SEXUAL ATTRACTION, MATING BEHAVIOR, AND DEMONSTRATION OF A FEMALE SEX PHEROMONE IN THE HESSIAN FLY, *MAYETIOLA DESTRUCTOR* (SAY) (DIPTERA: CECIDOMYIIDAE)

by

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INTRODUCTION

The discovery of insect sex pheromones and the potential use of chemical sex attractants in insect control have generated much research on the reproductive behavior of insects, particularly the role of pheromones in sexual behavior. Sex pheromones are defined as chemicals produced by either males or females that elicit one or more behavioral reactions in the opposite sex (Shorey 1973). Sexual behavior act as stimuli-response patterns to insure the fertilization of the ova (Barth and Lester 1973). These mechanisms include all activities proceeding from the attainment of sexual receptivity and terminating with the separation of the sexes following mating. Therefore, sex pheromones serve as chemical stimuli that elicit a sequence of behavioral responses to complete mating.

Sex pheromones have been demonstrated in over 100 species representing the orders Orthoptera, Homoptera, Coleoptera, Lepidoptera, Hymenoptera, and Diptera (Glass 1975). Since 1975 additional sex pheromones have been found. In the past few years, many of the sex pheromones have been isolated and characterized. However, studies demonstrating the presence of sex pheromones in Diptera and the elucidation of their chemical nature are limited. Fletcher (1977) listed 15 species of Diptera in which a pheromone was used as a sex attractant. The pheromone was released by the females in 10 species and by the male in 5 species. The species belonged to only 6 families: Culicidae, Syrphidae, Tephritidae, Drosophilidae, Chloropidae, and Muscidae.

There has been little effort to determine the role of sex pheromones in the gall midges, Cecidomyiidae. The gall midges comprise one of the largest groups of Diptera and several species are economically important pests worldwide. The Hessian fly, *Mayetiola destructor* (Say) is one of the most important pest species in this group and has been a major economic pest of wheat in the United States since the insect was first introduced in the late 1700s. Although numerous studies have been made on many facets of its biology, there have been few observations and experiments detailing the sexual attraction or mating behavior of the Hessian fly. Knowledge of the insect's sexual behavior and the role of pheromones in controlling behavior are important from the standpoint of understanding the reproductive biology of the species.

Previous observations of the adult biology and behavior of the Hessian fly provide some clues to suggest that a sex pheromone is involved in sex attraction and mating. Cartwright (1922) demonstrated that Hessian fly females attracted males in the field with the greatest attraction occurring in the early morning. McColloch (1923) observed that adult flies mated soon after emergence, often before the wings and body had assumed the usual dark color, and oviposition began in the morning from 1 to 3 hours after emergence, Enoch (1891) noted that shortly after eclosion virgin females usually hung from the undersides of leaves with their ovipositors fully extended in a "calling" position.

This study was undertaken with a broad objective to investigate the sexual attraction, mating behavior, and the presence of a sex pheromone in the Hessian fly. Specific objectives of the study were to (1) substantiate

and clarify the role of females in attracting males, (2) determine the role of ovipositor extension as an element of mating behavior, (3) determine the effect of female age and time of day on mating behavior and sex attraction, and (4) identify pheromone activity in various chemical extracts of virgin females.

PART I

SEXUAL ATTRACTION AND MATING BEHAVIOR OF THE HESSIAN FLY

Methods and Materials

Insect Rearing. Hessian flies used in these studies were obtained from a culture that had been reared in a greenhouse for about 10 generations. The culture was predominantly the Great Plains biotype and originated from collections made in Phillips County, Kansas. Rearing methods were similar to those described by Cartwright and LaHue (1944) and Gallun et al. (1961). All rearings were made in a greenhouse or environmental growth chambers. Temperatures were maintained between 21° and 24°C.

Male and female flies were bulk mated and females were caged individually on wheat plants grown in 10 cm diam. plastic pots. Females were allowed to oviposit on the plants for 2 days. Eggs hatched in about 5 days at 21°C and larvae migrated down the leaves, behind the sheaths to the base of the seedlings. The duration of the feeding stage of larvae is about 15 days. The larvae then developed into the puparia stage which lasted about 10 days. Adults of individual progenies emerged for about 7 to 10 days. Thus, the Hessian fly completed its life cycle in about 30 days under these rearing conditions (Cartwright and LaHue 1944).

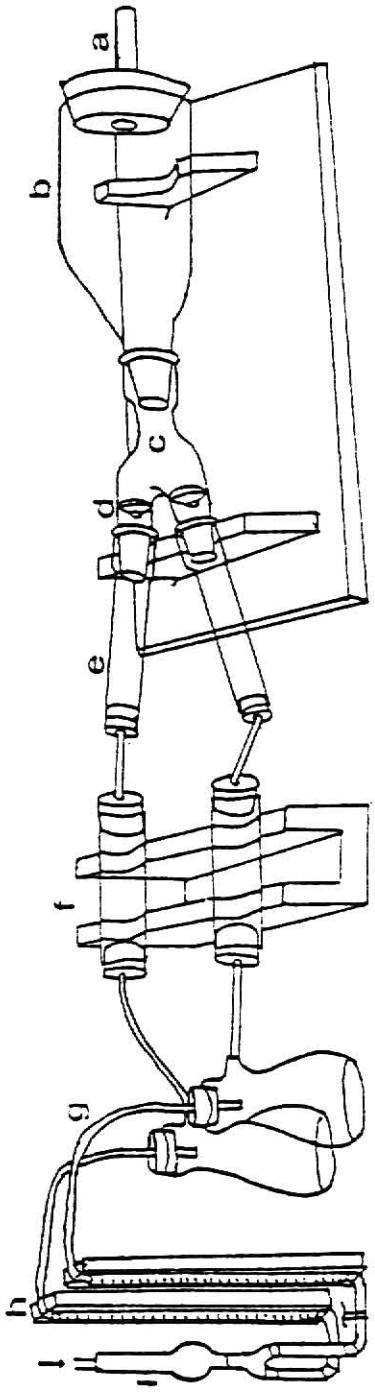
Prior to adult emergence, a clear plastic cage was placed over each pot to confine the adults. The Hessian fly breeds by unisexual families, i.e., most progenies of individual females are either male or female (Painter 1930). Thus, virgin females and males were readily obtained by

observing the sex of the progenies. Numerous matings (100 to 200) were made at various times during the studies to ensure that adequate numbers of virgin adults were available.

Attractiveness of Live Flies. A Y-tube olfactometer similar to the one designed by Chaudhury et al. (1972) was used to determine the sexual attractiveness of adult Hessian flies. The olfactometer consisted of a glass Y-tube (Kimax® 29/42) and two arms of straight glass tubes (Kimax® 29/42) (Figure 1). A glass chamber (6.3 cm diam x 19 cm) formed the base of the olfactometer and was attached to the Y-tube which served as a 2-choice chamber. The chamber was closed at its base by a rubber stopper that contained holes. A center hole (2 cm diam) was used for holding a vial from which the responding flies were released into the chamber. Three other holes were covered with fine mesh brass screen for air passage. Each arm of the Y-tube contained a glass funnel with an opening 5 mm in diam. which functioned to trap the "responding" flies. Each arm was connected by a Tygon® tubing to a glass cylinder (4.1 cm diam) or holding chamber. One of the glass cylinders was used to hold the "attracting" flies and the other one was used as the control or "blank" side. The overall length of the olfactometer in operation was 68 cm.

