doors were closed and the filter
papers were removed. The number of moths in each response chamber was
recorded, but for tests during the scotophase, counts were deferred until
the following photophase. The moths were discarded, release chambers were
washed with hot soapy water, and the entire apparatus was "cleaned" by
passing filtered air through it.

All males were tested at an adult age of 42-74 hr. Fifteen replicates
of 30 males each were made of both pheromone and control tests at each of
the 6 test times.

Results

Males displayed a daily periodicity in response to 1 FE of female
extract, supporting the hypothesis that the mating rhythm is due primarily
to a rhythm of male response to pheromone (Table 5). Male response had a
more gradual periodicity than the mating rhythm and more pheromone response
occurred after the onset of light than would be expected by the earlier
mating observations. The response elicited in the control chambers had a
periodicity in phase with that of the pheromone-exposed moths; this may
have been due to slight contamination of control chambers in spite of
efforts to minimize residual pheromone in the test apparatus. The bias

that such an effect would create is eliminated by subtracting the control

response from that occurring in the pheromone-treated chambers to yield a

net response to the 1 FE of female extract.

Table 5. Number of males responding out of 30 exposed to 1 FE of female extract for a period of 1 hr at 6 times in a 12L:12D photoperiod with dawn and dusk corresponding to 6 AM and 6 PM respectively. P=pheromone exposure; C=control; Net=(P-C).

	Phase and time in photoperiod											
Replicate	Light 8 AM		Light 12 N		Light 4 PM		Dark 8 PM		Dark 12 M		Dark 4 AM	
	P	C	P	C	P	C	P	C	P	C	P	C
1	12	5	8	1	8	0	1	0	19	13	16	10
2	20	3	10	0	14	0	1	0	13	11	19	5
3	21	8	15	7	3	0	0	0	13	5	24	8
4	19	1	12	2	9	0	1	2	14	8	19	7
5	20	3	11	0	5	0	3	0	19	4	20	4
6	16	3	12	5	5	0	0	0	10	11	21	11
7	16	2	17	2	7	0	0	0	14	9	22	12
8	17	6	18	0	9	0	1	0	15	12	23	4
9	23	3	14	1	5	0	4	0	9	5	23	8
10	25	4	12	0	9	0	1	0	19	5	18	9
11	16	3	16	2	10	3	5	1	11	5	20	8
12	22	4	14	6	4	0	3	0	11	7	21	9
13	20	6	12	1	10	1	1	0	10	11	20	10
14	21	4	10	4	7	0	3	0	18	10	18	8
15	15	4	8	4	9	0	1	2	12	4	21	5
Total	283	59	189	35	114	4	25	5	207	120	305	118
Net	224		154		110		20		87		187	

The rhythm of net pheromone response by males (Fig. 3, B, plotted by eye) appears out-of-phase with the mating rhythm; mating occurs earlier in the scotophase and not as late in the photophase as would be indicated by the data on male response to pheromone. Male response data, however, were based on the behavior of moths under very different conditions than those in which mating observations were made. In the mixed population used for mating observations, males stimulated to mate were free to do so; in these tests, males were isolated from females. It is suggested that the effect of isolation from females was a prolonged state of pheromone-responsiveness resulting in the observed phase shift.

From both mating and pheromone response data (Tables 2 and 5), it appears that pheromone response by males (and subsequent mating in mixed populations) essentially begins after the onset of darkness and increases during the scotophase. In situations where mating is prevented, such as the pheromone response tests, it seems reasonable that those males that would have responded and mated earlier probably would respond at a later time; where mating is not prevented, nocturnal mating would probably inhibit subsequent mating until the following scotophase.[*] Thus, pheromone response data probably represent cumulative values after the onset of darkness rather than mutually exclusive events. The response at 12 M probably included some males that would have responded (and mated in a mixed population) at 8 PM; the responses at 4 and 8 AM included males that would have responded earlier during the scotophase; and the responses at 12 N and 4 PM were probably

[*] Keys (1965 KSU unpubl.) indicated that male and female S. cerealella mate only once per day and, as supported by Simmons and Ellington (1933), rarely remain in copulo for longer than 3 hr; therefore, the matings recorded at 4-hr intervals in the earlier study of mating periodicity were essentially mutually exclusive events.

comprised almost entirely of males that would have responded earlier.

Based on the hypothesis that the male response data represent cumulative values after dusk, the net response at each time after the onset of darkness was subtracted from the net response at the next test time. These manipulations yielded data on the actual increase in male response at 4-hr intervals rather than the proposed accumulations of response (Table 6).

