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PHEROMONE-MEDIATED BEHAVIOR OF
A GREENHOUSE FUNGUS GNAT,
BRADYSIA IMPATIENS (JOHANNSEN)
(DIPTERA: SCIARIDAE)

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PHEROMONE-MEDIATED BEHAVIOR OF A GREENHOUSE FUNGUS GNAT, BRADYSIA IMPATIENS (JOHANNSEN) (DIPTERA: SCIARIDAE)

By

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ABSTRACT

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A GREENHOUSE FUNGUS GNAT,
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The existence of a pheromone system in Bradysia impatiens is established, and the factors involved in its production and male response were studied. Non-homogenized 30 F.E. extracts of female legs elicited the greatest amount of male wing fanning at 1900 and orientation at 1500-2100 hours in olfactometer tests. Extracts of females older than 12 hours elicited the greatest male orientation response; male wing fanning response was largest with 1 and 2 day-old females. Maximum orientation occurred with males older than 1 day of age. Age of the male did not affect wing fanning; fertilization of the females did not affect male orientation or wing fanning. Videotape recordings of the mating activity determined an ordering of the male's behaviors in the sequence. Males tested in the wind tunnel demonstrated long-distance, upwind zig-zag flight to the pheromone source using an optomotor feedback.

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The author wishes to express sincere thanks to Drs. M. Keith Kennedy and Ring Cardé and the committee members Drs. Fred Stehr and Will Carlson for their guidance and support.

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INTRODUCTION

Sciarid larvae have long been known to be destructive to plants. The larvae have been reported as pests on the roots of such crops as wheat (Coquillot 1895; Hungerford 1916; Johannsen 1912), corn (Forbes 1896; Johannsen 1912) and potatoes (Johannsen 1912; Gui 1933). Sciarid larvae attack a variety of potted plants in greenhouses, including geraniums, nasturtiums and carnations (Ellisor 1934), lettuce (Chittenden 1901), cucumbers (Forbes 1896; Chittenden 1901; Speyer 1923) and peas (Chittenden 1901). The larvae feed not only upon decaying plant material (Johannsen 1912; Speyer 1923; Steffan 1966), but also on apparently healthy plant tissue (Hungerford 1916; Ellisor 1934).

Sciarid larvae are commonly found in mushroom houses, where they feed upon the spawn and mycelium and tunnel throughout the fruiting body of the mushroom (Johannsen 1909; Thomas 1931; Austin and Jary 1933; Hussey et al. 1969). Sciarid adults have been suspected of vectoring plant pathogens (Charles and Popenoe 1928), and larval feeding injury may provide entry points for plant pathogenic organisms (Wilkinson and Daugherty 1970).

One species of sciarid which is frequently found in greenhouses in the eastern US is Bradysia impatiens (Johannsen). The larvae of this species in greenhouses feed upon decaying organic material (Steffan 1966) and healthy plant tissue in contact with the soil, such as root hairs, small rootlets and leaves of rooted cuttings and seedlings (Kennedy 1976).

The biology of B. impatiens was investigated by Wilkinson and Daugherty (1970). The eggs are laid on the soil surface in cracks, under organic debris or under dead adults, and hatch in approximately 4 days into translucent white larvae with black head capsules. The first 3 of the 4 instars are increasingly larger, but similar in appearance; the fourth larval instar can be distinguished by the appearance of dorsal prothoracic imaginal eye discs. During the onset of the prepupal stage, the larvae turns from its partially transparent condition to opaque white and seeks a drier area in which to pupate. The adult emerges in approximately 4 days, with the males emerging 1 to 2 days prior to the females. Development is completed in approximately 3 weeks at 25^{o} C.

The male appears to actively search for the female near the surface of the soil. When near a female, the male begins wing fanning and approaches her in jerky movements. The male curls his abdomen forward underneath his body as he approaches the female, while opening and closing his

claspers. After grasping the female's abdomen with his claspers, the male pivots 180° and couples in an end to end position (Wilkinson and Daugherty 1970).

Although several cultural control methods have been tried for Sciarids (Thomas 1931; Ellisor 1934), and many parasites and predators have been recorded (Hungerford 1916; Ellisor 1934; Madwar 1937; Thomas 1942; Poinar 1965; Steffan 1966), these have not prevented crop damage. The only insecticide registered for use on Sciarids is resmethrin, which is aimed at control of the adults rather than the damaging larval stage.

Due to the lack of an effective control, an alternate method is needed, and control utilizing the pheromone system of B. impatiens may prove to be successful. Pheromone systems have been reported for several species of Diptera. However, relatively few dipteran pheromones have been isolated and studied (Fletcher 1977). Fletcher lists 15 species of flies in which a pheromone evidently mediates attraction of the opposite sex.

The pheromone system of a species closely related to B. impatiens, Lycoriella mali (Fitch) was investigated by Kostelc (1977). He suggested that the pheromone was a series of n-alkane hydrocarbons, of which n-heptadecane elicited the best response. N-heptadecane was found in the abdomens of females, with a lesser amount on the males' abdomens.

The purpose of this study was to establish the possible existence of a pheromone system in B. impatiens and determine its role in the reproductive behavior of the fly. The factors which affect its production in the female and its response in the male were also studied. The experiments chosen were aimed at aiding the eventual use of the pheromone in a greenhouse pest management program.

METHODS AND MATERIALS

Culture Technique

A culture of dark-winged fungus gnats, B. impatiens was reared using a method modified after Kennedy (1973). Shell vials (25 x 95mm) were filled with 10 ml of a 2% non-nutritive agar solution, slanted to harden at an angle of 30° and sealed with a soft plastic stopper. To decrease fungal growth inadvertantly introduced into the test tubes on adult flies, granular agar, instead of finely-ground alfalfa as described by Kennedy (1973), was sprinkled over the solidified agar in each tube. The granules absorbed any excess surface moisture that could entrap the flies. To aid in removal of fungal spores on the flies' tarsi before being placed into the vials, adult flies were placed for a minimum of 1 hour into 15 by 60 mm petri dishes lined with damp filter paper. An adult female and 2 adult males were immobilized by cooling and placed into a shell vial, where mating and oviposition took place. Larvae were fed a 1 : 4 mixture of Brewer's yeast and finely-ground alfalfa, the latter serving as a spreader for the yeast. The culture was maintained at 25±1°C with a 16: 8 L:D light dark cycle in

a controlled environmental chamber with lights on at 0430 hours.

