Mating Behavior and Evidence of a Female Sex Pheromone in the Hessian Fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae)\(^1\)

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**ABSTRACT** In laboratory bioassays, the ovipositors of virgin females of the Hessian fly, *Mayetiola destructor* (Say), released a sex pheromone that attracted males. Pheromone release appeared to be regulated by extension and retraction of the ovipositor. Males were highly attracted to females with extended ovipositors. Females mated only once. After mating, females were unattractive to males. Female sexual behavior was manifested in a diurnal rhythm. Female attractiveness and mating activity were highest from 0600 to 1000 hours and lowest from 1400 to 1800 hours. Female age up to 72 h had little effect on sexual attractiveness or mating success.

ALTHOUGH the sex pheromones of Diptera have been researched extensively, information on the existence and role of sex pheromones in sexual behavior in the Nematocera remains limited. In the Nematocera, sex pheromones have been demonstrated for only a few species of culicids (Downes 1966, Kliewer et al. 1966, Gjullin et al. 1967) and two species of sciarids, *Lycoriella mali* Fitch (Kostelc et al. 1975) and *Bradyria impatiens* (Johnsen) (Alberts et al. 1981). Sex pheromones have not been identified for any species of Cecidomyiidae. Moreover, few detailed studies of the reproductive behavior of cecidomyiids have been reported, presumably because adults of most species are short lived and difficult to observe in the field or rear in the laboratory.

The cecidomyiid Hessian fly, *Mayetiola destructor* (Say), has been a serious pest of wheat in the United States since its introduction in the late 1700's. Because of its economic importance, the biology of the Hessian fly has been studied extensively. However, there is little information on its sexual behavior, although previous observations suggest that a female sex pheromone may attract males. Cartwright (1922) reported that Hessian fly females attracted males from 3 to 5 m in the field, with the greatest attraction occurring in early morning. Enoch (1891) observed that females extend their ovipositors soon after eclosion and hang in a calling position from leaves of wheat plants. McColloch (1923) reported that adults mate soon after eclosion and oviposition begins in the mornings 1 to 3 h after emergence.

The objectives of our study were to determine whether a sex pheromone is present in the female Hessian fly and elucidate some of the factors affecting female sexual behavior.

**Materials and Methods**

**Test Insects and Rearing Methods.** Hessian flies used for this study were of the Great Plains bio-type originally collected from Phillips County in Kansas, and were reared in a greenhouse for 10 generations. Rearing methods were the same as those described by Cartwright and LaHue (1944) and Gallun et al. (1961). Male and female flies were mated and individual females were confined for oviposition on wheat seedlings grown in plastic pots (10 cm diam). A clear plastic cage was placed over each pot before emergence of F\(_1\) adults. The Hessian fly breeds by unisexual families—i.e., most progenies are either all male or all female (Painter 1930). Thus, virgin adults were obtained readily by determining the sex of the progenies. All progenies used in the experiments were reared in an environmental chamber at 21 ± 1°C and a 12-h photoperiod from 0600 to 1800 hours. On this regime, females eclosed from 0530 to 0830 hours, whereas males eclosed during the same period, and also from 1630 to 1830 hours.

**Bioassay Procedure.** Sexual attraction of Hessian flies was studied using a Y-tube olfactometer similar to the one described by Chaudhury et al. (1972). The olfactometer consisted of a glass Y-tube (2.9 cm ID) and two arms of straight glass tubes. A glass chamber (19.0 cm long and 6.3 cm diam) formed the base and was attached to the Y-tube. The chamber was closed by a rubber stopper with four holes. The center hole (2 cm diam) held a glass vial from which "responding" flies were released; the others were screened for air passage.

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\(^1\) This article reports research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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Each arm of the Y-tube contained a glass funnel (5 mm opening), which functioned as a trap for responding flies. The distance from the release site of responding flies to the funnel trap was 30 cm. Each arm of the Y-tube was connected by plastic tubing to a glass cylinder (15 cm long and 4 cm diam), which held the "attracting" flies or hexane washes of virgin females. The olfactometer was washed with acetone, rinsed with distilled water, and air-dried before use. Air was filtered through three activated charcoal-glass wool filters and humidified by passing through a filter flask containing water. Air entered each arm of the olfactometer at a rate of 260 ml/min. The air speed through the funnel was ca. 22 cm/sec. The olfactometer was operated in an environmental chamber held at 21 ± 1°C and 16,000 lux.