Air was forced through the olfactometer by a vacuum-compression pump. The air generated by the pump passed through three chambers of activated charcoal and glass wool for cleaning and then split into two streams. Each air stream passed through a calibrated Roger Gilmont® flowmeter, a 250 ml Erlenmeyer flask, and then via the holding tube into the arms of the Y-tube. The two air streams came together at the base of the Y-tube.

Figure 1. Diagram of Y-tube olfactometer. A - release vial; B - chamber; C - Y-tube; D - glass funnel; E - arm; F - holding tube; G - Erlenmeyer flask; H - flowmeter; and I - charcoal filter.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The flasks contained 100 ml of distilled water for maintaining uniform humidity. Plastic tubing connected the pump, flowmeters, and flasks to the two holding chambers of the olfactometer.

For the attractiveness tests the olfactometer was operated in an environmental growth chamber programmed for 21° $\pm$ 1°C and full illumination (1500 ft -c). The rate of air flow through the arms of the olfactometer was calibrated at 260 ml/min. The air speed through the funnel was calculated to be about 22 cm/sec or about 0.5 mph. The olfactometer was washed with acetone and rinsed with distilled water after each use.

Series of bioassays were made to evaluate the attraction of adult male or female flies to the same or opposite sex. Virgin females, mated females, mated males, and virgin males (attracting flies) were tested for their attractiveness to virgin males (responding flies). The attracting flies were tested in groups of 40 with 20 responding flies (2:1 ratio). The attracting flies were collected and placed in a cylindrical, fine mesh, stainless steel cage. The cage was placed into one of the holding tubes of the olfactometer. In the test of mated females, the females were caged with males and allowed to mate for 30 minutes prior to the test. In the test of mated males, the males were allowed to mate with virgin females for 10 minutes prior to the test. Virgin males (attracting flies) were also tested for their attractiveness to virgin females (responding flies). After the attracting flies were placed in the holding tube, 20 responding flies were released from a glass vial at the base of the olfactometer. The flies were free to move upwind into either arm of the Y-tube and were allowed to respond for 20 minutes. Any fly that passed

through the funnel was trapped and considered to have made a choice. The number of responding flies trapped in the arms was recorded. Each attractiveness test was replicated four times. All tests were conducted between 0800 and 1000 hours. The males used in the tests were generally less than 12 hours old and all females were approximately 1 to 3 hours old.

Chi-square tests for significance and for heterogeneity were used. If the chi-square test for heterogeneity was not significant at the P> 0.05 level, the pooled chi-square was used for the significance tests. Nonsignificance for heterogeneity implies that variation in responses of flies among replications was minimal. If there was no attraction, the proportion of flies responding in a test should remain consistent with a theoretical ratio. The theoretical ratio tested was 1:1, i.e., equal numbers of flies should respond to each arm of the olfactometer. If flies failed to respond equally but preferred one arm to the other, the chi-square test would measure the significance of the departure. Comparisons among the different attracting flies were made by using a 2 x 2 contingency table (Snedecor and Cochran 1978).

Ovipositor Extension as an Element of Mating Behavior. Twenty newly emerged females were collected at 0600 hours and caged on wheat plants in a 10 cm diam.plastic pot. Females were observed for ovipositor extension or withdrawal during a 12 hour period in the absence of males. Observations were made immediately after collection at about 0630 hours and at 0800, 1000, 1200, 1400, and 1800 hours when females were about 1/2, 2, 4, 6, 8,

and 12 hours old, respectively. Observations of the same females were repeated for 2 more days at the same time periods.

Ten newly emerged females were also placed one at a time in a cage containing 5 virgin males. The female behavior of extending or withdrawing the ovipositor was observed prior to mating, during copulation, and after mating. The position of the ovipositor after mating was observed until the females began to oviposit. Ten single female-male pairs were also observed to determine whether females mate more than once.

Observations on the flies were made in a modified growth chamber in the laboratory. Temperatures in the laboratory ranged between 21° and 24°C. The growth chamber was equipped with six fluorescent and three 150 watt incandescent lights.

Effect of Female Age and Time of Day on Mating and Sex Attraction. Unisexual male and female progenies of bulk matings were held for emergence in a growth chamber programmed for 21° $\pm$ 1°C, 60 to 80% R.H., and 12 hour photophase from 0600 to 1800 hours. Collection of virgin females for mating tests began one day after the onset of emergence. At 0600 hours the females were collected and discarded. Females that emerged between 0600 and 0800 hours were collected in groups of 10 to 12 every 10 to 30 minutes. Each group of females was held on a pot of wheat plants, covered with a cheesecloth tent and labeled for date and time of eclosion.

The ages of females that were selected for the mating tests were: 1/2, 2, 4, 8, 12, 24, 26, 28, 32, 36, 48, 50, 52, 56, 60, and 72 hours. The corresponding times of day for matings in relation to female ages were 0630, 0800, 1000, 1400, and 1800 hours for three 24 hour periods.

When each group of females reached the specific age, 10 to 15 males were released into the covered pot. Only males less than 12 hours old were used for matings. Flies were allowed to mate for 30 minutes. At the end of the mating period, 10 females were removed and placed individually on wheat plants grown in 10 cm diam. plastic pots. Each pot was covered with a cheesecloth tent and placed in a growth chamber programmed as described previously. The females were allowed to oviposit for 3 days after which time the tents were removed, since the egg laying period lasted only 1 or 2 days for most females (McColloch 1923).

After 6 days the pots were removed from the growth chambers and the wheat plants were examined for larvae. Egg hatch and migration of larvae to the base of the plants normally occur in 5 days at 21° $\pm$ 1°C (Foster and Taylor 1975). Plants were removed from the pots and the leaf sheaths were teased away from the plants with a pair of fine-tipped forceps. Plants were examined under a stereoscopic microscope (10X) for presence of larvae at the base of the plants. If at least one larva was found, the female was recorded as successfully mated. If no larvae were found on any of the plants, the female was recorded as unmated.

The mating tests consisted of four replications conducted over a 6-week period. A replicate consisted of mating determinations on 10 females of each age. However, some of the 48 to 72 hour old females died before males were released so it was not always possible to make mating determinations on 10 females per replicate. The total number of mating determinations made for each female age ranged between 36 and 40. Analysis of variance tests were performed on the data and significance of the means

was tested at the P=0.05 level by the Duncan's new multiple range test. The means represented the percentage of successful matings for each female age.