Orientation Tube Experiments

An olfactometer comprised of 10 glass orientation tubes 1 m in length and 3 cm in diameter was used in all bioassays. Each glass tube was fitted on one end with a 105° connecting tube, which in turn connected to a glass manifold that distributed air equally to each of the 10 orientation tubes in the olfactometer. Wind velocity during the trials was 1.9 m/min in each of the 10 tubes. The air flow was forced through a water-filled Erhlenmeyer flask to maintain high humidity. Additional moisture was supplied by placing a 20 mm x 10 mm piece of moistened cotton dental gauze in each orientation tube.

Male and female flies for behavioral tests were isolated by sex at least 4 hours prior to testing.[1] Before each experiment, flies were introduced into the downwind end of the orientation tube and allowed to acclimate to the tube conditions for at least 15 minutes. In each experiment the orientation tubes contained 6 males, except for the test establishing male attraction to live females, in which there were 3 males per tube.

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[1] B. impatiens is monogenic (Metz 1925), so that most flies emerging in each shell vial were the same sex. However, an occasional adult of the opposite sex was found.

A double thickness of cotton gauze placed on the far end and a small circle of copper screening fitted into a rubber washer placed on the tube end nearest the pheromone prevented flies from escaping.

A triangular piece of filter paper (1.5 cm base and 2.5 cm sides) held on a piece of wire positioned into cork was used to dispense the test substance. At the beginning of an experiment, pheromone-impregnated paper was placed into the orientation tube air stream by inserting the cork into the top arm of a Y-shaped connecting tube. The piece of filter paper was removed after each treatment in the orientation tube and inserted into the air stream of the subsequent orientation tube. Each filter paper was used for 5 treatments, and a pheromone dosage of 30 F.E. was used in all experiments except the dose response series. Both orientation and wing fanning (rapid wing vibration) responses were monitored at intervals of 15, 30 and 60 seconds. A positive orientation response was defined as upwind movement of a fly into the first 10 cm of the tube. The activity of the fly was monitored for 60 seconds prior to the experiment in each individual tube as a control period. Percentage orientation response was calculated using the formula:

$$\frac{\text{Stimulus Response-Background Response}}{\text{n - Background Response}} .$$

No wing fanning response was observed during the control in any of the experiments.

All tests except the diel periodicity experiment were run at 1900 or 1930 hours. For each trial, flies were used only once. All glassware and equipment was thoroughly rinsed in acetone before use.

Testing for the Presence of a Pheromone

To substantiate the presence of a pheromone released by the female to attract the male, 5 live females were placed into a copper wire mesh cage, approximately 25 mm in length and inserted into the Y-shaped tubes. The cage was removed at the end of the 60 second time period and inserted into the air stream of the next tube so the same female flies were used in all trials. A total of 73 male flies was tested in the experiment.

To test for possible female attraction to males, 4 male flies were placed into the wire mesh cage and introduced into the air stream. The same cage of male flies was used for all orientation tubes in the olfactometer, and a total of 57 females was tested in the experiment. The ages of both male and female flies were variable, as individuals of both sexes were chosen randomly from the culture.

Homogenized vs. Non-homogenized Female Extract

To determine the effect of homogenization of the female extract on male response, homogenized and non-homogenized female extracts were compared. The pheromone extract was prepared by placing 100 female flies in methylene chloride for 2 hours. The extract was filtered and evaporated under N_2 to 1 ml. For this experiment, 1 of the 2 groups was first blended in a glass homogenizer before being placed into the methylene chloride. The other group of females was soaked uncrushed. A total of 83 male flies was tested with the homogenized extract and 80 flies with the non-homogenized extract.

Dose Response Experiments

To determine the effect of pheromone on male wing fanning and orientation, 5 different concentrations of extract were tested. One hundred females of varying ages were used in making 1 ml of non-homogenized female extract. However, in this experiment, redistilled methylene chloride was poured over live females. The extract was allowed to stand for 25 minutes, filtered and evaporated with N_2.

Pheromone doses of 1, 5, 10, 30 and 50 F.E. were placed on filter paper and tested in the orientation tubes using a randomized complete block design. Total numbers of males tested for each treatment were 61 at 1 F.E., 61

at 5 F.E., 84 at 10 F.E., 71 at 30 F.E. and 67 at 50 F.E.

Diel Periodicity of Pheromone Responsiveness

Males were tested every 4 hours starting at 1900. An additional time 1 half hour after lights out was included to determine the effect of lights-off. The total number of males tested at each time period was 117 at 0700, 109 at 1130, 118 at 1500, 106 at 1900, 115 at 2100, 113 at 2300 and 118 at 0300. Wing fanning and orientation responses were recorded as the maximum number of males responding at either the 15, 30 or 60 second time periods. Light with luminosity below full moon level (0.05 lux) and a low intensity light filtered with a Kodak Wratten filter No. 29, which eliminates light below 6100Å, allowed observation of fly activity during periods of darkness.

Site of Pheromone Production on the Female

To determine the possible origin of the pheromone, extracts of various female body regions were tested for male response. Live females were placed in a wire mesh cage and lowered into the air stream of an orientation tube containing male flies. Females which elicited no male orientation or wing fanning response were not used in the extracts. Each female chosen was immobilized by cooling and was dissected into the following 4 body

regions: head, legs, thorax and abdomen and placed into methylene chloride. Legs were dissected so that each leg included as much of the coxa as possible.

Based on results in the diel periodicity experiments, the females were dissected between 1900 and 2400 hours. The dissected body regions remained in the methylene chloride for 25 minutes after the last of the 100 females was dissected. At the end of this procedure, the first females dissected may have been in the solution for up to 5 hours. The extracts were tested between 24-70 hours after preparation. The experiments were run in a randomized complete block design with 94 males tested with the head extract, 96 with the abdomen extract, 91 with the thoracic extract and 96 with the leg extract.

Age of the Male

Males 1, 2, 3 or 4 days-old were tested to determine if age affects response. The experiment was run in a completely random design with a total of 81 1 day-old males, 78 2 day-old males, 75 3 day-old males and 65 4 day old males.

Age of the Female

Newly-emerged adults (less than 12 hours-old), 1, 2 and 3 day-old females were tested to determine the effect of the age of the female on pheromone production. Females older than 3 days were not used due to the

decrease in available numbers. The experiment was run in a randomized complete block with a total of 63 males tested with the extract from the females less than 12 hours-old, 65 males with the 1 day-old extract, 68 males with the 2 day-old extract and 64 males with the 3 day-old female extract.