Attractiveness of Live Flies. The attractiveness of virgin females, mated females, virgin males, and mated males to virgin males and the attractiveness of virgin males to virgin females were tested in separate bioassays. Groups of 40 attracting flies were tested against 20 responding flies. Attracting flies were collected in a cylindrical, fine mesh, stainless steel screen cage and placed into one of the holding chambers of the olfactometer. The other holding chamber was used as a control (blank). Virgin females and virgin males were confined for mating with the opposite sex for 30 and 10 min, respectively, before the bioassays. Females were confined with males for 30 min to ensure that most were mated before testing. Males were confined with females for only 10 min to avoid possible loss of pheromone that may have been transferred during copulation. After the attracting flies were placed in the holding chamber, responding flies were released and allowed to respond for 20 min; the numbers of flies trapped in the arms were recorded. All tests were conducted between 0800 and 1000 hours. Each attraction test was replicated 4 to 10 times using new groups of flies for each replication. Results were analyzed using \( \chi^2 \) tests for significance and heterogeneity. A pooled \( \chi^2 \) was used when heterogeneity was nonsignificant at \( P \leq 0.05 \). Differences in attractiveness of females and males to virgin males were determined by \( \chi^2 \times 2 \times 2 \) contingency table tests (\( P \leq 0.05 \)).

Attractiveness of Hexane Washes of Female Whole Bodies and Excised Ovipositors. Hexane washes of virgin female whole bodies, excised ovipositors, and whole bodies without ovipositors were bioassayed to compare their attractiveness to virgin males. Groups of 40 virgin females were collected at 0800 hours and cooled at 5°C in a refrigerator for several min. Females with extended ovipositors were placed individually on clean, glass slides. Ovipositors were fully extended by gently pressing the abdomens, then excised with a scalpel and pulled away from the bodies. The ovipositors and the bodies of 40 females were soaked separately in 300 μl of hexane for 5 min. Both washes were pipetted onto separate filter paper discs (2.2 cm diam) and air-dried for 3 min. Discs were pinned to stainless steel screens (3.8 cm diam) and placed into the holding chambers of the olfactometer. Attractiveness of the ovipositor wash and the body wash was tested against each other in the same test. Hexane solvent and hexane washes of female whole bodies (40 FE) also were bioassayed in separate tests. These tests were conducted between 0800 and 0930 hours. Additional bioassays were conducted between 1800 and 2100 hours to compare the attractiveness of hexane washes of female whole bodies made in the morning with those made in the evening. Methods (number of females and wash preparations) were the same as those in previous tests. Washes prepared in the morning were held in a refrigerator at 5°C until tested in the evening. (These bioassays were conducted in the evening to determine if males respond late in the photoperiod.) All tests were replicated four or five times, using washes of new females for each replicate. Twenty virgin males were used for each replicate and allowed to respond for 20 min. Differences in attractiveness were analyzed by \( \chi^2 \) tests for significance and heterogeneity.

Role of Ovipositor Extension in Attraction and Mating Behavior. Twenty newly emerged females were collected at 0600 hours and confined by a clear plastic cage on wheat seedlings. Females were observed for ovipositor extension or retraction at 0630, 0800, 1000, 1400, and 1800 hours for three 24-h periods. Ten newly emerged females also were placed one at a time in a cage with five virgin males. Females were observed for ovipositor extension or retraction before mating, during copulation, and after mating until oviposition began. Observations also were made on 10 single female-male pairs to determine if females mate more than once. All observations were made in an environmental chamber at 21 ± 1°C, 60 to 70% RH and a photoperiod of LD 12:12 (0600–1800 hours).

Effect of Female Age and Time of Day on Mating and Attraction. Females that emerged between 0600 and 0800 hours were collected in groups of 10 every 10 to 30 min. Each group was caged on wheat seedlings and labeled for date and time of eclosion. Females were held in an environmental chamber at 21 ± 1°C, 60 to 80% RH, and a photoperiod of LD 12:12 (0600–1800 hours) until they reached a specific age. Fifteen males (<12 h old) were released with each group when females were at the ages shown in Fig. 1 (mating success). The times of day of the matings were ca. 0630, 0800, 1000, 1400, and 1800 hours for three 24-h periods and were equivalent to female ages. After a 30-min mating period, individual females were caged on wheat seedlings for oviposition. Mating success of each female was determined 7 days later by examining plants for larvae. Foster and Taylor (1975) showed that egg hatch and migration of larvae to the base of the plants required 4 days at 21 ± 1°C. Mating success was deter-
Table 1. Response of virgin male Hessian flies to virgin females, mated females, mated males, and virgin males