The sexual attractiveness of virgin females of different ages and at different times of day was also evaluated in olfactometer tests. The olfactometer operations were the same as those previously described. The ages of females that were selected for testing were: 2, 4, 8, 12, 24, 36, 48, 60, and 72 hours. The corresponding times of days that attractiveness was tested in relation to female ages were: 0800, 1000, 1400, and 1800 hours for the first 24 hour period and 0800 and 1800 hours for second and third 24 hour periods. The methods of obtaining females of known age and holding them until they reached the specific age were the same as those described in the mating tests.

Females were collected and tested in groups of 40 for each female age. The same group of 40 females was used for the 2 and 4 hour age test, but different groups were used for all other ages. When groups of females reached the desired age, females were collected in a glass vial and transferred to a small, fine mesh, stainless steel cage. The cage was then inserted into one of the holding chambers of the olfactometer. Twenty virgin males less than 12 hours old were then released at the base of olfactometer and allowed to respond for 20 minutes. The number of responding males trapped in the arms was recorded.

The test for attractiveness for each female age and time of day was replicated four times. Chi-square tests for significance and for heterogeneity were performed on the number of males responding to females and

and the control (blank). Comparisons among responses of males to different age females and time of day were made using a 2 x 2 contingency table (Snedecor and Cochran 1978).

Results

Attractiveness of Live Flies. Responses of males to virgin females, mated females, mated males, and virgin males are summarized in Table 1. Bioassays showed that males were highly attracted to virgin females. About 70% of the total male population responded to virgin females. The chi-square test for heterogeneity was nonsignificant. Males were not attracted to mated females. The numbers of males that responded to mated females and the blank (control) were not significantly different. About 83% of the males failed to respond to either the mated females or the control. Mated males attracted significantly more virgin males than the control even though a high percentage of males responded to the control (23%) or failed to respond (38%). Also, the chi-square test for heterogeneity was significant, an indication that males did not respond uniformly among replications. There was no significant attraction of virgin males to virgin males. Similar numbers of virgin males responded to virgin males and the control.

Comparison tests indicated that only mated females and virgin males were not significantly different in the number of males responding to them. Virgin females and mated males were significantly more attractive with virgin females having significantly greater attraction than mated males.

Table 1. Responses of Virgin Hessian Fly Males to Virgin Females, Mated Females, Mated Males and Virgin Males in a Y-tube Olfactometer.

Attractiveness Test	Number of Replications	Total No. Males Responding to A	Total No. Males Responding to B	Total No. Males Not Responding	X^2	P	X_H^2 [1]	P
Virgin Females (A) vs. Blank (B)	10	139	10	51	111.68 p [2]	<.005	12.10	N.S. [3]
Mated Females (A) vs. Blank (B)	4	6	8	66	0.286 p	N.S.	3.64	N.S.
Mated Males (A) vs. Blank (B)	8	62	37	61	27.85	<.005	23.00	<.05
Virgin Males (A) vs. Blank (B)	8	17	15	128	0.125 p	N.S.	13.08	N.S.

1/ X_H^2 represents chi-square value for heterogeneity.

2/ p represents pooled chi-square value.

3/ N.S. = P>.05.

Ovipositor Extension as an Element of Mating Behavior. Observations on a group of 20 females conducted over a 3 day period showed that females began to extend their ovipositors within 30 minutes after eclosion. Ovipositor extension was observed in all females at 0800 and 1000 hours when females were approximately 2 and 4 hours old. Brief observations between 1200 and 1300 hours showed some females had withdrawn their ovipositors. Ovipositor withdrawal was noted in all females at 1400 and 1800 hours when females were about 8 and 12 hours old. A similar pattern of ovipositor extension and withdrawal was observed on the second and third day, i.e., females extended their ovipositors early in the morning and kept them in the extended position until about mid-day. Females progressively withdrew their ovipositors and kept them retracted for the remainder of the photophase until 1800 hours. Observations were not made during scotophase.

Female behavior of extending or withdrawing the ovipositor was observed prior to mating, during copulation, and after mating was completed. When females were introduced into the cage with males, most females crawled upon the wheat plants and remained motionless with their ovipositors extended. When a male located a female, he usually approached her from the side or rear and positioned himself along her side. The male attempted to copulate almost immediately after making physical contact with the female. Neither the female nor male seemed to exhibit any precopulatory courtship behavior. The females were receptive to the males once contact was initiated. When the pair was locked in copulation for 3 or 4 seconds, the female withdrew her ovipositor for the remainder of the copulation period, which

usually lasted for 10 to 20 seconds. Receptive females seemed to be highly attractive to other males just prior to and during copulation. Several males would approach the copulating pair and attempt to mate with the female. In one case, three males attempted to mate at the same time with a single female until one was finally successful in engaging the female. After copulation was completed and the male disengaged, females remained quiescent with their ovipositors withdrawn. At this point, females were apparently no longer attractive since none of the males approached or made physical contact with a mated female.

One female began ovipositing one hour and 45 minutes after copulating. Other females began ovipositing a short time later. Males were not attracted to ovipositing females.

Observations on several single pairs revealed that most males were able to locate the females within 4 minutes from the time the males were released. After the male located and approached the female, the mating behavior was similar to that described previously. When copulation was completed, the male took to the wing while the female remained passive and did not fly. Only once did a male approach a female a second time, but no attempt to copulate was made.

Effect of Female Age and Time of Day on Mating and Sex Attraction. The mean percentage of successful matings of females of different ages and at different times during the day are summarized in Table 2. The optimum time for mating was 0630 to 1000 hours. There were no significant differences in the percentage of successful matings among females of different ages at these times, with the exception of the 28 and 72 hour

Table 2. Mean Percentage of Successful Matings of Hessian Fly Females of Different Ages and at Different Times During Photophase, 0600-1800 Hours.

Age of Females (Hours ± 1/2 Hour)	Time of Mating[1/] (Hours ± 1/2 Hour)	Mean Percentage of Females Successfully Mated
1/2	0630	80.0 ab[2/]
24		85.0 a
48		71.4 ab
72		61.7 b
2	0800	82.5 ab
26		62.5 ab
50		83.8 ab
4	1000	68.6 ab
28		37.6 c
52		65.0 ab
8	1400	2.5 d
32		5.6 d
56		13.8 d
12	1800	0.0 d
36		2.5 d
60		2.5 d

1/ Approximate time males were released with females.

2/ Means with the same letter are not significantly different.

old females. The number of successful matings of 72 hour old females was only significantly different than that of the 24 hour old females at 0630 hours. Thus, the effect of aging on successful mating was only slight. There was no apparent explanation for the significant decrease in the number of successful matings of 28 hour old females at 1000 hours since there was no significant difference between the 4 and 52 hour old females at this same mating time. The number of successful matings decreased significantly at 1400 and 1800 hours. There were no significant differences among female ages at these mating times.

Results of the attractancy tests of virgin females of different ages and at different times during the day are shown in Table 3. Based upon male responses, females showed a pattern of attraction similar to that exhibited by mating activity in the previous experiment. The greatest response of males (81%) was to 2 hour old females at 0800 hours. Males also showed strong responses to 4 hour old females at 1000 hours and to 24 and 48 hour old females at 0800 hours. Males showed a weaker but significant response to 72 hour old females at 0800 hours. There were no significant responses of males to 8, 12, and 36 hour old females at 1400 and 1600 hours during the first and second 12 hour period. Heterogeneity tests for 60 and 72 hour old females were significant, indicating a varying degree of response among replications.