Virgin vs. Mated Females

One day-old virgin females were divided into 3 groups of 100 flies. Males were introduced into 2 of the 3 groups 28 and 4 hours before testing and allowed to mate. Females in the 3rd group remained unmated. Thus, 3 types of females were tested: 1) virgin females, 2) females mated for 4 hours and 3) females mated for 28 hours.

The experiment was run using a completely randomized design, with a total of 60 males tested with the virgin female extract, 66 with the extract from females mated for 4 hours and 65 males with the extract from females mated for 28 hours.

Mating Sequence

To establish possible ordering of behaviors involved in mating, mating sequences of 20 virgin females were recorded on videotape. The females were immobilized by cooling and placed in a 15 x 60 mm plastic petri dish

lined with moistened filter paper. When the females regained activity, 1 or 2 males were placed into the petri dish and the videotapes were analysized with the aid of a stopwatch.

Windtunnel Observations of Attraction

Males 2 days-old were separated from females for at least 6 hours prior to testing and were released from a platform 9 cm from floor level into a Plexiglas^{R} and aluminum windtunnel (1.4 x 0.8 x 2.8 meters) equipped with a movable floor of alternating green and white stripes (Cardé and Hagaman 1978). Wind velocity in the windtunnel was 38m/min. A moistened filter paper was placed on the platform as an incentive for the males to remain on the platform, as the males seem to require high humidity. As a control, males were tested for upwind flight before the introduction of the pheromone. The pheromone extract of 30 F.E. was introduced into the windtunnel 1.35 m upwind of the males. Duration of response was defined as the time the fly initiated flight until landing on the platform with the pheromone source. The role of visual cues in modulation of upwind flight was tested by introducing males into the windtunnel and intermittantly moving the floor backwards to maintain flight around 0.7 m from the pheromone source for

approximately 4 minutes. The potential duration of upwind flight given an optomotor feedback was not tested.

RESULTS

Orientation Tube Trials

Testing for the Presence of a Pheromone

Male wing fanning and orientation was significantly ($p<.05$) greater in the presence of live females than the control without females at 15, 30 and 60 seconds (Figure 1). When presented with the caged females, 83.6% of the males showed an initial wing fanning response at 15 seconds. This decreased significantly to 64.4% at 30 seconds and 63.0% at 60 seconds. Orientation increased significantly from 12.3% at 15 seconds and 17.8% at 30 seconds to 31.5% at 60 seconds ($p<.05$).

There was no evidence of female attraction to live males. The females remained motionless during the control period and after the live males were introduced upwind in the olfactometer.

Homogenized vs. Non-homogenized Extract

The male response of wing-fanning and orientation to the non-homogenized extract was significantly greater than the homogenized extract at 15, 30 and 60 seconds (Figure 2). Wing fanning for the homogenized extract

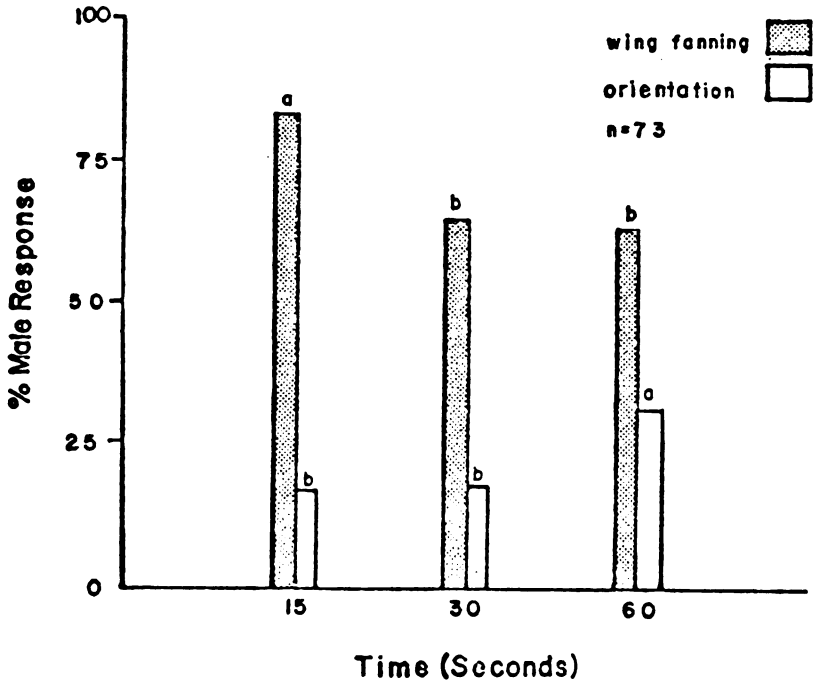

Figure 1.--Percentage of male wing fanning and orientation responses at 15, 30 and 60 seconds to live females. All wing fanning or orientation responses are significantly different ($p<.05$) from the control. All percent responses for wing fanning or orientation are not significantly different from the percent responses in other observation periods for that particular behavior if the behavior is followed by the same letter by Chi square 2 x 2 test of independence.

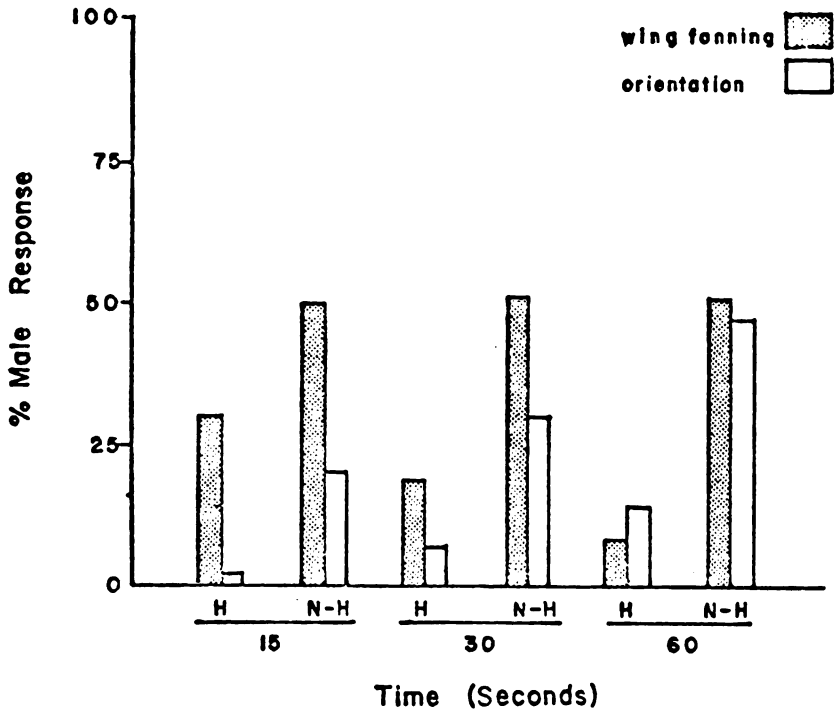

Figure 2.--Percentage of wing fanning and orientation responses of males at 15, 30 and 60 seconds to homogenized vs. non-homogenized female extracts. All percent responses for non-homogenized wing fanning or orientation responses are significantly different ($p<.05$) from the percent responses of the homogenized wing fanning or orientation responses by Chi square 2 x 2 test of independence.

reached a peak at 15 seconds with 30.1% of the males responding, while the non-homogenized extract had 51.3% wing fanning at both 30 and 60 seconds. The greatest amount of orientation for both the non-homogenized (47.5%) and the homogenized female extracts (14.5%) occurred at 60 seconds.