Table 6. Temporal organization of male response to 1 FE of female extract. Increase in response after dusk computed by considering net response data to be cumulative after the light-off stimulus (see text).

Phase and time in photoperiod		Net response	% of total net response	Increase in net response after dusk	% of total increase in net after dusk
light	8 AM	224	28.6	37	16.5
light	12 N	154	19.7	0	0
light	4 PM	110	14.1	0	0
dusk	6 PM				
dark	8 PM	20	2.6	20	8.9
dark	12 M	87	11.1	67	29.9
dark	4 AM	187	23.9	100	44.7
		782	100.0	224	100.0

The last column of Table 6 represents the percents of pheromone response which would presumably have occurred had responsive males had an opportunity to mate, thus inhibiting subsequent response until the next dark phase. These response frequencies show a remarkable similarity to the actual mating frequencies observed earlier (Table 2), and support the hypothesis

that the mating rhythm is due substantially to the male rhythm of pheromone response. The relationship between the percents of increase in response after dusk and the mating rhythm is shown by superimposing their representative curves on the same coordinates (Fig. 3, B, plotted by eye).

DISCUSSION

To infer causality from correlation is often dangerous; however, it appears justifiable to conclude that the mating rhythm, at least in dense populations, is probably determined by a rhythmicity in male responsiveness to female pheromone and not by a rhythm of pheromone release by females. Apparently the pheromone level in an enclosed environment does not fluctuate daily but presumably increases gradually as the female population increases.

For an insect which has adapted to passing its entire life cycle in relatively enclosed environments, it would seem of little or no adaptive significance for each female to compete with others (usually nearby) by producing an attractant with which to lure males for mating. Although the sex pheromone, under exposed field conditions, may function to attract males to females, the observed male response in the population under study consisted of apparently random trial-and-error copulation attempts directed toward any moths regardless of their sex. This behavior indicates the absence of actual chemical orientation mediated by the pheromone, although there is no doubt that chemical communication occurs.

A volatile sex _attractant_ would be of little functional significance in an enclosed environment, such as the laboratory cages or most grain storage containers, in which air currents are minimal and population

Figure 3. A: mating rhythm

B: mating and male pheromone-response rhythms
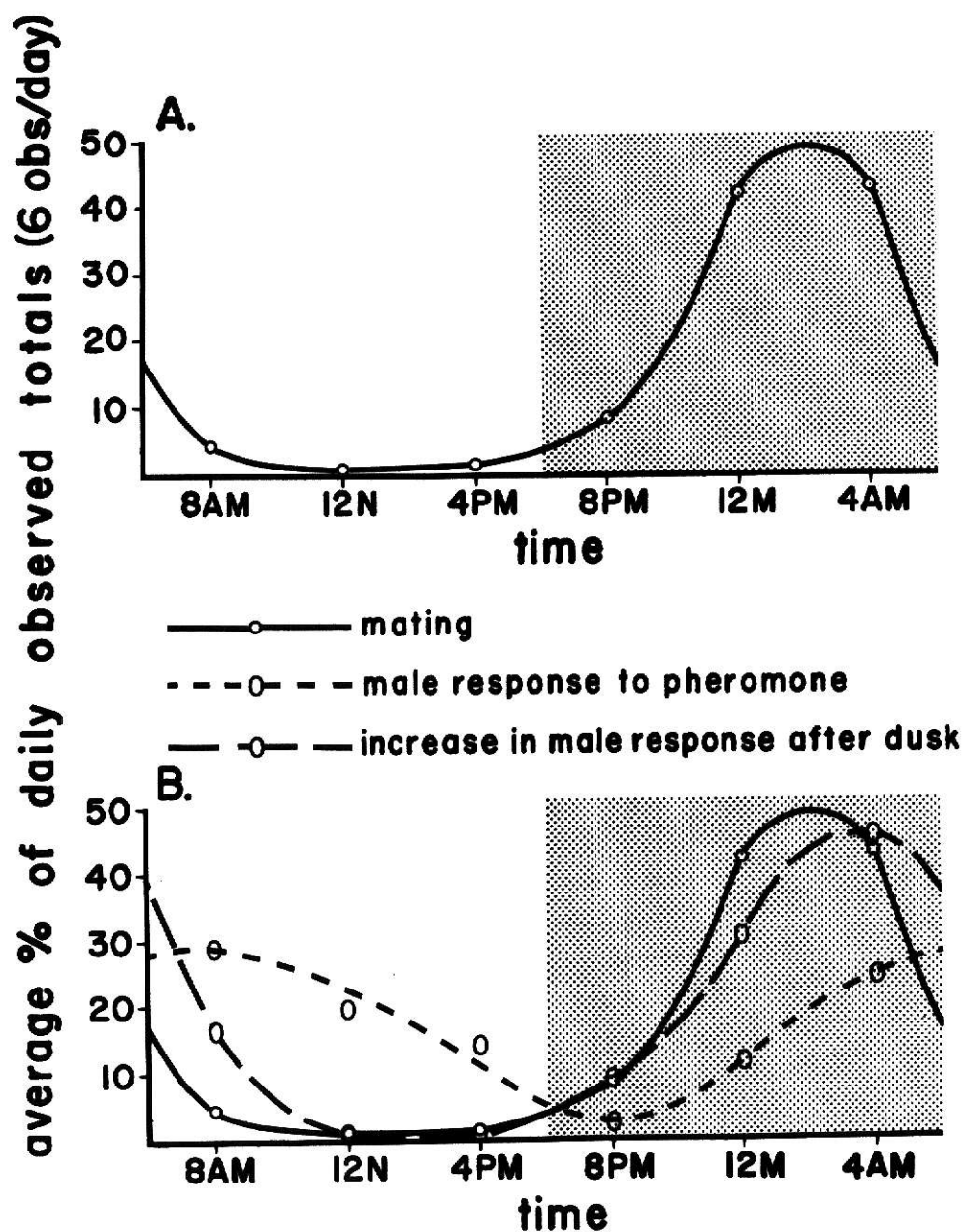
densities may be high. To regulate a daily mating time by a photoperiodic pheromone release rhythm would be difficult in such a situation because the pheromone would not be as subject to dilution and distribution by air currents. The possibility of functionally fluctuating pheromone production by female Angoumois grain moths is not negated; in low population densities or exposed field conditions a pheromone release rhythm may operate. If such a rhythm exists in a high density, enclosed population, it is made ineffective by the characteristics of the environment. In addition, female S. cerealella have not been observed to adopt a characteristic pheromone release posture common to those species for which a specific pheromone release period has been demonstrated (Jacobson 1965, Traynier 1970, Sower et al. 1971).