The attractiveness of 4, 24, and 48 hour old females was not significantly different in 2 x 2 contingency table tests. Likewise, 8, 12, 36, and 60 hour old females were not significantly different in the number of males responding to them. Both the 2 and 72 hour old females were significantly different from all other age groups. The bioassays indicated

Table 3. Responses of Virgin Hessian Fly Males to Virgin Females of Different Ages and at Different Times During Photophase, 0600-1800 Hours, in a Y-tube Olfactometer.

Age of Females (Hours)	Time of Test (Hours)	Number of Replications	Total No. Males Responding to A	Total No. Males Responding to B	Total No. Males Not Responding	X^2	P	X^2_H [1]	P
2 hr (A) vs. blank (B)	0800	4	65	4	11	53.93 p [2]	<.005	5.51	N.S. [3]
4 hr (A) vs. blank (B)	1000	4	51	2	27	45.31 p	<.005	4.86	N.S.
8 hr (A) vs. blank (B)	1400	6	16	13	91	.31 p	N.S.	10.57	N.S.
12 hr (A) vs. blank (B)	1800	4	5	6	69	.09 p	N.S.	4.13	N.S.
24 hr (A) vs. blank (B)	0800	4	53	7	20	35.27 p	<.005	4.25	N.S.
36 hr (A) vs. blank (B)	1800	4	7	7	66	0 p	N.S.	5.16	N.S.
48 hr (A) vs. blank (B)	0800	4	46	7	27	28.70 p	<.005	4.62	N.S.
60 hr (A) vs. blank (B)	1800	6	19	14	87	16.13	<.01	15.74	<.01
72 hr (A) vs. blank (B)	0800	4	38	10	32	22.90	<.005	9.96	<.025

1/ X^2_H represents chi-square value for heterogeneity.

2/ p represents pooled chi-square value.

3/ N.S. = P>.05.

that virgin females were most attractive to males shortly after eclosion and maintained a fairly high level of attractiveness the following two days but only during the morning hours.

Discussion and Conclusions

Results of the bioassay studies demonstrate that male Hessian flies are strongly attracted to virgin females and suggest the presence of a sex pheromone in the female fly. Sexual attraction in the Hessian fly was first observed by Cartwright (1922) when he showed that caged females attracted males from a distance of 3.0 to 4.8 m. It appears that Hessian fly females use a pheromone as a distant attractant for males. The lack of attraction of females to males indicated that the male has no influence on the orientation behavior of the female fly. Also, virgin males apparently do not exhibit any orientation behavior toward other virgin males. However, the significant attraction of virgin males to mated males suggest that some of the sex pheromone from females was transferred to males by contact with females during copulation.

The lack of response of virgin males to mated females indicated that pheromone emission ceases after females were successfully mated. Because Hessian fly adults are non-feeding and live for only 3 or 4 days and females apparently only mate once, the termination of pheromone production in mated females would appear to have some adaptive significance. Mated females no longer attractive to males could direct their energies toward the search for suitable host plants and deposition of fertilized eggs, and males no longer attracted to mated females could expend their energies toward the search of virgin females. Enoch (1891) found that one male could impregnate from one to six females.

The termination or reduction of pheromone production after mating has been reported in several insects. Shorey (1976) stated that females of some species that mate only once may produce or release little or no additional sex pheromone following a successful mating. Doane (1968) showed that female gypsy moths, *Porthetria dispar* (L.), usually lose their attractancy within the first 15 minutes after the onset of copulation. By retracting the ovipositor the gypsy moth female can effectively stop release of the pheromone. Happ and Wheeler (1969) showed that mated female *Tenebrio molitor* L. were highly attractive to males until the first cycle of oocyte growth was completed after which the rate of pheromone release declined.

Attractiveness of Hessian fly females and their receptivity to males appeared to be associated with the female's behavior of extending and withdrawing her ovipositor. Extension of the ovipositor was the only precopulatory behavioral element observed in females and may serve as a means of releasing the sex pheromone. Virgin females exhibited a behavioral rhythm of extending their ovipositors that coincided with periods of highest mating activity and sexual attraction. The rhythm began soon after females eclosed in early morning and stopped about mid-day, the period that females were most attractive and receptive to males. Virgin females began to withdraw their ovipositors in early afternoon and by late afternoon nearly all females had withdrawn their ovipositors. A similar rhythm of ovipositor extension and withdrawal was observed for two successive days. The period that females had their ovipositors withdrawn coincided with the time of day that females were least attractive and mating

activity was lowest. Thus, the female behavior of extending and withdrawing the ovipositor may be a mechanism for controlling pheromone release.

McConnell (1921) noted that the Hessian fly female contains her normal allotment of mature oocytes at the time of eclosion. Metcalfe (1935) observed that females copulated soon after eclosion and began ovipositing 15 minutes after mating. Observations of mating in the present study also showed that females were highly attractive and receptive to males soon after eclosion. All females had their ovipositors extended at the time males were attracted and just prior to copulation. Females withdrew their ovipositors during copulation and retained them in this position after copulation until oviposition began. The behavior of withdrawing the ovipositor after mating has been observed in other gall midges (Metcalfe 1933, Spince 1969), which suggests that the behavior may be common to cecidomyiids. The fact that Hessian fly females were observed to mate only once and appeared unattractive to males after mating suggests that the extension and retraction of the ovipositor may be a behavioral mechanism used by the female to control pheromone release. Also, the fact that females begin ovipositing soon after mating and are unattractive to males indicates a well defined change in female behavior from one of mating to one of ovipositing. This change apparently occurs within a rather short time span.

These observations and experiments clarify some aspects of the reproductive behavior of the Hessian fly. The mating behavior of the adults seem to be inherent of the biological characteristics of the species.

The short life span of the non-feeding adults requires an effective communication system for bringing the sexes together within the shortest time span possible. The females reproductive system that produces mature eggs at eclosion and requires only a single copulation is also favorable for a short life span. A female-produced sex pheromone that is released soon after emergence and used for long- and close-range orientation and for stimulation of the males increases the probability of mating. Behavioral mechanisms that allow the female to control pheromone release at times most favorable for mating also favors mating success. A behavior that terminates production of the attractant pheromone after a female has successfully mated facilitates the completion of her reproductive cycle and conserves males for additional matings. These behavioral patterns evolved in the Hessian fly and play a major role in the reproductive success of the species.

PART II

DEMONSTRATION OF A FEMALE SEX PHEROMONE IN THE HESSIAN FLY

Methods and Materials

Series of bioassays were conducted to determine the attractiveness of various chemical extracts of virgin Hessian fly females to males. The sexual attractiveness of the extracts were evaluated in an olfactometer. The operation of the olfactometer was the same as that described in Part I. Each attractiveness test consisted of four or more replications. Twenty virgin males less than 12 hours old were used as responding flies in each replicate. As before, males were allowed to respond to the attractant for 20 minutes. The quantity of extract evaluated in the tests was equal to 40 female equivalents (FE) unless stated otherwise.