Dose Response Experiments

The pheromone concentration of 30 F.E. elicited the highest response for both orientation and wing fanning at 60 seconds, although wing fanning was not significantly different from 50 F.E. (Figure 3). Wing fanning increased from 3.3% at 1 F.E. to 49.3% at 30 F.E. Orientation increased from 11.5% at 1 F.E. to 56.3% male response at 30 F.E. A significant decrease in orientation to 16.4% occurred when the extract was increased to 50 F.E. Similar results were noted at 15 and 30 seconds. At 15 seconds 30 F.E. elicited the most orientation response. At 30 seconds, 30 F.E. elicited a significantly greater proportion of wing fanning and orientation than the other concentrations.

Diel Periodicity Tests

The results in Figure 4 show that both male wing fanning and orientation increased in activity during the day, with a peak in male activity (29.2%) occurring in

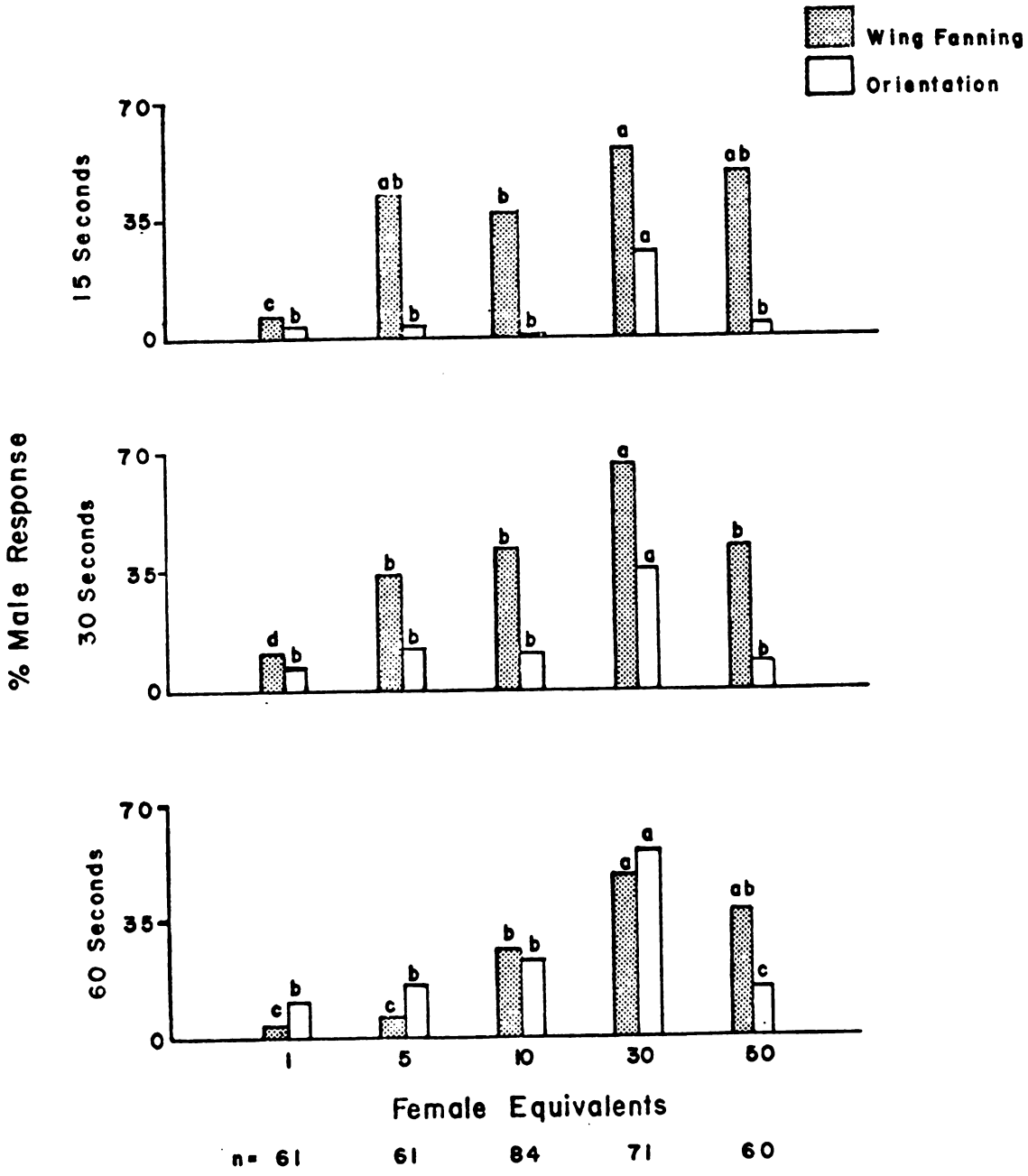

Figure 3.--Percentage of wing fanning and orientation responses at 15, 30 and 60 seconds for B. impatiens males to various concentrations of female extract. Percent responses of wing fanning or orientation are not significantly different ($p<.05$) by a Chi square 2 x 2 test of independence if percentage responses for a particular behavior are followed by the same letter.