In an enclosed environment, the photoperiodic male rhythm of pheromone response probably determines the mating period even if the ambient pheromone concentration is constant. The nocturnal mating period thus determined probably occurs at the peak of female receptivity and in temporal association with other behavioral and physiological rhythms of both sexes. Such a mating rhythm, therefore, would be adaptive for a population and subject to selective pressures.

In a high density population in an enclosed environment, orientation by the pheromone, if such exists for S. cerealella, appears to be replaced by an increased incidence of male copulatory attempts elicited by a high ambient pheromone level. Mating periodicity seems to be temporally determined by changes in the sex pheromone responsiveness of males, constituting an endogenous rhythm entrained by photoperiodic cues.

CONCLUSION

A definite photoperiodically entrained mating rhythm was observed for the caged laboratory population of S. cerealella held under a 12L:12D photoperiod. Ninety-four percent of the total observed mating was nocturnal with the highest incidence observed at 12 M and 4 AM (mid- to late scotophase). The rhythm phase-adjusted to a reversed photoperiod within 3 days. Bioassay did not detect a fluctuating pheromone level in the environment, but the mating rhythm was correlated with daily changes in pheromone responsiveness by males.

Males isolated from females were highly responsive to female extract from mid-scotophase to mid-photophase. It is suggested, however, that in a mixed population, nocturnal mating would inhibit subsequent response to pheromone until the following scotophase producing, in effect, an essentially nocturnal rhythm of sex pheromone response by males. The data are in agreement, therefore, with the hypothesis that the mating rhythm is the result of a photoperiodic rhythm of male response to the female sex pheromone.

ACKNOWLEDGEMENTS

Special acknowledgement is due Dr. Robert B. Mills who, as major professor, offered particular quidance in the planning and execution of research and the preparation of this paper. I also wish to thank Dr. Charles W. Pitts and Dr. Carl W. Rettenmeyer, who, as members of the Graduate Supervisory Committee, provided suggestions on the research and aided in the preparation of the thesis without approving all of my interpretations.

LITERATURE CITED

Barth, R. 1937. Herkunft, Wirkung und Eigenschaften des weiblichen Sexualduftstoffes einiger Pyraliden. Zool. Jahrb., Abt. Zool. Physiol. Tiere 58:297.

Brady, U. E. 1969. Response of male Sitotroga to the sex pheromones of Sitotroga and Pectinophora females and to propylure and deet, deet analysis in Sitotroga females. J. Georgia Entomol. Soc. 4:11-14.