The results of all tests were analyzed with chi-square tests for significance and heterogeneity. A pooled chi-square was used when the test for heterogeneity was nonsignificant at $P<0.05$ level. Comparisons between tests were analyzed with a 2 x 2 contingency table.

Attractiveness of Hexane Rinses of Whole Bodies, Macerated Bodies, and Excised Ovipositors of Females. Groups of 100 virgin females with ovipositors extended were collected in the morning and rinsed with 500 µl of hexane for 3 minutes. Two hundred µl (40 FE) of the hexane ranse were pipetted onto a 2.2 cm diam. glass filter paper (GFP) disc and air-dried for 3 minutes. As a control, 200 µl of hexane were pipetted onto a GFP disc and air-dried for 3 minutes. Each disc was pinned to a fine mesh, stainless steel screen (3.8 cm diam.) and placed separately in the holding tubes of the olfactometer for testing.

The same groups of 100 females that were rinsed with hexane were allowed to dry, macerated, and then rinsed in 500 µl of hexane for 3 minutes. Two hundred µl (40 FE) were pipetted onto a GFP disc and air-dried for 3 minutes. A hexane control was prepared in the same manner as the hexane rinse. The discs were placed in the olfactometer and tested as described previously.

A second attractiveness test was conducted in an attempt to determine more precisely the source of a sex pheromone in females. Forty females were collected at 0800 hours and immobilized by placing in a refrigerator for a few minutes. Females were then placed on microscope slides and positioned under a dissecting microscope. The ovipositors were excised with a scalpel and pulled away from the body. Most of the females had their ovipositors extended, however, in some cases the abdomen had to be pressed gently to extend the ovipositor. The bodies and the excised ovipositors were placed in separate vials each containing 300 µl of hexane. After all of the females were dissected, the vials were shaken gently and the mixtures were allowed to stand for a few minutes. Then the liquid contents of both vials were pipetted onto GFP discs, air-dried for 3 minutes, and placed separately in the holding tubes of the olfactometer. The attractiveness of the ovipositor rinse and the body rinse was tested against one another.

A third test was conducted to determine if the time of day that hexane rinses of females were made affected their attractiveness. Rinses of 100 newly emerged virgin females were prepared by rinsing whole bodies in 500 µl of hexane for 3 minutes, and later rinsing the macerated bodies in

another 500 µl for 3 minutes. These rinses were made between 0700 and 0800 hours. After rinsing, the extracts were pipetted into screw cap vials and held at -2.2°C in a refrigerator until about 1800 hours. The vials were then removed and allowed to warm to room temperature. Then, 200 µl each of the whole body and the macerated body rinses were pipetted onto GFP discs. Each rinse was tested against a hexane control between 1800 and 2100 hours.

Other groups of 100 virgin females collected in early morning were caged on wheat plants and held in a growth chamber until about 1800 hours. The growth chamber was programmed for 21 ± 1°C and full lights (1500 ft-c). Relative humidity was maintained between 60 and 70%. Hexane rinses of these females were prepared between 1830 and 1930 hours. Both whole bodies and macerated bodies were rinsed with 500 µl of hexane for 3 minutes. As before, rinses of macerated bodies were made from the same females and after the whole body rinses were made. The attractiveness of the whole body rinse and the macerated body rinse was tested separately against a hexane control. The tests were conducted between 1900 and 2100 hours.

A choice test was also conducted in the olfactometer using rinses made between 0800 and 0900 hours and those made between 1900 and 1930 hours. Tests of both whole body and macerated body rinses were made between 1900 and 2100 hours.

Attractiveness of Hexane and Benzene Fractions Eluted from Hexane Rinses of Whole Body Females and Excised Ovipositors. One hundred virgin females were collected in the early morning and rinsed with 500 µl of hexane for 3 minutes. The rinse was pipetted into a screw cap vial and

placed in a refrigerator. A crude column was set up by filling a Pastuer pipette with hexane. About 1.3 cm of BioSil HA® (Bio-Rad Lab), a -325 mesh silicic acid used for lipid chromatography, was added to the column. Most of the hexane was pressed out to remove air bubbles and to compact the BioSil HA® material. The hexane whole body rinse was then added to the column followed by the addition of more hexane to elute the hydrocarbon fraction from the rinse. Two mls of the eluate were collected in 1-ml vials. Benzene was then added to the column to elute other lipid fractions. Again 2 mls of the eluate were collected in 1-ml vials. The vials were placed in the refrigerator at -2.2°C. At the time of the test, the vials were allowed to warm to room temperature and 800 µl (40 FE) of each fraction were pipetted onto GFP discs. Discs of hexane and benzene were prepared in the same way and used as controls. After air drying for 3 to 5 minutes, the discs were placed in the holding tubes of the olfactometer for testing.

Ovipositors from 200 newly emerged females were excised over a period of 3 days. Ovipositors were placed in a vial containing hexane. The vial was kept in a refrigerator and removed only during the time ovipositors were excised. After the 200 ovipositors were collected and rinsed with hexane, the rinse was passed through a BioSil HA mini column as previously described. Hexane was added to elute the hydrocarbons and benzene was later used to elute the remaining fractions. Three mls of each extract were collected in 1-ml vials. Forty FE (600 µl) of each extract and the appropriate solvent control were prepared and tested in the olfactometer as described above.

Attractiveness of Various Chemical Fractions Obtained from Thin Layer Chromatography. For the thin layer chromatography (TLC) procedure, a set of standards representing the various lipid fractions present in a surface rinse were prepared. The standards were n-octacosane representing the hydrocarbon group, cholestryl palmitate and palmitic acid methyl ether for the ester group, tripalmitin for the triglycerides, palmitic acid as a fatty acid and cholesterol. To prepare these for use in the TLC, 10 mg of each material were placed in screw cap vials and 1 ml of benzene was added to each vial. A mobile phase consisting of 9 parts hexane to 1 part diethyl ether was prepared.

Four thousand virgin females previously collected and frozen over a nine month period were used to prepare the rinse for the TLC tests. Females were rinsed with 50 mls of diethyl ether for several minutes. This mixture was then poured through a sintered-glass filter to separate the liquid from the female bodies. The rinse was filtered into a dry beaker. At the end of the filtration process the beaker containing the rinse was placed inside a larger beaker containing several mls of warm water. A stream of nitrogen gas was directed through a pipette into the smaller beaker holding the rinse. After the rinse had evaporated, the lipid material was then rerinsed with 1 ml of benzene. The redissolved rinse was pipetted into a small screw cap vial.

A silica gel plate (Eastman®) was used for the TLC. Small pencil dots were marked along a line about 2.5 cm above the base of the plate. Using a disposable 50 µl applicator, 100 µl (about 400 FE) of the benzene rinse was banded on 2/3 of the plate on top of the dots. The six standards were spotted on the other 1/3 of the plate using a 1 µl pipette.