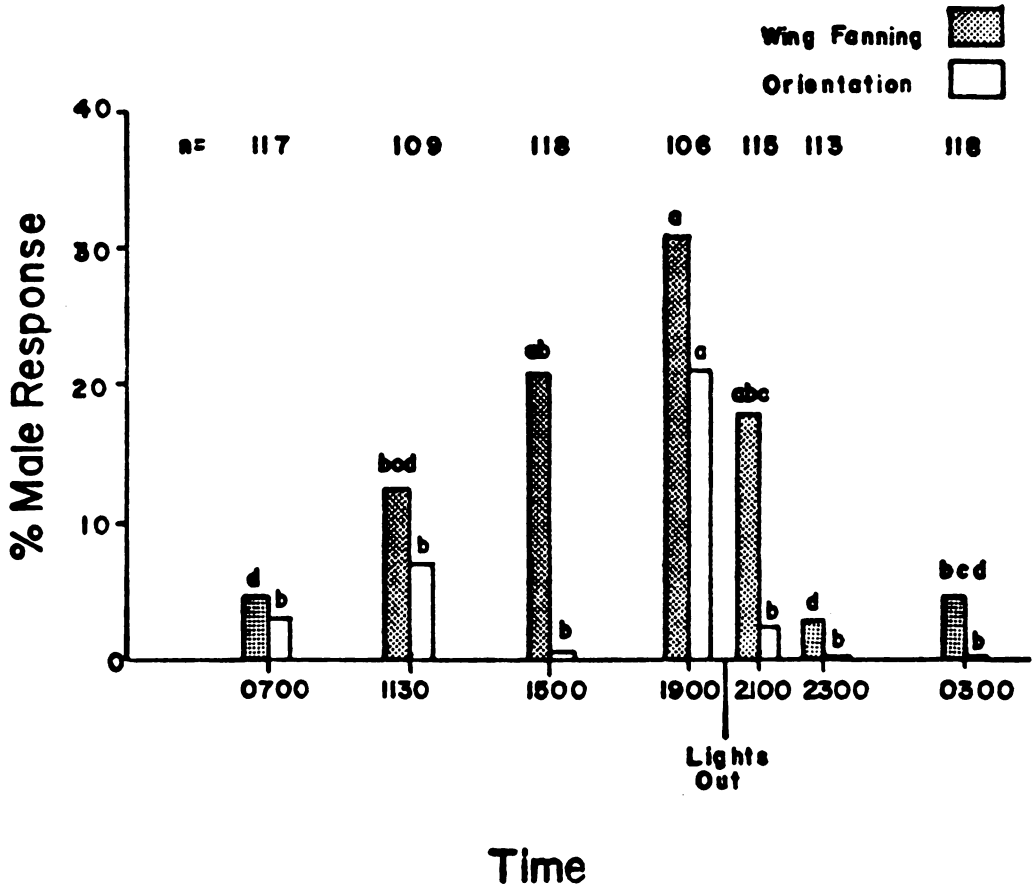

Figure 4.--Percentage of male wing fanning and orientation responses for the diel periodicity experiments. Responses of wing fanning or orientation are not significantly different ($p<.05$) by a Chi square 2 x 2 test of independence if percentage responses for a particular behavior are followed by the same letter.

the early evening at 1900 hours, followed by a decline in response after lights-out at 2030 hours. The lowest proportion of wing fanning occurred at 0700 hours with only a 4.3% male response. The lowest percentage of orientation was recorded at 3400 and 0300 hours, both of which had no responding males, while the maximum amount occurred at 1900 hours (21.7%).

Site of Pheromone Production on the Female

Dissection and testing of the 4 female body regions showed that males responded most strongly to the 30 F.E. extract of the females' legs (Figure 5). Male wing fanning and orientation responses to the legs at 60 seconds were 55.2% and 52.1%, which were significantly higher than the other treatments. Female thoracic extracts elicited the second highest orientation response (36.3%), followed by the response to the abdomens (22.9%). The percent wing fanning response for the thoracic extract (16.9%) was not significantly different from the abdomens (11.6%). There was no wing fanning response, and 8.5% orientation to the extract of the heads.

Age of the Male

Orientation reached a peak of 46.2% response at 60 seconds with 2 day-old males, which was significantly higher than 1 and 3 day-old males (16% and 29.3%), but

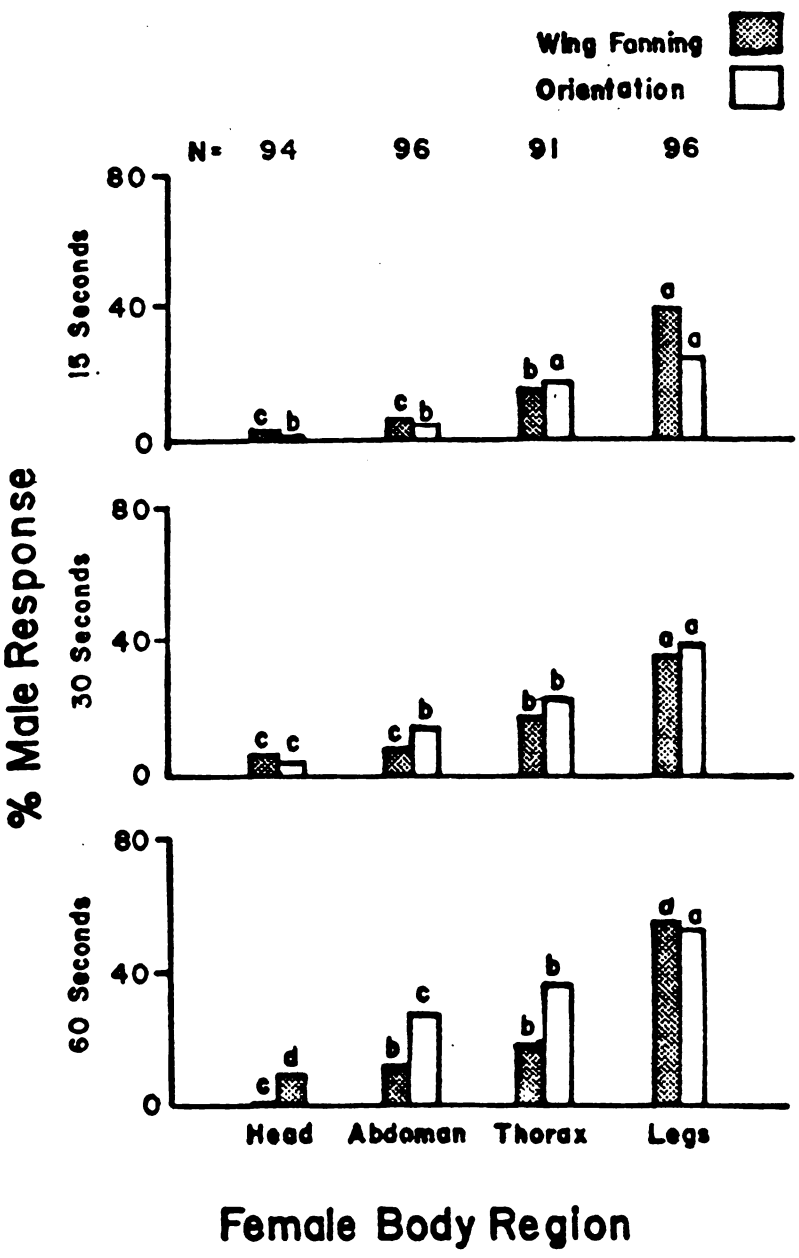

Figure 5.--Percentage of male wing fanning and orientation response at 15, 30 and 60 seconds to various female body regions. Percent responses of wing fanning or orientation are not significantly different ($p<.05$) by a Chi square 2 x 2 test of independence if percentage responses for a particular behavior are followed by the same letter.

not 4 day-old males (30.8%, Figure 6). Orientation response from 2 day-old males (33.35%) was significantly higher than all other treatments at 30 seconds, but the 2 day-old males at 15 seconds (23.1%) were not significantly higher in orientation than the 4 day-old males (10.8%).