Brady, U. E., and E. B. Smithwick. 1968. Production and release of sex attractant by the female Indian-meal moth, Plodia interpunctella. Ann. Entomol. Soc. Am. 61:1260-1265.

Bünning, E. 1964. The Physiological Clock. Academic Press, New York. 145 p.

Duhamel du Monceau, H. L., and M. Tillet. 1762. History of an insect which devours the grains of Angoumois. Paris. (Trans. by Elizabeth D. Simmons, 1925 typewritten, 59 p.)

Götz, B. 1951. Die Sexualduftstoffe an Lepidopteren. Experientia 7:406-418.

Jacobson, M. 1965. Insect Sex Attractants. Interscience Publ., New York. 154 p.

Jones, W. A., and M. Jacobson. 1968. Isolation of n, n-diethyl-m-toluamide (deet) from female pink bollworm moths. Science 159:99.

Keys, R. E. 1967. A study of the mating behavior and mating patterns of male and female Angoumois grain moths, Sitotroga cerealella (Oliv.). Undergraduate report, K. St Univ., Dept. Entomol.

Keys, R. E., and R. B. Mills. 1968. Demonstration and extraction of a sex attractant from female Angoumois grain moths. J. Econ. Entomol. 61:46-49.

Roelofs, W. L., and A. Comeau. 1969. Sex pheromone specificity: taxonomic and evolutionary aspects in Lepidoptera. Science 165:398-400.

Shorey, H. H., and L. K. Gaston. 1964. Sex pheromones of noctuid moths. III. Inhibition of male response to the sex pheromone in Trichoplusia ni (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 57:775-779.

Shorey, H. H., and L. K. Gaston. 1965. Sex pheromones of noctuid moths. V. Circadian rhythm of pheromone-responsiveness in males of Autographa californica, Heliothis virescens, Spodoptera exigua, and Trichoplusia ni (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 58:597-600.

Simmons, P., and G. W. Ellington. 1933. Life history of the Angoumois grain moth in Maryland. USDA Tech. Bull. 351. 34 p.

Sower, L. L., H. H. Shorey, and L. K. Gaston. 1971. Sex pheromones of noctuid moths. XXV. Effects of temperature, and photoperiod on circadian rhythms of sex pheromone release by females of Trichoplusia ni. Ann. Entomol. Soc. Am. 64:488-492.

Struble, D. L. 1970. A sex pheromone in the forest tent caterpillar. J. Econ. Entomol. 63:295-296.

Struble, D. L., and L. A. Jacobson. 1970. A sex pheromone of the red-backed cutworm. J. Econ. Entomol. 63:841-844.

Tamaki, Y., H. Noguchi, and T. Yushima. 1969. Mating behavior of the smaller tea tortrix, Adophyes orana Fisher von Roslerstamm and evidence of sex pheromone production. (Abstr.) Biol. Abstr. 1969:no. 99057.

Traynier, R. M. M. 1970. Sexual behavior of the Mediterranean flour moth, Anagasta kuhniella: some influences of age, photoperiod, and light intensity. Can. Entomol. 102:534-540.

A PHOTOPERIODIC MATING RHYTHM FOR THE ANGOUMOIS GRAIN MOTH,
SITOTROGA CEREALELLA (LEPIDOPTERA: GELECHIIDAE), AND THE ROLE OF
MALE RESPONSE TO THE FEMALE SEX PHEROMONE IN ITS DETERMINATION

by

JOHN C. GUERBER

B. S., Albright College, 1969

———————————————

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements of the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1971

A definite photoperiodically entrained mating rhythm was observed for
a caged laboratory population of Angoumois grain moths, <u>Sitotroga</u> <u>cerealella</u>,
reared under alternating 12 hr light, 12 hr dark. Ninety-four percent of the
total observed mating was nocturnal with the highest incidence observed at
12 M and 4 AM (mid- to late scotophase). The rhythm phase-adjusted to a
reversed photoperiod within 3 days. Bioassay did not detect a fluctuating
pheromone level in the environment, but the mating rhythm was correlated with
daily changes in pheromone responsiveness by males.

Males isolated from females for 42-74 hr were responsive to an ethanol
extract of 2-3 day old virgin females from mid-scotophase to mid-photophase.
It is suggested, however, that in populations of both sexes, nocturnal
mating would probably inhibit subsequent male response until the following
scotophase producing, in effect, an essentially nocturnal rhythm of sex
pheromone response. The data are, therefore, in agreement with the
hypothesis that the mating rhythm is the result of a photoperiodic rhythm
of male response to the female sex pheromone.