The plate was placed into the mobile phase for about one hour. After the plate had been removed from the mobile phase and dried, the side of the plate containing the standards was cut off. This section of the plate was then sprayed with 2'7'-dichlorofluorescein 0.2% in MeOH. The plate was placed under a UV light and the location where the standards had moved up the plate were circled in pencil. This section of the plate was used to indicate the locations of the various fractions in the female-rinse portion of the plate. Bands corresponding to the hydrocarbon, ester, and triglyceride fractions of the female rinse were scraped off the plate and placed in small beakers. Each beaker was initially rinsed with 500 µl of diethyl ether after the fractions were added. One ml of ether was then added and the mixture was poured through a sintered-glass filter. This process was repeated two more times. Each fraction was rinsed and filtered in this manner. The filtrates were collected in 5 ml vials. Three mls of ether were pipetted into a separate 5 ml vial to use as a control. The ether in each fraction was evaporated down to a volume of about 150 µl. The evaporation process involved warming the vial by placing it in a large beaker holding 150 mls of warm water and passing a stream of nitrogen through a pipette onto the fraction. The various fractions were either tested directly after fractionating or refrigerated overnight to test the following morning.

A series of four materials was tested for each TLC plate. They were: ether only, the hydrocarbon fraction, ester fraction, and triglyceride fraction. Ether was tested against a blank control and the fractions were tested against ether controls. About 100 µl of ether and of each fraction

were pipetted onto a GFP disc. For the three fractions, 100 µl of ether was pipetted onto a second GFP disc. After drying for 4 to 5 minutes the discs were placed in the olfactometer for testing. Each fraction was tested against an ether control.

Three TLC plates were prepared using the rinse obtained from the 4000 females. One of the plates was placed in an old mobile phase which resulted in poor separation of the fractions. The fractions from this plate, when tested in the olfactometer, gave poor results and were not analyzed.

Another 2000 females collected over several days were rinsed with 25 mls of ether and this was poured through a sintered-glass filter. The rinse was dried using the same method as mentioned earlier and the dried rinse was redissolved in a total of 500 µl of benzene. Between 100 and 200 µl of benzene was added at three separate times to dissolve the lipid material. The rinse was removed each time and placed into a small screw cap vial.

A TLC plate was prepared and again 100 µl (400 FE) of the benzene rinse were banded on the plate. The plate was treated in the same manner as mentioned earlier. The hydrocarbon, ester, and triglyceride fractions were removed and prepared as before. Tests were conducted in the olfactometer. Two plates were prepared from the rinse of the 2000 females.

Results

Attractiveness of Hexane Rinses of Whole Bodies, Macerated Bodies, and Excised Ovipositors of Females. Responses of virgin males to hexane,

whole body rinses, macerated body rinses, and ovipositor rinses of virgin females are summarized in Table 4. Hexane rinses of whole bodies and macerated bodies tested between 0800 and 0930 hours were highly attractive to males. About 75% of the males responded to the whole body rinses and macerated body rinses whereas only 2% of the males responded to the hexane control. Excised ovipositors rinsed in hexane and tested between 0930 and 1030 hours were also highly attractive. The rinses of whole bodies without the ovipositors were not attractive to males when tested against ovipositors. Less than 4% of the males responded to the rinses of whole bodies without ovipositors whereas about 81% of the males responded to the ovipositor rinse. The responses of males to the whole body, macerated body, and ovipositor rinses were not significantly different ($P<0.05$). Comparisons of these three tests to the original bioassay test of live females in Table 1 indicated that there was no significant difference ($P<0.05$) between these rinses and live females.

Hexane tested separately was not attractive to males. The heterogeneity was due to a preference reversal in one of the four replications.

Responses of males to whole body and macerated body rinses made at different times during the day are shown in Table 5. Hexane rinses of virgin females, both whole body and macerated body, made between 1830 and 1930 hours (ca. 12 hrs old) were not attractive to males compared to hexane rinses made between 0700 and 0800 hours. Only 9% of the males responded to the whole body rinse and 13% responded to the macerated body rinses. Few males responded to the hexane control, 5% in the whole body test and 15% in the macerated body rinse test. When the attractiveness of

Table 4. Resonses of Virgin Hessian Fly Males to Hexane. Whole Body Rinses, Macerated Body Rinses, and Ovipositor Rinses of Virgin Females in a Y-tube Olfactometer.

Attractiveness Test	Number of Replications	Total No. Males Responding to A	Total No. Males Responding to B	Total No. Males Not Responding	X^2	P	X^2_H [1]	P
Hexane (A) vs Blank (B)	4	3	10	67	3.77	N.S. [2]	13.03	<.005
Whole Body Rinse (A) vs. Hexane (B)	5	74	2	24	68.21 p [3]	<.005	2.78	N.S.
Macerated Body Rinse (A) vs. Hexane (B)	4	60	1	19	57.07 p	<.005	3.06	N.S.
Ovipositor Rinse (A) vs. Whole Body Rinse w/o Ovipositors (B)	4	65	3	12	56.35 p	<.005	3.25	N.S.

1/ X^2_H represents chi-square value for heterogeneity.

2/ N.S. = p>.05.

3/ p represents pooled chi-square value.

Table 5. Responses of Virgin Hessian Fly Males to Whole Body Rinses and Macerated Body Rinses of Virgin Females Tested Between 1800-2100 Hours in a Y-tube Olfactometer.

Attractiveness Test	Time Rinses were made (Hours ± ½)	No. of Replications	Total No. Males Responding to A	Total No. Males Responding to B	Total No. Males Not Responding	X^2	P	X^{2}_{H} [1]	P
Whole Body Rinses (A) vs. Hexane (B)	0715	4	35	6	39	20.51 p [2]	<.005	2.90	N.S. [3]
Whole Body Rinses (A) vs. Hexane (B)	1900	4	7	5	68	.33 p	N.S.	3.42	N.S.
Macerated Body Rinses (A) vs. Hexane (B)	0730	4	40	8	32	21.33 p	<.005	2.84	N.S.
Macerated Body Rinses (A) vs. Hexane (B)	1930	4	11	12	57	.04 p	N.S.	1.50	N.S.
Whole Body Rinses (A) vs. Whole Body Rinses (B)	0830 1900	4	40	11	29	16.49 p	<.005	7.54	N.S.
Macerated Body Rinses (A) vs. Macerated Body Rinses (B)	0900 1930	4	46	8	26	26.47 p	<.005	3.32	N.S.

1/ X^2_H represents chi-square value for heterogeneity.

2/ p represents pooled chi-square value.

3/ N.S. = p>.05.

rinses made at different times were compared in the same test, the rinses made in early morning were significantly more attractive than those made in the evening. Comparisons of whole body rinses and macerated body rinses made in the evening showed both rinses were equally unattractive to males and were not significantly different ($P<0.05$).