Wing fanning at 30 and 60 seconds was not significantly different among the 4 treatments. However, in the 2 day-old group of males, there was an additional percentage which flew up and down along the surface of the screening. This flying activity was not exhibited in any of the other age groups and would represent only an additional 12.8% of the 2 day-old males at 60 seconds or 4.0% at 30 seconds. Wing fanning at 15 seconds for the 1 day-old males (26.0%) was significantly lower than the 2-day old (44.9%) and the 4 day-old males (40.0%).

Age of the Female

There was a significant increase in the amount of both male wing fanning and orientation in response to extracts of females 1 day-old and older compared to newly emerged (less than 12 hours old) females (Figure 7).

There was no difference in male response to the extracts of the 1, 2 and 3 day-old females for orientation at 60 seconds, and all 3 age classes elicited a higher orientation response than the females less than

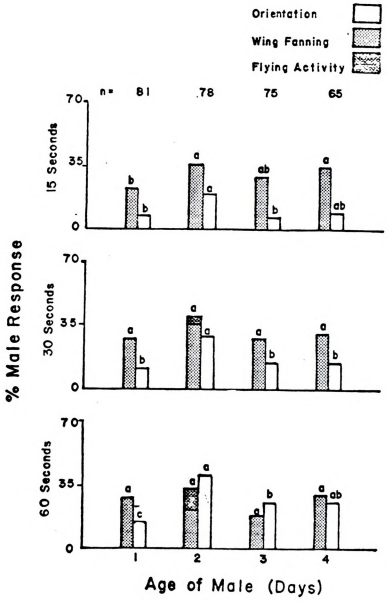

Figure 6.--Percentage of male wing fanning and orientation at 15, 30 and 60 seconds using various ages of the males. All percent responses for wing fanning or orientation are not significantly different ($p<.05$) by a Chi square 2 x 2 test of independence if percentage response for a particular behavior are followed by the same letter.

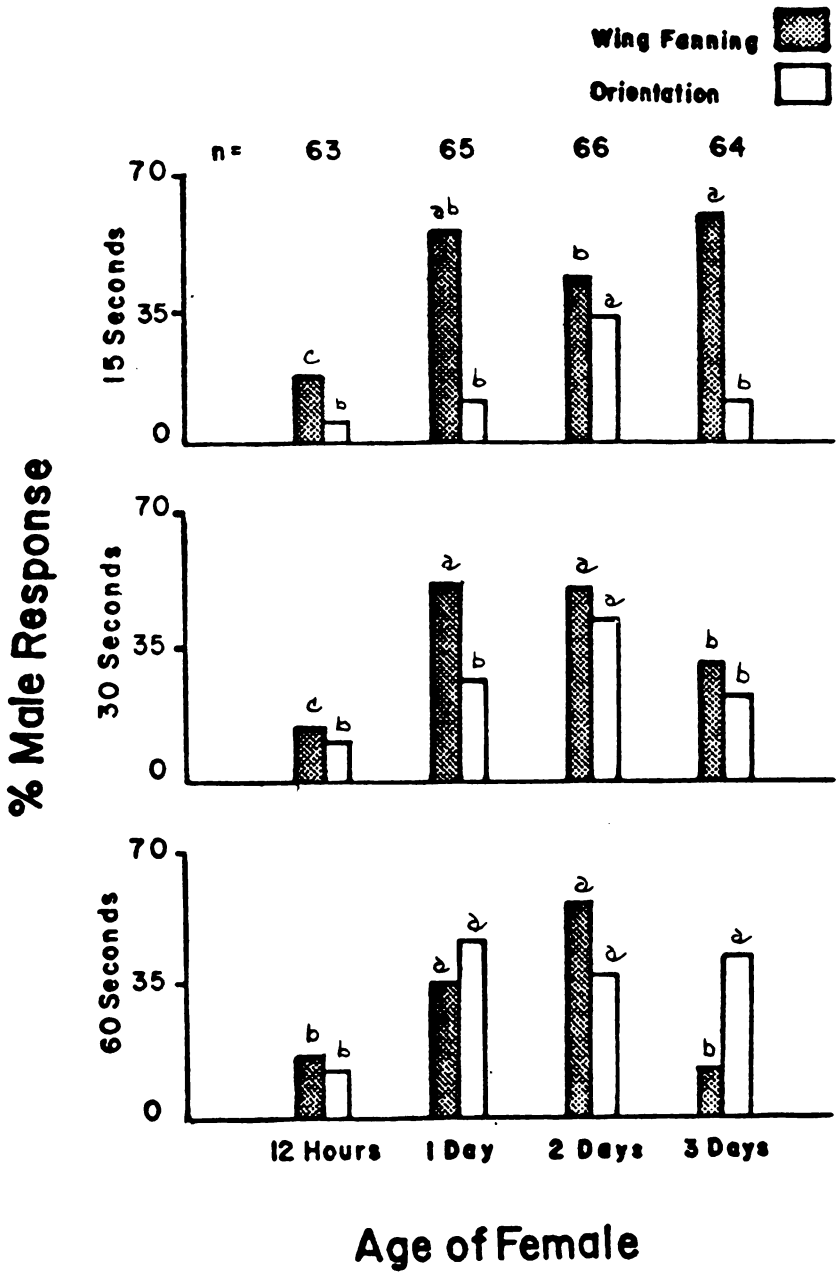

Figure 7.--Percentage of male wing fanning and orientation at 15, 30 and 60 seconds using extracts from various ages of the females. All percent responses for wing fanning or orientation are not significantly different ($p<.05$) by a Chi square 2 x 2 test of independence if percentage responses for a particular behavior are followed by the same letter.

12 hours old. Extracts from 2 day-old females elicited the highest male orientation at 15 and 30 seconds.