<table>
<thead>
<tr>
<th>Attraction test</th>
<th>No. of replicat-</th>
<th>Total no. of δ♀ responding to:</th>
<th>χ² Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ionsa</td>
<td>A vs. B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virgin 99 (A)</td>
<td>10</td>
<td>139 10</td>
<td>111.68b</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>vs. control (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mated 99 (A)</td>
<td>4</td>
<td>6 8</td>
<td>0.29b</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>vs. control (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mated δ♀ (A)</td>
<td>8</td>
<td>62 37</td>
<td>27.85</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>vs. control (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virgin δ♀ (A)</td>
<td>8</td>
<td>17 15</td>
<td>0.13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>vs. control (B)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

a Each replicate represents 40 females or males as attracting flies and 20 males as responding flies.
b Pooled χ² value.

Results and Discussion

Attractiveness of Live Flies. Responses of virgin males to virgin females, mated females, mated males, and virgin males are presented in Table 1. Males were highly attracted to virgin females with ca. 70% response. Only 5% of the males responded to the control and 25% failed to respond. Most of the males responded by flying to the source. Males were not responsive to flying to the source. Males were not responsive to flying to the source. Mated males attracted significantly more virgin males than the control. Heterogeneity among tests was significant, an indication that the mated males varied in their attractiveness. There was no significant attraction of virgin males to virgin males. Virgin males did not attract virgin females used as responding flies.

Virgin females were significantly (P ≤ 0.05) more attractive to virgin males than were mated females. Mated males were significantly (P ≤ 0.05) more attractive than virgin males. Thus, these results strongly indicated the presence of a female sex pheromone which elicits male response. Mat- ing may suppress pheromone release or perhaps production. The attraction of virgin males to re- cently mated males suggested that the pheromone was transferred to males during copulation.

Attractiveness of Hexane Washes of Female Whole Bodies and Excised Ovipositors. Male responses to hexane washes of female whole bodies and excised ovipositors made and tested between 0700 and 0930 hours are shown in Table 2. Male response to hexane alone was not significantly differ- ent than to the control. Significant heterogene- ity of hexane versus control was due to reversal of male response in one replicate. The hexane wash of female whole bodies was highly attractive to males with 74% of the males responding. Only 2% of the males were trapped in the control tube. In bioassays of excised ovipositor wash versus the wash of whole bodies without ovipositors, males showed a significantly greater response to the ovipositor wash (81%). Male response to the wash of whole bodies without ovipositors (4%) was similar to male response to the hexane solvent (2%). Thus, these results suggest that the ovipositor is the pheromone release site and possibly the source of production.

The comparative attractiveness of female whole body washes made in the morning and those made in the evening are shown in Table 3. Heteroge- neity was not significant in any of the tests. The whole body wash made at 0715 hours was significantly more attractive to virgin males than was

Table 2. Response of virgin male Hessian flies to hexane washes of whole bodies and excised ovipositors of virgin females

<table>
<thead>
<tr>
<th>Attraction test</th>
<th>No. of replicat-</th>
<th>Total no. of δ♀ responding to:</th>
<th>χ² Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ionsa</td>
<td>A vs. B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexane solvent (A)</td>
<td>4</td>
<td>3 10</td>
<td>3.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>vs. control (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole body wash (A)</td>
<td>5</td>
<td>74 2</td>
<td>68.21b</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>vs. hexane solvent (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovipositor wash (A)</td>
<td>4</td>
<td>65 3</td>
<td>56.35b</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>vs. whole body w/o ovipositor wash (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

a Each replicate represents 40 female equivalents as the attractant and 20 males as responding flies.
b Pooled χ² value.
the hexane solvent. In contrast, the attractiveness of the whole body wash made at 1900 hours was not significantly different than the hexane control. In the comparison tests, whole body washes made at 0830 hours were significantly more attractive to males than to the whole body washes made at 1900 hours with 50 and 14% of the males responding, respectively. Differences in the attractiveness of washes made in the morning and evening suggest that pheromone production and release may be controlled via a diurnal rhythm. Males do not appear to have a daily rhythm of sexual behavior, because they responded to specific washes in both morning and evening tests.

Role of Ovipositor Extension in Attraction and Mating Behavior. Female behavior of extending and retracting her ovipositor also showed a diurnal rhythm. Ovipositor extension occurred within 30 min after female eclosed. Ovipositors were constantly extended until ca. 1000 hours. Females began retracting their ovipositors between 1200 and 1300 hours. Ovipositors were constantly retracted between 1400 and 1800 hours.