Hexane rinses made in the morning between 0700 and 0800 hours and refrigerated until 1800 hours were attractive to males. About 50% of the males responded to the whole body rinses and also to the macerated body rinses. The males responding to the hexane control comprised only about 8% and 10% of the total males, respectively. Comparisons of the macerated body and whole body rinses indicated there was no significant difference ($P<0.05$) in attractiveness. The comparisons of whole body rinses and macerated body rinses made in the morning showed that those tested between 0800 and 0930 hours (from Table 4) were more attractive than those tested between 1800 and 2100 hours (Table 5). Males used in the evening tests were considerably younger than those used in the morning tests and this may have affected the results.

The test comparing rinses prepared in the morning with those made in the evening showed a significantly greater response by males to rinses made in the morning. This was true for both the whole body and macerated body rinses. Results of a choice test in the olfactometer between the two preparation times of the rinses confirmed the 2 x 2 contingency test results. As shown in Table 5, whole body rinses made in the morning attracted 50% of the males while only 13% of the males responded to rinses made in the evening. Likewise, 58% of the males responded to macerated

body rinses made in the morning and only 10% were attracted to rinses made in the evening. The numbers of males responding to rinses made in the evening were comparable to the numbers responding to hexane controls in other tests. Thus, evening rinses of females apparently contained little of the sex attractant.

Attractiveness of Hexane and Benzene Fractions Eluted from Hexane Rinses of Whole Body Females and Excised Ovipositors. Responses of males to hexane eluted hydrocarbons and benzene fractions are shown in Table 6. Fractions from whole body rinses obtained from the BioSil HA® column indicated that both the hydrocarbons and benzene fractions were highly attractive to male flies. A significantly greater number of males responded to the hexane eluted hydrocarbons (59%) and the benzene fractions (61%) than the control. Only 6% responded to the control solvent in both cases. The heterogeneity in the benzene fraction tests was due to a varying degree of response among the four replications. Comparisons of the two fractions showed no significant difference ($P<0.05$) in the number of males responding to the hexane and benzene fractions. Comparisons of the hexane fraction in this test with the hexane rinses of whole bodies in Table 4 showed that the hexane fractions were significantly less attractive ($P<0.05$). In contrast, there were no significant differences ($P<0.05$) in the attractiveness of the benzene fractions and the hexane rinses of whole bodies.

Fractions of ovipositor rinses obtained from the BioSil HA® column confirmed the attractiveness of both hexane and benzene fractions to males. Forty-five percent of the males were attracted to the hexane fractions and

Table 6. Responses of Virgin Hessian Fly Males to Benzene, Hexane Eluted Hydrocarbons, and Benzene Fractions of Whole Body Rinses and Ovipositor Rinses of Virgin Females in a Y-tube Olfactometer.

Attractiveness Test	Type of Rinse	Number of Replications	Total No. Males Responding to A	Total No. Males Responding to B	Total No. Males Not Responding	X^2	P	X_H^2 [1]	P
Benzene (A) vs. Blank (B)	Control	4	11	14	55	.36 p [2]	N.S. [3]	3.93	N.S.
Hexane Fractions (A) vs. Hexane (B)	Whole Body	4	47	5	28	33.92 p	<.005	3.65	N.S.
Hexane Fractions (A) vs. Hexane (B)	Ovipositor	4	36	7	37	19.56 p	<.005	3.41	N.S.
Benzene Fraction (A) vs. Benzene (B)	Whole Body	4	49	5	26	49.25	<.005	13.04	<.005
Benzene Fraction (A) vs. Benzene (B)	Ovipositor	4	44	4	32	33.33 p	<.005	4.14	N.S.
Hexane Fraction (A) vs. Benzene Fraction (B)	Whole Body	4	6	54	20	38.40 p	<.005	1.69	N.S.

1/ X_H^2 represents chi-square value for heterogeneity.

2/ p represents pooled chi-square value.

3/ N.S. = P>.05.

55% to the benzene fractions. Again, only a small percentage of the males responded to the control solvents, 9% and 5%, respectively. The attractiveness of the hexane and benzene fractions from ovipositor rinses were not significantly different ($P<0.05$).

The lower response of males to fractions from ovipositor rinses was not apparent when compared to their counterpart fractions eluted from whole body rinses. Hexane fractions from ovipositor and whole body rinses were not significantly different ($P<0.05$) in a 2 x 2 contingency test. The same was true for the benzene fractions. However, both the hexane and benzene fractions of ovipositor rinses were significantly less attractive ($P<0.05$) than that of the hexane rinses of ovipositors in Table 4. Some loss of activity may have resulted in preparing the fractions of the ovipositor rinses.

Although the hexane fractions were attractive when tested alone, they were not attractive when tested against the benzene fractions. The benzene fractions attracted 68% of the males while the hexane fraction attracted only 8% of the males.

Attractiveness of Various Chemical Fractions Obtained from Thin Layer Chromatography. The responses of males to the fractions obtained by the TLC method are summarized in Table 7. The ester fraction was highly attractive with about 75% of the males responding to it. The ester fraction was also uniformly attractive in all replications as revealed by the nonsignificant chi-square value for heterogeneity.

Table 7. Responses of Virgin Hessian Fly Males to Ether, Hydrocarbon, Ester, and Triglyceride Fractions Isolated by Thin Layer Chromatography from Virgin Females in a Y-tube Olfactometer.

Attractiveness Test	Total Number of Replications	No. of Males Responding to A	No. of Males Responding to B	Total No. Males Not Responding	X^2	P	X^2_H [1]	P
Ether (A) vs. Blank (B)	4	12	19	49	1.58 p [2]	N.S. [3]	1.84	N.S.
Ether (A) [4] vs. Blank (B)	4	20	40	20	15.54	<.005	10.03	<.025
Hydrocarbon (A) vs. Ether (B)	4	27	20	33	14.64	<.005	13.88	<.005
Esters (A) vs. Ether (B)	4	60	7	13	41.93 p	<.005	4.34	N.S.
Triglycerides (A) vs. Ether (B)	4	16	22	42	27.56	<.005	27.33	<.005

1/ X^2_H represents chi-square value for heterogeneity.

2/ p represents pooled chi-square value.

3/ N.S. = p>.05.

4/ Represents the ether tested with the fractions.

There was considerable variability in the responses of males to the other two fractions. Both the hydrocarbon and triglyceride fractions had significant heterogeneity among replications. There was little attractancy to the hydrocarbon and triglyceride fractions removed from the TLC plates. The significance in the chi-square tests were caused by the heterogeneity present among the replications of these two fractions.

The responses of males to the ether control were also variable. The first of the ether tests represented a separate test conducted to determine if ether was attractive or repellent. One hundred µl of ether was used for each replication and in this test no attractancy or repellency was observed. Both the chi-square for significance and for heterogeneity were nonsignificant.

The second ether test involved testing the ether which had been dried down with the other fractions and was tested the same morning as the fractions. Again around 100 µl were tested. There is probably some repellency present as suggested by the results, but again, the male responses between replications were variable.