The 1, 2 and 3 day-old female extracts were significantly greater than the females less than 12 hours-old in percentage of wing fanning at 15 and 30 seconds, and the 1 and 2 day-old extract elicited a greater wing fanning response than the 12 hour-old females at 60 seconds.

Fertilized vs. Virgin Females

No significant difference in male response was found between 30 F.E. extracts from virgin females, females mated for 4 hours and for 28 hours (Figure 8).

Sequence of Mating

The typical mating sequence (with the percentage of occurrence in parentheses, n = 20) of B. impatiens occurred as follows: 1) the male ceased movement (100); 2) the antennae were raised (100); 3) the male wing fanned while walking in jerky movements (100); 4) the male approached the female (still wing fanning) while curling his abdoment forward beneath his thorax and opening and closing his claspers (100); 6) the male pivoted 180^{o} while clasping the female (100); 7) mating occurred (95) the male released the female (100); and 9) the male ceased moving (100). The 10th step was 1 of

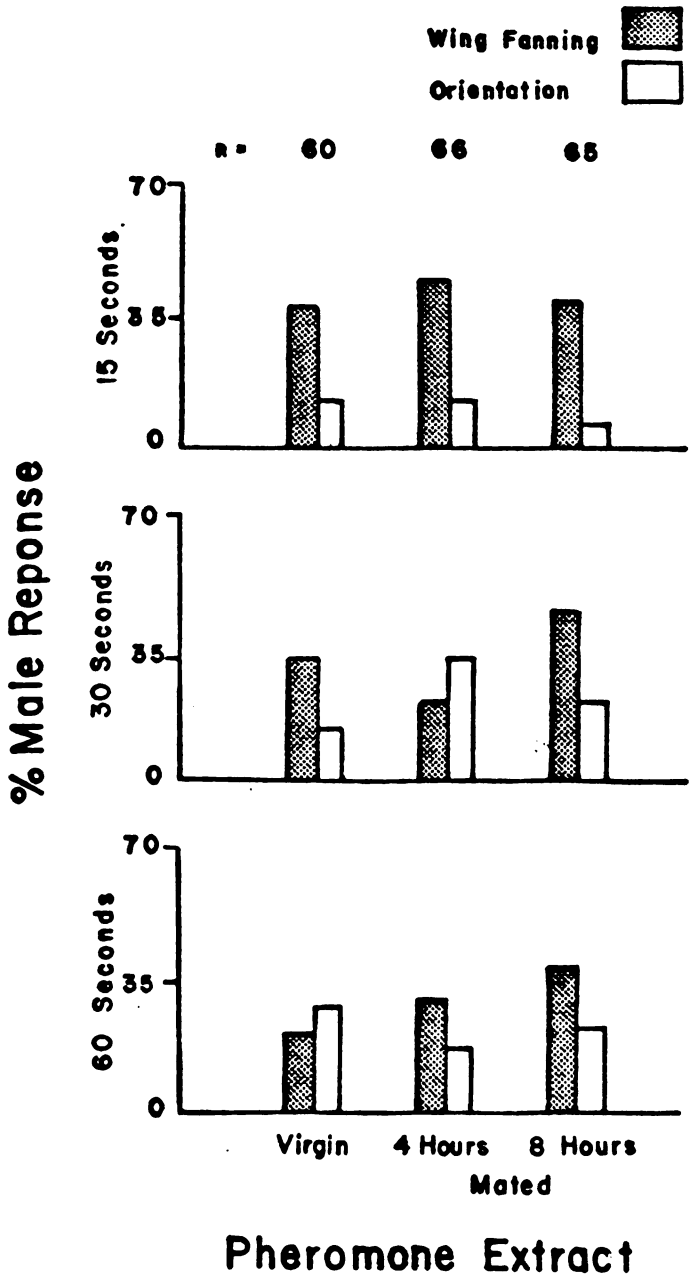

Figure 8.--Percentage of male wing fanning and orientation at 15, 30 and 60 seconds using extracts from virgin and mated females. All percent responses for wing fanning or orientation are not significantly different ($p<.05$) by a Chi square 2 x 2 test of independence.

the following behaviors: 1) grooming, where the wings were wiped on the filter paper and the abdomen was cleaned with the metathoracic legs (52.6), 2) wing fanning behavior (26.3) or 3) apparently random walking throughout the petri dish (21.1). The female remained motionless during the majority of the mating sequence. However, in 8 of the 20 sequences the female walked forward as the male approached while curling his abdomen underneath his thorax. In each case, the male followed the female while continuing to curl his abdomen.

The mating sequences were timed for duration of the behavior in the sequence in which mating occurred, although 4 males actually mated on the 2nd attempt, and 1 male mated after the 3rd try. One male did not mate after 4 attempts, but its mating sequence of events began in a similar fashion. After the 4th attempt, the male began apparently random walking throughout the dish. Percentages of responses and duration of these main behaviors in the sequence are shown in Table 1.

Windtunnel Observations of Attraction

The percentage of males flying upwind 1.35 m to the pheromone was 69.3% (n = 29). Males which did not respond within 2 minutes were not included.

The flight durations ranged from 27 to 215 seconds, with a mean of 90.7 seconds. When the males were given

Table 1.--Percentage of occurrence of male responses and duration of the main behaviors in the mating sequence of B. impatiens.

Behavior	% Occurrence	Duration Range	Mean Duration
Antennal response	100	---	---
Wing fanning	100	1.5 - 59 sec	17.9 ± 21.9 sec
Wing fanning and abdomen curling	100	0.5 - 15 sec	3.6 ± 0.05 sec
Mating	95	187 - 500 sec	285.7 ± 0.05 sec
Pause	100*	4 - 120 sec	24.3 ± 28.5 sec
Grooming	52.6*	---	---
Wing fanning	26.3*	---	---
Random movement	21.1*	---	---

*Percent from those successfully mating.

an optomotor feedback by moving the ground pattern backwards, however, the mean flight time increased to 271 seconds (Table 2).

Flight upwind to the pheromone extract ranged from a slow, zig-zag pattern with wide oscillations throughout the pheromone plume to a faster, more direct flight with less zig-zag motion. Males tended to remain approximately 5 - 10 cm from the floor of the windtunnel in both cases, moving upwards to the stimulus only shortly before reaching it. Landed males wing fanned after reaching the paper.

When the ground pattern was moved, males tended to fly approximately 15 - 25 cm above the windtunnel floor. Although all males were released 1.35 meters from the pheromone, males could be manipulated by floor pattern movement backwards almost 2 m and still remain in apparently oriented flight in the plume.