Ovipositors were extended before and during the initiation of mating. Males were attracted only to females with extended ovipositors. In single-pair matings, males located the female and initiated mating within 4 min. Males, usually in flight, approached females directly from the rear or side and mated in a side-to-side position. Males did not show any courtship behavior before mating and females were nearly always receptive on the first contact with males. Females retracted their ovipositors 3 to 4 s into copulation. Copulation lasted between 10 and 20 s. After flies disengaged, females did not re-extend their ovipositors and usually remained quiescent until oviposition. Similar postmating behavior has been reported for other gall midges (Metcalfe 1933, Spince 1969). Thus, this behavior may be common in cecidomyiids. Females that we observed mated only once and began ovipositing on the wheat plants within 2 h after copulating. Males did not attempt to mate with mated and ovipositing females. This observation supports the results of attraction tests (Table 1), which showed that mated females were not attractive to males.

Effect of Female Age and Time of Day on Mating and Attraction. Mating success and male response to virgin females of different ages and at different times during the photoperiod are shown in Fig. 1. Mating activity and sexual attractiveness of females showed a distinct, daily rhythm coinciding with that of ovipositor extension and retraction. Highest mating success was between 0600 and 1000 hours. Numbers of successful matings during this period were not significantly different among female ages, except for the 28- and 72-h-old females. The decrease in mating success of 28-h-old females cannot be explained since mating successes of 4- and 52-h-old females also at 1000 hours were high and not significantly different. The 24-h-old females had a significantly higher mating success (85%) than the 72-h-old females (62%). Thus, in the laboratory environment, female age up to 72 h had little effect on mating success. Mating activity showed a marked decline at 1400 and 1800 hours. Mating successes during this period were not significantly different among female age groups and ranged from 0% for 12-h-old females to 13% for 56-h-old females.

Female attractiveness as measured by male response in olfactometer tests also showed a daily

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**Table 3. Response of virgin male Hessian flies to hexane washes of whole bodies of virgin females made at different times of day and tested between 1800 and 2100 hours**

<table>
<thead>
<tr>
<th>Attraction test</th>
<th>Time of wash (hour)</th>
<th>No. of replications</th>
<th>Total no. M responding to:</th>
<th>( \chi^2 ) Value</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body wash (A) vs. hexane solvent (B)</td>
<td>0715</td>
<td>4</td>
<td>45 vs. 6</td>
<td>20.51</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Whole body wash (A) vs. hexane solvent (B)</td>
<td>1900</td>
<td>4</td>
<td>7 vs. 5</td>
<td>0.33</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Whole body wash (A) vs. whole body wash (B)</td>
<td>0830</td>
<td>4</td>
<td>40 vs. 11</td>
<td>16.49</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

* Each replicate represents 40 female equivalents as the attractant and 20 males as responding flies.

* Pooled \( \chi^2 \) values.

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**Fig. 1.** Effect of female age and time of day on mating success and female attractiveness as measured by male response. Shaded areas indicate scotophase.
rhythm. Males were most responsive to 2-, 4-, 24-,
and 48-h-old females at 0800, 1000, 0800, and 0800
hours, respectively. Males showed a weaker but
significant response (48%) to 72-h-old females at
0800 hours. Males were not attracted to 8-, 12-,
and 36-, and 60-h-old females at 1400, 1800, 1800
hours, and 1800 hours, respectively. Heterogeneity
was significant for male response to 60- and 72-h-
old females. In comparison tests, 4-, 24-, and 48-
h-old females were similarly attractive early in the
photophase, and 8-, 12-, 36-, and 60-h-old females
were similarly unattractive late in the photophase.
Attractiveness of 2- and 72-h-old females were sig-
nificantly different than all other female age
groups. These results support the results of pre-
vious olfactometer tests (Table 3), which showed
that hexane washes of virgin females made in the
evening were unattractive to males, and are fur-
ther evidence that pheromone release occurs at
specific times during the day.

These results clearly demonstrate the presence
of a female sex pheromone in the Hessian fly and
indicate that the ovipositor is the site of phero-
mones release. Pheromone release may be con-
trolled by the female extending or retracting her
ovipositor, a behavior that appears to be manifest-
ed in a diurnal rhythm. These results and earlier
observations on sexual attraction of Hessian fly fe-
male (Cartwright 1922) suggest that the phero-
mones attract males from a distance and stimulates
mating.

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alomya hirtipes O.S. (Diptera:Cecidomyiidae) on So-

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