Discussion and Conclusions

The results of these experiments demonstrate conclusively and support behavioral evidence in Part I that female Hessian flies produce a sex pheromone or a mixture of sex pheromones to attract and to stimulate males. The sex attractant(s) was successfully removed from females with the hexane solvent either as whole body or macerated body extracts. This suggests that the active component(s) is most likely a non-polar neutral lipid(s) since hexane is a solvent with low polarity.

The strong responses of males to hexane rinses of excised ovipositors is further evidence that the ovipositor may be the release site and possibly the production site of the pheromone, as was suggested in Part I. Female sex pheromone glands have been isolated from the terminal abdominal segments of several lepidopteran species. Among others, pheromone activity was demonstrated in extracts of abdominal tips in the European leafroller (Roelofs et al. 1976), clover cutworm (Underhill et al. 1976), European corn borer (Klun and Junk 1977), and the naval orangeworm (Coffelt et al. 1979). Nation (1972) found an abdominal gland involved in the pheromone production of male Caribbean Fruit flies. Other dipteran males which produce a sex pheromone in an abdominal gland include *Dacus tryoni* (Fletcher 1969), the Oriental fruit fly and Melon fly (Kobayashi, et al. 1978).

The attractiveness of hexane rinses of females made in the morning and the lack of attractiveness of those made in the evening indicate a daily rhythm of pheromone release by the female. Because males responded in the evening to rinses made in the morning, the lack of attraction of evening rinses is more likely due to a rhythm of pheromone production and/or release by the female rather than a rhythm of responsiveness by the male. This conclusion is also supported by the results obtained in Part I that showed a rhythm was associated with mating activity, sexual attractiveness of females, and female behavior of extending the ovipositor. Thus, it appears that the release and production of the sex attractant may be shut off completely in the afternoon and evening. However, another plausible explanation is that the sex attractant may be depleted in a

female by afternoon or evening. In either case, an apparent rhythm seems to be associated with sexual attractiveness of females.

Although males seem to respond to a sex attractant at anytime, at least during photophase, males did show a stronger response to rinses when tested in the morning and this may be an effect of their age. Males used in the morning were about 12 hours old whereas males used in the evening were 4 to 6 hours old, an indication that responsiveness may increase with age. Hessian fly males emerging in the afternoon and evening would not normally encounter actively calling females. Thus, their sensitivity to the sex pheromone may be lower in the evening which would possibly explain why the response of younger males was not as strong as that of older males.

Further isolation of lipid fractions from the hexane surface rinses of females using the BioSil HA® column showed that both hexane eluted hydrocarbons and benzene eluted esters and other lipids are attractive to males. The attraction of hydrocarbon fractions when tested alone in the olfactometer indicated the presence of an active component which affects the sexual behavior of males. However, when the hexane fraction was tested against the benzene fraction, the attractiveness of the hexane fraction apparently was overridden by the much stronger attractiveness of the lipids eluted by benzene. Responses in most olfactometers measure only a few centimeters, therefore, no distinction can be made between long- and short-range attractants (Fletcher 1977). The loss of the attractiveness of the hexane fraction in the presence of benzene lipids suggests that the hexane hydrocarbon component may be a mating-stimulant

pheromone or a short-range attractant for males. Hydrocarbons that act as contact pheromones or as short-range attractants have been identified in several dipterans. *Fannia pusio* (Weidemann) and *F. femoralis* (Stein) utilize the same hydrocarbon as a short-range attractant (Uebel et al. 1978 and Uebel et al. 1978). Contact hydrocarbon pheromones were also found to stimulate mating in the stable fly (Uebel et al. 1975) and in the tsetse fly (Carlson et al. 1978).

Fletcher (1977) stated that distant attractants often involve a different blend of chemicals than those used for close-range orientation and are often associated with specialized mating habits in Diptera, such as mating on emergence. Observations by Cartwright (1922) that Hessian fly females attract males as far away as 5 m and the fact that females mate soon after emergence suggest a long-range attractant is involved. Thus, the active component in the ester fraction, which was strongly attractive to males, may act as the long-range sex attractant. The hydrocarbon component then may act alone or as synergist with the ester component to attract or sexually excite males at short distances. The results of these experiments suggest that the sexual attractiveness of Hessian fly females is probably a multicomponent pheromone system with the individual components possibly having different functions. Further research is needed to isolate and identify these components and to clarify the functions of each component.

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VITA

Patricia A. McKay was born September 11, 1956, in Albuquerque, New Mexico. She is the only daughter and the youngest of four children of Mr. and Mrs. Robert J. McKay. She received her elementary and secondary education in Albuquerque and graduated from high school in 1974. She attended Midland College in Fremont, Nebraska from 1974 to 1977 where she majored in biology. In 1977 she transferred to the University of Wyoming at Laramie, Wyoming. In 1978 she received a B.S. degree in Entomology. She entered Kansas State University's graduate school in 1979 where she was a teaching assistant in economic entomology.

SEXUAL ATTRACTION, MATING BEHAVIOR, AND DEMONSTRATION OF A FEMALE SEX PHEROMONE IN THE HESSIAN FLY, *MAYETIOLA DESTRUCTOR* (SAY) (DIPTERA: CECIDOMYIIDAE)

by

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AN ABSTRACT OF A MASTER'S THESIS

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Laboratory and greenhouse studies were conducted to investigate the sexual attraction, mating behavior, and presence of a female sex pheromone in the Hessian fly, *Mayetiola destructor* (Say).

Results of bioassays utilizing a Y-tube olfactometer showed that virgin females were highly attractive to males. Mated females were not attractive to males. Virgin females exhibited a calling behavior by extending their ovipositors during morning hours, but retracted their ovipositors during the afternoon and during copulation and pre-ovipositional periods. The behavior of extending and withdrawing the ovipositor appeared to be associated with the female's attractiveness and her receptivity to males. The period that females extended their ovipositors coincided with the time of day that mating activity and sexual attractancy were highest. The period of highest mating activity as measured by the percentage of successful matings occurred between 0630 and 1000 hours. Sexual attractiveness of females as measured by responses of males in the olfactometer was also greatest during morning hours. Maximum attraction of males occurred shortly after female eclosion.

Hexane rinses of whole females and macerated females were highly attractive to males. Rinses prepared in the morning were significantly more attractive to males than rinses prepared in the evening when females were about 12 hours old. Hexane rinses of excised ovipositors were significantly more attractive to males than rinses of females without ovipositors, an indication that the ovipositor may be the release site of the sex pheromone.

Hexane rinses of virgin females were fractionated on silicic acid (BioSil HA®) columns into hydrocarbons eluted with hexane and esters and sterols eluted with benzene. Both hexane and benzene fractions from hexane rinses were attractive to males when tested separately in the olfactometer. However, when tested against each other, the benzene fraction containing the esters was significantly more attractive than the hexane fraction containing the hydrocarbons.

Bioassays of hydrocarbon, ester, and triglyceride fractions seperated on thin-layer chromatography plates showed the ester fraction was significantly more attractive to males than the hydrocarbon and triglyceride fractions. The results of these experiments suggest that the sex pheromone of the Hessian fly is not a single compound but probably a multicomponent system, with the main attractive component in the ester fraction.