Table 2.--Comparison of backward motion of the windtunnel floor pattern to no floor movement on the flight duration of B. impatiens males. The mean flight times are significantly different using the t-test ($p < .01$).

Flight in Seconds	
Stationary Floor Pattern	Moving Floor Pattern
195	200
215	270
98	505
106	305
146	195
27	275
27	268
32	247
34	239
75	252
84	240
49	243
	255
	320
	251
$\bar{x}$ = 90.7 ± 65.0 sec	$\bar{x}$ = 271 ± 72.5 sec

DISCUSSION

The results in Figure 1 demonstrated the presence of a sex attractant emitted by the females of B. impatiens. Other apparent sex attractants have been reported in Diptera. Fletcher (1977) listed 10 species of flies in which the female releases a "sex attractant" for the male and 5 others in which the male "attracts" the female.

Results from this study indicated that the non-homogenized female extract was more effective in eliciting male response in B. impatiens, suggesting the pheromone may be cuticular in origin, instead of glandular. However, the homogenization of the females might result in the emission of other compounds which could either lower responsiveness or retard volatilization.

Although Dipteran pheromones are found both in glands and in cuticular waxes, flies which possess a pheromone system effective in distance (<1 m) communication generally release the pheromone from a gland (Fletcher 1977). The communication system of B. impatiens can be defined as a long-distance sex attractant, since the male responds to the extract from distances over 1 m.

Orientation tube tests with B. impatiens indicated a greater male wing fanning and orientation response to the thoracic and leg extracts than to extracts of the abdomens and heads of the females (Figure 5). The leg extract was significantly greater in orientation than all the other extracts, and males exposed to the leg extract responded with more direct flight orientation and remained active longer than those exposed to the other extracts. However, the experiments can not conclusively determine the region of pheromone production in the female. The female's activity of rubbing her legs over her abdomen during preening when the male is near could affect pheromone distribution.

B. impatiens females showed a peak of pheromone production on the 2nd day after eclosion. For both glandular and cuticular sources of the pheromone in some Diptera, female flies may not respond to or release a pheromone until attainment of sexual maturity (Fletcher 1977). Murvosh (1965) found in Musca domestica L. that mature male house flies were attracted to females 7 days old, but not to newly emerged flies between 1 and 7 hours of age.

Similar peaks in the amount of male response due to increasing age of the males have been reported in Diptera. Maximum response in the male sheep blowfly,

Lucillia cuprina (Wied.), occurred on the 4th day after eclosion (Bartell at el. 1969). Murvosh (1974) reported that male M. domestica will not mate for at least 16 hours after eclosion, and most males mated when they were 26 hours old. B. impatiens males exhibited a higher level of orientation after 1 day of age.

Mating of the female did not affect pheromone production of B. impatiens. Rogoff (1964) and Silhacek et al. (1972) found no significant difference in male responsiveness between virgin and mated females of M. domestica.

The range of dosages of F.E. used in Dipteran pheromone studies is very broad. Tests run by Haniotakis et al. (1977) on the sex attractant of the olive fruit fly Dacus oleae (Gmelin) used concentrations of 50 F.E. Kostelc (1977) found, however, that in Lycoriella mali 10^{-1} to 1.F.E. were optimum and 10^{2} F.E. seemed to have a "repellent" effect. No response in the male was elicited with B. impatiens in the orientation tubes at concentrations lower than 1 F.E., and while male wing fanning and orientation response diminished at the concentration of 50 F.E., no "repellent" effect was noticed. The discrepancy between B. impatiens and L. mali male response may be due to the differences in the methods of behavioral assay. Kostelc's (1977) olfactometer was a Plexiglas^{R} box 8.2 cm^{3}, which involves a smaller distance to the

stimulus than the 1 m tubes used with B. impatiens. The assay time of Kostelc's (1977) tests (no time stated) could suggest that the pheromone's effect also could be related to a locomotory "arrestant."

B. impatiens males, in the greenhouse, are sexually responsive during many times of the day and mating occurred in confined conditions, such as in the shell vials or petri dishes, throughout the day. The results of the periodicity trials, however, indicated an increase in activity in the late afternoon and early evening, with a maximum at 1900 hours. Diurnal mating is known to occur in other Diptera.

Females of Dacus tryoni (Frogg), the Queensland fruit fly, are attracted to the pheromone released by the male during the late afternoon and early evening (Fletcher 1977), while the female Hessian fly Phytophaga destructor (Say) is most active in the early morning (Cartwright 1922). The periodicity of upwind attraction for B. impatiens may be narrower than that of the mating rhythm.

The duration of the precopulatory behaviors in the mating sequence varied considerably. Males frequently paused in wing fanning after the initial wing fanning response before continuing with the next step in the sequence. Time spent in abdominal curling appeared dependent upon the action of the females. If the female was

receptive to the male, the male clasped the female on the 1st attempt. However, if the female would move forward slowly after the male was near, the male frequently followed her curling his abdomen. One male was observed to continue this behavior for 15 seconds as he pursued the female around the petri dish.

In the petri dishes, wing fanning preceded orientation in every mating sequence. In the orientation tubes, wing fanning was also found to be a precursor to orientation. Using the male responses in wingfanning and orientation collected during the experiments to determine the effect of the age of the males on male response, there was no significant difference from the number of males wing fanning at 15 seconds and those oriented at 60 seconds. Wing fanning is always a part of the male mating sequence of B. impatiens, and it could serve as a "key" response for behavioral bioassays. However, the pheromone may be a blend of compounds with the reactions of wing fanning and upwind walking or flight elicited by different blend combinations (Cardé et al. 1975).

Although Fletcher (1977) listed other species of flies which release sex attractants, he stated that most of the attraction occurred in olfactometers involving a distance of only a few cm of "attraction." These assays were usually conducted over comparatively

long intervals and involved the teleological categories of "attraction" and "arrestment." The actual locomotory reactions remain undefined as pointed out by Kennedy (1978). Kellogg et al. (1962) demonstrated the modulation in Dipteran upwind flight using an optomotor feedback of a moving floor pattern in Drosophila melanogaster Meigen to a food-odor stimulus. B. impatiens, however, is the first Dipteran in which long-distance upwind flight using evident optomotor anemotaxis coupled with reversing anemomenotaxis to a sex attractant has been demonstrated.

Because of the high percentage of males orienting to the pheromone extract in the windtunnel (79.3%), a good possibility exists for the eventual use of B. impatiens pheromone traps once the pheromone has been isolated, identified and synthesized.

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